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Increasing trends in faecal pollution revealed over a decade in the central Adriatic Sea (Italy)

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ABSTRACT

Faecal contamination of the coastal sea poses widespread hazard to human and environmental health and is predicted to rise in response to global change and human pressure. For better management and risk reduction it is thus imperative to clarify and predict trends of faecal pollution over spatial and temporal scales, and to assess links with climate and other variables. Here, we investigated the spatio-temporal variation in the Faecal Indicator Bacteria (FIB) *Escherichia coli* and enterococci, over a time frame spanning 11 years (2011–2021) along a coastal area covering approximately 40 km and 59 bathing sites in the Marche region (Adriatic Sea, Italy), characterized by intense beach tourism, high riverine inputs, resident population, maritime traffic and industrial activities. Our analysis, that considers 5,183 measurements during the bathing season (April to October), shows that FIB abundance varied significantly among years. A general, although not significant, increase over time of both FIB was observed, mainly due to a general reduction of structural zeros (i.e., zeros originated from the actual absence of the response variable) over the examined time period. FIB abundances displayed their maxima and minima in different years according to the municipality, with overall peaks recorded in different months (May-June or September), whereas the lowest values were always observed in October. FIB levels were not significantly related neither to rainfalls nor to river discharge, but the activation of combined sewer overflows (CSOs), typically occurring after intense rainfall events, appeared as a necessary condition for the high faecal contamination levels. Considering climate change scenarios predicting significant increases in extreme weather events, our findings support the usefulness of analysing long-term trends to identify pollution sources, and the prioritization of control strategies to better manage the release of microbial pollutants from combined sewer overflows in coastal waters to reduce human risks.

1. Introduction

Faecal contamination of coastal water causes major public health concern, with new challenges necessitating the urgency in developing fast and reliable methods to monitor contaminants and aimed at preventing human exposure (Griffith et al., 2009; Holcomb and Stewart, 2020). Humans exposed to contaminated water and sediments in coastal areas are at increased risk of waterborne infections such as gastroenteritis, dermatitis and other illnesses (Bonilla et al., 2007; Heaney et al., 2012). Globally, an estimated ca. 120 million cases of gastrointestinal diseases and ca. 50 million cases of respiratory diseases are caused each year by swimming in wastewater-polluted waters (H. Shuval, 2003). Faecal pollution leads to poor quality bathing water and causes major economic losses, that include increased water treatment costs to ameliorate source water quality, beach closures and compromised shellfish fisheries (Rabinovici et al., 2004). In light of all these concerns, the need is prompted to better understand trends and factors affecting faecal pollution, also over large spatial and temporal scales and to improve managing microbial pollution in the coastal sea.

Faeces-associated microorganisms reach the coastal sea via specific ("point"; e.g., discharge from wastewater treatment plants or run-off from farms) and diffuse ("non-point"; e.g., stormwater runoff and

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sediment resuspension, transport of faecal and potentially pathogenic bacteria by bathers) sources (Elmir et al., 2007; Solo-Gabriele et al., 2016; Unno et al., 2018), that convey treated or untreated faecal matter to waterways (Molina et al., 2014; Basili et al., 2022 and 2023). Faecal pollution is often the result of storm water overflows of sewage or water draining from farms and farmland (Niu and Phanikumar, 2015). Such pollution increases during heavy rains and floods, when pollutants are transported into rivers and seas (Ahmed et al., 2019). High touristic fluxes and increased bather densities at recreational beaches, as well as urbanisation (Elmir et al., 2007; Cahoon et al., 2016; Weiskerger et al., 2019), are also significant contributors to faecal pollution in coastal ecosystems. Despite the role played by these factors has been linked to the trends and magnitude of faecal pollution (Parker et al., 2010; Powers et al., 2020), inter-sites variations may still occur depending on the hydrological, geographical and urbanisation-related features of the interested areas. This highlights the need of further efforts to identify sources of faecal bacteria, as well to understand the behaviour of faecal pollutants over long temporal scales.

The expected alterations in temperature, precipitations, sea level and storm intensities worldwide due to the climate change (IPCC, 2018) are predicted to have profound impacts on microbial quality of water resources (Weiskerger et al., 2019; Burge et al., 2014; Levy et al., 2016; Kapoor et al., 2018; Yu et al., 2018; Miller and Hutchins, 2017), with direct consequences on the infectious disease burden from exposure to pathogens in recreational waters (Semenza and Menne, 2009; Sterk et al., 2013). Climate change has the potential to influence levels of waterborne pathogens and FIB by increasing inputs of pollutants, and by altering transport pathways and environmental stressors (Weiskerger et al., 2019). The level of faecal bacteria may increase in soils and streams by the end of this century (Jeon et al., 2019), with consequent effects on the contamination of the coastal sea (Sterk et al. 2016). Extraordinary strong storm events have become more frequent in many areas in the last decade and are predicted to further rise in their frequency. During these events, combined sewer overflows (CSOs) discharging high loads of faecal bacteria in the receiving water bodies have become common (Al Aukidy and Verlicchi 2017; Botturi et al. 2020), especially when a dry period occurs before the event (Manini et al., 2022), triggering pollution events that lead on the short term to exceedance of the bathing water quality standards (Ferrarin et al., 2021). Such events might thus lead to likely, yet unpredictable, consequences on persistence and distribution of faecal bacteria over long temporal and spatial scales and might exacerbate contamination of large coastal areas. In this regard, indications of increasing trends in faecal pollution are emerging in the US. Tomenchok et al. (2021), observed that faecal contamination in recreational beaches across south Florida increased during 2015–2019 compared to the prior 15-year period. Also Powers et al. (2021) recently provided the first comprehensive decadal assessment of faecal pollution across coastal Texas, reporting an increase in pollution related to population size and sea level. In another long-term study in California, a six-years long water quality data set was analysed and the discharge from a local lagoon found to be a significant source of faecal pollution (Riedel et al., 2015). Similar studies on decadal trends in faecal pollution in areas of the Mediterranean Sea, a highly anthropized basin subjected to high levels of pollution, haven't been performed yet.

Routine surface water quality monitoring is mainly based on the cultivation of the Faecal Indicator Bacteria (FIB) *Escherichia* (*E.*) *coli* and intestinal enterococci (Field and Samadpour, 2007), which indirectly indicate the possible presence of pathogenic microorganisms. In the EU, the provisions regulating bathing water quality (Bathing Water Directive, BWD, 2006/7/EC which indicates, for marine bathing waters, contamination levels below 200 CFU or MPN/100 ml for enterococci and below 500 CFU or MPN/100 ml for *E. coli*) involve monitoring of these two bacterial indicators to determine if surface waters impacted by sewage are safe for swimming and other recreational activities. Although limitations of using FIB monitoring have long been acknowledged (Field and Samadpour, 2007; Stewart et al., 2008), FIB provide a simple and reasonably reliable tool to assess bathing water quality (Holcomb and Stewart, 2020). The huge amount of data on the FIB levels released by public monitoring Agencies represents thus an invaluable wealth of information to assess spatial patterns and trends of faecal contamination in anthropized coastal areas. However, only rarely these long-term datasets have been exploited to better understand the trends, distribution and fate of faecal pollutants into shoreline waters, or have been related to trends in environmental and social variables. Characterizing long-term trends in coastal water quality is critical given the current and projected rates of population growth, human pressure on the coast and climate change (Parker et al., 2010).

With a total number of 5524 bathing waters in 2021 over the total of 21,859 bathing waters in Europe (EEA 2022), Italy ranks number one across the EU in terms of bathing water sites, with a number of waters (122, corresponding to 1.8 %) displaying poor water quality. Located in the Adriatic Sea, the coast of the Marche Region reaches the total length of ca. 180 km and is characterized by several urban settlements and popular bathing locations, that represent a resource of great economic and environmental importance (Penna et al. 2021; Ferrarin et al., 2021). However, given the high number of rivers that collect urban, zootechnical and industrial pollutants and discharge them into the sea (Basili et al., 2021), problems persist at bathing waters having poor quality or bathing waters that are often affected by short-term pollution, where exceedances of contamination levels are sometimes experienced leading to beach closures. Because of all these features, this area represents an ideal site where to study long-term trends of water quality and identify human and environmental drivers of long-term pollution.

In this study, we retrospectively evaluated and analysed a large set of data on FIB abundances along a coastal area of the Marche region (about 48 km) over 11 years (2011–2021), and related them to precipitations levels, rivers' flow and CSOs, with the primary objectives of: i) assessing the spatio-temporal variation of the faecal contamination over a decade, ii) testing the hypothesis that FIB levels increase over the analysed period and iii) exploring the role of potential factors able to explain the faecal contamination, including precipitation intensity (also considering events of extreme rainfall), river level and CSOs. Because of the magnitude of the dataset analysed, we expect our study will improve our understanding of the future variability of faecal contamination in the coastal sea, as well as in identifying more effective management plans to contain microbial pollution in coastal waters.

2. Materials and methods

2.1. Site description

The study has been conducted in the Marche Region (Central Adriatic sector) in four main areas that are grouped according to the municipality (Ancona, Falconara Marittima, Montemarciano and Senigallia) and encompass an overall length of about 48 km of coastline. For each municipality, 12 to 18 sampling points were monitored in the period comprised between May 2011 to October 2021 (18 in Ancona, 13 in Falconara Marittima, 12 in Montemarciano, 16 in Senigallia), for a total of 59 sampling stations each corresponding to a bathing site (Supplementary Table S1). Considering that a low tidal excursion occurs along the Marche Region coastline, water inputs from rivers in this region are subjected to a variety of physical drivers (e.g., wind, currents), which have been extensively described in Baldoni et al. (2022). The study area has a consolidated tourism-based economy and represents a popular summer holiday destination. However, in certain areas during summer, sewage overflows do occur, causing short term pollution events that lead to beach closures caused by the faecal contamination of bathing water, which consequently negatively impacts on tourism and related economic activities (Penna et al., 2021). Our study area is under the influence of two rivers, the Esino (close to Falconara Marittima) and the Misa (close to Senigallia) representing sources of chemical and microbial pollutants to the coastal sea (Basili et al., 2021). Other sources of pollution in the studied area are the city of Ancona with its large harbour, one of the largest in the Adriatic Sea hosting an intense touristic, commercial and fishery-related ship traffic (already reported to be severely contaminated by FIB; Luna et al., 2019), and Falconara Marittima that hosts an oil refinery (Capotondi et al., 2015).

2.2. Sample collection and analysis of FIB

Data on faecal pollution were provided by ARPA Marche (Marche Regional Agency for Environmental Protection) and are the same data deposited in publicly available databases (www.arpa.marche.it; www.portaleacque.salute.gov.it/PortaleAcquePubblico/homeBalneazione.do?lang=it). Concentrations of *E. coli* and intestinal enterococci were measured by the reference culture methods as detailed in the National and European Regulations (D. Lgs. 116/08 and Decreto 30 March 2010; Bathing Water Directive, BWD, 2006/7/EC). Data are available as concentrations of *E. coli* (n*/100 ml) and e nterococci (n*/100 ml), where ”n” refers to CFU (Colony Forming Units, as for the membrane-filtration-based methods EN ISO 9308–3 for *E. coli* and EN ISO 7899–1 for enterococci) or MPN (Most Probable Number, as for the MPN-based methods ISO EN ISO 9308–3 for *E. coli* and EN ISO 7899–1 for enterococci). For MPN-based methods, the limit of quantification (LOQ) is 10 cells/100 mL; in case of MPN data <LOQ, based on the indications provided by the Italian Ministry of Health and the Istituto Superiore di Sanità, data are treated as following: MPN $<$ 10 cells/100 mL $=$ 10 cells/100 mL. Surface seawater samples for the analysis on FIB concentrations were collected following the monitoring procedure used by ARPAM as requested by the abovementioned National and European Regulations. According to the Bathing Water Directive, compliant contamination levels for recreational activities in marine waters are set below 200 CFU or MPN/100 ml for enterococci and below 500 CFU or MPN/100 ml for *E. coli*. Sampling activities, planned *a priori*, typically covered the bathing season, starting at the beginning of May until the end of September-beginning of October, depending on the year. A monthly sampling was performed in the selected stations, except for more critical points where sampling efforts were usually increased at two sampling events each month. In the Marche Region, in case of intense rainfall events, when the amount of runoff exceeds the capacity of the system, CSO events occur and local authorities are alerted by remote (automatic) monitoring. If the alert occurs during the bathing season, a bathing water closure order is issued with the aim of preserving bathers' health. Some of the stations experienced FIB concentrations that exceeded the admitted threshold in some instances. Seawater samples were collected at bathing coastal sites with an average depth between 80 and 120 cm, as required by current regulations.

2.3. Meteorological, hydrological and CSO events

The meteorological and hydrological data were extracted from the online service SIRMIP (Regional Meteo-Hydro-Pluviometric Information System), managed and made available upon registration by the Marche Region - Civil Protection Service. (http://app.protezionecivile.marche.it/sol/indexjs.sol?lang=en). The stations of interest for the present study are listed in Supplementary Table S2. The data extracted are the cumulative daily precipitation and the hydrometric level (valid flow data were not available). The CSO events employed in this study were provided by the Marche Region - Water Protection Directorate. According to current regional directives, the local integrated water service management bodies report the activation of the CSOs. In correspondence to these events, as a precaution, a bathing ban order is issued by the mayor and ARPAM checks the faecal contamination levels. When the FIB abundancies reach compliant levels, recreational bathing waters are re-opened.

2.4. Statistical analysis

The presence of outliers in both *E. coli* and enterococci data was firstly assessed using the cleveland dotplot (Chambers et al., 1983). As their number was very limited ($n = 3$ in the whole dataset, see Figure S1), these values were nonetheless included in the following analyses. The correlation between *E. coli* and enterococci concentrations was assessed both with the Pearson correlation coefficient *r*, that assumes the occurrence of a linear relationship between two normally distributed variables, and with the Kendall rank correlation coefficient *tau*, that assumes neither a linear relationship nor a normal distribution for the two variables (Stanton, 2012). As the FIB data analysed (i.e., abundances of *E. coli* and enterococci) were “zero-inflated”, i.e., with a quite high number of zeros, exceeding that of any standard distributions (Heilbron 1994; Martin et al. 2005), in order to assess the possible associations between factorial covariates (i.e., Year, Month, Municipality) and response variables (i.e., *E. coli* and enterococci abundances), we performed the non-parametric Kruskal-Wallis Analysis of Variance (ANOVA) followed by the Dunn *post-hoc* tests with the Bonferroni correction for multiple comparison. The Dunn test was chosen as it is appropriate when the cell sizes are unbalanced and is less conservative and more powerful than the Steel-Dwass post-hoc test (Zar, 2014). For the temporal trend, it was assumed that the zeros in FIB values were originated both from the actual absence of the response variable, hereafter referred to as “structural zeros”, and from the incapability of detecting the response variable, hereafter referred to as “sampling zeros”. Sampling zeros were therefore considered to belong to the same statistical distribution of the positive values (counts) of the respective response variable. The temporal trend of the zero-inflated FIB data was analysed using the *zeroinfl* function from the R package *pscl* (Jackman, 2024). As time covariate we used the number of weeks elapsed since the first sampling. The *zeroinfl* function allows to separate the structural zeros from the sampling zeros and to assess, separately, their relationship with the time covariate (Zeileis et al., 2008). To separate structural and sampling zeros, the function *zeroinfl* applies a mixture of two distributions: a binomial distribution to separate the structural from the sampling zeros and the distribution describing the counts (Zeileis et al., 2008; Martin et al., 2005; Feng, 2021).

For the count distribution, the negative binomial was chosen as count model family. Statistical analyses were performed in the R environment (RStudio Team, 2020) (version 4.2.3). The list of all R packages used in this study is reported in Supplementary Table S3. For the statistical tests, a p value $\leq$0.05 was considered statistically significant. In this study, in order to present the results in a more informative way, we decided to use the means instead of the medians, although the latter are more appropriate in case of skewed data, as the medians were very often equal to zero due to the zero-inflation occurring in the data.

3. Results

3.1. Exploratory data analysis

Exploratory analyses of the entire dataset highlighted the presence of few outlier values for both *E. coli* and enterococci abundances (Figure S1, Panel A and B, respectively). Such data were however considered for further analyses. When considering the whole dataset, concentration of *E. coli* and enterococci were significantly and positively correlated with each other (Pearson $r = 0.791$, Kendall tau $= 0.568$) (Figure S1, Panel C).

3.2. Variations in FIB abundances according to year

The non-parametric omnibus ANOVA analysis indicated that *E. coli* abundances changed with the year ($\chi^2 = 1092.8$, df $= 10$, $p < 0.001$) (see Supplementary Table S4 for the Dunn's post-hoc tests; summary statistics in Supplementary Table S5). Mean values of *E. coli* abundances ranged from 10 to 20 CFU/100 ml between 2011 and 2014; a sharp increase up to 94 CFU/100 ml was observed in 2015 (no. data = 491), whereas a drop to 51 CFU/100 ml in 2016 (no. data = 553), followed by an "hump" with a maximum of 80 CFU/100 ml in 2018 (no. data = 583, were recorded (Fig. 1A). A small rebound can be noted in 2021 (Fig. 1A).

Similarly, for enterococci, the non-parametric omnibus ANOVA analysis showed a significant effect of the factor year ($\chi^2 = 303.98$, df = 10, $p < 0.001$; see Supplementary Table S6 for Dunn's post-hoc tests; summary statistics in Supplementary Table S7). In Fig. 1A, the mean values of enterococci as a function of the year are shown. The mean values of enterococci abundances (including also all the zero values) showed an apparent increasing trend is clear with some up and down along the period. The peak values, about 45 CFU/100 ml, were reached in year 2017 (no. data = 513) and 2019 (no. data = 537), while the minimum was recorded at the beginning of the sampling.

3.3. Variations in FIB abundances according to month

The non-parametric omnibus ANOVA analysis showed a significant effect of the factor "month" on *E. coli* abundances ($\chi^2 = 74.773$, df = 6, $p < 0.001$; see Supplementary Table S8 for Dunn's post-hoc tests; summary statistics in Supplementary Table S9). *E. coli* reached the highest abundance values in May and June (Fig. 1B) (about 60 CFU/100 ml, with the number of data equal to 1009 and 1002, respectively); a decreasing trend was observed up to October, when the mean value was even lower than in April (which typically anticipates the beginning of the touristic season).

Similar results were found also for enterococci ($\chi^2 = 41.168$, df = 6, $p < 0.001$; see Supplementary Table S10 for Dunn's post-hoc tests; summary statistics in Supplementary Table S11). The highest values of enterococci abundances (about 65 CFU/100 ml) were observed in May (no. data = 291), with a decreasing trend afterward (Fig. 1B).

3.4. Variations in FIB abundances according to the municipality

Abundances of *E. coli* and enterococci significantly differed according to the municipality. The non-parametric omnibus ANOVA results were: $\chi^2 = 483.9$, df = 3, $p < 0.00$ for *E. coli*, and $\chi^2 = 651.82$, df = 6, $p < 0.00$ for enterococci, respectively (Supplementary Tables S12, S13 for *E. coli* and Tables S14 and S15 for enterococci. The most polluted municipality was Falconara Marittima (avg. *E. coli* 90.19 CFU/100 ml [no. data = 1720] and enterococci 47.23 CFU/100 ml [no.data =,1719]), followed by Ancona (avg. *E. coli* 44.56 CFU/100 ml [no. data = 1738]) and enterococci 25.75 CFU/100 ml [no. data = 1738]), Montemarciano (avg. *E. coli* 11.89 CFU/100 ml [no. data = 817] and enterococci 5.48 CFU/100 ml [no. data = 817]) and Senigallia (avg. *E. coli* 13.94 CFU/100 ml [no. data = 1104] and enterococci 6.61 CFU/100 ml [no. data = 1104]), with the latter two displaying a comparable level of contamination (Fig. 2, panels A-C).

3.5. Spatial variations of FIB over time

A more detailed analysis according to the municipality showed that years of minima and maxima of *E. coli* abundances differed among municipalities. In more detail, in Ancona, 2017 and 2019 displayed the highest *E. coli* values (avg. values 87.95 [no. data = 168] and 88.65 CFU/100 ml [no. data = 178], respectively) and 2013 the lowest (4.05 CFU/100 ml, [no. data = 126]); in Falconara Marittima, 2015 displayed the highest *E. coli* values and 2013 the lowest (211.83 [no. data = 176] and 26.54 CFU/100 ml [no. data = 105], respectively); in Montemarciano and Senigallia, 2019 displayed the highest *E. coli* values (35.28 [no. data = 72] and 31.74 CFU/100 ml [no. data = 96], respectively) and 2020 the lowest (0.94 [no. data = 72] and 1.15 CFU/100 ml [no. data = 96], respectively). Considering FIB data according to the municipality and month, we observed that lowest values of *E. coli* were reported, at all municipalities, in October (Ancona: 6.56 CFU/100 ml [no. data = 18]; Falconara Marittima: 0.62 CFU/100 ml [no. data = 13]; Montemarciano: 1.25 CFU/100 ml [no. data = 12]; Senigallia: 1.25 CFU/ml [no. data = 16]). Conversely, maxima of this FIB differed at the different locations, with May being the month with the highest values of *E. coli* at Ancona (85.86 CFU/100 ml [no.data = 334]), June at Falconara Marittima (109.88 CFU/100 ml [no. data = 366]) and September at Montemarciano and Senigallia (28.65 CFU/100 ml [no. data = 132] and 25.48 CFU/100 ml [no. data = 178], respectively). *E. coli* abundances typically displayed constant values during summer months, i.e., between May-June and August.

Years of minima and maxima of enterococci significantly differed among municipalities. In more detail, 2011 represented the year with lowest enterococci values in Falconara Marittima and Senigallia (avg., 8.20 CFU/100 ml [no. data = 93] and 0.32 CFU/100 ml [no. data = 96], respectively), whereas 2013–2014 displayed the minima for this FIB in Ancona (about 5.4 CFU/100 ml [no.data = 126 and 138, respectively]) and 2020 in Montemarciano (avg., 0.86 CFU/100 ml [no. data = 72]). Similarly, the highest values for enterococci were recorded in different

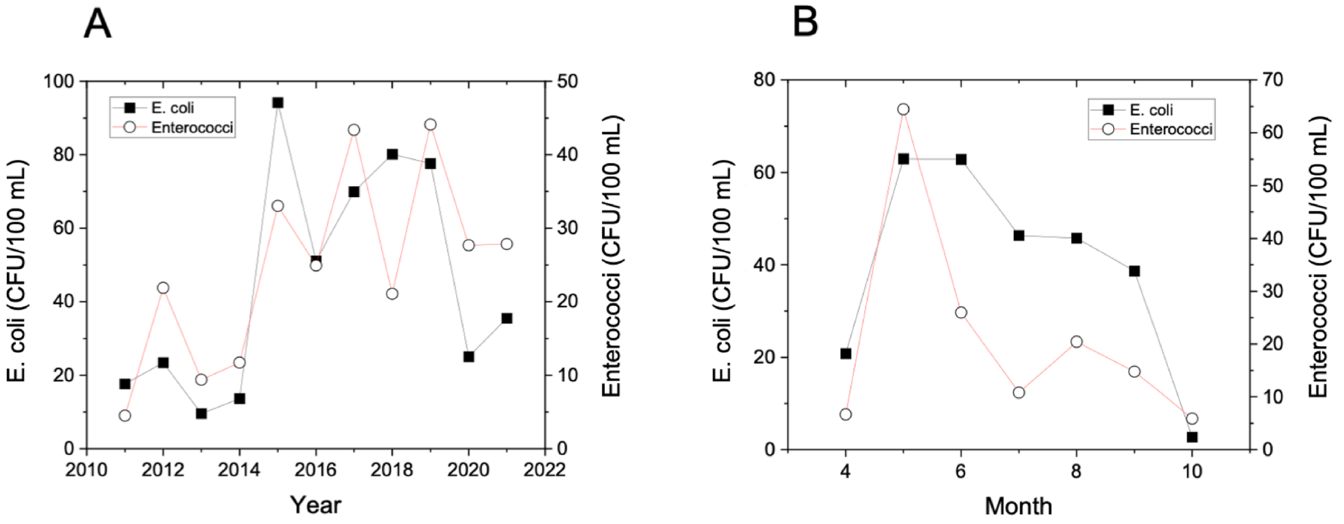

Fig. 1. Plots showing the mean values (CFU/100 mL) of *E. coli* (left y-axis) and enterococci (right y-axis) as a function of the year (panel A) and month (panel B; on the x-axis, "4" = April, "6" = June; "8" = August, "10" = October). The mean values were calculated including all the values, i.e., also all the zero values (see also Supplementary Table S5 and S7).

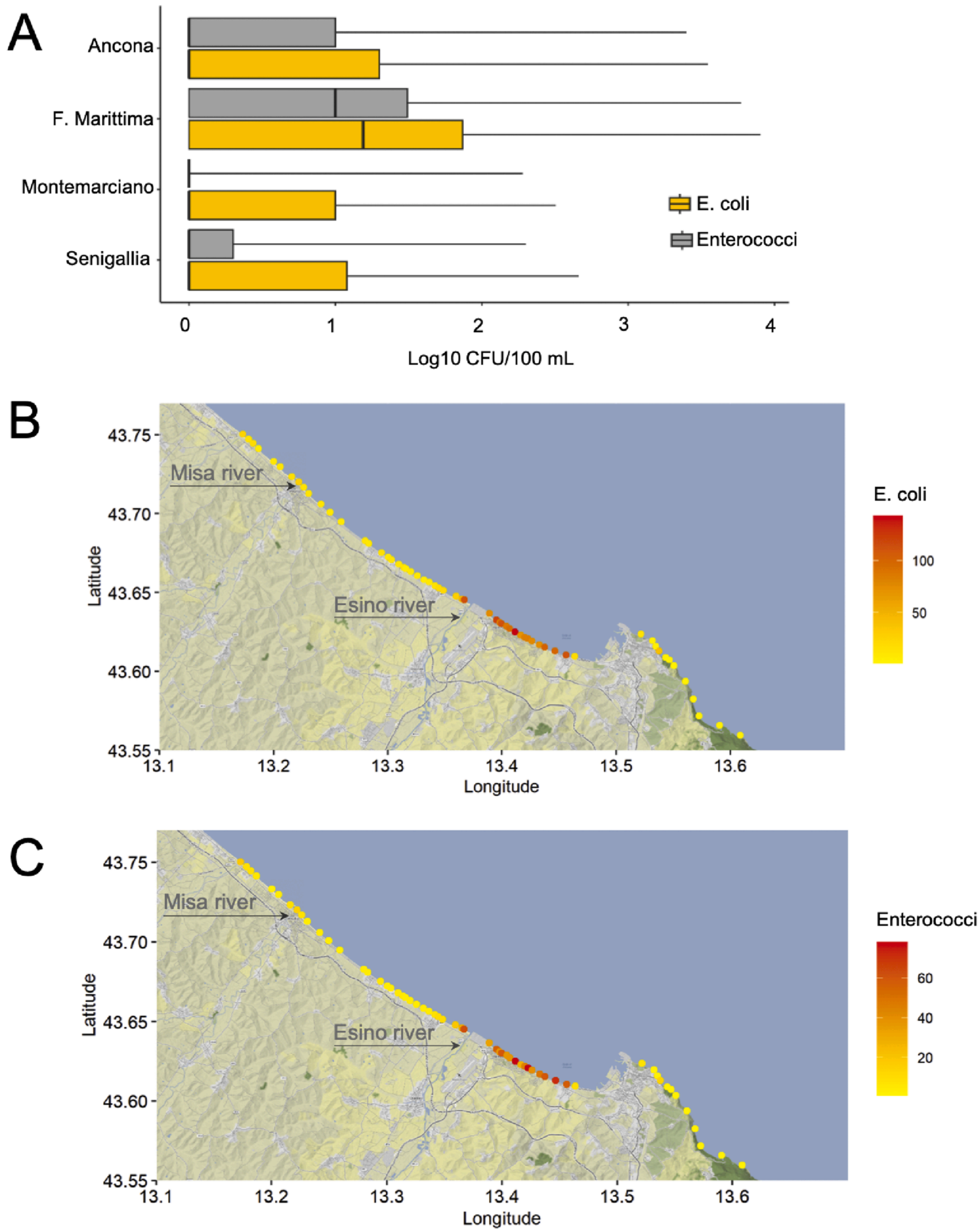

Fig. 2. Mean level of faecal contamination in the four municipalities, as resulting from the analysis of *E. coli* and enterococci abundances of the 59 sampling stations analysed in this study. Values in the legend are reported as CFU/100 ml.

years according to the municipality, with 2017 being the maximum in Ancona (avg., 60.31 CFU/100 ml [no. data = 168]), 2015 in Falconara Marittima (avg., 79.97 CFU/100 ml [no. data = 176]), 2019 in Montemarciano (avg., 23.35 CFU/100 ml [no. data = 72]) and 2012 in Senigallia (avg., 17.84 CFU/100 ml [no. data = 96]). About municipality-month, the lowest abundances were always recorded in October at all municipalities (Ancona: avg., 5.89 CFU/100 ml [no. data = 18]; Falconara Marittima: avg., 0.54 CFU/100 ml [no. data = 13]; Montemarciano: avg., 0.50 CFU/100 ml [no. data = 12]; Senigallia: avg., 1.31 CFU/100 ml [no. data = 16]). Conversely, maxima were reported in May for Ancona and Falconara Marittima (avg., 64.48 CFU/100 ml [no. data = 334] and avg., 64.43 CFU/100 ml [no. data = 334], respectively), in September for Montemarciano (avg., 15.52 CFU/100 ml [no. data = 132]), and September and May for Senigallia (avg., about 10.9 CFU/100 ml [no. data = 178 and 197, respectively]). Similar to what was observed for *E. coli*, enterococci abundances didn't vary significantly during summer months, i.e., between May and August.

FIB pollution data were also analyzed considering FIB exceedances of water quality criteria in order to clarify changes occurring the considered time period in the selected area. Overall, we found that, at Senigallia and Montemarciano, FIB values were always below the limits indicated by the BWD, whereas variable trends in the number of exceedances were found in Ancona and Falconara Marittima. Interestingly, an overall increase in FIB abundances exceedance were observed in Ancona over time, with particular reference to *E. coli* abundances (Figure S2) and with the highest number of exceedances ($n = 11$ and 9 for enterococci and *E. coli*, respectively) observed in 2019 for both FIB. In Falconara Marittima, two main peaks in the number of exceedances were observed in 2015 ($n = 10$ and 18 for enterococci and *E. coli*, respectively) and 2019 ($n = 13$ and 14 for enterococci and *E. coli*, respectively), although periods showing low to very low numbers of exceedances were displayed before and after these peaks (Figure S2). Overall, the observed peaks reflected the minima and maxima of FIB abundances observed over time.

3.6. *Temporal regression*

The temporal regression analyses showed that the average of counts in *E. coli* (including sampling zeros) did not increase significantly over the analysed eleven years of data ($p = 0.553$; Supplementary Table S16). However, the same analysis revealed that, in the same period, the structural zeros decreased ($p < 0.001$), with a weekly decreasing rate of 1.16 % ($\exp[-0.011707] = 0.9883613$). The same temporal regression analyses showed that enterococci abundances did not increase over time ($p = 0.059$; Supplementary Table S17), whereas structural zeros decreased in the same period (p value <0.001), with a weekly decreasing rate of 1.05 % ($\exp[-0.010530] = 0.9895252$). It must be pointed out that these analyses should not be compared with those presented in Fig. 1, where all zeros were considered indistinctly, in addition to a different "granularity" in time. In this time regression, the statistical function *zeroinfl* separated the structural zeros from the sampling zeros, with only the latter being incorporated in the count model.

3.7. *Drivers of FIB abundances: analysis of precipitation data, river discharge and CSOs*

To identify the drivers influencing the observed variability across space and time, we further analysed the relationships between FIB abundance and precipitation, rivers discharge and CSOs.

Precipitation data were analysed in relation to faecal pollution based on data recorded at three municipalities, namely Ancona, Falconara Marittima and Senigallia, where such data were available. As shown in Fig. 3, we found that, in general, many peaks in FIB did not correspond to precipitation events, even when considering a few (0, 1, 2, 3, and 4) days lag. In addition, heavy precipitation events were often not associated with peaks or increase in FIB (Fig. 3, Panel A-F). Nevertheless, only in Senigallia, a closer relationship between precipitation levels and FIB abundance was observed (Fig. 3, panels E-F).

FIB concentrations were further analyzed in relation to hydrometric level data from the two most important rivers discharging in coastline here considered, i.e., the Esino river, discharging in proximity to Falconara Marittima, and the Misa river, discharging in proximity to Senigallia. The analyzed rivers' hydrometric level data start from 2016. As shown in Fig. 4, rivers' hydrometric level was not related to the increase of faecal contamination in none of the analyzed areas (Fig. 4, Panel A-D).

Finally, FIB concentrations were analyzed in relation to events of CSOs close to the Ancona and Falconara Marittima municipalities. Both for data collected in Ancona and Falconara, a high correspondence between peak values in FIB and CSOs events (all corresponding to CSOs activation) was found (Fig. 5). CSOs events appeared to be a necessary (but not sufficient) condition to observe high levels of FIB contamination, i.e., the occurrence of a CSO event didn't necessarily correspond to a peak in FIB abundances, whereas peaks in FIB abundances were always observed in correspondence to a drainage basin discharge.

4. Discussion

Due to the presence of diverse sources of human-related contaminants, coastal ecosystems, and especially those located in proximity to urbanized areas, face heightened vulnerability to faecal pollution. However, the analysis of large datasets on FIB collected within monitoring programs has been relatively infrequent, despite a large number

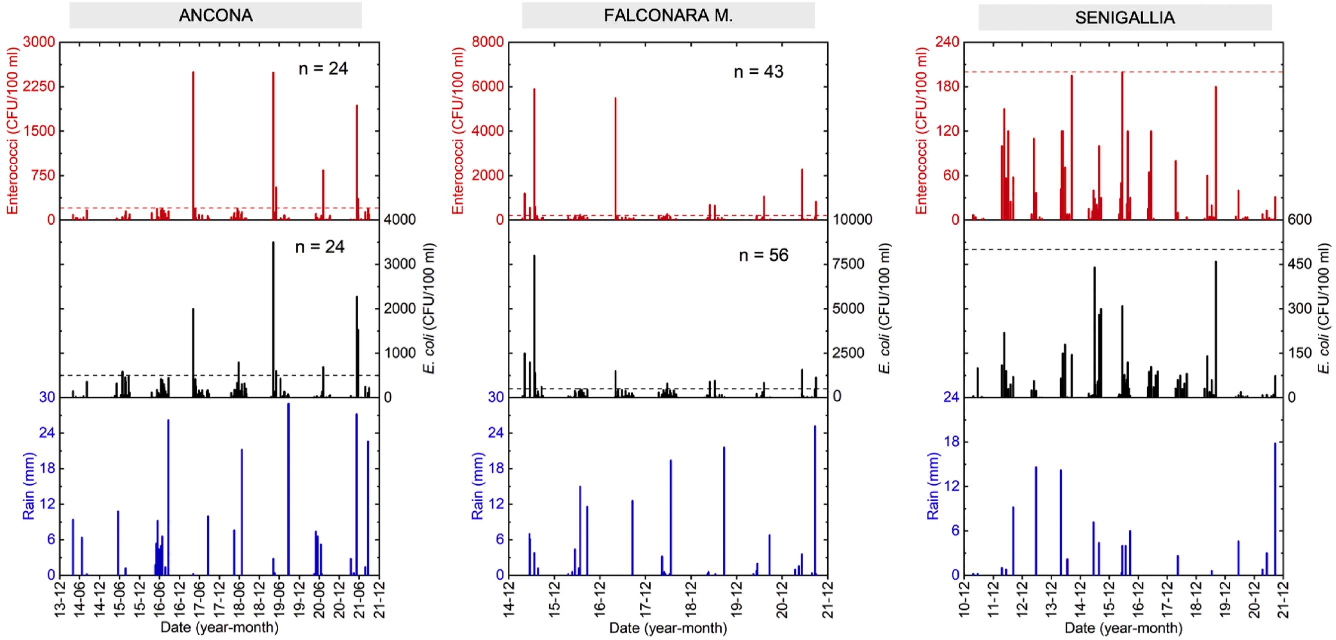

Fig. 3. Relationships between precipitation data and FIB in the analyzed dataset. Concentrations of enterococci or *E. coli* in relation to time (format yy-mm) are reported in red (upper panels) and black (mid panels), respectively. Horizontal dashed red and black lines represent the threshold for marine bathing waters as for the BWD, 2006/7/EC for enterococci and *E. coli*, respectively. The number of events of FIB exceedances of water quality criteria are reported as "n" within the relevant panel. Blue lines (lower panels) represent the level of precipitations in correspondence to the date on which precipitation data were recorded; the length of the blue line is proportional to the daily cumulative rainfall on that date. Left panels refer to Ancona, mid and right panels to Falconara Marittima and to Senigallia, respectively.

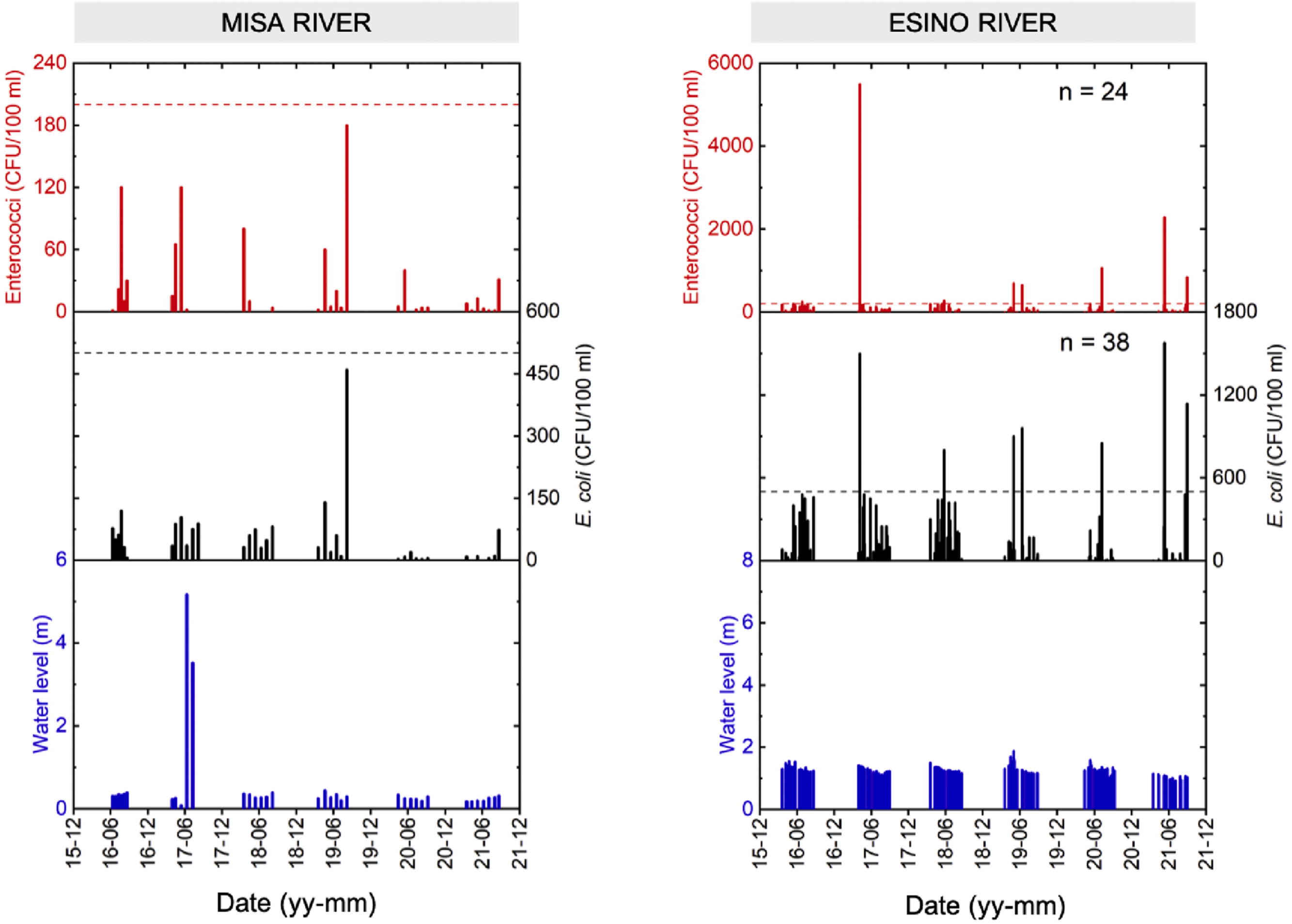

Fig. 4. Relationships between river hydrometric level data and faecal contamination in the analysed dataset. Concentrations of enterococci or *E. coli* in relation to time (format yy-mm) are reported in red (upper panels) and black (mid panels), respectively. Horizontal dashed red and black lines represent the threshold for marine bathing waters as for the BWD, 2006/7/EC for enterococci and *E. coli*, respectively. The number of events of FIB exceedances of water quality criteria are reported as "n" within the relevant panel. Blue lines (lower panels) represent river hydrometric level data; the length of the blue line is proportional to the hydrometric level on that date. Left panel= Esino river; right panel Misa river.

of data is available due to decades spent in monitoring water quality at bathing beaches for public protection purposes. Thus, it appears crucial to exploit more these datasets to comprehend the spatio-temporal trends, and to identify the key drivers of faecal contamination in coastal areas (Tomenchok et al., 2021). This objective seems especially pertinent in light of the current and predicted global change consequences (including, e.g., more frequent and intense extreme weather events, potentially leading to malfunctioning or damage of current sewage infrastructures) and the corresponding adaptive changes in microorganisms (D. Coumou and Rahmstorf, 2012; Teixeira et al., 2020). Additionally, while it has been less prevalent so far, integrating long-term data with modelling - as proposed by Coulliette et al. (2009) - remains a useful strategy to predict contamination levels once the main environmental drivers have been identified in a specific area.

By considering 11 years of FIB abundance data over a 48 km coastal strip covering 59 bathing sites, our study shows that FIB abundance in the analysed period varied significantly across time and space. Our data confirm previous findings indicating the Ancona (highly significant for tourism, commerce, and fishing) and the Falconara Marittima (a “site of national interest” due to the presence of an oil refinery) urbanized areas as those displaying the highest contamination values, in accordance to the previously suggested high influence of both anthropogenic (Capotondi et al., 2015) and zootechnical contamination sources in these two areas. The higher levels of FIB contamination observed in Ancona and Falconara Marittima, in comparison to Montemarciano and Senigallia, also suggest that different sources of contamination inputs, as well as different dynamics of FIB dispersion once released in the marine environment, occur along the analysed portion of the Marche region coastline. Indeed, several morphological characteristics differentiate Falconara Marittima and Ancona from the other sites, including the release of sewage discharge from CSOs, and, for Falconara, the presence of artificial breakwavers barriers along its coasts, which limit hydrodynamic circulation hindering a rapid dispersal of faecal contaminants.

Despite variable FIB load peaks identified according to both year and site, we observed a general, although not statistically significant, increasing trend of faecal pollution along the coastline of the Marche region here investigated. This overall increase is primarily driven by a general reduction of structural zeros over the time-period under examination. Thus far, only a small number of researches have drawn attention to the trends of faecal contamination over relatively extended time periods. A recent study analysing data collected in South Florida between 2000 and 2019 reported an increased number of advisories issued for recreational beaches, highlighting the role of the county population, air temperature, sea level, and *Sargassum* abundance as the main factors associated with such increase (Tomenchok et al., 2021). Similarly, Laureano-Rosario et al. (2021) identified precipitation, sea level anomalies, and wave height as the main environmental conditions influencing FIB abundances in Costa Rica over 15 years of collected data; however, no indications of whether FIB abundances changed over time were provided. In a more recent study, Cira et al. (2022) analysed

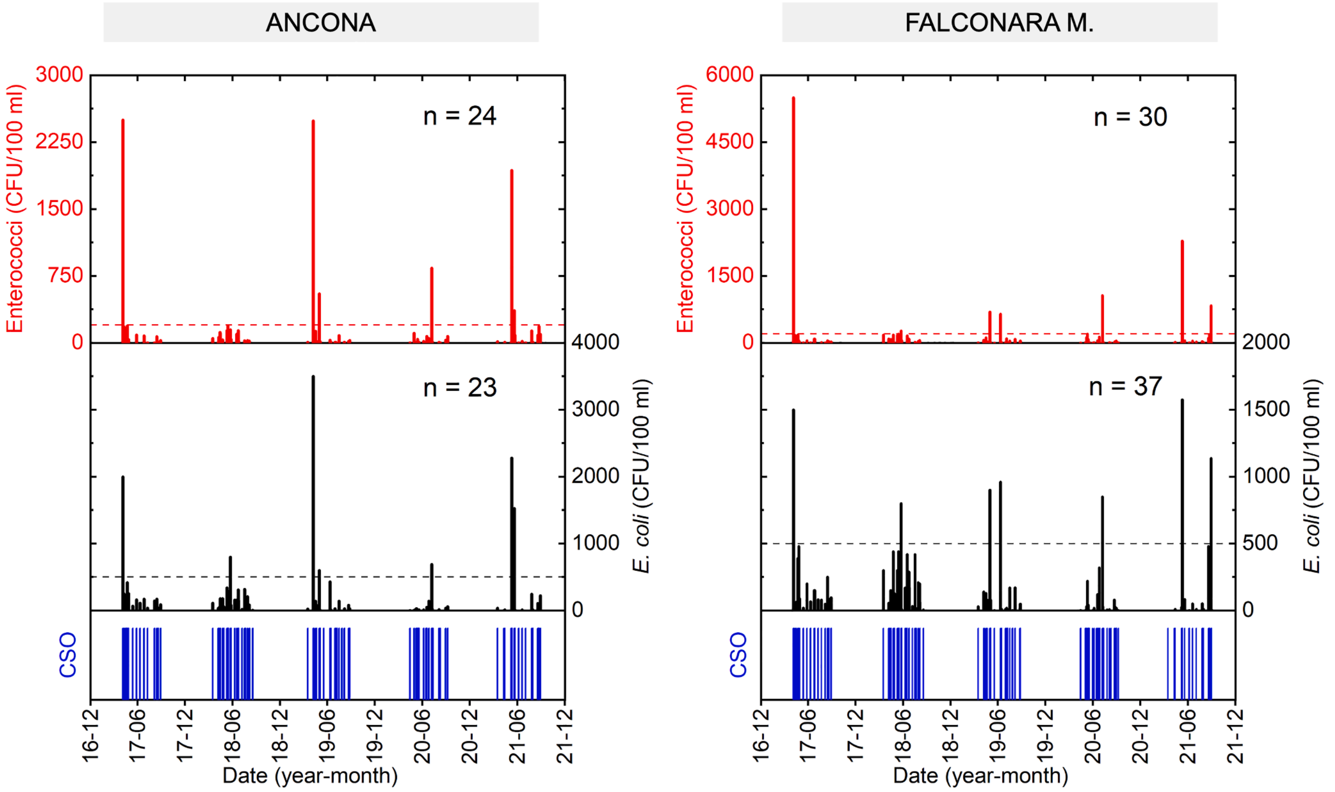

Fig. 5. Relationships between CSOs discharge events data and faecal contamination in the analysed dataset. Concentrations of enterococci and *E. coli* in relation to time (format yy-mm) are reported in red (upper panels) and black (mid panels), respectively. Horizontal dashed red and black lines represent the threshold for marine bathing waters as for the Bathing Water Directive, BWD, 2006/7/EC for enterococci and *E. coli*, respectively. The number of events of FIB exceedances of water quality criteria are reported as "n" within the relevant panel. Blue lines (lower panels) represent the date on which drainage basins were allowed to discharge and it is not a quantitative variable. Left panels = enterococci and *E. coli* abundances in relation to CSOs events in Ancona; right panels = enterococci and *E. coli* abundances in relation to CSOs events in Falconara Marittima.

long-term trends (2008–2020) in California coastal waters and found significant increase in FIB following a large fire, suggesting that sediment and microbial inputs to coastal ecosystems increased substantially post-wildfire at levels relevant to public and environmental health. The paucity of such kind of studies underlines the need for more efforts of long-term analyses of faecal contamination in coastal ecosystems. Furthermore, the general conclusion that faecal contamination is rising with time is supported by the overall increase in FIB exceedances of water quality parameters occurring during the investigated time period in the analysed area, particularly in Ancona. As Kelly et al. (2018) recently reported, a number of factors, primarily related to beach geomorphology and beach grooming activities, may influence or explain FIB exceedances at recreational beaches. The same study calls for broadening the understanding of FIB exceedances in future studies of very large public FIB databases, an aspect that is certainly worth investigating in larger portions of the coast and for larger temporal datasets available for the Marche Region.

Interestingly, faecal contamination was found to be significantly related to the sampling month in our dataset. However, conversely to what expected, no correlation was found between higher loads of faecal bacteria and summer months, when the Marche region's beaches typically experience a large number of (bathing) tourists. Our findings indicate that May showed the highest values of FIB, suggesting that other sources than tourism *per se* drive increasing faecal pollution in the analysed portion of the Marche region coasts.

Identifying environmental variables driving spatial and temporal FIB distribution is crucial to determine which of them are more likely associated to FIB variability, and to clarify the combined effects of these variables on microbial contaminants' concentrations (Tomenchok et al., 2021) also to elaborate mitigation strategies. In addition, predictive models that incorporate considerations of all respective parameters should allow for more timely beach advisories that reduce public health risks. To this end, in our study, we focused on a those variables that are expected to be relevant in our study area (i.e., precipitation intensity, river level and CSOs) and that are often reported in the recent scientific literature as those most likely determining higher loads of faecal contaminants in aquatic environments (Brandão et al., 2022; Weiskerger et al., 2019; Ahmed et al., 2018; Penna et al., 2023; Basili et al., 2021; Tomenchok et al., 2021; Manini et al., 2022).

Conversely to other studies (You et al., 2023), our approach was based on the analysis of microbial loads peaks events in correspondence to precipitations, river level increase and CSOs, rather than on correlation analyses between large sets of environmental and FIB data. In our study, heavy precipitation events were often not associated with peaks or increase in FIB, thus revealing that precipitation data alone were unable to explain FIB variability in our study area. Contrasting data have been reported on the influence of precipitations on FIB levels, with most of them suggesting an impact of rainfall levels of faecal contamination (Laureano-Rosario et al. 2021; You et al., 2023; Ackerman and Weisberg, 2003; Silva et al., 2014; Beversdorf et al., 2007; Heaney et al., 2014; Ahmed et al., 2018; Aragonés et al., 2016; Islam et al., 2017; Manini et al., 2022), and others indicating an influence only on one of the bacterial indicators (Penna et al., 2023) or according to season (Ndione et al., 2022). Stormwater runoff associated with rainfall events is well known to represent often a significant source of faecal pollution, especially in urbanized bays (Parker et al., 2010; Powers et al., 2020). Despite the analyses herein performed suggest a lack of a direct relationship between precipitations and faecal bacteria levels, we hypothesize that faecal pollution in coastal environments after precipitation events are also be influenced by other factors, such as the local surface water circulation (Baldoni et al., 2022), that may have interacted with rainfall in determining the observed FIB levels in our study area.

Similarly, data on river discharge (often linked to the level of precipitations) in the analysed area did not suggest the occurrence of a relationship with FIB levels. However, in the same Marche region, microbial pollution has been linked to the presence of river estuaries, showing variable contamination levels according to the overall pollution status of the considered river (Basili et al., 2021; Penna et al., 2023). We therefore hypothesize that river discharge impacts on FIB levels only above certain thresholds, and especially in response to particularly intense precipitation events.

This hypothesis seems to be supported by the results obtained from the analysis of FIB contamination in relation with CSOs. CSOs data were available and analysed for the two more polluted sites (Ancona and Falconara Marittima). Interestingly, our data indicate that the extreme precipitation events, which lead to CSOs, were highly related to FIB peaks, suggesting their role as main contributors to faecal pollution maxima in the studied area. This result identifies better controlling of CSOs as the focus for faecal bacteria loads reduction in the analysed area, underlining how wastewater management practices have a profound impact on the health of coastal ecosystems and the well-being of individuals relying on these water resources for livelihood and recreational activities (Wear et al., 2021). Future, better management practices will have to be oriented to avoiding, especially in urbanized areas like those here investigated, massive pulses of polluted stormwater to reach the coastal sea, as these pulses are recognized mechanisms of land-to-sea transport of microbial pollutants (Sauer et al., 2011).

5. Conclusions

There is an urgent need for comprehensive, long-term assessments of faecal pollution on a coastwide scale to pinpoint the drivers of bacterial contamination. Such assessments are crucial for informing proactive management and supporting efforts in watershed restoration, as underscored by Powers et al. (2021). The strength of our study comes from 11 years of monitoring data of faecal bacteria, to provide valuable insights into the dynamics of microbial pollutants in our study area. Our main findings are that the area under investigation displays a general increase over time of faecal bacteria (evident in terms of a reduction of structural zeros over the examined timeframe), and that CSOs represent a significant source of microbial pollutants causing peaks in FIB. Increases in CSOs may exacerbate bacterial exceedances and beach advisories, which emphasizes the need to further evaluate global factors that may influence coastal water quality. Finally, predictive models that incorporate considerations of all respective parameters should allow for more timely beach advisories, that reduce public health risks.

CRediT authorship contribution statement

Grazia Marina Quero: Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Conceptualization. **Stefano Guicciardi:** Writing – review & editing, Visualization, Methodology, Formal analysis. **Pierluigi Penna:** Writing – review & editing, Formal analysis. **Giorgio Catenacci:** Writing – review & editing, Data curation. **Milena Brandinelli:** Writing – review & editing, Data curation. **Luigi Bolognini:** Writing – review & editing, Conceptualization. **Gian Marco Luna:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2024.122083.

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