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Short-term impacts of a large cultural event on the microbial pollution status of a pre-alpine river

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ABSTRACT

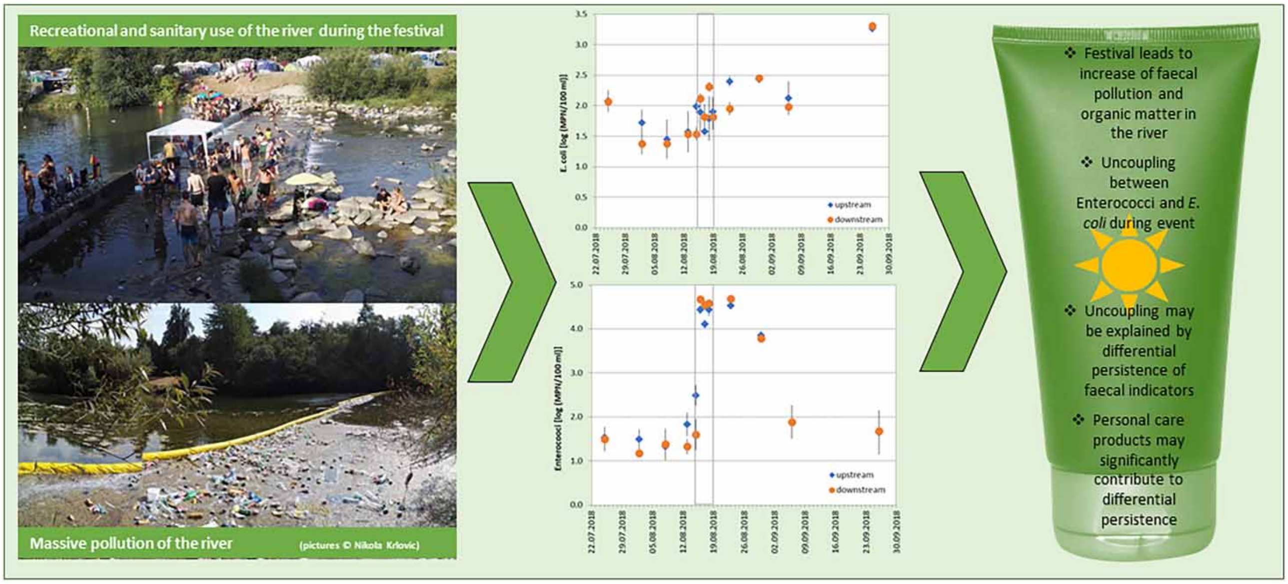
Rivers are impacted by microbial faecal pollution from various sources. We report on a short-term faecal pollution event at the pre-alpine Austrian river Traisen caused by the large cultural event FM4 Frequency music festival, with around 200,000 visitors over 4 days. We observed a massive increase of the faecal indicator bacteria (FIB) intestinal enterococci during the event, while *Escherichia coli* concentrations were only slightly elevated. This increase poses a significant potential health threat to visitors and people recreating downstream of the festival area. A plausible explanation for the uncoupling of the two FIBs may have been a differential persistence caused by a combination of factors including water temperature, solar radiation, and the excessive presence of personal care products (PCPs) in the river water. However, a potential impact of PCPs on FIB assay performance cannot be ruled out. Our observations are relevant for other intensively used bathing sites; detailed investigations on persistence and assay performance of the FIB in response to different ingredients of PCPs are highly recommended. We conclude that for future festivals at this river or other festivals taking place under similar settings, a more effective management is necessary to reduce deterioration in water quality and minimise health risks.

Key words: faecal pollution, persistence, personal care products, river water, short-term event

HIGHLIGHTS

- Strong impact of a cultural event on microbial faecal river pollution.
- Uncoupling of *E. coli* and enterococci concentrations during the pollution event.
- Plausible explanation for uncoupling is the differential persistence of faecal indicators.
- Personal care products may significantly contribute to differential persistence of faecal indicators.

GRAPHICAL ABSTRACT



INTRODUCTION

Many rivers are continuously impacted by microbial faecal pollution from point sources such as wastewater treatment plants or untreated sewage discharges (Servais *et al.* 2007; Kirschner *et al.* 2017). Therefore, the extent of faecal river pollution is generally dependent on the available wastewater treatment infrastructure. Rivers or river sections where state-of-the-art wastewater treatment is implemented should have low to moderate faecal pollution levels, while the absence of wastewater treatment infrastructure can result in critical to excessive faecal pollution levels (Kirschner *et al.* 2009, 2017). Nevertheless, even in countries with state-of-the-art wastewater treatment, short-term events can lead to extreme microbial contamination of rivers. It is well known that heavy rain events in particular lead to substantial increases in faecal indicator levels (Tornevi *et al.* 2014) which are caused by combined sewer overflows (CSOs) (Passerat *et al.* 2011; Mascher *et al.* 2017; McGinnis *et al.* 2018) or faecal run-off from manured fields, live-stock pastures or areas with wildlife droppings (Fernández-Alvarez *et al.* 1991; Aitken 2003; Gilfillan *et al.* 2018; Mushi *et al.* 2021). Such storm and flood events can also lead to increased faecal contamination due to resuspension of bacteria deposited in river sediments (Chu *et al.* 2011; Bradshaw *et al.* 2016; Reitz *et al.* 2021).

Less well-studied causes of short-term faecal pollution in rivers are recreational activities (e.g., bathing and water sports) and cultural activities, which can also have significant impacts on microbial water quality. Religious mass bathing events, after which outbreaks of faecal-borne diseases have been recorded, have been reported specifically for India (Vortmann *et al.* 2015; David & Roy 2016; Purohit *et al.* 2020). To the best of our knowledge, there are no studies examining the impact of mass cultural events on microbial water quality of rivers in other parts of the world.

Here, we assessed the impact of the large FM4 Frequency music festival on the microbial quality of the Traisen River, a pre-alpine tributary of the Danube River in Austria. An approximately 2-km long stretch of the river is part of the festival area; during the 4 days of the festival, people are excessively using the river for recreation and personal hygiene, bringing faecal bacteria and chemicals from personal care products (PCPs) such as sunscreen, soap, and shampoo into the water environment (Supplementary material, Figure S1) (Harjung *et al.* 2020). As pollution parameters, we determined the concentrations of two standard faecal indicators, enterococci and *E. coli* according to the Austrian Bathing Water Act (Austrian Bathing Water Act 2009) and the concentrations of organic and inorganic nutrients, in the water upstream and downstream of the festival site. In this way, we were able to demonstrate that large music festivals can have a significant short-term impact on microbial water quality in rivers when they are an integral part of the cultural event.

MATERIALS AND METHODS

Site description and sampling

The Traisen River, a pre-alpine tributary of the Danube River, is located in the province of Lower Austria. The catchment area consists of agricultural, riparian, and urban areas. The river itself has been subjected to intensive river regulation measures (Haidvogl *et al.* 2018), such as the construction of shallow cross-section dams dividing the river into basins of 200–400 m length. The man-made river bed consists mainly of coarse gravel and cobbles. In this study, we investigated the short-term impact of the large cultural event FM4 Frequency music festival on a 2-km long and 40-m wide section of the river in the immediate vicinity of the city of St. Pölten (approximately. 60,000 inhabitants), which is most affected by the music festival with ~200,000 visitors over the course of 4 days (up to 50,000 per day). From 18 km upstream to 32 km downstream of the festival area, no wastewater treatment plant effluents are discharged into the river. Additional samples taken over 1 year (October 2020–October 2021, $n = 2 \times 5$) at one site 17 km upstream and one site 15 km downstream showed low to moderate faecal pollution levels in the river throughout the year (upstream site: 2.24 ± 0.55 log10 *E. coli*/100 ml, downstream site: 1.25 ± 0.15 log10 *E. coli*/100 ml).

In the present study, samples were collected between July 25 and September 29, 2018 at 13 points in time in the middle of the river downstream (48°09′39.9″N, 15°37′45.7″E) and upstream (48°11′33.2″N, 15°38′00.0″E) of the festival area (Figure 1). We selected the exact location of the upstream site based on available information that it reflects a site that would not be impacted by festival attendees. The location of the downstream site was chosen to take into account the overall impact of the festival on the river. We sampled in weekly intervals in the pre- and post-festival period to monitor background pollution levels in the observed section. Shortly before, during and shortly after the festival (August 13 until August 19) the sampling frequency was increased (daily to 3-day intervals). During this period, the river basins are heavily frequented by the festival participants for recreation and personal hygiene. This is accompanied by a high input of visible waste (compostable, metallic, plastic and cardboard; Supplementary material, Figure S1). Although portable toilets were installed on the festival grounds, there was also misuse of the river for sanitary purposes. Many visitors were already present and pitched their tents a day before the official start of the festival, and some visitors stayed a few days beyond the festival. Unexpectedly, visitors also used river basins upstream of our upstream site, as many of them were obviously aware of the pollution hazard in the festival area. Apart from the festival event, the river with its sub-basins serves as a recreational area for humans and domestic animals all year round. More than 100 bird species were recorded along this river stretch, 75 of which breed along the shores (Seehofer *et al.* 2011).

At each sampling occasion, quadruplicate water samples were collected by hand from a depth of 30 cm below the water surface in autoclaved 250-ml glass bottles at both the upstream and downstream sites. Sampling took place between noon and 1 p.m. The bottles were kept in a cool bag during transport to the laboratory, where they were stored at 4 °C and processed within 24 h.

Chemophysical parameters

During each sampling event, water temperature, oxygen content, electrical conductivity, and pH were measured on-site with handheld devices (Xylem Analytics Germany Sales GmbH & Co. KG, Weilheim, Germany: pH WTW 33110, conductivity WTW 3310, dissolved oxygen and temperature WTW Multi 3510 IDS). To assess the potential input of dissolved organic matter, dissolved organic carbon (DOC) was analysed. DOC-free vials were filled by releasing river water from a DOC-free syringe with a sterilised glass microfiber GF/F filter on the tip. The vials were then placed in the cooling box and stored at 4 °C until analysis (within 1 week after sampling). DOC analysis was performed on a GE-Sievers 900 TOC analyzer (SUEZ Water Technologies & Solutions, Trevose, PA USA) equipped with a persulfate oxidation and an inorganic carbon removal unit (LOD 0.1 mg/l). Total phosphorus (TP) was measured after wet combustion of the raw water sample with a spectrophotometer at 890 nm (Hach-Lange DR 2800; LOD 0.5 μg/l) according to the Austrian standard OENORM EN ISO 6878. Discharge data were obtained from the office of the provincial government of Lower Austria.

Faecal indicator bacteria (FIB)

Escherichia coli and intestinal enterococci were analysed according to international standards (ISO 1998A, 1998B). MUG/MUD microtiter plates (Biorad Austria GmbH, Vienna, Austria), four replicates each, were incubated at 44 ± 0.5 °C for 36–42 h. After incubation, the plates were analysed in a dark room using a handheld UV light-source. The fluorescent wells were counted and the most probable number (MPN) was determined according to the manufacturers MPN table. By

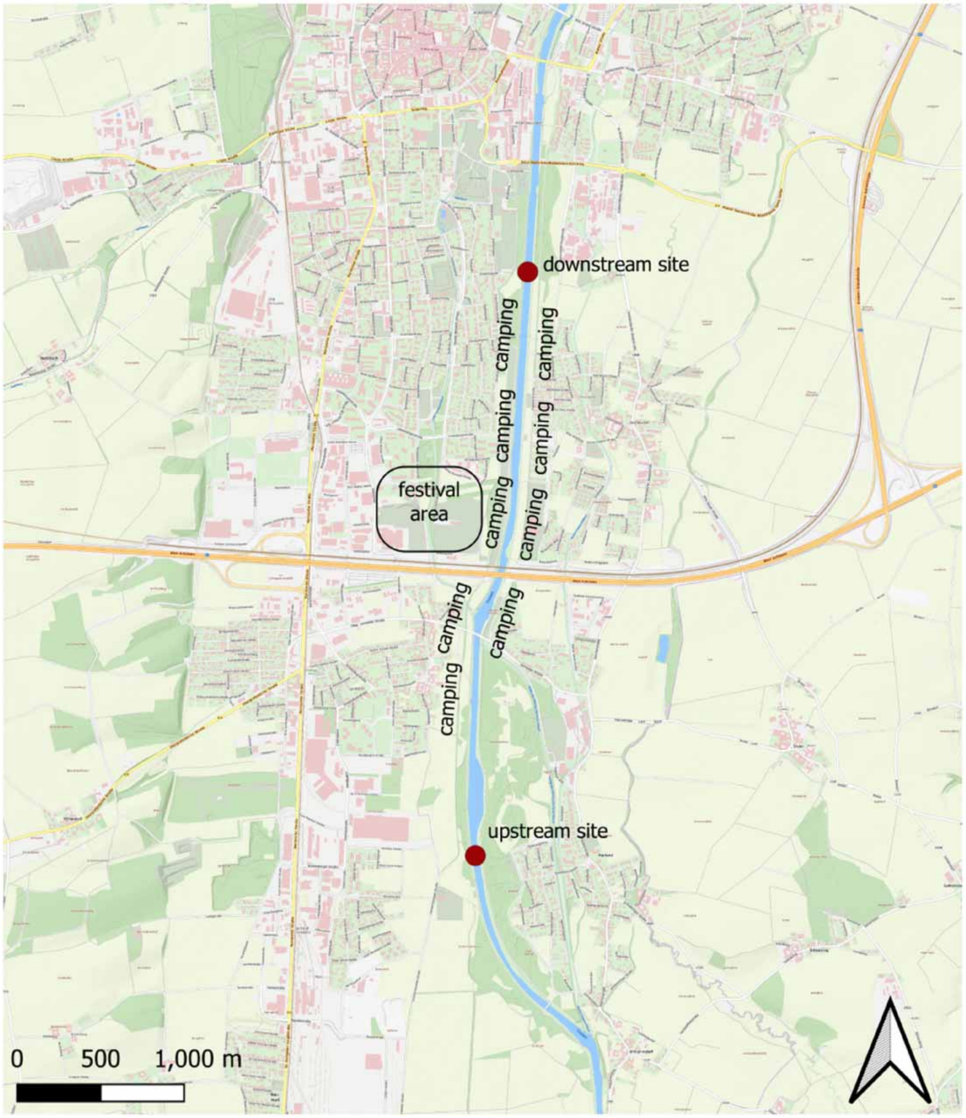


Figure 1 | Overview of the festival area, the camping sites, and the upstream and downstream sampling sites.

applying two dilutions (original and 1:20 dilution), as recommended for bathing waters, the limit of detection of both microtiter plate methods were 15 MPN/100 ml (1.18 log10 MPN/100 ml).

Statistical analysis

For statistical analysis, *E. coli* and enterococci concentrations were log10 transformed. Differences between sites were tested by Students *t*-test for paired samples. For assessing temporal differences, the investigation period was divided into a pre-festival stage (before 13 August), the festival stage (15–19 August) and the post-festival stage. Differences between stages were assessed by one-way ANOVA and Tukey post hoc test was applied. Analyses were performed with IBM programme SPSSv24; a significance level of $p < 0.05$ was chosen for significant differences.

RESULTS AND DISCUSSION

Temporal patterns of *E. coli* and enterococci

In the pre-festival stage, *E. coli* and enterococcci concentrations were at similar low levels around 1.5 log10 MPN/100 ml at both the upstream and the downstream site of the festival area (Figure 2). No significant differences between upstream and

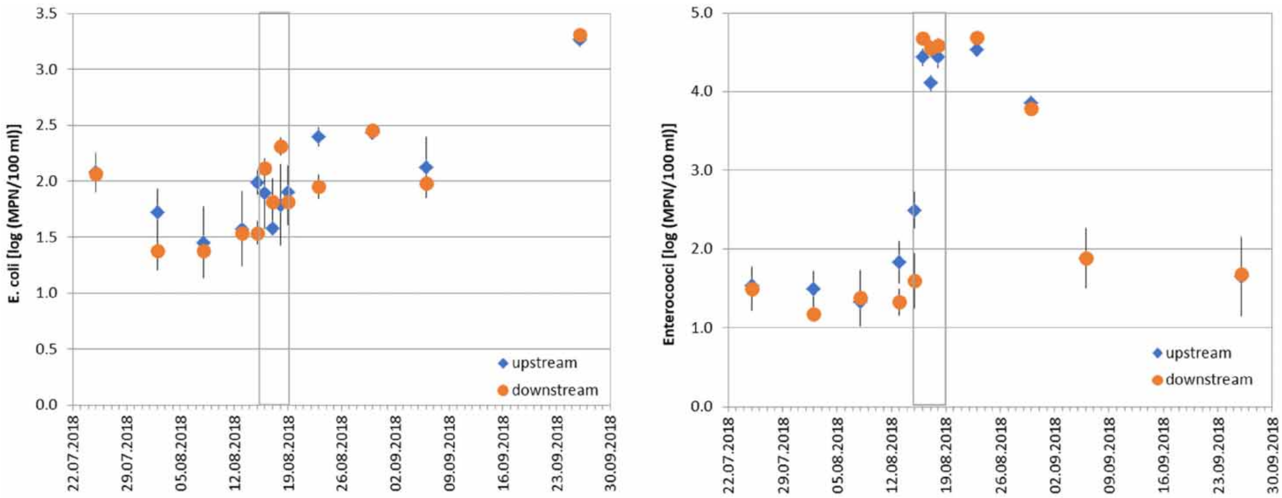


Figure 2 | Course of *E. coli* and enterococci concentrations in Traisen River water before, during (grey box), and after the festival event. Data are expressed in log10 (MPN per 100 ml). Symbols and error bars represent mean scores and standard deviation of four replicates.

downstream sites were observed (*t*-test; $p > 0.05$). While *E. coli* concentrations increased only slightly during the festival (up to 2.3 log10 MPN/100 ml at the downstream site), enterococci exhibited a massive increase up to 4.7 log10 MPN/100 ml ($=5 \times 10^4$ MPN/100 ml) during the festival (Figure 2), far above the limit values for short-term pollution events in bathing waters according to Austrian legislation (400 MPN/100 ml (Austrian_Bathing_Water_Act 2009)). The increases started on the first day of the festival and were statistically significant only for the enterococci, both at the upstream and at the downstream site (ANOVA; Tukey post hoc, $p < 0.001$). Slightly higher values were observed downstream of the festival area (4.5–4.7 log10 MPN/100 ml) in comparison to the upstream site (4.1–4.5 log10 MPN/100 ml), but the difference was not statistically significant (*t*-test, $p > 0.05$). Three weeks after the festival, the enterococci concentrations dropped back down to levels that were similar to what was observed before the festival (ANOVA; Tukey post hoc, $p < 0.001$). In contrast, *E. coli* concentrations remained at the same level and exhibited the highest value at the end of the study period.

It seems unlikely that a differential shedding of the two FIBs took place and more enterococci than *E. coli* entered the river water during the festival. In contrast, faeces from humans (Wright 1982; Farnleitner *et al.* 2010) and waterfowl (Kirschner *et al.* 2004) usually contains higher average concentrations of *E. coli* than enterococci. A plausible explanation for the observed differential increase could have been a different persistence of the two FIBs. Such an uncoupling of enterococci and *E. coli* values has been observed previously for saline environments (Kirschner *et al.* 2004), with *E. coli* showing lower persistence at higher salinities (Sagarduy *et al.* 2019).

Solar irradiation and water temperature are other key factors influencing the decay of FIB, which may affect *E. coli* vs. enterococci differently (Brooks & Field 2016; Sagarduy *et al.* 2019). We observed only a slight increase in river water salinity during the festival event at the downstream site, as measured by electrical conductivity (from 430 to 445 μS/cm, Figure 3), which precludes a significant effect on *E. coli* persistence. Water temperature (Supplementary material, Figure S3) and solar radiation were high throughout the festival and may have exerted a strong influence on the FIB. The water levels of the river were very low (see Supplementary material, Figure S2), and water stayed longer in the separate river basins formed by the cross-sectional shallow dams, which allowed the water to warm up quickly and sunlight to penetrate to the river bottom. Alternatively to the hypothesised differential persistence of the faecal indicators, a possible impact of the PCPs on the assay performance cannot be completely excluded. The two fluorogenic substrates 4-methylumbelliferyl-β-D-glucuronide (MUG) for *E. coli* and 4-methylumbelliferyl-β-D-glucoside (MUD) for enterococci could theoretically be differently impacted by certain compounds contained in PCPs, although no data are available in the literature or from the manufacturer (Biorad, Marnes-la-Coquette, France, personal communication).

Another interesting finding was that the steep increase in enterococci occurred at both sampling sites, upstream and downstream of the festival area. One explanation for this could be that visitors of the festival also used sub-basins of the river further upstream, as they were aware of the high contamination levels in the festival section of the river. Additionally, the

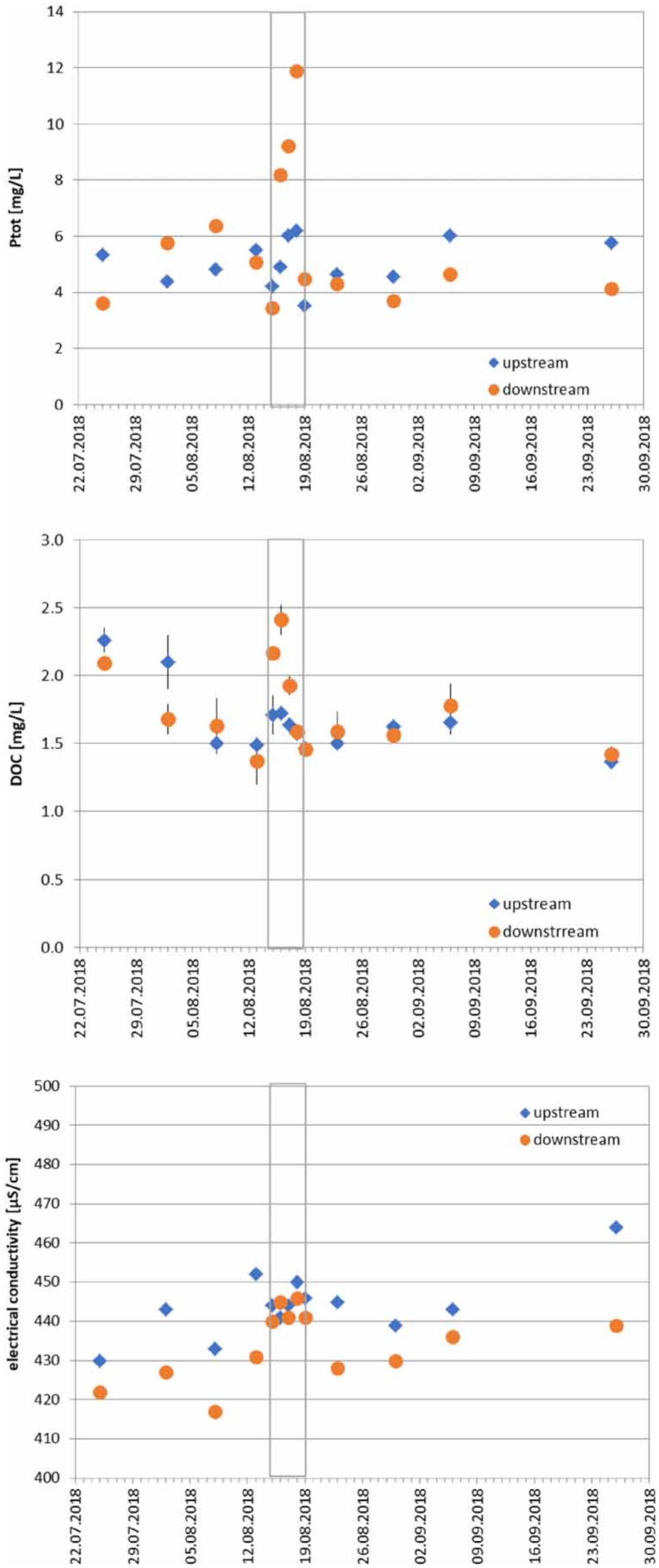


Figure 3 | Course of DOC and total phosphorus (P_{tot}) concentrations as well as electrical conductivity values in Traisen River water before, during (grey box) and after the festival event. Symbols and error bars of DOC data represent the mean and standard deviation of four replicates, P_{tot} and conductivity was analysed from single samples.

faecal pollution upstream may have been caused by waterfowl displaced to these undisturbed areas. This urban river section usually hosts more than 70 breeding bird species (Seehofer *et al.* 2011). The festival significantly disturbs wildlife inhabiting the banks and flood retention areas. The animals are displaced and use both upstream and downstream areas as surrogate habitats, where they feed and defaecate. To distinguish between these two sources of faecal pollution (human vs. birds), we recommend applying host-associated microbial source-tracking markers (Green *et al.* 2012; Kirschner *et al.* 2017) for future studies on this event. Although sediment and biofilm resuspension from visitors cannot be excluded, high loadings of faecal indicators from the sediment into the water column are unlikely, because in a recent study at a comparable site 16 km downstream of the festival area, *E. coli* in biofilms were mostly undetectable (unpublished data).

In comparison to other short-term events, the observed elevated faecal indicator levels during the festival were surprisingly high. Combined sewer overflow events in rivers with state-of-the-art wastewater treatment have been reported to result in increased faecal indicator concentrations that are at least two orders of magnitude higher than during dry weather. In the Seine River (France), directly below a CSO outfall, maximum concentrations of 2.9×10^5 *E. coli* and 7.6×10^4 intestinal enterococci per 100-ml were recorded (Passerat *et al.* 2011), and in the Mohawk River (New York; USA) an activated CSO resulted in *E. coli* and enterococci concentrations above their upper detection limit of $>2.4 \times 10^4$ colony forming units/100 ml (Lininger *et al.* 2022). The intestinal enterococci concentrations found in our study (5×10^4 MPN/100 ml) were thus in a comparable order of magnitude to CSOs in rivers. To the best of our knowledge, scientific literature on the short-term impacts of cultural events on river water quality is limited to mass bathing events in India. Vortmann *et al.* (2015) reported that total coliform concentrations in the Ganga River increased from 1.5×10^4 to 5×10^4 per 100 ml during a mass bathing event at a downstream site. Much higher levels were reported for the Kshipra River in Central India, where an increase from 3.7×10^5 to 5.1×10^6 *E. coli* per 100 ml was observed during such an event (Purohit *et al.* 2020). Since no information on enterococci was provided in these studies, and rivers in India generally display higher faecal pollution levels due to poor wastewater treatment, a direct comparison is not appropriate.

Physical-chemical water quality parameters

Next to the increased microbial faecal pollution, we measured a significant increase of DOC and P_{tot} concentrations at the downstream site during the festival (Figure 3). Similarly, electrical conductivity was also elevated during the festival, with significantly higher values at the downstream site. Immediately after the festival ended, DOC and P_{tot} dropped to values comparable to the pre-festival period. The other measured physico-chemical parameters temperature, dissolved oxygen, and pH values did not change significantly during the cultural event (Supplementary material, Figure S3).

The observed input of organic and inorganic matter was likely caused by the presence of festival participants and potentially originating from urine, faecal matter but also from various kinds of PCP. We hypothesise that the massive presence of certain PCPs in the river water (Harjung *et al.* 2020) also contributed to the differential persistence of the FIB. The effects of PCPs at both upstream and downstream sites of the festival area was likely enhanced by the low water levels observed during the festival (see above), which increased the exposure time of the FIBs to the PCPs. Indeed, Harjung *et al.* (2020) reported a dramatic increase in chemically stable UV-B filter phenylbenzimidazole sulphonic acid (PBSA) during the same festival, indicating that organic compounds in sunscreens and other PCPs are the sources of elevated DOC.

E. coli as a Gram-negative bacterium might be more sensitive to components of PCPs than Gram-positive bacteria such as enterococci, due to the lipopolysaccharide characteristics of their outer cell membrane. For example, EDTA (Voss 1967) and dodecyldiethanolamine (Lambert & Smith 1976), substances added to cosmetic products, have been reported to have effective bactericidal activity against *E. coli* and other Gram-negative bacteria. More recently, nanoparticles in sunscreen products were shown to have inhibitory and toxic effects on *E. coli* (Baek *et al.* 2017). Also antibacterial agents such as triclosan or triclocarban that are often present in PCPs, have been found in rivers such as in Italy (Palmiotto *et al.* 2018) or Spain (Carmona *et al.* 2014) and could have a differential effect on the persistence of the two faecal indicators investigated.

However, due to the large number and diversity of ingredients in PCPs, an enhanced effect of combinations of different chemicals on Gram negatives – in contrast to Gram positives – can only be speculated. To date, the presence of PCPs in rivers has generally been associated with their emission from sewage effluents (van Wijnen *et al.* 2018; Homem *et al.* 2022; Rapp-Wright *et al.* 2023). Concentrations in the magnitude of several hundred ng L^{-1} have been recorded for various substances, significantly exceeding predicted no effect concentrations (PNEC) (Homem *et al.* 2022). One study indirectly linked the elevated occurrence of PCPs (particularly ingredients of sunscreen products) in alpine rivers to the number of tourists and residential population in the region, showing that the highest concentrations up to 6 μg L^{-1} occurred in summer

(Mandaric *et al.* 2017). To the best of our knowledge, the study by Harjung *et al.* (2020) is the only one that directly links the input of such PCPs into river water to bathing guests/festival visitors. PBSA concentrations of 45 μg L^{-1} measured during the festival by far exceeded those found in other rivers and lakes (Harjung *et al.* 2020), corroborating our hypothesis of a potential impact on *E. coli* and enterococci detection.

CONCLUSIONS

We demonstrated that a large cultural festival event triggered a significant short-term faecal pollution event in a medium-sized pre-alpine tributary of the Danube River. The specific situation at the festival area and the intensive use of the river for recreational and hygienic purposes by festival participants led to a remarkable rise in enterococci concentrations in the river water, far above the limits for short-term pollution events in bathing waters according to Austrian legislation. This poses a significant potential threat to the health of the festival participants and people recreating in the festival area and downstream who could become infected with pathogens concomitantly shed into the water. In contrast to the enterococci, there was only a minimal increase in *E. coli* concentrations. This uncoupling could have been caused by the different persistence of the two FIB investigated in relation to high water temperatures, solar radiation and the presence of potentially large amounts of PCPs. Alternatively, an impact of the PCPs on the assay performance cannot be excluded. More detailed investigations on the persistence of FIB in response to different PCP ingredients and on the potential impact of these products on the performance of the two assays are highly needed. Such scenarios could also be relevant for other intensively used marine and freshwater bathing sites. We further conclude that more effective management is necessary for upcoming festivals at this river or other festivals taking place under similar settings, to prevent deterioration in water quality in terms of faecal pollution and to minimise the health risk to visitors and recreational users downstream. Management measures could include improved sanitation infrastructure, better waste management and campaigns to raise awareness of the problem among festival visitors.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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