Cross-tracking of faecal pollution origins, macronutrients, pharmaceuticals and personal care products in rural and urban watercourses

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ABSTRACT

This study describes microbial and chemical source tracking approaches for water pollution in rural and urban catchments. Culturable faecal indicator bacteria, represented by *Escherichia coli*, were quantified. Microbial source tracking (MST) using host-specific DNA markers was applied to identify the origins of faecal contamination. Chemical source tracking (CST) was conducted to determine contaminants of emerging concern (CEC) of human/anthropogenic origin, including pharmaceuticals and personal care products (PPCPs) and endocrine-disrupting chemicals (EDCs). In addition, the eutrophication-causing macronutrients nitrogen and phosphorus were studied. MST tests revealed both anthropogenic and zoogenic faecal origins, with a dominance of human sources in the urban stream; non-human/environmental sources were prevalent in the rural creek. CST analyses revealed a higher number of CECs in the urban stream than in the rural watercourse. Positive correlations between PPCPs and both *E. coli* and the human DNA marker were uncovered in the urban stream, while in the rural creek, PPCPs were only highly correlated with the anthropogenic marker. Interestingly, macronutrients were strongly associated with primary faecal pollution origins in both watercourses. This correlation pattern determines the main pollutant contributors (anthropogenic or zoogenic) to eutrophication.

Key words | *Bacteroidales* 16S rRNA gene markers, *Escherichia coli*, faecal water contamination, microbial and chemical source tracking, nitrogen and phosphorus, pharmaceuticals and personal care products

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HIGHLIGHTS

- Microbial source tracking is essential to define the origins of faecal contamination.
- Chemical source tracking supports microbial source tracking faecal pollution results.
- Pharmaceuticals and personal care products correlate with the human DNA marker.
- Dominant faecal origin reveals primary sources of macronutrient pollution in water.

INTRODUCTION

All terrestrial and aquatic ecosystems are directly or indirectly impacted by faecal contamination, which creates serious problems for public and environmental health. Human and animal faeces are the main sources of enteric pathogens responsible for food- and waterborne disease outbreaks (Paruch & Paruch 2018). The mortality and morbidity associated with faecal water contamination represent nearly 10% of the total burden of human disease worldwide (WHO 2020). Therefore, the identification of faecal origins and tracking the sources of contamination become crucial for water quality

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management (Pascual-Benito *et al.* 2020), protection strategies for aquatic ecological environments (Paruch *et al.* 2019), treatment options for point and non-point/diffuse sources (Chen *et al.* 2020), the development of remediation regimes (Ahmed *et al.* 2020) and the potential health risk assessment for humans and the environment (Salim Dantas *et al.* 2020).

Faecal indicator bacteria (FIB), represented usually by *Escherichia coli* (*E. coli*), have been extensively examined to evaluate water quality associated with faecal contamination. The presence of FIB indicates the occurrence but not the origin of faecal pollution largely due to its low host specificity, replication in the environment and geographic and temporal variability (Tran *et al.* 2015). Therefore, various other microbes, indicators and markers have been selected and validated for microbial source tracking (MST) of faecal pollution. These include host-associated molecular markers derived from *Bacteroidales* 16S rRNA genes, which exhibit high host specificity and geographical stability; thus, they have been extensively applied worldwide in diverse water-quality-related studies focused on urban and agricultural watersheds (Sowah *et al.* 2017), natural surface and ground waters (Tran *et al.* 2015), drinking water sources (Paruch *et al.* 2020), coastal and marine aquatic environments (Jardé *et al.* 2018) and recreational beaches (Molina *et al.* 2014). The MST tests based on quantitative polymerase chain reaction (qPCR) have been proven to achieve rapid detection and accurate quantification of specific microbial genetic markers developed for different hosts, e.g. humans, ruminants, pigs, horses, dogs and others (Paruch *et al.* 2015; Staley *et al.* 2016; Sowah *et al.* 2017; Haramoto & Osada 2018).

In addition to MST, chemical source tracking (CST) has been implemented to determine the origins of faecal water contamination. Various CST identifiers/markers have been used (e.g. caffeine, faecal sterols and stanols, bile acids, laundry brighteners, fragrances and pesticides) and among them, pharmaceuticals and personal care products (PPCPs) are the trace contaminants most frequently detected in close association with anthropogenic activities (Yang *et al.* 2020). Hence, they have been employed as chemical markers to detect and evaluate human faeces in water bodies. PPCPs are of high concern in terms of the direct or indirect impact on human and environmental health since they and their metabolites constitute substantial sources of contaminants of emerging concern (CEC) in aquatic environments (Su *et al.* 2020).

Different measures based on MST host-specific genetic markers and CST identifiers have been developed and applied in tracking faecal sources in contaminated waters. MST and CST have usually been applied separately as independent methods, but recently, the combined usage of MST and CST has been advocated to support a more robust result (Staley *et al.* 2016; Devane *et al.* 2019). However, to date, some important technical questions have not yet been well addressed, e.g.: (i) How, in practice, should MST and CST methods be incorporated, especially under different circumstances? (ii) How can the results from these two methods be reasonably integrated and validated in order to achieve more robust and reliable results?

Therefore, in the current study, we attempted to shed some light on these aspects rather than making side-by-side comparisons of the two methods (i.e. pros and cons), since those have been explored in detail in earlier investigations (Staley *et al.* 2016; Devane *et al.* 2019). We implemented both MST and CST methods to address faecal contamination (microbial pollution and PPCP contamination) in two differently representative water types: urban and rural watercourses. Moreover, nutrient levels (mainly nitrogen – N and phosphorus – P) are drastically elevated due to faecal water pollution (e.g. wastewater discharges and agricultural run-off) causing eutrophication and the deterioration of the quality of aquatic ecosystems (Blankenberg *et al.* 2016); therefore, we also monitored nutrient parameters and linked them to the MST studies. We hypothesised that quantitative microbial source tracking (QMST) can also facilitate the source identification of N and P in water environments. To the best of our knowledge, this is a pioneering study, as it focuses on the integrated cross-tracking of various micro-, macro- and emerging water contaminants; hence, it provides valuable input for better interpreting the results of MST and CST tests, as well as novel insights for defining the origins of faecal pollutants in aquatic environments.

MATERIALS AND METHODS

Study sites and water sampling

This study was conducted in two watercourses (a rural creek and an urban stream) located in agricultural and urban catchment areas of neighbouring municipalities, Ås and Nordre Follo, on the border of south-east Oslo, Norway

(Figure 1). The rural creek, Grytelandsbekken, is approximately 2.5 km long and runs east of the town of Ås (approximately 11,000 people). It has a catchment (also known as the Skuterud catchment) covered mostly with farmland (60%) and forest/marshland (31%). The first study site was located at Grytelandsbekken (G), which is downstream of this catchment and at the mouth of the creek. The urban stream, Blåveisbekken, runs through the city of Ski (approximately 20,000 residents) and is partially culverted (Figure 1). The second study site was situated at the end of the culvert in Blåveisbekken (B). Water samples were collected at G and B at quarterly intervals for over 2 years (November 2014 to March 2017). In total, 80 samples, 40 per study site (20 for CEC, 10 for macropollutants and 10 for microbial contaminants), were collected during 10 sampling events (Nov. 2014, Feb. 2015, Jun. 2015, Sep. 2015, Dec. 2015, Mar. 2016, Jun. 2016, Sep. 2016, Dec. 2016, Mar. 2017).

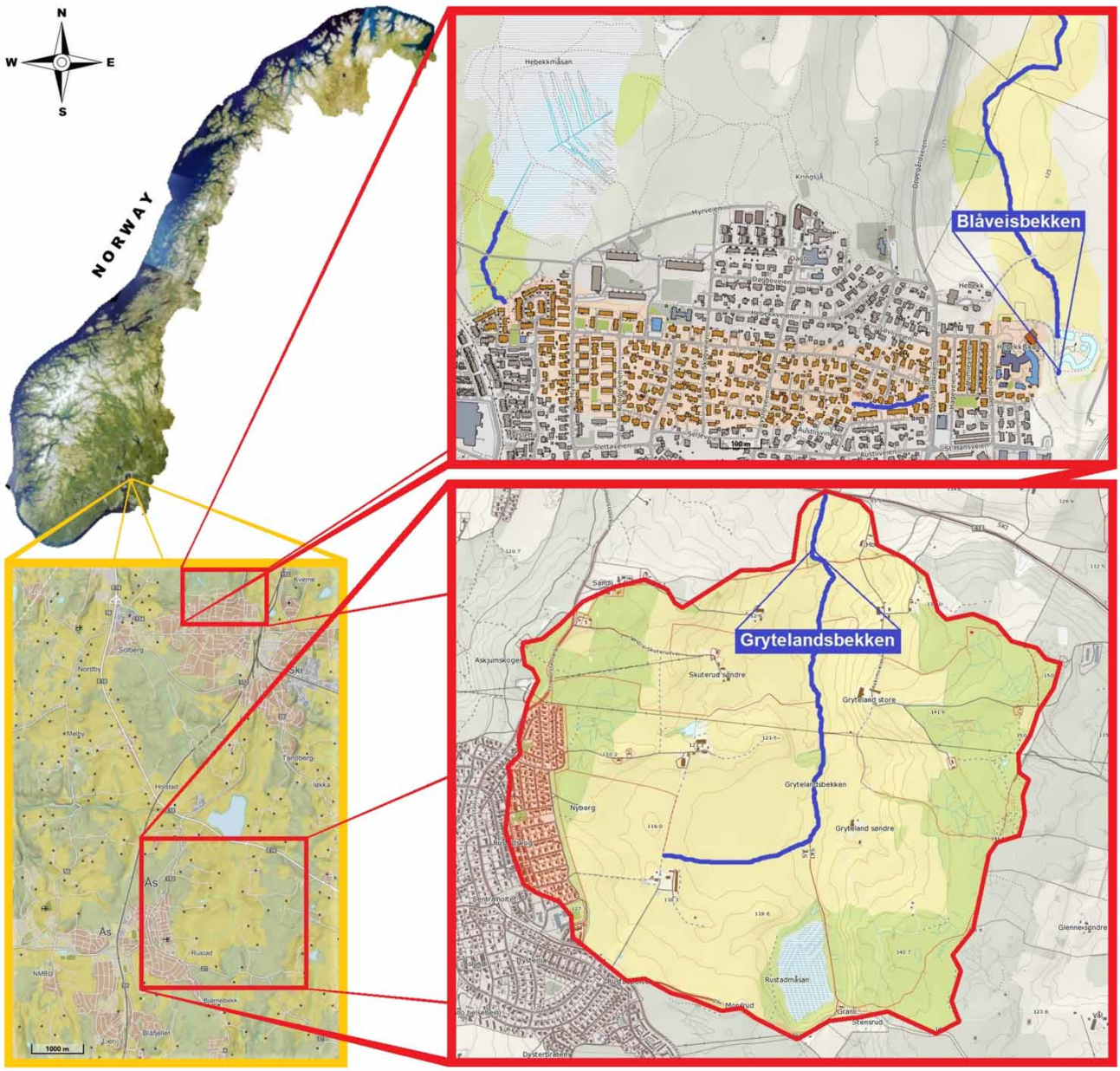

Figure 1 | Location of the study sites G (Grytelandsbekken rural creak) in Ås, and B (Blåveisbekken urban stream) in Ski. The satellite image and maps were obtained from NIBIO's primary map service, Kilden (https://kilden.nibio.no).

Water quality examination

Standard water quality control measures were conducted to determine some of the most common macropollutants, such as organic matter content expressed as chemical oxygen demand (COD), total dissolved solids (TDS) and eutrophication-causing macronutrients (total nitrogen – TN and total phosphorus – TP). All macropollutants and pH were analysed in an accredited laboratory of the ALS Laboratory Group Norway AS according to the respective ISO and national standards for COD (ISO 15705), TDS (CSN 757346, CSN 757347, EN 16192), pH (ISO 10523, EPA 150.1, EN 16192), TN (EN 12260) and TP (ISO 6878, ISO 15681-1).

Chemical source tracking (CST)

The study sites were examined for CEC, particularly PPCPs, including their metabolites, and endocrine-disrupting chemicals (EDCs). These compounds were tested by the Water Management Laboratory Plzeň, Povodi Vltavy, State Enterprise following national, ISO and EPA standards (CSN ISO 20179, CSN ISO 25101, EPA 1694 and EPA 535). All detectable (above limits of quantitation – LOQ) CECs were recorded and used for data analysis in this study.

Microbial source tracking (MST)

Microbial examination of faecal contamination were carried out using FIB tests, i.e. enumeration of *E. coli*. These tests were performed in the Laboratory of Aquatic Microbial Biotechnology (LAMB) administrated by the Norwegian Institute of Bioeconomy Research (NIBIO) and were executed using the Colilert 18 method (IDEXX Laboratories, Inc., Westbrook, Maine, USA) constituting the worldwide ISO standard 9308-2:2012. This method determines concentrations of *E. coli* as the most probable number (MPN) of organisms in 100 ml of examined water. The MPN counts were supported by 95% confidence limits estimated by the IDEXX MPN Generator Ver. 1.4, Quanti-Tray® and Quanti-Tray®/2000 MPN Table Program (©2020 IDEXX Laboratories, Inc., Westbrook, Maine, USA).

The faecally contaminated (positive for *E. coli*) water samples were further analysed using molecular tools in order to determine the anthropogenic/human and zoogenic/environmental (e.g. pet, livestock and wild animal) sources of faecal pollution. For this purpose, each polluted water sample (200 ml) was concentrated in an ultrafiltration unit and the solid material obtained was subjected to microbial genomic DNA extraction using a DNeasy PowerWater Kit (QIAGEN GmbH, Hilden, GERMANY) following the protocol provided by the manufacturer. The purified DNA material was used in a real-time quantitative polymerase chain reaction (RT-qPCR) for the detection and quantitation of host-specific *Bacteroidales* 16S rRNA genetic markers implemented in the QMST technique. The markers applied and methodology of this technique have been previously described in detail (Paruch *et al.* 2015, 2017).

Toolkit determining faecal dominant origin and source

The microbial and molecular analyses constitute the integrated components of the testing toolkit developed at NIBIO for defining faecal pollution origins and determining the dominant source of pollution. The established toolkit consists of three interrelated methodological steps: (1) microbial examination for screening faecally contaminated water samples; (2) molecular diagnostic of the contaminated samples, i.e. QMST testing; (3) source apportionment profile of the genetic markers detected in the faecally polluted sample. Based on the QMST outcomes, a faecal source apportionment profile (FSAP) was established. The FSAP presents the percentage distribution of DNA-based markers; thus, it determines the dominant faecal origin and source in the examined water. The scientific basis and methodological procedures of this toolkit have been described in depth elsewhere (Paruch *et al.* 2020).

Statistical analysis

A redundancy analysis (RDA) was conducted to determine the correlations between biotic (*E. coli* and the dominant faecal sources) and abiotic components (chemical parameters) based on Pearson's correlation test, with a statistical significance level above 95% ($p < 0.05$). This was performed using the MPN counts, faecal source abundance data, amount of total PPCPs (including detected metabolites and bisphenol A – BPA) and indicative PPCPs (i.e. the most frequently detected, such as ibuprofen, gabapentin, paracetamol and caffeine), and content of macropollutants. In addition, the biotic and abiotic factors were applied to generate the Pearson's correlation heat maps, distinguishing between the positive and negative data (correlation coefficients between 1 and −1). The RDA and the correlation analysis were performed using XLSTAT-ECOLOGY statistical software package version 2019.1.1 (Addinsoft 2020, Boston, USA, https://www.xlstat.com).

RESULTS AND DISCUSSION

The standard water quality control measures exposed higher contents of both organic matter and macronutrients in Grytelandsbekken (study site G; 9–40 mg O_2/l, 0.02–0.23 mg TP/l and 2.56–9.68 mg TN/l; Table 1) than in Blåveisbekken (study site B; 5–30 mg O_2/l, 0.03–0.16 mg TP/l and 1.5–4.07 mg TN/l; Table 2). These outcomes reflect the different characters of the studied catchments (rural and urban, Figure 1) and are associated with agricultural practices (e.g. mineral and organic N/P fertilisers) where agrarian run-off is recognised as the main source of eutrophication-causing macronutrients in freshwater bodies. Firmansyah *et al.* (2016) revealed that run-off, erosion and leaching in agricultural systems contribute significantly to N/P loss. Blankenberg *et al.* (2016) reported that run-off from agricultural areas was the main source of nutrients in surface waters. In addition, an earlier study by Paruch *et al.* (2019) on aquatic microbial community composition in Grytelandsbekken demonstrated a dominance of archaeal populations that were positively correlated with fertiliser use and farmyard manuring.

A side effect of the use of manure is the contamination of soil and water with animal faecal matter. However, this is not the only faecal source in agricultural catchments; wild animals, livestock farming and human settlements can also contribute to faecal pollution (Paruch *et al.* 2017). The microbial investigations of both study sites demonstrated constant faecal water contamination, with much higher *E. coli* concentrations (expressed as MPN with 95% confidence limits) in the urban stream than the rural creek (Figures 2 and 3). The molecular tests unveiled that these contaminations were both anthropogenic and zoogenic (Figures 2 and 3).

The QMST tests determined that zoogenic faecal sources dominated in the rural site G, as demonstrated by FSAP ranging from approximately 80% to 99% (Figure 2). Only one case showed a prevalence of an anthropogenic faecal impact, though under the lowest *E. coli* concentration. This could have been a consequence of some sporadic leakage from decentralised/on-site wastewater treatment systems, which have been previously localised in nearby scattered rural settlements in the catchment of Grytelandsbekken (Blankenberg *et al.* 2016).

In contrast to the rural water at site G, a high proportion of anthropogenic faecal sources was detected in the urban water at site B (Figure 3). This finding is in line with a general opinion that urban faecal water contamination is anthropogenic. It derives principally from sanitary and/or combined sewer systems (sewage leakages, wastewater discharges and overflows from pumping stations and storm and drainage systems). However, this opinion is not entirely

Table 1 | Overview of the standard water quality control conducted in water samples from the rural creek, Grytelandsbekken

Parameter	Nov. 2014	Feb. 2015	Jun. 2015	Sep. 2015	Dec. 2015	Mar. 2016	Jun. 2016	Sep. 2016	Dec. 2016	Mar. 2017
Chemical oxygen demand – COD (mg O_2/l)	28	15	22	40	26	24	9	29	27	27
Total dissolved solids – TDS (mg/l)	139	107	185	133	140	124	177	194	131	153
Total phosphorus – TP (mg P/l)	0.05	0.03	0.23	0.06	0.05	0.02	0.03	0.06	0.05	0.12
Total nitrogen – TN (mg N/l)	6.19	3.06	3.37	4.42	9.68	2.56	3.98	3.91	7.48	3.83
pH	7.34	7.53	7.43	7.09	6.91	7.46	7.67	7.45	7.35	7.01

Table 2 | Overview of the standard water quality control conducted in water samples from the urban stream, Blåveisbekken

Parameter	Nov. 2014	Feb. 2015	Jun. 2015	Sep. 2015	Dec. 2015	Mar. 2016	Jun. 2016	Sep. 2016	Dec. 2016	Mar. 2017
Chemical oxygen demand – COD (mg O_2/l)	25	6	20	29	22	5	6	28	11	30
Total dissolved solids – TDS (mg/l)	148	228	187	120	144	200	181	188	174	161
Total phosphorus – TP (mg P/l)	0.04	0.05	0.04	0.16	0.03	0.03	0.03	0.15	0.06	0.06
Total nitrogen – TN (mg N/l)	3.69	3.05	1.50	2.85	3.91	1.72	2.56	4.07	3.70	2.88
pH	7.68	7.63	8.40	7.40	7.70	7.60	7.66	7.63	7.74	7.39

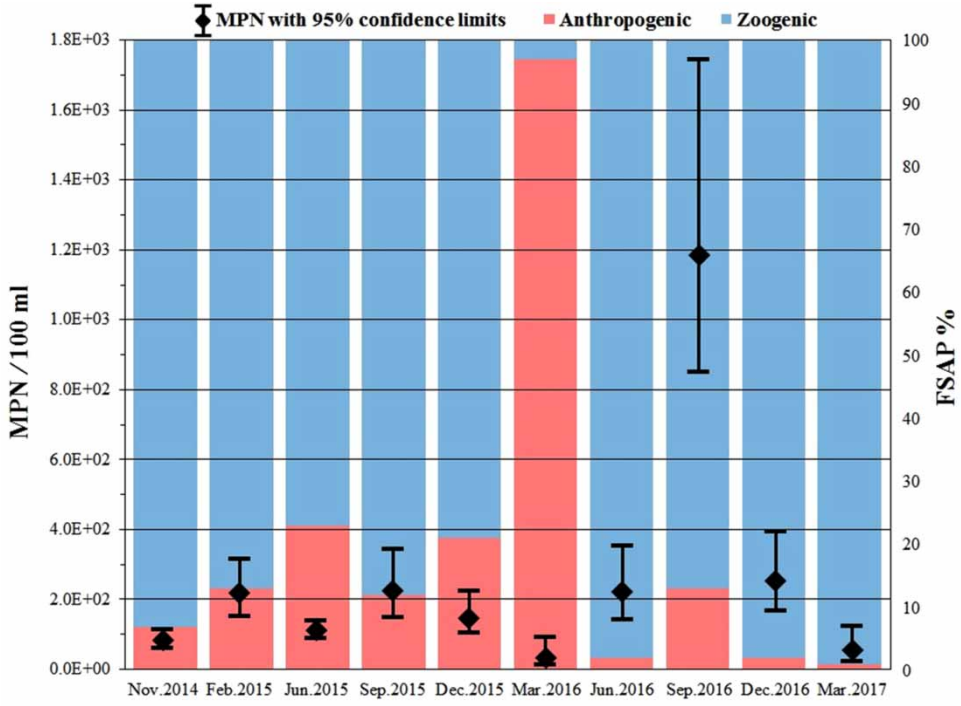

Figure 2 | Concentrations of *E. coli* as MPNs with 95% confidence limits and FSAP in water samples from the rural creek Grytelandsbekken.

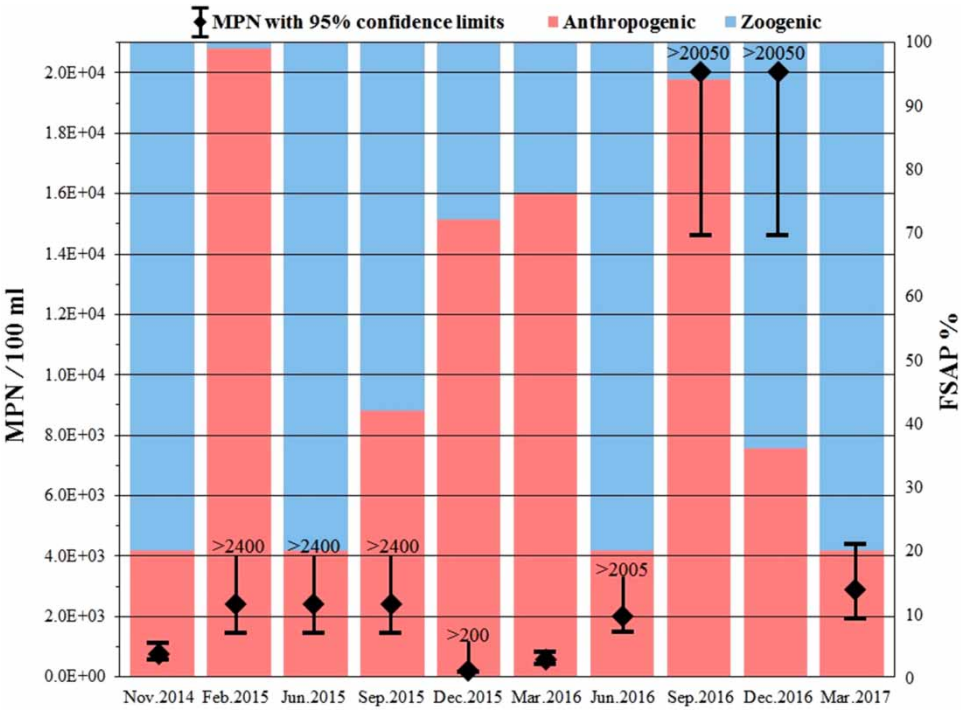

Figure 3 | Concentrations of *E. coli* as MPNs with 95% confidence limits and FSAP in water samples from the urban stream, Blåveisbekken.

true, especially nowadays with the 'blue-green' development trend of urban areas, which are favoured and inhabited by various wildlife species (Gallo & Fidino 2018; Partridge & Clark 2018). The urban fauna, represented mostly by birds (e.g. pigeons, crows, ravens and gulls), in particular waterfowl (swans, geese and ducks), and mammals (e.g. rats, bats, raccoons, foxes and beavers), contribute directly and/or indirectly (e.g. via urban run-off) to faecal water contamination (Paruch & Paruch 2018). The city sewerage harbours a flow of pollution generated by a variety of urban life (i.e. human and animal); hence, not all that comes from sewerage has an anthropogenic origin. Therefore, it is time to update the fact that faecal pollution in urban catchments has also substantial footprints of zoogenic origins, as documented at study site B (Figure 3). To this end, we implemented QMST to distinguish the major (human or non-human) faecal pollution source in urban water.

Source tracking and discrimination between anthropogenic and zoogenic sources of faecal water contamination is of vital importance in safeguarding and mitigating public health risks and sustaining functional aquatic microbial ecology (Paruch *et al.* 2019, 2020). The major health threats are incurred by enteric pathogens, especially pathogens of emerging concern (emerging pathogens). These are primarily responsible for severe waterborne zoonoses because zoonotic pathogens comprise 75% of emerging infectious diseases (Bolin *et al.* 2004). In addition, impaired human and environmental health effects are caused by emerging chemical pollutants derived preliminary from anthropogenic practices. Therefore, urban watersheds are highly vulnerable to CEC contamination; wastewater, sewage and sludge are the major reservoirs of PPCPs and EDCs in the environment (Su *et al.* 2020; Yang *et al.* 2020). Similarly, in our study, the CST tests revealed that the highest levels of CEC were detected in the urban stream (Table 3). These included a range of PPCPs (ibuprofen, gabapentin, paracetamol and caffeine being the most frequently detected), their metabolites,

Table 3 | Concentrations of emerging contaminants (ng/l) along with their LOQ in water samples from the urban stream, Blåveisbekken

Compound	LOQ	Nov. 2014	Feb. 2015	Jun. 2015	Sep. 2015	Dec. 2015	Mar. 2016	Jun. 2016	Sep. 2016	Dec. 2016	Mar. 2017
Ibuprofen	20	170	130	<20	93	35	140	28	290	<20	300
Gabapentin	10	10	71	67	31	31	42	58	920	29	12
Paracetamol	10	90	520	88	150	120	370	230	7,500	2,400	120
Caffeine	100	170	680	580	500	460	1,000	1,200	2,800	510	260
Saccharin	50	94	110	62	84	52	83	170	2,000	430	<50
Naproxen	50	<50	160	<50	<50	<50	<50	51	550	<50	<50
Tramadol	10	<10	<10	<10	<10	<10	<10	<10	47	63	<10
Chloramphenicol	20	<20	<20	<20	<20	<20	<20	<20	<20	<20	32
Carbamazepine	10	<10	<10	<10	<10	<10	<10	<10	15	<10	<10
Diclofenac	20	<20	<20	<20	<20	<20	<20	<20	29	<20	<20
Atenolol	10	<10	<10	<10	<10	<10	<10	<10	58	<10	<10
Ketoprofen	10	<10	<10	<10	<10	<10	<10	<10	17	<10	<10
Metoprolol	10	<10	<10	<10	<10	<10	<10	<10	74	<10	<10
Hydrochlorothiazide	50	<50	<50	<50	<50	<50	<50	<50	68	<50	<50
Erythromycin	10	<10	<10	<10	<10	<10	<10	<10	<10	250	<10
Furosemide	50	<50	<50	<50	<50	<50	<50	<50	<50	160	<50
Carboxyibuprofen	20	n.t.	n.t.	n.t.	26	80	130	100	1,400	<20	<20
2-hydroxyibuprofen	30	n.t.	n.t.	n.t.	<30	31	54	59	630	<30	<30
4-hydroxydiclofenac	20	n.t.	n.t.	n.t.	<20	<20	25	<20	<20	<20	<20
O-desmethylnaproxen	20	n.t.	n.t.	n.t.	<20	<20	<20	<20	26	<20	<20
Venlafaxine	10	n.t.	n.t.	n.t.	<10	<10	<10	<10	12	<10	<10
Iohexol	50	n.t.	n.t.	n.t.	<50	<50	570	<50	1,300	<50	<50
BPA	50	n.t.	n.t.	n.t.	<50	<50	<50	1,200	51	<50	<50

In the periods where some compounds were not tested, the concentrations are marked n.t.

and BPA (a known endocrine disruptor). Much lower amounts of CEC were recorded in the rural creek (Table 4); ibuprofen, gabapentin, paracetamol and caffeine were also the PPCPs most often found in water.

Regardless of the primary faecal contamination origin (Figures 2 and 3), all detected CECs in both rural and urban watercourses were solely correlated with anthropogenic faecal pollution (Figures 4 and 5). As revealed by the RDA,

Table 4 | Concentrations of emerging contaminants (ng/l) along with their LOQ in water samples from the rural creek, Grytelandsbekken

Compound	LOQ	Nov. 2014	Feb. 2015	Jun. 2015	Sep. 2015	Dec. 2015	Mar. 2016	Jun. 2016	Sep. 2016	Dec. 2016	Mar. 2017
Ibuprofen	20	270	120	<20	<20	<20	2,500	160	140	46	<20
Gabapentin	10	30	18	40	21	37	79	<10	13	<10	14
Paracetamol	10	<10	28	24	<10	<10	<10	360	60	89	12
Caffeine	100	<100	<100	280	<100	<100	390	280	160	220	<100
Saccharin	50	<50	<50	80	<50	<50	<50	140	<50	70	<50
Naproxen	50	<50	<50	<50	<50	<50	<50	100	<50	<50	<50
Tramadol	10	<10	<10	<10	<10	<10	<10	86	24	24	<10
Chloramphenicol	20	<20	<20	<20	<20	<20	<20	<20	<20	<20	890
Carboxyibuprofen	20	n.t.	n.t.	n.t.	<20	<20	34	940	150	72	<20
2-hydroxyibuprofen	30	n.t.	n.t.	n.t.	<30	<30	76	1,100	430	110	68

In the periods where some compounds were not tested, the concentrations are marked n.t.

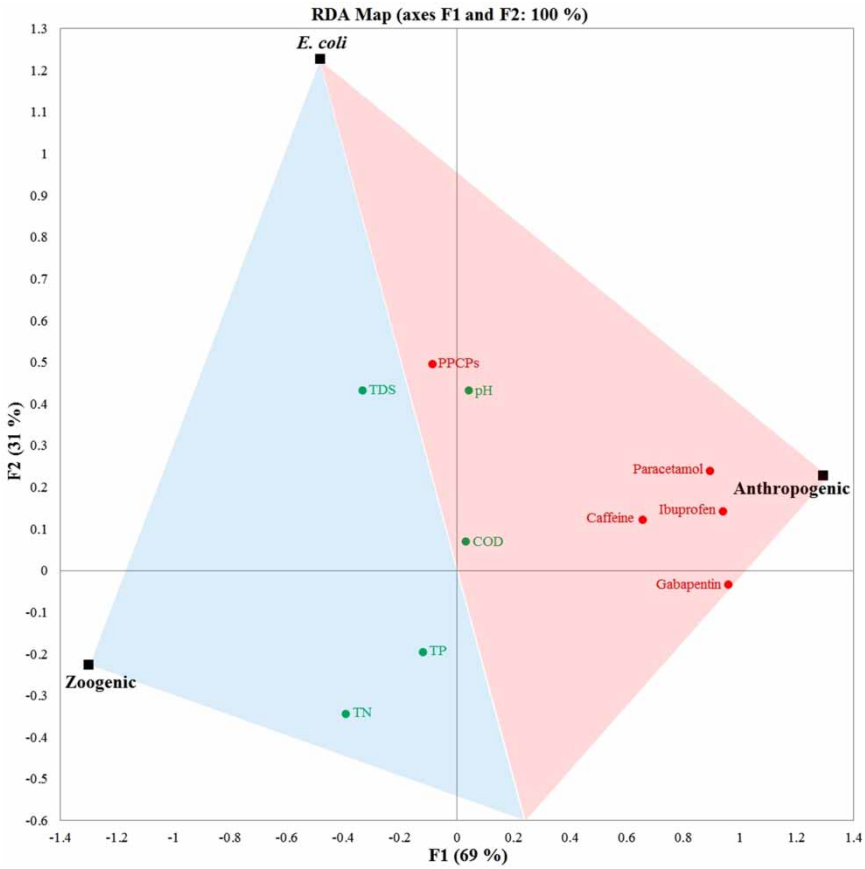

Figure 4 | RDA map of significant ($p < 0.05$) relationships between biotic (*E. coli* and the dominant faecal sources, i.e. zoogenic and anthropogenic) and abiotic (chemical parameters including PPCPs) components in the rural creek, Grytelandsbekken.

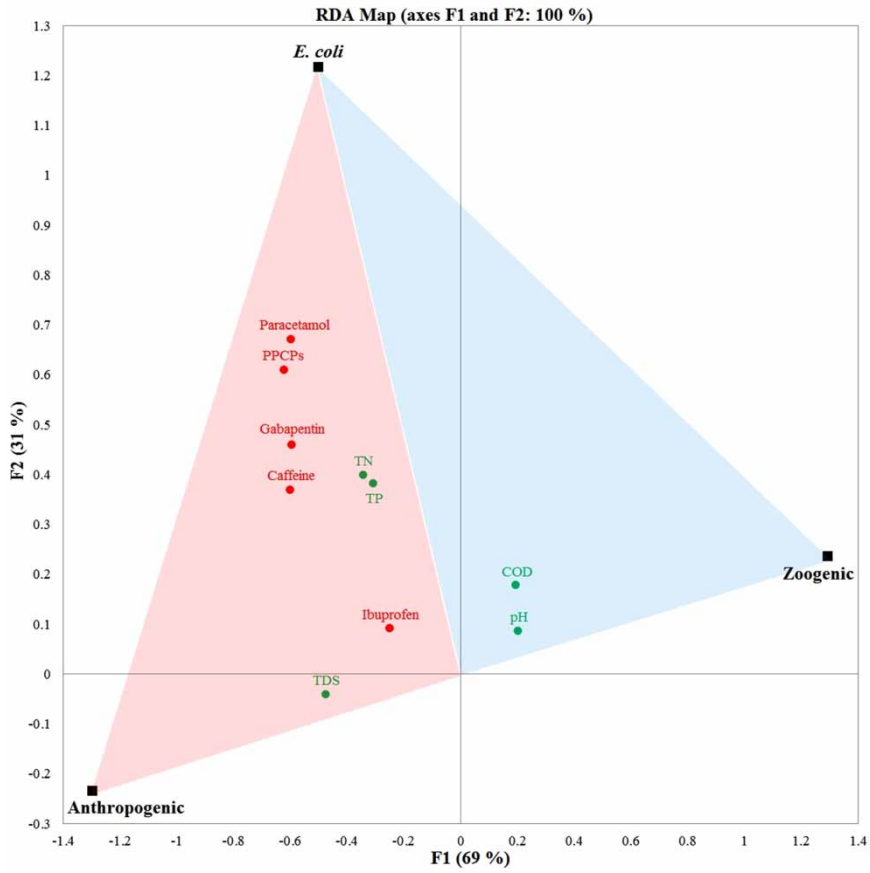

Figure 5 | RDA map of significant ($p < 0.05$) relationships between biotic (*E. coli* and the dominant faecal sources, i.e. zoogenic and anthropogenic) and abiotic (chemical parameters including PPCPs) components in the urban stream, Blåveisbekken.

strong statistically significant ($p < 0.05$) relationships were established between faecal contamination from anthropogenic sources and the total and most frequently detected PPCPs. This supports the application of PPCPs as chemical indicators for anthropogenic source tracking. Furthermore, Pearson's coefficient matrices indicated positive correlations between the total and most frequent PPCPs with both *E. coli* and a human DNA marker in the urban stream (Figure 6). The same trend was observed in the rural creek; however, *E. coli* was positively correlated only with the total PPCPs. Interestingly, the correlation patterns between the individual PPCPs and the anthropogenic DNA marker were much stronger in the rural creek (e.g. coefficient 0.949 for ibuprofen, 0.938 for gabapentin and 0.667 for caffeine) than in the urban stream, where the highest positive correlation (coefficient 0.85) was between *E. coli* and paracetamol (Figure 6). Yet, in the urban stream, the correlation patterns were stabilised among the faecal anthropogenic pollution origin and the total and most frequent PPCPs.

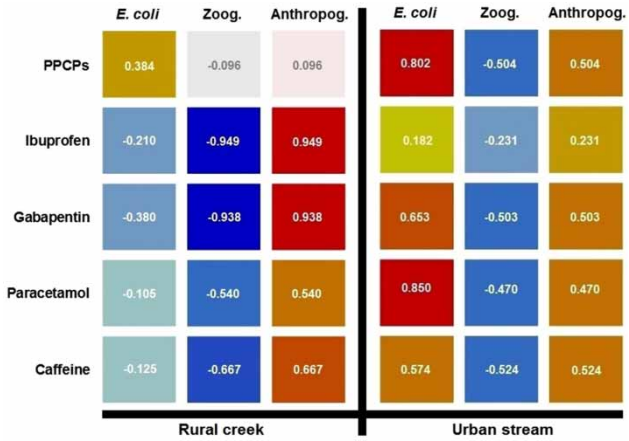

Figure 6 | Pearson's correlation matrix between *E. coli*, zoogenic (Zoog.) and anthropogenic (Anthropog.) faecal sources and total and selected PPCPs in the rural creek, Grytelandsbekken (left plot), and the urban stream, Blåveisbekken (right plot). The correlation heat map uses a hot–cold colour scale to display the correlation close to 1 (hot colours) and −1 (cold colours). Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wst.2020.603.

We also discovered that the main eutrophication-causing macronutrients (TN and TP) and TDS were significantly ($p < 0.05$) associated with the primary faecal pollution origins in the studied watercourses studied, i.e. with animal/environmental contamination sources in the zoogenic dominated rural creek (Figure 4) and human pollution sources in the anthropogenic dominated urban stream (Figure 5). This trend was also supported by Pearson's correlation matrices, which indicated the strongest links between TDS and *E. coli*, and TN and the zoogenic DNA marker in the rural creek, and between TP/TN and *E. coli*, and TDS and the anthropogenic DNA marker in the urban stream (Figure 7).

These findings imply that the QMST technique could open a new avenue of source identification of macronutrient pollution in water. It provides more 'real-time' and accurate information than currently applied statistics-based methods for point source pollution and model-based systems for non-point/diffuse source pollution (Strokal *et al.* 2015; Yi *et al.* 2017). Some studies have also implemented a stable isotope approach in nutrient source tracking (Zhang *et al.* 2014), but due to the high cost, sophisticated techniques, limited analysis capacities and challenges when multiple nutrient sources are present, this method is still under development and improvement (Grimmeisen *et al.* 2017; De Bondt *et al.* 2018). In comparison, QMST is a well-established methodology that can provide more biologically characterised information on the primary pollution source, i.e. anthropogenic or zoogenic, rather than merely geographical localisation of the sources. In this regard, QMST demonstrates a practical potential for rapid tracking of eutrophication-causing macronutrients in aquatic ecosystems.

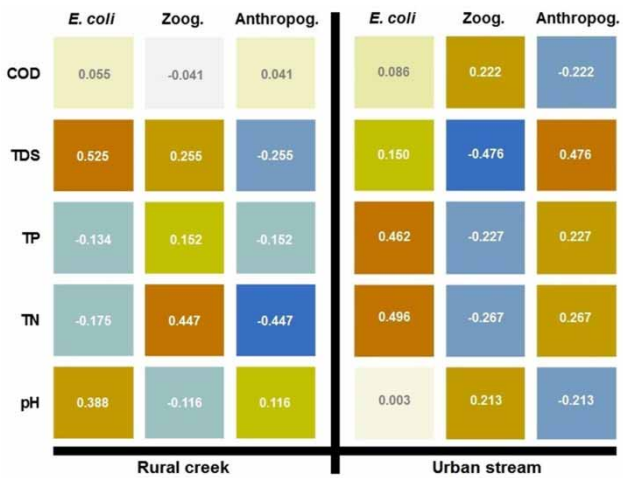

Figure 7 | Pearson's correlation matrix between *E. coli*, zoogenic (Zoog.) and anthropogenic (Anthropog.) faecal sources and macropollutants in the rural creek, Grytelandsbekken (left plot), and the urban stream, Blåveisbekken (right plot). The correlation heat map uses a hot–cold colour scale to display the correlation close to 1 (hot colours) and −1 (cold colours). Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wst.2020.603.

CONCLUSIONS

Overall, there are limited published records revealing an integrated cross-tracking of various faecal water contaminants and aligning them with primary anthropogenic or zoogenic pollution origins. We have integrated the outcomes of MST and CST tests and discovered that CEC (represented by PPCPs, their metabolites and BPA) correlates significantly with both *E. coli* and the human DNA marker. However, CST could not identify the primary pollution source of faecal water contamination. Source attribution was only attained through the QMST analysis together with derived FSAP, which enabled the pinpointing of the dominant faecal origin in the examined waters. The CST method, albeit supportive in validating QMST findings, is relevant for cases with apparent anthropogenic impact. The identified primary faecal pollution origins were significantly associated with the main eutrophication-causing macronutrients. Such findings underpin the key role of QMST in monitoring and targeted mitigating of water-related health risks and deterioration of aquatic environments. The results of our study highlight the need to prioritise QMST for regular water quality control, not only to track down faecal origins but also to aid the source attribution of macropollutants in water. In this context, further research with regard to the apportionment profile of various zoogenic sources and their associations with waterborne contaminants would be interesting.

FIGURES

Figure 1 was generated from a satellite image and topographical map of Norway provided by the NIBIO. The Institute's geographical data is archived in its primary map service, Kilden (https://kilden.nibio.no). All maps and images stored in Kilden are open source and can be saved or printed (https://www.nibio.no/en/subjects/soil/national-land-resource-map?locationfilter = true).

Figures 2–7 were created using tools in the Addinsoft (2019) XLSTAT statistical and data analysis solution (Boston, USA. https://www.xlstat.com).

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DATA AVAILABILITY STATEMENT

The datasets generated during this study are available from the corresponding author upon reasonable request.

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