A Randomised Controlled Trial Assessing Infectious Disease Risks from Bathing in Inland Recreational Waters

Edward K Lam [1], Márta Vargha [2], Daniel Thomas [3], Roland Salmon [4], Maria José Figueras [5], Julii Brainard [1], Jamie Bartram [6], Zsofia Barna [7], Paul R Hunter [1]

[1] Norwich Medical School, University of East Anglia, Norwich, NR4 7TJ, UK
[2] National Center for Public Health and Pharmacy, Budapest 1097, Hungary
[3] Communicable Disease Surveillance Centre, Public Health Wales, Cardiff, CF10 4BZ, UK
[4] Consultant Epidemiologist (Retired)
[5] Department of Basic Medical Sciences, Faculty of Medicine, University Rovira and Virgili, 43201 Reus, Spain

[6] School of Civil Engineering, University of Leeds, Leeds, LS2 9BT, UK
Department of Environmental Science and Engineering, School of Public Health, University of North Carolina at Chapel Hill, USA

[7] Independent consultant on water hygiene, https://legionellakontroll.hu/

* Corresponding author: Edward KS Lam (Edward.Lam@uea.ac.uk)

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ORCID numbers
Edward Lam 0000-0002-2115-9331
Márta Vargha 0000-0002-6426-2056
Julii Brainard 0000-0002-5272-7995
Paul R Hunter 0000-0002-5608-6144
Maria J Figueras 0000-0002-2268-8980
Daniel Rhys Thomas 0000-0002-2426-5893
Jamie Bartram 0000-0002-6542-6315

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Available at https://github.com/edwardkslam/Epibathe.

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Highlights

- Large RCT quantifying the health risk from recreational inland bathing.
- Standardised bathing and concurrent water sampling enabled dose-response analysis
- Bathers had higher risk of gastrointestinal and skin ailments.
- Gastrointestinal illness risk increased with *E. coli* and coliphage densities.
- Findings support *E. coli* and coliphage densities as indicators of freshwater quality.

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Abstract

Evidence to determine the suitability of water quality standards to prevent illness from recreation exposure in inland waters is limited. We report findings from four Hungarian freshwater study sites included in the Epibathe study, a large randomised controlled trial. A total of 2,368 participants were randomly allocated either to bathe for ten minutes undertaking at least three head immersions, or to remain on the shoreside without water contact. Concurrent water sampling quantified individual-level faecal indicator organism densities and health outcomes were assessed at one-week follow-up.

Generalised estimating equation models quantified the relative risk for adverse health outcomes for bathers versus shoreline-only (non-bather) participants, as well as the change in risk per unit increase in FIO concentration; crude, covariate-adjusted and covariate-adjusted models with multiple imputation are reported. Higher concentrations of *Escherichia coli* and somatic coliphage were each associated with increased risk of gastrointestinal illness (adj. RR = 1.73; 95% CI 1.13–2.65 and RR = 1.46; 95% CI 1.04–2.04 respectively). Bathing itself, independent of any individual microbial indicator, was associated with increased risk for skin ailments (adj. RR = 2.17; 95% CI 1.55-3.03). Low prevalence of eye, ear or respiratory infections precluded reliable estimation of exposure-response relationships for these outcomes.

These findings confirm the value of *E. coli* and potential of somatic coliphage densities as indicators of freshwater quality relevant to recreation-associated gastrointestinal illness risk. In freshwater settings, *E. coli* and coliphages appear to be more informative than enterococci as predictors of gastrointestinal illness, contrasting with evidence from marine waters.

Key words:
Open water swimming; rivers; lakes; *Escherichia coli*, faecal enterococci, somatic coliphages, gastrointestinal illness

1. Introduction

Outdoor recreation on inland-waterways is a popular pastime (University of Brighton *et al.* 2015, RYA 2023). Although engagement with natural "blue spaces" has been associated with diverse health and economic benefits (Gascon *et al.* 2017, White *et al.* 2020, Cromley & Mackintosh 2021, Geneshka *et al.* 2021), direct contact with, and in particular ingestion of, untreated freshwater may also pose a risk for waterborne infections. Microbial contamination remains a persistent problem for many freshwater bathing sites (Binns 2023) and recreational exposure has been linked to disease outbreaks. For example, a single open water river swimming event on the River Thames, UK, in October 2012 was associated with 338 cases of gastrointestinal illness linked in part to *Cryptosporidium* and *Giardia* infections (Hall *et al.* 2017).

Human and animal faecal material may enter freshwater systems from a range of sources, including sewage discharges, agricultural and urban surface runoffs, wildlife and human activities, including bathing itself. Assessment of recreational water quality typically relies on the quantification of faecal indicator organisms (FIOs), which act as proxy measures for faecal contamination and the potential presence of enteric pathogens. There is a strong evidence base for FIO-based monitoring in marine contexts, where randomised controlled trials have shown robust associations between concentrations of faecal streptococci and gastrointestinal illness (Cabelli *et al.* 1982, Dufour 1984, Ferley *et al.* 1989, Kay *et al.* 1994).

Although FIO-based monitoring is widely used by regulatory frameworks, such as the European Bathing Water Directive (2006/7/EC ; European Union 2006) and the World Health Organisation's Standards for Recreational Water Quality (WHO 2021), there remains uncertainty regarding how well FIO concentrations predict health risks in freshwater environments dominated by diffuse, non-point contamination (Kozak *et al.* 2025). Observational cohort studies, comparing illness outcomes among self-selected bathers and non-bathers, have attempted to address this uncertainty. Whilst informative, these studies are vulnerable to selection and confounding bias, especially when baseline behaviours and susceptibility differ between groups and are not adequately controlled for (Kay *et al.* 1994). Additionally, individual-level exposure is often poorly characterised, with limited use of concurrent microbiological

measurements. As a result, cohort studies have reported inconsistent associations between exposure to FIO concentrations and illness risk (Fewtrell & Kay 2015).

Addressing this evidence gap requires studies that pair individual-level FIO exposure measurements with health outcomes under randomised controlled conditions; to date, however, only one such randomised controlled trial (RCT) has been published (Wiedenmann *et al.* 2005).

Here we report findings from the four freshwater study sites included in the Epibathe study, a large randomised controlled study carried out in 2006/2007 at bathing sites in Europe. Participants were prospectively recruited and randomly allocated to bathing or non-bathing exposure groups. All bathers had comparable bathing duration and immersion behaviours. Concurrent water sampling enabled the characterisation of individual-level FIO exposure and health outcomes were assessed at one and three-week follow-up. Following Wiedenmann et al. (2005), we restricted subsequent analyses to outcomes reported at one-week follow-up, consistent with the acute time course expected of water-associated illnesses. Using population-average generalised estimating equation models that adjusted for socio-demographic covariates and accounted for clustering by study site, we quantified associations between specific faecal indicator concentrations and subsequent illness risk. Drawing upon data from four study sites spanning a broad microbial exposure gradient, these findings strengthen the epidemiological basis for *E. coli* and somatic coliphages as indicator organisms for recreational water-quality guidelines in freshwater environments affected by diffuse contamination.

2. Methods

A detailed experimental 'manual' for undertaking the Epibathe study was developed and revised as the trials proceeded. The manual contained instructions and guidance for trialists and support staff, including lessons learned, steps taken to ensure data quality, reasons for aspects of the protocol and other protocol information. This manual is available in Supplementary Text 1 (Supplementary Materials). A shorter summary of

trial implementation is below. Ethical clearance for this component of the Epibathe study was granted by the Hungarian Scientific and Research Ethics Committee in 2006.

2.1 Study sites

The Hungary field studies were a mixture of four riverine and lacustrine locations. They were chosen in order to capture the natural variability in water movement, mixing, usage and potential for microbial contamination across bathing sites in Central Europe. All four sites met EU bathing water standards. The trial sites are described individually and specifically in the Methods (next paragraphs) but their respective results are only indicated by non-identifiable numbers otherwise (numbered from 10 to13). Further details and maps are available in Supplementary Text 3.

Dömsöd and Fadd bathing sites were predominantly lacustrine in nature, situated on fossil reaches of the River Danube: dead-arm channels that are hydrologically connected to, but largely isolated from, the main river. The Fadd site was more hydrologically enclosed and exhibited no direct water exchange with the main Danube channel, but there was limited exchange at the Dömsöd site, particularly during flooding. Neither site received direct effluents from wastewater treatment facilities, but both sites were subjected to diffuse sources of contamination associated with local human settlements, recreational huts and waterborne activity, including bathing and fishing.

Tiszakécske and Csongrád riverine sites were situated along the Tisza River, a transboundary river that flows through Romania and Ukraine before entering Hungary. In contrast with the partially isolated fossil reaches in Dömsöd and Fadd, these riverine bathing areas are directly connected with upstream waters, with continuous freshwater flow and less opportunities for stagnation. The Tisza River from the Tiszakécske site receives secondary treated sewage effluent both directly and indirectly through small water inflows (Fózer *et al*. 2024). Both human and animal (bovine and porcine) faecal input have been identified (Rusiñol *et al*. 2014). At the Csongrád site, the Tisza River receives further input from the Körös River, a major tributary originating from Romania that is prone to pollution events. Diffuse pollution from agricultural activity, holiday homes, unofficial bathing sites, bathing and fishing may have also occurred.

2.2 Microbiological Testing

All study sites were divided at ten meter intervals into six bathing zones. Within each zone, water samples were collected every twenty minutes at chest depth throughout the trial. These samples were analysed by the laboratory of the Hungarian National Public Health Center for *E. coli*, Intestinal Enterococci and Somatic coliphage: levels were quantified using the microtitre 96 well Most Probable method (EC96) (ISO9308-3:1998), microtitre 96 well Most Probable method (IE96) (ISO7899-1:1998) and plaque assay (ISO10705-2:2000) respectively.

2.3 Recruitment and randomisation of participants

Volunteers were predominantly recruited from the local communities situated within 15-50km of each study site. Recruitment was facilitated by internet and radio advertisements, poster campaigns and through collaboration with local public health offices. Standardised baseline questionnaires collected from volunteers included pertinent information on demographics, recent medical history and potential behavioural confounders. All answers from were scanned into a participant dataset using optical character reading (OCR). Steps taken to ensure data quality are documented in the trial manual in Supplementary Text 1. Volunteers were allowed to participate if they gave informed consent, appeared to be generally in good health, were willing to bathe at the sites and said that they were able to comply with study procedures. Each volunteer who gave complete information (filled in all questionnaires) was eligible to receive 40 € compensation in shopping vouchers.

On the trial day, volunteers were again interviewed to account for any changes in recent medical history or potential behavioural confounders since recruitment. Using a computer-generated allocation schedule in Microsoft Excel, volunteers were randomly allocated to one of two groups: bathers or non-bathers. Bathers and non-bathers were separated and identified distinctively by specific colour wrist bands or t-shirts. All bathers were required to bathe once for ten minutes within their designated zone, making at least three full facial immersions. Individual exposure levels (of faecal indicators) were assigned based on their zone and nearest time zone where bathing

took place; the mean value was used when exposure occurred exactly between two time zone. Non-bather volunteers were instructed not to bathe one week before and after the trial; during the trial, they were not allowed to bathe at all. In the non-bather zone alternative activities were provided, such as a bouncy castle, football and climbing walls. Non-bathers had to stay on the beach for three hours while the bathers had their immersions. Only bathers were allowed into the bather area, but every volunteer could go into the non-bather area. Both bathers and non-bathers were supplied with the same sandwich lunch options.

2.4 Health outcomes

Seven days after the trial, participants were interviewed in person or by telephone to assess for post-exposure health conditions and symptoms. Following the approach of Wiedenmann *et al*. (2005), we defined the five primary health outcomes (below) using Boolean combinations of reported symptoms (operators in capitals). These symptom definitions mirrored those used in Wiedenmann *et al*. to facilitate comparability.

(i) **Gastrointestinal illness** (GI): *diarrhoea* OR *vomiting* OR (*nausea* AND *fever*) OR (indigestion AND fever)

(ii) **Acute febrile respiratory illness**: (*headache* OR *joint pain* OR *blurred vision* OR *loss of appetite* OR *tiredness* OR *dizziness* OR *pins & needles* OR *muscle cramps*) AND (*sore throat* OR *dry cough* OR *productive cough* OR *shortness of breath* OR *runny nose*)

(iii) **Ear infection**

(iv) **Eye infection**

(v) **Skin Ailment**: *skin rash* OR *skin ulcer* OR *itching*

2.5 Predictor Variables

For each primary health outcomes, we estimated the independent association with each of the following exposure variables:

(i) **Bathing group status**

(ii) Microbial water-quality indicators (log-transformed titres):

   a. ***E. coli***

   b. **Enterococci**

c. Coliphages

These exposures were analysed independently, using separate generalised estimating equation (GEE) models. Additional covariates were considered with each model:

(i) **Prior illness** (three weeks before water exposure): to adjust for pre-existing background prevalence, each model included an indicator of prior illness matching the case definition of the outcome, i.e. prior GI illness when modelling GI illness outcomes.

(ii) **Age group**: categorised as 4-10, 11-20, 21-30, 31-40 and >40 years.

(iii) **Potential confounders**: we evaluated several behavioural variables that were potentially associated with gastrointestinal infections and other health outcomes, e.g. raw or unpasteurised milk consumption, stomach remedy and alcohol intake (see Tables S1-5 in Supplementary Text 2). Covariates demonstrating evidence of association with the health outcome at the $p<0.10$ level were considered potential confounders and were retained for inclusion in subsequent multivariate generalised estimating equation (GEE) models. For rare health outcomes, some behavioural variables exhibited perfect separation and were thus excluded from subsequent multivariate GEE models.

2.6 Generalised Estimating Equations

To estimate the association between bathing-water exposure and adverse health outcomes, we used GEE models to obtain population-average (marginal) relative risks (GEE; Liang & Zeger 1986). This approach accounts for the clustering of participants within study sites and potential geo-temporal variations in underlying illness prevalence. Unlike random-effects models, GEEs directly estimate marginal effects without requiring assumptions about the distribution of site-level effects or their independence from exposure variables: such assumptions would be violated in environmental studies, where site characteristics are intrinsically liked to microbial water quality.

All analyses were conducted in R version 4.5.1 using the glmtoolbox package (version 0.1.12). All data and code needed to reproduce the analyses are provided in the project Github repository (https://github.com/edwardkslam/Epibathe). Significance threshold was set at $p < 0.05$.

2.6.1 Model Structure

Let Y_{ik} denote the binary health outcome, e.g. gastrointestinal illness at one-week follow-up, for participant i in study site (cluster) k:

$$Y_{ik} \sim Bernoulli(p_{ik})$$

With p_{ik} being the probability of illness for participant i in site k.

We modelled this probability using a log-linked binomial GEE, so that

$$\log(p_{ik}) = \beta_0 + \beta_{exp}X_{exp,ik} + \beta_1 X_{1,ik} + \beta_2 X_{2,ik} + \cdots + \beta_p X_{p,ik}$$

where $\beta_{exp}X_{exp,ik}$ and $X_{1,ik,}, X_{2,ik}, \cdots, X_{p,ik}$ denotes the exposure and covariate values (see above) respectively for participant i in site k. Under the log-link, exponentiated coefficients yield the relative risks:

$$RR_j = e^{\beta j}$$

Which represents the multiplicative change in risk associated with a one-unit increase in predictor X_j.

Participants within the same site are likely to experience similar environmental factors and baseline exposure to background illness. To account for non-independence of individual responses within each study site, we specified an exchangeable working correlation, meaning that all pairs of participants within the same site share a common correlation parameter α:

$$Corr(Y_{ik,}Y_{i'k}) = \begin{cases} 1 & i = i' \\ \alpha & i \neq i' \end{cases}$$

Robust (sandwich) standard errors (Huber 1967, White 1980) were used to ensure valid inference in case the working correlation structure was mis-specified.

2.7 Multiple Imputation

To assess for robustness to missing data, we imputed missing covariate values using multiple imputation. Missing data were limited to covariate information (Table S6 in Supplementary Text 2); the fraction of missing information differed across the health outcomes considered, with the highest proportion observed in the GI models (1.65%; n=39). Following the rule of thumb recommended by White *et al.* (2011), whereby the number of imputations should exceed the percentage of incomplete cases to ensure reproducible standard errors, we generated $m = 5$ imputed data sets. These were performed under a fully conditional specification model with the Missing At Random (MAR) assumption using the mice package (version 3.18.0) in R. These imputation models included all covariates, exposure variables and health outcomes, in order to preserve underlying associations between predictors and outcomes.

For each imputed data set $d = 1, \cdots, m$, we fitted GEE models and obtained robust estimates for the regression coefficient $\hat{\beta}^{(d)}$ and variance $\hat{V}^{(d)}$. Pooled parameter estimates were derived using Rubin's Rules (Rubin 1996):

$$\bar{\beta} = \frac{1}{m}\sum_{d=1}^{m} \hat{\beta}^{(d)} \text{ z}$$

$$\bar{V} = \frac{1}{m}\sum_{d=1}^{m} \hat{V}^{(d)}$$

It is necessary to further inflate within-imputation variance $\bar{V}$ with between-imputation variation B to reflect the effect of missing data and Monte Carlo error.

$$B = \frac{1}{m-1}\sum_{d=1}^{m} \left(\hat{\beta}^{(d)} - \bar{\beta}\right)^2$$

Total (pooled) variance T is therefore given as:

$$T = \bar{V}\left(1 + \frac{1}{m}\right)B$$

Subsequent results from imputed analyses were highly consistent with complete-case estimates (Table 2); given the low proportion of missingness and restriction of

imputation to covariates, rather than outcomes, additional sensitivity analyses were not pursued.

3. Results

3.1 Study population

Across the 2006 and 2007 bathing seasons, a total of 2368 persons participated in bathing trials and subsequent one-week follow-up across four freshwater bathing sites in Hungary. Of these, 1,133 (47.8%) were randomised to the bather group and 1,235 (52.5%) to the non-bathers. Baseline demographic and behavioural characteristics were well-balanced between bathers and non-bathers (Table S7 in Supplemental Text 2): no statistically significant differences were observed across key variables, including prior illnesses. With regards to problem health outcomes, gastrointestinal (n=48; 2.03%) and skin (n=47; 1.98%) illnesses had the highest prevalences (Table 1).

Health problem	Overall (n=2368)	Non-bather (n=1235)	Bather (n=1133)
Gastrointestinal	48 (2.03%)	24 (1.94%)	24 (2.12%)
Skin	47 (1.98%)	16 (1.30%)	31 (2.74%)
Respiratory	9 (0.38%)	2 (0.16%)	7 (0.62%)
Ear	13 (0.55%)	5 (0.40%)	8 (0.71%)
Eye	13 (0.55%)	4 (0.32%)	9 (0.79%)

Table 1: Frequency of primary health outcomes by bather status

Initially, 2,724 participants were recruited, of whom 2,368 completed the one-week follow-up interview, representing an overall retention rate of 86.9% (Table S12 in Supplementary Text S2). Substantial attrition occurred between initial recruitment and the bathing trial (162 across all four sites), most notably at site 13 (645 to 537; -21.4%). This pattern may reflect site-specific recruitment or logistical challenges. Reassuringly, post-bathing follow-up completion was high across all four sites: sites 10 (679 to 664; -2.2%), 11 (616 to 607; -1.5%), 12 (597 to 592; -0.8%) and 13 (507 to 505; -0.4%). This

suggests that outcome ascertainment bias from differential dropout after exposure is unlikely to be a major concern in the analysis.

3.2 Microbiological Titres

For bathing participants, we examined study site-specific exposure to the three microbial indicators, which were quantified using standardised laboratory methods (see Methods). Overall, we observed substantial between-site heterogeneity in microbial concentrations (Figure 1 and Table S8 in Supplementary Text 2).

For sites 10 to12, *E. coli*, Intestinal Enterococci and somatic coliphage titres were mostly right-skewed, with most samples clustered near the lower detection limit and relatively few high-titre observations (Figure 1 and Table S8 in Supplementary Text 2). Notably, somatic coliphages were not detected at site 11, with all measurements at or below the plaque assay detection limit. In contrast, site 13 exhibited markedly higher and more variable concentrations, particularly for *E. coli* and somatic coliphages. This variability supports the use of site-clustered statistical approaches, in order to assess the associations between microbial indicators and health outcomes across a broad exposure gradient.

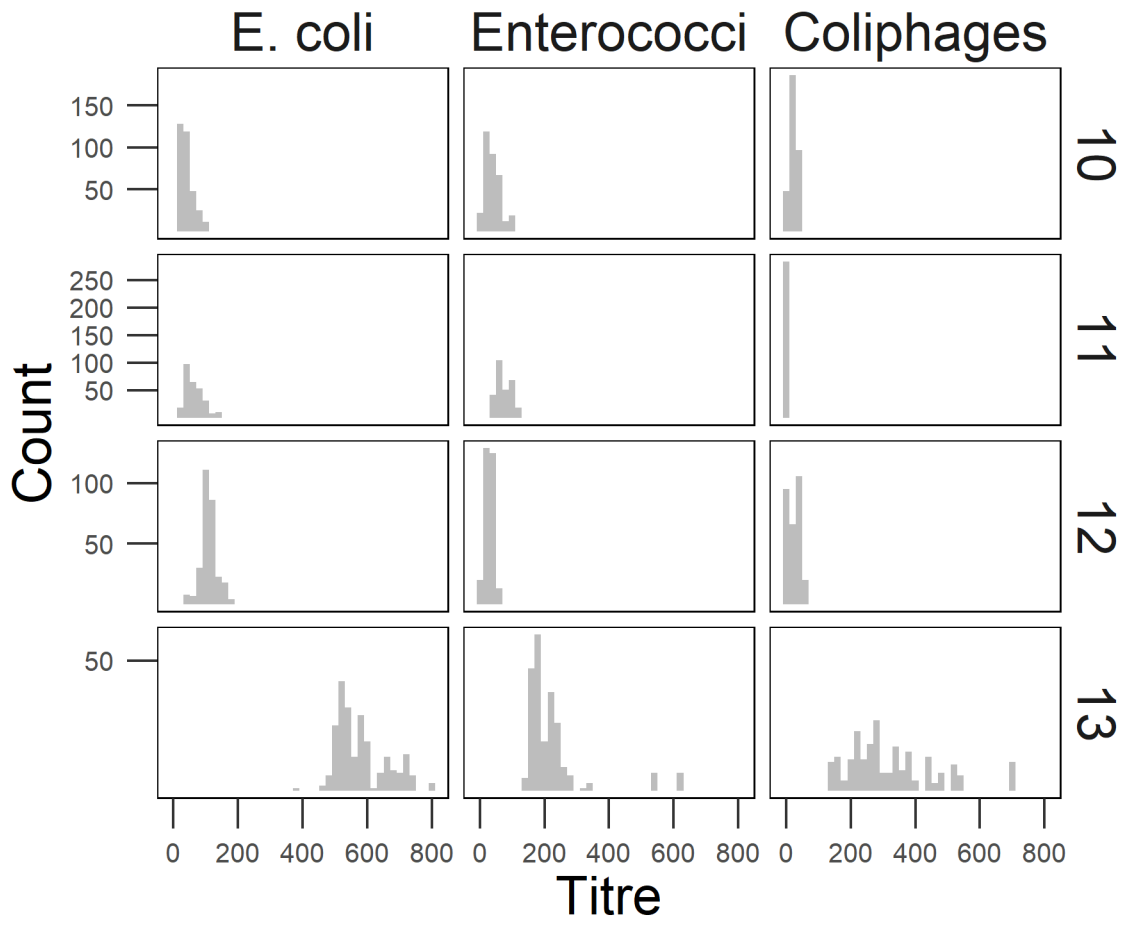

Figure 1: The distribution of *E. coli*, Intestinal Enterococci and Somatic coliphage titres across the four Hungarian study sites. The three indicators were quantified using standardised microbiological methods (see Methods).

3.3 Gastrointestinal Illness

We examined the effect of each of the four exposure variables on gastrointestinal illness in separate GEE models, using only complete cases. After adjusting for recent GI illness, age group and additional behavioural confounders (Table 2, Adjusted Model, middle column), we observed statistically significant positive associations ($p<0.05$) between GI illness and the microbial indicators *E. coli* (RR = 1.77 per one-unit increase in log-transformed indicator density; 95% CI 1.15-2.73) and somatic coliphage (RR = 1.48; 95% CI 1.05-2.06). In contrast, neither bathing status nor Intestinal Enterococci concentrations showed no such statistically significant associations with GI illness. Notably, the estimated effect of *E. coli* increased after adjustment for age, indicating negative confounding by age, whereby younger participants, particularly 11-20 and 21-

30 year olds (Table S9 in Supplementary Text 2), had a higher underlying risk of GI illness than other age groups.

We assessed for potential age-specific heterogeneities in illness risk by incorporating interaction terms between log-transformed E. coli concentrations and age group in the GEE model (Table S10 in Supplementary Text 2). Although age-specific interaction coefficients were individually significant ($p<0.05$), the overall interaction term was not (Joint Wald test; $\chi2(3)=7.41$; $p=0.06$). Age group-specific average marginal effects (AME) showed modest heterogeneity in the exposure-response relationship (Table S11 in Supplementary Text 2), with larger increases in predicted GI illness risk observed amongst the 11-20 year- (AME=0.03; 95%CI 0.02-0.03) and 21-30 year- (AME=0.02; 95%CI –0.01-0.05) old cohorts.

To assess the robustness against missing data, we repeated our analyses with multiple imputation: a total of 39 missing covariate values (1.65% of cases) were imputed, generating 5 replicate datasets, which were subsequently pooled (see Methods). The imputed GEE models (Table 2, Adjusted+MI Model, rightmost column) yielded estimates for the effect size for *E. coli* (RR = 1.73; 95% CI 1.13–2.65) and Somatic coliphage (RR = 1.45; 95% CI 1.05–2.04) that were highly consistent with our initial complete-case analyses, suggesting limited impact from missing data on inference.

3.4 Skin Ailments

Skin ailments were the second most common adverse health outcome (n = 47). Co-variate adjusted GEE models showed that bathing status was positively associated with increased risk for skin ailments across both complete-case (RR = 2.17; 95% CI 1.55-3.03) and imputed analyses (RR = 2.30; 95% CI 1.57-3.37; Table 2). In contrast, none of the microbial indicators were associated with increased risk of skin ailments (at $p<0.05$).

3.5 Acute Febrile Respiratory Illness

Respiratory illness was a rare outcome (n = 9) within this study cohort. Because of this low incidence, several covariates, including prior respiratory illness and egg

consumption, exhibited perfect separation, meaning that no participants with these characteristics experienced the outcome. This separation prevented stable convergence of covariate-adjusted GEE models, resulting in non-estimable or unreliable relative risk estimates, as was the case for *E. coli*, in both complete-case and imputed (Table 2) analyses.

To address the issues arising perfect separation, we evaluated crude, unadjusted associations between each exposure variable and respiratory illness. We re-fit GEE models excluding all covariates (Table 2). These crude, simplified models, which only retained the exposure variable of interest and accounted for clustering within study sites, converged successfully; however, the rarity of the outcome inherently resulted in wide confidence intervals. Consequently, it was difficult to determine whether exposure to microbial indicators truly resulted in any additional risk for respiratory illness beyond background levels.

3.6 Ear Infections

Ear infection was also a rare outcome (n=13). Similar to the respiratory illness GEE models, several covariates, including prior ear infection and raw milk consumption, exhibited perfect separation again. As a result, covariate-adjusted GEE models for all microbial indicators failed to converge, producing unreliable relative risk estimates in both complete-case and imputed analyses. However, GEE did converge for bathing status for both complete and imputed (Table 2) analyses, but the associations (both RR = 1.64; 95% CI 0.91-2.95) were not statistically significant (p = 0.10).

Crude, unadjusted models similarly failed to converge for the microbial indicators and bathing status did not have a statistically significant association with ear infections.

3.7 Eye infections

Eye infections were likewise rare (n = 13). As with the other rare outcomes, several covariates, including prior eye infection and raw milk consumption, exhibited perfect separation. The covariate-adjusted GEE models again failed to converge for all

microbial indicators failed to converge in both complete-case and imputed analyses. Bathing-status models converged; however, for both complete-case and imputed (Table 2) analyses, bathing was not significantly associated ($p = 0.10$) with increased risk of eye infection at one-week follow-up. Again, the wide confidence intervals highlighted the limited reliability of these estimates.

Crude GEE models, which excluded all covariates to avoid separation, also failed to identify any statistically significant associations between exposure variables and eye infection risk.

GI Illness	Crude			Adjusted			Adjusted +MI		
Indicator	RR	95% CI	p-value	RR	95% CI	p-value	RR	95% CI	p-value
Non-bathers	1	-	-	1	-	-	1	-	-
Bathers	1.11	0.61-2.02	0.73	1.13	0.63-2.02	0.68	1.12	0.63-2.00	0.7
Log. *E. coli*	1.48	0.98-2.25	0.06	1.77	1.15-2.73	0.01	1.73	1.13-2.65	0.01
Log. I. Enterococci	1.25	0.67-2.35	0.48	1.20	0.75-1.94	0.45	1.18	0.74-1.88	0.49
Log. S. phage	1.55	1.18-2.04	0.002	1.48	1.05-2.06	0.02	1.45	1.05-2.04	0.03

Skin Ailments	Crude			Adjusted			Adjusted +MI		
Indicator	RR	95% CI	p-value	RR	95% CI	p-value	RR	95% CI	p-value
Non-bathers	1	-	-	1	-	-	1	-	-
Bathers	2.12	1.47-3.06	0.00	2.17	1.55-3.03	0.00	2.30	1.57-3.37	0.00
Log. *E. coli*	0.70	0.36-1.39	0.31	0.61	0.26-1.44	0.26	0.50	0.21-1.16	0.11
Log. I. Enterococci	0.53	0.17-1.58	0.25	0.63	0.24-1.68	0.36	0.50	0.19-1.28	0.15
Log. S. phage	1.38	0.89-2.13	0.15	1.31	0.88-1.93	0.18	1.26	0.79-2.00	0.34

Respiratory Illness	Crude			Adjusted			Adjusted +MI		
Indicator	RR	95% CI	p-value	RR	95% CI	p-value	RR	95% CI	p-value
Non-bathers	1	-	-	1			1		
Bathers	3.81	0.85-17.1	0.08	Did not converge			Did not converge		
Log. *E. coli*	2.52	1.12-5.65	0.03	3.02	1.42-6.42	0.00	3.01	1.41-6.43	0.00
Log. I. Enterococci	7.95	0.98-64.7	0.05	Did not converge			Did not converge		
Log. S. phage	1.76	1.14-2.72	0.01	Did not converge			Did not converge		

Ear Infection	Crude			Adjusted			Adjusted +MI		
Indicator	RR	95% CI	p-value	RR	95% CI	p-value	RR	95% CI	p-value
Non-bathers	1	-	-	1	-	-	1	-	-
Bathers	1.76	0.87-3.56	0.11	1.64	0.91-2.96	0.10	1.64	0.91-2.96	0.10
Log. *E. coli*	Did not converge			Did not converge			Did not converge		
Log. I. Enterococci	Did not converge			Did not converge			Did not converge		
Log. S. phage	Did not converge			Did not converge			Did not converge		

Eye Infection	Crude			Adjusted			Adjusted +MI		
Indicator	RR	95% CI	p-value	RR	95% CI	p-value	RR	95% CI	p-value
Non-bathers	1	-	-	1	-	-	1	-	-
Bathers	2.46	0.97-6.21	0.06	2.29	0.85-6.22	0.10	2.29	0.84-6.2	0.10
Log. *E. coli*	1.20	0.90-1.61	0.21	Did not converge			Did not converge		
Log. I. Enterococci	0.73	0.34-1.59	0.43	Did not converge			Did not converge		
Log. S. phage	1.55	0.95-2.52	0.08	Did not converge			Did not converge		

Table 2: Model results for infection risks

Notes: Relative risks for microbial indicators correspond to a one-unit increase in log-transformed indicator density. RR = Relative risk; Log. = natural logarithmic transformation of raw values of stated indicator; MI = multiple imputation; "Did not converge" means the model could not be constructed; I. Enterococci refers to Intestinal Enterococci; S. phage refers to somatic coliphages.

4. Discussion

Across all health outcomes considered, GI illness was most consistently associated, as theoretically could be expected, with faecal contamination (Cabelli *et al.* 1982, Dufour 1984, World Health Organization 2021). In particular, higher concentrations of *E. coli* and somatic coliphages were each associated with increased risk (RR = 1.73; 95% CI 1.13–2.65 and RR = 1.45; 95% CI 1.05–2.04 respectively), whereas Intestinal Enterococci showed no such association. Independent of microbial concentrations, bathing itself was associated with an increased risk of skin ailments (RR = 2.30; 95% CI 1.57-3.37). *E. coli* appeared to be associated with respiratory illness (RR=3.01; 95% CI 1.41-6.43), with a wide confidence interval as the result of a low prevalence of disease (n=7). Bathing status nor any of the FIOs were robustly associated with ear or eye infections: stable model convergence and reliable inference for these outcomes were limited by low case numbers (n=8 and 9 respectively).

These results suggest that *E. coli* and somatic coliphages counts are better predictors for GI illness in freshwater environments than enterococci, unlike the case in marine waters (Cabelli *et al.* 1982, Dufour 1984, Kay *et al.* 1994, World Health Organization 2011) . Experimental studies have shown that environmental drivers, such as sunlight and interactions with indigenous microbiota, can contribute to differential persistence of FIOs in freshwater and marine waters (Fujioka & Narikawa 1982, Davies 1989, Korajkic *et al.* 2013): notably, *E. coli* and somatic coliphages decay more slowly and persist longer than enterococci in freshwater environments (Anderson *et al.* 2005), implying that the former may better retain a signal of recent faecal contamination and predict GI health risks. This pattern differs from marine environments, where enterococci exhibit greater environmental stability (Anderson *et al.* 2005) and have been shown to be reliable predictors of illness, whereas *E. coli* performs poorly as an indicator (Cabelli *et al.* 1982, Dufour 1984, Ferley *et al.* 1989, Kay *et al.* 1994).

Taken together, enterococci are likely poor indicators of faecal contamination and associated health outcomes in freshwater settings, emphasising the need for appropriate microbial indicators for inland recreational waters. Concurrently, freshwater and marine systems may require different indicator organisms and compliance regimes to account for the contrasting performance of indicators between

environments. The absence of a single unified standard presents regulatory challenges, including the financial and administrative costs of individually classifying bathing locations and assessing them against different standards. Such clear dichotomisation would also fail to provide practical guidance for many popular bathing sites situated within transitional environments, such as estuarine beaches. In these settings, the coexistence of freshwater and marine influences may further complicate the behaviour and predictive value of microbial indicators.

The association between bathing status and skin ailments, in the absence of a corresponding relationship with FIO concentrations, suggests that bathers are exposed to authoctonous microorganisms or other contaminants, which are independent of human faecal pollution. One plausible mechanism is the exposure to other non-human pathogens, which would not be expected to correlate with human FIO concentrations. Avian schistosomes are parasitic trematodes shed in the faeces of waterfowl and can cause cercarial dermatitis (swimmer's itch) through direct skin penetration (Horák *et al*. 2015). Whilst clinical cases of cercarial dermatitis are sparsely reported in Hungary (Juhász *et al*. 2022), the true prevalence may be underestimated, as mild cases are likely to resolve spontaneously without prompting medical consultation. Alternatively, whilst our study sites were not directly affected by wastewater treatment effluents, agricultural or industrial runoffs could have contaminated the freshwater with chemical irritants.

An RCT design addresses the key methodological limitations of observational cohort studies in freshwaters by enabling robust, exposure-response based relative risk estimation, under controlled conditions. The Epibathe study thus addressed a critical evidence gap surrounding the use of FIOs to monitor freshwater quality. Wiedenmann *et al*. (2005) remains the only previously published RCT to have examined the health risks following controlled freshwater exposure with concurrent measurement of FIOs. Similar to our findings, (Wiedenmann *et al*.) reported positive associations between increasing *E. coli* concentrations and GI illness risk. However, their No Observed Adverse Effect Level (NOAEL) framework relies upon a single exposure threshold, above which presents increased risk of illness: whilst readily applicable for regulatory purposes, this fails to quantify risk across the full exposure-response gradient. Instead, our analyses provide population-average relative risks across a broad range of FIO

exposures, enabling a more detailed epidemiological characterisation of such exposure-response relationships.

In sites dominated by point-source sewage contamination, the association between FIO concentrations and the risk of GI illness is well established, forming the basis for existing regulatory standards (Cabelli *et al.* 1983, Office of Water Quality 2012). In contrast, evidence for the health impact of non-point contamination, which is largely derived from cohort studies, has been inconsistent, resulting in uncertainty surrounding the predictive value of FIOs in such settings (Fujioka *et al.* 2015). With the superior study design of a RCT, our findings demonstrate statistically significant associations between GI illness and both *E. coli* and somatic coliphages across freshwater environments characterised by diffuse contamination. By combining site-clustered analyses across a variety of freshwater environments and a broad exposure gradient, this Epibathe trial robustly extends the conclusions of Wiedenmann *et al.* (2005): the consistency of findings across the different freshwater systems of Hungary and Germany respectively provides a strong empirical foundation for the wider applicability of these exposure-response relationships. Further studies will be necessary to confirm these findings in other climatic and environmental settings and thereby strengthen the epidemiological evidence base for specific FIOs, relevant to recreational water quality guidelines internationally.

Limitations for our study include: several non-gastrointestinal outcomes were rare, limiting statistical power and preventing robust inference for respiratory, ear and eye infections. Additionally, while FIO measurements were temporally proximate to faecal exposure, they remain indirect proxies for the presence of human enteric pathogens: our microbiological methodologies could not exclude the possibility that elevated FIO concentrations originated from non-human faecal contamination. These constraints highlight the need for larger RCTs with greater statistical power and environmental diversity, as well as the evaluation of a broader panel of FIOs, including source-specific markers, that may better reflect health risks to humans.

5. Conclusion

The Epibathe trial quantified GI illness risk across a broad range of FIO exposures in freshwater recreational environments. Our findings reinforce the value of *E. coli* as a FIO for GI illness and highlight the growing importance of somatic coliphages as indicators of bathing water quality, whilst providing evidence that Intestinal Enterococci have limited predictive value in freshwater contexts. These results are consistent with those from the only previously published similar RCT epidemiological study in freshwater (Wiedenmann *et al.* 2005).

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References

Anderson, K. L., J. E. Whitlock and V. J. Harwood (2005). "Persistence and differential survival of fecal indicator bacteria in subtropical waters and sediments." Applied and Environmental Microbiology **71**(6): 3041–3048.

Binns, K. (2023, Aug 10). "When water pollution is rife in the UK, where's safe for wild swimming?", from https://www.nationalgeographic.com/travel/article/water-pollution-uk-where-wild-swimming.

Cabelli, V., A. Dufour, L. McCabe and M. Levin (1982). "Swimming-associated gastroenteritis and water quality." American Journal of Epidemiology **115**(4): 606–616.

Cabelli, V., A. Dufour, L. McCabe and M. Levin (1983). "A marine recreational water quality criterion consistent with indicator concepts and risk analysis." Water Pollution Control Federation **55**(10): 1306–1314.

Cromley, V. and C. Mackintosh. (2021). "The Economic Impact of Outdoor Recreation in the UK: The Evidence." from https://researchonline.ljmu.ac.uk/id/eprint/1579/1/Reconomics%20-%20The%20Evidence%20PDF%202014.pdf.

Davies, C. M. (1989). The effect of sunlight and other factors on the survival of starved enteric bacteria in natural waters, Newcastle University.

Dufour, A. P. (1984). "Health effects criteria for fresh recreational waters." Environmental Protection Agency. August. from
European Union (2006). Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC. Document 32006L0007. European Union, European Union,. **Document 32006L0007**.

Ferley, J., D. Zmirou, F. Balducci, B. Baleux, P. Fera, G. Larbaigt, E. Jacq, B. Moissonnier, A. Blineau and J. Boudot (1989). "Epidemiological significance of microbiological pollution criteria for river recreational waters." International Journal of Epidemiology **18**(1): 198–205.

Fewtrell, L. and D. Kay (2015). "Recreational water and infection: a review of recent findings." Current Environmental Health Reports **2**(1): 85–94.

Fózer, M., M. T. Nagy, E. Csépes, M. Cserháti and E. Kaszab (2024). "Potenciális szennyezők monitorozása a Közép-Tisza szakaszon vízhigiénés szempontból." Journal of Central European Green Innovation **12**(1-2): 66–75.

Fujioka, R. and O. Narikawa (1982). "Effect of sunlight on enumeration of indicator bacteria under field conditions." Applied and Environmental Microbiology **44**(2): 395–401.

Fujioka, R. S., H. M. Solo-Gabriele, M. N. Byappanahalli and M. Kirs (2015). "US recreational water quality criteria: a vision for the future." International Journal of Environmental Research and Public Health **12**(7).

Gascon, M., W. Zijlema, C. Vert, M. P. White and M. J. Nieuwenhuijsen (2017). "Outdoor blue spaces, human health and well-being: A systematic review of quantitative studies." International Journal of Hygiene and Environmental Health **220**(8): 1207–1221.

Geneshka, M., P. Coventry, J. Cruz and S. Gilbody (2021). "Relationship between green and blue spaces with mental and physical health: a systematic review of longitudinal observational studies." International Journal of Environmental Research and Public Health **18**(17): 9010.

Hall, V., A. Taye, B. Walsh, H. Maguire, J. Dave, A. Wright, C. Anderson and P. Crook (2017). "A large outbreak of gastrointestinal illness at an open-water swimming event in the River Thames, London." Epidemiology & Infection **145**(6): 1246–1255.

Horák, P., L. Mikeš, L. Lichtenbergová, V. Skála, M. Soldánová and S. V. Brant (2015). "Avian schistosomes and outbreaks of cercarial dermatitis." Clinical Microbiology Reviews **28**(1): 165–190.

Huber, P. J. (1967). The behavior of maximum likelihood estimates under nonstandard conditions. Proceedings of the fifth Berkeley symposium on mathematical statistics and probability, Berkeley, CA: University of California Press.

Juhász, A., G. Majoros and G. Cech (2022). "Threat of cercarial dermatitis in Hungary: A first report of Trichobilharzia franki from the mallard (Anas platyrhynchos) and European ear snail (Radix auricularia) using molecular methods." International Journal for Parasitology: Parasites and Wildlife **18**: 92–100.

Kay, D., F. Jones, M. Wyer, J. Fleisher, R. Salmon, A. Godfree, A. Zelenauch-Jacquotte and R. Shore (1994). "Predicting likelihood of gastroenteritis from sea bathing: results from randomised exposure." The Lancet **344**(8927): 905–909.

Korajkic, A., B. R. McMinn, V. J. Harwood, O. C. Shanks, G. S. Fout and N. J. Ashbolt (2013). "Differential decay of enterococci and Escherichia coli originating from two fecal pollution sources." Applied and Environmental Microbiology **79**(7): 2488–2492.

Kozak, S., A. Roiko, K. Gutjahr-Holland, W. Ahmed, C. Veal, P. Fisher, S. Toze, M. Weir and H. Stratton (2025). "The use of faecal indicator organisms to manage microbial health risks in recreational waterways not impacted by point sources of sewage: a systematic review of the epidemiological evidence." Journal of Water and Health **23**(5): 563–586.

Liang, K.-Y. and S. L. Zeger (1986). "Longitudinal data analysis using generalized linear models." Biometrika **73**(1): 13–22.

Office of Water Quality (2012). "Recreational Water Quality Criteria." from https://www.epa.gov/sites/default/files/2015-10/documents/rwqc2012.pdf.

Rubin, D. B. (1996). "Multiple imputation after 18+ years." Journal of the American statistical Association **91**(434): 473–489.

Rusiñol, M., X. Fernandez-Cassi, A. Hundesa, C. Vieira, A. Kern, I. Eriksson, P. Ziros, D. Kay, M. Miagostovich and M. Vargha (2014). "Application of human and animal viral

microbial source tracking tools in fresh and marine waters from five different geographical areas." Water Research **59**: 119–129.

RYA. (2023). "Watersports participation remains buoyant according to latest Watersports Participation Survey." from https://www.rya.org.uk/news/watersports-participation-remains-buoyant-2023.

University of Brighton, Exegesis SDM Ltd, Rubicon Associates, Plumpton College and G. a. L. Hughes. (2015). "Enjoying Water." from https://assets.publishing.service.gov.uk/media/5a7ba5f440f0b638d61be082/gean0609bqda-e-e.pdf.

White, H. (1980). "A heteroskedasticity-consistent covariance matrix estimator and a direct test for heteroskedasticity." Econometrica: journal of the Econometric Society: 817–838.

White, I. R., P. Royston and A. M. Wood (2011). "Multiple imputation using chained equations: issues and guidance for practice." Statistics in Medicine **30**(4): 377–399.

White, M. P., L. R. Elliott, M. Gascon, B. Roberts and L. E. Fleming (2020). "Blue space, health and well-being: A narrative overview and synthesis of potential benefits." Environmental Research **191**: 110169.

WHO (2021). Guidelines on recreational water quality. Volume 1: coastal and fresh waters, World Health Organization.

Wiedenmann, A., P. Krüger, K. Dietz, J. M. López-Pila, R. Szewzyk and K. Botzenhart (2005). "A randomized controlled trial assessing infectious disease risks from bathing in fresh recreational waters in relation to the concentration of Escherichia coli, intestinal enterococci, Clostridium perfringens, and somatic coliphages." Environmental Health Perspectives **114**(2): 228.

World Health Organization. (2011). "Guidelines for Drinking-water Quality." from https://iris.who.int/server/api/core/bitstreams/b437749a-43f7-472f-a6d9-42596e8ac0ae/content.

World Health Organization (2021). Guidelines on recreational water quality. Volume 1: Coastal and fresh waters, World Health Organization.