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

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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.

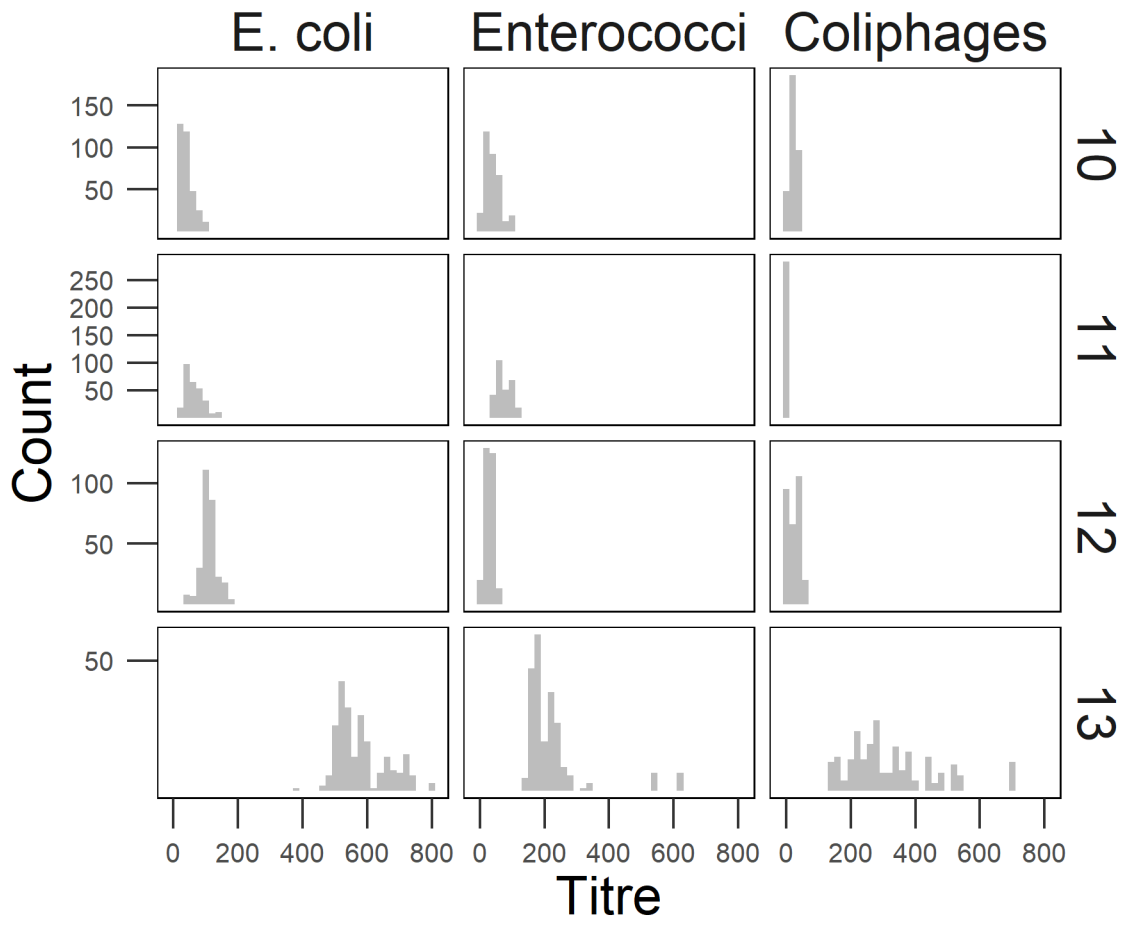

Figure 1: The distribution of *E. coli*, Intestinal Enterococci and Somatic coliphage titres across the four Hungarian study sites. The three indicators were quantified using standardised microbiological methods (see Methods).

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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EpiBathe

A randomized control trial for the assessment of health effects of natural bathing waters

Manual for trial design and management

Supplementary Text 1

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Introduction

The following document summarizes the methods of organizing field trials for the project Epibathe. Experience and lessons learnt from the 4 field trials conducted in Hungary in the bathing season of 2006 and 2007 are summarized.

All throughout the manual, the following "starting conditions" should be kept in mind

- Main organizer on the Hungarian side was the Water Microbiology Department of the National Institute for Environmental Health
- Majority of management work was carried out by two researchers (permanent staff). One additional person was hired in year 1 for a 4 month period.
- One researcher (permanent staff) was responsible for the coordination of lab-work.
- One IT technician was responsible for data handling.
- Other personnel (permanent and temporary) are listed at appropriate points of text.

I.Design and Management

Site selection

Timing: 4 month before the trial

Factors to be considered:

- Water quality – past 4 years
- Water type (river/lake/reservoir etc.)
- Is site prone to unexpected events that influence water quality (flood, drought, algal bloom)?
- Logistics
  - Large enough for 6 bathing zones
  - Place to accommodate non-bathers and additional programs
  - Easy to separate trial site from the rest of the beach
  - Easy to separate bathers from non bathers
  - Ample parking space for cars and buses
  - Distance from laboratory is no more than 2 hours by selected means of transport (Other option is to use mobile lab, or find a collaborating lab (e.g. regional public health office lab) near site. We have opted to use our own accredited, well maintained lab and equipment, and our reliable laboratory staff.)

Visiting the site:

It is necessary before choosing a site to visit and check for the above parameters. It is useful to take samples at this point to check water quality using the same methods and lab conditions as during the trial (see section on Microbiology).

Contacting local authorities

Timing: 3-4 month before the trial

Staff requirement: main organizing group (2-3 senior members)

Who to contact:

- Mayor/municipality
- Local public health office (it is a good idea to contact them during the site selection process)
- Proprietor/operator of the beach

Issues to discuss:

Mayor: The mayor should to be informed about and consent to the trial. Date should be agreed on (no other major local event on the day of the trial OR combine with other event – we have not tried that).

Local public health officers: Since the trial covers public health issues, and the organizing team also belongs to the National Public Heath Service, the local public health offices were the "local headquarters" of the trial. They were extremely helpful in all cases, and their knowledge of the site and the community was invaluable. They advised on local media to use for advertisements, on interview sites, accommodation for helpers for interview days, etc. Local public health officers are encouraged – but not requested - to participate as helpers (interviewers, etc.), and bring family members as well (see section on Interviewers).

Proprietor/operator of the beach: The consent of the operator is necessary to use the beach. Conditions need to be discussed. They can decide whether to close the beach for the trial day or a section of the beach can be selected and separated for the trial purposes. Trial date also needs to be discussed (preferably no other event on beach). Other issues include lifeguards, availability of potable water and electricity on the beach, accommodation near the beach for organizing team members.

Ideally, all of the involved local authorities should be briefed and consulted in a single meeting.

Selecting trial dates

Conclusions of the above discussions were used to decide.

Sunday as trial day was found convenient (must be week-end, when more people have free time, even in summer). Sunday is more convenient for lab personnel than Saturday, as otherwise evaluation of microbiological results would fall on Sunday.

Spacing of the trials in time: With the number of staff working on the project, parallel organization of two trials was not feasible, and it also easier to mix up data that way. Busiest time of organizational work is the week before and the two weeks after the trial. Therefore trials at least 4 weeks apart were chosen in our scheme. In Hungary, weather restricted potential dates between the end of June and mid-August, therefore too far apart is again not practical, besides, we fall out of practice. So 4-6 weeks were optimal in our view.

Throughout the manual, trial day is referred to as Day 0, all dates will be given in relation to trial day.

Ethical clearance

Ethical clearance was given by the Hungarian Scientific and Research Ethics Committee. The Committee had only minor comments; removal of two questions bearing special sensitivity was requested. As none of the questions were critical for the aims of the trial, the questionnaires were modified accordingly. Some difficulty has arisen from the fact that the Committee mainly deals with clinical (e.g pharmaceutical) trials, this type of study was unprecedented. Bad reputation of Hungarian bathing waters as a possible outcome of the trial was also raised as a concern on the behalf of the Committee. After giving supplemental information all objections were cleared. Documents requested by the Committee: a request form including project details (name, contract number, time frame), list of project participants (international), list of involved personnel, project summary; copy of documents used during the trial (information sheet, informed consent sheet, questionnaires); a declaration that the personnel has no financial interest in the outcome of the project; declaration on data confidentiality.

Publicity and recruitment

Publicity and recruitment is a two-step process.

Phase 1 Press release/press conference 2-3 months before the trial. The public is made aware of the trial without specifically targeting potential recruits.

Phase 2 Active recruitment starting about 5 weeks before the trial. Forms of publicity (in order of their usefulness in our experience)

- Personal recruitment **by local public health officers** or municipality officers – "spreading the word" through locally known and respected people is the most reliable method. These people provide personal guaranty for the project. Especially in poorer regions, offering financial reward for something that does not look like work may otherwise raise suspicion.
- National or local associations – such as association of large families, sport clubs etc. One contact person within the association can reach and motivate many people.
- Local radio or television ads – especially in smaller communities people tend to listen to local radio stations/watch local television programs rather than national ones
- Website – very useful both for providing detailed information, and as a site for volunteering (see below)
- Posters and flyers – useful in combination with the above. Raises attention, and directs potential volunteers to other sources of information, such as the website or the local public health office
- Newspaper ads (daily news paper) – not as efficient, at least if appears only one day - cost/benefit ratio is poor (was not used in year 2)

- Recruitment in shopping malls – high drop-out rate (50 %) among people recruited in shopping malls, very time consuming, and less efficient (was not used in year 2)
- Where to recruit?
- Location: optimal distance from trial site – close enough for easy access, but not as close as people would visit the beach on a regular basis. In our experience this is 20-50 km from the site, but this depends on means of transport, popularity of the beach etc.
- Size of the community: 20000-100000 is optimal. Large enough to supply the targeted 500 volunteers, but small enough for the trial to excite sufficient interest. This is where the local contacts might have more influence. In year 1, first location was close to the Budapest, with 2 million inhabitants, and recruitment was focused there, however, turn-out rates were not higher than in smaller communities.
- Number of targeted communities: in our experience, 2 sites give enough flexibility, but can be handled with a team of our size. With a larger team, more recruiting/interview locations can be handled. (In year 1, 4 interview locations were selected, but it was found too complicated, and not efficient.)

Who to recruit?

- Age limit: above 4 years, no upper age limit
- Healthy individuals – required for participation
- Participation of children is encouraged
- Targeting families is a good option. Financial reward adds up to a more substantial sum, and thus it is more motivating. With additional programs (see there) the trial can be a tempting family event as well. (Civil associations of large families were very convenient way of reaching families. Through one contact person, many families were mobilized. In one case, the local association was so agile, that they alone recruited over 200 volunteers, provided interviewers and organized their own transportation.)
- Targeting school groups or university students is more questionable – might result in undesirably distorted age-distribution

Staff requirement of recruitment

Depends on the choice of methods. Personal recruitment requires two persons/site/day, at least one of whom should be part of the main organizing staff. Finding and contacting family association, sport clubs, etc., keeping in contact with the local public health offices – about a full time job for one staff member between 6 to 4 weeks before the trial. In the local public health offices, usually one or two volunteering local officer was in charge of the local recruitment. One person was contracted to design print-quality fliers, posters, later to produce photos and videos for documentation. At local radios and televisions, the production of advertisements was included in the price.

Registration of volunteers

Means of registration

- Registration through the website – online registration form
  - convenient for recruits
  - convenient for organizers – data is directly exported from the online form to the volunteer database, no manual entry is involved, less chance of introduced errors
  - however, it is not available to everyone, restricts participation to those with internet access and appropriate skills
- Paper-based registration
  - Registration forms and detailed information sheets are made available at a central location of the community (e. g. the municipality). In our practice it was usually the local public health office.
  - People obtained sheets from this location, and returned them to the same place
  - Data was entered manually to the database (double-checked)
- Registration forms (both online or paper copy, see Annex 1) contain the following information:
  - Personal data: name, gender, date of birth
  - Contact information: address, phone number, email address
  - Information for randomization: whether volunteer needs accompanying person (compulsory under 10 years of age, optional between 10-14, above 14 only allowed for special cases, i.e. handicapped persons). Name of potential accompanying person(s). Since during randomization, randomization status will be manually changed to match minors and accompanying persons, it is very helpful if volunteers give the name of all potential accompanying persons (e.g. both parents, elder brothers or sisters, or other participating relatives).
  - Logistic information: location, date and time of first interview, location, date and time of 3rd interview, means of getting to trial site (individually, or by the provided buses)

Combination of the two methods worked well in our experience. Proportion of participants registering through internet varied from site to site.

Closing the enrolment

Online enrolment was usually closed 1 day before the first interview to give enough time to produce participant lists. Paper-based registration was closed 2-4 days earlier to allow shipping and data entry.

Staff requirements for the enrolment of participants

Once the web-page for registration was designed, the on-line registration required no further man-hours. Data entry was carried out by one staff member, and double-checked by an additional person.

Database

Assembling and management of the participant database is a crucial point of trial execution. A sample database is attached in Microsoft Excel and OpenCalc format. OpenCalc is shareware, 95 % compatible with Microsoft Excel (charts and diagrams tend to get distorted if transferred, but data remains intact). At least as user-friendly as Microsoft Excel, some functions work even better.

The primary participant database includes the following information:

- Identification information:
  - Volunteer number (a running numbering, given in the order of enrolment)
  - First name
  - Last name
  - Gender
- Logistic information:
  - Location, date and time of the 1st interview (chosen by the volunteer upon enrolment)
  - Location, date and time of the 3rd interview (chosen by the volunteer upon enrolment)
  - Chosen means of getting to the bathing site (buses were provided for volunteers)
    - if by bus: from where, when
  - Q4 by post or email (in year 1, option was provided, year 2, only postal)
- Contact information:
  - Address
  - Home phone
  - Work phone
  - Mobile (cell) phone
  - Email address
  - Preferred method of contact (any of the above) (usually phone or email was used, if it was necessary to reach someone)
- Information for randomization:
  - birth date
  - bathing alone/with accompanying person/as a accompanying person
  - name of potential accompanying persons (as many as possible)
- Randomization
  - Columns of randomization calculation (method of randomization: see below)
  - Assigned bathing status
  - Assigned bathing zone
- Interviews
  - Completed Q1 (yes/no)
  - Completed Q2 (yes/no)
  - Completed Q3 (yes/no)
  - Completed Q4 (yes/no)
  - Signed informed consent

- Bathing data:
  - Actual bathing status
  - Actual bathing zone
  - Start of bathing
  - End of bathing
  - Time zone
  - Number of head immersions
  - Activity
- Voucher data (may/may not receive voucher, picked it up or not)
- Notes

Data that was included in the beginning, but omitted later, as not useful: title, work address, general practitioner name, GP address and phone number, interviewers name and number for each personal interview – all of these can be picked up from the questionnaires if necessary.

Challenges in database management during the process

- Pulling out the required subset of information at different stages of the trial. Since the database is really large, this problem was first handled by hiding unnecessary columns, and reorganizing the necessary ones, but this is a potential source of user-introduced errors, especially if there is more than one person managing the database. Therefore, preset sub-databases were defined for special requirements. E.g. to produce a list of participants arriving for 1st interview at a certain location, sub-database would only include the following fields: volunteer number, first name, last name, birth date, chosen time of interview, a field to check if present, and a field for signature. Sub-databases were read-only, data could only be entered into the primary (full) database. For additional subsets, see the attached sample database. Other possibility is to use blocks of columns in the primary database, which allows for instance all contact information to be pulled out or hidden simultaneously.
- Sorting data: data need to be sorted by various fields for different purposes (e.g by name to find identical names or by zone and time of bathing to assign water quality data). This is again a potential source of error. Part of the problem is resolved by fixing options for various fields (e. g. yes/no, male/female, post/email/phone etc.) This seems self-evident, but very often alternative wording was used by different persons (e.g by post instead of post), or accidental misspellings caused trouble. Automatic error messages were built in to notify user if she tried to enter text into a numerical field, etc.
- Duplicate names: the problem of two (or even more) participants with the same name was resolved by checking the birth-date, and even including it into the last name field. This was necessary, as duplicates were not always evident at first glance – e.g. if data was sorted by volunteer number, or if they went to different interview locations. It was necessary to check for duplicates at every phase – before the first interview, before randomization and after the bathing.

Interviewers/helpers

Recruiting interviewers

Interviewers were from three different "sources".

1. organizing staff members – most well acquainted with the project and most dedicated
2. local public health officers and their friends/family members – usually motivated, skilful and agile
3. student summer workers – in general, less active and less careful

In our experience, it is good to find interviewers who have some kind of personal involvement in the project, such as public health officers, or their acquaintance. Student helpers hired through student work organization in general, were not as proficient, especially in Trial 1. From Trial 2, only those who proved to be efficient in the previous trial(s), were contracted. It is very helpful to use the same people in more than one trial, they are more experienced, and need less training. If that is not possible, it is good if interviewers can at least come for all three personal interviews of a trial, which is again more difficult if working with hired student helpers. Students were finishing secondary school or college students.

Number of required interviewers

1st interview	30 interviewer total 1 main organizing staff member/location 2 persons at the registration desk/ location
Bathing Day	24 interviewer total 3-3 persons at the registration desk both in the "bather" and the "non-bather" area 36 other helpers
3rd interview	30 interviewer total 1 main organizing staff member/location 2 persons at the registration desk/ location
Handling and correcting the questionnaires	3 persons – 3-4 weeks

Training the interviewers

Training was usually performed 1 day before the 1st interview. One of the main organizers held the training, it usually took 1,5-2 hours. Important and often missed issues were repeated again before each interview during a short briefing (15 minutes).

Training material consisted of a powerpoint presentation, sample questionnaires and a training manual. Final version of the training material is attached (Annex X.)

Procedure of the training:

- Presentation (approx. 25 min)
  - introduction of the study (background, aims, participants)
  - study procedure (step-by-step, with emphasis on the task of helpers at each stage)
  - general method of interviewing (elimination of interviewer bias)
  - procedure of the interviews
  - safety and data confidentiality
- Introduction of the questionnaires (approx. 20 min)
  Interviewers received sample copies of Q1-Q3. The trainer took them through the questionnaires question by question, clarifying questions that caused confusion previously, pointing out potential sources of error (for details, see questionnaires section).

- Test interviews (approx. 40 min)
  Interviewers filled out all questionnaires in pairs, interviewing each other. In the training for Trial 1, they only filled out 1 each, but experience showed that it was necessary for every interviewer to do a test interview of each, so potential problems arise during training and not during the real interview (though interviewers were encouraged to ask during real interviews rather than proceed with what they presumed to be the correct interpretation).

- Discussion (approx. 15 min)
  Problems, ambiguous questions were discussed.

Repeatedly raised problems

(general, for problems concerning specific questions, see Questionnaires section)

- Questionnaires need to be filled out with black pen to enable OCR processing. In Hungary, generally any official document has to be filled out with blue, so this had to be emphasized on every occasion.
- Legibility – neat handwriting in open answer entries is necessary for the optical reading. Cross should not extend outside the box. Special problem in Hungary is the slightly different method of writing some numbers (1, 7 and 9). Training interviewers

to write numbers differently is not easy, usually care was taken to print at least the volunteer number - as the most important numerical entry - the English way.

- The importance of reading the entire question and all potential answers to the participant before accepting an answer, especially the questions concerning symptoms or illnesses. Participants tend to give a straight "no" without considering each of the symptoms.
- The importance of standardized interviewing procedure had to be stressed on every occasion (i.e. read every question verbatim, do not "help out" the volunteer with an answer, wait for the participant's answer even at recurrent questions). As participants were also impatient to get the questionnaires done and sometimes complained about long questions or having to answer some questions twice, interviewers were advised to say "I know we have been through this before, but please understand that this is a standard procedure" or something similar.
- It is necessary to check whether every question has been filled out while the participant is still there; it is much easier to fill any gaps then.
- Interviewer sometimes questioned the usefulness of certain questions, e.g. nobody would swim often in sea-water in Hungary, and sea-water swimming pools are unknown. It was explained that this is an international trial and questionnaires were standardized for comparability.
- Interviewing minors: as lower age limit was 4 years, some volunteers were too young to answer the questions reliably. Though individual cases might vary, general procedure was: <10 years the parent would answer for the child (or help the child with the answer); 10-14 years the parent is present to correct or assist, but the child answers the questions; 14-18 years volunteers answer for themselves.

Questionnaires

Questionnaires were prepared strictly following the original questionnaires of the UK studies by David Kay. Translation was as verbatim as possible. However, there were some expressions (e.g certain foods or sports) which could not be translated, do not have equivalent or have different meaning in Hungarian context.

Specific examples:

- Foods, such as meat pie – was substituted by Hungarian food that carries similar risk of gastrointestinal disease
- Sports – kayaking in Hungary means invariably flatwater kayaking, which is different type of exposure than wild water kayaking. Similar with sailing- always dinghy sailing, as Hungarian lakes will not accommodate anything much larger.

Two questions were removed on the request of the ethical committee.

Pre-exposure questionnaire, Q19 point 4. How often do you go to church? – still a sensitive question in post-socialist Hungary.

Postal questionnaire Q29. What newspaper do you read regularly? – preferred daily paper is an indicator of political standing, it was banned as unrelated to the study.

Problematic questions

Questions that were ambiguous or confusing in our experience (every problem that was raised more than once, even if it seems self-explanatory):

Pre-exposure questionnaire

Q3. (occupation) Many volunteers had problems fitting their occupation in the given categories. A lot of them were on pension, unemployed or self-employed.

Q4. Definition of "living in a household".

Q6. "Symptoms of an infection" – volunteers were given examples (throat ache, diarrhoea, vomiting, etc.)

Q7. "Chronic illness or condition" – important to read all questions, as most participants would not consider e.g. short-sightedness as a "chronic illness", and would answer no

Q10c. Length of illness – boxes are given for weeks and days. It was often asked whether to fill out both (e.g. write 3 weeks and also 21 days). Answer was to put either weeks or days (or e.g. 3 weeks and 2 days)

Q11. point 1. definition of fever was agreed to be "above 37.5 °C"

Q12c. "How much do you smoke?" It was asked whether to put the number of pieces or packs of cigarette – answer was to put units (= 1 cigarette or 1 cigar or 1 pipe)

Q13. "When did you stop smoking?" Option "never smoked" was necessary to be included in Year 2 (it was not in the original questionnaire). In Year 1, instruction was to leave the question blank if the answer is "never smoked".

Q14b. Interviewers were instructed on how to calculate alcohol units.

Q16. It was often asked whether to include oral contraceptives or dietary supplements. Instruction was yes to the former and no to the latter. (Dietary supplements count as "drugs" to many people, as they could only be obtained in pharmacies until recently.)

Q17c. This question was repeatedly found confusing. Separate lines refer to separate trips.

Q19. point 2. It was often asked whether a family birthday gathering counted as a "party" or not. (In Hungarian, party is mostly used in a stricter sense of large gathering with music,

dancing and drinking.) Instruction was to include every occasion where a lot of people are present and consume food together.

Q20. Some points include more than one activity. Instruction was given to underline the one that applies.

Q21b. “When you swim, how much? (length of a pool is 25 m)” It was confusing whether to put meters or lengths of pool. The correct option was meters.

Q21c. Separate lines refer to separate places where the participant bathed.

Exposure Day questionnaire

Q1c-d. It was necessary to point out that “chicken” is every food made with chicken and “eggs” mean every food made with eggs (rephrased accordingly in Year 2). “Home made” in this case means that the food was prepared at home and not the chicken was raised or the eggs produced at home. “Turkey” also counts as “chicken” in this case.

Q1e. Only the mayonnaise that was produced from eggs and oil counts as “fresh”, nothing in a jar or a tube.

Q1h. “Salad” was replaced by “any kind of salad (e.g. cucumber, tomato, etc.)” to clarify that not only lettuce salad counts. (Lettuce and salad are expressed with the same word in Hungarian.)

Q1i. The expression “raw milk” meant “uncooked milk” to many, it was replaced with “unpasteurized milk” to clarify.

Q4. “What was the first symptom?” First symptom observed, not first on the list

Both food consumption and symptom related questions refer to period of 3 days before the questionnaire.

Post-exposure questionnaire

Same notes apply as with Exposure Day questionnaire, except that all questions refer to the time “since Bathing Day”.

Post-exposure postal questionnaires

- About 25 % used blue pen, even though instruction to use black was on the cover page in bold.

- The date when the questionnaire should be filled out was also on the cover page; still a lot of people filled it out as soon as they got it, while a few only several days later. (Questionnaires were posted to arrive 2-3 days before the required date.)
- Detailed instructions on how to fill out the questionnaires were included. Q14 was the only one what people found too difficult.
- There was some problem with people who did not put their name on the questionnaire and we had to refer to the envelope. Some parents who filled out questionnaire for their small children as well, put their own name on both questionnaires.
- Most confusion was caused by the volunteer number, even though it was included in the instructions that this entry will be filled out by the organizers. Still a lot of people called to ask what was their number or even worse, wrote in what they thought was their number (quite often missing it.)
- The information hotline (a mobile phone that was always with one of the main organizer staff) was very useful for people who had problem with filling out the questionnaires.

Layout of the questionnaires

- Questionnaires were colour-coded – printing on different coloured paper is the most convenient method
- Double-paged leaflet format (Year 2) was easier to handle than single-sided stapled questionnaires.
- Page-breaks should be positioned without breaking up questions otherwise it is easier to miss some sections.
- Pre-exposure questionnaire has a cover page of personal information which needs to be removed before scanning. It is convenient to make this easily removable (e.g. perforate)
- In Year 1, postal questionnaire was also prepared in an electronic format, a word document that was identical to the printed version but was protected so only the entry fields could be modified. It was emailed as an attachment. We had problems with the format, some people could not open it, others could not send it back electronically, or it would fall apart when we tried to open it, but otherwise it seemed to be a good option. Other possibility would be an online form – we did not try it, but that would help to warn participants to fill out every field. Sometimes questions were missed both in the electronic and in the paper format.
- Copy numbers: target numbers were 500 volunteers/trial, maximum number of attendance was over 700. Thus 800 copies of each questionnaire per trial were necessary.

Interviews

Pre-exposure interview

Timing: 2 and 3 days before the trial (Day -2 and 1-3), 12AM to 8 PM.

Location: Potential sites for interviews are schools, municipalities, local cultural facilities in the communities where recruitment was focused. Interview locations should be sufficiently spacious to accommodate up to 20 interviewers (exact numbers depend on the number of locations and volunteers), and the volunteers. Additional space is needed for those who wait. Central location is preferred for easy access. In Year 1, 4 locations were available for each trial, in Year 2, 2 locations for each trial. Interview sites were organized to have a registration desk in front, and (preferably) separate tables for each interviewer.

Staff requirements

- Supervisor (1-2 persons/interview location). Should be (an) experienced member(s) of the main organizer team. Task: rehearse training for the interviewers, greet and register volunteers, assign volunteers to interviewers, supervise interview process.
- 2 helpers/interview site for administration. Task: enter volunteer data (e.g. attendance) to the electronic database. Revise questionnaires on site.
- Interviewers. Number depends on the time-frame of the interview and the number of volunteers. (A total of 30 interviewers were necessary for approximately 600 volunteers, but not all interviewers worked all day.)

Equipment:

- Questionnaires. Copy numbers: expected number of volunteers + 30 %. (The more interview locations, the more extra copies are needed. Volunteers choose interview location upon registration, and that helps previous calculations, but some tend to change their mind and go to another location, especially if they are close to each other.)
- List of volunteers (printed copy, 1 copy/location). In year 1, separate lists with people who registered for a certain location were prepared. It was found more useful to print full lists, as people do not always stick to their original choice of location, and it is also easier to spot duplicates. List is sorted by name (alphabetical order). Pre-exposure interview list contained the following fields: volunteer number, last name, first name, birth date, chosen location of the interview, chosen date and time of the interview, signature. Pre-exposure interview was also a good occasion to register all possible accompanying persons,
- Ample supply of black pens
- Study information sheet
- Informed consent sheets
- Parental permission sheets
- Boxes for the completed questionnaires

- Laptop computer – not indispensable, but very helpful. Database can be kept up to date by entering the data on site. Care should be taken when merging databases created at different interview locations.

Interview process

- The volunteer lists are prepared the previous day
- The interviewers arrive to the interview site 30 minutes prior to starting time
- The supervisor briefly revises the key points of the training
- Volunteers proceed to the registration desk upon arrival. They are given an information sheet and an informed consent sheet to sign (pre-exposure interview only).
- The supervisor finds the volunteer on the list. If not on the list (did not register beforehand), assigns a running volunteer number. (Care should be taken not to assign the same numbers at the different interview sites.) The volunteer signs the list. The supervisor enters the volunteer number to the appropriate field on the questionnaire. (This was always done by the supervisor i. to minimize administrative errors ii. to make sure that at least volunteer number is written "the English way".) As errors are likely to occur even with utmost care, it is advisable to put the volunteers name on the **back** of the questionnaire. (This way it will not be scanned, thus will not compromise anonymity, but it will be still possible to link the questionnaire to the volunteer, should it be needed at a later stage).
- The volunteer is assigned to one of the interviewers.
- Interview is performed.
- Interviewer checks the questionnaire for completeness before the volunteer leaves.
- Volunteers are reminded to their next task (bathing day at the first interview)
- One of the administrative helpers double-checks the questionnaire (checks for completeness, legibility, double-checks volunteer number). It is good to have the second check on site, since it gives and immediate feed-back on the performance of the interviewers. Those interviewers, who had repeated problems of legibility or accuracy, were given a different task.
- Questionnaires are sorted in numerical order by volunteer number (continuous).
- Attendance data and newly registered volunteers are entered into the electronic database (continuous).
- The supervisor takes time to walk around and check the performance of the individual interviewers during the interview process (continuous).

Exposure Day interview:

Timing: on Bathing Day (Day 0), during the trial (10 AM to 2 PM)

Location: Exposure Day interviews were performed in tents set up on trial site. Non-bather and bather interview locations were separated.

Staff requirement:

- 1-1 supervisor in the bather and non-bather area

- 2-2 administrative helpers in the bather and non-bather area
- 12-12 interviewers in the bather and non-bather area

Equipment:

- Questionnaires. Exposure Day questionnaires (800 copies). Pre-exposure questionnaires (100 copies) for new volunteers.)
- List of volunteers (printed copy, bather list/non-bather list). List is sorted by name (alphabetical order). Exposure interview list contained the following fields: volunteer number, last name, first name, birth date, competed Q1 (yes/no), signature.
- Black pens
- Study information sheet (for new volunteers)
- Informed consent sheets (for new volunteers)
- Parental permission sheets (for new volunteers)
- Boxes for the completed questionnaires
- Laptop computer

Procedure:

- The volunteer lists are prepared the previous day (see section on Randomization)
- Volunteers report to the bather/non bather supervisor desk after registration.
- The supervisor finds the volunteer on the list. The supervisor enters the volunteer number to the appropriate field on the questionnaire, and puts the volunteer's name on the back of the questionnaire. If the participant is a new volunteer, signs the informed consent sheet.
- The volunteer is assigned to one of the interviewers. One ore more interviewer (depending on the number of new volunteers) is assigned for new participants who need to complete both the pre-exposure and the exposure day questionnaire.
- Interview is performed.
- Interviewer checks the questionnaire for completeness before the volunteer leaves.
- Volunteers are reminded to their next task (post-exposure interview)
- Volunteers receive a mark or a stamp on their wrist-bands upon the completion of the interview.
- One of the administrative helpers double-checks the questionnaire.
- Questionnaires are sorted in numerical order by volunteer number (continuous).
- Attendance data and newly registered volunteers are entered into the electronic database (continuous).
- The supervisor takes time to walk around and check the performance of the individual interviewers during the interview process (continuous).

For more detail on the process, see section on Bathing Day

Post-exposure interview:

Timing: 1 week after the trial (Day 7), 8 AM to 8 PM

Location: Same as the pre-exposure interview locations

Staff requirement:

- Supervisor (1-2 persons/interview location). Should be (an) experienced member(s) of the main organizer team. Task: rehearse training for the interviewers, greet and register volunteers, assign volunteers to interviewers, supervise interview process.
- 2 helpers/interview site for administration. Task: enter volunteer data (e.g. attendance) to the electronic database. Revise questionnaires on site.
- Interviewers. Number depends on the time-frame of the interview and the number of volunteers. (A total of 30 interviewers were necessary for approximately 600 volunteers, but not all interviewers worked all day.)

Equipment:

- Questionnaires. Copy numbers can be more accurately determined, as the maximum number of expected volunteers is known.
- List of volunteers (printed copy, 1 copy/location). Post-exposure interview list contained the following fields: volunteer number, last name, first name, birth date, postal address, chosen location of the interview, chosen time of the interview, completed Q1, completed Q2, signature.
- Black pens and other stationary
- Boxes for the completed questionnaires
- Laptop computer

Procedure:

- The volunteer lists are prepared the previous day. Only those with completed Q1 and Q2 may come for the post-exposure interview.
- Volunteers report to the supervisor desk after registration.
- The supervisor finds the volunteer on the list. Double-checks the postal address (important for sending the postal questionnaires). The supervisor enters the volunteer number to the appropriate field on the questionnaire, and puts the volunteer's name on the back of the questionnaire.
- The volunteer is assigned to one of the interviewers.
- Interview is performed.
- Interviewer checks the questionnaire for completeness before the volunteer leaves.
- Volunteers are reminded to their next task (postal questionnaire)
- One of the administrative helpers double-checks the questionnaire.
- Questionnaires are sorted in numerical order by volunteer number (continuous).
- Attendance data is entered into the electronic database (continuous).
- The supervisor takes time to walk around and check the performance of the individual interviewers during the interview process (continuous).

Interviews by phone

It was only possible at the post-exposure interviews. Volunteers who did not come for the interview were called the next day, and were offered the possibility to do the interview through phone. It required 1-2 interviewers, depending on the number of volunteers.

Postal questionnaires

Timing:

- Posted at Day 16 to arrive Day 19-20
- Requested date to fill out the questionnaire Day 21
- Return the questionnaires Day 22-30

Staff requirement: 2-3 persons to prepare and post the envelopes, and to handle completed questionnaires.

Procedure:

- Cover page was prepared for the postal questionnaires, containing the following information:
  - the date when the questionnaire should be filled out (Day 21)
  - detailed instructions on how to complete the questionnaire, including the instruction to use black pen and to leave volunteer number field empty (most frequently occurring problem)
- Addressed answer envelope was included (with stamps).
- Questionnaire was posted for every participant with completed Q1 and Q2. For families, questionnaires were posted in one envelope.
- Completed questionnaires were received between Day 22 and 30. If it did not arrive, participants were called to remind them on the deadline.
- Volunteer number was written on the questionnaires. Participants with identical names were sometimes difficult to identify, sender's address (if given) was very helpful.
- Questionnaires were sorted by number.

Randomization

Timing: Day -1. It is easy – in our experience – to underestimate the time requirement of this process. At the first trial, it took over 12 hours, which gradually decreased as we gained experience.

Staff requirement: 1-3 senior members of the main organizing team, 1-2 administrative helpers.

Procedure

1. The volunteer database was finalized. Newly registered volunteers were entered into the database. It is of choice whether to delete those who registered, but did not come to the first interview – we decided to keep them on the list as they might still come to the Bathing Day. Empty lines (with volunteer number only) were included for those who register at the trial site.
2. Data was sorted by volunteer number.
3. Random numbers between 0 and 1 were generated by inserting a random number function to the appropriate column of the database.
4. A bathing status was assigned to the numbers, e.g. <0.5 bather, >0.5 non-bather. This was also done as a preset function in the database. Care needs to be taken to have the bathing status fixed (e.g. by copy and paste special/value), the random number function in Excel will recalculate continuously.
5. Once random bathing status was assigned, it was necessary to check minors and their accompanying persons whether they have the same status, and change one of them if not. This is the most tedious part in our experience. First, everyone who was labelled "bathing with accompanying person" and "accompanying person" was selected. List was sorted by last name to have families together (though not always, as many women in Hungary keep their maiden name). Going down the list, the bathing status of each minor and all the listed accompanying persons were checked. If any of the potential accompanying persons had a matching status, he/she was selected and the others were deleted. If none of them matched then bathing status of the child or the parent was changed. Rule of the thumb was to change to bather and to non-bather alternately. Exception was taken if one accompanying person was assigned to more than one child (e.g one parent with children). In this case, it was done to involve the least possible changes (e.g. if the parent was a bather, and all the children non-bathers, then the parent was changed to non-bather).
6. The bathers were assigned to bathing zones. All bathers were listed and sorted in numerical order. Minors and accompanying persons were assigned to two selected zones with shallow water (always chosen on trial site). All others were assigned a letter A-F, going down the list.
7. Once randomization was completed, lists were prepared for Bathing Day. Five full lists for the registration desks at the entrance, bathers' lists for the bather supervisor, non-bathers' list for the non-bather supervisor, and bathing zone lists (six, A-F) for the bathing zone supervisors (see section on Bathing Day).

Organization of the trial site

Timing: Day -1, and the morning of Day 0

Staff requirement: In our case, technical part of the site preparation was hired out to an event/party organizer team. Their staff was approx. X people, including the animators for the games. One senior member of the main organizing team was present to supervise preparations.

Equipment for site preparation

- Fences to separate bather and non-bather area
- Party tents (total of X) for the interviews, for changing, for refreshments (with coolers) etc
- Tables and chairs (X) for the interviews and registration
- Chairs and sunshades for the bathing zone supervisors
- Signs posted around the area and on the major roads to show site location
- Signs for the trial site (bathers, non-bathers, interview, etc.)
- Buoys to divide the swimming zones
- Posts for the swimming zones (A-F)
- Mobile toilettes
- Equipment for games and other entertainment (optional)

Site plan:

- Separate area was assigned for bathers and non-bathers. Only bathers were allowed into the bather area, but every volunteer could go into the non-bather area
- Registration desks were setup at the entrance. 4 (general registration) +1 (new volunteers, minors, and everyone who had any problem) registration desks.
- Swimming zones were only separated in the water
- Separate interview tents were setup for bathers and non-bathers
- In the bather area, tents were setup to serve as changing room
- Refreshment tents were setup in both the bather and non-bather area

Bathing Day

Timing:

5:00 Site preparation (continued from Day -1)

7:00-8:00 main organizers arrive to site

8:00-8:30 the interviewers and the helpers arrive to site

8:30 Briefing for the helpers. Site plan explained, general information given, helpers are assigned to individual tasks (see below).

9:00 Briefing for samplers.

10:00 start of the trial. First bather group usually started bathing between 10:10 and 10:30. First sample was taken 10 minutes after the first group started bathing.

13:00 End of the trial (scientific part)

14:00 End of entertainment

14:00-17:00 Packing up.

Staff requirement:

Task	Nr. of required helpers
Registration	5
Interviewers – bathers	12
Interviewers – non-bathers	12
Interview supervisor (bather and non-bather interviews)	2
Interview administrator (bather and non-bather interviews)	4
Bathing zone supervisor	12
Water sampler	6
Water sampling coordinator	2
Information/errand boys	2
Main organizing staff (super-supervisors)	3
Photos and video recording	1
Transport of water samples	3
Life guards	2
Medical personnel	1
Handing out T-shirts and lunch (bather and non-bather area)	6

Equipment (apart from what was listed in the previous section):

- Equipment for the interviews (see there)
- Volunteer lists. Five full lists for the registration desks at the entrance, bathers' lists for the bather supervisor, non-bathers' list for the non-bather supervisor, and bathing zone lists (six, A-F) for the bathing zone supervisors.
- Diary sheets for the bathing zone supervisors to record bather data (see annex X.)
- Laptop computer
- Stationary. Black pens, paper, clips, folders.
- Colour-coded wrist-bands for volunteers (blue –bathers, green – non-bathers, 400 each)

- Colour T-shirts. Different colour was used for main organizers, helpers, bathing zone supervisors. Volunteers received T-shirts as well (blue for bathers, brown for non-bathers)
- Swimming caps for the bathers (different colour for each zone)
- Sandwiches and bottled water for the volunteers
- Sampling equipment (sampling bottles, cooling boxes, ice, thermometers) (see section on Laboratory)

Bathing Day procedure

- Volunteers arrive to site. The buses are arranged for transport were scheduled to arrive at 10:00, 11:00 and 12:00. (approximately 50 %of the participants used the buses)
- Volunteers arrive to the registration desks. New participants, minor and their family and everyone with a problem is referred to the "special" registration desk. (It is good to have someone experienced and competent sitting there).
- Volunteers are informed about their bathing status. They receive the colour-coded wrist-bands (blue – bathers, green – non-bathers, and informed about their tasks. Volunteer number is written on the wrist-bands.
- New participants who registered but did not come to the first interview get their original volunteer number, and the bathing status randomized to that number. They proceed the same, except that they have to fill out the pre-exposure questionnaire on site as well.
- New participants who did not register get the next empty volunteer number on the list and the bathing status randomized to that number. They proceed the same, except that they have to fill out the pre-exposure questionnaire on site as well.
- Bathing status for minors is given according to the process detailed in the randomization section.
- **It is not allowed to change the bathing status for anyone**. If it is absolutely necessary (e. g. someone cannot bathe for health purposes), then it is better to change one person than to swap bathing status for two, as it would mean more bias to the randomization.
- Non-bathers move on to the non-bather area. They need to fill out the exposure day questionnaire during the trial time, otherwise they are free to spend their time as they wish. They report to the non-bather interview supervisor who finds them on the non-bather list (this is a double-check against errors at the registration), and assigns them to an interviewer. There are interviewers specially assigned to interview new participants (as the pre-exposure questionnaire is quite long, this way average waiting time is shortened. Upon completion of the interview they receive a sign or a stamp on their wrist-bands. It is only possible to pick up their free sandwich lunch and their t-shirts if they have this sign.
- Bathers move on to the bather area. They have two tasks: to bathe and to fill out the exposure day questionnaire. The order of the two is unimportant, but they need to perform both.
    - To do the interview, they report to the non-bather interview supervisor who finds them on the non-bather list (this is a double-check against errors at the

registration), and assigns them to an interviewer. There are interviewers specially assigned to interview new participants (as the pre-exposure questionnaire is quite long, this way average waiting time is shortened. Upon completion of the interview they receive a sign or a stamp on their wrist-bands.
    - To take part in the bathing, they report to their assigned bathing zone supervisor. The zone supervisor sends volunteers to bathe in groups of 2-5 in 10 min intervals. They record in the diary sheets the start and end of bathing, the number of head immersions, and their general activity. Bathers are asked to submerge their heads 3 times, and encouraged to actively bathe rather than stand in knee-deep water. It is enough to submerge their face, not necessary to wet their hair (often a problem for the ladies). Upon completion of the interview they receive a sign or a stamp on their wrist-bands.
    - It is only possible to pick up their free sandwich lunch and their t-shirts, if they have both signs.
    - Once they completed both tasks, they are free to go to the non-bather area and take part in the programs.
- Non-bathers are not allowed to bathe at all, not even after the trial. Bathers are only allowed to bathe the prescribed 10 min period. Children are often difficult to get out from the water; in this case it is important to have the exact start and end of bathing recorded.
- Sandwiches and t-shirts are distributed in tents in the bather and the non-bather zone.
- At the end of the trial, volunteers are transported back to their city by buses or leave on their own. Volunteers are not requested but encouraged to stay on trial site during the whole trial (10:00-13:00).
- Additional programs/activities are not indispensable for the study. However, as we targeted families during the recruitment, we intended to have an additional attraction in the form of these programs. It also facilitates the presence of both bathers and non-bather during the entire trial

Sampling

- Samples were taken every 20 minutes. First bather started bathing 10 min before the first sample; last bather finished bathing 10 minutes after the last sample.
- Sampling coordinator gave sign for simultaneous sampling. Samples were taken at the middle of each bathing zone, at chest depth (140 cm), except in the zone for the smallest children, where it was taken at 90 cm depth (place of exposure).
- Samples were taken in sterile 1 L flasks, from 20 cm below surface.
- Samples were stored and transported in cooler-boxes, on ice. Sample temperature was controlled by continuously recording digital thermometer in simulated samples.
- Samples were usually transported to the laboratory in 3 sets, depending on the distance from the laboratory (samples from sampling 1-2, 3-5, 6-9).

Insurance and safety

- All participants were insured for the days of the interviews and the trial under a bulk insurance plan by a commercial insurance company
- Ambulance car was present on the trial site with appropriate medical personnel (required for events with more than 500 participants by Hungarian legislation). There was no major accident during the 4 trials, one person cut his foot on a shell.
- Lifeguards were present on trial site. Their intervention was not necessary at any of the trials
- Civil guards were usually provided by the municipality to ensure orderly execution of the trial. They helped to prevent non-bathers entering to the bather area.

Compensation of the volunteers

Volunteers received 40 € in shopping vouchers for their time and effort. Only those who completed all 4 questionnaires could receive the vouchers. Everyone had to pick up the vouchers in person, except for minors.

II. Microbiology

Methods

Detection of intestinal Enterococci

Membrane filtration method

Method was performed according to ISO xxx standard. Water sample aliquots were concentrated on 0.45 µm pore size mixed cellulose ester membrane filters by vacuum filtration. Filters were placed on Slanetz-Bartley agar plates, and incubated for 44 hours at 36 ˚C. Following the inubation, >1 mm colonies, showing pink to dark red colouration were enumerated as presumptive enterococci. Filters were transferred on Bile aesculin agar plates and incubated for 2 h at 44 °C. The presumptive enterococci colonies showing the characteristic brown-black discloration of the media were counted as confirmed enterococci.

Microtiter MPN method

Method was performed according to ISO xxx standard. Water sample aliquots were diluted 2-fold by DSM diluent. 96 well microplates containing MUD dehydrated media were inoculated by 200 µL/well diluted water sample. Plates were incubated for 44-48 h at 44 ˚C. Following the incubation period, plates were read on a UV transilluminator. Wells showing fluorescent singal and reddish precipitation on the bottom of the well were counted as positive for *Enterococcus*. In case of ambiguous results, microplates were incubated for an additional 2 h at 44 ˚C, and re-read.

Detection of Escherichia coli

Membrane filtration method

Method was performed according to ISO xxx standard. Water sample aliquots were concentrated on 0.45 µm pore size mixed cellulose ester membrane filters by vacuum filtration. Filters were placed on TSA/TBA double-layer agar plates, incubated for exactly 4 hours at 36 ˚C, then transferred to 44 ˚C, and incubated for 16-20 h*. Following the incubation period, James reagent was used to identify indol positive colonies. A few drops of the reagent were placed into an empty Petri-dish, the filter was placed on top to allow it to wet through. Purple colonies with a purple ring (indol positives) were enumerated as confirmed *E. coli*.

*The second incubation time is specified as 20-22 h in the standard. However, in our experience, fresh water samples were prone to develop a deep purple background when the reagent was added to the filter, thus making the positive colonies unidentifiable. This phenomenon was tentatively attributed to an indol positive microflora present in the freshwater samples. Reducing the second inubation time to 16 h seemed to overcome the problem.

Microtiter MPN method

Method was performed according to ISO xxx standard. Water sample aliquots were diluted 2-fold by DSM diluent. 96 well microplates containing MUG dehydrated media were inoculated by 200 µL/well diluted water sample. Plates were incubated for 44-48 h at 44 ˚C. Following the incubation period, plates were read on a UV transilluminator. Wells showing fluorescent singal were counted as positive for *E. coli*. In case of ambiguous results, microplates were incubated for an additional 2 h at 44 ˚C, and re-read.

Somatic coliphage

Method was performed according to ISO xxx standard. Double-layer pour-plate method was used. MSA plates were prepared 15 cm Petri dishes. Host strain WG5 was grown in MSB to 10^8 cfu/mL (measured by turbidimetry). Aliquots of the water sample were mixed with

1 mL WG5 culture and 120 µL $CaCl_2$ xx M solution. Mixture was briefly vortexed, poured on the MSA plate, overlayered with 20 mL 45 °C LMSA, mixed inmediately, and allowed to solidify. Plates were incubated for 20-24 h at 36 ˚C. Following the incubation, plaques >1 mm were counted.

Pre-tests

Two sets of testing were performed prior to Trial Day.

One set of samples were taken in the planning phase when contemplating the site as a potential trial location, to assess whether the water quality is suitable for the trial. Usually 2 samples were taken at the same time but on different sections of the beach. Samples were processed by the above methods.

Second set of samples was taken 1 week before the trial to determine aliquot size to use during the trial sample processing. Usually 2 samples were taken at the same time but on different sections of the beach. Samples were processed by the above methods using various sample volumes/dilutions. The sample size that gave optimal results was selected.

Lab preparations

Preparation of the sampling equipment

1 L sampling bottles were used for sampling. 6 sampling sites, 9 sampling time – a total of 54 bottles plus 6 spares were prepared. Bottles were labelled (letters A-F for the sampling zone, numbers 1-9 for the sampling time). Each bottle was wrapped individually in autoclavable bag, and sterilized by autoclaving (121 °C, 15 min). Bottles were packed in cooler boxes by 6 – separate cooler box for each sampling time to minimize the possibility of sample mix-up.

Preparation of media

Slanetz-Bartley agar, Bile aesculin azid agar, MSA plates, MSB media and DSM diluent were prepared 2-3 days before trial day. LMSA was prepared 2 days before the trial and aliquoted in tubes (20 mL/tube). TBA plates were prepared 1 day before the trial. TSA was layered on top of TBA plates no more than 2 hours prior to use. All media were quality controlled (positive and negative control).

Inoculation of WG5 strain into MSB media

Sample processing

Transport

Samples were transported on melting ice in cooling boxes to the lab. Samples were refrigerated on arrival to the lab. Sample temperature was monitored with a continuous thermometer in a simulated sample.

Samples were refrigerated upon arrival until processing.

Processing

Samples were processed in sets of six – samples from one sampling at a time.

Time and staff requirement of sample processing is inversely proportional to each other. In our case, permanent staff involved in water sample processing is 5 persons, each of them experienced in general lab techniques. Our decision was not to introduce new members to the team whose performance is not known. With the given staff, most samples were processed within 6 hours of sampling, and all of them were processed within 9.

Somatic coliphage detection and the microtiter MPN methods were performed in 3 parallels. Membrane filtration was done using 3 different volumes, 3 parallels each.

Result evaluation

Day 1:

- Somatic coliphage plates were read. Plaques >1 mm were counted.
- TSA/TBA plates were read using James reagent as described in the Methods section.
- Every plate was read at least twice by 2 persons independently.
- Result evaluation on Day 1 was x hours for z persons

Day 2:

- Slanetz-Bartley agar plates were read to enumerate presumptive enterococci. Filters were transferred on BEAA plates. Confirmed Enterococcus counts were recorded after 2 h incubation at 44 °C.
- MUG and MUD microplates were read on a UV transilluminator
- Every plate was read at least twice by 2 persons independently.
- Result evaluation on Day 1 was x hours for z persons

Data recording and calculation

- Raw data was recorded on Result sheets and entered to an electronic Result database
- Final counts for each parameter were calculated
- Membrane filtration results were calculated as total counts/total sample volumes using all 9 results from the same sample. For Enterococci, both presumptive and confirmed counts were calculated.
- Microplate MPN results were calculated using the MPNcalc software provided by Prof. Albrecht Wiedemann

- Somatic coliphage counts were calculated as total plaque count/total sample volume of the 3 parallels.

Quality control

- All media were quality controlled using positive and negative control strains
- Incubator temperature was recorded during the entire incubation period using a continuously recording thermometer
- For intra- and inter laboratory performance evaluation an AQC protocol was employed. AQC was performed on each day when trial samples were processed, using low and high count E. coli and Enterococcus lenticules and somatic coliphage culture with known phage titer.

III.Data processing

Questionnaires

- Upon completion of all 4 questionnaires, all were checked for correct volunteer number. This was 3 weeks for 3 persons full time in Year 1. In Year 2, when questionnaires were checked immediately on the interview site, it was still 2 weeks for 1 person full time.
- Questionnaires were scanned by optical character reading (OCR)

Volunteer database

- Details from bathing (bathed or not, which zone, beginning of bathing, end of bathing, number of head immersions, activity)
- Assigned bathing status (the original bathing status after randomization) was compared to the actual bathing status (bathed or not). Changes in bathing status were entered as a separate field (0=did not change status; 1=changed from bather to non-bather; 2=changed from non-bather to bather). Changes initiated by the organizers (i.e changing to match the accompanied minor's status) or initiated by the volunteer were not differentiated.
- After double-checking the database, all personal data (name, contact information etc.) was removed.
- Volunteers with only one completed questionnaire (Q1) were deleted.
- The final volunteer database contained the following fields: volunteer number, birthdate, gender, bathing status, bathing zone, beginning of bathing, end of bathing, number of head immersions, changes of bathing status, number of missing questionnaires.

Assigning microbiological data to volunteers

- 9 time periods were defined based on the sampling times. Time period 1 was the interval 10 min before and after the 1st sampling etc.

- Each period was characterized by the water quality data of the sampling within the period.
- Volunteers were assigned to time periods based on the start and the end of bathing. If someone started and finished bathing within a time period, then was obviously assigned that one.
- Microbiological data belonging to the given period was assigned to the volunteer.
- If the bathing time fell into more than one period, then the bather was assigned the period which covered larger share of the bathing time (i.e if someone bathed from 10:18 to 10:28, and Period 1 was 10:00-10:20 and Period 2 was 10:20-10:40 then the bather was assigned Period 2).
- If the bathing time was divided equally between 2 time periods, then the bather was assigned the mean of two values for each of the water quality parameters.

Supplementary Text 2

		GI Illness Risk		
Variable		**RR**	**95% CI**	**p-value**
Bathing Status:	**Non-bathers**	1	-	-
	Bathers	1.11	0.61-2.02	0.73
Gender:	**Female**	1.00	-	-
	Male	0.64	0.37-1.13	0.12
Log. *E. coli*		1.48	0.98-2.25	0.06
Log. I. Enterococci		1.25	0.67-2.35	0.48
Log. S. phage		1.55	1.18-2.04	0.00
Immersed Head		1.01	0.95-1.07	0.69
Prior GI Illness		3.12	1.44-6.73	0.00
Prior Respiratory Illness		Did not converge	Did not converge	Did not converge
Prior Ear Infection		1.82	0.34-9.66	0.48
Prior Eye Infection		Did not converge	Did not converge	Did not converge
Prior Skin Ailment		1.86	0.82-4.23	0.14
Current Medication Use		1.29	0.92-1.82	0.14
Laxatives Use		Did not converge	Did not converge	Did not converge
Stomach Remedy Use		3.93	1.39-11.1	0.01
Antibiotic/Steroid Use in Prior 4 weeks		2.56	1.31-5	0.01
Alcohol Consumption:	**<1 a week**	0.79	0.38-1.62	0.52
	≥1 a week	0.75	0.37-1.52	0.43
Mayonnaise Consumption		0.95	0.81-1.11	0.51
Sandwich Consumption		1.40	0.48-4.04	0.54
Chicken Consumption		1.16	0.8-1.68	0.44
Eggs Consumption		0.79	0.71-0.87	0.00
Hotdogs Consumption		1.54	0.99-2.42	0.06
Raw Milk Consumption		2.49	1.4-4.42	0.00
Cold Meat Consumption		1.69	1.33-2.15	0.00
Seafood Consumption		1.03	0.42-2.57	0.94
Illness in Household		0.71	0.21-2.33	0.57
Additional bathing		1.94	1.28-2.95	0.00

Table S1: Univariate GEE Model Results for gastrointestinal illness outcome. Correlation structures were specified to account for clustering within study sites and robust (sandwich) standard errors were used.

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.

Skin Ailment Risk				
Variable		**RR**	**95% CI**	**p-value**
Bathing Status:	**Non-bathers**	1	-	-
	Bathers	2.12	1.47-3.06	0.00
Gender:	**Female**	1.00	-	-
	Male	1.01	0.66-1.54	0.96
Log. *E. coli*		0.70	0.36-1.39	0.31
Log. I. Enterococci		0.53	0.17-1.59	0.25
Log. S. phage		1.38	0.89-2.13	0.15
Immersed Head		0.92	0.79-1.06	0.23
Prior GI Illness		2.19	1.08-4.45	0.03
Prior Respiratory Illness		6.10	1.57-23.67	0.009
Prior Ear Infection		2.01	0.41-9.99	0.39
Prior Eye Infection		1.16	0.33-4.09	0.816
Prior Skin Ailment		3.18	1.36-7.43	0.01
Current Medication Use		2.16	1.49-3.15	0.00
Laxatives Use		Did not converge	Did not converge	Did not converge
Stomach Remedy Use		1.23	0.46-3.26	0.68
Antibiotic/Steroid Use in Prior 4 weeks		1.03	0.18-5.76	0.98
Alcohol Consumption:	**<1 a week**	1.36	1.12-1.64	0.00
	≥1 a week	1.68	1.33-2.13	0.00
Mayonnaise Consumption		1.37	1.01-1.86	0.04
Sandwich Consumption		1.04	0.4-2.68	0.94
Chicken Consumption		1.28	1.09-1.5	0.00
Eggs Consumption		4.51	1.82-11.21	0.00
Hotdogs Consumption		1.60	1.34-1.9	0.00
Raw Milk Consumption		0.78	0.66-0.93	0.01
Cold Meat Consumption		1.10	0.62-1.95	0.74
Seafood Consumption		2.76	0.89-8.54	0.08
Illness in Household		Did not converge	Did not converge	Did not converge
Additional bathing		1.10	0.54-2.24	0.79

Table S2: Univariate GEE Model Results for skin ailment outcome. Correlation structures were specified to account for clustering within study sites and robust (sandwich) standard errors were used.

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.

Respiratory Illness Risk

Variable		RR	95% CI	p-value
Bathing Status:	**Non-bathers**	1	-	-
	Bathers	3.81	0.85-17.13	0.08
Gender:	**Female**	1.00	-	-
	Male	0.57	0.17-1.92	0.12
Log. *E. coli*		2.52	1.12-5.65	0.03
Log. I. Enterococci		7.95	0.98-64.67	0.05
Log. S. phage		1.76	1.14-2.72	0.01
Immersed Head		1.02	1.01-1.03	0.00
Prior GI Illness		Did not converge	Did not converge	Did not converge
Prior Respiratory Illness		Did not converge	Did not converge	Did not converge
Prior Ear Infection		Did not converge	Did not converge	Did not converge
Prior Eye Infection		Did not converge	Did not converge	Did not converge
Prior Skin Ailment		5.10	2.29-11.32	0.00
Current Medication Use		1.59	0.43-5.93	0.49
Laxatives Use		Did not converge	Did not converge	Did not converge
Stomach Remedy Use		Did not converge	Did not converge	Did not converge
Antibiotic/Steroid Use in Prior 4 weeks		Did not converge	Did not converge	Did not converge
Alcohol Consumption:	**<1 a week**	0.47	0.2-1.12	0.09
	≥1 a week	0.00	0-0	0.00
Mayonnaise Consumption		1.30	0.29-5.75	0.73
Sandwich Consumption		1.30	0.14-11.69	0.81
Chicken Consumption		0.78	0.07-9.03	0.84
Eggs Consumption		0.40	0.14-1.14	0.08
Hotdogs Consumption		1.42	0.14-14.01	0.76
Raw Milk Consumption		0.61	0.26-1.41	0.25
Cold Meat Consumption		1.32	0.17-10.52	0.79
Seafood Consumption		Did not converge	Did not converge	Did not converge
Illness in Household		2.88	0.38-21.96	0.31
Additional bathing		3.52	1.22-10.11	0.02

Table S3: Univariate GEE Model Results for respiratory illness outcome. Correlation structures were specified to account for clustering within study sites and robust (sandwich) standard errors were used.

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.

Ear Infection Risk				
Variable		**RR**	**95% CI**	**p-value**
Bathing Status:	**Non-bathers**	1	-	-
	Bathers	1.76	0.87-3.56	0.12
Gender:	**Female**	1.00	-	-
	Male	0.21	0.05-0.90	0.03
Log. *E. coli*		0.58	0.20-1.69	0.32
Log. I. Enterococci		Did not converge	Did not converge	Did not converge
Log. S. phage		Did not converge	Did not converge	Did not converge
Immersed Head		Did not converge	Did not converge	Did not converge
Prior GI Illness		Did not converge	Did not converge	Did not converge
Prior Respiratory Illness		Did not converge	Did not converge	Did not converge
Prior Ear Infection		45.51	14.23-145.54	0.00
Prior Eye Infection		4.38	1.45-13.22	0.01
Prior Skin Ailment		2.81	1.13-7.01	0.03
Current Medication Use		1.24	0.4-3.89	0.71
Laxatives Use		Did not converge	Did not converge	Did not converge
Stomach Remedy Use		Did not converge	Did not converge	Did not converge
Antibiotic/Steroid Use in Prior 4 weeks		Did not converge	Did not converge	Did not converge
Alcohol Consumption:	**<1 a week**	0.92	0.41-2.06	0.84
	≥1 a week	0.39	0.05-2.9	0.36
Mayonnaise Consumption		1.31	0.53-3.23	0.55
Sandwich Consumption		0.99	0.5-1.94	0.97
Chicken Consumption		1.94	0.64-5.93	0.24
Eggs Consumption		1.04	0.52-2.07	0.91
Hotdogs Consumption		0.91	0.13-6.68	0.93
Raw Milk Consumption		6.88	2.24-21.16	0.00
Cold Meat Consumption		0.54	0.25-1.14	0.10
Seafood Consumption		Did not converge	Did not converge	Did not converge
Illness in Household		3.49	0.28-44	0.33
Additional bathing		Did not converge	Did not converge	Did not converge

Table S4: Univariate GEE Model Results for ear infection outcome. Correlation structures were specified to account for clustering within study sites and robust (sandwich) standard errors were used.

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.

Eye Infection Risk

Variable		RR	95% CI	p-value
Bathing Status:	**Non-bathers**	1	-	-
	Bathers	2.46	0.97-6.21	0.06
Gender:	**Female**	1.00	-	-
	Male	0.55	0.11-2.83	0.48
Log. *E. coli*		1.20	0.9-1.61	1.20
Log. I. Enterococci		0.73	0.34-1.59	0.73
Log. S. phage		1.55	0.95-2.52	1.55
Immersed Head		0.88	0.8-0.97	0.88
Prior GI Illness		Did not converge	Did not converge	Did not converge
Prior Respiratory Illness		Did not converge	Did not converge	Did not converge
Prior Ear Infection		Did not converge	Did not converge	Did not converge
Prior Eye Infection		4.38	0.79-24.21	0.09
Prior Skin Ailment		1.43	0.2-10.07	0.72
Current Medication Use		0.89	0.22-3.52	0.86
Laxatives Use		Did not converge	Did not converge	Did not converge
Stomach Remedy Use		Did not converge	Did not converge	Did not converge
Antibiotic/Steroid Use in Prior 4 weeks		Did not converge	Did not converge	Did not converge
Alcohol Consumption:	**<1 a week**	0.61	0.11-3.4	0.57
	≥1 a week	1.19	0.35-4.05	0.78
Mayonnaise Consumption		1.25	0.91-1.71	0.16
Sandwich Consumption		0.59	0.15-2.28	0.45
Chicken Consumption		0.80	0.25-2.57	0.71
Eggs Consumption		0.78	0.36-1.68	0.52
Hotdogs Consumption		0.89	0.18-4.36	0.89
Raw Milk Consumption		1.68	1.16-2.43	0.01
Cold Meat Consumption		0.54	0.2-1.49	0.24
Seafood Consumption		Did not converge	Did not converge	Did not converge
Illness in Household		3.00	0.44-20.52	0.26
Additional bathing		1.31	0.41-4.15	0.65

Table S5: Univariate GEE Model Results for eye infection outcome. Correlation structures were specified to account for clustering within study sites and robust (sandwich) standard errors were used.

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.

Variable	Missing (Non-bather)	Missing (Bather)
Age Group	0	0
Gender	0	0
Current Medication Use	10	10
Laxatives Use	1	0
Stomach Remedy Use	1	0
Antibiotic/Steroid Use in Prior 4 Weeks	0	0
Alcohol Consumption	23	19
Mayonnaise Consumption	0	0
Sandwich Consumption	0	0
Chicken Consumption	0	0
Eggs Consumption	0	0
Hotdogs Consumption	0	0
Raw Milk Consumption	0	0
Cold Meat Consumption	0	0
Seafood Consumption	0	0
Additional bathing	0	0
Prior GI Illness	14	12
Prior Respiratory Illness	12	8
Prior Ear Infection	14	8
Prior Eye Infection	13	6
Prior Skin Ailment	12	11
Log. *E. coli*	-	4
Log. I. Enterococci	-	4
Log. S. phage	-	4
GI Illness Outcome	0	0
Respiratory Illness Outcome	0	0
Ear Infection Outcome	0	0
Eye Infection Outcome	0	0
Skin Ailment Outcome	0	0

Table S6: Number of missing covariate and primary health outcome cases for bathers and non-bathers.

Notes: I. Enterococci refers to Intestinal Enterococci; S. phage refers to somatic coliphages.

Variable		Non-bather (n=1235)	Bather (n=1133)	df	Chi-squared Statistic	p value
Age Group:	**4-10yrs**	142 (11%)	136 (12%)			
	11-20yrs	374 (30%)	329 (29%)	4	2.24	0.69
	21-30yrs	196 (16%)	176 (16%)			
	31-40yrs	217 (18%)	185 (16%)			
Gender	**Female**	664 (54%)	600 (53%)	1	0.12	0.72
Current Medication Use		417 (34%)	368 (32%)	1	0.37	0.54
Laxatives Use		4 (0%)	2 (0%)	-	-	0.69
Stomach Remedy Use		24 (2%)	21 (2%)	1	0.00	0.99
Antibiotic/Steroid Use in Prior 4 weeks		61 (5%)	34 (3%)	1	5.27	0.02
Alcohol Consumption:	**<1 a week**	528 (43%)	475 (42%)	2	0.43	0.81
	≥1 a week	203 (16%)	182 (16%)			
Mayonnaise Consumption		328 (27%)	306 (27%)	1	0.04	0.84
Sandwich Consumption		1051 (85%)	966 (85%)	1	0.00	0.96
Chicken Consumption		904 (73%)	832 (73%)	1	0.01	0.93
Eggs Consumption		846 (69%)	753 (66%)	1	1.03	0.31
Hotdogs Consumption		106 (9%)	91 (8%)	1	0.17	0.68
Raw Milk Consumption		335 (27%)	316 (28%)	1	0.14	0.71
Cold Meat Consumption		1041 (84%)	994 (88%)	1	5.51	0.02
Seafood Consumption		49 (4%)	49 (4%)	1	0.11	0.74
Illness in Household		21 (2%)	16 (1%)	1	0.07	0.79
Additional bathing		313 (25%)	308 (27%)	1	0.94	0.33
Prior GI Illness		31 (3%)	21 (2%)	1	0.91	0.34
Prior Respiratory Illness		5 (0%)	3 (0%)	-	-	0.73
Prior Ear Infection		15 (1%)	9 (1%)	1	0.68	0.41
Prior Eye Infection		18 (1%)	27 (2%)	1	2.19	0.14
Prior Skin Ailment		74 (6%)	69 (6%)	1	0.00	0.99

Table S7: Baseline demographic and behavioural characteristics for bathers and non-bathers. Categorical variables were compared using Pearson's chi-squared tests; Fisher's Exact test was used for Laxatives Use and Prior Respiratory Illness, where counts were low and ≤5.

Study Site	Microbial Indicator	Median (IQR)
10	*E. coli*	39 (25-53)
	I. Enterococci	35 (21-57)
	S. Coliphages	20 (13-33)
11	*E. coli*	57 (46-76)
	I. Enterococci	68 (61-91)
	S. Coliphages	0 (0-0)
12	*E. coli*	110 (95-130)
	I. Enterococci	28 (21-35)
	S. Coliphages	27 (7-33)
13	*E. coli*	570 (520-640)
	I. Enterococci	200 (180-240)
	S. Coliphages	283 (230-381)

Table S8: Median and Interquartile Ranges for Microbial Indicator Titres by study site.

Notes: I. Enterococci refers to Intestinal Enterococci; S. coliphage refers to somatic coliphages.

GI Illness Risk

Indicator	Covariate	Adjusted			Adjusted +MI		
		RR	95% CI	p-value	Pooled RR	95% CI	p-value
Bathing Status	Non-bathers	1.00	-	-	1.00	-	-
	Bathers	1.13	0.63-2.03	0.68	1.12	0.63-2.00	0.70
	Age group 4-10yrs	1.00	-	-	1.00	-	-
	Age group 11-20yrs	1.33	0.54-3.32	0.54	0.38	0.14-1.04	0.06
	Age group 21-30yrs	2.61	0.96-7.13	0.06	0.51	0.39-0.66	0.00
	Age group 31-40yrs	0.89	0.33-2.40	0.81	0.34	0.13-0.90	0.03
	Age group 40yrs plus	0.50	0.16-1.57	0.24	0.19	0.08-0.45	0.00
	GI Illness 3wks before	2.32	1.36-3.97	0.00	2.32	1.38-3.92	0.00
	Stomach Remedy	4.92	2.09-11.57	0.00	4.93	2.09-11.63	0.00
	Drugs 4 weeks before	2.04	0.97-4.27	0.06	2.04	0.97-4.30	0.06
	Eggs	0.90	0.84-0.98	0.01	0.90	0.83-0.96	0.00
	Cold Meat	1.50	1.10-2.05	0.01	1.50	1.10-2.05	0.01
Log. *E. coli*	Log. *E. coli*	1.77	1.15-2.73	0.01	1.73	1.13-2.65	0.01
	Age group 4-10yrs	1.00	-	-	1.00	-	-
	Age group 11-20yrs	2.39	0.48-11.94	0.29	0.39	0.07-2.16	0.28
	Age group 21-30yrs	2.62	0.47-14.77	0.27	0.90	0.61-1.34	0.61
	Age group 31-40yrs	0.78	0.60-1.02	0.07	0.30	0.04-2.03	0.22
	Age group 40yrs plus	0.68	0.17-2.78	0.59	0.26	0.17-0.39	0.00
	GI Illness 3wks before	2.65	1.01-6.99	0.05	2.67	1.03-6.93	0.04
	Stomach Remedy	4.56	1.38-15.08	0.01	4.66	1.41-15.43	0.01
	Drugs 4 weeks before	1.06	0.22-5.13	0.94	1.08	0.22-5.31	0.92
	Eggs	1.26	0.88-1.80	0.21	1.25	0.88-1.77	0.22
	Cold Meat	1.32	0.33-5.31	0.70	1.33	0.33-5.35	0.69
Log. I. Enterococci	Log. I. Enterococci	1.20	0.75-1.94	0.45	1.18	0.74-1.88	0.49
	Age group 4-10yrs	1.00	-	-	1.00	-	-
	Age group 11-20yrs	2.31	0.48-10.99	0.29	0.42	0.08-2.21	0.31
	Age group 21-30yrs	2.39	0.46-12.54	0.30	0.95	0.67-1.37	0.80
	Age group 31-40yrs	0.76	0.54-1.07	0.12	0.32	0.05-2.17	0.24

	Age group 40yrs plus	0.65	0.17-2.57	0.54	0.27	0.19-0.40	0.00
	GI Illness 3wks before	2.78	1.02-7.57	0.05	2.82	1.04-7.62	0.04
	Stomach Remedy	5.02	1.65-15.31	0.00	5.10	1.67-15.59	0.00
	Drugs 4 weeks before	1.08	0.21-5.45	0.93	1.10	0.21-5.61	0.91
	Eggs	1.29	0.92-1.82	0.14	1.28	0.92-1.79	0.14
	Cold Meat	1.34	0.34-5.29	0.67	1.35	0.35-5.28	0.66
Log. S. phage	Log. S. phage	1.48	1.06-2.06	0.02	1.46	1.04-2.04	0.03
	Age group 4-10yrs	1.00	-	-	1.00	-	-
	Age group 11-20yrs	2.16	0.45-10.29	0.33	0.42	0.08-2.19	0.31
	Age group 21-30yrs	2.40	0.47-12.33	0.29	0.89	0.63-1.26	0.53
	Age group 31-40yrs	0.72	0.50-1.02	0.06	0.30	0.04-1.99	0.21
	Age group 40yrs plus	0.62	0.16-2.37	0.49	0.26	0.17-0.39	0.00
	GI Illness 3wks before	2.58	1.05-6.33	0.04	2.48	0.98-6.27	0.05
	Stomach Remedy	4.45	1.33-14.96	0.02	4.62	1.33-16.01	0.02
	Drugs 4 weeks before	1.04	0.21-5.24	0.96	1.07	0.21-5.40	0.94
	Eggs	1.22	0.84-1.77	0.30	1.20	0.84-1.72	0.33
	Cold Meat	1.23	0.34-4.40	0.75	1.24	0.35-4.40	0.73

Table S9: Expanded GEE Model Results for gastrointestinal illness outcome. Correlation structures were specified to account for clustering within study sites and robust (sandwich) standard errors were used.

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; I. Enterococci refers to Intestinal Enterococci; S. phage refers to somatic coliphages.

GI Illness Risk

Indicator	Covariate	Adjusted		
		RR	95% CI	p-value
Log. *E. coli*	Log. *E. coli*	0.09	0.01-0.84	0.03
	Age group 4-10yrs	1.00	-	-
	Age group 11-20yrs	0.01	0-0.2	0.00
	Age group 21-30yrs	0.01	0-0.32	0.01
	Age group 31-40yrs	0.04	0-0.57	0.02
	Age group 40yrs plus	0	0-0.05	0.00
	GI Illness 3wks before	2.65	0.93-7.53	0.07
	Stomach Remedy	4.6	1.35-15.66	0.01
	Drugs 4 weeks before	1.1	0.24-5.08	0.9
	Eggs	1.27	0.9-1.78	0.17
	Cold Meat	1.36	0.36-5.19	0.65
	Log. *E. coli* * Age group 11-20yrs	24.44	2.07-288.36	0.01
	Log. *E. coli* * Age group 21-30yrs	20.06	1.54-261.4	0.02
	Log. *E. coli* * Age group 31-40yrs	5.47	1.36-22.01	0.02
	Log. *E. coli* * Age group 40yrs plus	22.58	2.34-218.23	0.01

Table S10: GEE Model (with effect interactions between Log. *E. coli* and Age group) Results for gastrointestinal illness outcome. Correlation structures were specified to account for clustering within study sites and robust (sandwich) standard errors were used.

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. Log. *E. coli* * Age group 11-20yrs denotes the interaction between Log. *E.coli* counts and the 11-20 year age group, representing the multiplicative difference in relative risk, compared to the reference 4-10 year age group.

	Log. E. coli		
Age group	**Average Marginal Effect**	**95% CI**	**p value**
4-10yrs	-0.05	-0.05--0.02	0.00
11-20yrs	0.03	0.02-0.03	0.00
21-30yrs	0.02	-0.01-0.05	0.11
31-40yrs	-0.01	-0.02-0.01	0.33
40yrs plus	0.01	0.00-0.01	0.00

Table S11: Age group-specific Average Marginal Effect for Log. *E.coli*. Average Marginal effects represent the change in predicted probability of gastrointestinal illness associated with a one unit increase in log-transformed *E. coli* concentration, for each age group from the GEE model (Supplementary Table 10) including interaction terms between *E. coli* and age group.

Number of Participants

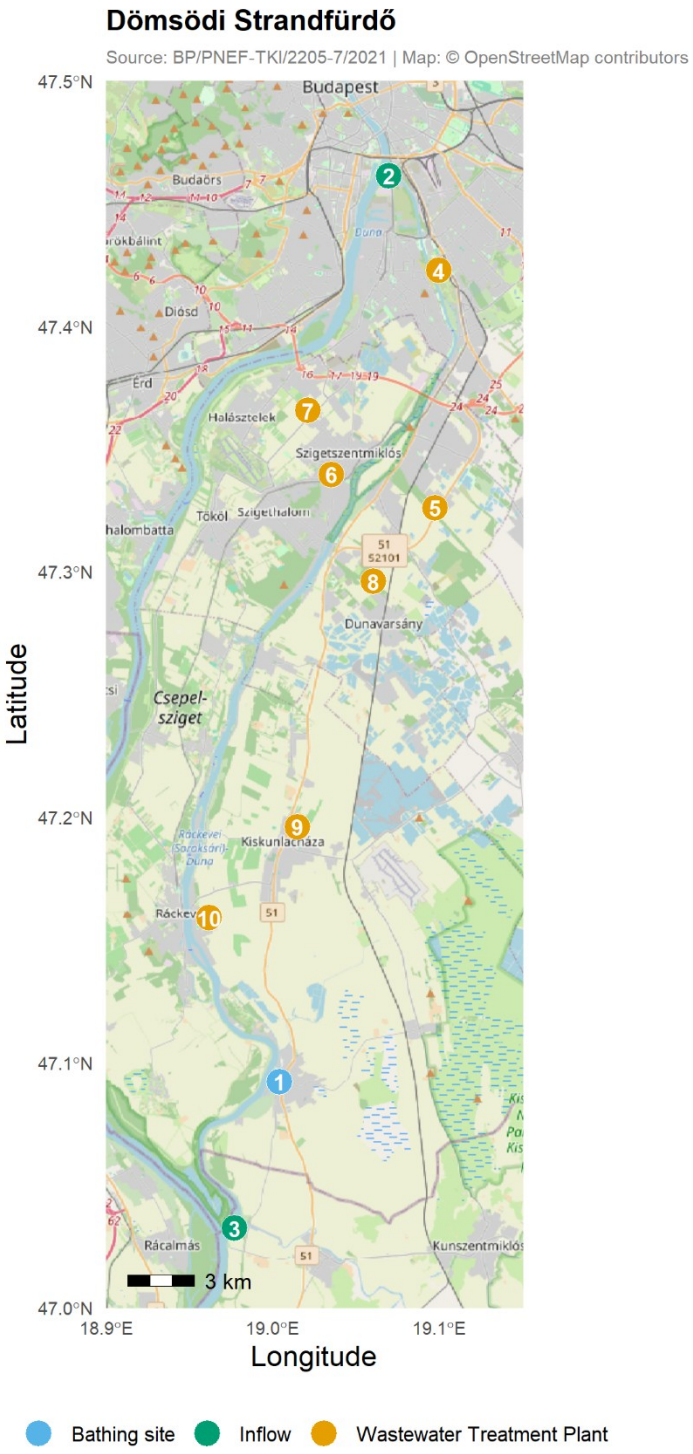
Stage	Study Site				Total
	10	11	12	13	
Recruitment	705	698	676	645	2724
Pre-trial Baseline Interview	706	689	630	537	2562
Bathing Trial	679	616	597	507	2399
One Week Follow-up	664	607	592	505	2368

Table S12: Participant retention by study site and stage.

Supplementary Text 3

The following summaries were primarily derived from official bathing water profiles (https://nnk.gov.hu/index.php/furdovizprofilok.html) and Hungarian government sources. Google Maps, Google Reviews, OpenStreetMaps and open source photographs were used to geolocate specific features.

Maps were constructed in R version 4.5.1 using the maptiles (version 0.11.0) and sf (version 1.1-0) packages: all code needed to reproduce the maps are provided in the project Github repository (https://github.com/edwardkslam/Epibathe).



The Dömsöd bathing site (1; bathing water identifier HUBW_01408) is a 110m long sandy and partly grassy beach. Water depth is 2.3-4m with a gravel and sand bed; the average summer water temperature is 22°C.

Waterbody

The bathing site is situated at the southern end of the Ráckevei (Soroksári) Duna (RSD; VOR water body code AIQ014), a 57.3km long closed backwater of the Danube with no natural inflows.

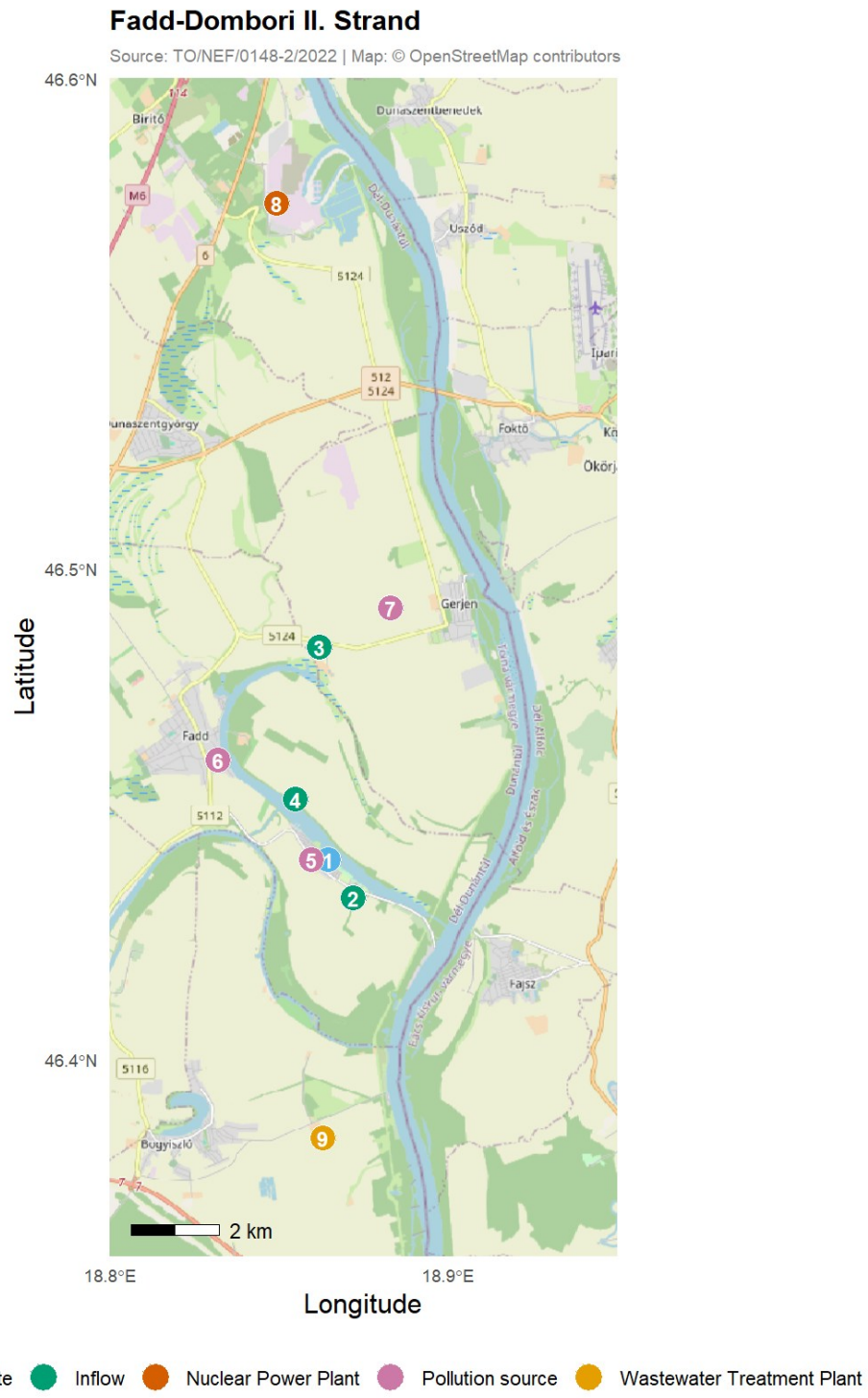
Inflows

Water exchange with the Danube occurs once every 1.5-2.5 weeks in the summer at two controlled sluices at either end of the (RSD): Kvassay (2) 47km upstream and Tassi (3) 10 downstream of the bathing site.

Pollution sources

There are no specific point or diffuse sources identified at the site or its vicinity. The official bathing-water profile notes six wastewater treatment plants (WWTPs) that may influence the water quality of the RSD more broadly. Only the Budapest Dél-Pest (4) WWTP discharges treated municipal and industrial effluent directly into the RSD, approximately 33km upstream from the bathing site. ÁTI-SZIGET WWTP (6) discharges treated industrial effluent directly into the RSD 21km upstream. Dunavarsány (8) WWTP discharges industrial cooling water directly into the RSD 18km upstream. Dunaharaszti (5) and Kiskunlacháza (9) WWTPs discharge into the Duna-Tisza and I. Árapasztó side-canals respectively, which connect indirectly to the RSD 24 and 10km upstream.

Szigetszentmiklós (6) and Ráckeve (9) are noted to only discharge treated municipal effluents into the main Danube.



The Fadd bathing site (1; bathing water identifier HUBW_01701) consists of a 150m long sandy, gravelly beach with partly concreted and naturally grassed areas. The Water depth is 2-2.5m with a silty gently sloping bed and average summer water temperatures of 23°C (minimum and maximum of 18 and 29.5°C respectively).

Waterbody

The bathing site is situated on the south-east bank of the Faddi Holt-Duna (Fadd Oxbow Lake; VOR water body code AIH066): a completely enclosed former meander of the Danube. The lake is 10km long, 100-350m in width and 0.5-3.5m deep. All water supply is artificial, via two engineered systems. The lake is eutrophic with a history of cyanobacterial blooms, most notably in 2006 and 2017.

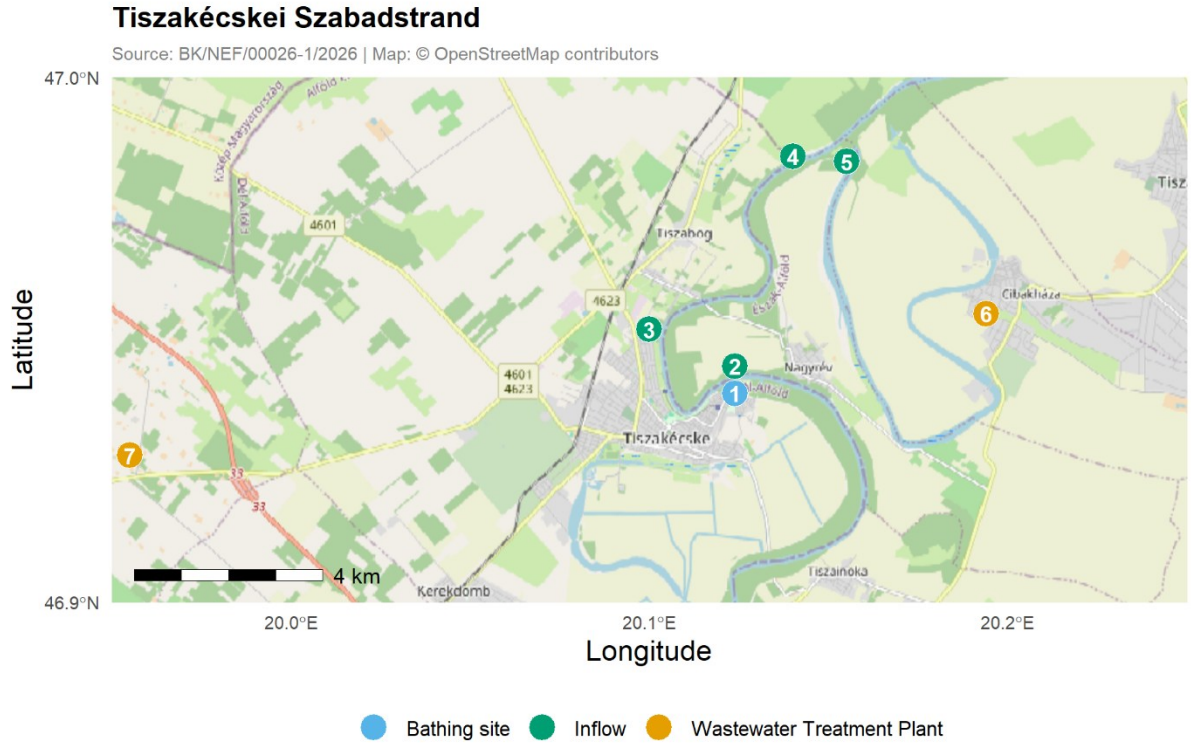
Inflows

The lake receives water supply from two artificial inflows: the Dombori flood-gate (2) and Paks-Faddi supply canal (3). The Dombori flood-gate is immediately adjacent to the beach, enabling top-up from the Danube at high water and drainage in reverse. The Paks-Faddi supply canal carries cooling water from the Paks Nuclear Power Plant (7), as well as upland catchment runoff, through a reed-bed biological filter before entering the lake at the northern end (3). There is also a sluice (4) that regulates the water level between the northern and southern ends of the lake.

Pollution Sources

No sewage treatment plants discharge into the oxbow: all settlements in the catchment discharge treated effluent to the main Danube at the Bogyiszló wastewater treatment plant (9), which is 6km downstream from the lake.

Identified pollution risks include runoffs from lakeside houses and local agricultural land use. Along the western shores of the lake, there are 12 permanently houses (6) that are not connected to the sewer network. These have been identified as the most likely active point-source risk of pollution near the beach. The resort (5) adjacent to the beach does not have a stormwater drainage system and contributes rainwater runoffs, especially during heavy rainfall. The surrounding arable land and Gerjen Lajos livestock farm (7) contribute diffuse agricultural runoffs from fertilisers, pesticides and animal faecal matter.



The Tiszakécske bathing site (1; bathing water identifier HUBW_00312) is 350m long sandy riverside bathing area. The river bed has a steep profile; water depth is highly considerably with river level and flow conditions. The average summer water temperatures is 24°C (minimum and maximum of 18 and 28°C respectively). The site has separated toilets, handwashing facilities, showers, water fountain taps and waste bins.

Waterbody

The bathing site is situated on the right bank of the Tisza river (VOR water body code AEQ060) within the Tisza sub-catchment (HUAEP 182) of Danube basin district. The Tisza is a flowing river system at this location. Within 10km upstream, there are four recognised surface water inflows.

Inflows

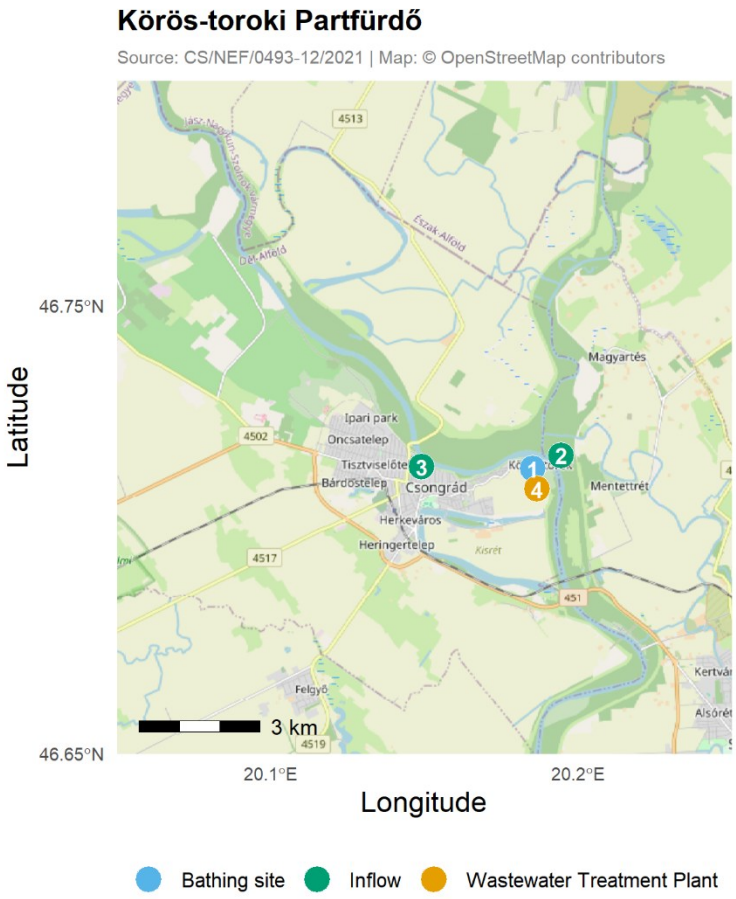
Facilities at the bathing site discharge treated thermal water directly into the Tisza (2). The Peitsik , Pereghalmi II and Cibakházi-Holt-Tisza lecsapoló canals drain into Tisza at

(3), (4) and (5) respectively. Drainage into the Tisza occurs seasonally, typically during periods of high-water or significant rainfall.

Pollution Sources

There are two wastewater treatment plants identified as the principal point sources of pollution. Both WWTPs discharge treated municipal effluents: the Cibakháza WWTP (6) directly discharges into the Tisza approximately 200m upstream from (5) and 11km upstream of the bathing site whilst the Szentkirály WWTP (7) discharges into the Peitsik canal, which enters the Tisza about 3.5km upstream at (3). Since the Szentkirály plant discharges modest volumes of around 100m^3 per day and the Peitsik canal only intermittently drains into the Tisza, this WWTP is considered unlikely to pose a major risk to bathing water quality.

Agricultural diffuse pollution and industrial sources are also noted as general background influences within the wider Tisza catchment area.



The Csongrád bathing site (1; bathing water identifier HUBW_00604) is a 400m long sandy riverside bathing area. The riverbed is composed of clay and silt, dropping steeply to a depth of 10m; the average summer water temperature is 22°C (minimum and maximum of 18 and 26°C respectively). The site is also used for recreational activities, such as boating and pedal boats

Waterbody

The bathing site is situated on the right bank of the Tisza river (VOR water body code AEQ056), at its confluence (2) with the Hármas-Körös river, which enters from the left bank. At this location, the Tisza is influenced by a very large upstream catchment (157,000 km^2).

Inflows

The defining hydrological feature is the Hármas-Körös confluence (2), which lies immediately opposite to the bathing area. More broadly, the site is influenced by numerous upstream tributaries and by surrounding inland drainage canals discharging

into the Tisza, although no local inflows were specified in the official bathing water profile. A sluice (3) is also present nearby.

Pollution Sources

No specific sources were identified at or near the site. The official bathing water profile highlights broader catchment-scale risks, including diffuse phosphorus loading from agricultural land use and solid waste transported during floods from illegal upstream floodplain dumping sites.

The Csongrád municipal WWTP (4) is located approximately 250 m downstream of the bathing site and would therefore not be expected to pose an upstream risk under normal flow conditions.