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RESEARCH ARTICLE

Early detection and environmental drivers of sewage fungus outbreaks in rivers

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Abstract

1. Sewage effluent is a major ongoing threat to water quality and biodiversity in freshwater environments. It can cause outbreaks of sewage fungus (fungus-like bacteria which form macroscopic masses) but, until now, these were only qualitatively recorded from visual inspection, ignoring microscopic forms.
2. Here, we used an innovative method that combines machine learning, microscopy and flow cytometry, to rapidly and efficiently quantify the presence and abundance of sewage fungus in rivers. Our study involved 11 rivers with ($n = 6$) and without ($n = 5$) sewage input in England over four sampling occasions.
3. We were able to detect and enumerate the filaments before masses became visible to the naked eye and, as expected, we found a higher number of filaments downstream of sites where treated sewage was offloaded into the river. Therefore, our detection method could be used as a 'canary in the coal mine' for future outbreaks allowing early intervention.
4. Combining our quantitative data on filaments with data on the physical and chemical parameters of the rivers, we found that high conductivity, sulphate, nitrates and TDS were associated with the presence and proliferation of sewage fungus. This information can be extremely useful for regulatory bodies and water companies to develop mitigating strategies and action to prevent future outbreaks.

KEYWORDS
automated technique, freshwater ecology, machine learning, river pollution, sewage fungus, wastewater

1 | INTRODUCTION

Sewage effluent is one of the most important contributors to pollution of aquatic ecosystems on a global scale (Turner et al., 2003; Whelan et al., 2022). Rivers, in particular, are heavily affected by sewage discharge, with potentially severe impacts on ecosystem functioning and natural capital (Oliveira & Goulder, 2006). Rivers are crucial parts of the global water cycle and essential for human health, connecting inland watersheds to the marine environment and providing drinking water for most of the global population (Koelmans et al., 2016). Thus, investigating the effects of sewage effluent on river ecosystem processes and water quality is paramount

not only for the environment, but also for local human populations (e.g. Eregno et al., 2016).

Water companies in the UK are permitted to release treated wastewater into rivers, and during heavy rainfall conditions, untreated wastewater is also released (Geatches et al., 2014). Although treated sewage is diluted by river water, the discharge often influences the ecosystem by altering water quality, which has well-recognised consequences for freshwater organisms (Hilario Garcia et al., 2017; Riechel et al., 2016; Worrall et al., 2019). For instance, the high nutrient load in sewage discharge favours algal growth causing blooms. When the algae die, they boost microbial activity resulting in oxygen depletion and mass fish mortality (Aubertheau et al., 2017; Gonzalo & Camargo, 2013; Mor et al., 2019). Additionally, sewage effluent is of public health significance, as it can contain bacteria and dangerous pathogens. This poses a threat for human well-being if water is then used for drinking, recreational or agricultural purposes (Robertson et al., 2006; Zafar et al., 2017).

Sewage effluent is a complex mixture of inorganic nutrients, organic matter and microorganisms (Marti et al., 2004). These microorganisms can be divided into two groups: those that convert organic matter into inorganics and are a fundamental part of wastewater treatment processes (Oliveira & Goulder, 2006), and those which are informally and collectively called 'sewage fungus'. Sewage fungus comprises several species of bacteria (e.g. *Sphaerotilus natans*, *Beggiatoa alba*, *Carchesium polypinum*, *Flexibacter* spp.), often with a filamentous, 'fungus-like' shape in surface waters (Figure 1a,b, Kator & Rhodes, 2003). Despite appearing like fungi, the filaments are actually single rod-shaped cells that are mostly Gram-negative, obligate aerobes that divide by binary fission and do not branch (Kator & Rhodes, 2003; Oliveira & Goulder, 2006). These filamentous bacteria form macroscopic masses when there are significant amounts of organic nutrients, which not only cause unpleasant smells but severely reduce oxygen levels in water that can adversely affect all river biota (Curtis, 1969). For instance, *Sphaerotilus* sp, a common genera of sewage fungus worldwide, smothers fish eggs, stopping them from hatching (Gaufin & Tarzweu, 1955; Smith & Kramer, 1963). Therefore, early detection of sewage fungus in rivers is paramount to allow intervention and to avoid extensive outbreak events (i.e. when massive growths of sewage fungus can completely cover submerged surfaces, causing organic pollutions in rivers, Curtis & Harrington, 1971).

Despite the potential impacts of sewage fungus in freshwaters, current approaches to detect its presence and quantifying its abundance in UK rivers only comprise a simple in situ visual, qualitative inspection by managers (Curtis & Harrington, 1971; Geatches et al., 2014). In standard environmental monitoring protocols (Geatches et al., 2014), only the sewage fungus surface coverage and water turbidity are recorded for a random area of the river, employing four qualitative categories ("none", "localised", "widespread" and "extensive" for surface coverage; "clear", "slight", "moderate"

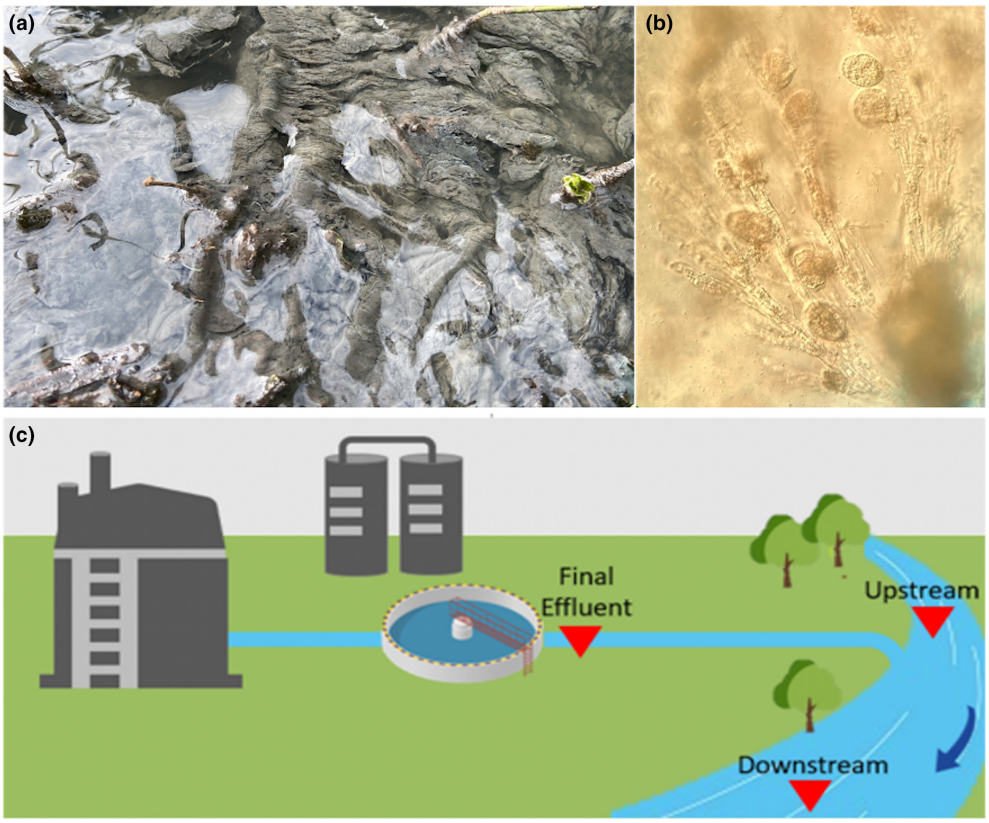

FIGURE 1 (a) Sewage fungus mat found in the downstream area of one river sampled in this study. (b) Sewage fungus under a Zeiss compound microscope (Zeiss Axioskop 2 Plus). (c) Locations of the sampling sites (red triangles) at the impact rivers: final effluent from the treatment plant and upstream and downstream in the receiving river. The blue arrow indicates the flow of the river.

and "high" for turbidity; Geatches et al., 2014). This is a substantial limitation because visual assessment means that only large masses at the inspection point and in the human field of view are recorded, and small, less dense outbreaks are excluded. Sewage fungus outbreaks are, therefore, only subjectively detected once they are large enough to be having negative effects on the river community. Armed with such innovative approach, we developed an effective and fast method to detect and facilitate early prevention of sewage fungus in freshwaters, a method that can be used to create crucial strategies of intervention for monitoring and management of our rivers.

For successful intervention, there is also a need to better understand the physical and chemical variables which may promote growth, such as temperature, pH, oxygen and nutrients (Hulsen et al., 2016; Kaleli & Islam, 1997; Zhang et al., 2018). These factors might change in different areas of the river which are directly (i.e. downstream of the outflow) or not (i.e. upstream of the outflow) affected by the discharge of sewage effluent into rivers (e.g. Curtis & Harrington, 1971; Geatches et al., 2014). Understanding which environmental variables influence sewage fungus growth will inform practitioners of the most efficient sewage treatment methods and their impacts on river health. Our objectives are, therefore, to (i) efficiently quantify the abundance of sewage fungus in rivers to allow early detection; (ii) investigate the environmental factors that increase the chance of a sewage fungus outbreak; and (iii) recommend approaches to pre-empt future sewage fungus outbreaks.

2 | MATERIALS AND METHODS

2.1 | Study area

Four sampling campaigns were undertaken at 11 rivers in England, UK, in 2021 (June, August, October and November). Six rivers received treated wastewater and had previously recorded outbreaks of sewage fungus (replicated impact sites), whereas the remaining five rivers did not have a sewage outlet (replicated control sites). All rivers were surveyed to detect the presence and abundance of sewage fungus and characterise the physical habitat and water chemistry. For each of the impact river, we sampled at two sites: 5–10 m upstream and 5–10 m downstream of the sewage outflow. Additional water samples were collected at the final effluent (FE) point (Figure 1c) in the water treatment sites. Control rivers were randomly sampled at one site only. Anonymity of the river locations has been intentional to protect the identity of the wastewater treatment company.

2.2 | Sewage fungus sampling and processing

Two water samples from each site in each sampling month were collected randomly using 50 mL tubes. The water samples were preserved using three drops of 25% glutaraldehyde and kept refrigerated until processed in the Aquatic Laboratory at the University of Oxford. Here, subsamples of 10 mL of water were visually analysed under a compound microscope (Zeiss Axioskop 2) to identify the sewage fungus. For the enumeration of sewage fungus filaments, we used 20 mL subsamples and a Bench Top FlowCAM® 8000 (Fluid Imaging Technologies, Inc.) and its particle analysis software (VisualSpreadsheet©, version 4). FlowCAM® 8000 is an imaging flow cytometer developed for rapid particle detection and enumeration. It uses a combination of imaging and laser light to detect particles within a fluid sample, enabling the capture of particle images for image analysis. The FlowCAM settings were: 4× objective lens, 0.16 cm inner diameter tubing, field of view 300 flow cell (300 µm depth, 3000 µm width), an imaging rate of 21 frames/s and a flow rate of 0.15 mL/min. AutoImage mode was used to process the images captured (Fluid Imaging Technologies Inc., 2011; Poulton, 2016). Image libraries of sewage fungus were created prior to the experiment, adding to the machine a known sample with sewage fungus and identifying the filaments based on their shapes and forms. Libraries used as a reference for identification. In this way, once the sample was photographed, images were auto identified by the VisualSpreadsheet© software. Manual post-processing control was performed to ensure the accuracy of the software. Once images were correctly sorted, the total number of images (i.e. density of the filaments) was recorded.

2.3 | Physio-chemical measurements and water sampling

Physical parameters of the rivers were recorded for all 4 months of sampling, while the chemical parameters were tested in August, October and November 2021. At each sampling site in each of the 11 rivers the water temperature, pH, total dissolved solids (TDS), total dissolved oxygen (DO) and electrical conductivity were measured using a hand-held Combo pH and EC Hanna probe or a LT Lutron probe. Additionally, three replicates of 100 mL of water were collected to analyse fluoride, chloride, bromide, nitrite (NO_2^-), nitrate (NO_3^-), phosphate (PO_4^{3-}), sulphate (SO_4^{2-}) and total organic carbon (TOC). Water samples were kept refrigerated and analyses were undertaken in the laboratory of the School of Geography and the Environment at the University of Oxford (Table S1).

2.4 | Data analysis

We first tested if filament presence was significantly different in our control rivers and the upstream sites of our impact rivers using a binomial generalised linear model. No differences were detected (Table S2, Figure S1) and, therefore, we considered the upstream sites as controls in further analyses. To test for differences in filament numbers between upstream, downstream and final effluent, we used a linear mixed effect model (the *lmer* function in the package "lme4" in R, which was used for all analysis; R Core Team

TABLE 1 (a) Result of the linear mixed effect model to test the difference in sewage fungus filaments between different sampling sites and months. (b) Post-hoc analysis to test the difference in the presence of fungus filaments between location pairs.

(a)	Sum of square	Mean	df	*F*	Pr (>*F*)
Sampling month	13.68	4.562	3	8.958	<0.05
Area	532.35	266.175	2	522.716	<0.05
Sampling month: Area	8.61	1.435	6	2.817	<0.05

(b)	Estimate	SE	Z value	Pr (>z)
FE—Downstream	0.805	0.206	3.908	<0.05
Upstream—Downstream	−5.323	0.206	−25.842	<0.05
Upstream—FE	−6.128	0.206	−29.750	<0.05

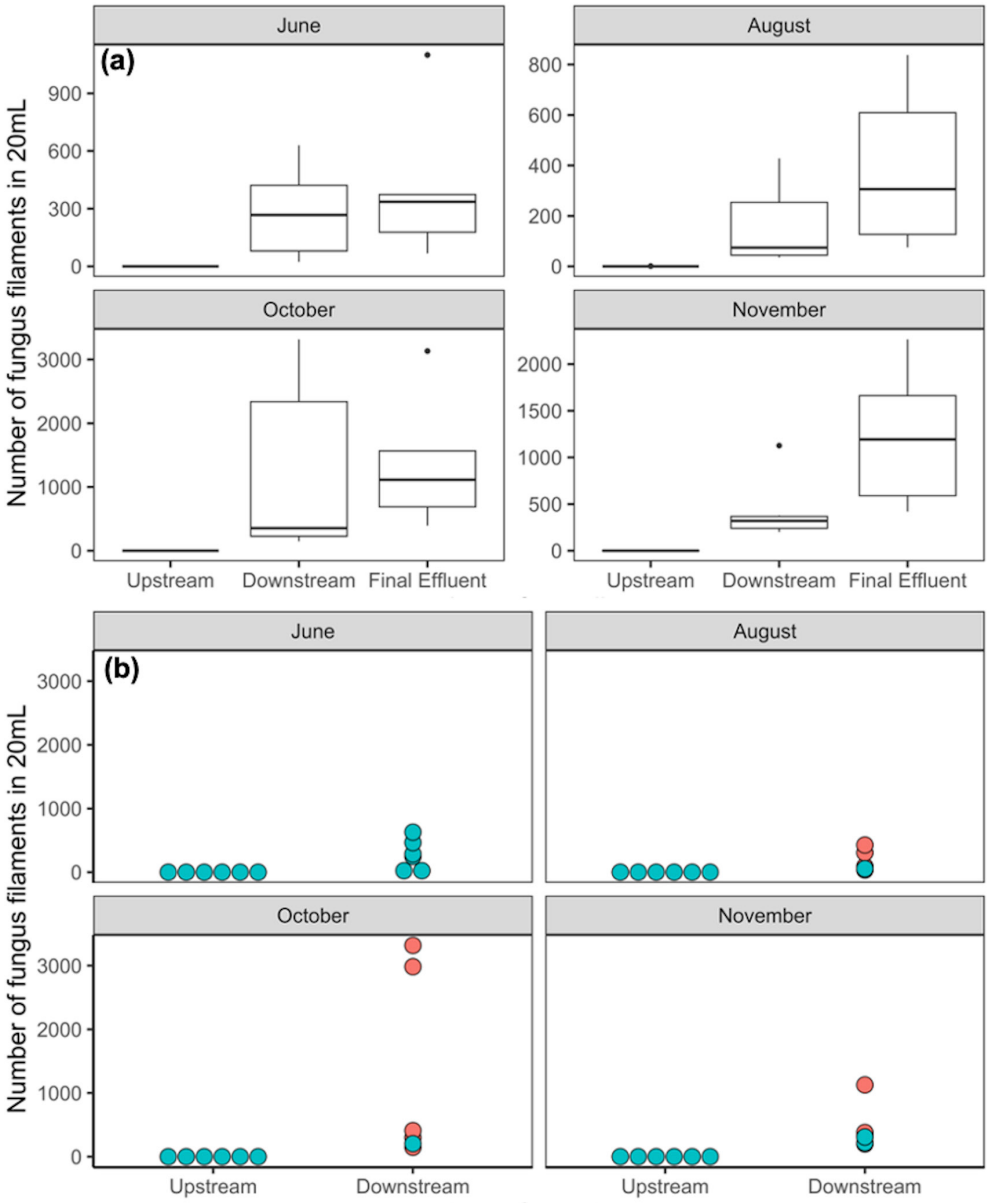

FIGURE 2 (a) Sewage fungus abundance for all river sites (filaments in 20 mL) in each sampling area (upstream, downstream and final effluent) and for four sampling months. (b) Sewage fungus abundance (filaments in 20 mL) in upstream and downstream sampling areas, for all river sites, for four sampling months. Pink dots denote the samples with sewage fungus detected both with a visual qualitative inspection of the river and with the FlowCam analysis. Turquoise dots are the samples with sewage fungus detected only with the FlowCam but not visible in the river.

version 1.4.17, Bates et al., 2015), with log+1 data transformed to meet the assumption of the model. Sampling month and site were set as explanatory variables, and the river was considered as a random effect. We performed a post-hoc test using the *glht* function in the "multcomp" package. We also determined if filament counts were a good predictor of visible sewage fungus presence and outbreaks. We obtained data on outbreaks reported to the Environment Agency (EA) by the water company during 2021, in addition to our own visual records of sewage fungus growing in the sample rivers.

For each sampling month, we used multivariate analysis of variance based on permutations (PERMANOVA, Anderson, 2017) to test for chemical and physical differences between upstream, downstream and final effluent areas. PERMANOVAs were performed using the "adonis" function in the "vegan" package (Oksanen, 2020), with a Bray-Curtis distance matrix and a α-level of 0.05 and setting 999 permutations for the test (due to a technical limitation in processing, we removed two impact sites downstream and two impact sites upstream from the dataset). Further, similarity percentage analysis (SIMPER) was used for identifying which environmental parameters contributed the most to differences between sampling sites. We then performed a regression analysis with the most influential physical and chemical parameters identified by the SIMPER analysis to test if they had a significant effect on the abundance of sewage fungus filaments who were present in the downstream river areas. Data were log transformed to achieve the normality of the residuals.

Physical and chemical dissimilarity between upstream and downstream areas was visualised with a non-metric multidimensional scaling (NMDS) ordination on a Bray-Curtis distance matrix, using the function *metaMDS* in the "vegan" package (Oksanen, 2020). The stress of each model was obtained by the distance matrix and provided assessments of the model fit (i.e. if the 2-axis created by the NMDS are sufficient to explain the data). We considered the reduced-dimension representations of our data to be acceptable if NMDS stress scores were ≤0.2 (Fetzner & Taylor, 2018). Here, we omitted FE since physical measures were not recorded. NMDS of chemical variables only, which includes FE, are included in the SI. All plots were made using the "ggplot2" package (Wickham, 2016).

3 | RESULTS

We found that sewage fungus was significantly higher in water samples downstream of final effluent input, and that filament numbers were an appropriate predictor of pollution events. Filament numbers were associated with conductivity, sulphate, nitrates and TDS.

3.1 | Quantification of sewage fungus

Upstream sites had significantly lower numbers of filaments compared to downstream sites (e.g. mean filaments per 20 mL sample per sites: 0.5 ± 0.4 SE), which in turn had fewer filaments than the FE (Table 1a,b, Figure 2a). Sites where we visibly saw the presence of fungus mats had a higher number of filaments than those where we did not see outbreaks (Figure 2b).

3.2 | Environmental drivers of sewage fungus filament numbers

Environmental variables were statistically different between upstream, downstream and final effluent sites in all sampling months (Table 2; Figure S2). Furthermore, there was clear clustering between upstream and downstream sites in the ordination, with the former having higher oxygen levels and pH, and lower nutrient pollution (Figure 3). SIMPER analysis revealed that conductivity, TDS, nitrates and sulphates were the primary drivers of environmental variability (Table S3; Figure S3). All the environmental variables we tested (TDS, conductivity, pH, total dissolved oxygen, nitrates and sulphates) had a significant effect on the sewage fungus abundance (Table 3). The best fit model was conductivity, suggesting that this variable is most closely associated with sewage fungus outbreaks.

4 | DISCUSSION

In this study, we demonstrate that a novel method which combines machine learning, microscopy and flow cytometry, is a fast and effective approach to quantitatively detect sewage fungus in rivers. Overall, the abundance of sewage fungus filaments was high when outbreaks

TABLE 2 Results of the PERMANOVA analysis on the difference between environmental parameters for upstream, downstream, and final effluent areas.

	df	Sum of square	R^2	Pseudo-*F*	Pr (>*F*)
June					
Area	1	0.032	0.122	4.7333	<0.05
Residuals	34	0.233	0.877		
Total	35	0.266	1.000		
August					
Area	2	1.971	0.734	66.415	<0.05
Residuals	48	0.712	0.265		
Total	50	2.683	1.000		
October					
Area	2	1.462	0.747	48.836	<0.05
Residuals	33	0.494	0.252		
Total	35	1.957	1.000		
November					
Area	1	0.095	0.241	10.8	<0.05
Residuals	34	0.302	0.758		
Total	35	0.397	1.000		

FIGURE 3 Non-metric multidimensional scaling (NMDS) graph on the axes of a two-dimensional solution based on Bray-Curtis dissimilarities of environmental parameters in the upstream (green) and downstream (orange) sampling area. Different panels show the different sampling months (June, August, October and November). Vectors represent strength and direction of correlation of each parameter relative to the two NMDS axes. All the NMDS converged on a two-dimensional solution with an acceptable stress level (<0.2).

were visible (Figure 2b), suggesting that regular monitoring of their abundance will allow for early detection in, and efficient management of, wastewater treatment plants to pre-empt the visible mats that are considered a pollution event and can have negative effects on fish and in-stream ecosystems (Willoughby & Roberts, 1991).

For the first time, we were able to detect and enumerate sewage fungus filaments even when not visible to the naked human eye. Traditional sampling methods and visual inspection are not able to forecast future outbreaks or to determine which filaments are viable for proliferation. Our novel approach is an effective and efficient solution to pre-empt and intervene before a sewage fungus outbreak occurs, although it is important to note that the detected filaments might not always be viable. Despite this, the advantages of swiftly identifying and quantifying filaments outweigh the slight potential for overestimation. This new technique, therefore, has the potential to be used in regular sampling by water companies and other regulatory organisations (such as the Environmental Agency) to monitor rivers and inland waters.

Sewage fungus densities varied with the sites sampled, with higher concentrations of filaments in final effluent samples. A reasonable explanation of this is that river water dilutes the highly concentrated FE. Variation between downstream sites might be explained by other sources of pollution such as agricultural discharge and other forms of chemical and physical contamination (e.g. pharmaceuticals and microplastics, Fan et al., 2022; Gray, 1985; Luo et al., 2014; Whelan et al., 2022). However, we found a clear clustering between upstream and downstream sites in the ordination suggesting that the major physical and chemical

TABLE 3 Linear model analysis showing the effects of relevant environmental parameters on sewage fungus abundance (log transformed +1), for the different sampling months.

	df	Sum of square	Mean square	*F* value	Pr (>*F*)
Conductivity					
Conductivity	1	47.421	47.421	98.215	<0.05
Sampling month	2	7.787	3.894	8.064	<0.05
Conductivity: Sampling month	2	8.017	4.009	8.302	<0.05
TDS					
TDS	1	37.264	37.264	50.223	<0.05
Sampling month	2	15.288	7.644	10.302	<0.05
TDS: Sampling month	2	0.2566	0.283	0.381	0.685
pH					
pH	1	11.659	11.658	18.5211	<0.05
Sampling month	2	40.341	20.170	32.042	<0.05
pH: Sampling month	2	5.505	2.752	4.372	<0.05
O_2					
O_2	1	16.440	16.440	35.272	<0.05
Sampling month	2	40.437	20.218	43.379	<0.05
O_2: Sampling month	2	18.177	3.500	7.509	<0.05
Nitrates					
Nitrates	1	45.313	45.313	79.802	<0.05
Sampling month	2	9.890	4.945	8.708	<0.05
Nitrates: Sampling month	2	4.707	2.354	4.145	<0.05
Sulphates					
Sulphates	1	27.249	27.249	69.107	<0.05
Sampling month	2	32.740	16.370	41.517	<0.05
Sulphates: Sampling month	2	6.688	3.343	8.480	<0.05

differences are caused by the input to rivers of treated sewage. Sewage fungus was present in areas of the rivers that were characterised by low oxygen and pH, and high temperature, nutrient loadings, TDS and conductivity. This is in line with established findings in previous studies (e.g. Curtis, 1969; Curtis & Harrington, 1971; Passell et al., 2007). These environmental variables are closely related to each other; for instance, TDS combines the sum of all ion particles including organic nutrients (such as hydrocarbons and urea) and salt ions (Carey & Migliaccio, 2009). In addition, conductivity is a measure of the capacity of water to pass electrical flow which is directly related to the concentration of ions (e.g. chlorides, sulphides and carbonate compounds) in the water (Carey & Migliaccio, 2009; Ekka et al., 2006; Haggard et al., 2005). In this study, we found that sites where sewage fungus occurred in significant abundance were associated with waters with high conductivities (e.g. 900 μs/cm). We also found that high levels of sulphates and nitrates, typical products of waste water treatments, were associated with presence of sewage fungus. This could have negative implications for primary production, as stream cycling of N can affect the nutrient supply available for phytoplankton and aquatic plants (Palmer-Felgate et al., 2010). Moreover, high sulphate concentrations can imbalance the natural sulphur cycle (Hulshoff Pol et al., 1998; Silva et al., 2002), causing acidification of surface waters and substrates, and resulting in a decrease of the species diversity and the vitality of many freshwater ecosystems (Silva et al., 2002).

Anthropogenic stressors of water quality threaten biodiversity globally, a growing crisis that is particularly acute in freshwater ecosystems (Tickner et al., 2020). In the context of global change, this study is a key baseline to mitigating river ecosystem degradation. By creating a quantitative method which can be developed and used to predict and prevent future outbreaks, together with future research to identify 'tipping point' densities of sewage fungus, it will be possible to efficiently monitor and regulate sewage fungus.

5 | CONCLUSIONS

In summary, we developed a promising quantitative and forecasting approach for the detection and enumeration of sewage fungus using a FlowCAM, which combines innovative (machine learning) and more traditional (microscopy and flow cytometry) techniques. This approach (a) performs better than the qualitative visual inspections currently adopted by regulators and the water industry, (b) robustly and rapidly quantifies sewage fungus presence even when sewage fungus is not visible to the naked eye, and (c) can be used as a powerful prediction tool by regulatory bodies such as the Environment Agency and by water companies aiming to identify possible thresholds of sewage fungus abundance which can lead to an outbreak. A further outcome of the research was to identify key environmental variables (such as conductivity, nitrates and sulphates) associated with the presence and proliferation of sewage fungus in rivers in the south-west of the UK.

AUTHOR CONTRIBUTIONS

Michelle C. Jackson obtained the funding and conceived the idea. Dania Albini, Michelle C. Jackson, Jocelyne M. R. Hughes, Lauren Lester and Philip Sanders collected the data in the field. Dania Albini analysed the data with input from Michelle Jackson. Dania Albini led the writing of the first draft of the manuscript. Michelle C. Jackson, Jocelyne M. R. Hughes and Lauren Lester contributed to manuscript editing and all authors gave the final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Michelle Jackson is an Associate Editor of Ecological Solutions and Evidence, but took no part in the peer review and decision-making processes for this paper.

DATA AVAILABILITY STATEMENT

Data are publicly available in the Dryad repository at the following link: https://doi.org/10.5061/dryad.ffbg79d12 (Albini et al., 2023).

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REFERENCES

Albini, D., Lester, L., Sanders, P., Hughes, J., & Jackson, M. (2023). Data from: Early detection and environmental drivers of sewage fungus outbreaks in rivers. *Dryad Repository*. https://doi.org/10.5061/dryad.ffbg79d12

Anderson, M. J. (2017). Permutational multivariate analysis of variance (PERMANOVA). *Wiley StatsRef: Statistics Reference Online*, 557–574.

Aubertheau, E., Stalder, T., Mondamert, L., Ploy, M. C., Dagot, C., & Labanowski, J. (2017). Impact of wastewater treatment plant discharge on the contamination of river biofilms by pharmaceuticals and antibiotic resistance. *The Science of the Total Environment*, *1*, 1387–1398.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*, 1–48.

Carey, R. O., & Migliaccio, K. W. (2009). Contribution of wastewater treatment plant effluents to nutrient dynamics in aquatic systems. *Environmental Management*, *44*, 205–217.

Curtis, E. J. C. (1969). Sewage fungus: Its nature and effects. *Water Research*, *3*, 289–311.

Curtis, E. J. C., & Harrington, D. W. (1971). Occurrence of sewage fungus in rivers in the United Kingdom. *Water Research*, *5*, 281–296.

Ekka, S. A., Haggard, B. E., Matlock, M. D., & Chaubey, I. (2006). Dissolved phosphorus concentrations and sediment interactions in effluent–dominated Ozark streams. *Ecological Engineering*, *26*, 375–391.

Eregno, F. E., Tryland, I., Tjomsland, T., Myrmel, M., Robertson, L., & Heistad, A. (2016). Quantitative microbial risk assessment combined with hydrodynamic modelling to estimate the public health risk associated with bathing after rainfall events. *Science of the Total Environment*, *548*, 270–279.

Fan, Y., Zheng, J., Deng, L., Rao, W., Zhang, Q., Liu, T., & Qian, X. (2022). Spatiotemporal dynamics of microplastics in an urban river network area. *Water Research*, *212*, 118116.

Fetzner, J. W., & Taylor, C. A. (2018). Two new species of freshwater crayfish of the genus *Faxonius* (Decapoda:Cambaridae) from the Ozark Highlands of Arkansas and Missouri. *Zootaxa*, *4*, 491–520.

Fluid Imaging Technologies Inc. (2011). *FlowCAM manual* (3rd ed.). Yarmouth, Maine, USA.

Gaufin, A. R., & Tarzweu, C. M. (1955). Environmental changes in a polluted stream during winter. *The American Midland Naturalist*, *54*, 78–88.

Geatches, T., Gething, J., & Rutt, G. (2014). *Sewage fungus' a field and microscopic guide*. Version 3, https://www.Charvalley.org.

Gonzalo, C., & Camargo, J. A. (2013). The impact of an industrial effluent on the water quality, submersed macrophytes and benthic macroinvertebrates in a dammed river of Central Spain. *Chemosphere*, *93*, 1117–1124.

Gray, N. F. (1985). Heterotrophic smiles in flowing waters. *Biological Reviews*, *60*, 499–548.

Haggard, B. E., Stanley, E. H., & Storm, D. E. (2005). Nutrient retention in a point-source-enriched stream. *Journal of the North American Benthological Society*, *24*, 29–47.

Hilario Garcia, A. L., Matzenbacher, C. A., Santos, M. S., Prado, L., Picada, J. N., Premoli, S. M., Correa, D. S., Niekraszewicz, L., Dias, J. F., Grivicich, I., & da Silva, J. (2017). Genotoxicity induced by water and sediment samples from a river under the influence of brewery effluent. *Chemosphere*, *169*, 239–248.

Hulsen, T., Barry, E. M., Lu, Y., Puyol, D., & Batstone, J. (2016). Low temperature treatment of domestic wastewater by purple phototrophic bacteria: Performance, activity, and community. *Water Research*, *100*, 537–545.

Hulshoff Pol, L. W., Lens, P. N. L., Stams, A. J. M., & Lettinga, G. (1998). Anaerobic treatment of sulphate-rich wastewaters. *Biodegradation*, *9*, 213–224.

Kaleli, H. A., & Islam, M. R. (1997). Effect of temperature on the growth of wastewater bacteria. *Toxicological & Environmental Chemistry*, *59*, 111–123.

Kator, H., & Rhodes, M. (2003). Handbook of water and wastewater microbiology.

Koelmans, A. A., Bakir, A. G., Burton, A., & Janssen, C. R. (2016). Microplastic as a vector for chemicals in the aquatic environment: Critical review and model-supported reinterpretation of empirical studies. *Environmental Science & Technology*, *50*, 3315–3326.

Luo, Y., Guo, W., Ngo, H. H., Nghiem, L. D., Hai, F. I., Zhang, J., Liang, S., & Wang, X. C. (2014). A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Science of the Total and Environment*, *473*, 619–641.

Marti, E., Aumatell, J., Gode, L., Poch, M., & Sabater, F. (2004). Nutrient retention efficiency in stream receiving inputs from waste water treatment plants. *Journal of Environmental Quality*, *33*, 285–293.

Mor, J.-R., Dolédec, S., Acuña, V., Sabater, S., & Muñoz, I. (2019). Invertebrate community responses to urban wastewater effluent pollution under different hydro-morphological conditions. *Environmental Pollution*, *252*, 483–492.

Oksanen, J. (2020). *Vegan: Community ecology package*. R package version 2. http://cran.r-project.org/package=vega

Oliveira, M. A., & Goulder, R. (2006). The effects of sewage-treatment-works effluent on epilithic bacterial and algal communities of three streams in northern England. *Hydrobiologia*, *568*, 29–42.

Palmer-Felgate, E. J., Mortimer, R. J. G., Krom, M. D., & Jarvie, H. P. (2010). Impact of point-source pollution on phosphorus and nitrogen cycling in stream-bed sediments. *Environmental Science and Technology*, *44*, 908–914.

Passell, H. D., Dahm, C. N., & Bedrick, E. J. (2007). Ammonia modelling for assessing potential toxicity to fish species in the Rio Grande 1989-2002. *Ecological Applications*, *17*, 2087–2099.

Poulton, N. J. (2016). FlowCam: Quantification and classification of phytoplankton by imaging flow cytometry. *Methods in Molecular Biology*, *1389*, 237–247. https://doi.org/10.1007/978-1-4939-3302-0_17

Riechel, M., Matzinger, A., Pawlowsky-Reusing, E., Sonnenberg, H., Uldack, M., Heinzmann, B., Caradot, N., von Seggern, D., & Rouault, P. (2016). Impacts of combined sewer overflows on a large urban river—Understanding the effect of different management strategies. *Water Research*, *105*, 264–273.

Robertson, L. J., Hermansen, L., & Gjerde, B. K. (2006). Occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in sewage in Norway. *Applied and Environmental Microbiology*, *72*, 5297–5303.

Silva, A. J., Varesche, M. B., Foresti, E., & Zaiat, M. (2002). Sulphate removal from industrial wastewater using a packed-bed anaerobic reactor. *Process Biochemistry*, *37*, 927–935.

Smith, L., & Kramer, R. (1963). Survival of walleye eggs in relationship to wood fibres and Sphaerotilus natans in the Rainy River, Minnesota. *Transactions of the American Fisheries Society*, *92*, 220–234.

Tickner, D., Opperman, J. J., Abell, R., Acreman, M., Arthington, A. H., Bunn, S. E., Cooke, S. J., Dalton, J., Darwall, W., Edwards, G., Harrison, I., Hughes, K., Jones, T., Leclère, D., Lynch, A. J., Leonard, P., McClain, M. E., Muruven, D., Olden, J. D., … Young, L. (2020). Bending the curve of global freshwater biodiversity loss: An emergency recovery plan. *Bioscience*, *70*, 330–342.

Turner, R., Rabalais, N., Justic', D., & Dortch, Q. (2003). Global patterns of dissolved N, P and Si in large rivers. *Biogeochemistry*, *64*, 297–317.

Whelan, M. J., Linstead, C., Worrall, F., Ormerodd, S. J., Durance, I., Johnson, A. C., Johnson, D., Owen, M., Wiik, E., Howden, N. J. K., Burt, T. P., Boxall, A., Brown, C. D., Oliver, D. M., & Ticknerb, D. (2022). Is water quality in British rivers "better than at any time since the end of the Industrial Revolution"? *Science of the Total Environment*, *843*, 157014.

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. Springer-Verlag. ISBN 978-3-319-24277-4.

Willoughby, L. G., & Roberts, R. J. (1991). Occurrence of the sewage fungus *Leptomitus lacteus*, a necrotroph on perch (*Perca fluviatilis*) in Windermere. *Mycological Research*, *95*, 755–758.

Worrall, F., Howden, N. J., Burt, T. P., & Bartlett, R. (2019). The importance of sewage effluent discharge in the export of dissolved organic carbon from UK rivers. *Hydrological Processes*, *33*, 1851–1864.

Zafar, S., Kahan, A., Ullah, H., Khan, M. S., Khan, I., Hammed, A., Rehman, S. U., & Yasmeen, G. (2017). Risk assessment of Giardia from a full scale MBR sewage treatment plant caused by membrane integrity failure. *Journal of Environmental Sciences*, *30*, 252–258.

Zhang, H., Feng, J., Chen, S., Li, B., Sekar, R., Zhao, Z., Jia, J., Wang, Y., & Kang, P. (2018). Disentangling the drivers of diversity and distribution of fungal community composition in wastewater treatment plants across spatial scales. *Frontiers in Microbiology*, *18*(9), 1291.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Methods used to analyse water chemistry samples.

Table S2. Results of the generalised linear model: presence or absence of fungus between control and upstream area, divided by sampling months.

Table S3. Results of the SIMPER analysis to test which environmental parameter has more influence on the presence of fungus. The analysis is divided by month.

Figure S1. Sewage fungus abundance in upstream and control rivers.

Figure S2. NMDS divided by chemical and physical parameters, for the different sampling sites and months. Upstream samples are in blue, downstream in yellow and final effluent in green. Stress values are indicated to show the good fit of the model.

Figure S3. Relationship between the most influential physical and chemical parameters identified by the SIMPER analysis, on the abundance of sewage fungus filaments.

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