

# Dynamics of Freshwater Microbial Communities in Two Different Rivers Exposed to Sewage Effluent in Novel In Situ Mesocosms

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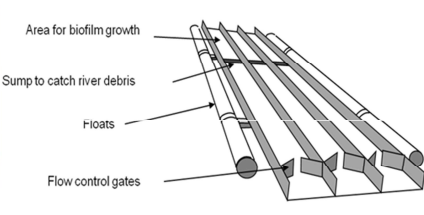
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## Background

Wastewater Treatment Plant (WWTP) discharge has been a well-known cause of eutrophication in many rivers, and has prompted widespread introduction of tertiary treatment (Wakelin, *et al.*, 2008, Gücker, *et al.*, 2006). It is also a well known source of pharmaceutical and other chemicals substances, and of bacteria that have either originated in the WWTP or have passed through the system. In large rivers, dilution, photodegradation and biotransformation remove some of that contamination (Fono, *et al.*, 2006), but decreased flow scenarios and higher discharge rates might change the balance. In addition, bacterial communities exposed to sewage sludge have been shown to have a higher prevalence and abundance of class 1 integrons, genetic elements that carry antibiotic resistance genes (Gaze, *et al.* 2011). The impact of this on riverine microbial systems remains under-researched.



Fig. 1: Construction details of mesocosm and placement in the River



## Materials & Methods

### Experiment 1 - Laboratory Microcosms:

- Two periphyton biofilm communities grown in the 'pristine' River Lambourn (ML) in Boxford, and the sewage effluent-impacted River Kennet (MK) in Mildenhall, UK, were transferred to 16 microcosms (2 ml) containing autoclaved water from the Lambourn augmented with organic matter retrieved from the river.
- Sewage Effluent (SE) was added to four of the microcosms containing replicates of ML and to four of those containing MK, adjusting the total SE volume to 25%.
- The communities were left to grow for four days in the dark at 17°C.
- The community DNA from each microcosm was extracted, amplified, profiled by tRFLP and processed in Genemarker.



### Experiment 2 - In-situ Mesocosms:

- Novel in-river mesocosms (Fig. 1) that allow for the replication and manipulation of river conditions were used to investigate the effect of Sewage effluent (SE) on periphyton biofilm in two UK chalk rivers, one relatively pristine (River Lambourn, Boxford, UK), the other impacted by pollution (River Kennet, Mildenhall, UK).
- Sewage effluent was dripped continuously into four of the channels to maintain  $\geq 2.5$  ppm.
- Periphyton biofilms were grown for 9 days on limestone tiles in 4 SE and 4 control channels. On day 9 tiles were taken from all replicate channels.
- DNA from all tiles in both experiments was extracted, amplified, sequenced on the 454 Titanium platform (Roche), processed in Qiime on CloVR and divided into bacterial and eukaryote communities.
- The samples were analyzed for Class I integron prevalence to investigate sewage-impact on a molecular level

### Microbial Community Analysis:

- Hierarchical clustering was used to investigate patterns in the data. The data is clustered by group averages calculated from a distance matrix based on Bray Curtis similarities.
- ANOSIM (Analysis of Similarity) was used to provide a multivariate test statistic. ANOSIM analyzes differences in multivariate community ecology data, comparing dissimilarity matrices. It is not appropriate for analyzing beta-diversity itself, but it can be used to analyze beta-diversity variation between sites (Legendre 2005), as is the case in this study. The ANOSIM statistic is useful because it does not only provide a p-value to show that differences are significant, it also provides the R-statistic, which on a scale of 0 to 1 shows increasing differences in community composition.

### Naming Conventions used in Fig. 2 & 3:

Community	Abbreviation	Treatment	Abbreviation	Replicate/treatment	Abbreviation
Microcosm/Lambourn	ML	Sewage Effluent	S	Up to four	1,2,3,4
Microcosm/Kennet	MK	Control	C		
Kennet	K				
Lambourn	L				

## Results

- The Kennet microcosms showed no significant difference between communities exposed to sewage effluent (SE) and the controls (Fig.2, Table 1)
- The Lambourn microcosm results show that the sewage-impacted community is very different from the control community. The samples cluster neatly (Fig 2), the R-value is high and the result is significant (Table 1).
- The Lambourn and Kennet mesocosm results show distinctly different communities per river, but no clustering patterns (Fig. 3) or significant community differences (Table 1) between the SE-impacted biofilms and controls.
- Class I Integron prevalence in the communities exposed to SE is greater than in the control channels (Fig.4), and is generally much higher in the Kennet than in the Lambourn.

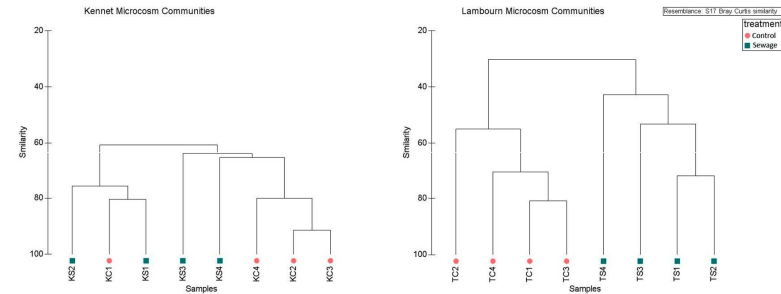


Fig. 2. Clustering patterns of Kennet & Lambourn Microcosm communities, with and without SE exposure.

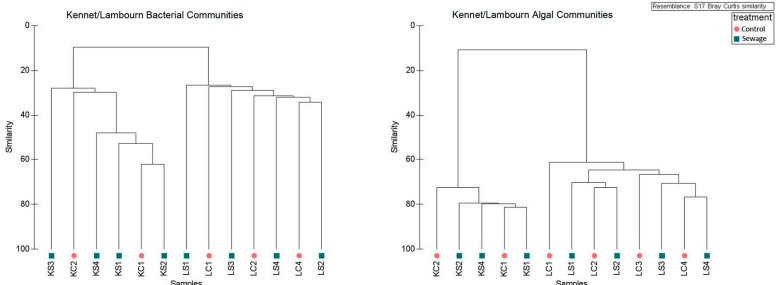


Fig. 3. Kennet & Lambourn Macrocosm samples showing distinctly different per river, but not per treatment

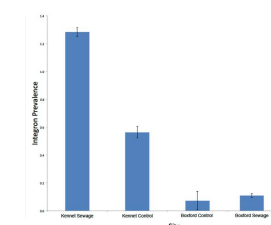


Fig. 4: Class I Integron prevalence in Kennet and Lambourn Mesocosms

### Anosim results:

	R-statistic	p-value
Microcosm/Lambourn	0.969	0.029
Microcosm/Kennet	0.333	0.11
Kennet/ Lambourn	1	0.003
Kennet/ treatments	not significant	
Lambourn/ treatments	not significant	

Table 1: ANOSIM values for the Microcosm and Mesocosm experiments. Higher R-values show bigger community differences

- In the microcosm experiment, the Lambourn community, grown in near-pristine water, was much more impacted by SE-addition than the Kennet community. This is likely due to the fact that the Kennet communities were adjusted to high levels of SE already. It is therefore also not surprising that the mesocosm study in the Kennet did not show significant differences in community composition between SE-addition and control channels.
- The Mesocosm Lambourn community was not changed by the presence of SE. Bacterial seeding from upstream is the likely cause of resilience in the open mesocosm system.
- Higher Class I integron prevalence in both the Kennet and Lambourn SE-addition channels show that the sewage addition has had an impact if not on the community level, then at a molecular level. The higher level of integron prevalence in the Kennet in general marks it out as the more sewage-impacted river (Gaze et al 2011).

## Conclusion

Open freshwater systems show some resilience to the impact of sewage effluent on a species level. Even in systems in which changes to the microbial system are masked by bacterial seeding from upstream, the impact of sewage can be shown by a higher prevalence and abundance of class I integrons. This change, which brings about greater antibiotic resistance – and all the problems this entails - should not be ignored. Heavily sewage impacted rivers like the Kennet might have a microbial system that has already adjusted to continuous heightened SE input.

Future work will investigate the effect of different tertiary treatment options on class I integron prevalence in effluent, and the limits of resilience of riverine biofilms.

### Acknowledgements

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