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NATURAL ENVIRONMENT RESEARCH COUNCIL

Effects of an Influenza Pandemic: A Freshwater Microbiology Story

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Background

Exposure to pharmaceutical pollution originating from sewage effluent is an unavoidable component of freshwater microbial life. Despite this 'home truth', the extent to which these pollutants influence the composition and function of freshwater microbial systems remains unclear. Tamiflu is an antiviral with unprecedented projected use patterns during an influenza pandemic (Singer et al. 2008; Singer et al. 2011). The impact this pulse of bioactive drug will have on the resilience of freshwater microbial communities remains unexplored.

Fig. 1. Mesocosm situated in the River Lambourn.

Materials & Methods

Experiment 1 – Laboratory Microcosms:

- Two periphyton biofilm communities grown in the River Lambourn in Boxford, UK, were transferred to 16 microcosms (2 ml) containing autoclaved water from the Lambourn augmented with organics.
- Tamiflu was added to 4 of the microcosms containing "Community 1" & 2 at a concentration of 1000 ng/L.
- The communities were grown for 4 days in the dark at 17 C after which DNA was extracted, amplified and profiled by tRFLP.

Experiment 2 - River Mesocosms:

- Novel in-river mesocosms (Fig 1) that allow for the replication and manipulation of river conditions were used to investigate the effect of Tamiflu on periphyton biofilm in a relatively pristine UK chalk river (River Lambourn, Boxford, UK).
- Tamiflu was dripped continuously into four of the channels to maintain a minimum concentration of 1 ug/L.
- Periphyton biofilms were grown for 9 days on limestone tiles. On day 5, 7 & 9, tiles were taken from all eight experimental channels.
- DNA from all tiles was extracted, amplified and sequenced on the 454 Titanium platform (Roche) and preprocessed in Qiime on CloVR and divided into eukaryote and bacterial communities.

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. The ANOSIM statistic provides a p-value to show that differences are significant, and the R-statistic, which on a scale of 0 to 1 shows increasing differences in community composition.

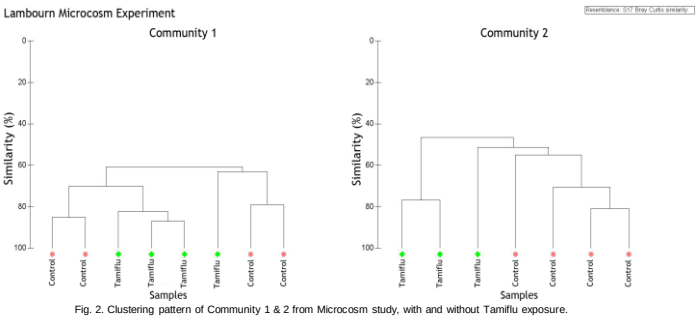
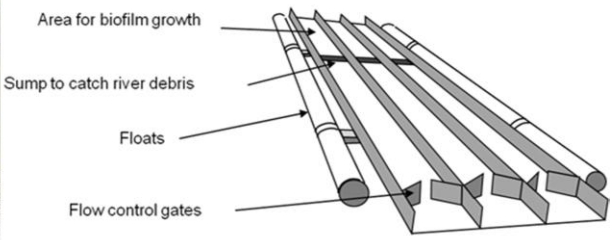


Fig. 2. Clustering pattern of Community 1 & 2 from Microcosm study, with and without Tamiflu exposure.

Results

- In the microcosm study (Fig. 2), the microbial communities exposed to Tamiflu were significantly changed when compared to the unexposed controls (ANOSIM R-Statistic 0.64 and 0.70 respectively; p-values 0.029)
- The mesocosm study showed that statistically significant overall community shifts can be observed from timepoint to timepoint (Fig. 3), but not between treatments.
- A comparison of dominance curves (DOMDIS analysis) for all samples across all time points shows that the abundance patterns of the Tamiflu replicates in the mesocosm study differ from the controls (Fig. 4; ANOSIM R=0.142, p=0.06).



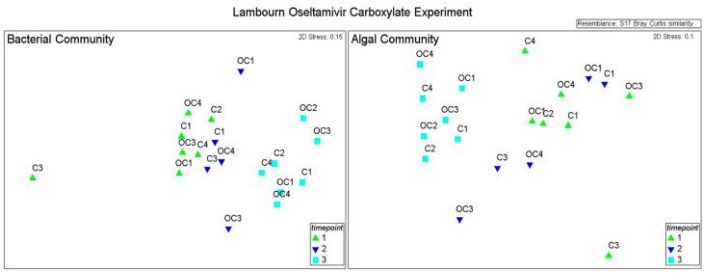


Fig. 3. NMDS of bacterial and algal communities from Mesocosm study, with and without Tamiflu exposure, at three different time points.

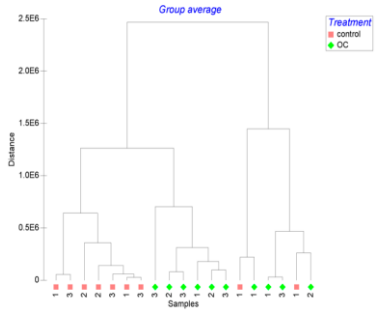


Fig. 4. Dominance curves (DOMDIS analysis) for all samples across all time points in mesocosm study.

Conclusions

The results suggest that an open freshwater system might be more resilient to change as compared with the closed laboratory microcosm environment. The lack of immigration (ie. re-seeding events) in the laboratory system might be best able to explain the discrepancy between the two studies. Despite the realism provided by the in-river mesocosms, the batch microcosm studies might be more indicative of acute ecotoxicity owing to the fact that the unit of measurement, i.e. the bacterial community, is not masked by bacterial immigration. In conclusion, the absence of suitable immigration to buffer the effects of Tamiflu might lead to a less well functioning downstream freshwater ecosystem, which could be a problem during an influenza pandemic.

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