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qPCR (quantitative polymerase chain reaction) for the quantification of bacteriophages in stream water samples to investigate hydrological processes: a proof-of-concept study in the Huewelerbach experimental catchment (Luxembourg)

Marta Antonelli (1), Mukundh Narayanan Balasubramanian (2), Leslie Ogorzaly (1), and Laurent Pfister (1)

(1) Luxembourg Institute of Science and Technology (LIST) 5, avenue des Hauts-Fourneaux L-4362 Esch/Alzette , (2) Biosistemika Ltd. Pot za Brdom 102, 1000 Ljubljana, Slovenia

Albeit recent technological developments (e.g. field deployable instruments operating at high temporal frequencies), experimental hydrology is a discipline that remains measurement limited. From this perspective, trans-disciplinary approaches may create valuable opportunities to enlarge the amount of tools available for investigating hydrological processes.
Bacteriophages have been widely used in hydrology as biological tracer for investigating colloid transport and contamination of ground water systems. However, there are only a few studies focusing on the employability of bacteriophages as surface water tracers (i.e. phage transport, system functioning).
Here, we present a proof-of-concept study carried out in the Huewelerbach catchment in Luxembourg in December 2015. The aim of this study was to investigate how viral particles can be used to detect hydrological connectivity between the riparian zone/river bank and the stream during rainfall events. Moreover, this study is one of the first attempts for applying the qPCR (quantitative polymerase chain reaction) technique for the quantification of bacteriophages in stream water samples to investigate hydrological processes. This technique is very sensitive and has a large dynamic range - enhancing ease and speed of phage detection.
We used two different male-specific coliphages (GA phage, genogroup II and SP phage, genogroup IV). Two litres of GA phage were injected directly in the stream as a slug injection and two litres of SP phage were poured next to the river bank (alluvial deposition) close to the injection point. We also added NaCl (200 g) to both phage suspensions. We collected stream water samples 100 m and 500 m downstream (i.e. catchment outlet) of the injection point for one week. Phages were concentrated through ultracentrifugation of 100 ml of water sample followed by quantification via qPCR. Conductivity in stream water was monitored for the entire duration of the experiment. Discharge was monitored both immediately upstream of the injection point and at the catchment outlet.
Preliminary results show that at the catchment outlet, the GA-phage injected in stream displayed almost complete mass recovery ($\sim$93 %), in contrast to the partial recovery ($\sim$12%) of the SP phage that was introduced on the river bank. Additionally, the amount of GA phages detected 100 m downstream of the injection point evolved back to its background level after six days. We could not observe a similar evolution for the SP phage. At the outlet, the amount of both phages did not return to background levels after six days. This can be due to a combined action of the occurrence of preferential flowpaths and the behaviour of colloids. During the monitored rain event we observed a dilution effect on both phages and a slight increase of the quantity of SP phage right after the peak of discharge. This finding suggests a release of viral particles from the river bank. Overall, we have demonstrated with this proof-of-concept study the value of phages as eco-hydrological tracer.