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Protocol for quantitative tracing of surface water with synthetic DNA

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Based on experiments we carried out in 2010 with various synthetic single stranded DNA markers with a size of 80 nucleotides (ssDNA; Foppen et al., 2011), we concluded that ssDNA can be used to carry out spatially distributed multi-tracer experiments in the environment. Main advantages are in principle unlimited amount of tracers, environmental friendly and tracer recovery at very high dilution rates (detection limit is very low). However, when ssDNA was injected in headwater streams, we found that at selected downstream locations, the total mass recovery was less than 100%. The exact reason for low mass recovery was unknown. In order to start identifying the cause of the loss of mass in these surface waters, and to increase our knowledge of the behaviour of synthetic ssDNA in the environment, we examined the effect of laboratory and field protocols working with artificial DNA by performing numerous batch experiments. Then, we carried out several field tests in different headwater streams in the Netherlands and in Luxembourg.
The laboratory experiments consisted of a batch of water in a vessel with in the order of 10^{10} ssDNA molecules injected into the batch. The total duration of each experiment was 10 hour, and, at regular time intervals, 100 μl samples were collected in a 1.5 ml Eppendorf vial for qPCR analyses. The waters we used ranged from milliQ water to river water with an Electrical Conductivity of around 400 μS/cm. The batch experiments were performed in different vessel types: polyethylene bottles, polypropylene copolymer bottles , and glass bottles. In addition, two filter types were tested: 1 μm pore size glass fibre filters and 0.2 μm pore size cellulose acetate filters. Lastly, stream bed sediment was added to the batch experiments to quantify interaction of the DNA with sediment.
For each field experiment around 10^{15} ssDNA molecules were injected, and water samples were collected 100 – 600 m downstream of the point of injection. Additionally, the field tests were performed with salt and deuterium as tracer. To study possible decay by sunlight and/or microbial activity for synthetic DNA, immediately in the field and for the duration of the entire experiment, we carried out batch experiments. All samples were stored in a 1.5 ml Eppendorf vial in a cool-box in dry ice (-80°C). Quantitative PCR on a Mini Opticon (Bio Rad, Hercules, CA, USA) was carried out to determine DNA concentrations in the samples.
Results showed the importance of a strict protocol for working with ssDNA as a tracer for quantitative tracing, since ssDNA interacts with surface areas of glass and plastic, depending on water quality and ionic strength. Interaction with the sediment and decay due to sunlight and/or microbial activity was negligible in most cases. The ssDNA protocol was then tested in natural streams. Promising results were obtained using ssDNA as quantitative tracer. The breakthrough curves using ssDNA were similar to the ones of salt or deuterium. We will present the revised protocol to use ssDNA for multi-tracing experiments in natural streams and discuss the opportunities and limitations.