



## Microbial source tracking and transfer hydrodynamics in rural catchments.

Sinead Murphy (1), Niamh Bhreathnach (1), Vincent O'Flaherty (1), Philip Jordan (2), and Stefan Wuertz (3)

(1) Microbial Ecology Lab., Dept of Microbiology & ECI, NUI Galway, Ireland (s.murphy41@nuigalway.ie), (2) Agricultural Catchments Programme, Johnstown Castle, Teagasc, Wexford, Ireland., (3) Department of Civil and Environmental Engineering, University of California, Davis.

In Ireland, bacterial pathogens from continual point source pollution and intermittent pollution from diffuse sources can impact both drinking water supplies and recreational waters. This poses a serious public health threat. Observing and establishing the source of faecal pollution is imperative for the protection of water quality and human health. Traditional culture methods to detect such pollution via faecal indicator bacteria have been widely utilised but do not decipher the source of pollution. To combat this, microbial source tracking, an important emerging molecular tool, is applied to detect host-specific markers in faecally contaminated waters. The aim of this study is to target ruminant and human-specific faecal Bacteroidales and Bacteroides 16S rRNA genes within rural river catchments in Ireland and investigate hydrological transfer dependencies. During storm events and non-storm periods, 1L untreated water samples, taken every 2 hours over a 48-hour time period at the spring (Cregduff) or outlet (Dunleer), and large (5-20L) untreated water samples were collected from two catchment sites. Cregduff is a spring emergence under a grassland karst landscape in Co. Mayo (west coast of Ireland) and Dunleer is a mixed landuse over till soils in Co. Louth (east coast). From a risk assessment point of view, the catchments are very different. Samples were filtered through  $0.2\mu\text{m}$  nitrocellulose filters to concentrate bacterial cells which then underwent chemical extraction of total nucleic acids. Animal and human stool samples were also collected from the catchments to determine assay sensitivity and specificity following nucleic acid extraction. Aquifer response to seasonal events was assessed by monitoring coliforms and *E. coli* occurrence using the IDEXX Colisure® Quanti Tray®/2000 system in conjunction with chemical and hydrological parameters. Autoanalysers deployed at each catchment monitor multiple water parameters every 10 min such as phosphorus, nitrogen (nitrate), turbidity, conductivity and flow rate. InStat V 3.06 was used to determine correlations between chemical and microbial parameters ( $P < 0.05$  considered significant). There was a positive correlation between *E. coli* and phosphorus in Cregduff during rain events ( $p=0.040$ ) & significant correlation for a non-rain periods ( $<0.001$ ). There was a positive correlation between *E. coli* and turbidity in Dunleer during rain events ( $p=0.0008$ ) and in Cregduff during non-rain periods ( $p=0.0241$ ). The water samples from Dunleer have a higher concentration of phosphorus than in Cregduff. Host specific primers BacCow-UCD, BacHum-UCD, BacUni-UCD and BoBac were then assayed against both faecal and water extracts and quantified using PCR. BacUni-UCD, BacCow-UCD and BoBac detected faecal contamination in three of the four sample sites in Dunleer and BacHum-UCD detected faecal contamination in one of the sites. The concentrations of the BacUni-UCD qPCR assay were higher in the water samples taken from Dunleer outlet than those taken from Cregduff spring. BacCow-UCD and BacHum-UCD qPCR detected low and very low concentrations, respectively, in water from the Dunleer outlet. The concentrations can be seen changing over the hydrograph event. None of the host-specific assays detected pollution in Cregduff. From the results, it can be seen that Dunleer is more subject to contamination than Cregduff.