

# ***E. coli* interactions with stream colloids**

**Lisa Avery, Andy Vinten and Bill  
Crooks**



***Craigiebuckler, Aberdeen, AB15 8QH, UK***

## ***E. coli*, coliforms or O157?**

- ***E. coli* coliforms:**
- **Indicators of faecal contamination**
- **Regulation based on these**
- **Surrogate for pathogen behaviour**
- **O157 – because behaviour can differ!**



## ***E. Coli O157***

- **Main environmental reservoir: CATTLE (non-toxigenic) and other farm, domestic and wild animals.**
- **Food poisoning associated with undercooked beef, other foods including fruit, salad & vegetables and contaminated drinking water.**
- **Illness also associated with direct environmental contact e.g. recreational use of water, petting farms, camping.**



# What make serotype O157 different?

- ***E. coli* O157 produces toxins**
  - verocytotoxins
  - Shigella like toxins
- ***E. coli* O157 hangs on gut wall**
  - fimbrae
- **It has a low infective dose**
  - 10 to 50 cells cause symptom development
- **Cat III organism**



# Pathogen isolates from humans in Scotland, per 100,000 population (Reilly, 2002)

	North East Scotland	South West Scotland
<i>E.coli</i> O157	13.4	6.7
Campylobacter	153.6	48.1
Cryptosporidium	46.2	3.2
Salmonella	32.7	11



# What does *E. coli* O157 do to humans?

- Haemorrhagic colitis (bloody diarrhoea)
- Haemolytic-uraemic syndrome (HUS)
- Thrombotic thrombocytopenic purpura (TTP)



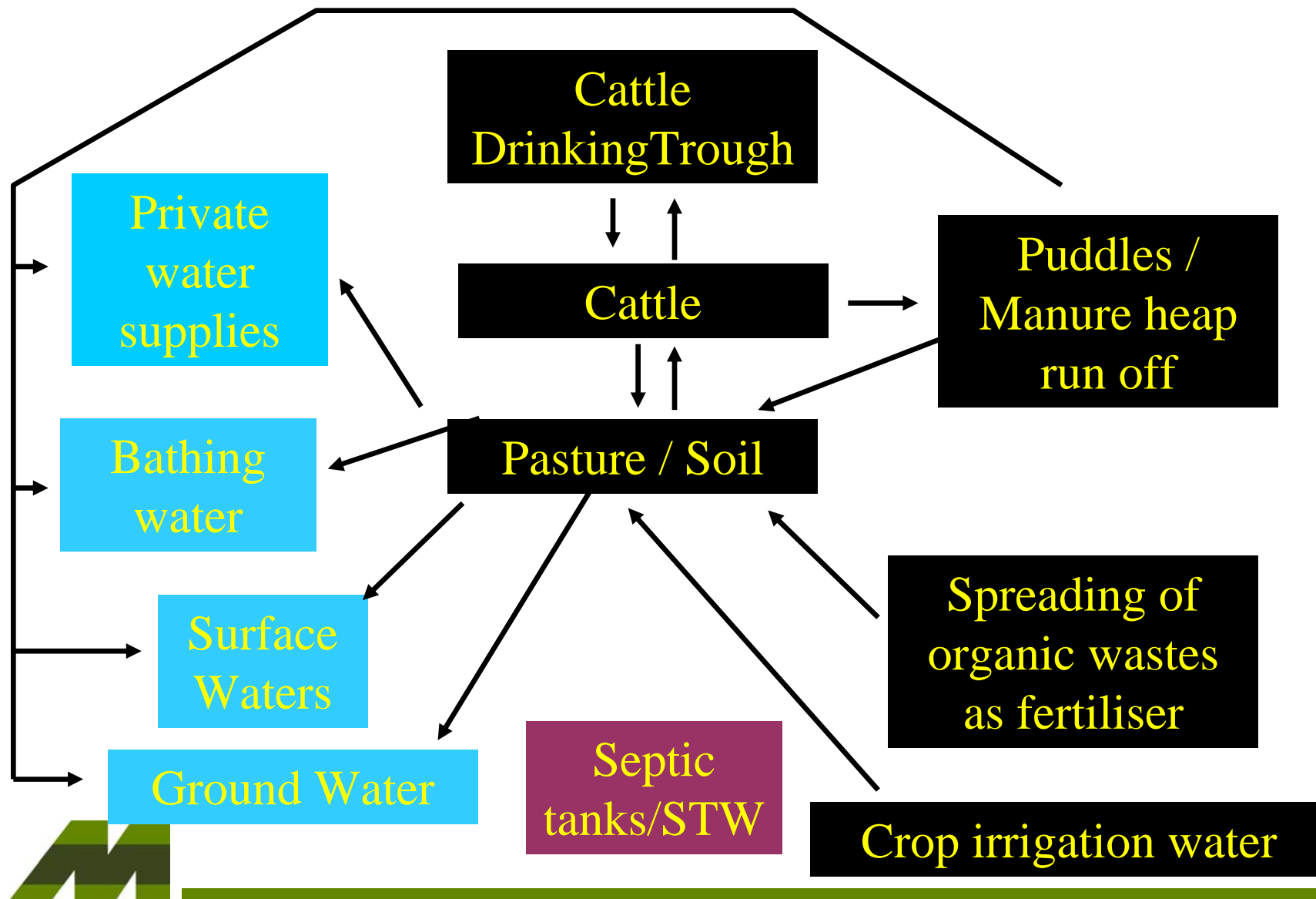
- \* 10% develop HUS
  - \* 70% require blood transfusions
  - \* 50% suffer kidney failure
  - \* 5% mortality rate
-

## ***E. coli* - an environmental problem...**

- **Non-compliance with EU microbiological standards for recreational bathing waters**
- **e.g. Ayrshire, SW Scotland**
- **Intensive dairy farming**
- **High livestock densities,**
- **Cool, moist summers**
- **Limited effective storage of livestock wastes on farms**
- **Steading areas vulnerable to direct loss by runoff**
  
- **Microbially contaminated water → streams (ultimately bathing water)**



# The ecological cycle of *E. coli* O157 in the farm environment



# The ruminant bioreactor

- A cow produces 50 litres of faeces a day
- Cow faeces contain  $10^7$  bacteria  $\text{ml}^{-1}$
- Cow faeces contain  $10^2$ - $10^5$  *E. coli* O157  $\text{ml}^{-1}$
- Human infective dose is 10-50 cells

Add in survival and transport, link to human exposure and determine risk



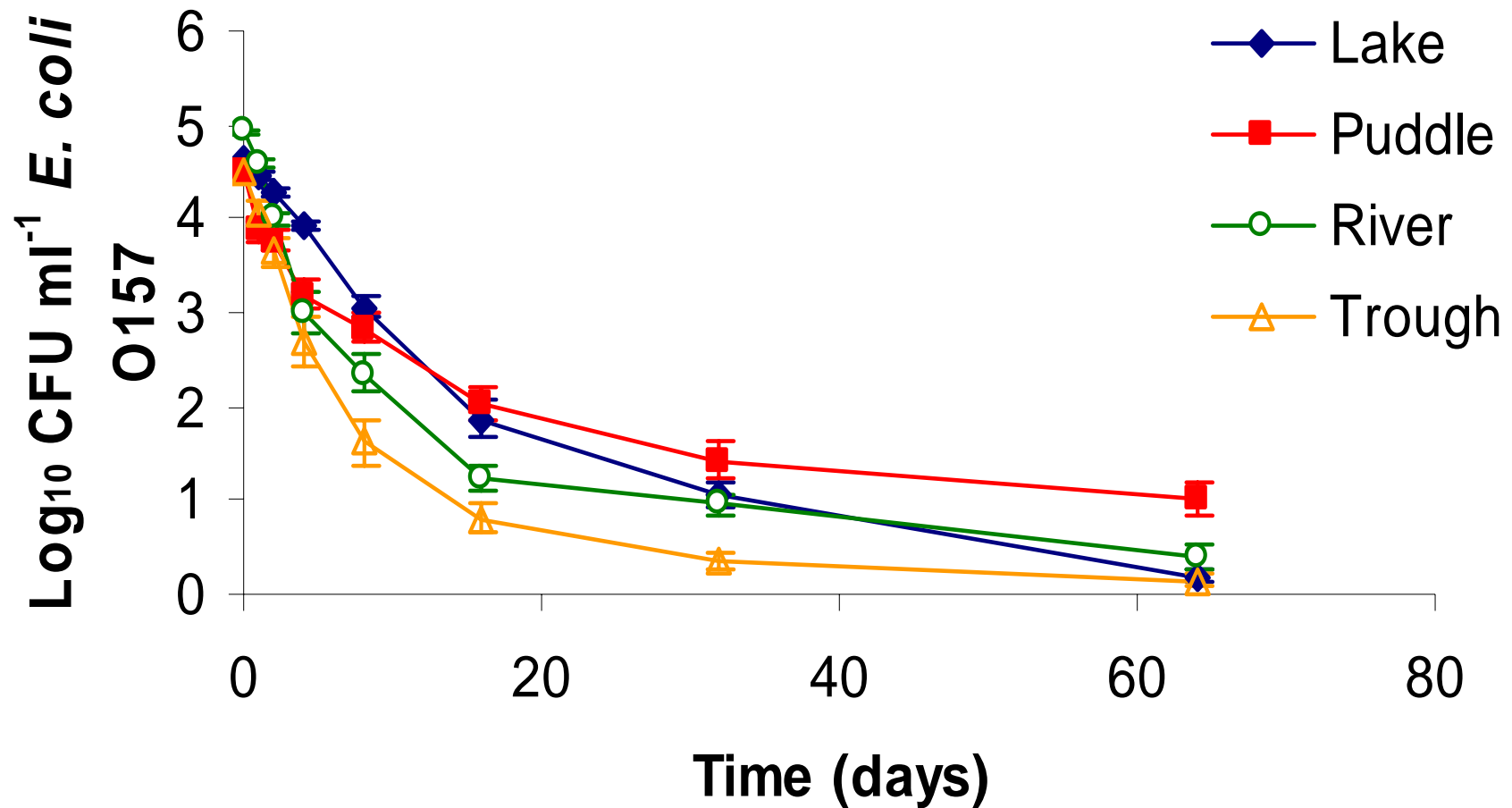
# Survival in Soils

- 99 days (Bolton *et al.*, 1999)
- Soil type
- Moisture (Artz *et al.*, 2003)
- Temperature
- Competing microflora/predation
- VBNC

Soil Properties	T <sub>99</sub> (d)
Clay loam	19.6
Silty loam	20
Silty clay loam	30
Peaty loam	35
Peaty loam	51.4
Peaty loam	>60



	Trough	River	Puddle	Lake
<b>T<sub>99</sub> value</b>	11.45	10.6	64	19.4
	11.35	10.4	32.05	18.2
	7.1	5.7	13.9	13.6
	6.15	5.5	12.05	12.8
	4.95	3.8	7.45	12.1
	3.9	3	6.5	9.2
	2.75	2.8	3.75	9.2
	2.3	*	2.5	9
<b>Mean ± SEM</b>	6.24 ± 1.26	5.97 ± 1.16	17.78 ± 7.38	12.49 ± 1.43
<b>One-way ANOVA</b>	Single factor effect of water type <i>P</i> = 0.034			



2-way ANOVA: Time x water type interaction  
( $P > 0.001$ )



## 0157 in water:

- ***E. coli* O157:H7** persisted for over 2 months in one faecally contaminated puddle water and one month in another.
- Range of survival times in other waters from 2-19 days.
- Significant effect of water type on survival.
- But no correlation with specific characteristics



# What we don't know

- **Significance of colloids on transport**
- **Significance of colloids – infectivity**
- **VBNC?**
- **Dynamics of colloid-E. coli interactions**
- **Where are the bugs??!**
- **Strain-dependent differences**
- **Experiments are based on inoculations**
- **Little real environmental data**
- **Poor understanding of survival mechanisms**



# Transport

- **Sources?**
- **What conditions lead to transport**
- **What conditions lead to sedimentation and die off?**
- **Microbial concentrations in streams greatest at high discharges**
- **Assumption - direct runoff is the main source.**
- **Resuspension of sediment?**
- **To what extent do colloid-bacterial interactions mediate transport processes?**



# *E.coli*:colloid interactions

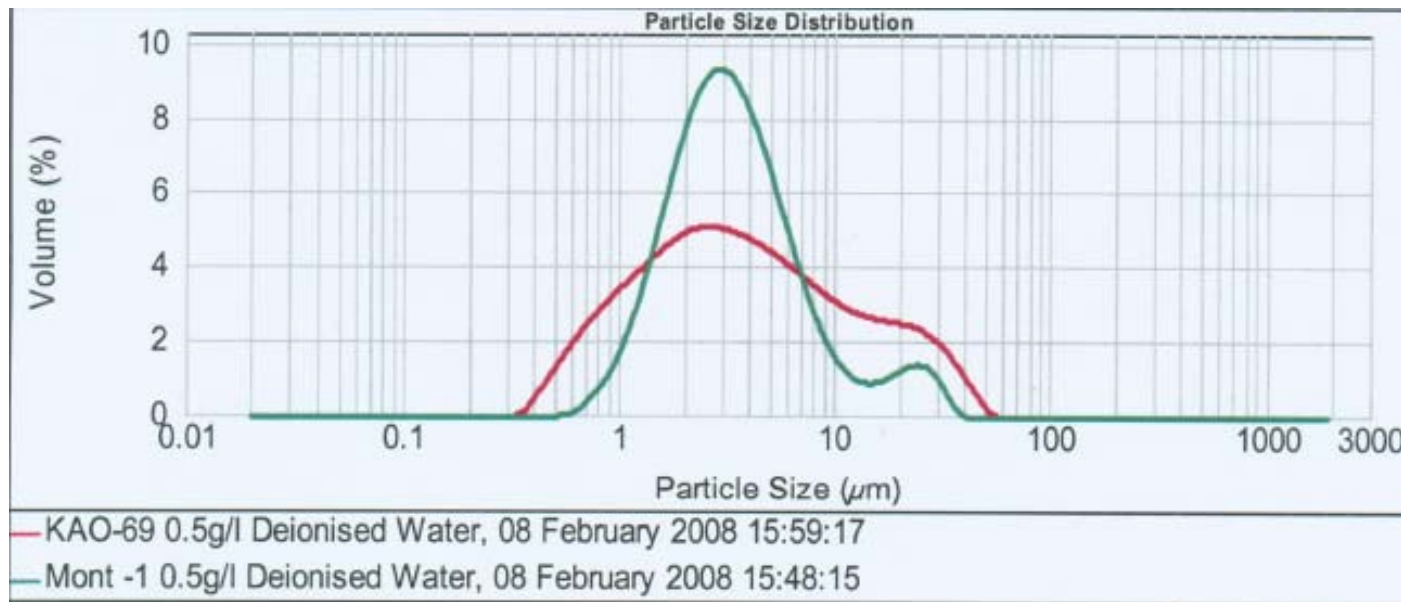
- **Aim:**

**To understand which factors influence bacterial binding to colloids and ultimately effects on transport linked to colloid types**



# Approach

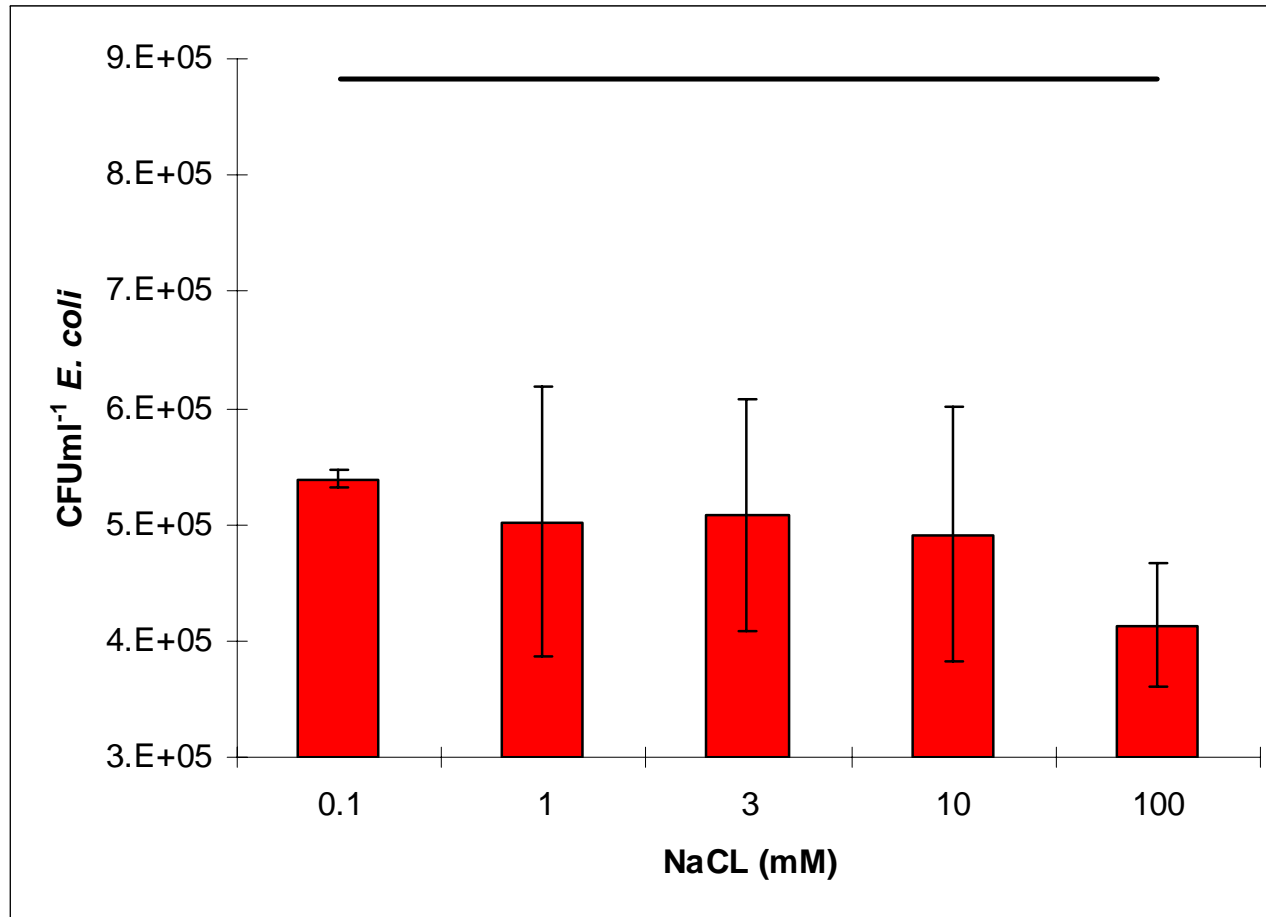
- “Model” colloids – Mont-1 and KaO- 69



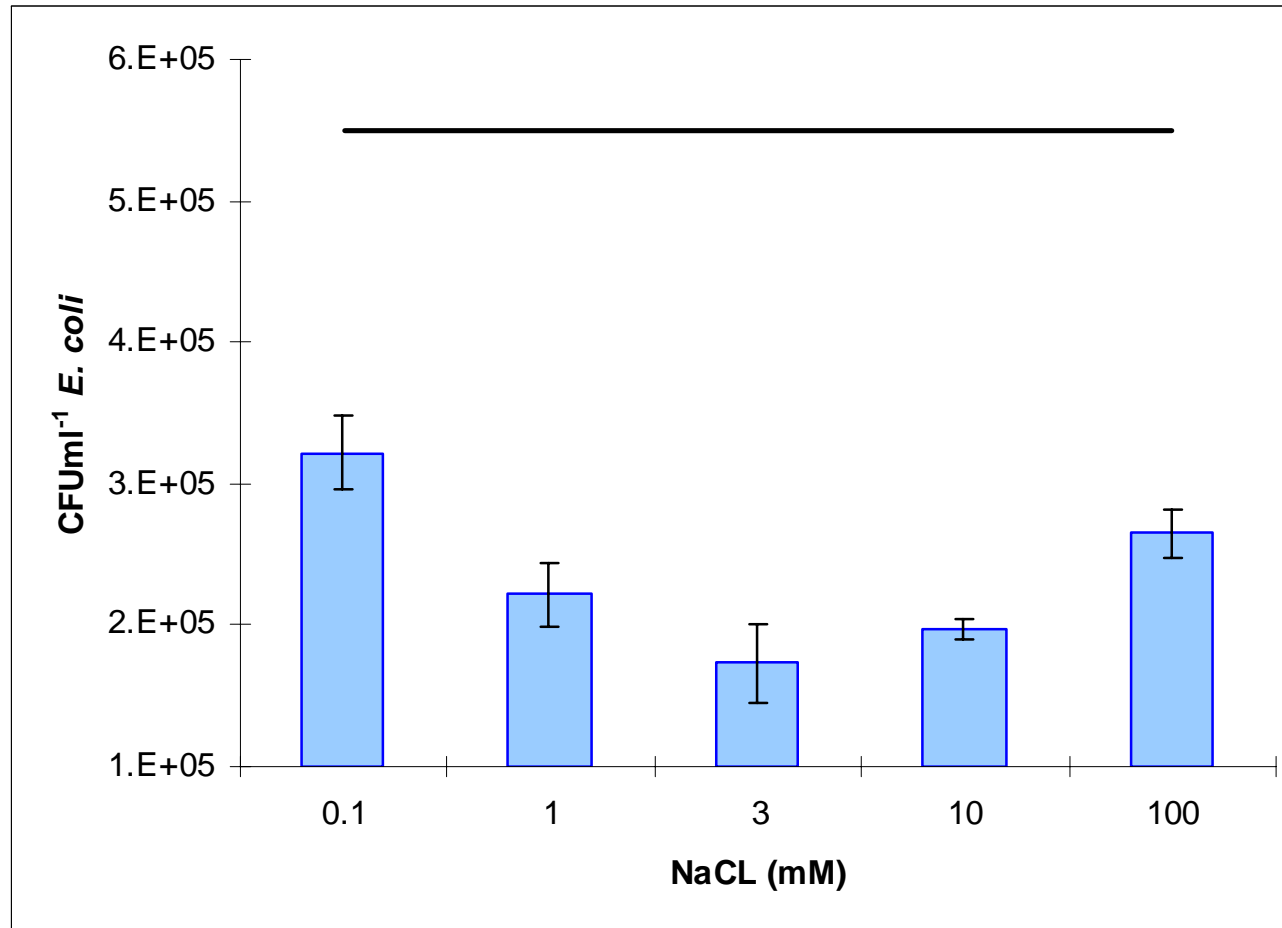
- Lab culture *E. coli*



# *E. coli* + Montmorillonite

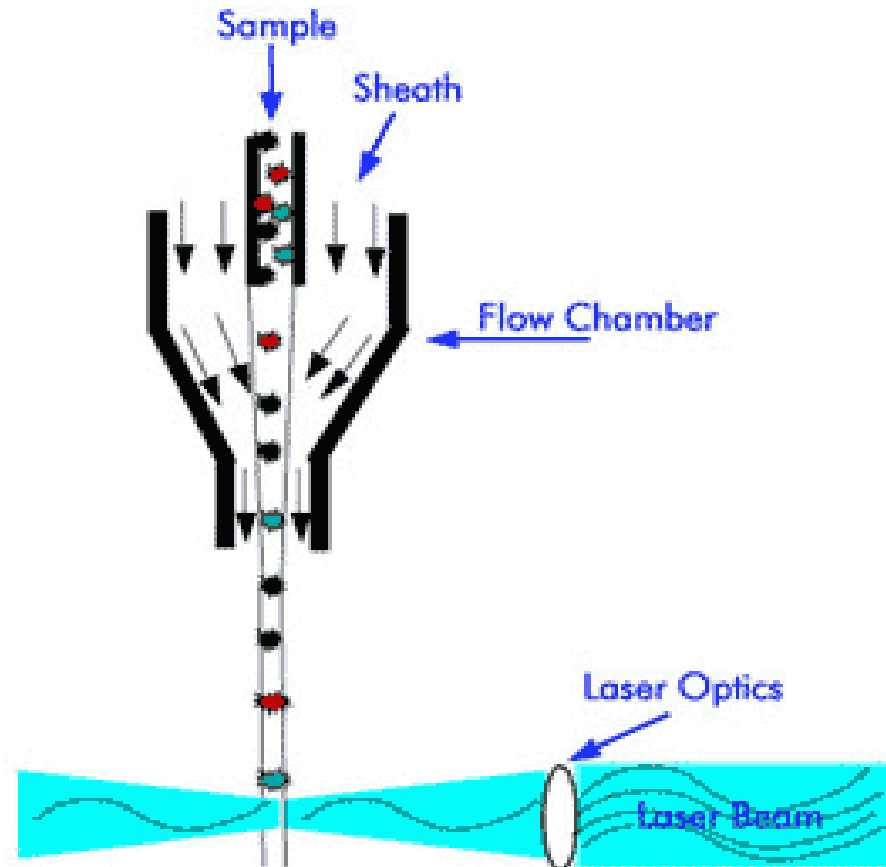


# *E. coli* + Kaolinite

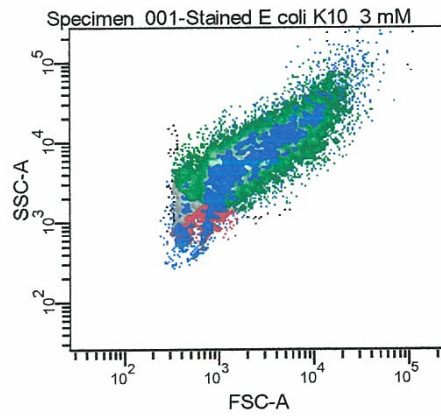
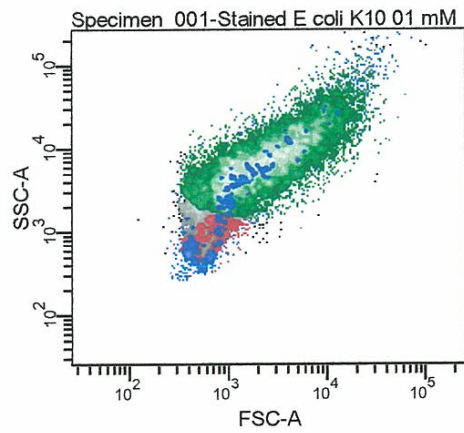
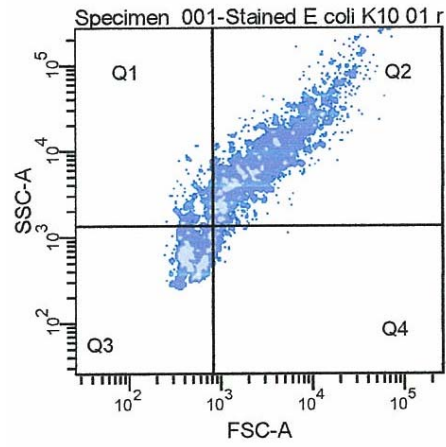
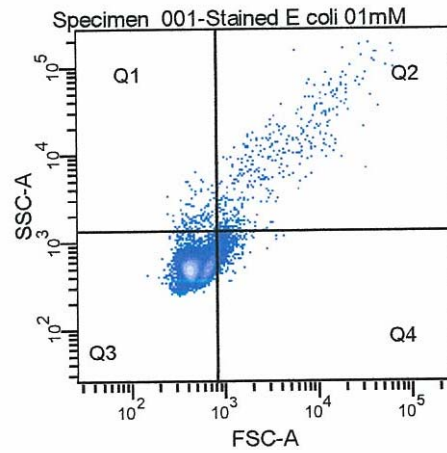


# Flow Cytometry

- Cells focussed into a buffer sheath
- Presented to a laser and light scatter patterns detected
- Staining



# Flow cytometry

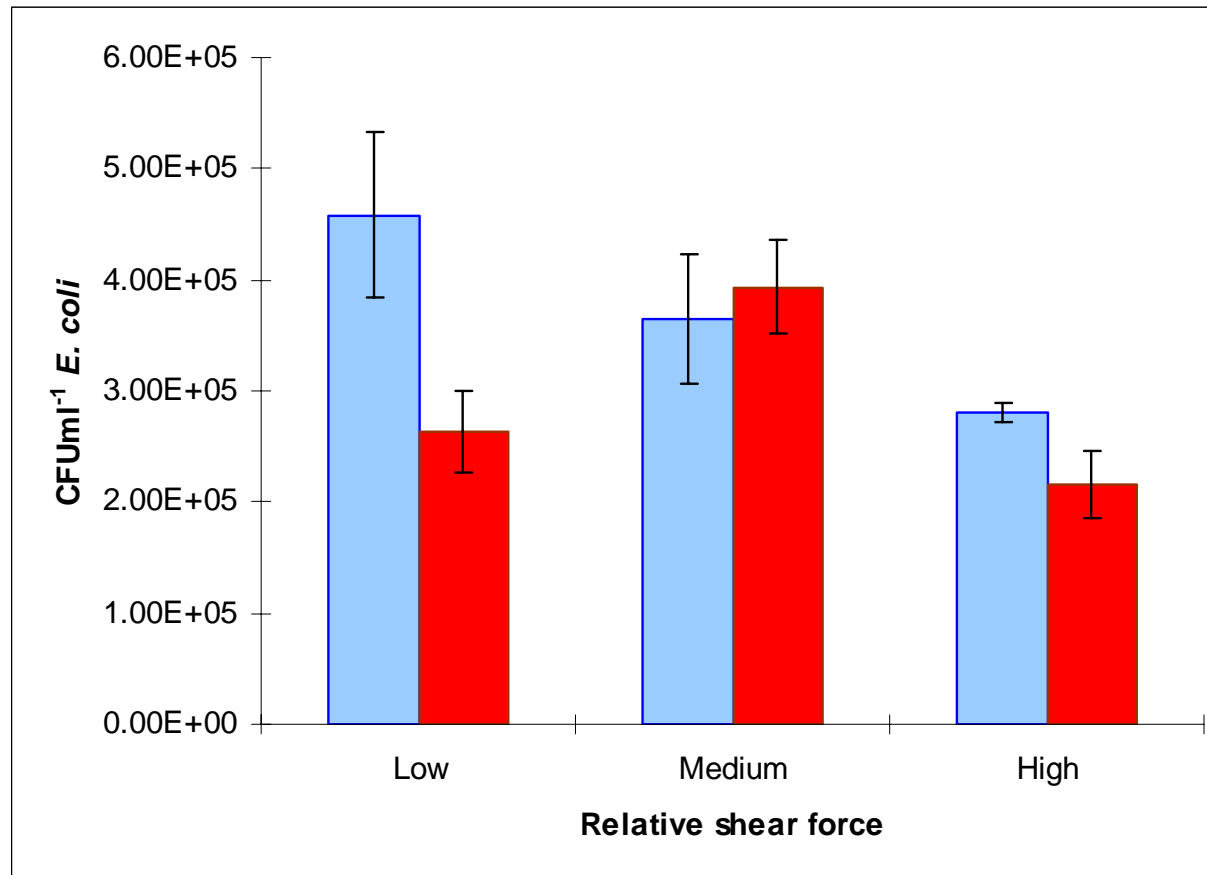


# Summary

- Flow cytometry shows that stained cell populations shift towards increased particle size and complexity upon addition of Kaolinite indicating aggregation with colloids.
- Shift towards larger more complex particles with increased salt concentration.
- With Montmorillonite – fewer free cells as NaCl concentration increased, with Kaolinite – fewer free cells as NaCl concentration increased from 0.1-3 mM, but less aggregation from 10-100mM
- Increasing shear forces led to more aggregation

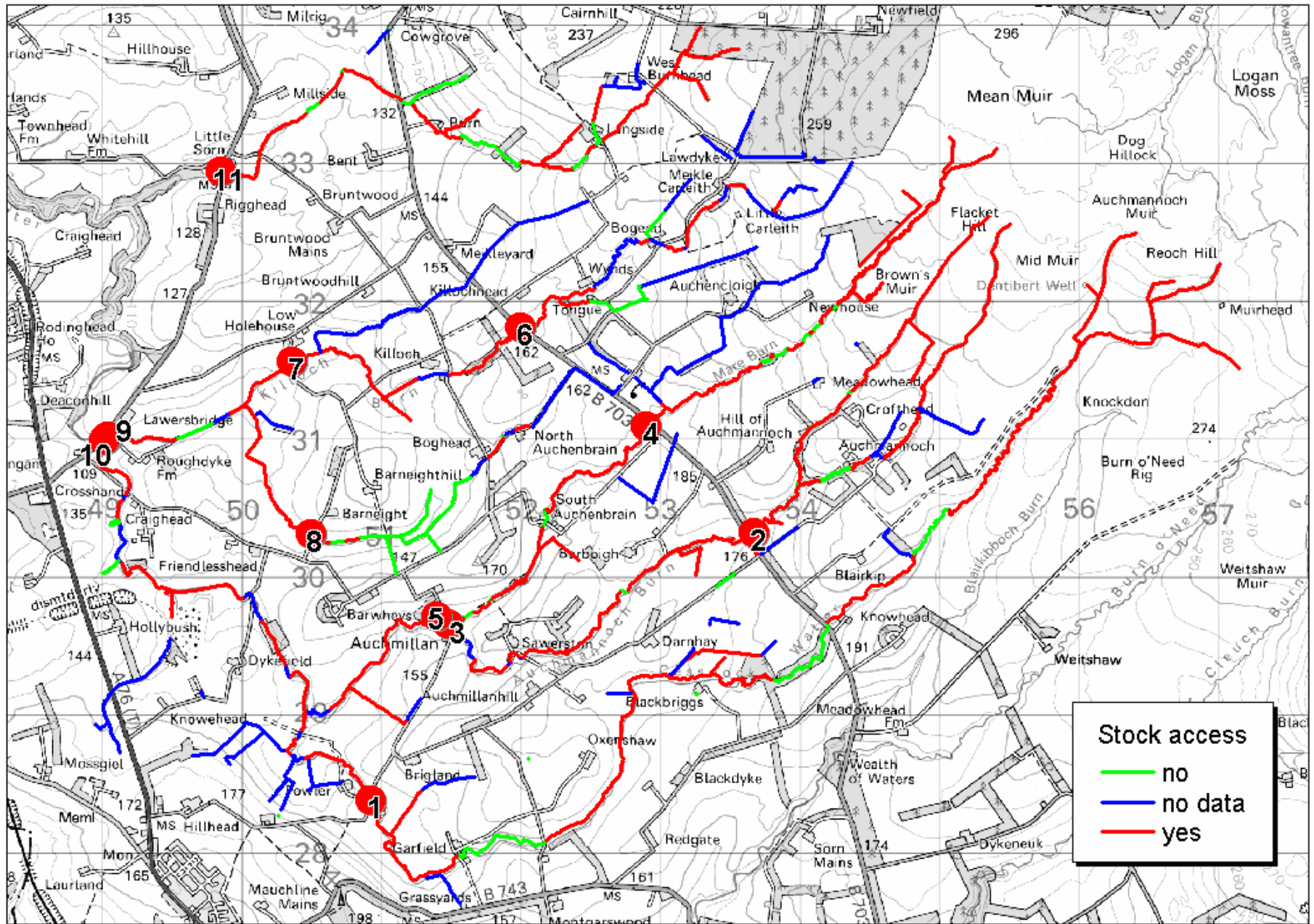


# Shear force - Montmorillonite



- **Blue bars**  
**1mM NaCl**
- **Red bars** **100**  
**mM NaCl**
- **Significant**  
**interaction of**  
**shaking speed**  
**and NaCl**  
**concentration**  
***P*=0.005**

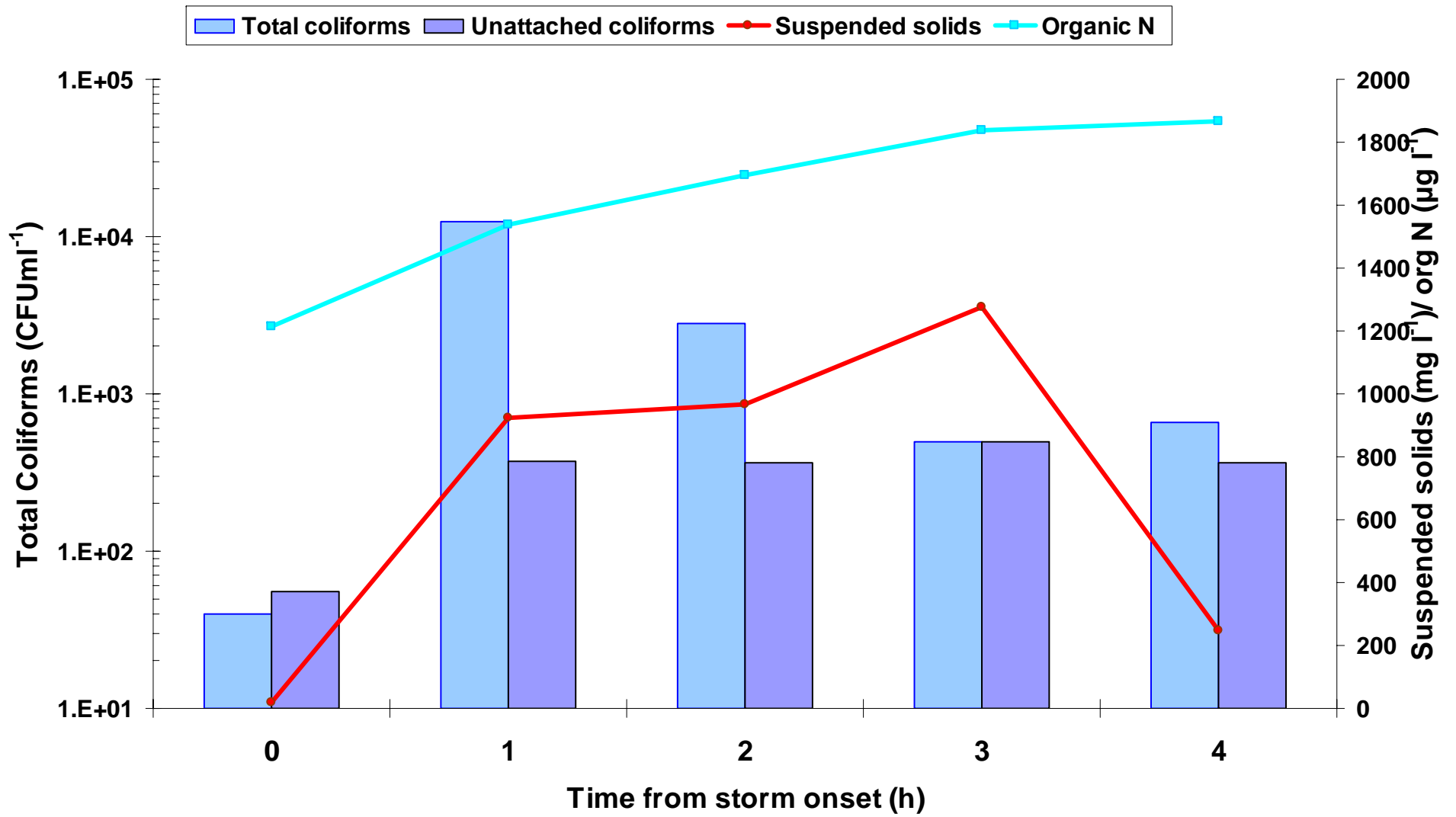




## Method

Stream samples were collected during a storm event on the Killoch Burn, downstream of Low Holehouse farm (a 55 ha farm with approximately 150 dairy cows and store cattle), within the Cessnock catchment in Ayrshire. The sampling was carried out using ISCO automatic samplers on a 1 hour fixed time interval as part of the Environmental Focus Farm project, led by SAC.





# Future work II – understanding the mechanisms

- **Micro & mesoscale**
  - How does *E. coli* interact with stream sediment?
  - Adsorption and desorption (AFM, Flow cytometry)
- **Catchment scale:**
  - How does this affect transport and subsequent bathing water quality
  - Impact of land use/characteristics and mitigation measures on *E.coli* / O157 numbers in surface waters



# Acknowledgements

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