

## Real-time E. coli monitoring in a large river system using the Proteus



### Summary

RS Hydro were commissioned by a UK government agency to undertake a trial of the Proteus for E. coli measurement. The key goal of the study was to compare the real-time measurement provided by the Proteus with traditional laboratory measurements of E. coli. Measurements were undertaken in River Lagan, Northern Ireland at 5 sites covering a rural to urban gradient. The Proteus performed extremely well and displayed a strong correlation ( $r = 0.95$ ) with the laboratory data. The utility of the sensor as a tool for rapid assessment of bathing water quality was also highlighted. The sensor successfully classified 90% of samples into the 'correct' bathing water category based on the laboratory data. These findings highlight that the Proteus can provide repeatable, accurate, reliable and high frequency measurements of microbial contamination and is highly suitable for environmental monitoring and bathing water quality monitoring.

### Background

RS Hydro were commissioned by a UK government agency to undertake a trial of the Proteus for coliform measurement. The key goal of the study was to compare the real-time measurement provided by the Proteus with traditional laboratory measurements of presumptive coliform and E. coli analysis. Such data was considered invaluable for future design of any larger catchment investigations where the purchase of a number of units to provide highly resolved (in time and space) indications of bacteria loading and inform bathing water alerts and feed into shell fish ....

### Proteus BOD Specification

The Proteus (Table 1 & 2 and Figure 1) is a multi-parameter optical sensing platform consisting of (in this case): (1) tryptophan like fluorescence sensor (TLF), (2) a turbidity sensor and (3) a temperature sensor; that can provide users with a real-time indication of total coliforms or E. coli. The TLF measurement is compensated for temperature and turbidity interference to provide a robust indication of total coliforms or E. coli counts. The sensor platform can also house a suite of additional sensors (optical and electrochemical). For this trial electrical conductivity, dissolved oxygen and fDOM (fluorescent coloured organic matter) sensors were also fitted to the unit. The addition of fDOM was expected to provide users with information on the total organic load as it measures fluorescence of humic and fulvic materials.

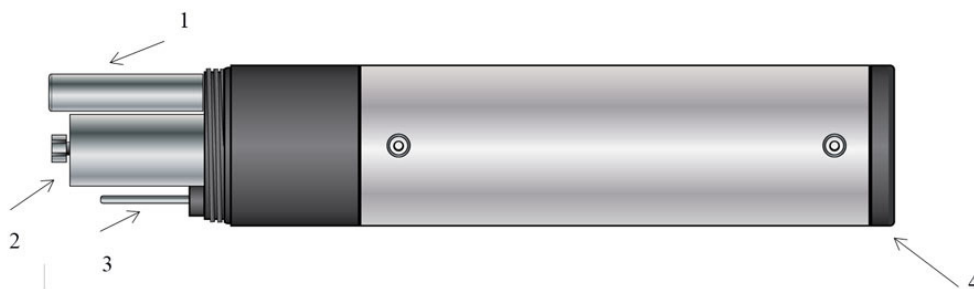


Figure 1. Schematic of a standard configuration Proteus BOD

Table 2. Proteus mechanical, optical and physical specifications

Proteus specifications	
Dimensions	OD 76 mm L 460 mm
Weight	1.36 Kg
Operating range	-5° C - 50° C
Depth rating	200 m
Supply voltage range	5-15 Vdc
Signal output	SDI 12 & RS232 (Ethernet, TCP-IP, modbus, 4-20mA & relays with optional controller (AC or DC).
Communications	RS232/ Bluetooth
Sample rate	1 Hz
Internal memory	4 MB; >1,000,000 readings
Warranty	12 - 24 months
Fluorometer specifications	
Excitation (nm) ± bandpass (nm)	285 ± 10
Exmission (nm) ± bandpass (nm)	350 ± 55
Detection limit (ppb)	3.00*
Dynamic range (ppb)	0 - 20000*
Accuracy	±1% of reading (0 - 10000 ppb)
Coliform resolution (CFU/100ml)	0.1
Temperature compensation	Automatic (flexible- user defined)
Turbidity sensor specifications	
Turbidity range	0 -3000 NTU
Accuracy	0- 600 NTU (±1%) / 600 -3000 NTU (±2%)
Temperature compensation	Automatic (fixed)
Temperature sensor specifications	
Temperature range	-5° C - 50° C
Accuracy	± 0.1 ° C

## Methods

### Calibration, validation and maintenance

All sensors were calibrated prior to installation across the following ranges:

Turbidity: 0 – 1000 NTU

Tryptophan fluorometer: 0 – 1000 ppb (synthetic tryptophan standard)

fDOM fluorometer: 0 – 1000 ppb (quinine sulphate standard)

Dissolved oxygen: 0 – 100 %

Electrical conductivity: 0 – 1413  $\mu$ S

### Sampling sites

For this trial, measurements were undertaken in a freshwater river environment (River Lagan, NI, See Figure 1a and 1b) at a number of sites covering a rural to urban gradient. The sites sampled were:

Site 1 Blackstaff outflow, a site close to Gas Works, Belfast (Figure 2 a & b).

Site 2 Gas Works Culvert, a site close to Gas Works, Belfast (Figure 2 a & b).

Site 3 Shaws Bridge, Newforge lane, Belfast (Figure 2 a & b).

Site 4 Lisburn Civic Centre, Lisburn (Figure 2 a).

Site 5 Lagan Upstream Dromore (Figure 2 a & b).



Figure 2. Installation location for the duration of the trial.

The catchment of the River Lagan at the lowest monitoring location is large (~500 km<sup>2</sup>) with a considerable agricultural activity in the headwaters (~15% of catchment area) and significant urban cover in the lower catchment (i.e. around the Belfast area). For sampling sites (1-3) there are several major Wastewater Treatment Works (WwTW) imputing effluent into the river system, historical levels of *E. coli* have been recorded between 300 and 30,000 CFU /100ml at Site 1 with peak discharge from these treatment works is between 7:00 – 9:00 and 18:00 – 21:00 daily.

## Sampling protocol

At all sites the Proteus was suspended from a pole which extended 1-2 metres from the bank to ensure the probe was in the main flow of the river and clear of bank sediment. All parameters were logged to the internal memory on the Proteus at 5 min intervals. Grab samples for laboratory analysis were collected at between 10 and 15 min intervals and synchronized with the Proteus logging intervals. All samples were 1 litre in size and were collected at the approximate depth of the probe. In all, 49 catch samples were collected and subjected to presumptive coliform (MSLB medi, 37°C) and *E. coli* (TBX, 30°C for 4 hrs, 44°C 16 hrs) enumeration. All samples were stored overnight (4°C) prior to analysis.

## Results

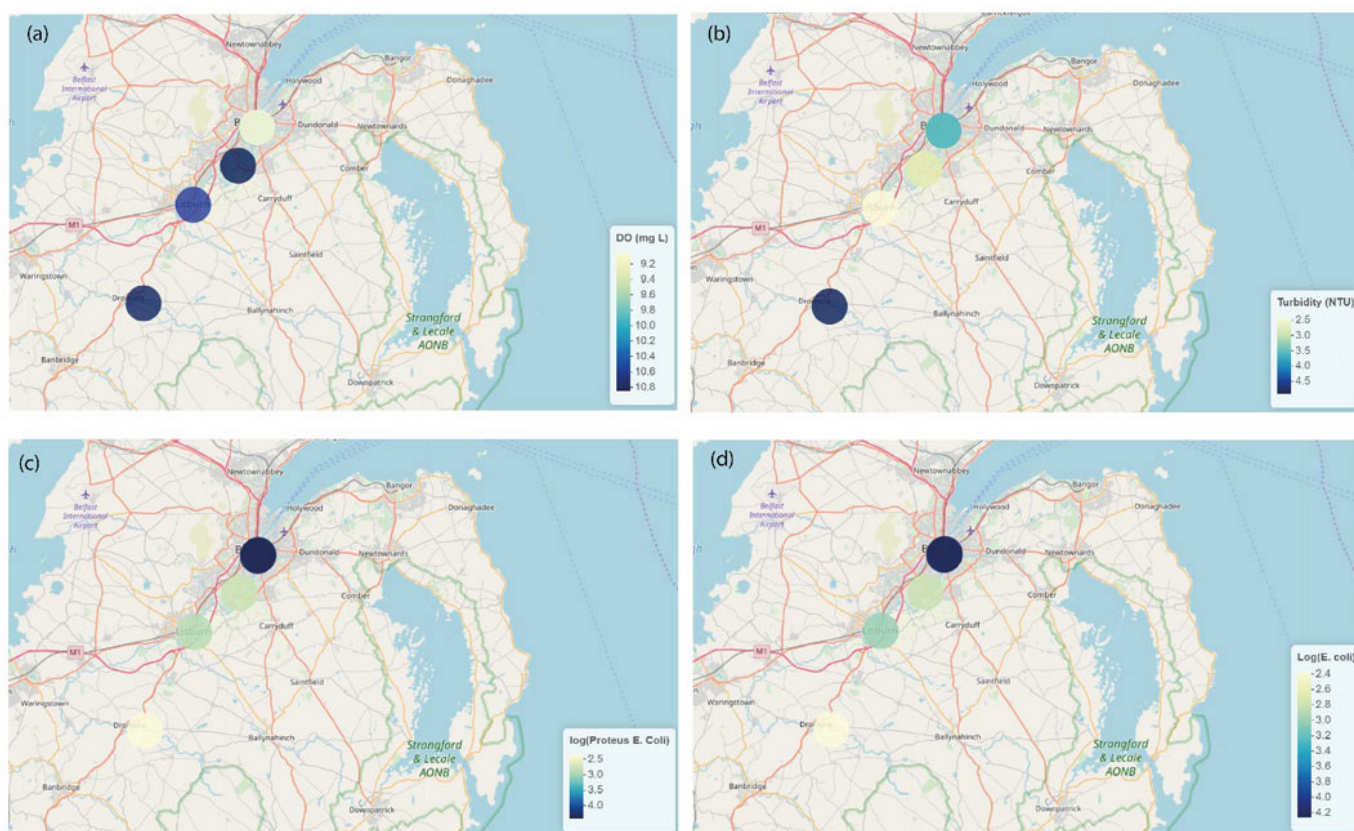


Figure 3. Average measurement of key parameters recorded at all sites (a) dissolved oxygen, (b) turbidity, (c) log transformed Proteus measurements of *E. coli* and (d) log transformed laboratory measurements of *E. coli*.

## Spatial patterns in water quality and *E. coli* counts

In-situ water quality and laboratory measurements displayed clear spatial patterns with decreasing dissolved oxygen concentration with increasing distance downstream (Figure 3a). The highest dissolved oxygen concentration  $10.82 \pm 0.01$  mg L<sup>-1</sup> (mean  $\pm$  SD) was recorded at Site 5, Upstream of Dromore and lowest,  $9.06 \pm 0.92$  mg L<sup>-1</sup>, was recorded at Site 1, Black Staff Outflow, Central Belfast. Turbidity was low across the monitoring sites with the lowest measurements from Site 4, Lisburn Civic Centre,  $2.43 \pm 0.42$  NTU and highest at Site 5,  $4.90 \pm 0.36$  NTU. Laboratory determinations of *E. coli* varied by 2 orders of magnitude across the urban – rural gradient with the highest counts recorded at Site 1 (Urban),  $22,962 \pm 19,570$  CFU 100ml<sup>-1</sup>, and lowest at Site 5,  $250 \pm 76$  CFU 100ml<sup>-1</sup>. The in-situ measurements made with the Proteus displayed similar patterns to the laboratory measurements with highest values at Site 1;  $25,298 \pm 19,109$  CFU 100ml<sup>-1</sup> and lowest value  $241 \pm 51$  CFU 100ml<sup>-1</sup>.

## Relationship between Proteus E. coli and lab E. coli measurements

There was a strong linear relationship between Proteus E. coli and laboratory measurements of E. coli when all data points, or the full data set (FD) were considered (Figure 4 a). A regression model was fitted separately to the low range data (LD:<1250 CFU 100 ml<sup>-1</sup>) and a reasonable linear relationship was apparent (Figure 4b; R<sup>2</sup> > 0.6). Additional goodness of fit metrics were calculated for FD, ~300 – 25,000 CFU 100 ml<sup>-1</sup> and low range samples (LD) <1250 CFU 100 ml<sup>-1</sup>. Mean error :FD = 116 CFU 100 ml<sup>-1</sup> LD =32 116 CFU 100 ml<sup>-1</sup>, Pearson's correlation coefficient FD = 0.95, LD = 0.83 percent bias FD =1% and LD = 0.1%. The laboratory and Proteus E. coli data were classified following the WFD inland bathing water quality standards, Excellent (< 500 CFU 100 ml<sup>-1</sup>), good (500-1000 CFU 100 ml<sup>-1</sup>) and poor (1000 > CFU 100 ml<sup>-1</sup>). Good overall classification accuracy was achieved with the Proteus with 90% of samples correctly classified into bathing water quality classes (Table 3).

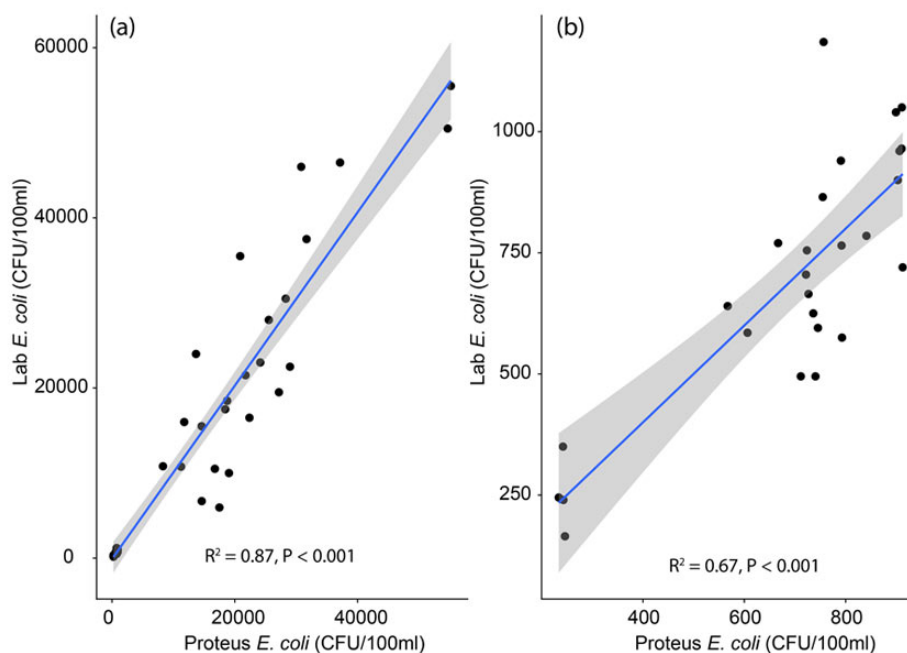


Figure 4 (a) Relationship between the Proteus measurement of E. coli and laboratory measurements for all samples. (b) Relationship between the Proteus measurement of E. coli and laboratory measurements for low range samples

Table 3. Confusion Matrix showing agreement between bathing water classification based on Proteus measurements and laboratory measurements of E. coli. The laboratory and Proteus E. coli data were classified following the WFD inland bathing water quality standards, Excellent (< 500 CFU 100 ml<sup>-1</sup>), good (500-1000 CFU 100 ml<sup>-1</sup>) and poor (1000 > CFU 100 ml<sup>-1</sup>). Agreement between the Proteus and lab classification is highlighted in grey and mis-classification is highlighted in red.

Lab \ Proteus	<501 (CFU)	501-1000 (CFU)	1000> (CFU)
<500 (CFU)	4	0	0
500-1000 (CFU)	2	17	3
1000> (CFU)	0	0	24

## Conclusions and recommendations

The Proteus performed well during this rigorous trial assessing E. coli measurement accuracy. The sensor showed a strong correlation ( $r = 0.95$ ) with the laboratory data. The utility of the sensor as a tool for rapid assessment of bathing water quality was reinforced here. The Proteus successfully classified 90% of samples into the 'correct' bathing water category based on the laboratory data. This study has highlighted that the Proteus is able to provide repeatable, reliable and high frequency monitoring of microbial contamination and is suitable for environmental monitoring and bathing water quality monitoring.