1 Protocol design and quality assurance

The overall aims of this project were to:

1. acquire new empirical data on faecal indicator loading in catchment streams before and after a series of remedial measures or BMPs were implemented on 48 livestock farms in Scotland, thus, to facilitate an;
2. evaluation of the effectiveness of the remedial measures implemented.

The data reported here were acquired over a period spanning October 2002 to October 2004. A complementary investigation at Brighouse Bay (situated approximately 25 km to the west of Sandyhills) involved environmental sampling spanning the period October 2003 to August 2004. All sampling was undertaken in targeted, intensive campaigns by field staff located in the study catchment to facilitate opportunistic sampling in response to high flow events.

The sampling campaign timing for each of the sites and the sample numbers analysed are presented in Table 1.1.

This Section describes the approach to sample site selection and the field and laboratory methods used. Readers seeking the results of each regional study are directed to Sections 2 (Page 10) to 5, which contain summary and site-by-site data for all regions. The approach to the data analysis is outlined in Section 1.5 (Page 8).

Table 1.1 Study periods and samples analysed

Study site	Samples analysed	Fieldwork period 2002 to 2004 dd/mm/yr	
Ettrick Bay	420	14/10/02	10/11/02
	313	28/07/04	16/08/04
Killoch Burn	417	11/11/02	07/12/02
	341	31/07/04	17/08/04
Sandyhills	797	09/12/02	22/01/03
	879	05/07/04	30/09/04
River Nairn	456	23/01/03	31/03/03
	621	26/08/04	07/10/04

1.1 Sample site selection

For each study region, a site visit and detailed consultation with local SAC staff was undertaken to define the planned, or likely, remediation measures in each subcatchment. Sampling sites were then chosen to define the hydrological subcatchments which would be affected by a range of remediation measures. For one study region, the Killoch Burn catchment within the Cessnock drainage basin, a previous investigation conducted during June and July 2002 (Edwards *et al*., 2004), provided comparative 'summer' bacteriological and hydrological monitoring data. In this study region, one of the sampling sites (Killoch site 2002) was selected in the present study to facilitate the acquisition of comparative 'autumn' data.

This comparative analysis is presented in Section 3 of this report (see Figure 3.4 Page 21).

The fieldwork protocol design for the Ettrick Bay investigation was developed following a joint site visit by SAC and CREH staff prior to detailed negotiation and formal agreement between SAC and any of the farmers who later became involved in the programme. Thus, the design of the pre-remediation sampling programmes was based on the best judgement of the SAC and CREH staff and their understanding of the likely nature and location of remedial measures.

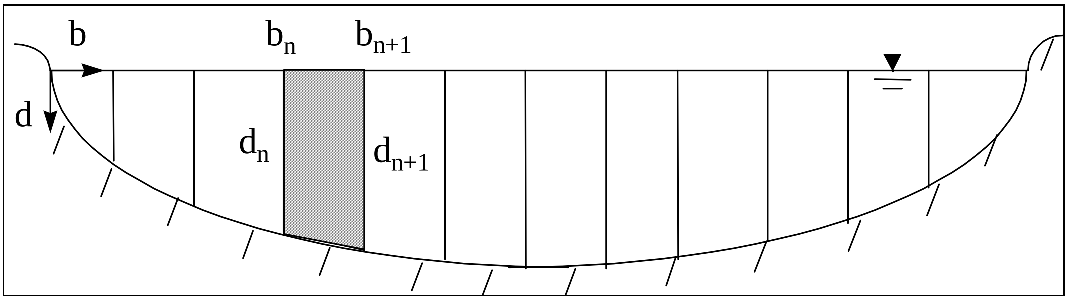
A geographical information system (GIS) was constructed for each region. The GIS was used to define watershed boundaries and their areas based on an associated digital elevation model. The GIS also allowed more precise location for each site to be defined, theoretically to +/- 5 metres in the north-south (y) or east-west (x) directions.

1.2 Hydrology

The discharge measurements reported here employed a uniform implementation of the 'normal velocity area' approach (see Richards, 1982 Page 127; Buchanan and Somers, 1969, British Standards Institute, 1964 and ISO, 1996, Environment Agency, 2003). The 'normal velocity area' approach was used for comparable sized streams in the summer 2002 studies which provide useful baseline data from the Macaulay/CREH work at Killoch site 2002 and SAC/SEPA work at Killoch site 2001.

At locations where a faecal indicator budget calculation was required, the reach was first surveyed for a distance of 25 m upstream and downstream of the sampling site to define the best cross section for measurement. The ideal location would be a straight reach with a symmetrical cross-section above a stable bed form, which would form a control cross section for the flow regime i.e. a rock shelf or stable boulders. Often, flow constraints such as bridges or culverts provide such a stable controlled cross-section. The chosen cross-section was first marked to facilitate multiple measurements and a stage board was located by stake and/or attachment to existing structures immediately upstream of the cross-section. The field team undertook velocity measurements at each cross-section using the 'mean section' method (Environment Agency, 2003) illustrated in Figure 1.1.

Figure 1.1 Diagram illustrating the mean section method



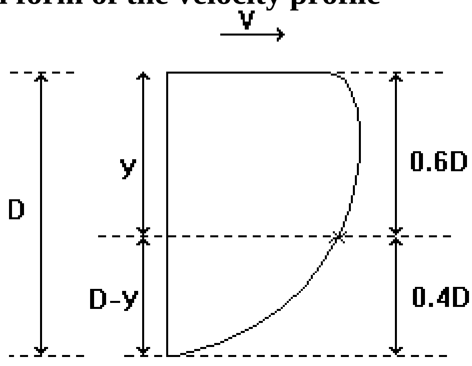
The flow in the shaded panel is calculated as follows:

$$Q = \left(b_{n+1} - b_n\right)\left(\frac{d_{n+1} + d_n}{2}\right)\left(\frac{\bar{v}_{n+1} + \bar{v}_n}{2}\right)$$

where $\bar{v}$ is the average velocity in each vertical.

Mean velocity in each vertical was measured at 0.6 of the depth from the water surface as recommended for depths of less than 1m in Environment Agency (2003) (Figure 1.2).

Figure 1.2 Classical form of the velocity profile



Where	V	=	velocity
	D	=	total depth
	y	=	position relative to water surface

(*Source* Environment Agency, 2003)

White plastic metre rules were used for depth measurement and a 30 m tape was employed to locate the verticals. Velocity was measured using SENSA RC2 Velocity Meters, calibrated by Aqua Data Services Ltd using the Distance/Time approach in accordance with ISO 3455. This electromagnetic instrument has no moving parts and is designed to measure flows from zero m.sec^{-1} to 4 m.sec^{-1}, with an accuracy of +/-

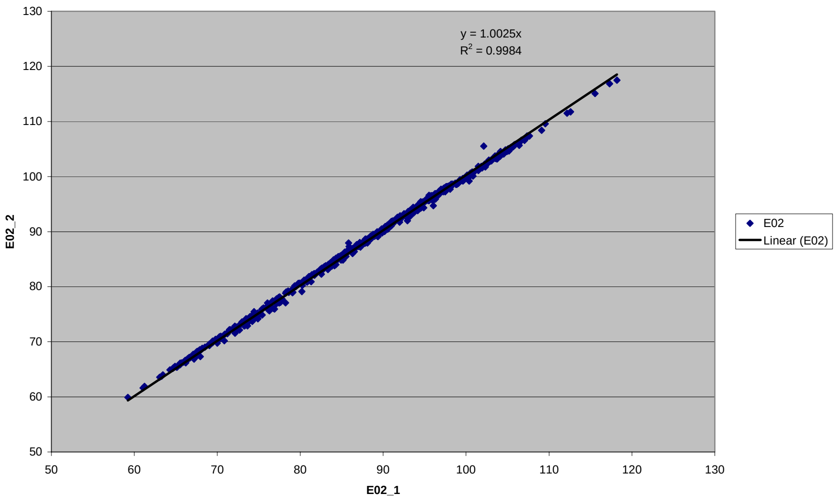
0.001 m.sec^{-1} at a flow velocity of <0.2 m.sec^{-1} (i.e. normally encountered stream velocities) to 0.02 m.sec^{-1} at flow velocities of 2.0 m.sec^{-1} to 4.0 m.sec^{-1}.

Level recording devices were installed at 55 of the principal faecal indicator flux locations. For sites where a stage discharge relationship was not obtained because the cross section proved unstable or was heavily vegetated, the flux calculations are based on adjacent flows scaled by catchment area calculated from the GIS. AOTT™ water level recording systems were deployed in each regional study. These horizontal paper chart recorders were used to provide a visual stage record for the sampling team to allow them to assess whether streams were in high or low flow at the time of sampling. Van Essen Instruments Divers® (electronic, recording pressure transducers) were installed in the streambed at each measurement location. A further three Divers® were deployed in the open atmosphere to record atmospheric pressure during each of the regional studies. Data from these three units was used to compensate the data from the streambed units to provide a continuous 15-minute interval time series of stream level through each study period.

It is important when defining the relationship between stream level (stage) and discharge, to acquire discharge data over a range of stream levels. Where possible, this was done by the field team who collected water samples then returned to the field and continue with hydrological measurement over a range of flows.

The reliability of the Diver® stream level logging system was checked by comparing records from pairs of units installed at each location in the Ettrick study for the pre-remediation period (see Figure 1.3).

Figure 1.3 Correlation between diver 1 and diver 2 at site 1002



Each stream level record was examined in detail before the discharge plots illustrated in Appendix I were prepared. Ground truth data were provided by the stage readings recorded at each sampling event. Some discontinuities were evident in the records caused either by: (i) physical disturbance by the sampling team checking the divers (e.g. 19th November 2002 in the Killoch catchment around noon); or (ii) rare but observed disturbance by flood events causing the diver to move as bed load was transported down the channel (e.g. Killoch site 2006 was observed to have been dislodged by a flood on 19th November 2002 and at Ettrick site 2002 the flood of 11th August 2004 caused the loss of monitoring equipment). In such cases, the subsequent trace was recalibrated against the fixed stage board reading recorded at each sampling event and the subsequent trace amended accordingly.

The fixed stage boards provide the relevant stream level reading from which discharge is calculated for each site. Relationships between stream level values from the Diver® trace at the time of corresponding stage board readings were defined using regression models of the form:

$$Y=b + aX$$

where:

Y = stage board reading
X = Diver® trace reading
and a and b are slope and intercept constants respectively

These equations were used to predict the time series of stage board values between the physical stage board readings, recorded by the field team at times of sampling, from the Diver® trace.

To facilitate re-visitation of sites, a discreet but permanent height mark (i.e. either a notch in a convenient location made with a stone grinder or double horizontal drill holes marked by washers and screws) has been made at each site. The permanent mark can be related back to the stage board height used in this investigation, full details of which have been logged for potential use in the future.

1.3 Water quality data acquisition

The importance of acquiring water quality analyses under a range of flow conditions has been demonstrated in previous investigations in Ayrshire and beyond (Kay *et al*., 1999; Wyer *et al*., 1997, 1999). To facilitate this, a mobile field laboratory was deployed within each study area. Two field laboratories were used in this study. These were located on facilities owned by Scottish Water (Killoch, Sandyhills and Nairn (pre-remediation)) or on caravan parks (Ettrick and Nairn (post-remediation)).

1.3.1 Sampling

Bacteriological samples were taken aseptically using sterile, disposable, 150ml sample pots. Samples were stored on ice packs in a covered cool box, then transported back to the mobile laboratory.

On receipt at the laboratory, samples were stored in plastic boxes containing melting ice cubes and water to ensure good thermal conductivity and, hence, rapid sample cooling. All samples were analysed as soon as possible and in chronological order. All samples were analysed within 24 hours as recommended in (Environment Agency, 2000).

1.3.2 Analysis

Enumeration of coliforms (TC), *Escherichia coli* (*E. coli*, (EC)) and intestinal enterococci (IE) was carried out using membrane filtration, following methods detailed in Environment Agency (2000). At least two sample dilutions were performed for all three determinands to ensure that an accurate count could be made. In addition, in the first sample run at each site, samples were filtered at three dilutions to minimise 'greater than' and 'less than' values in the data set. All concentrations reported are presumptive organisms expressed as colony forming units (cfu) 100 ml^{-1}. Turbidity (NTU) and pH measurements were completed on the sample remaining after bacteriological analysis and always within 24 hours of the sample being taken.

1.3.3 Quality Control

With each batch of samples, one sample was analysed in duplicate for indicator organisms and appropriate positive and negative controls were included with each incubation batch. The temperature of all incubators and refrigerators was monitored with a calibrated thermometer and recorded on each day of laboratory use.

The statistical significance of differences between sample and duplicate results was examined using a paired t-test. For each pair the duplicate result was subtracted from the sample result and the mean difference compared with a mean of zero (i.e. the mean difference should not be significantly different from zero). Exemplar results of this analysis are shown in Table 1.2. In all cases the significance value, *p*, is never less than 0.05 indicating that at the 95% confidence level, the mean differences are not significantly different from zero.

Turbidity and pH meters were also checked on a daily basis, and re-calibrated at least once a week.

1.4 Rainfall measurements

Onset™ data logging rain gauges were deployed at key sites for each of the studies. These are interrogated using BoxCar™ software which can be set up to provide cumulative (i.e. tips from start of recording) and/or rainfall rate (i.e. tips per hour) data which can be exported directly to a spreadsheet. Each tip represents 0.2mm of rainfall.

Table 1.2 Exemplar analysis of duplicate quality control samples to test the hypothesis that the original samples and their replicates derive from different populations

Site	**Test**	**Mean**	**St Dev**	**t-value**	***p*-value**
Ettrick	TC only	-0.0140	0.1253	-0.37	0.7190
	EC only	-0.0200	0.1643	-0.39	0.7010
	IE only	0.0172	0.2559	0.22	0.8280
	All 3	-0.0055	0.1846	-0.17	0.8660
Killoch	TC only	-0.0198	0.2441	-0.27	0.7930
	EC only	-0.0004	0.0838	-0.02	0.9870
	IE only	0.0455	0.1766	0.85	0.4130
	All 3	-0.0085	0.1504	-0.32	0.7520
Sandyhills	TC only	0.0008	0.1856	0.01	0.9890
	EC only	-0.1290	0.3740	-1.04	0.3300
	IE only	0.0136	0.0938	0.32	0.7630
	All 3	-0.0453	0.2609	-0.85	0.4030
Nairn	TC only	0.0101	0.0847	0.32	0.7630
	EC only	-0.1000	0.2510	-0.98	0.3740
	IE only	-0.1840	0.4430	-0.93	0.4060
	All 3	-0.0804	0.2716	-1.26	0.2260
All 4 sites	TC only	-0.0075	0.1710	-0.27	0.7850
	EC only	-0.0536	0.2286	-1.43	0.1620
	IE only	-0.0229	0.2354	-0.54	0.5920
	All 4	-0.00737	0.04912	-1.55	0.1240

1.5 Approach to the data analysis

This study aimed to compare stream faecal indicator concentrations pre- and post-remediation or between modified (test) and ‘control’ sites. However, the extreme seasonal variability evident in the data, which is outlined above, makes direct ‘before and after’ comparisons difficult. Table 1.3 exemplifies the general pattern observed using the Sandyhills data. It contains geometric mean values for the three faecal indicator species in all samples taken pre- and post-remediation.

Table 1.3 Geometric means (GM) and sample numbers acquired for all Sandyhills faecal indicator organism data pre- and post-remediation

Parameter and flow condition	Pre-remediation Dec-Feb		Post-remediation July-Sept	
	n	**GM**	***n***	**GM**
TC High Flow	237	3,349	382	8,993
EC High Flow	237	791	382	5,864
IE High Flow	237	474	382	1,139
TC Low Flow	476	624	487	1,064
EC Low Flow	471	148	487	570
IE Low Flow	477	92	487	177

The pre-remediation Sandyhills data were acquired in the mid-winter period and exhibit lower geometric mean values than the post–remediation ‘summer’ samples. At face value, this might be taken to infer a catchment-wide deterioration in water quality despite the remedial measures deployed. This elevation in faecal indicator concentration in the summer high flow samples is repeated in the other study areas.

This seasonality in the faecal indicator geometric mean values ‘confounds’ the pre- and post-remediation comparison of stream water quality. It might be expected that the seasonality would lead to an increase in catchment-wide geometric mean values whilst the remedial measures tend (hopefully) to produce a reduction. In the Brighouse Bay investigation, a very similar problem was encountered (Dickson *et al*., 2005). In the absence of ‘standard’ methods for the analysis of such seasonality, the Brighouse Bay stream water quality data acquired in the pre- (autumn) and post-remediation (summer) phases were compared to the water quality in an adjacent stream chosen as a control catchment prior to the commencement of the study. Figure 1.4 illustrates this approach. The control catchment was unmodified by any catchment measures and it exhibited an increase in FIO concentrations between the pre- and post-remediation sampling phases (shown as b in Figure 1.4). The remediated catchments also exhibited an increase in FIO geometric mean values between pre- and post-remediation monitoring (shown as a in Figure 1.4). Thus, all streams exhibit a ‘seasonal shift’ in faecal indicator geometric mean values between the two sampling phases. If the measures designed to reduce pollution have a positive effect, then this shift in the remediated catchments should be less than the shift in the unmodified control catchment. In addition, the change in $\log_{10}$ mean concentration due to remediation can be calculated as in Figure 1.4. A similar approach has been taken in this report to compare the effects of the remedial measures. It is important to note, however, that, in this study, the original protocol design did not incorporate preselected control catchments. In fact, catchments where water quality data were acquired, but no (or very few) remedial measures were implemented in the upstream catchment were defined as control sites retrospectively. This is far from ideal because control catchment choice was not *a priori* and bias may be introduced if, for example, the control site(s) tend to be in headwater areas, with different morphometric, soil, land cover and land management characteristics compared to the remediated catchments.

Given these reservations, this analysis should be considered exploratory and any predictions treated with extreme caution. Where possible, the intensity of measures implemented in terms of fencing or steading investment has been compared to the difference in shift between individual remediated catchments and selected controls. Again, caution is appropriate in the interpretation of this analysis. It would certainly be inappropriate to infer a direct relationship between the intensity of remedial measures and the shift difference using data from the four study areas alone.

Figure 1.4 Graphical representation of the log_{10} shift analysis