SARF013/SAMS Report No. 256
RISK FACTORS IN SHELLFISH HARVESTING AREAS

Final Project Report

Prepared for

Scottish Aquaculture Research Forum

Shona Magill[1], Kenny Black[1], David Kay[2], Carl Stapleton[2], Simon Kershaw[3], David Lees[3], James Lowther[3], Carol Francis[4] John Watkins[4] and Cheryl Davies[4]

.

[1] Scottish Association for Marine Science
Dunstaffnage Marine Laboratory
Dunbeg
Oban, Argyll
PA37 1PA
U.K.

[2] Centre for Research into Environment and Health
University of Wales
Lampeter
Ceredigion
SA48 7ED
U.K.

[3] CEFAS
The Nothe
Barrack Road
Weymouth
DT4 8UB
U.K.

[4]CREH Analytical Ltd
Hoyland House
50 Back Lane
Horsforth
Leeds
LS18 4RS
U.K.

SCOTTISH ASSOCIATION *for* MARINE SCIENCE

CREH
CENTRE FOR RESEARCH INTO ENVIRONMENT AND HEALTH

Cefas

Recommendations

Context

This study assessed the risk factors associated with cultured shellfish. The study was carried out on Loch Etive and offers a number of lessons for other sea lochs on the west coast of Scotland. Results from the three elements of this study indicate that *E. coli* non-compliance issues within the Loch Etive shellfish production areas appear primarily to be summer high flow event driven. Therefore, determining the human (sewage)/ animal (diffuse) mix of pollution impacting on shellfish harvesting areas under high flow events is crucial to the design of remediation measures to prevent impairment of 'protected areas'. Although there is significant human settlement around the sealoch, the 'sewage' contribution to the bacterial compliance parameter loadings during periods of peak input to Loch Etive were tiny when compared to the diffuse catchment flux derived from livestock grazing areas. Some small agricultural catchments were found to generate disproportionately high loadings to the loch which offers scope for clearly targeted remediation effort.

1. A programme of sanitary surveys is currently being carried out across Scottish shellfish growing areas. Such surveys provide an essential detailed assessment of potential pollution sources impacting harvesting areas. However, in rural environments such as Loch Etive, sanitary surveys of shellfish harvesting areas which quantify only anthropogenic microbial hazards will provide little useful information on the reasons for non-compliance with faecal indicator compliance parameters**. Thus, for waters at risk of non-compliance a quantitative microbial source apportionment exercise is an essential foundation for any sanitary survey exercise.**
2. Attention solely focused on reduction of point source discharges to any rural loch system would be unlikely to produce significant reductions in total bacterial loading. **Thus, design of any remediation strategy to reduce sewage fluxes to impaired waters should be undertaken only after a source apportionment study as suggested above is undertaken.**
3. Quantification of the diffuse faecal indicator loading requires measured or, if this is not feasible, modelled high flow flux information from all catchment stream inputs and any sewage infrastructure point source discharges. **Source apportionment requires targeted high flow sampling (i.e. responsive aseptic sampling capacity on a 24 hour basis) and this should be built in to any sampling programme to inform management decisions designed to effect improvement.**
4. Where reduction of pathogen presence (e.g. norovirus) in shellfish flesh is the principal management objective: i.e. when the harvesting is compliant with the coliform parameter but still exhibits virus–positive periods, **then additional evaluation of specific anthropogenic point source discharges of sewage would be prudent.**
5. Historical routine stream monitoring data (if available) will be biased to 'low flow' conditions and use of such data to estimate flux from catchment systems will produce erroneous and dangerously optimistic conclusions concerning the total pollutant flux to adjacent harvesting waters. **Thus, historical monitoring data should not be used in isolation to estimate pollutant loadings unless it**

contains information on high flow water quality or is augmented by additional targeted sampling.

6. The sanitary survey results determine the selection of the routine monitoring points. The process requires full cooperation from all relevant stakeholders and relies on the availability of accurate and comprehensive data on both anthropogenic point sources and diffuse catchment sources. **Information on all domestic sources (including raw sources, private and public sewage systems) should be centralised and readily available. In addition, access to more detailed livestock census data and management information at the catchment, or even individual farm, level would facilitate a greater evaluation of diffuse sources.**
7. Regular monitoring programmes may improve characterisation of *E. coli* contamination patterns. However, full characterisation following high flow events may only be achieved through higher resolution sampling. **High intensity sampling events should be included in the microbiological assessment phase of the sanitary survey in order to a) aid selection of routine monitoring points and b) assess the impact of high flow events on shellfish production areas (particularly new designations and areas at risk from non compliance).**
8. The level of norovirus detected in this study was lower than previously seen in other UK studies. The actual risk presented to humans consuming norovirus contaminated shellfish, is currently unknown. **Research aimed at quantifying the relationship between norovirus levels in shellfish and the associated health risk is vital in order to truly evaluate any potential risks. Further, standards and guidelines are required both for regulators and industry to assess the suitability of areas for shellfish production.** CEFAS is currently leading a working group aimed at method development for accreditation by the European Committee for Standardisation (CEN) for viruses in food. Development of standards is targeted for 2012.
9. *E. coli* and FRNA+ bacteriophage were found to be poor indicators of norovirus in Loch Etive shellfish. This is likely to be the case in other large catchments where the influence from human sewage is small. *E. coli* may act as a better norovirus indicator in shellfish from more urban/human impacted catchments. **Thus, neither *E. coli* nor FRNA+ bacteriophage should be considered as reliable quantitative risk indicators of norovirus contamination in shellfish on the west coast of Scotland. However, research to clarify the potential of these indicators in areas thought to be under greater influence from domestic sewage is required.**
10. Further to source apportionment assessment, further studies may be required to establish connectivity between key sources, the receiving waters and harvested shellfish. This may include tracer studies or surface hydrodynamic studies. The present study did not investigate potential 'connectivity' between the present treated sewage discharge point in Dunstaffnage Bay (or the CSO in Connel) and the harvesting areas in the outer basin and/or the flux of faecal; indicators travelling westward from the inner basin. **We would recommend that both investigations are completed to (i) clearly establish the risk of human virus contamination from the sewage infrastructure and (ii) discount the possibility of a major, but unmeasured, flux of faecal pollution from the upper basin**. Potential protocols for both investigations have been discussed by the project Steering Group.

Executive Summary

Sanitary survey

1. Shellfish cultivation has seen a substantial increase in recent decades, and is an important industry for coastal rural communities. Molluscan shellfish are efficient filter feeders and suitably sized particles may become concentrated at 100 times the background levels. Suspended particles in the water column can contain faecal bacteria (such as *E. coli*) and viral pathogens (such as the enteric norovirus). Consumption shellfish containing harmful microorganisms may pose a significant health risk. The objectives of this study were to identify key pollution sources and conditions contributing to viral and bacterial contamination of cultured shellfish. The project consisted of three key elements – a sanitary survey; 12 month monitoring programme; a source apportionment study of faecal indicator bacteria.

2. The study site, Loch Etive, is a large sealoch on the west coast of Scotland and is divided into two main basins, upper and lower. The loch has a large catchment area (approximately 1350 km^2) with a number of freshwater inputs to the system. The immediate catchment area of the study has a low human population density (approximately 2500 dwelling on the immediate shoreline) with a further 1000 (approximately) within 4 km of the mouth of the loch. In common with many sealoch coastlines of the west coast of Scotland, the catchment consists of a number of small villages and many rural dwellings.

3. Approximately 53 % of the immediate population is served by public network sewage facilities. Accurate detailed information could not be gathered on all private domestic sewage systems in the catchment but it is estimated that a large number of dwellings are served by septic tank systems. However, in a number of areas shoreline dwellings discharge raw sewage into the loch, both outside and within the Shellfish Growing Waters.

4. One Waste Water Treatment Works (secondary treatment) facility operates within the village of Taynuilt, serving around 60 % (approximately 700) of the local population in that area. Three public network septic systems operate within the area. The two largest systems discharge to marine outfalls outwith the main lower basin of the loch. There are Combined Sewer Overflow and Emergency Outfalls within the loch itself, but are outside the Shellfish Growing Waters (SGW). A small network in the village of Bonawe discharges raw sewage directly into the loch within the SGW. This network is small in capacity (23 dwellings) but is in close proximity to the shellfish production sites.

5. Agriculture in the catchment is dominated by livestock grazing for sheep and cattle. In excess of 5000 ewes and 500 cattle graze within the immediate catchment. Sheep numbers during the summer months, when lambs are present, may be approximately double this estimate. Much of the grazing areas are in close proximity to the shoreline. Livestock census data for the

agricultural parishes in the catchment area indicate that sheep numbers have decreased by approximately 10 % from 2000 to 2005.

6. Statistical analysis of the relationship between historical *E. coli* levels and environmental parameters (local wind, rainfall, river flow and tidal datasets) revealed a variation in response. This response may partly be driven by the variation in *E. coli* levels across the sites. Seawater temperature was significantly positively correlated with *E. coli* levels at two of the production sites. This indicates that *E. coli* levels are elevated during warmer summer months and less prevalent during winter months.

7. Examination of available historical classification data collected by Fisheries Research Services (FRS:1999-2000), and Food Standards Agency Scotland (FSAS: 2000-2006) indicated that on a number of occasions there was a degree of variation in *E. coli* levels between the production sites in Loch Etive West, despite the close proximity of the sampling sites. It should be noted that the historical data used in the Sanitary Survey was collected prior to the implementation of more stringent sampling protocols by FSAS in 2007. These protocols required sampling to be taken from fixed monitoring points by designated Environmental Health Officers (EHOs). Potential variations in sampling points used prior to 2007, means that the interpretation of historical data within the Sanitary Survey must be viewed with caution.

Twelve month monitoring programme

8. Two sites in Loch Etive West were chosen to assess faecal bacterial and viral contamination in cultivated shellfish tissue over a 12 month period. One site was located in Achnacloich Bay and one site near Airds Point. Mussels were sampled weekly at each site. More intensive seven day daily sampling episodes were carried out three times within the 12 months in order to characterise contamination levels following heavy rainfall events.

9. At Site A 43.3 % of the *E. coli* results fell in the A classification band (<230 Mean Probable Number (MPN) 100 g^{-1}). At Site B 50.8 % of the *E. coli* results were in the A classification band. Five results were returned as a C classification (4600 – 46000 MPN 100 g^{-1}) at Site A and 9 at Site B. However, at each site only one C result occurred during normal weekly sampling, most C results occurred during event sampling.

10. Microbiological data were similar at the two sites, indicating that the two sites were subject to similar contamination patterns. Mean bacterial levels (geometric mean) were similar at the two sites.

11. Approximately one third of the samples at each site tested positive for norovirus. At Site A 31.3 % of samples tested positive for norovirus genogroup I (GI) while 29.7 % tested positive for genogroup II (GII). At Site B 33.3 % of samples tested positive for GI and 31.8 % positive for GII. Combined positivity for both genogroups was 41.7 % at Site A and 46.9 % at Site B.

12. Of the positive norovirus results, many were observed close to the limit of detection. The vast majority of samples returned less then 25 Polymerase Chain Reaction (PCR) units - Site A 95.5 % (GI) and 86.4 % (GII); Site B 90.0 % (GI) and 95.5 % (GII). Maximum counts of 50.8 (GI) and 85.1 (GI) were observed at Site A and B respectively. All results from the study were returned a combined (both genogroups) maxima of less than 100 PCR units.

13. The rates of positivity and PCR counts found in this study are lower than have been observed in other harvesting areas in the UK. For example, positivity rates of 52-69 % for both genogroups was reported for two oyster harvesting sites in the UK. The same study reported combined (both genogroups) maximum PCR counts of more than 500 units for both areas.

14. *E. coli* and faecal coliform (FC) levels varied seasonally. Higher concentrations occurred during the summer and levels showed a high degree of fluctuation from week to week. Winter months were characterised by lower concentrations and little fluctuation from week to week, particularly in *E. coli* levels. A number of factors may influence the seasonal variation in *E. coli* and faecal coliform levels, such as changes in livestock numbers; movement of livestock and seasonal farming practices; changes in the human population during the main tourist season; decreased survival rate of *E. coli* and FC at colder temperatures.

15. The differences observed in seasonal pattern of coliform and norovirus prevalence suggests that the coliform compliance parameters may not always offer a good indicator system for predicting the risk of illness transmission caused by norovirus infection.

16. Norovirus was predominantly present over the winter months, being either absent or present only at very low levels during the summer. This observation was supported by a significant negative correlation between surface (Site A norovirus GI) and 9 m (Site A and B norovirus GI) seawater temperature and some of the winter norovirus parameters. The observed winter occurrence of enteric viruses in shellfish is in agreement with the natural seasonal prevalence patterns of these viruses within the human population of Northern Europe.

17. *E. coli* levels during the June event sampling show an increase from the weekly levels up until that point. However, in general over the monitoring programme, the levels detected during the event sampling were within the levels detected during the weekly sampling. Both the June and August sampling events detected some *E. coli* 'C' class results. *E. coli* levels did increase during the winter event sampling, but levels remained within the 'B' classification. Therefore there is some evidence that shellfish tissue *E. coli* compliance issues may be event driven in the summer. Winter events produce some increase in tissue *E. coli* levels, but to a lesser extent than during the summer.

18. *E. coli* levels at Site B were negatively correlated with norovirus GI and GII levels during the winter, although the relationship is not highly significant

(0.05>p>0.001). The relationship was not replicated at Site A. It is possible that Site B is under greater influence of sewage from the local human population. The inconsistency in significant relationships between the two sites may also be a consequence of the low levels of norovirus detected at the study sites.

19. Tissue FRNA bacteriophage levels did not show a seasonal pattern of occurrence and there was no correlation with norovirus levels, at either site. This suggests that FRNA bacteriophage would not act as a good indicator of norovirus contamination in cultured shellfish in Loch Etive. Other studies have reported a significant relationship between the two parameters.

20. Norovirus levels showed no response during the summer event sampling weeks. Levels of norovirus showed little sign of increase during the winter event sampling, with the majority of results returned as less than 25 PCR units. The maximum norovirus GI level (50.8 PCR units) at Site A was detected during the winter event, but this still represents a low level compared with maxima recorded at other sites in the UK.

21. Dunstaffnage 7 day average rainfall was correlated to tissue *E. coli* levels at both sites during the summer months. River Strae flow (24 and 48 hr average flow rate) was also correlated with tissue *E. coli* at both sites. The resultant correlations for these parameters were highly significant at Site B. This indicates that *E. coli* summer compliance issues in cultured shellfish from the study site are likely to be driven by rainfall/riverflow events. In general, a greater number of significant relationships, some being highly significant, were observed between summer tissue *E. coli* datasets and rainfall/river flow parameters were observed for Site B. This may indicate that such environmental factors have a stronger influence on faecal bacterial levels at Site B, which is in closer proximity to the major freshwater inputs to the loch.

22. The results of the weekly sampling programme indicate a degree of fluctuation in the concentration of enteric bacteria in shellfish tissue from week to week. This highlights the need for regular monitoring to fully capture rapidly changing levels in microbiological parameters in cultivated shellfish.

23. Designated sampling points for EHO classification purposes should be fixed throughout the monitoring season. These should facilitate better characterisation of monitoring site and produce more robust data for classification purposes. This was implemented by FSAS in early 2007

Source apportionment for faecal indicator fluxes into Loch Etive outer basin

24. The source apportionment study investigated faecal indicator organism (FIO) budgets from sewage and riverine sources draining to the outer basin of Loch Etive, Scotland above the Falls of Lora road crossing. The budgets were constructed to provide an indication of the relative contribution of 26 out of 34 identified sewage and stream inputs. This report does not include study of the

fate and transport of faecal indicators within the waters of the loch itself that might infer a link from any discharge to compliance monitoring points. This would require a separate microbial tracer exercise ideally linked with a hydrodynamic modelling investigation to quantify the impacts of individual inputs at compliance locations.

25. It should be noted that this study considered a summer condition when other investigations have suggested that agricultural sources of faecal indicators in Scottish streams are at their maximum. Similar previous studies have largely been driven by bathing water compliance considerations and have, thus, centred on summer fluxes. It may be more appropriate to understand the full annual cycle of faecal indicator fluxes where shellfish hygiene is the principal driver but this would require winter and summer conditions to be characterised.

26. Twenty-nine river and stream sample sites (including five sites upstream of the catchment outlet sample point) were selected to provide data for the budget calculations. The only continuous sewage discharge impacting the outer basin of the loch, Taynuilt wastewater treatment works (WwTW) was also sampled along with any combined sewage overflow effluents that spilled during the study period. Flow monitoring was undertaken using existing installations and temporary monitors.

27. Geometric mean (GM) FIO concentrations in river samples generally showed a statistically significant elevation, often in excess of an order of magnitude, during high flow compared to base flow conditions. Three catchments were notable for particularly high FIO concentrations at their tidal limits: Abhainn Achnacree, Inion Farm stream and Kenmore Bay stream. The high concentrations in Abhainn Achnacree may be caused by elevated levels in one of its tributaries, Allt nam Ban. Concentrations in the Inion Farm stream were lower upstream of the farmstead whilst those in Kenmore Bay stream were lower upstream of Ardchattan School.

28. Geometric mean concentrations of FIOs in the Taynuilt WwTW secondary treated final effluent (FE) showed statistically significant dilution during high flow events during the 2006 field survey period. However, data from the 2007 field survey period showed the more commonly observed pattern of increased GM concentrations during high flow events. Both the treated FE and the CSO effluent from Taynuilt WwTW displayed FIO concentrations lower than similar effluents sampled by CREH in previous studies.

29. The estimated FIO budgets for Loch Etive showed the largest contribution was derived from the River Awe, which accounted for up to 37% of the faecal coliform (FC) and intestinal enterococci (EN) loads estimated using 2006 data wherever possible, although a lower proportion (up to 25%) was estimated if using 2007 data wherever possible. This contribution was a function of the much greater discharge of the River Awe, which accounted for 88% of the freshwater input to the outer basin of the loch. However, its FIO concentrations were some of the lowest observed in the studied catchments. This source dominated FIO delivery during base flow periods, accounting for

up to 95% of the instantaneous flux of organisms, although its importance was diminished during high flow events, when inputs from other catchments were dominant.

30. Relatively large contributions to the FIO load discharged to the outer basin of Loch Etive during the study period were derived from the catchments of Abhainn Achnacree (FC: up to 15%, EN: up to 10%), Inion Farm stream (FC: up to 5%, EN: up to 19%) and Kenmore Bay stream (FC: up to 0.5%, EN: 16%). The proportional contribution of these sources to the instantaneous delivery of organisms in the two budget estimates peaked at 53% for FC in Abhainn Achnacree, 34% for FC and 54% for EN in Inion Farm stream and 39% for EN in Kenmore Bay stream. This further illustrates the counter-intuitive importance of small catchment sources and episodic pollution fluxes in the overall faecal indicator budget.

31. Other relatively large contributors to the FIO load discharged to Loch Etive were Lusragan Burn, which contributes a relative high proportion to both the base flow and high flow instantaneous flux of organisms, and the River Esragan, which is an important source later during high flow events when some of the more 'flashy' streams have returned to base flow.

32. Taynuilt WwTW FE was estimated to contribute between 2% and 5% of the FIO load to the loch. Data from 2006 showed the majority of this load was delivered during base flow conditions, when FIO concentrations in the effluent were generally higher than during high flow periods. However, the data from 2007 showed an increase in FIO concentrations during high flow events, with a greater proportion of the FE total load discharged during the high flow events. Although the overall contribution of the FE was relatively small, the two alternative budgets constructed for the current situation showed that the proportion of the instantaneous flux of FIOs represented by the FE peaked at 43% during the study period. The CSO at Taynuilt WwTW accounted for <1% of the total FIO input to the loch, although its instantaneous contribution could be as high as 9% when it is discharging.

33. Adjusted budgets, using the lower FIO concentrations observed upstream of potential sources in Abhainn Achnacree, Inion Farm stream and Kenmore Bay stream during the 2006 study period suggested that remediation measures in these small catchments alone could reduce the overall FC load by 14% and the overall EN load by 31%.

34. During the high flow periods, when compliance of the shellfish beds are compromised, the adjusted budget suggests there would be a 13% and 45% reduction in FC and EN delivery respectively. The flux of organisms during high flow accounted for 54% and 44% of the adjusted total FC and EN loads respectively.

35. Stream inputs clearly dominate the total faecal indicator flux to the basin during low and high flow conditions. One issue, not addressed by this investigation, is whether faecal indicators discharged from the septic tank to Dunstaffnage Bay could, under certain tidal conditions, contribute to

concentrations above the Falls of Lora. A microbial tracer exercise and sampling programme of locations close to shellfish harvesting areas would best address this question.

Table of Contents

1. Introduction

1.1 Shellfish cultivation in Scotland

In recent decades shellfish cultivation has become a major industry for coastal rural communities. The period 1995 – 2005 has seen a substantial increase in annual production figures for all cultivated shellfish species. Production figures (for table consumption) totalled 1136 tonnes (all species) in 1995 and this rose to 4464 tonnes in 2005 (FRS, 2005). Production is dominated by mussel cultivation, with 4135 tonnes produced in 2005 (from a figure of 882 tonnes in 1995). This figure was 2 % less than 2004 production figures for mussel cultivation (FRS 2004). In 2004 the total value of the Scottish shellfish production industry for table consumption was estimated at £6 million, with mussels accounting for £3.4 – 5.4 million.

Cultured Scottish shellfish species such as oysters (*Crassotrea gigas* and *Ostrea edulis*), mussels (*Mytilus edulis*) and scallops (*Pecten maximus and Chlamys opercularis*) are filter-feeding bivalve molluscs. During feeding, water, containing suspended particles, is drawn through a siphon and passed over the gills where particles are filtered from the water. Particles are then passed to the mouth by means of a mucus flow from the gill filaments. This process serves to concentrate micro-organisms and other particles of the optimum size. This can include harmful bacteria, viral pathogens and toxic chemicals if they are present in the water column of the shellfish growing waters. Suitable sized particles may be 100 times more concentrated in shellfish tissue than in the surrounding medium. Shellfish are often consumed raw or lightly cooked, thus the presence of toxic micro-organisms and chemicals in the tissue of the shellfish could pose a significant health risk.

In the EU, two main regulations govern the microbiological standards for shellfish cultivation, the EU Food Hygiene Regulations and the Shellfish Waters Directive. The Food Standards Agency for Scotland (FSAS) is the Competent Authority responsible for the implementation of shellfish hygiene regulations in Scotland. Water bodies used for production and harvesting of shellfish are designated as 'shellfish production areas' under the EU Food Hygiene Regulations (852/853/854). This set of regulations serve to ensure the safety of commercially harvested shellfish intended for human consumption and came into force in January 2006, and supersedes the Shellfish Hygiene Directive (91/492/EEC) (www.defra.gov.uk). Regulation EC (No) 854/2004 requires FSAS to classify designated shellfish production areas according to the level of faecal contamination. The standards are based on *Escherichia coli* concentration in shellfish tissue (Table 1.1), as determined by an accredited MPN (Mean Probable Number) method. All *E. coli* analysis for classification purposes must be carried out by suitably accredited laboratories. Bivalve molluscs are monitored from designated sampling sites within production areas according to a sampling programme set by FSAS. Currently, classification requires a minimum 6 samples to be tested in separate months over the year for a given area, although in the majority of cases monitoring is conducted on additional months throughout the year.

Production areas are then given a yearly classification based on the results obtained for the previous year.

Table 1.1. Details of shellfish classification microbiological criteria

Classification	***E. coli* (per 100g flesh and intervalvular fluid)**	**Requirements**
A	< 230	Approved for direct consumption
B	< 4600	Must be depurated, heat treated or re-laid to meet category A
C	> 4600 -46000	Must be re-laid for 2 months to meet category A or B. May also be heat treated by approved method

In Scotland there are 108 (www.defra.gov.uk, 2006) shellfish growing waters (SGW) regulated under the Shellfish Water Directive, 79/923/EEC. In October 2007 this Directive was amended to "Directive 2006/113/EC". This directive states that designated waters require protection to ensure the quality and productivity of shellfish and must meet minimum environmental quality standards. The parameter used to indicate faecal contamination of SGW's is faecal coliform (FC) concentration. However, at present, there is no mandatory standard set for faecal coliforms (FC) under this directive, but a guideline standard has been set at ≤ 300/100 ml of shellfish tissue and intervalvular fluid in 75% of samples. The designation and regulation of SGW is carried out by SEPA (Scottish Environment Protection Agency). Sampling for FC concentration in SGW's currently is carried out every three months (SEPA *pers. comm.*).

In addition to harmful bacteria, shellfish are known to concentrate viral pathogens present in the water column. Occurrences of enteric viruses such as norovirus, Hepatitis A and enterovirus in shellfish has been widely reported (Croci *et al*., 2007; Maekawa *et al.,* 2007; Nishida *et al.,* 2007; Phan *et al.,* 2007; Constantini *et al.,* 2006; Cheng *et al.,* 2005; Myrmel *et al.,* 2004; Pommepuy *et al.,* 2004; Formiga-Cruz *et al.,* 2002, 2003; Hernroth *et al.,* 2002; Le Guyader *et al.,* 2000; Green *et al.,* 1998). A number of viral outbreaks have been attributed to consumption of shellfish, including hepatitis A and enteric viruses such as norovirus (Webby *et al.,* 2007; Le Guyader *et al.,* 2006; Prato *et al.,* 2004; Lees 2000; Kohn *et al.,* 1995). Viruses are known to be more resilient than bacteria in the environment and are able to survive longer in the water column and sediments (Power & Collins 1990). Viral detection techniques have indicated that the presence of human enteric viruses has been highly correlated with *E .coli* concentration in growing waters affected by high levels of faecal contamination (Formiga-Cruz *et al.,* 2003). However, in cleaner areas (A and low B classification) this relationship is lost and enteric viruses can be isolated even when *E. coli* counts are low (Formiga-Cruz *et al.,*. 2003, 2002; Hernroth *et al.,*. 2002; Le Guyader *et al.,*. 2000). However, at present there are no standards, guidelines, or routine monitoring practices for viral contamination of shellfish requirements under current EU regulations. This has been driven by a lack of standardised methodology and quality assurance measures. It is expected that criteria for viral contamination will be set once these issues have been resolved. Bacteriophage strains have previously been used as indicators of enteric viral presence in some areas. FRNA bacteriophages associated with human faecal bacteria have been shown to have a

similar fate in the environment to that of human enteric viruses and may prove to be a useful indicator of viral infection in shellfish tissue (Havelaar *et al.,* 1986).

1.2 Study site – Loch Etive, West Coast of Scotland

Loch Etive has been an important site for mussel cultivation for some years, with production commencing in the early 1980's. With such a long history of mussel cultivation, the operators in Loch Etive have witnessed the rapid development of the industry in terms of technology, regulation and management techniques. From the 1980's production levels have grown steadily and, at its peak Loch Etive produced approximately 1000 tonnes of mussels (in 2000). This accounted for approximately one third of the total Scottish production for that year. There are a number of production sites in Loch Etive and several operators (Figure 1.1).

The majority of the Loch Etive area is administered by Argyll and Bute Council, who are responsible for the collection of shellfish hygiene classification samples (for FSAS). The loch is divided into two production areas – Loch Etive West and Loch Etive East, separated by the Bonawe Narrows (see below for description of area). Classification records are available for both production areas from 1999. For most of that period Loch Etive West has had three sampling sites. *E. coli* results indicate that most of the samples are classified as A or low B (Figure 1.2). However, it is apparent that more substantial faecal contamination of shellfish does occur, and on a number of occasions samples registered in excess of 4600 *E. coli* (per 100g MPN). A further complication exists, in that contamination events (as indicated by high B or C samples) do not appear to be synchronised across all sites in this production area. On several occasions a 'C' classification sample has been returned for one site, while the other two sites returned either 'A' or low 'B' samples (for example 29/9/1999, 10/9/2003, 15/11/2004, 20/7/2005 and 5/9/2005).

Loch Etive East has 5 sample sites, with one site being sampled regularly since 1999. Again, the majority of samples have returned 'A' or low 'B' results, but there are several occasions where more substantial contamination has been detected (Figure 1.3). The majority of these higher contamination occasions occur during the summer/early autumn (June to October), although 'C' class results have been returned outside that period. Contamination patterns seem to be more synchronised across the sites in Loch Etive East, than in Loch Etive West, with higher results tending to occur simultaneously across the production area.

The historical data appears to show an increase in the number of higher contamination events over time, particularly from 2003 onwards. This may, in part, be a result of more sample sites and more sampling occasions in the later years (Table 1.2). Loch Etive East's 1 sample site increased to 5 by August 2003 and Loch Etive West increased from 2 sites to three at the same time. A summary of the results (Table 1.2), in terms of the proportion of 'A' and 'C' class FSAS results does indicate a decrease in the proportion of 'A' samples in each production area. For both production areas, 'A' class results

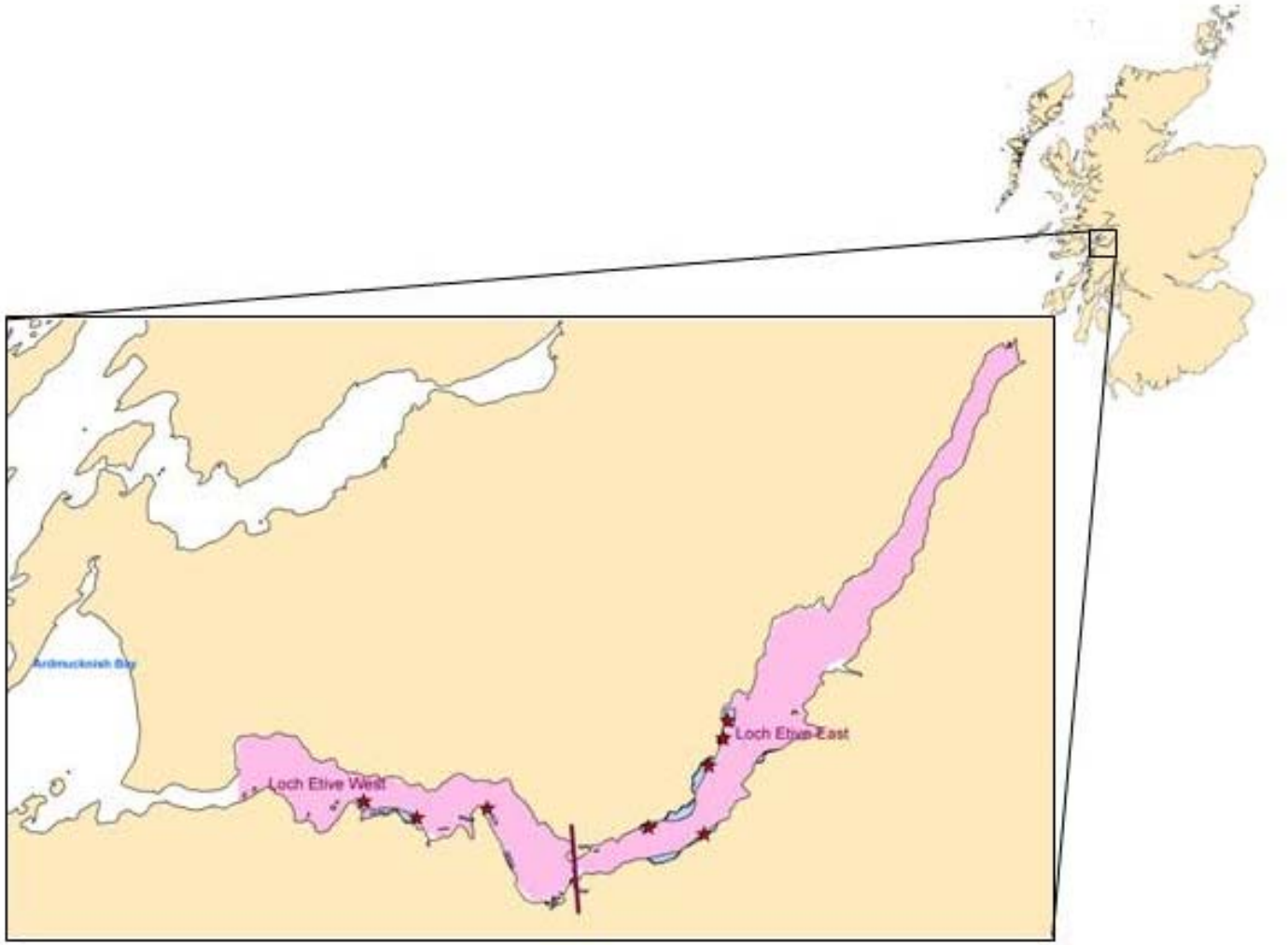


Figure 1.1 Location of Loch Etive, shellfish lease sites , FSAS Production Areas and sample sites, and shellfish growing waters

dominate the results each year, apart from 2005 in Loch Etive East when 0.43 of the results were 'A'. Each year over 50 % of Loch Etive West's results were 'A' and the lowest proportion occurred in 2004 at 0.51 (Table 1.2). There were no 'C' class results in Loch Etive East in the years 1999-2001 and in Loch Etive West in 2000 and 2001. Levels of 'C' class results peaked in Loch Etive East in 2004 at 10 %. Loch Etive West in had 17 % of results returned as 'C' in 1999, however, sample size is small (12). During the period 2002-2003 incidence of such results peaked at 14 % in 2005.

Table 1.2. Incidence of 'A' and 'C' *E. coli* classification results in Loch Etive production areas. Raw data courtesy of FSAS.

Year	Loch Etive East			Loch Etive West		
	No. of samples	Proportion of 'A' results	Proportion of 'C' results	No. of samples	Proportion of 'A' results	Proportion of 'C' results
1999*	3	0.67	0	12	0.67	0.17
2000	4	0.75	0	12	0.67	0
2001	7	0.57	0	11	0.82	0
2002	18	0.56	0.06	23	0.52	0.04
2003	39	0.67	0.08	26	0.61	0.12
2004	58	0.59	0.10	35	0.51	0.09
2005	44	0.43	0.07	28	0.54	0.14

*1999 production area monitoring was carried out under Fisheries Research Services (FRS), from 2000 onwards the monitoring was carried out under FSAS.

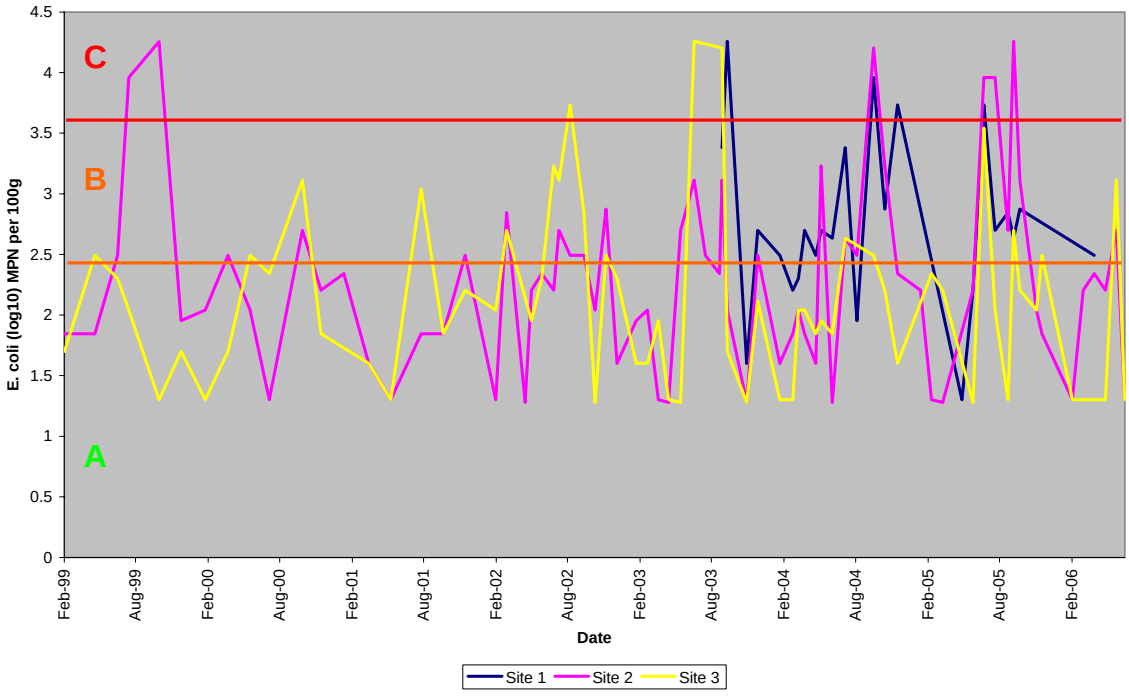


Figure 1.2. *E. coli* classification sampling results for Loch Etive West (courtesy of FSAS)

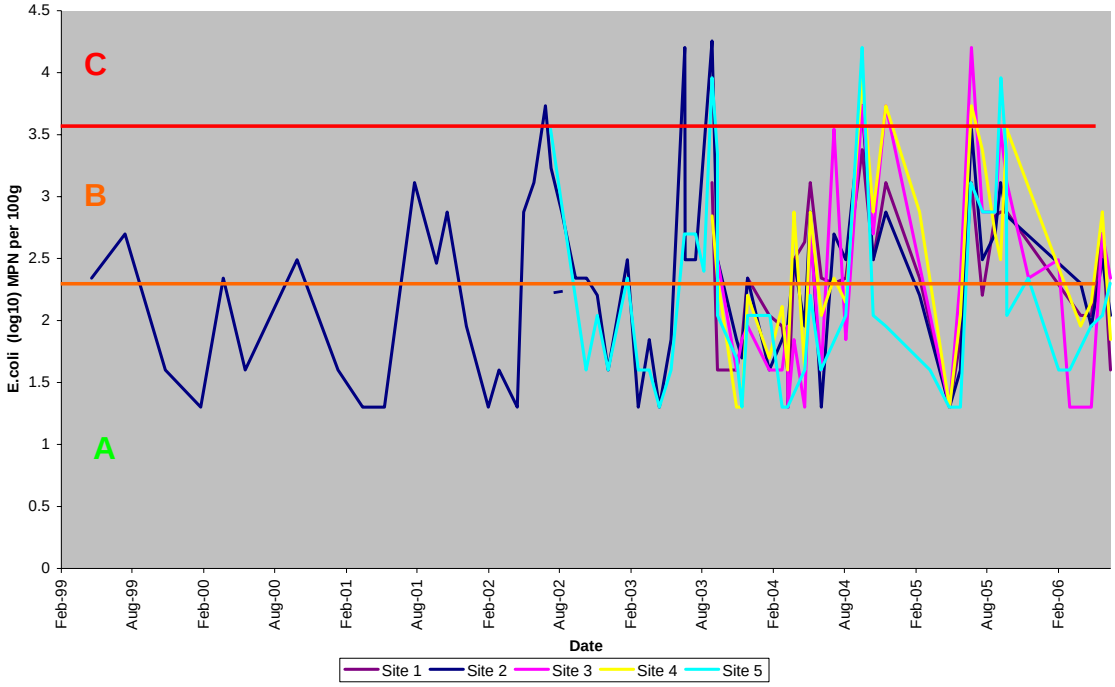


Figure 1.3. *E. coli* classification sampling results for Loch Etive East (courtesy of FSAS)

1.3 Objectives

The objectives were to identify key pollution sources and conditions contributing to viral and bacterial contamination of shellfish with the aim of determining critical environmental contamination triggers for shellfish production. The project consisted of three elements

- Sanitary survey
- 12 month monitoring programme
- Source apportionment study of faecal indicator bacteria

The Sanitary Survey was carried out in accordance with the US FDA National Shellfish Sanitation Programme Guide for the Control of Molluscan Shellfish 2003. The aim of the sanitary survey was to provide an in-depth evaluation of all the environmental factors that have a bearing on the water quality in a shellfish growing area. The 12 month monitoring programme aimed to assess the contamination levels of faecal bacteria and the viral pathogen norovirus in cultivated shellfish. Sampling was carried out on a weekly basis to provide high resolution data on these microbiological parameters in shellfish. Four week long sampling events, consisting of daily sampling, were carried out in addition to the weekly sampling programme. A source apportionment study was devised to provide characteristic riverine and effluent quality that could be combined with flow data to enable calculation of bacterial flux estimates.

2. Sanitary Survey of Loch Etive

Shona Magill

Scottish Association for Marine Science
Dunstaffnage Marine Laboratory
Dunbeg
Oban
PA37 1QA
United Kingdom

2.1 Introduction and Literature Review

The US National Shellfish Sanitation Programme (NSSP), introduced in 1925, states a number of criteria for the safe production of shellfish.

1. the area should be sufficiently removed from major sources of pollution so that the shellfish would not be subjected to faecal contamination in quantities which might be dangerous to human health
2. the area is free from even small amounts of fresh sewage
3. bacteriological examination does not ordinarily show the presence of coli-aerogenes group of bacteria in 1 ml dilutions of the growing area water.

The collective application of these criteria was known as the sanitary survey, and was used to determine if an area was safe for harvesting of shellfish intended human consumption.

Faecal contamination or pollution of water bodies can be categorised as point and non-point (diffuse). Point source refers to pollution that has a discrete or identifiable discharge point. Such sources are predominantly anthropogenic in origin and can be domestic, commercial or industrial. Discharges from waste water treatment works and from combined sewage outfalls are significant (point) sources of faecal contamination, particularly in urban areas. This pollution is largely controlled by a range of treatment options from septic tank (primary treatment) through to tertiary treatment involving sand filtration and UV disinfection, before the final effluent is discharged. In most urban areas, domestic sewage is collected and transported to WwTW (Waste Water Treatment Works) by the public sewage network. However, in small communities and rural areas, connectivity to the public network can be poor. Rural dwellings can be dispersed such that the cost of providing connection to the public network is prohibitive. Many such dwellings may have septic tanks, soak-away or small package treatment plants such as RBC (Rotating Biological Contactor) units. However, many older dwellings sited in close proximity to the shore line may discharge raw sewage directly into the water. Faecal coliform output estimates, provided by Scottish Water are summarised in Table 2.1.

Non-point-source pollution occurs where the source of pollution cannot be identified or pinpointed. Non-point source, or diffuse, pollution can enter the environment by a number of routes. Such sources can be difficult to monitor and control. Individually sources may be small, but in a large rural catchment collectively the impact can be substantial (DEFRA 2004). Diffuse pollution can be a significant contributor to bacterial and viral degradation of shellfish and recreational waters (www.environment-agency.gov.uk). This occurs through faecal contamination of coastal waters and catchment rivers and streams.

Table 2.1. Faecal coliform loadings from domestic sewage

Sewage treatment level	Estimate of faecal coliform output (per 100 ml of effluent)
Raw sewage	$1 \times 10^6 - 2 \times 10^7$
Primary	$1 \times 10^5 - 1 \times 10^6$
Biological (secondary)	$1 \times 10^4 - 1 \times 10^5$
Tertiary	$< 1 \times 10^3$

Non-point-source pollution largely occurs as a result of runoff and leaching from the land. The impact of diffuse sources is highly episodic, being strongly linked to heavy rainfall and storm events (Crowther *et al.,* 2002, 2001). Following heavy rainfall, or even snow melt, movement of water across the ground (soil and anthropogenic materials such as tarmac and concrete) can carry faecal material and pathogens, litter and chemical contaminants to drains, catchment rivers and streams. Contaminants are thus transported to inland water bodies and coastal waters where recreational and shellfish waters may be at risk. A recent UK study estimated that geometric mean concentrations (calculated as the antilog of the arithmetic mean of $\log_{10}$ concentrations) of faecal indicator organisms can be increased 10-fold during high flow conditions (Crowther *et al.,* 2003, 2002). In New Zealand, faecal indictor organisms were found increased by 2 orders of magnitude during the rising limb of a flood event (Nagels *et al.,* 2002)

Diffuse faecal pollution sources are, however, often difficult to identify and quantify as rivers and streams may receive diffuse inputs from a variety of sources. For instance, large catchment rivers may also contain sewage outputs from CSOs (Combined Sewer Overflows) and storm overflows following very high rainfall. Under high flow conditions the inflow to WwTWs can exceed design capacity and untreated inflow can then bypass treatment processes and be discharged directly to the environment. Heavy rainfall can also cause overflow events in septic tank systems. Faecal contamination of streams and rivers can also result from run-off from agricultural livestock grazing and terrestrial wildlife areas. Aquatic wildlife, such as birds (Wither *et al.,* 2005b; Jones & White 1984) and marine mammals also have the potential to make a significant contribution to faecal bacterial flux to coastal waters. A number of studies have further indicated that stream sediments may also act as a store for faecal indicator organisms (Wilkinson *et al.,* 2006; Muirhead *et al.,* 2004) which may be released during flood events.

Land and resource use can have a significant effect on non-point pollution sources. As populations increase in close proximity to rivers and coastal areas, natural and rural areas become replaced by man-made structures and surfaces such as concrete and tarmac. Such materials are impervious and unable to absorb storm water or filter contaminants found in run-off (Mallin *et al.,* 2000). This can result in rapid transport of contaminants to drains and catchment streams (Mallin *et al.,* 2001). Forestry management operations such as tree felling and ploughing disturb and expose large areas of soil. This affects the integrity of forest soils and large amounts of sediment can be lost into adjacent catchment streams and rivers, if activities are badly managed. In addition, the natural ability of these soils to absorb run-off and related contaminants can be altered such that run-off volume is increased (DEFRA, 2004). Higher levels of suspended solids and faecal coliforms were detected in a creek stream for 15 months following timber harvesting, despite a 10 m buffer zone from the stream (Ensign & Mallin 2001). In preparation for harvesting, forestry operators build forest roads which can be highly destructive to the forest floor and can further compromise the ability of the land to absorb and filter surface waters. Forestry activities are, however, highly regulated in the UK and all commercial forestry operators must adhere to strict guidelines in order to minimize the impact on the local catchment area (Forestry Commission 2003).

Faecal contamination of water courses and associated shellfish growing areas can originate from both human and animal (domestic and wild) sources (Cox *et al.,* 2005; Derlet *et al.,* 2004; Donnison & Ross, 1999; Parveen *et al.,* 1999). In large rural catchments faecal contamination may be predominantly non-human sourced (Carroll *et al.,* 2005). *E. coli* is largely found in the intestine of warm-blooded animals (Orskov & Orskov, 1981), thus its presence is not necessarily indicative of human-sourced pollution. Non-human sourced faecal bacteria and viral pathogens are known to originate from domestic livestock, aquatic and terrestrial wildlife (Griffin *et al.,* 2000; Orskov & Orskov, 1981). Potential *E. coli* loads originating from these sources are reported to be comparable, or greater than that of humans (see Table 2.2) (Wither *et al.,* 2005a). Faecal material from agricultural grazing land and rural catchment areas can be washed into streams and rivers and transported to open water bodies, where shellfish and recreational waters can become contaminated. In order to aid management strategies, a range of techniques have been employed to differentiate faecal contamination sources, including *E. coli* ribotype profiling (Parveen *et al.,* 1999), F+ specific RNA coliphage genotyping (Griffin *et al.,* 2000), DNA sequencing (Dombek *et al.,* 2000), antibiotic resistance analysis (Carroll *et al.,* 2005; Burnes 2003; Hagedorn *et al.,* 1999; Wiggins *et al.,* 1999) and sterol profiling (Suprihatin *et al.,* 2003). Sinton *et al.,* (1998) provides a comprehensive review of techniques employed.

Table 2.2. Estimated *E. coli* loadings from humans, selected domestic animals and birds.

	Faecal production (g per day)	*E. coli per g of faeces*	*E .coli* load per animal per day
Domestic chicken	182	1.3×10^6	2.4×10^8
Human	150	1.3×10^7	1.9×10^9
Gull	15	1.3×10^6	2×10^9
Cow	23600	2.3×10^5	5.4×10^9
Duck	336	1.3×10^8	1.1×10^{10}
Domestic sheep	1130	1.6×10^7	1.8×10^{10}

It is widely recognized that livestock agricultural practices can contribute significantly to the influx of faecal pathogens to inland and coastal surface waters (Meals & Braun 2006; Weaver *et al.,* 2005; Rodgers *et al.,* 2003; License *et al.,* 2001; Howell *et al.,* 1995; Fernandez-Alvarez *et al.,* 1991). Non compliance of water quality standards for inland rivers (Howell *et al.,* 1995) and recreational bathing waters has been attributed to agricultural run-off in a number of cases (Vinten *et al.,* 2004b). Collins *et al.,* (2005) reported *E. coli* concentrations of between 10^5 and 10^8 per m^2 transported to a catchment stream from grazing land following rainfall events. Seasonal contamination patterns have correlated well with pastoral stocking densities, with the highest faecal contamination observed in summer months (Hunter *et al.,* 1999). Vinten *et al.,* (2004a) also observed a seasonal pattern in *E. coli* contamination from grazing plots with the highest average counts (282 cfu ml^{-1}) in autumn. This study further concluded that following 7mm of rain approximately 14 % of the daily input of *E coli* from grazing animals was transported to the adjacent river. Grazing areas are often in close proximity to, or give livestock direct access to, field drains and catchment streams. As a result, faecal material and enteric pathogens can be shed directly into small rural waterways or deposited onto grazing areas where run-off can be contaminated after rainfall (Rodgers *et al.,* 2003). Davies-Colley *et*

al., (2004b) reported that peak *E. coli* concentrations of 50000 cfu 100 ml^{-1} were detected in a stream regularly used for cattle crossing. The same study also found a higher defecation rate at the stream than anywhere else, suggesting that cattle preferentially used the stream for defecation. Livestock aggregation points such as stream crossings, watering points and feedlots are likely to have an increased potential for faecal contamination (Miller *et al.,* 2004). Areas such as hard standings and farm yards can present an additional source of faecal material and bacteria (Rodgers *et al.,* 2003). Hard standings and farmyards can become fouled by manure and faecal material which can be readily washed into nearby streams during or following rainfall. Faecal bacterial flux to a small stream was observed to increase by 385% following input from a farmyard hardstanding area (D. Kay *pers. comm.*).

Particular agricultural practices have been linked to an increased potential for the bacterial contamination of catchment streams and rivers, including manure and slurry application to fields (Meals & Braun, 2006; Nunez-Delgado *et al.,* 2002) and intensive stocking practices (Aitken, 2003). A recent study indicated that application of slurry may result in an increase in run-off volume (Ramos *et al.,* 2006), thus potentially increasing the bacterial flux to streams and field drains. Significant increase in run-off bacterial flux was noted following such practices and this was particularly marked following rainfall events (Ramos *et al.,* 2006 - to between 1.9 x 10^4 and 1.1 x 10^6 Presumptive FC 100 ml^{-1}; Nunez Delgado *et al.,* 2002). In a study of 117 farms in two river catchments in south west Scotland, 50 % of farms were at risk of impacting the local water course through application of manure or by intensive stocking of livestock (Aitken, 2003). Aitken (2003) concluded that faecal indicator organisms were between 4 and 8 times higher in sub-catchments with high livestock stocking density than in sub-catchments with low stocking densities.

Faecal contamination from wild birds can be a significant non-point pollution source in surface waters (Kirschner *et al.,* 2004; Suprihatin *et al.,* 2003; Ricca & Cooney, 1998; Levesque *et al.,* 1993; Jones & White 1984). Birds, such as feral pigeons, seabirds, geese, ducks and wildfowl, can gather in large numbers near open water bodies and in close proximity to recreational and shellfish waters. These aggregations may relate to feeding ecology, migration patterns or breeding colonies. It has been estimated that 100 gulls can have the equivalent FIO (Faecal Indicator Organism) input to that of effluent from a secondary WwTW with a capacity for a population of 10,000 (Jones and White, 1984). In a study of the impact of bird populations on the water quality of the bathing waters of the Flyde Coast, UK, Wither *et al.,* (2005b) reports up to 30000 starlings roosting on piers. Seasonal concentration of faecal indicator organisms (and therefore water quality) was significantly correlated to spatial distribution of the birds in close proximity to the bathing waters. Fluctuation in faecal coliform counts in inland water bodies in Canada has been attributed to the migration pattern of Canada geese (*Branta canadensis*) (Wakelin *et al.,* 2003). Analysis of faecal material from wild birds has indicated up to 10^9 thermotolerant coliforms, 10^2 F-specific coliphages and 10^6 somatic coliphages per gram of faeces (Ricca & Cooney, 1998).

As warm-blooded animals, marine mammals are a potential non-point source of faecal contamination. Harbour seals (*Phoca vitulina*) are reported to contribute to contamination of a number of shellfish growing areas within the United States (Nash *et al.,* 2000). Analysis of weddell seal (*Leptomychotes weddellii*) faecal samples from Antarctic indicated that faecal indicator organisms (including faecal coliforms and *E. coli*) occurred at concentrations similar to that of untreated sewage (Lisle *et al.,* 2004). It would follow that seals could represent a substantial reservoir of faecal pathogens, in areas where they are found in large numbers.

There is little information available to suggest that fish are a source of faecal coliforms, such as *E. coli*, in the aquatic environment. *E. coli* may be ingested by fish from the surrounding environment (where it has been introduced from other sources) and the presence in the intestine has been taken as an indication that the fish has had passage through faecal contaminated waters (Geldreich & Clarke, 1966). More recent work has suggested that *E. coli* may be introduced to farmed fish via contaminated feed and water (Pal & Das Gupta, 1992). Al-Harbi, (2003) attributed coliforms found in tilapia to local pigeon populations contaminating the pond water. *E. coli* has been found to become established and to grow within the intestine of fish (Niemi, 1985). In a study of rainbow trout fed on *E. coli* contaminated feed, the bacteria was found to increase in the intestine and was still viable after 4 days at 15 ˚C and 2 days at 6 ˚C (Del Rio Rodrigues *et al.,* 1997). Farmed fish are generally confined to one site (therefore there is unlikely to be passage through contaminated water). It is possible that *E. coli* introduced via contaminated water (point or non-point source) or feed could increase in concentration on transit through the farmed fish, but any subsequent impact on the surrounding environment is likely to be small and localised. Merceron *et al.,* (2002) reported no detectable increase in faecal coliforms as a result of a brown trout farm on the French coast.

Environmental factors have the potential to influence the flux of faecal pathogenic bacteria and viruses to inland waterways and coastal waters. Under certain conditions this can lead to substantial faecal contamination of shellfish and recreational waters, particularly in rural areas where diffuse pollution sources dominate (Crowther *et al.,* 2001). Increased bacterial concentration in rural streams and rivers has been reported following heavy rainfall or under high flow (Collins *et al.,* 2005; Shehane *et al.,* 2005; Crowther *et al.,* 2001). Similarly, faecal coliform concentrations in coastal waters and cultured shellfish have been shown to increase following such events (Brock *et al.,* 1985). However, events that lead to contamination of shellfish beds are likely to be complex and involve the interaction of a number of meteorological, hydrodynamic and hydrological factors ((Lee & Morgan 2003). For instance a number of studies have noted the importance of season and tidal cycle (Lee & Morgan, 2003), sunshine hours and wind direction (Crowther *et al.,* 2001) in contamination of shellfish and recreational waters. Chigbu *et al.,* (2004) reported significantly inverse relationships between faecal coliform concentration in coastal waters and salinity and seawater temperature, while positive relationships were reported for both rainfall and river levels.

Seasonal faecal coliform contamination patterns in rural streams and rivers have been widely reported, particularly over the summer months (Hunter *et al.,* 1999). This could, in part, reflect increased bacterial load from a higher grazing stocking density or that increased rainfall events during winter months effectively 'clear out' faecal bacteria from soil and stream sediments more effectively. FRNA bacteriophage occurrence in shellfish has been shown to be highly seasonal, with peak numbers occurring in winter months (Formiga-Cruz *et al.,* 2003). Similarly, viral contamination of the marine environment has been reported to peak during winter months and was linked to hydraulic overload to a sewage treatment plant following heavy rainfall (Moissec *et al.,* 2000). The same study reported enterovirus and norovirus contamination of shellfish to be significantly related to seawater temperature. Viability and survival of viral particles is known to be influenced by temperature, with increased viability during winter months (Gantzer *et al.,* 1998; Girones *et al.,* 1989). Ultraviolet light may also contribute to seasonal contamination patterns described in coliforms and viral particles in seawater. UV light is reported to lead to inactivation of some viruses (Gantzer *et al.,* 1998) and could thus partly explain winter concentration peaks in the marine environment. *E. coli* has also been shown to be sensitive to visible light while in seawater (Gourmelon *et al.,* 1997) and can influence bacterial survival rates (Davis-Colley *et al.,* 1994).

2.2 Objectives

The aim of this sanitary survey was to provide an in depth evaluation of all the relevant environmental factors that could have a bearing on bacterial and viral contamination of shellfish and associated growing water. The main components of the sanitary survey were;

1. Baseline study of the area under study – to collect available data and information on the topography, terrestrial and aquatic resource use and population areas within the catchment area of the shellfish growing water
2. Shoreline survey - to identify and evaluate all the potential pollution sources which may have an impact on faecal bacterial and viral contamination of shellfish waters. Both point and non-point sources were identified.
3. Assessment of the relevant environmental factors that may influence contamination patterns in harvested shellfish – to gather all relevant meteorological, hydrographic and hydrological data and information available for the study area, in order to evaluate critical contamination triggers for shellfish production.

2.3 Baseline study

2.3.1 Loch Etive

Loch Etive (OS reference NM890340) is a tidal fjordic sealoch on the west coast of Scotland (Figure 2.1). The loch is approximately 30 km long with a coastline of approximately 65 km and an area of 29.5 km^2 at high water. The loch is characterised by several (6 in total) shallow water sills across the width of the loch. The major sills (Connel Bridge and Bonawe Narrows) divide the loch into two dominant basins – the

lower and upper basins. The sill at Connel Bridge (320 m long, 440 m wide at high water with a depth of approximately 7 m) marks the boundary of the lower basin and the outer, most seaward of the basins (Edwards & Sharples 1985). The large volume of water passing this sill during the tidal cycle creates the impressive Falls of Lora tidal rapids. The water body between Connel Bridge and the Bonawe Narrows is referred to as the lower basin and has a maximum basin depth of 68 m. The sill at Bonawe Narrows (680 m long, 220 m wide at high water and with a depth of 13 m) marks the boundary between the lower and upper basin. The upper basin stretches from the Bonawe Narrows to the head of the loch at Glen Etive and has a maximum depth of 145 m. The classified shellfish production areas Loch Etive West and Loch Etive East are situated in lower and upper basins respectively

Due to the shallow and often narrow nature of the sills, the tidal exchange of water across the sills can be severely restricted. This affects both the tidal amplitude and creates a lag time in the tidal phase within the loch (Gage, 1972). Tidal range outside the loch has a maximum of approximately 4 m, compared with a 2 m maximum range inside the loch (Edwards & Edelsten, 1977). Tidal phase lags approximately 1 hour 50 minutes behind the waters outside of the loch.

The hydrodynamics of Loch Etive are complex and currents vary significantly both horizontally and vertically across the loch. The loch has a substantial freshwater input compared to the tidal influence from the coastal waters outside the loch (see below). However, unlike many fjordic sealochs the main freshwater input is not from the head of the loch (River Etive) but enters laterally (River Awe) adjacent to the Bonawe Narrows in the upper basin. Loch Etive presents a combination of freshwater dynamics, tidal regime, shallow sills and deep basins, which results in a spatially and temporally complex water body. This fact has been supported by modelling approaches (POLCOMMS), even with the freshwater component not included (P. Gillibrand, SAMS *pers. comm.*). Figure 2.2 gives an over-view of the range of tidal excursion from high water in different areas of the loch. The predicted distance that specific areas of water are moved following high tide are indicated. This figure quite clearly shows how different areas of the water body move greater distances over a tidal cycle and therefore will be subject to a range of current speeds. Little information exists on current speeds within the lower basin. Some current data may exist for the two fin-fish farms on Loch Etive, however, since only one farm operates in the lower basin the data could not be extrapolated to accurately represent the entire water body (A. Henderson, SEPA *pers. comm.*).

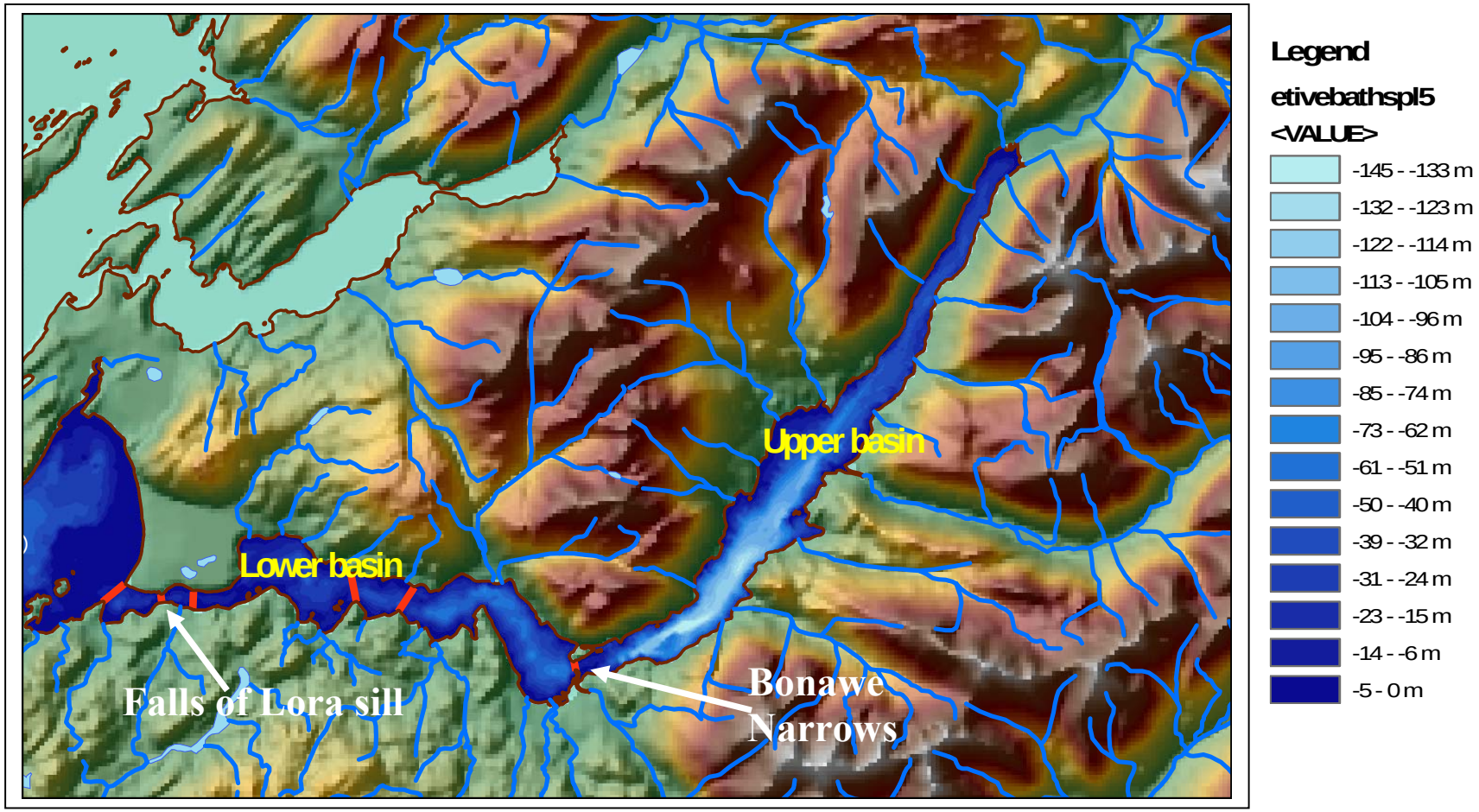


Figure 2.1 Loch Etive with bathymetry and sills indicated in red**.**

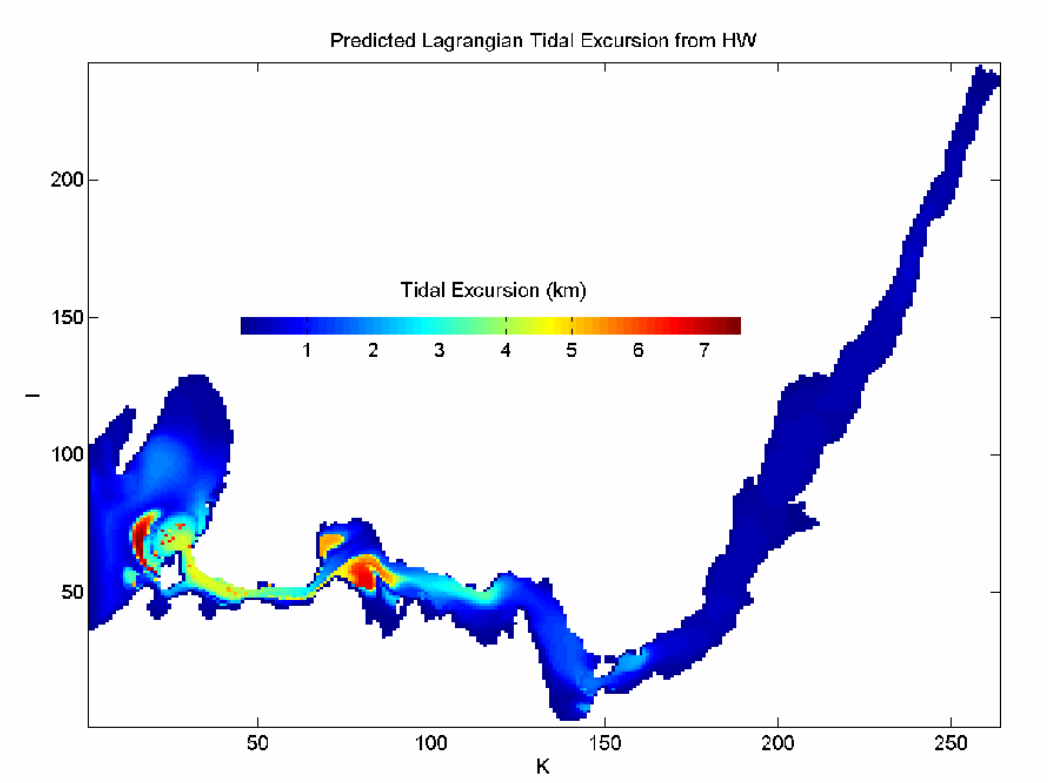


Figure 2.2 Simulated tidal excursion from high water, using POLCOMMS modelling approach. K and I are model grid cell units in increments of 100 m

2.3.2 Catchment, topography and land use

Loch Etive has a large catchment area of approximately 1350 km^2 (Edwards & Sharples 1985) with a number of major rivers draining to the loch (Figure 2.3, Table 2.3), including the rivers Awe, Kinglass and Noe in the upper basin and the rivers Nant, Esragan and Lusragan Burn in the lower basin. The River Awe in the upper basin connects Loch Etive to the freshwater Loch Awe. This is a substantial inland water body with an extensive catchment area. The catchment of Loch Awe accounts for more than half the total catchment area for the Loch Etive. A barrage operates along the River Awe, as part of a hydroelectric power scheme, and results in the flow of the river being largely controlled. Daily flow data for the barrage and the river is available from Scottish and Southern Energy, who operate the barrage. Loch Etive has a high fresh/tidal flow ratio of 4.1 (Edwards & Sharples, 1985) as a result of the substantial freshwater input relative to the total volume of the loch.

The Etive catchment has several areas designated as SSSI (Site of Special Scientific Interest). There are a number of sessile oak woodland areas, the Loch Etive Woods (grid reference NN040360), which are collectively designated (EU Habitats Directive) as an SAC (Special Area of Conservation) (http://www.jncc.gov.uk).

Table 2.3 Riverine inputs to Loch Etive, with estimated annual rainfall and flow details

River	Catchment area km^2	Annual rainfall mm	Mean flow m^3/s	Q95 [1] m^3/s	Q05 [2] m^3/s
Lower basin					
Lusragan	21.8	1847	0.99	0.08	3.06
Allt Nathais	10.3	2078	0.54	0.03	2.12
Allt na h-Airde	4.7	2047	0.25	0.03	0.94
Allt a' Bhile	45.0	2318	1.42	0.21	4.70
Nant	45.0	2318	2.8	0.19	9.56
Abhainn Achnacree	7.0	1878	0.34	0.03	0.99
Esragan	15.6	2512	1.12	0.05	4.17
Blacreen Burn	5.4	2174	0.34	0.03	1.18
Upper basin					
Awe	828.6	2540	49.67[3]	5.3	146.7
Noe	17.4	3235	1.63	0.13	5.87
Liver	10.6	3038	0.89	0.05	3.62
Kinglass	71.7	2929	5.89	0.26	24.4
Etive	151.2	2815	12.39	0.55	47.98
Allt Easach	14.7	2775	1.15	0.05	4.53
Abhainn Dalach	12.2	2728	0.95	0.04	3.99

[1] Q95 is the flow that is equaled or exceeded 95% of the time (i.e. a low flow). [2] Q05 is the flow that is equaled or exceeded 5% of the time (i.e. a high flow). Data courtesy of SEPA. [3] Estimated from daily discharge data for Awe barrage 2000-2006, raw data courtesy of Scottish & Southern Energy.

2.3.2.1 Upper basin

The land surrounding the upper basin of Loch Etive and Glen Etive is largely steep and mountainous, with the highest peak at 1126 m (Ben Cruachan which drains to the Rivers Noe and Awe). The basin has a catchment of approximately 350 km^2. The main rivers in the upper basin catchment are the Rivers Etive, Kinglass, Liver, Noe and Awe. The catchment area is largely used as hill sheep grazing land and has high numbers of wild deer (see below). Access is limited to a land rover track as far as Ardmaddy, near the mouth of the River Kinglass. The west shore of the upper basin is largely comprised of extensive areas of mixed open woodland and two areas of managed forestry. This shore is accessible by land rover as far as the lower stretches of Beinn Trilleachan. Apart from a few farms and remote dwellings there are no housing aggregations on the upper basin and human population numbers are small. There is a quarry operating outside of Bonawe on the north shore of the upper basin (Figure 2.4).

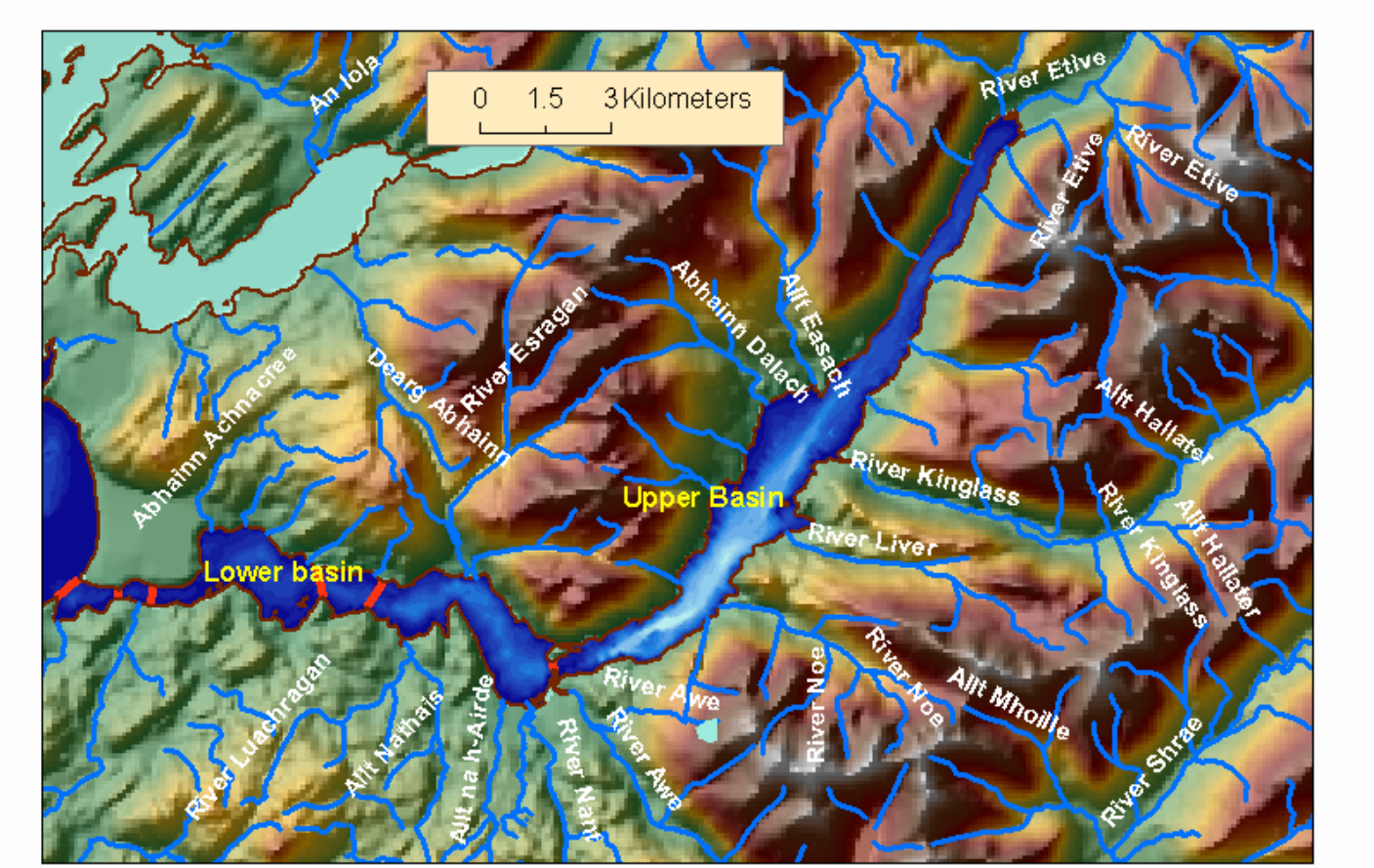


Figure 2.3 Immediate catchment of Loch Etive with major freshwater inputs indicated in white.

There are 3 areas of commercial forestry in the upper basin (Table 2.4 and Figure 2.4). There is also a stretch of woodland that is currently being managed as native oak woodland. Two of the areas are operated by the Forestry Commission and the third is a private concern (Forestry Commission 2003). In addition, there are several small areas of non-commercial mixed woodland. The Barrs and Cadderlie area is a young forest and as yet there have been no harvesting or major felling activities. A small ATV (all terrain vehicles) track has been built to give improved access to the area. Inverawe Forest has seen sustained harvesting activities over the past decade. The area has one main forest road, with another one planned in the near future (Forestry Commission *pers. comm.*).

Table 2.4 Commercial forestry areas in the immediate Loch Etive catchment

Site name	**Area**	**Operator**	**Felling activity**
Upper basin			
Barrs and Cadderlie	586 ha	Forestry Commission	No felling
Inverawe	420 ha	Forestry Commission	Consistent felling activity 2000-01, 2003-04, 2005-06
Glen Etive	1345 ha	Scottish Woodlands (private)	No details available
Lower basin			
Fearnoch	765 ha	Forestry Commission	Consistent felling from 2003 - 2006
Glen Nant	330 ha	Forestry Commission	Felling 1999, 2002-03, 2004, 2005
Barcaldine	290 ha	Forestry Commission	Felling 2002-03, Jun 2006
Ichrachan	n/a	Tilhill (private)	unknown

2.3.2.2 Lower basin

The topography of the lower basin is less dramatic than the upper basin. Here, the loch drains approximately 100 km^2 of lower elevated land. Major freshwater inputs include the Rivers Nant and Esragan, Lusragan Burn and Blacreen Burn. Outwith the main populated areas, the land is largely used for livestock grazing (sheep, cattle and deer) with a large area of managed forestry (see below) and scattered areas of mixed woodland. Much of the immediate lower basin shore is easily accessible via the A85 trunk road on the south and a minor coastal road on the north.

There are a number of commercial forestry locations within the area of the lower basin (Table 2.4). Fearnoch and Barcaldine Forests are both large forestry areas of which only parts are included in the Loch Etive catchment. Both forests are subject to regular harvesting activities. A large section of Fearnoch Forest is under 'constant cover' management. This means that harvesting is not carried out by clearing whole areas at a time. Such practices are likely to reduce soil erosion and the environmental impact of forestry activities. Glen Nant Forest is currently managed under a natural regeneration scheme. In addition, the lower basin has several areas of non-commercial mixed woodland.

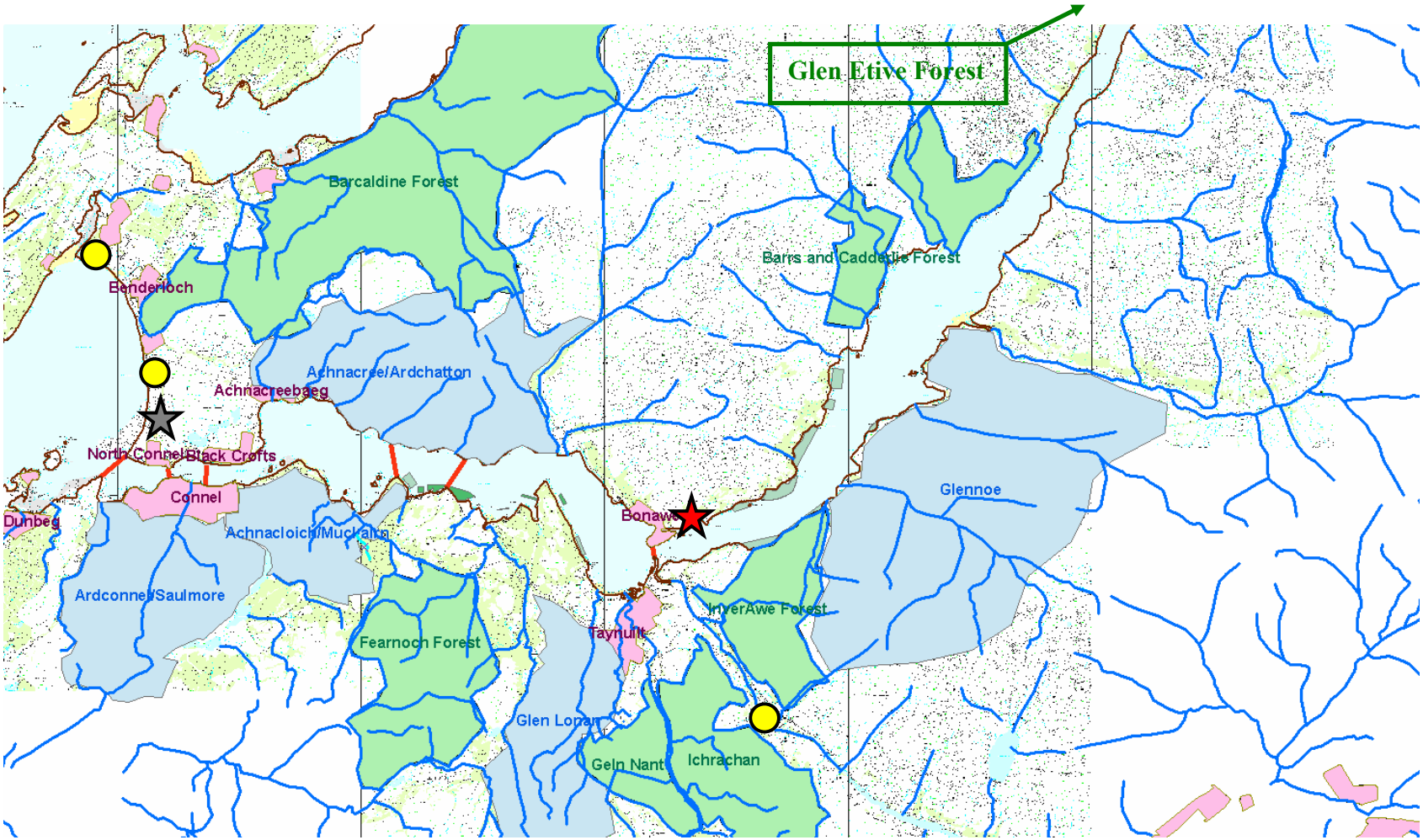
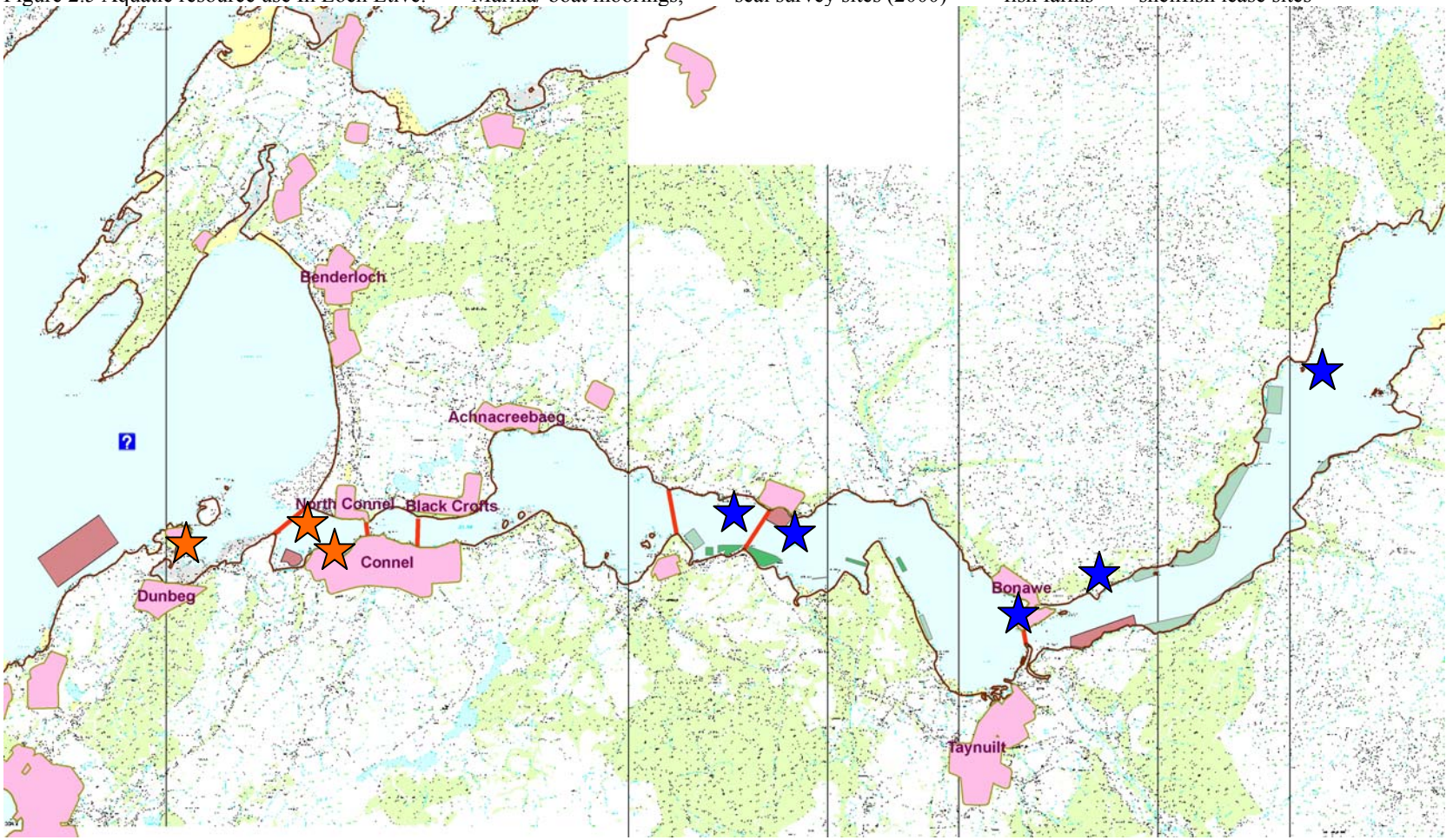


Figure 2.4 Loch Etive land resource use Commercial forestry Main agricultural areas Main population areas ● – Caravan and camping sites ★ – Bonawe Quarry ★ – Connel Airport

Figure 2.5 Aquatic resource use In Loch Etive. Marina/ boat moorings, seal survey sites (2000) fish farms shellfish lease sites



2.3.3 Aquatic Resource Use

Although not completely impassable, the tidal rapids of the Falls of Lora can act as a navigational hazard for commercial and recreational vessels. The majority of boat traffic within the loch is largely comprised of local commercial vessels, associated with fish farm activities and the tourist industry. Bonawe Quarry operates within the shellfish growing waters (SGW) and is regularly served by vessels involved in movement of quarry materials and equipment.

There are a small number of recreational vessels (small yachts and motor boats) moored within the lower basin (i.e. inside the Falls of Lora). The activity of recreational boats within and immediately outside the loch is highly seasonal, with greater activity in the summer months. Two small aggregations of moorings are found to the west of the Falls of Lora (outwith the SGW) with a capacity for approximately 30 vessels, although all the moorings are seldom occupied at any one time (see Figure 2.5 for main boat mooring areas). These moorings are used by small recreational and commercial vessels (fish farm service boats and small fishing boats). Dunstaffnage Marina is situated in Dunstaffnage Bay within close proximity of the mouth of the loch and 4.5 km from the SGW. The pontoon facilities have been extended in 2006 to increase capacity from 130 to 200 yachts. Again, use of this facility peaks during summer months.

Three marine fish farms operate within Loch Etive, all producing rainbow trout (*Oncorhynchus mykiss*). Two of the farms (Ardchattan in the lower basin, and Inverawe in the upper basin) are within the SGW. The third farm operates in Camas Bruaiche Ruaidhe Bay, to the west of the Falls of Lora and outwith the SGW. A larger fish farm producing Atlantic salmon (*Salmo salar*) operates in the Lynn of Lorne, approximately 3.3 km from the mouth of Loch Etive and 6.5 km from the SGW.

2.3.4 Population

All population data are estimated from official figures provided by the General Register Office for Scotland 2006. The south shore of Loch Etive has two main population areas (Figure 2.4), Taynuilt (population approximately 1100) and Connel (population approximately 540). On the north shore the main population area is North Connel (population approximately 560), the majority of which are spread out along a 4 km stretch of the Loch Etive shoreline. There is also a small population at Bonawe (population approximately 140). Taynuilt, Bonawe and part of North Connel lie within the SGW, while Connel is 1.5 – 3 km outside. Population numbers within the immediate catchment area is approaching 2500. The village of Dunbeg has a population of 900 and is approximately 5.5 km from the SGW, adjacent to Dunstaffnage Bay. Further afield is the village of Benderloch (population approximately 305), adjacent to Ardmucknish Bay. This takes the population in the area within and just outside the loch to approximately 3500. The town of Oban lies approximately 7.5 km from Connel and has a population of

around 8000. The main population centre on the shores of Loch Awe is the village of Dalmally with a population of approximately 300.

The area surrounding Loch Etive and its catchment is a popular tourist destination. The area is popular with walkers, scuba divers, cyclists and sightseers, particularly during the summer months. Thus, population numbers in the summer will be higher than during the winter. A number of hotels, guest houses and bed and breakfast accommodation operate within the area. An increase in population could place extra pressure on the local domestic sewage (both public and private) facilities. Precise data on tourist numbers in the immediate vicinity of Loch Etive is not available. An estimate of tourist numbers was obtained by looking at the number of beds available in the Visit Scotland official accommodation listings for the area. This method estimates maximum increase in population (i.e. when accommodation is fully booked) of 150 in Connel, 156 in Taynuilt, 52 in North Connel and 29 in Dunbeg. This represents a potential increase in population of approximately 379 people, 12 % of the resident population. This is likely to be a conservative estimate as some accommodation may not be included in the official listings. In addition, this does not include visitors to private residences.

Villages further afield also offer tourist accommodation. Benderloch has an estimated capacity of approximately 68. On the shores of Loch Awe, accommodation estimates are approximately 600, primarily in the villages of Dalmally, Lochawe and Kilchrenan.

There are three camping and caravan sites in the area, which can attract large numbers of tourists during the summer months. Crunachy Caravan Park lies adjacent to the River Awe and has a capacity for 80 caravans. Tralee caravan park has a range of chalets and static caravans, and has a capacity for 440 people, while Ledaig caravan park has a capacity for 140 mobile units. Both Tralee and Ledaig are found close to Benderloch (on the shore of Ardmucknish Bay), outwith the main water body of Loch Etive.

2.4 Shoreline Survey of Loch Etive and associated Shellfish Growing Waters

2.4.1 Objectives

The objective of the shoreline survey was to identify, describe and, where possible, quantify pollution sources with the potential to impact on Loch Etive, catchment area and the associated shellfish waters. This included point sources such as;

- Public network Wastewater Treatment Works (WwTW's)
- Combined sewer overflows (CSO) and emergency outfalls (EO)
- Industrial/commercial discharges
- Private septic tank systems
- Untreated outfalls

and non-point sources such as;

- Storm water run off
- Run off from agricultural livestock areas
- Run off from wildlife areas
- Aquatic wildlife (primarily birds and seals).

Following storms or high rainfall events, storm runoff, agricultural and wildlife runoff will affect flow levels in catchment streams and rivers. Such rivers and streams are likely to act as the main diffuse pollution source route to shellfish waters.

2.4.2 Point Source Pollution

2.4.2.1 Public network facilities

Public domestic sewage facilities are provided and maintained by Scottish Water. Given the rural and dispersed nature of the population around Loch Etive, a number of facilities, with varying levels of treatment, operate around Loch Etive. A significant proportion of the population are not connected to the public system. Public network facilities are described in Table 2.5 and indicated in Figure 2.6. The public network does not serve any dwellings in the upper basin

The main treatment works in the area is within the village of Taynuilt. This facility, established in 1978, is a secondary treatment plant (activated sludge works) (Figure 2.6, Table 2.5) which has a design capacity for a population of approximately 1400. Around 60 % of the local population is connected to the facility. The facility also has a CSO (combined sewage overflow). At present the CSO is not equipped with an event recording device. Therefore, time and quantity of CSO spillages cannot be determined. The main outfall for the WwTW and the CSO do not discharge directly into the SGW, but into the River Nant approximately 800 m from the mouth of the river (which is within the SGW).

The public facilities in the village of Connel consist of 3 small networks serving a total of 87 houses, a small school and a hotel/restaurant. Approximately 40 % of the population is on the public network. Up until July 2006 each of the networks discharged raw sewage into the loch, outside the SGW boundary (see Table 2.5). All three network facilities were upgraded with the installation of new pumping stations in early 2006. The works were completed in July 2006. Effluent is now pumped from each facility to a new septic tank facility at Saulmore (see Table 2.5 and below for further details) outwith the lower basin of Loch Etive. The new pumping stations have retained CSO and/or EO (Emergency Outfall) capability. Scottish Water estimates that significant CSO spills (greater than 50 m^3) from these facilities will be less than 10 per annum (Scottish Water Solutions 2005).

The village of Dunbeg lies out with the main Loch Etive basins, but in close proximity to the mouth of the lower basin. The Scottish Water network in Dunbeg serves around 90% of the population (approximately 673). In conjunction with improvements to the network in Connel the raw outfall in Dunstaffnage Bay has been upgraded to act as a pumping station, where effluent will be pumped to the new facility at Saulmore (see below). The facility will retain CSO and EO capability.

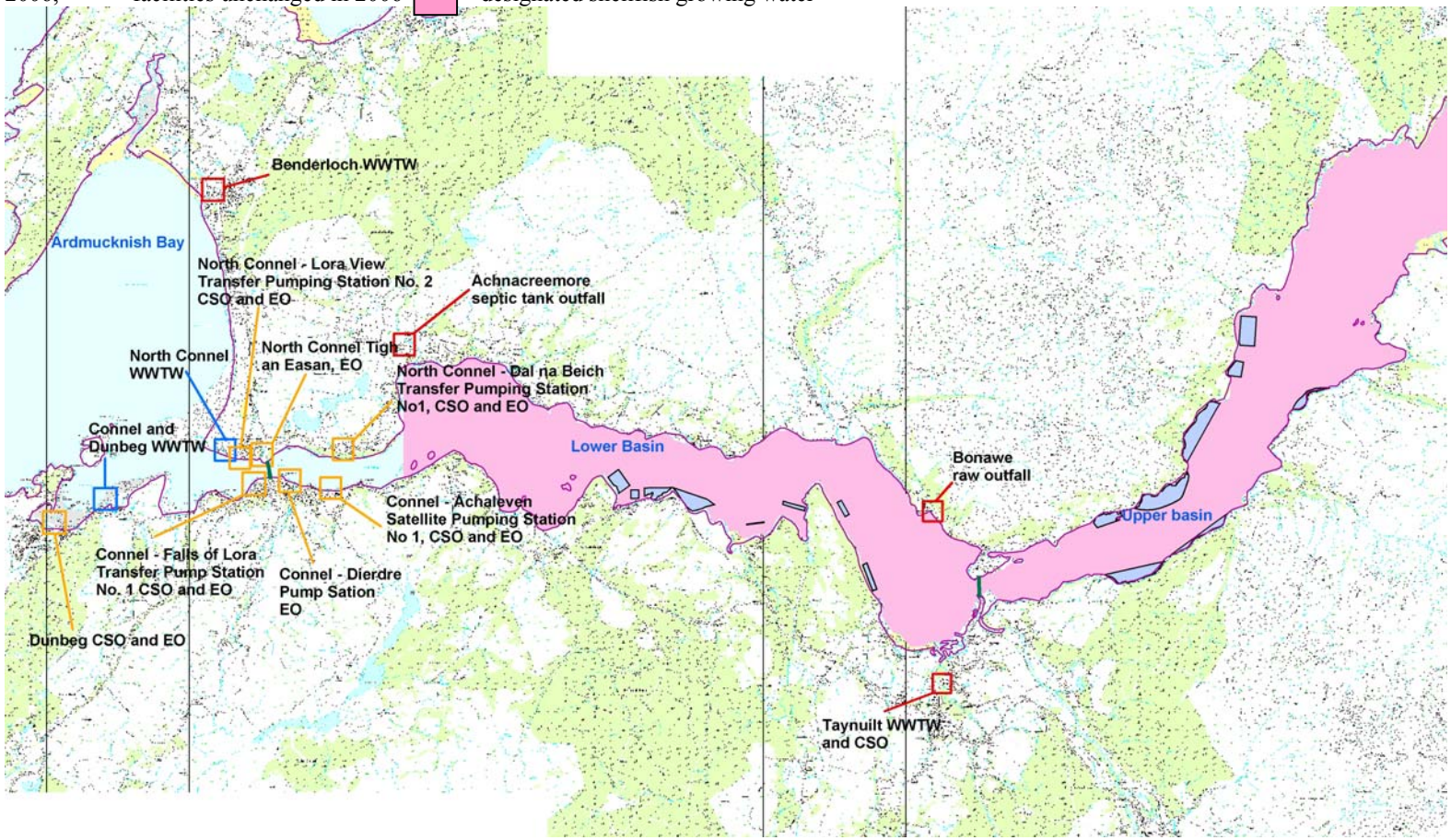
A new facility was constructed at Saulmore (Connel and Dunbeg WwTW). This facility provides primary (septic tank) treatment for Connel and Dunbeg dwellings currently on

the Scottish Water network, serving a population of approximately 959. This facility discharges through a marine outfall 13.65 m depth (from MLWS). This facility effectively brings the effluent from Dunbeg slightly closer (by a few hundred metres) to the mouth of Loch Etive. However, modelling studies carried during the planning of the new woks have indicated that the discharge meets the requirements as set out in relevant SEPA policy documents (Scottish Water Solutions 2005). The modelling further showed that for the two new works for Connel/Dunbeg and North Connel compliance in relation to the shellfish waters is achieved, assuming a mixing zone of 20 m (Scottish Water Solutions 2005).

Table 2.5 Public network domestic sewage facilities in the Loch Etive area, including upgrade details were applicable (courtesy of Scottish Water). New assets are noted in italics.

Location	Current type	Grid Reference	Upgrade details	No of dwellings connected	% population connected
Connel – Dierdre	Raw outfall outside SGW	NM 912 343	Pump Station. Intermittent - EO	27	
Connel - Falls of Lora	Raw outfall outside SGW	NM 909 344	Transfer Pump Station. Intermittent EO & CSO	29+ hotel	286 - 40%
Connel - Achaleven	Raw outfall outside SGW	NM 918 342	Pump Station. Intermittent EO & CSO	31+ school	
Dunbeg	Raw outfall outside SGW		Pump Station Intermittent EO and CSO		673 – 90%
Saulmore - Connel & Dunbeg WwTW	*New asset – not in operation outside SGW*	*NM 896 345*	*Collection and Primary treatment. Continuous & intermittent (EO & CSO)*		*959*
North Connel - Tigh an Easan	Raw outfall Outside SGW	NM 907 347	Intermittent EO	86	266
Dal-na-Beich (Black Crofts)	Raw outfall outside SGW	NM 909 344	Transfer Pump Station No.1 Intermittent EO & CSO	29	
North Connel - Lora View	*New asset Outside SGW*	*NM 906 346*	*Transfer Pump Station No.2 Intermittent EO & CSO*		*266*
North Connel WwTW	*New asset Outside SGW*	*NM 905 345*	*Continuous. Primary treatment (septic tank)*	*115*	
Achnacreemore	Primary Treatment Outside SGW		n/a	11	
Taynuilt WwTW	Secondary Treatment & CSO Outside SGW	NN 005 316	n/a	284	700 – 60%
Bonawe	Raw outfall Inside SGW	NN 002 338	n/a	23	60
Benderloch	Primary treatment (package works) Outside SGW	NM89958 38362	n/a	n/a	90%

Figure 2.6 Public network facilities around the Loch Etive catchment. —— - facilities that have been upgraded in 2006, —— new facilities, installed in 2006, —— - facilities unchanged in 2006 ▭ – designated shellfish growing water



Modelling studies carried out by Scottish Water Solutions indicate that once operational, the discharge from this facility will comply with microbiological standards for Shellfish Growing Waters (i.e. not exceeding 100 faecal coliforms per 100 ml, 90% of the time) as determined by SEPA (Scottish Water Solutions 2005).

The village of North Connel has two public network facilities, serving a total of 115 houses (Figure 2.6, Table 2.5). One network at Tigh an Easan, serves 86 dwellings, and one at Dal-na-beich, serves 29 houses. Both networks were upgraded in 2006. In both cases, sewage was previously collected and discharged raw into the Loch. The Tigh an Easan outfall is just outside the Falls of Lora at the mouth of the lower basin and approximately 2.5 km from the SGW. The Dal-na-beich outfall is within the lower basin, approximately 1.0 km from the SGW. The outfall at Tigh an Easan has been upgraded with the installation of a new pumping station – North Connel (Lora View) Transfer Pump Station No.2. There, the discharge is now pumped to a new septic tank treatment facility, North Connel WwTW (see Table 2.5 and below for details). The Tigh an Easan outfall has been retained as an EO for a small pump serving two dwellings that are below the level of the new system. The previous raw outfall at Dal-na-Beich has also been converted into a Transfer Pumping Station (North Connel, No.1), where the discharge is now diverted to the new North Connel WwTW. Dal-na-Beich has retained CSO and EO capabilities.

The North Connel WwTW is a new asset offering primary (septic tank) treatment for dwellings currently on the Tigh an Easan and Dal-na-Beich networks, serving a total of 115 houses and a population of 266. This facility has a marine outfall at a depth of 18.65 m (from MLWS). The depth and position of the outfall will mean that the outfall will comply with microbiological standards for Shellfish waters (i.e. not exceeding 100 faecal coliforms per 100 ml 90% of the time) as determined by SEPA (Scottish Water Solutions 2005). The outfall for this facility is approximately 3.5 km of the SGW boundary.

There is a small network of 11 dwellings, at Achnacreemore in North Connel, served by a Scottish Water septic tank. The outfall of this facility is outside the SGW, close to the Abhainn Achnacree, a river which discharges to Loch Etive in Achnacreemore Bay.

There is also a small public network, serving 23 houses in Bonawe (approximately 60 % of the population). The network collects and discharges (raw) directly into the loch, within the SGW. Scottish Water reported that a new first time sewerage scheme has been identified for Bonawe. This new scheme would include the existing properties in Bonawe, the school and adjacent schoolhouse, plus a proposed 5 property development. Options for location of any new treatment plant and the level of treatment are currently under consideration. The deadline for this provision is the end of March 2010 although the scheme is currently being progressed for delivery late 2007 (Scottish Water *pers. comm.*)

Scottish Water have provided an estimate of how the coliform loadings into Loch Etive will be affected once the new facilities (Connel and Dunbeg WwTW, and North Connel

WwTW) are fully operational. It is estimated that there will be an overall 99.2% reduction in loadings from the continuous public discharges (Table 8).

Table 2.6. Estimated reduction in continuous coliform loading from public systems (provided by Scottish Water)

Settlement	Population Served	Historic Loading	New Loading	% reduction in load
North Connel	266	1.12E+12	5.6E+9	99.5
Connel	286	1.2E+12	6.01E+9	99.5
Dunbeg	673	2.83E+12	1.41E+11	95.0

The village of Benderloch is approximately 3.5 km from North Connel. Although not directly on the Loch Etive shore line, and not strictly in the catchment area, the village has a small primary treatment WwTW which discharges into Ardmucknish Bay a few kilometers from the lower basin of Loch Etive. There is high connectivity to the public network, with around 90% of the population served by the WwTW.

2.4.2.2 Industrial/Commercial point sources

There are few significant commercial activities in the Loch Etive area. Most of the coastal stretches are rural and used for livestock grazing (see below) and forestry. On the north shore, in close proximity to the Bonawe Narrows is an active aggregate quarry (operated by Ennstone Thistle). There is no discharge point source associated with this site. Despite this, it is likely that sediment is discharged into the loch as a result of the quarry activities. In the village of Connel there is a small oil depot. A discharge license (SEPA) is held for this site but the details of the licence were not available.

As noted above the area surrounding Loch Etive is a popular tourist destination during the summer months. There are three large caravan camping sites in proximity to Loch Etive. None operate directly within the loch or the SGW. The caravan park at Tralee, near the village of Benderloch has capacity for 440 people and has a septic tank on site and is served by a long marine outfall (approximately 7.8 km from SGW) in Ardmucknish Bay, which is adjacent to the mouth of Loch Etive. South of the village of Benderloch there is a caravan and campsite with capacity for 120 tents or caravans and this is also served by a septic tank with a long marine outfall (approximately 6 km from SGW), also in Ardmucknish Bay. A campsite for mobile caravans and campervans operates adjacent to the River Awe (downstream from the River Awe barrage). This has a capacity for 80 mobile caravan units and is currently served by a soak-away system (SEPA, *pers. comm.*). This site is approximately 4.3 km from the SGW, along the River Awe.

2.4.2.3 Non-public network domestic sewage sources

Within the lower basin there are a number of dwellings that appear not to be connected to the public sewage network. Domestic sewage from such dwellings may be discharged to the environment as septic or raw waste. Sewage discharges from private dwellings into the marine environment are permitted under a discharge consent authorised by SEPA. However, according to SEPA's database, few private dwellings (outwith the public network) in the catchment of Loch Etive have discharge consent. This includes

approximately 115 dwellings in the village of Connel, 110 in North Connel, 20 in Bonawe and 120 in Taynuilt. Available resources for this project were not able to accommodate a full investigation into how domestic sewage is treated and disposed of in each of these dwellings. It is likely that the vast majority of dwellings, particularly more recent buildings and those that have undergone alteration in recent years have a septic tank or soak away unit. For instance, the majority of dwellings in North Connel and not on the public network are not situated immediately adjacent to the shore. Consultation with some local residents in the area indicates that most houses have a septic tank.

A number of dwellings discharge raw sewage into the loch. This situation is most often seen in older dwellings that are in close proximity to the shore. In Connel Bay, to the west of the Falls of Lora (outside SGW by approximately 2.5 km) a number of dwellings (approximately 25) on the south shore appear to discharge domestic waste directly into the sea. A walk along the accessible parts of the shoreline at low tide clearly reveals a number of pipes from these houses. A similar picture is seen in Achnacreemore Bay on the north shore of the lower basin, where a number of pipes run into the loch. Much of Achnacreemore Bay is within the SGW area.

2.4.3 Non-point sources pollution

2.4.3.1 Livestock

Livestock census data was obtained from RERAD (Scottish Government Rural Environment Research and Analysis Directorate). Data is collated by agricultural parish. The Loch Etive catchment area includes parts of 4 parishes, 'Ardchattan and Muckairn', 'Innishail', 'Kilchrenan and Dalavich' and 'Kilmore and Kilbride'. As explained above, Loch Awe itself has a substantial catchment area and also includes parts of the above parishes, as well as additional parishes. For the purposes of this study, only livestock downstream of the Loch Awe barrage are considered. The dominant parish is 'Ardchattan and Muckairn' which covers all of the north shore of the loch (both upper and lower basins) and the east shore of the upper basin, as well as a large area of the south catchment between Connel and Taynuilt.

Livestock figures for 2000-2005 are displayed in Table 2.7. It should be noted that these official parish figures pertain to the whole of each parish. It was not possible to obtain official census data for specific areas within each parish. Only data for livestock categories present in the parishes are presented.

Sheep are the most numerous livestock species in all four parishes, with a total of over 88000 sheep over all parishes. The data would also indicate that over the five year period shown, sheep numbers have reduced by approximately 10% from the 2000 figure of approximately 98000. This reduction in numbers is marked in the Ardchattan and Muckairn parish where sheep numbers have reduced by 18% in that period. Cattle numbers have changed little in that period and there has been some reduction in poultry numbers.

Table 2.7. Official livestock census data for agricultural parishes in the Loch Etive catchment area (courtesy of SEERAD)

	Total Poultry		Total Cattle		Total Sheep	
Parish/Year	**Units**	**Numbers**	**Units**	**Numbers**	**Units**	**Numbers**
2000						
Ardchattan and Muckairn	12	1559	38	1791	44	25487
Glen Orchy and Innishail	11	196	20	1167	27	34931
Kilchrenan and Dalavich	*	*	8	639	7	13926
Kilmore and Kilbride	7	140	20	862	33	23694
2001						
Ardchattan and Muckairn	15	1287	38	1807	47	24902
Glen Orchy and Innishail	11	233	20	1141	26	32563
Kilchrenan and Dalavich	*	*	7	547	7	13747
Kilmore and Kilbride	8	149	22	904	34	23394
2002						
Ardchattan and Muckairn	13	1336	40	1718	45	24014
Glen Orchy and Innishail	10	187	20	1062	28	35391
Kilchrenan and Dalavich	*	*	7	456	7	13531
Kilmore and Kilbride	9	319	24	834	33	22028
2003						
Ardchattan and Muckairn	13	1446	39	1622	42	24071
Glen Orchy and Innishail	8	150	21	1122	27	33275
Kilchrenan and Dalavich	*	*	9	463	8	13038
Kilmore and Kilbride	9	265	24	830	35	22662
2004						
Ardchattan and Muckairn	13	992	41	1879	43	22759
Glen Orchy and Innishail	11	174	20	1168	27	32980
Kilchrenan and Dalavich	*	*	8	396	7	13600
Kilmore and Kilbride	7	181	20	818	33	22791
2005						
Ardchattan and Muckairn	15	1106	43	1866	43	20866
Glen Orchy and Innishail	11	185	21	1121	30	32299
Kilchrenan and Dalavich	*	*	8	444	8	12823
Kilmore and Kilbride	6	151	19	883	37	22304

To prevent disclosure of individual holdings, entries relating to less than five holdings or those where two or less holdings account for 85 % of the information have been replaced with an asterisk.

For the purposes of this study a more accurate picture of livestock numbers within the Etive catchment alone, was desirable. Figures for individual farms were sought by contacting the farmers, where possible. It should be noted that data could not be obtained for all relevant livestock holdings.

There are approximately five main livestock holdings on the north shore of the lower basin. The grazing areas of this shore stretch from the Abhainn Achnacree catchment and east to the Blacreen Burn (Achnacree/Ardchattan area). All rivers and stream in this area have loch-side discharge points within the SGW. In total, approximately 1960 sheep and

270 cattle are grazed in this area. Individual farm figures were obtained outwith the main grazing season and only account for numbers of ewes (i.e. numbers of lambs are not included). It is likely that peak numbers of sheep during the main grazing season could be double that reported here. Thus the potential for faecal input to the SGW will be maximized when stocking numbers are at their peak. The impact of seasonal stocking density and stock movement patterns could have a substantial impact on the input of faecal material into shellfish waters. For instance, during the summer numbers of ewes and lambs is at a maximum, but the stock may be well dispersed throughout the catchment area. In late summer the stock is often moved closer to the farm, ready for market and thus stocking density is increased in that area, giving the potential for high faecal loads into local catchment streams. In the area of Loch Etive stock is often moved out of the area for over wintering as the grazing lands in the area can be marginal.

The south shore of the lower basin has a number of livestock holdings. The upper reaches of the Allt na h-Airde and the Allt a' Bhile streams have extensive grazing areas (Glen Lonan area). The main livestock holding there have approximately 800 ewes. Both streams discharge within the SGW. The Achnacloich area of the south shore holds approximately 1000 sheep and 150 cattle (Achnacloich/Muckairn area). A network of streams and small field drains are found in this area, the majority of which discharge within the SGW in close proximity to current shellfish lease sites. Outwith the SGW there are a number of holdings. However, livestock numbers could not be obtained for all holdings, particularly in the Lusragan Burn catchment.

The east shore of the upper basin appears to have one main farm covering the Glen Noe and Inverliver catchment areas, within the SGW. This holding reports approximately 1200 ewes and 40 cattle. The upper reaches of the River Kinglass and Etive have livestock grazing areas. However at the time of writing, estimates of livestock numbers for either area could not be obtained.

2.4.3.2 Wildlife

Regular wild bird counts are carried out in the Loch Etive area, however, due to the remoteness of the upper basin counts are generally confined to the lower basin, between Connel Bridge and the Bonawe Narrows (P. Daw *pers. comm.*). The counts do not reflect species or populations numbers in the extensive catchment area. Counts of many species are based on the breeding season or migration patterns. For instance, gull counts are made during the month of June while the birds are nesting and counts of gulls (including common gull, black headed gull, herring gull, great black-backed gull and artic tern) are generally not made during the rest of the year, although some birds are likely to be present in the area. Total numbers of gulls, geese and ducks, and waders are shown in Table 2.8, with an indication of when the maximum number is recorded, for the period 1997-2003. Available records for 2004 to the present are not complete and unlikely to reflect true population numbers. Gulls are the most numerous, and most prevalent in the summer months. Breeding gull numbers are generally around 1100, with an increase to 1345 in 2003. A number of species are included in the geese and ducks group, including, common eider, wigeon, mallard, greylag geese, little grebe and goldeneye. Many of the species in this group occur in greater numbers in late autumn and winter. Between 1997

and 2003 counts ranged from 250 to 741, with a maximum in 2001. Waders (mainly curlew, ringed plovers and turnstone) are generally less numerous in this area, with maximum numbers (110 in January 1998 and 161 in January 2000) seen in the winter months.

Table 2.8. Maximum population numbers of gulls, geese and ducks, and waders in the lower basin of Loch Etive. * All gull counts are made in June

Year	Gulls*	Geese and Ducks	Waders
1997	1345	291 (Dec)	
1998	938	407 (Dec)	110 (Jan)
1999	932	493 (Oct)	38 (Sep)
2000	1130	383 (Nov)	161 (Jan)
2001	1146	741 (Feb)	68 (Feb)
2002	1100	565 (Dec)	33 (Feb)
2003	1345	421 (Nov)	32 (Jan)

Wild deer are found around all shores of Loch Etive, but mostly associated with the more remote open hillsides of the upper basin. The dominant species in the northern stretches of the Etive catchment is the red deer, *Cervus elephus*, the UK's largest native land mammal. Smaller numbers of roe deer *Capreolus capreolus* are indigenous to all wooded areas of the catchment, and the southern reaches of Loch Awe also contain populations of sika deer, *Cervus nippon* (K. Black *pers. comm.*). Population estimates are not available for these two species, however, it is unlikely that significant numbers are present in the catchment. Population numbers of red deer are monitored regularly by the Red Deer Commission. Much of the catchment of the upper basin of Loch Etive falls under the remit of the Blackmount Deer Management Group. The most recent major survey was undertaken in 2000 and population numbers for the whole management area, were as follows; male – 2002, females 3803, calves 1207 (Deer Commission Scotland *pers. comm.*). Approximately two thirds of the population appears to be found in the catchment of the upper basin. Available literature suggests that although faecal micro-organisms such as *E coli* have been detected in wild deer populations, prevalence is low and cervids are unlikely to act as substantial reservoirs of pathogenic faecal organisms (Fischer *et al.*, 2001; Lillehaug *et al.*, 2005).

Two seal species are found on the west coast of Scotland – harbour seals and grey seals (*Halichoerus grypus*). Seal counts are carried out sporadically within Loch Etive by the Sea Mammal Research Unit (SMRU). In total 4 surveys (see Figure 2.5 for survey sites) were carried out between 1975 and 2000. There is a small population (Table 2.9) of resident harbour seals found within Loch Etive (C. Duck, SMRU, St Andrews *pers. comm.*).

Table 2.9. Historical Harbour seal count data for Loch Etive (courtesy of SMRU)

Date	Seal count	Basin
1975	40	lower
1990	72	lower
	18	upper
1996	44	lower
	21	upper
2000	34	lower
	32	upper

2.4.4 Aquaculture

There are four fish farm sites within the Loch Etive area. Rainbow trout (*Oncorhynchus mykiss*) is farmed at two sites within the SGW and another site just outside the mouth of the loch (Table 2.10 and Figure 2.5). Atlantic salmon (*Salmo salar*) is farmed at the Dunstaffnage site (see above for details) just outside Loch Etive. Maximum consented fish biomass for each fish farm site is set by SEPA, which aims to minimise the impact of organic fish farm effluent (such as waste feed and faecal material) on the surrounding sea bed and the total amount of nutrients to a semi-closed water bodies such as Loch Etive. In total the three trout farms are consented to produce 650 tonnes. The Dunstaffnage (salmon) site has a consented biomass of 700 tonnes, however the site is now closed. As discussed above fish farm sources are unlikely to represent a significant impact in terms of *E. coli* contamination (see section 2.1).

Table 2.10. Finfish cages operating in, or in close proximity to Loch Etive Shellfish Growing Waters

Location	**NGR**	**Within Shellfish Growing Water?**	**Consented biomass (t)**	**Species**
Inverawe	NN020328	Yes	250	Rainbow trout (*Oncorhynchus mykiss*)
Ardchatton Bay	NM973347	Yes	150	Rainbow trout (*Oncorhynchus mykiss*)
Camas Bruaiche Ruaidhe Bay	NM 89917 34064	No (approx 3 km)	250	Rainbow trout (*Oncorhynchus mykiss*)
Lynn of Lorn (Dunstaffnage)	NM 8658 3387	No (approx 6.5 km)	700	Atlantic salmon (*Salmo salar*)

2.5 Environmental Study

2.5.1 Objectives

The objective of the Environmental Study was to identify and assess the environmental factors that may contribute to contamination patterns in Loch Etive cultured shellfish species. Where possible this included relevant meteorological, hydrodynamic and hydrometric factors.

2.5.2 Data Sources

2.5.2.1 Bacteriological data

Mussel tissue bacteriological data was provided by FSAS for the Loch Etive West shellfish production area. This area includes three harvesting sites in the lower basin of the loch which are currently sampled by Local Authority (Argyll and Bute Council) Environmental Health Officers (EHO). Prior to 2007 all sampling was carried out by the harvesters themselves on behalf of the Local Authority and this pertains to all the data used in this environmental study. Data for Loch Etive West was available from 1999 until June 2006. During this time the sampling points employed were not always consistent

and likely to reflect harvesting activities. In addition, sampling at one site only began in Aug 2003 and ran until March 2006. National Grid References (NGR) for the sites are as follows; Site 1 – NM988 344; Site 2 – NM973 338; Site 3 – NM959 343 (Figure 2.7). EHO sampling of mussel tissue returns *E. coli* counts per 100g of tissue and intervalvular fluid, using the MPN (Mean Probable Number) approach. All counts for EHO sampling are carried out at UKAS (United Kingdom Accreditation Service) accredited labs.

It should be noted that each of the EHO sampling sites have associated NGRs. Shellfish companies will often operate a number of sites within a production area. During the data gathering phase of the current project, it became clear that samples submitted to the EHO were not always taken from the same location, and may therefore not always have been representative of the official NGR.

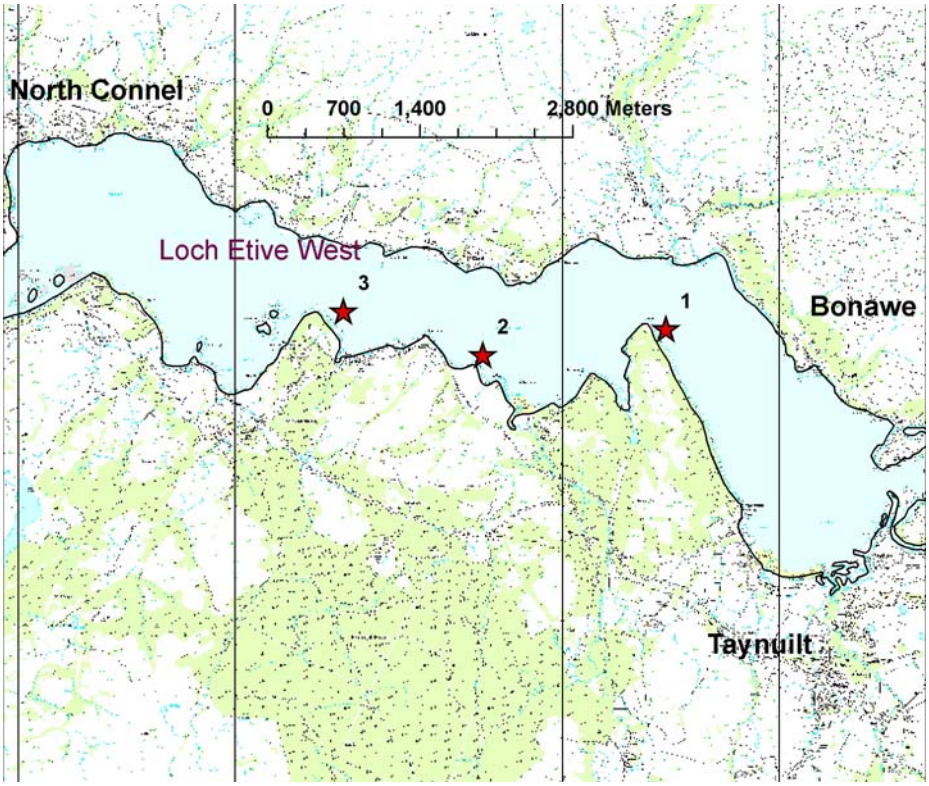


Figure 2.7. FSAS sampling sites in Loch Etive West

2.5.2.2 Environmental data

Seawater temperature data was measured for the surface waters of Loch Etive on each day of classification sampling and was measured using a hand held temperature probe (data provided by FSAS).

The nearest Met Office weather station to Loch Etive is situated at SAMS, Dunstaffnage Marine Laboratory in Dunbeg. At the most seaward and westerly end of Loch Etive, data from this weather station is a good indicator of weather patterns in the lower reaches of the Loch Etive catchment. Data for wind direction (degrees), wind speed and rainfall (mm) were obtained for this station for the period 2000 to June 2006. Rainfall data for 1999 were not available on a regular basis due to problems with on-site equipment. All data were collated into daily averages. A measure of sunshine hours would have been a valuable addition to the environmental analysis. However, the met station at SAMS does not have a sunshine recorder and the nearest available data is for Colonsay, approximately 65 miles south west of Dunbeg. Given the complex nature of the weather patterns on the west coast of Scotland, it was felt that data from this recorder was unlikely to be representative of sunshine hours in the Loch Etive area.

SEPA operate a number of rain gauges throughout Scotland. One is situated in Glen Strae, which is part of the Loch Awe catchment. Daily rainfall data (mm) was obtained for 2000 until June 2006. The rainfall for Glen Strae is a reasonable indicator of rainfall patterns in the upper reaches of the Loch Etive catchment. Rainfall data for both Dunstaffnage and Glen Strae are displayed in Figure 2.8.

The largest freshwater input to Loch Etive is from the River Awe. This waterway connects Loch Etive to Loch Awe, a large freshwater loch to the east of the study area. The waters of Loch Awe are part of a hydroelectric power generation operation on the north west region of the loch. The operation uses a barrage to control the level of water in the loch, and therefore the flow of water in the River Awe. Thus the flow in the river Awe is not always a reflection of recent rainfall events, as water is often retained within Loch Awe, particularly after a prolonged dry spell. Daily mean flow (cumecs – cubic metres per second) for the River Awe, for the period January 2000 to June 2006, was supplied by Scottish and Southern Energy.

SEPA operate a number of river gauging stations on the River Strae. This river does not discharge into Loch Etive directly but is likely to be a good proxy for river flow in the upper reaches of the Etive catchment area. Data for river flow (cumecs) was provided by SEPA for 1999 to June 2006. Daily flow data is displayed in Figure 2.8.

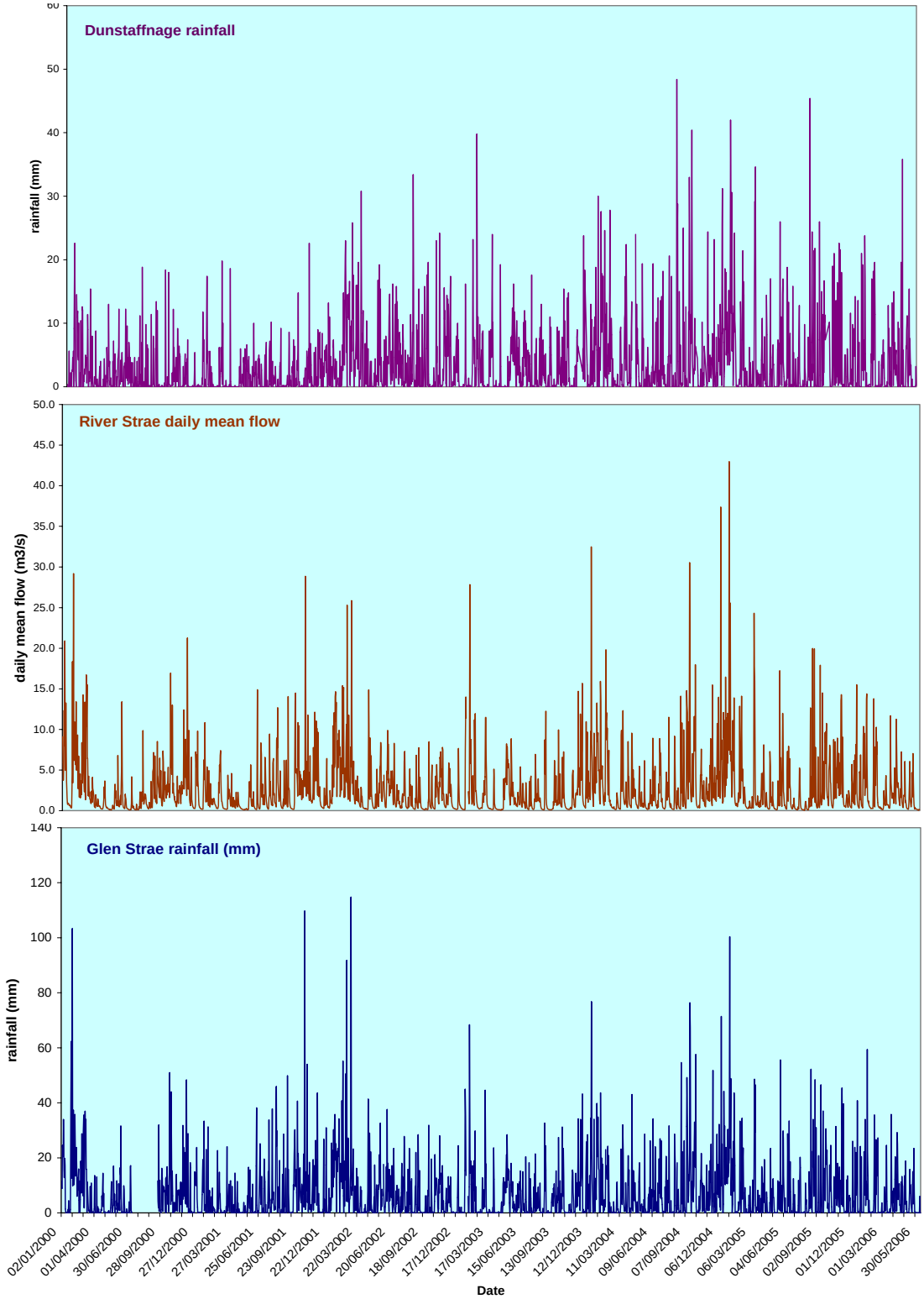


Figure 2.8 Rainfall for Dunstaffnage and Glen Strae, and River Strae mean daily flow.

The tidal cycle within Loch Etive is complex. The size and position of the sills creates a reduced tidal amplitude and a lag phase from that of the coastal seas outside the loch. The tidal phase within the lower basin of Loch Etive is synchronized with the tidal cycle at the Bonawe Narrows. However, freshwater input has a very strong influence in the loch and this can alter actual height and time of high and low water. The freshwater effect will be particularly pronounced following high rainfall throughout the catchment. Bonawe Narrows is a secondary port and all tidal predictions are based on Oban. Accurate tide height data outside of high and low water are not available for Bonawe Narrows. State of tide, at the time of EHO classification sampling, was expressed as the sine and cosine of the time in hours relative to last low water time divided by the length of the tidal cycle (12.4 h) x 2π. e.g. State of tide = sin (a-b/(12.4h)*2π), where a = time of sampling, b = time of low water.

2.5.3 Data analysis

E. coli counts (response variable) were $\log_{10}$ transformed prior to analysis in order to improve normality of distribution (Zar 2001). Pearson's correlation coefficient on ranked data was calculated to provide bivariate relationship between the *E. coli* counts and all potential predictor variables (wind direction, wind speed, seawater temperature, month, Dunstaffnage rainfall, River Strae flow, Glen Strae rainfall, tidal state). Stepwise regression was performed between *E. coli* counts (dependent variable) and all environmental variables described below. Stepwise regression is a multiple regression technique where potential predictor (independent) variables are added and subtracted to a regression model in order to identify a useful subset of variables that make a significant contribution to the observed variance in a dependant variable. For the purposes of the regression analysis all river flow data (River Strae and Awe) were $\log_{10}$ transformed in order to improve parametricity. All variables were analysed for basic distribution parameters and any variables with a skewness of ≥ 1.0 were $\log_{10}$ transformed. The probability of F for a predictor variable to enter the model was set at 0.05. The strength of the relationship between dependant and predictor variables was assessed by the coefficient of determination (r^2 expressed as a % and adjusted for degrees of freedom). All analyses were conducted using the Minitab 14.2 statistical software package.

Predictor variables may operate over a range of time scales. In order to account for this possibility data was collated to reflect a range of timescales as follows;

- **24 hr** – Mean or total data for the 24 hours immediately prior to sampling day. In the case of rainfall, wind direction and speed the 24 hour period ends at 0900hrs, for river flow data the 24 hour period runs until midnight of the day before sampling.
- **48 hr** - Mean or total data for the 48 hour period immediately prior to sampling day.
- **72 hr**, **96 hr** and **7 day** options follow as per the 24 hr and 48 hr options.

Individual days were also isolated as potential predictors as follows;

- **-2 days** – the mean for the 24 hour period 2 days before sampling day
- **-3 days** – the mean for the 24 hour period 3 days before sampling day
- **-4 days** – the mean for the 24 hour period 4 days before sampling day

2.5.4 Results and discussion

Correlation analysis between river flow data (Strae and Awe) and rainfall data (Dunstaffnage and Glen Strae), as expected, indicated a strong relationship between these environmental variables.

Results of the correlation analysis between environmental variables and bacteriological counts varied across the three sites in the Loch Etive West area and are shown in Table 2.11. Site 1 showed significant correlation with mean '24 hr' and '7 day' River Strae flow data. This site also showed a significant relationship with Glen Strae 24 hr, 96 hr and 7 day rainfall totals. Mean wind direction over the '7 day' period was also significant, as was the mean wind direction for '-4 days' prior to sampling. Mean flow at the River Awe barrage for the '48 hr' and '-2 days' variables was significantly related to the *E. coli* counts at this site.

At Site 2 seawater temperature returned a highly significant relationship with bacterial counts ($r = 0.399$, $p = 0.001$) (Table 2.11). Dunstaffnage rainfall variables were significantly related to bacterial counts. A number of the variables collated for Glen Strae rainfall and River Strae flow data showed significant relationships with the bacterial counts at this site. In particular, mean river flow data for '48 hr' and ($r = 0.353$, $p = 0.004$), '72 hr' ($r = 0.361$, $p = 0.004$) and the '– 2 days' options indicated highly significant relationships. All but one of the River Awe barrage flow variables showed a significant correlation with *E. coli* counts at Site 2. In particular, the '-2 days' variable was highly significant ($r = 0.353$, $p = 0.004$).

Site 3 correlation analysis indicated seawater temperature as having a significant relationship with bacterial counts (Table 2.11). Data for '24 hr' rainfall for both Dunstaffnage and Glen Strae ($r = 0.410$, $p = 0.002$) also returned significant relationships. Six of the River Awe barrage variables (not '24 hr' or '-4 days') were found to be significant with the '-2 days' variable highly significant ($r = 0.378$ $p = 0.003$)

Multiple stepwise regression analysis of the *E. coli* counts against the full range of environmental variables also revealed a difference in response across the three sites (Table 2.12). At Site 1, the only predictor variable which was entered into the regression model was the mean wind direction over 7 days (WD 7 days) and accounted for 23.7 % of the variance in bacterial counts. At Site 2, mean River Strae flow over 7 days (RSF 7 days) was the first variable to enter the model, with seawater temperature as the second variable. Indicating that *E. coli* concentration in mussel tissue was elevated following high flow conditions in the River Strae for the 7 day period prior to sampling and while the seawater temperature is increased. Together these two variables accounted for 34.7 %

of the variance. The stepwise regression results for Site 3 indicated five variables contributing significantly to the *E. coli* counts at that site. The first variable in the regression was seawater temperature, which accounted for 14.7 % of the variance. This again indicates the influence of warmer seawater temperatures on *E. coli* counts in mussel tissue. Glen Strae rainfall (24 hrs) was the next step entered into the model (step 1 and 2 combined r^2 of 34.7 %) indicating that rainfall events in the upper catchment may have some influence over *E. coli* results. Another three steps were entered into the regression model (see Table 2.11), however, r^2 values were low, thus the predictive value of such variables is low.

Both the correlation analysis and the multiple regression analysis indicate a variation in response in *E. coli* levels to the range of environmental parameters presented. Given the distance between the sites (approximately 3 km) and an initial look at terrestrial resource use and the proximity of the main populations this variation is surprising. The main freshwater inputs to the loch are some distance from the sampling sites employed for classification and the main population areas are approximately 3 km to the south east of Site 1 and 4.5 km west of Site 2. Resource use around the sites is a mix of forestry and livestock grazing. However, Loch Etive is hydrodynamically complex and it is possible that sampling sites may be subject to different hydrodynamic regimes. This could create variation in the delivery of faecal contaminated water to the cultured mussels at each site and/or alter the influence of environmental variables at different sites. Contamination patterns at the three sites where largely similar (see Figure 2 and 3), however, on a number of occasions substantial contamination events were not synchronised across all sites. This in part may support the hypothesis that spatial and temporal variation in waterbody dynamics can influence contamination patterns.

As described above, the classification samples were not always taken from the exact same location. Thus sampling results may not be fully representative of a particular site. This may reduce the effectiveness of assessing the influence of environmental factors on a particular location. This will be particularly relevant where the impact of environmental factors vary spatially across the loch area.

Table 2.11. Results from Pearson's Correlation on ranked historical *E. coli* counts for Loch Etive West and environmental variables as listed.

Site	**Wind Direction , WD (°)**								**Wind Speed, WS (knots)**								**Sin TDC**	**Cos TDC**	**Seawater, TEMP (° C)**
	24 hr	48 hr	72 hr	96 hr	7 day	- 2 days	- 3 days	- 4 days	24 hr	48 hr	72 hr	96 hr	7 day	- 2 days	- 3 days	- 4 days			
1				*	*			*											
2																			**
3																			*

Site	**Dunstaffnage Rainfall, DRF (mm)**								**Glen Strae rainfall, GSR (mm)**							
	24 hr	48 hr	72 hr	96 hr	7 day	- 2 days	- 3 days	- 4 days	24 hr	48 hr	72 hr	96 hr	7 day	- 2 days	- 3 days	- 4 days
1									*			*	*			
2	*	*	*	*	*					*	*	*	*			
3	*								**							

Site	**River Strae flow, RSF (cumecs)**								**River Awe flow, AWE (cumecs)**							
	24 hr	48 hr	72 hr	96 hr	7 day	- 2 days	- 3 days	- 4 days	24 hr	48 hr	72 hr	96 hr	7 day	- 2 days	- 3 days	- 4 days
1	*				*					*				*		
2	*	**	**	*	*	**	*		*	*	*	*	*	**	*	
3										*	*	*	*	**	*	

*- significant at <0.05 level, ** - significant at <0.005 level.

Table 2.12. Stepwise multiple regression results for log 10 transformed historical *E. coli* counts for Loch Etive West against independent environmental variables. See text for description of variable abbreviations.

Site	**Independent variable**	r^2 **(adjusted) %**	***p***
Site 1	WD 7 days	23.68	0.011
Site 2	RSF 7 days	15.47	<0.001
	Temp	34.70	<0.001
Site 3	Temp	14.65	0.003
	GSR 24	27.08	0.003
	GSR -3 days	32.74	0.026
	AWE 7 days	41.86	0.005
	RSF 24	46.19	0.031

3. 12 Month Monitoring Report

Shona Magill[1], Kenny Black[1], David Kay[2], Simon Kershaw[3] and David Lees[3] and James Lowther[3].

[1] **Scottish Association for Marine Science**
Dunstaffnage Marine Laboratory
Dunbeg
Oban
Argyll
PA37 1PA
United Kingdom

[2] **Centre for Research into Environment and Health**
University of Wales
Lampeter
Ceredigion
SA48 7ED
United Kingdom

[3] **CEFAS**
The Nothe
Barrack Road
Weymouth
DT4 8UB
United Kingdom

3.1 Objectives

The objectives of the 12 month monitoring programme were to;

- devise and carry out a weekly sampling programme in order to assess occurrence and magnitude of faecal bacteria, norovirus and FRNA bacteriophage in harvested shellfish in Loch Etive, west coast of Scotland
- assess the relationship between bacterial and viral parameters detected in mussel tissue
- assess the relationship between faecal bacteria in surface waters and in mussel tissue
- examine potential weather-driven trigger events on observed bacterial and viral contamination patterns in mussel tissue
- examine the relationship between on-site environmental conditions and microbiological parameters in mussel tissue

3.2 Material and Methods

3.2.1 Study site

Loch Etive, on the west coast of Scotland was chosen as the study site. Loch Etive has been an important site for mussel cultivation for some years, with production commencing in the 1980's. Production levels have grown steadily and, at its peak in 2000, Loch Etive produced approximately 1000 tonnes of mussels. This accounted for approximately one third of the total Scottish production for that year.

Loch Etive (OS Ref NM890340) is a tidal fjordic sealoch, about 30 km in length (Figure 2.1). It is characterised by a series of sills, dividing the loch into basins. Two of the sills are particularly shallow and the exchange with coastal waters outside of the loch is severely restricted, affecting both tidal amplitude and timing. The hydrodynamics of the loch are complex and currents vary significantly both horizontally and vertically throughout the loch. The loch has a large catchment area (1350 km^2) (Edwards & Sharples, 1985) with a number of major rivers draining to the loch. The largest freshwater input is from the River Awe, which enters the loch laterally in close proximity to the Bonawe Narrows in the upper basin. This river connects Loch Etive to the freshwater Loch Awe, which itself has a large catchment area (828.6 km^2). River Awe flow is controlled by a hydroelectric barrage at the exit to Loch Awe.

The loch is divided into two classified production areas – Loch Etive West and Loch Etive East, separated by the Bonawe Narrows (Figure 3.1). Each production area has a number of operators. Loch Etive West is situated in the lower basin of the loch, while Loch Etive East is in the upper basin. The current classification (2007/2008) for Loch Etive West is A for April 2007, B from May to December 2007 and A for January to March 2008, while classification for Loch Etive East is B for April to December 2007 and A for January to March 2008 (www.food.gov.uk/scotland).

The monitoring programme was carried out at two sites in Loch Etive West. Loch Etive West is more accessible than Loch Etive East, therefore the logistics of sampling and transportation of samples to the analysis laboratories was more easily

accommodated. Site A (NGR NM 963 340) is situated in Achnacloich Bay (Figure 3.1). The residential areas of Connel (population approximately 540) and North Connel (population approximately 560) are approximately 4.5 – 5.0 km away. Site B (NGR NM 991 338) is situated close to Airds Point. The village of Taynuilt (population 1100) is 2.8 km away and the small community of Bonawe is 1.3 km away.

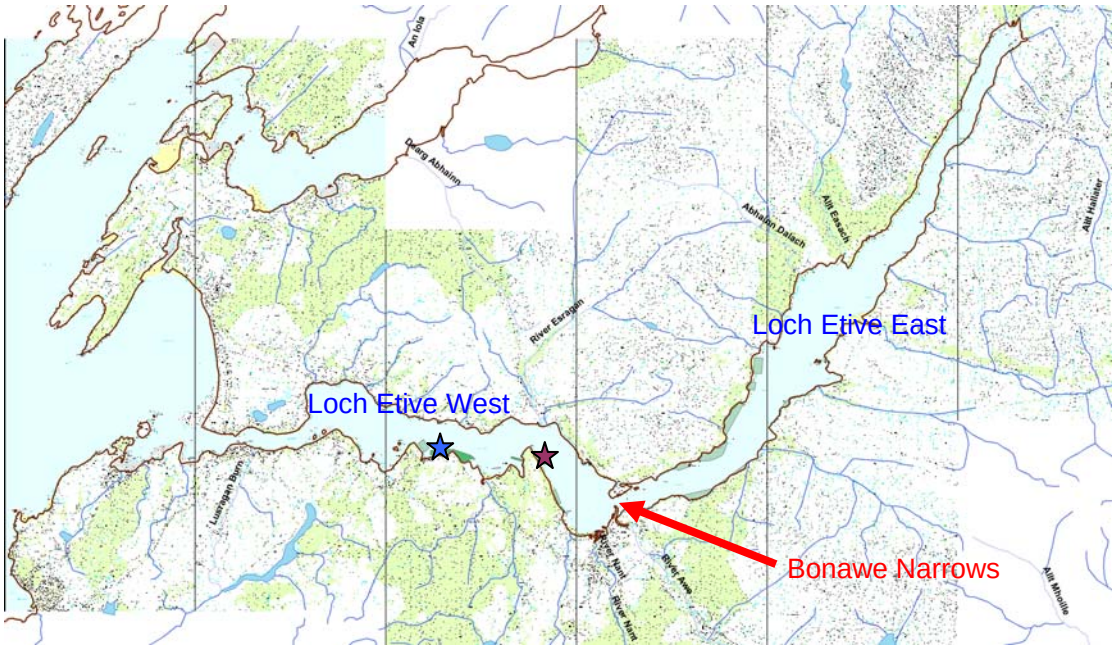


Figure 3.1. Loch Etive and monitoring programme study sites. ★ Site A, ★ Site B

3.2.2 Sampling methodology

All mussels sampled as part of the monitoring programme were grown on commercial grade mussel lines and attached to a floating raft. Within Loch Etive, stratification generally produces higher FIO concentrations in the upper part of the water column where surface freshwater influence is greatest, therefore all samples were taken from the top 1 m of the mussel lines. On each sampling occasion approximately 5 kg of mussels were removed from each site. All mussels > 50 mm were removed from the mass, then rinsed lightly in potable water and blotted dry. Four samples of 25-30 mussels were selected at random for each station and then placed in food standard plastic sample bags. The mussels were double-bagged, sealed using cable ties and labelled accordingly. The bags were wrapped in thick tissue and placed in insulated polystyrene boxes (rated to 2-8 ˚C for 24 hrs) along with 4 small ice-packs.

On each sampling occasion, a surface water sample was taken from each site. Samples were taken using a 250 ml sterilised glass bottle attached to a 1.5 m sampling pole. This allowed the surface sample to be taken away from the side of the boat. During transportation back to shore, the bottles were stored in a small insulated cool bag in order to protect the samples from sunlight. On shore, the samples were wrapped in bubble wrap and placed in the appropriate insulated box awaiting shipment.

Samples were transported to the appropriate analysis lab via TNT next day delivery service. Analysis details are provided in Table 3.1.

Table 3.1. Details of bacterial and viral analysis carried out.

Laboratory	**Analysis carried out**
Glasgow Scientific Services (Glasgow)	Mussel tissue – *E. coli* MPN
SEPA (East Kilbride)	Mussel tissue – faecal coliforms Water samples – faecal coliforms and faecal streptococci
CEFAS (Weymouth)	Mussel tissue – norovirus and FRNA bacteriophage

Weekly sampling was initiated on 11th April 2006 and was completed on 10th April 2007. Weekly sampling did not occur over the Christmas period due to the logistics of transportation and analysis over that period. During the course of the monitoring programme 3 week-long event sampling episodes were undertaken. On these occasions sampling took place daily for a period of 7 days. Such sampling events were carried out in order to provide greater detail on bacterial and viral contamination patterns following heavy rainfall events. The start dates for the three event samples were, 19/6/06, 28/8/06 and 28/2/07.

3.2.3 Environmental data collection

3.2.3.1 Hydrodynamic data

Two microcat temperature salinity loggers (Seabird SBE 37) were placed at each sampling location for the duration of the sampling programme. The loggers were deployed on standard 14 mm rope with one placed at approximately 1 m depth in order to capture the influence of freshwater on the surface layer and one at 9 m in order to indicate the depth of the fresher surface layer (Figure 3.2). Salinity and temperature readings were logged every hour. Additional temperature loggers were placed at 3.5 m and 6.5 m. The loggers were deployed on the 17th of March 2006 and serviced regularly.

On one maintenance visit it became apparent that one of the loggers (surface logger at Site A) had malfunctioned. Further examination revealed debris lodged in the logger sampling tube. This was corrected and the logger redeployed. From the readings it is difficult to determine the exact date of malfunction but the problem is likely to have affected the period between 16/5/06-13/7/06. The temperature readings appear to be affected for a shorter period (27/6/06-13/7/06), perhaps indicating that the logger still retained some functionality up until 27/6/06. Readings for the affected period were discounted.

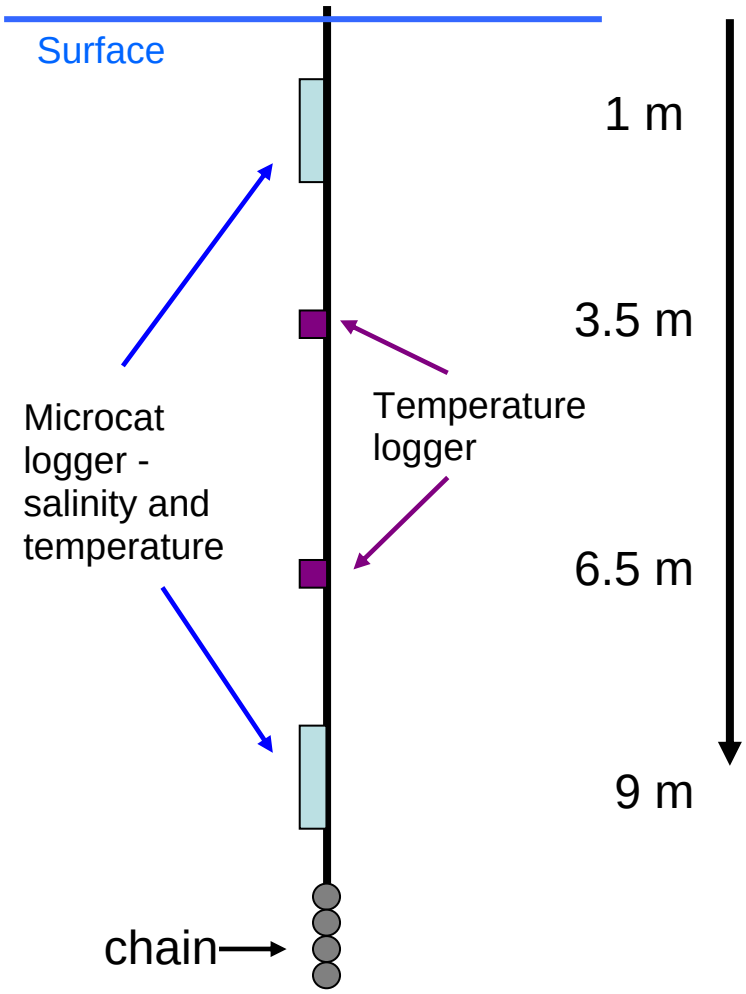


Figure 3.2. Schematic of the logger array at each sampling site.

For analysis purposes the average temperature and salinity for the 24 hour period immediately prior to sampling was collated for each logger. The salinity at the time of each sampling was also collated.

The tidal cycle within Loch Etive is complex. The size and position of the sills creates a reduced tidal amplitude and a lag phase from that of the coastal seas outside the loch. The tidal phase within the lower basin of Loch Etive is synchronized with the tidal cycle at the Bonawe Narrows. However, freshwater input has a strong influence in the loch and can alter actual height and time of high and low water. The freshwater effect will be particularly pronounced following high rainfall throughout the catchment. Bonawe Narrows is a secondary port and all tidal predictions (as calculated by POLTIPs v3.0 tidal prediction software) are based on Oban. For analysis purposes the state of tide (ebb or flow) was determined for the time of sampling.

3.2.3.2 Meteorological Data

The nearest Met Office weather station to Loch Etive is situated at SAMS, Dunstaffnage Marine Laboratory in Dunbeg (Figure 3.3), at the most seaward and westerly end of Loch Etive. Data from this weather station is a good indicator of weather patterns in the lower reaches of the Loch Etive catchment. Daily rainfall

(mm) data, for the duration of the study, were obtained for this station from the Meteorological Office.

SEPA operate an automatic rain gauge in Glen Strae (NGR NN 146 294). The River Strae does not flow directly into Loch Etive but runs parallel (at approximately 10 km away) to the upper basin of the loch and is part of the catchment for Loch Awe (Figure 3.3). The surrounding terrain is similar in both areas. For the purposes of this study, rainfall data for Glen Strae were considered to be a reasonable indicator of rainfall patterns in the upper catchment of Loch Etive. Daily rainfall data (mm) were obtained for the duration of the monitoring programme.

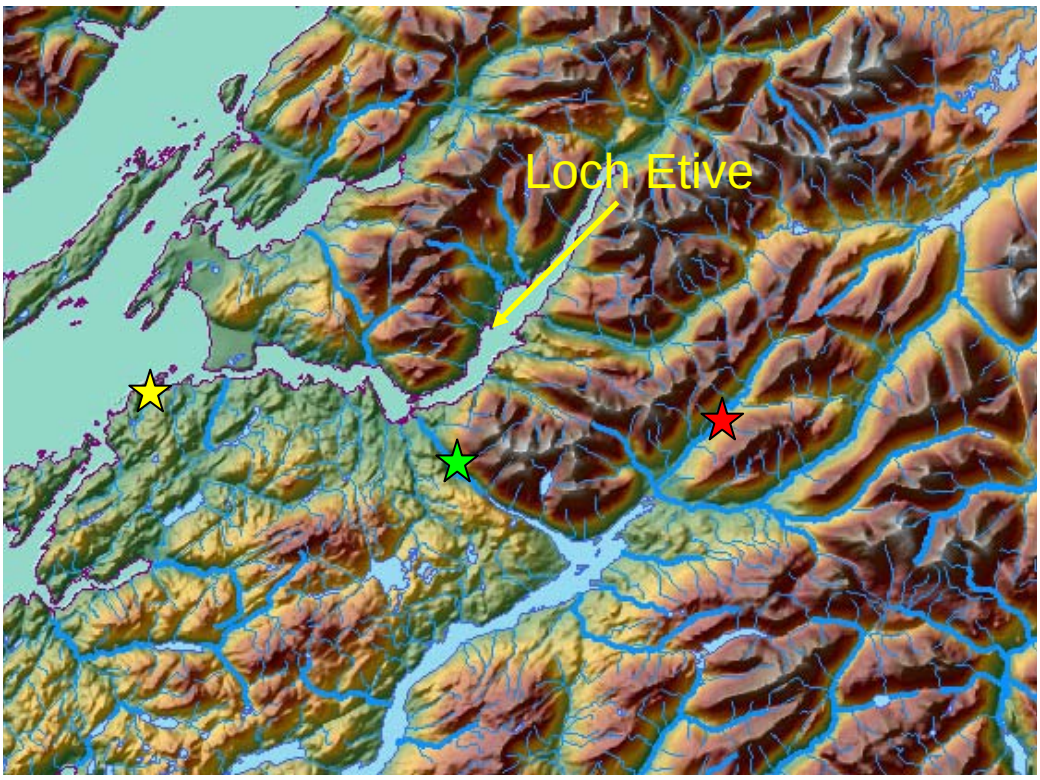


Figure 3.3. Location of rainfall and river flow stations. ★ Dunstaffnage, ★ Glen Strae, ★ River Awe Barrage.

Solar irradiance has been reported to affect the concentration of culturable intestinal bacteria in a water body (Troussellier et al., 1998). Sunshine strength data were available for Dunstaffnage, (close proximity to the Met Office weather station). No other sunshine recorder data were available for the study area. The sunshine data (Watts per metre^2- W/m^2) were logged every 5 minutes, which were then combined to give an average reading for the three hour period prior to sampling.

Air temperature data were also measured at Dunstaffnage. The air temperature at the time of sampling was collated for the study period.

3.2.3.3 Hydrometric data

The largest freshwater input to Loch Etive is from the River Awe. Flow on the river is controlled by a barrage, as part of a hydroelectric power scheme (barrage location is indicated in Figure 3.3). The flow in the River Awe is not necessarily a reflection of recent rainfall events, as water is often retained, particularly after a prolonged dry

spell. Daily mean flow (cumecs – cubic metres per second) for the River Awe was supplied by Scottish and Southern Energy.

SEPA operates a river gauging station on the River Strae (see above and Figure 3.3 for location and explanation). Despite the fact that this river does not discharge into Loch Etive directly, it was considered to be a reasonable indicator for river flow in the upper reaches of the Etive catchment area.

3.2.4 Data analysis

Initial data exploration, descriptive statistics and correlations were performed using MINITAB v14.0. Tissue *E. coli* (MPN $100g^{-1}$), faecal coliform (FC) (MPN $100g^{-1}$) and surface water faecal coliform (WFC) and streptococci (WFS) (colony forming units, cfu, per 100 ml of seawater) were log_{10} transformed prior to analysis. Bacteriophage data (pfu $100g^{-1}$) were also log_{10} transformed. Pearson's correlation analysis was used to assess the bivariate relationship between a) microbiological datasets (mussel tissue *E. coli*, FC, FRNA bacteriophage and norovirus GI and GII and surface water WFC and WFS) from the two monitoring sites, b) the microbiological datasets and all potential environmental predictor data (seawater temperature and salinity, Dunstaffnage rainfall, River Strae flow, Glen Strae rainfall, River Awe flow, sunshine level and air temperature data). Initial data exploration revealed a seasonal pattern in concentration of *E. coli*, faecal coliforms and norovirus GI and GII. In order to assess the role of catchment environmental parameters, the microbiological datasets were split into summer (May-October) and winter (November to April) datasets. The majority of the summer/winter microbiological datasets were normal on log_{10} transformation. The norovirus GI and GII data did not always (but did in some cases) conform to normal distribution (as determined by the Shapiro-Wilks test, Minitab v14.0), even after transformation. Parametric analysis is more powerful than non-parametric analysis. It was also essential that the analysis results were comparable therefore it was decided to proceed with parametric analysis (Pearson's correlation) for the entire dataset.

Predictor variables may operate over a range of time scales. In order to account for this possibility, hydrometric and rainfall data were collated to reflect a range of timescales as follows;

- **24 hr** – 24 hour mean immediately prior to sampling day. In the case of rainfall the 24 hour period ends at 0900hrs, for river flow data the 24 hour period runs until midnight of the day before sampling.
- **48 hr** - mean for the 48 hr period prior to sampling day.
- **72 hr** - mean for the 72 hr period prior to sampling day.
- **96 hr** - mean for the 96 hr period prior to sampling day.
- **120 hr** – mean for the 120 hr period prior to sampling day
- **144 hr** – mean for the 144 hr period prior to sampling
- **7 days** - mean for the 7 day period prior to sampling day.

Individual days were also isolated as potential predictors as follows;

- **-2 days** – the mean for the 24 hr period 2 days before sampling day
- **-3 days** – the mean for the 24 hr period 3 days before sampling day
- **-4 days** – the mean for the 24 hr period 4 days before sampling day

- **-5 days** - the mean for the 24 hr period 5 days before sampling day
- **-6 days –** the mean for the 24 hr period 6 days before sampling day
- **-7 days –** the mean for the 24 hr period 7 days before sampling day

3.3 Results

3.3.1 Microbiological data

Table 3.2 provides a summary of the main bacterial and viral results. *E. coli* levels (as determined by the accredited MPN method) in mussel tissue for both sites over the monitoring period are shown in Figure 3.4. The results indicate that the two sites exhibit a similar pattern of *E. coli* concentration over the study period.

Table 3.3 indicates the number of results occurring in each category for both sites. Half of the results from Site B returned 'A' class results, with 13.5% of the results at 'C' level. At Site A, 43% of the results were 'A' class and only 7.5% returned as 'C' class. Only one result per site was retuned as 'C' outside of the event sampling periods (Figure 3.4). Classification sampling (10 samples) for the same period returned indicated 44.4% of samples were returned in the A band and the B band, while 11.15 were in the C band (i.e. just one sample).

Table 3.2. Summary of bacterial and viral data from the two monitoring sites. Summary covers both weekly sampling data and event sampling data.

	Site A			**Site B**		
Bacterial parameters	**Maximum**		**Geometric mean**	**Maximum**		**Geometric mean**
E. coli (MPN $100g^{-1}$)	18000		319	16000		304
Faecal coliform (MPN $100g^{-1}$)	180,000		1148	180,000		1532
FRNA phage (pfu $100g^{-1}$)	513,000		207	1,290,000		206
Viral parameters	**Maximum**		**mean**	**% positive results**		**Maximum**
Norovirus GI (PCR units)	50.78	3.98	31.3	85.07	**mean**	**% positive results**
Norovirus GII (PCR units)	31.28	3.31	29.7	62.48	1.76	33.3

Table 3.3. Mussel tissue *E. coli* levels in terms of shellfish classification bands for monitoring sites.

	Number of samples. Percentage of total samples given in parenthesis		
	Site A (n=67)	Site B (n=65)	Classification sampling results for Loch Etive West (n=9, 1 sample)
≤ 230 (A)	29 (43.3%)	33 (50.8%)	4 (44.4%)
> 230 (B)	33 (49.3%)	23 (35.4%)	4 (44.4%)
> 4600 ≤ 46000 (C)	5 (7.5%)	9 (13.8%)	1 (11.1%)

There is strong correlation between *E. coli* levels at the two sites during the summer (r =0.576 p=0.002) (Table 3.4), but not during the winter (r-0.300, p=0.175). Both sites show seasonal variation in levels with higher concentrations detected during the summer months. The winter months are characterised by lower *E. coli* levels, with most results returned in the A classification band. Maximum *E. coli* levels at the two sites are similar (Site A – 18000, Site B – 16000) as are the geometric means.

Table 3.4. Pearson's Correlation analysis results between microbiological parameters at the two monitoring sites. * Not tested

	Summer		Winter	
Tissue	r	p	r	p
E. coli	0.572	0.002	0.300	0.175
Faecal coliforms	0.605	0.001	0.806	<0.001
FRNA bacteriophage	0.750	<0.001	0.657	0.001
Norovirus GI	*	*	0.614	0.004
Norovirus GII	*	*	0.650	0.022
Surface water				
Faecal coliform	0.592	0.002	0.881	<0.001
Faecal streptococci	0.660	0.003	0.634	0.006

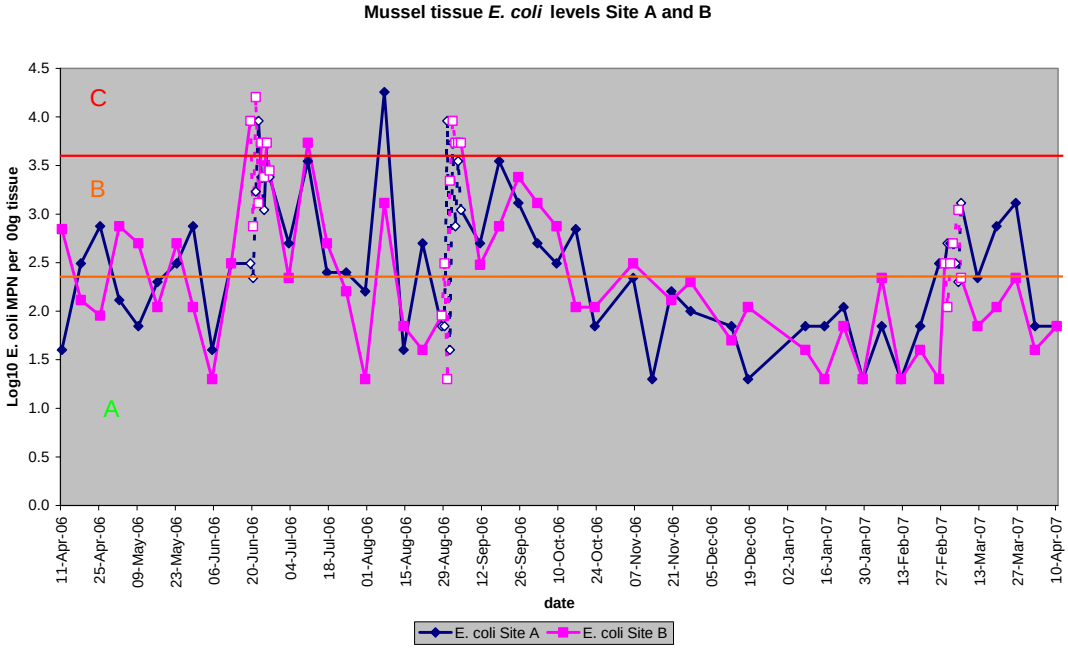


Figure 3.4. Time series plot of *E. coli* levels at Sites A and B. Event sampling days are indicated by white centred data points and dashed lines.

Mussel tissue faecal coliform (FC) levels follow a similar pattern at both sampling sites (Figure 3.5). FC levels at the two sites was highly correlated (summer r=0.605, p=0.001, winter r=0.806, p<0.001) (Table 3.4). Maximum FC levels of 180000 were returned for both sites (Table 3.2). The geometric mean for Site B (1532) was slightly higher than Site A (1148). At both sites there was a strong correlation between *E. coli* levels and mussel tissue FC levels during the summer (Site A - p<0.001, Site B – p=0.005) (Table 3.5). During winter there was a correlation between tissue *E. coli* and FC levels at Site A (p=0.025) but not at Site B (p=0.187) (see Table 3.5).

Faecal coliform (WFC) and streptococci (WFS) levels in surface waters were also found to show a similar pattern at each site, with no evidence of increased contamination at one site (Figure 3.6 and 3.7). There was a high correlation between WFC/WFS data at the two monitoring sites, in both summer and winter datasets (Table 3.4). WFC showed no correlation with either *E. coli* or FC at Site A, in either season. There was a significant correlation between WFC and *E. coli* levels at Site B during the summer (r=0.647, p<0.001) (Table 3.5). WFS correlated significantly with FC (winter – p=0.016; summer – p=0.015) at Site A only (Table 3.5).

Table 3.5. Correlation results between bacterial counts at monitoring sites. Bold indicates significant correlations (i.e. p<0.05).
Upper values are correlation coefficients and lower values are p values.

	Site A						Site B					
	EC	FC	WFC	WFS	FRNA	NV G1	EC	FC	WFC	WFS	FRNA	NV G1
Winter												
FC	**0.475**						0.300					
	0.025						0.187					
WFC	-0.310	0.067					-0.121	0.180				
	0.160	0.761					0.621	0.461				
WFS	0.100	**0.506**	0.417				0.218	0.443	0.441			
	0.666	**0.016**	0.053				0.400	0.075	0.076			
FRNA	**0.545**	-0.090	**-0.504**	-0.369			0.046	-0.314	-0.161	0.028		
	0.009	0.699	**0.020**	0.109			0.842	0.177	0.522	0.918		
NV GI	-0.144	-0.295	-0.242	-0.373	0.096		**-0.466**	-0.064	-0.235	-0.164	0.140	
	0.534	0.194	0.291	0.105	0.689		**0.029**	0.783	0.334	0.528	0.545	
NV GII	0.104	0.045	0.267	0.409	-0.085	-0.326	**-0.663**	-0.118	0.035	-0.396	-0.487	-0.146
	0.655	0.848	0.241	0.073	0.722	0.139	**0.019**	0.715	0.913	0.203	0.109	0.651
Summer												
FC	**0.635**						**0.533**					
	<0.001						**0.005**					
WFC	0.302	0.232					**0.647**	0.207				
	0.152	0.276					**<0.001**	0.320				
WFS	0.227	**0.538**	0.213				0.343	0.111	0.224			
	0.336	**0.015**	0.368				0.139	0.641	0.343			
FRNA	-0.133	-0.063	0.088	0.025			0.287	0.076	0.059	0.251		
	0.525	0.764	0.683	0.916			0.164	0.719	0.778	0.285		
NV GI	*	*	*	*	*	*	0.142	-0.093	0.124	-0.133	-0.120	
							0.489	0.650	0.554	0.575	0.567	
NV GII	0.002	-0.061	-0.106	0.131	0.068	*	-0.219	-0.270	-0.314	0.031	0.089	0.215
	0.991	0.770	0.630	0.593	0.751		0.282	0.182	0.127	0.898	0.671	0.292

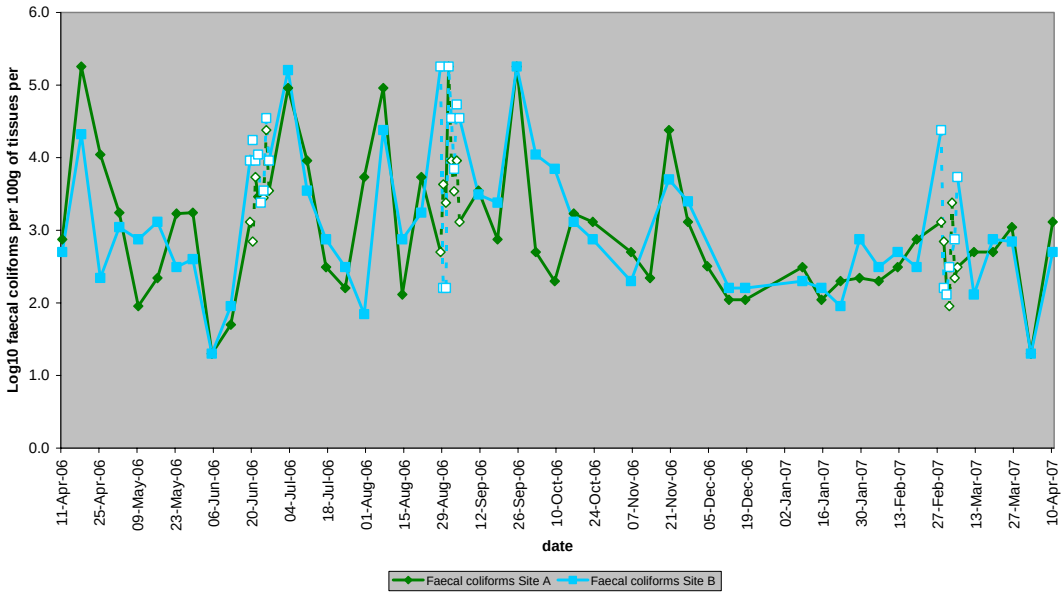


Figure 3.5. Time series plot of mussel tissue faecal coliform levels at Sites A and B. Event sampling days are indicated by white centred data points and dashed lines.

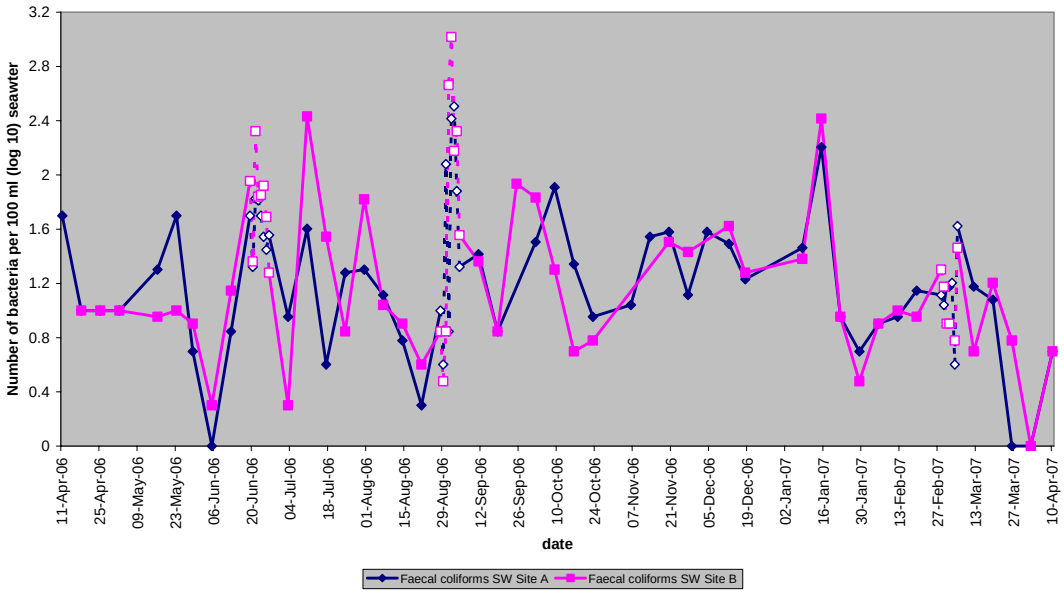


Figure 3.6. Time series plot of surface water faecal coliform levels. Event sampling days are indicated by white centred data points and dashed lines

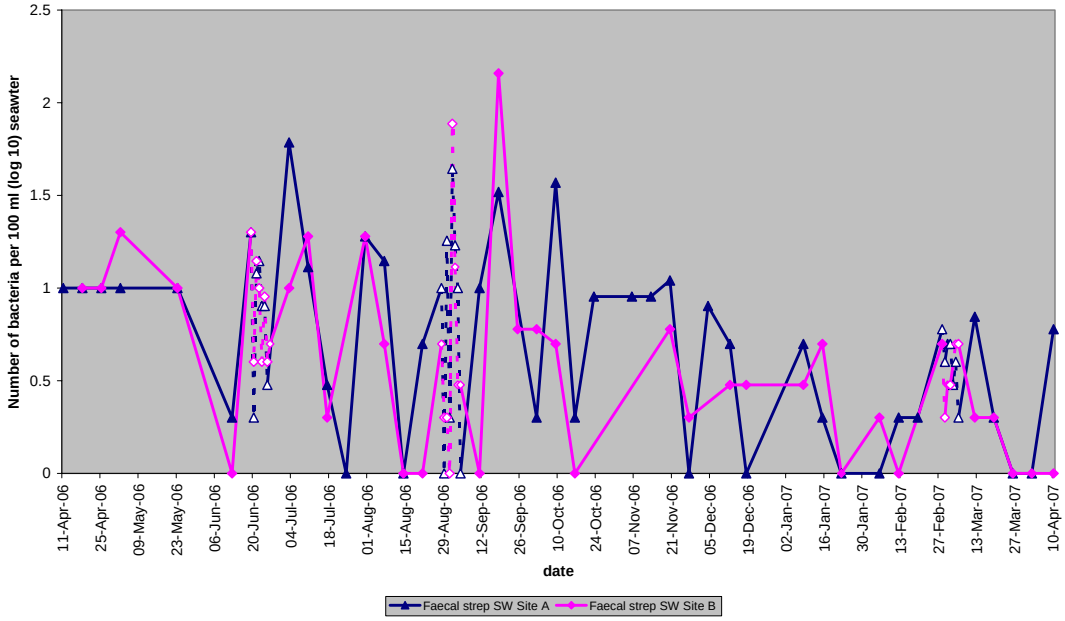


Figure 3.7. Time series plot of surface water faecal streptococci levels. Event sampling days are indicated by white centred data points and dashed lines.

FRNA bacteriophage levels were relatively consistent throughout the sampling programme at both sites (Figure 3.8), with the exception of 2 high results detected at both sites during May (Figure 3.8). There was a strong correlation between the levels at the two sites (summer - $p<0.001$, winter $p=0.001$, Table 3.4). Correlations were also observed between FRNA bacteriophage and tissue E. *coli* data from Site A during the winter($r=0.545$, $p=0.009$) (Table 3.5).

Norovirus genogroup I (GI) and genogroup II (GII) levels varied markedly with season (Figures 3.9, 3.10). Positive norovirus results were predominantly limited to the winter months. Positivity was fairly constant, ranging from 29.7 % (Site A GII) to 33.3 % (Site B GI) (Table 3.2). Combined positivity for both genogroups was 41.7 % at Site A and 46.9 % at Site B. For those samples that tested positive for norovirus the detection levels remained below 100 PCR units (including combined counts for both genogroups) (Figures 3.9 and 3.10). At Site A approximately 95.5 % of GI and 86.4 % of GII results were below 25 PCR units. At Site B 90.9 % of GI and 95.5 % of GII results were below 25 PCR units.

Presence of norovirus GI in winter correlated significantly between the two sites ($p=0.004$, Table 3.4), correlation between GII at the two sites was less significant ($p=0.022$, Table 3.4). There was some temporal variation in detection of different norvirus genogroups which could indicate circulation of different norovirus strains in the community. Norovirus levels GI and GII at Site A were not correlated with any other microbiological parameter in either summer or winter (Table 3.5). The Site B winter *E. coli* dataset was negatively correlated with GI ($r=-0.466$, $p=0.029$) and GII ($r=-0.663$, $p=0.019$) (Table 3.5).

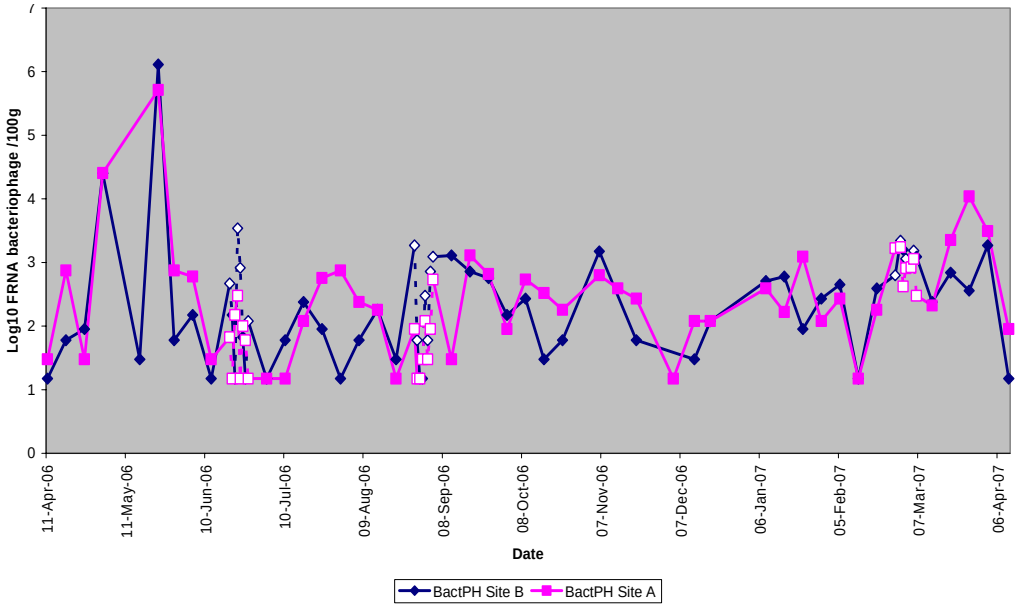


Figure 3.8. Time series plot of FRNA bacteriophage. Event sampling days are indicated by white centred data points and dashed lines.

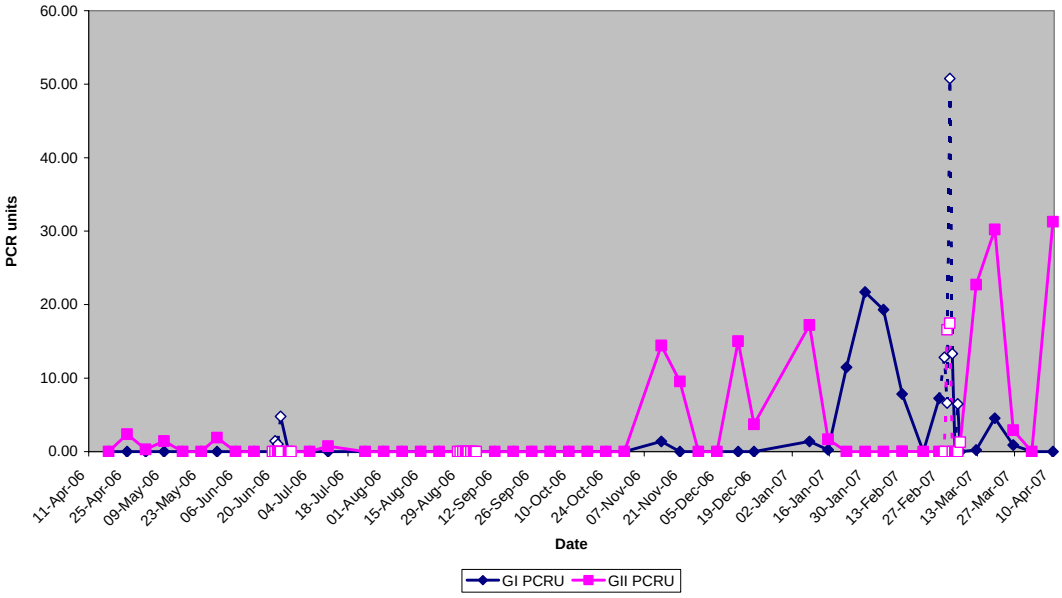


Figure 3.9. Time series plot of norovirus GI and GII PCR units for Site A. Event sampling days are indicated by white centred data points and dashed lines

3.3.1.1 Event sampling episodes

Prior to the June sampling event tissue *E. coli* levels at both sampling sites had been below 1000 MPN/100g (Figure 3.4). During this event week *E. coli* levels at Site A increased from low levels (equivalent of very low B and A classification) to levels in excess of 4600 MPN/100g. Levels then decreased slightly but remained above 1000 MPN/100g for the remainder of the week (Figure 3.11). There was a greater fluctuation in tissue FRNA bacteriophage levels at Site B during this event week (Figure 3.11). However, there was no indication that there was an increase in tissue FRNA bacteriophage levels above levels already observed in the weekly sampling (Figure 3.8).

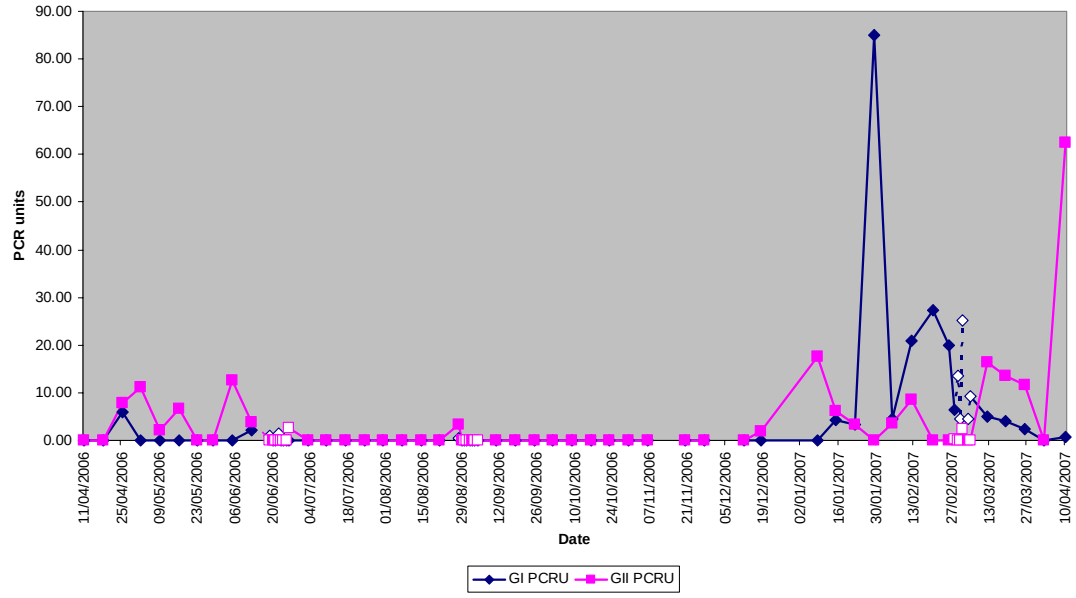
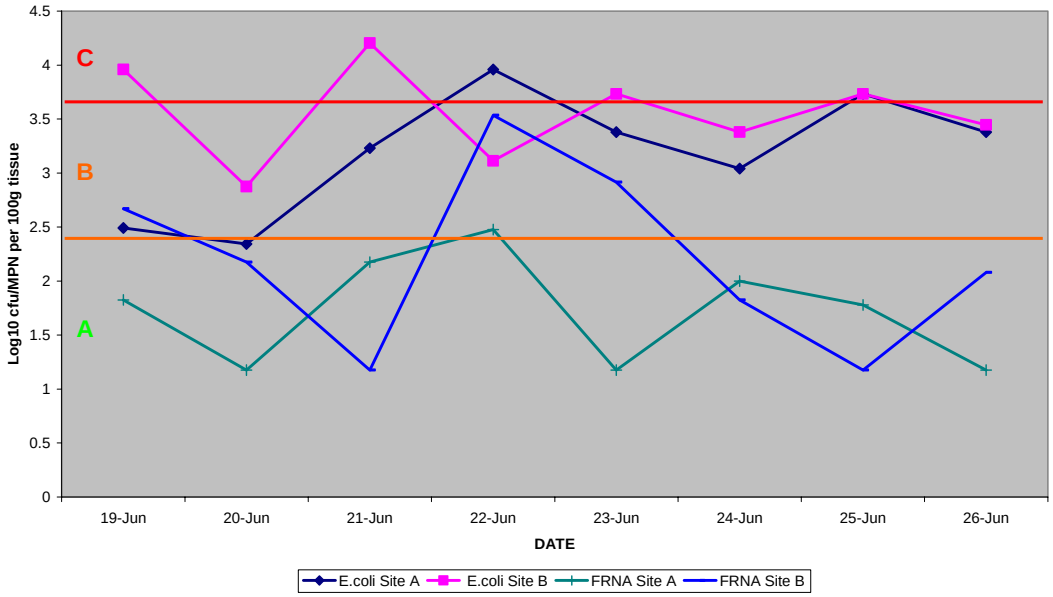


Figure 3.10. Time series plot of norovirus GI and GII PCR units for Site B. Event sampling days are indicated by white centred data points and dashed line.

WFC levels were similar at the two sites during this event (Figure 3.11). WFC levels during the June event were similar at the two sites. However, the levels observed at Site A were higher than had been observed up until that point in the sampling programme (Figure 3.6). The levels remained high and then had decreased by the following week.

Tissue *E. coli* levels at the beginning of the August event sampling week were below 100 MPN/100g. Site A levels exceeded 4600 MPN/100g twice over the course of the week and then remained at levels equivalent to a high 'B' classification. Site B *E. coli* increased to levels in excess of 4600 and remained at this level until the end of the week (Figure 3.12). By the following weeks sampling *E. coli* levels had returned to a low 'B' value (Figure 3.4). Tissue FRNA bacteriophage levels during the August event (Figure 3.12) did not increase above the levels observed in the weekly sampling (Figure 3.8). Levels were consistently higher at Site A than Site B. WFC levels during the August event show a high degree of fluctuation (Figure 3.12), much greater than observed during weekly sampling (Figure 3.6). Levels appeared to increase part way through the sampling week and then start to decrease towards the end of the week

During the February event tissue *E. coli* levels at Site A remained relatively stable. Levels at Site B increased from a low 'A' level to a low 'B' over the course of the week. Samples obtained during this event week indicate that tissue FRNA bacteriophage levels at both sites are within the levels previously seen in weekly sampling (Figure 3.8). FRNA bacteriophage levels at the two sites are similar and stable throughout the sampling week (Figure 3.13). WFC levels are lower than in the two previous events (Figure 3.6). Levels are initially stable and while levels increase at the end of the week at Site A, there is a decrease at Site B (Figure 3.12).Throughout the February event norovirus levels largely remained below 25 PCR units. Norovirus GI at Site A did increase to approximately 50 PCR units on the 2nd March but had fallen back to 13 PCR units by the next day and remained low for the rest of the week. GI at Site B was highest on the 3 rd March at 25 PCR units. Sampling could not be carried out at Site B on the 4th March as a result of bad weather.



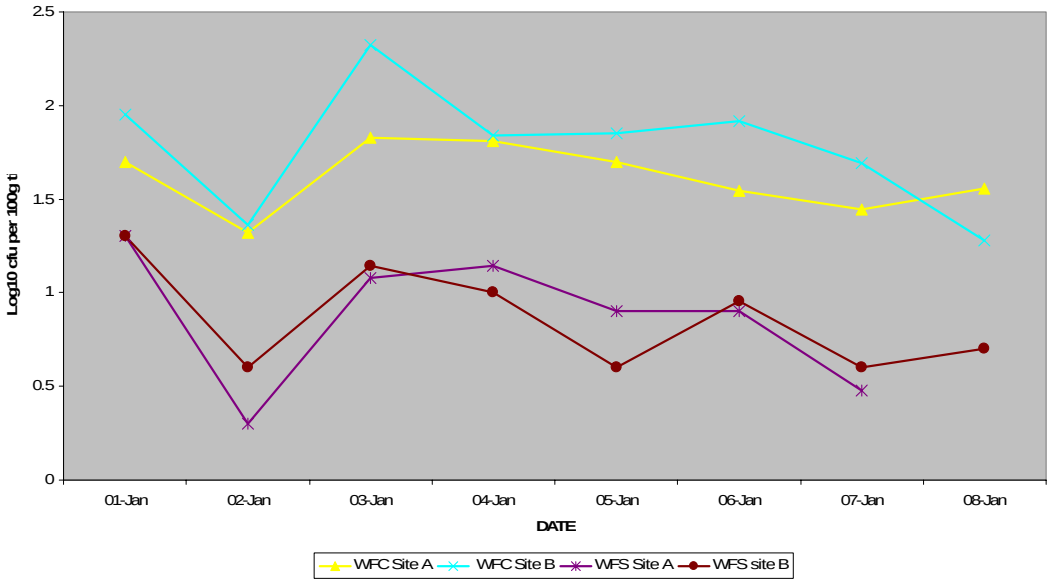


Figure 3.11. Levels of selected microbiological data during the June event sampling week. 'A', 'B' and 'C' levels pertain to *E. coli* classification levels (see Table 1.1).

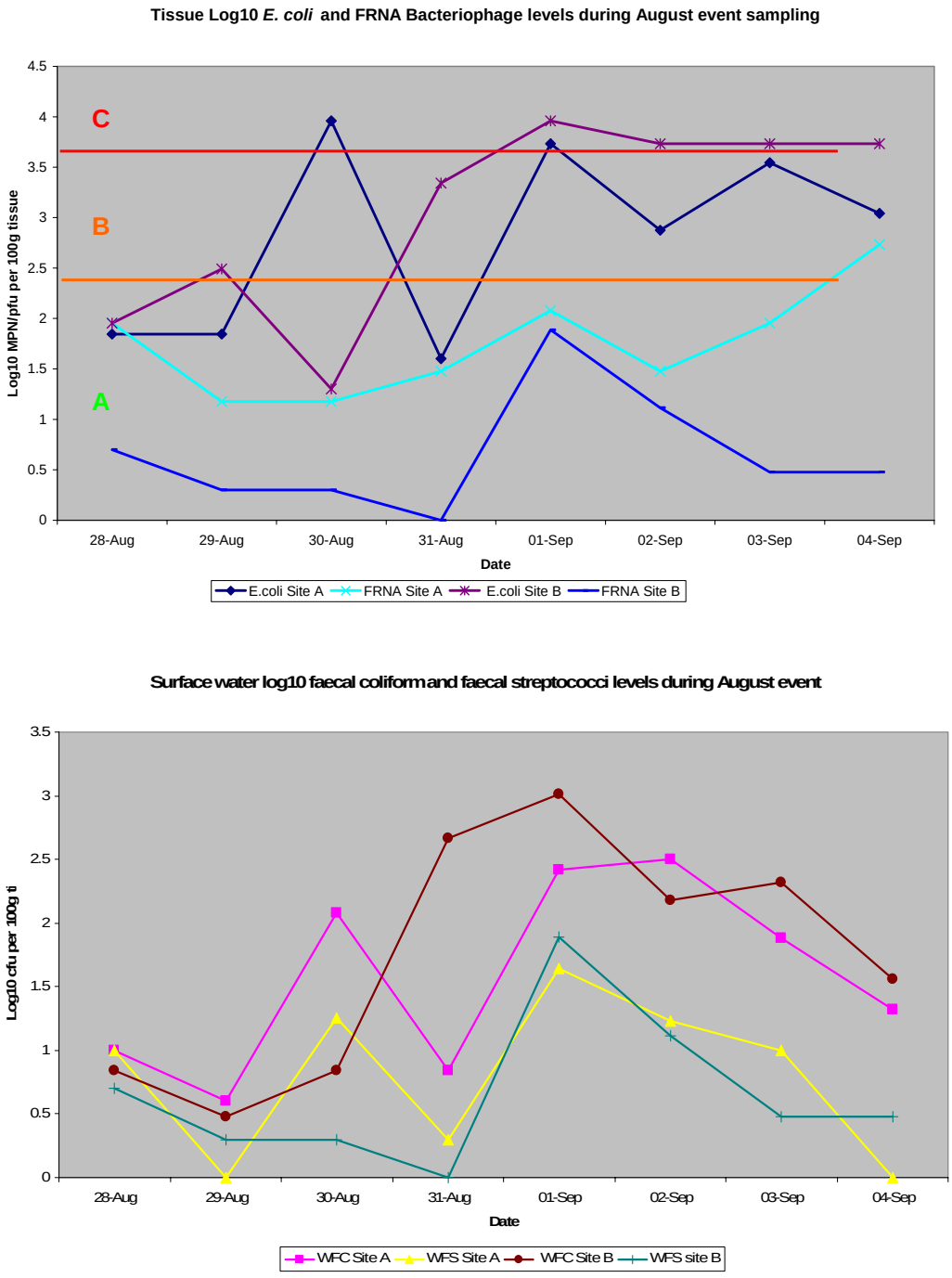


Figure 3.12. Levels of selected microbiological data during the August event sampling week. 'A', 'B' and 'C' levels pertain to *E. coli* classification levels (see Table 1.1).

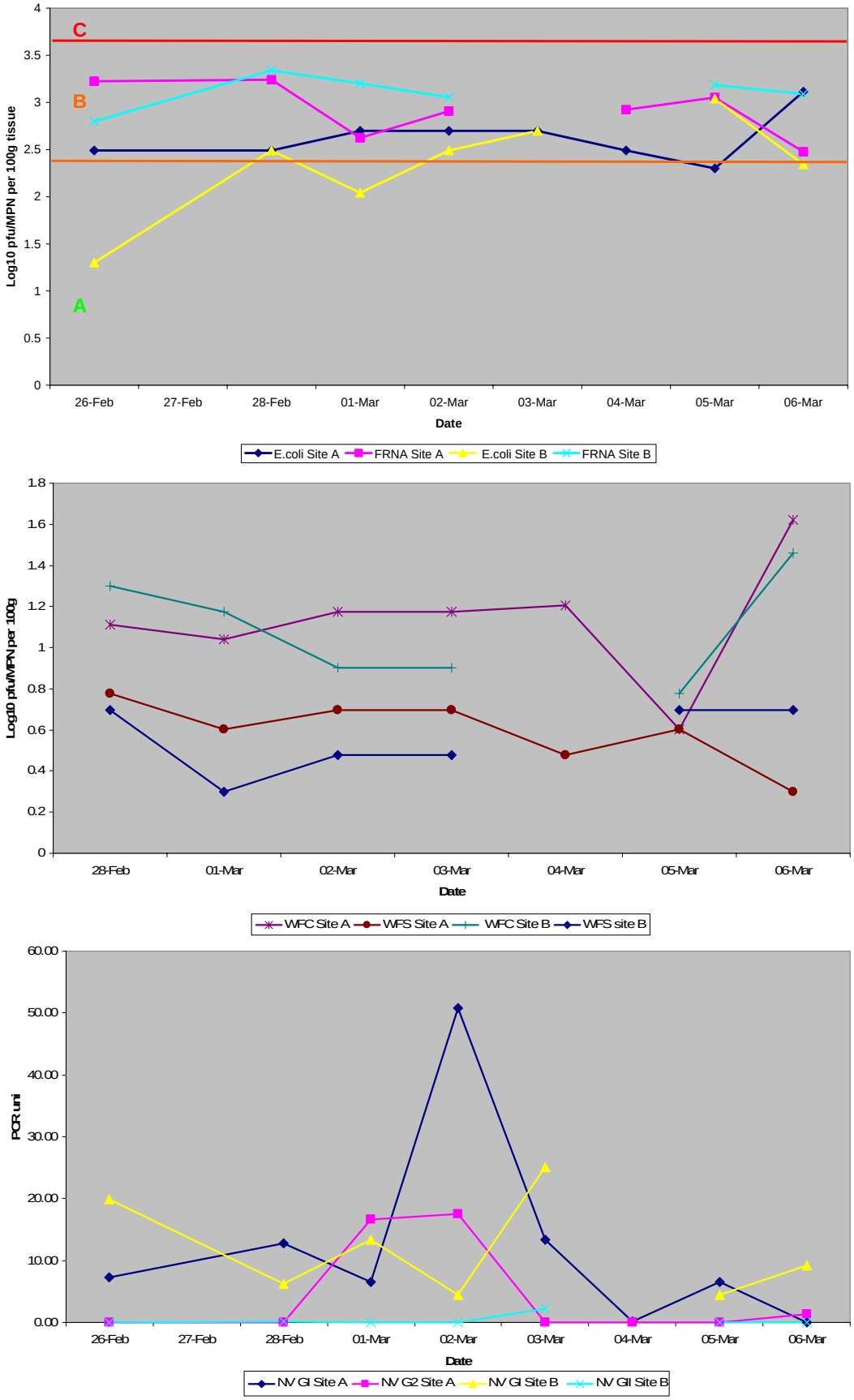


Figure 3.13. Levels of selected microbiological data during the February event sampling week. 'A', 'B' and 'C' levels pertain to *E. coli* classification levels (see Table 1.1)

3.3.2 Environmental data

3.3.2.1 Hydrodynamic data

Seawater temperature data shows an obvious seasonal trend at the surface and at 9 m, at both sites (Figures 3.14 and 3.15). The surface water temperature range was 5.5 – 16.7 ˚C at Site A and 5.1 – 17.4 ˚C at Site B. the temperature range at 9 m was 6.3 – 14.7 ˚C at Site A and 6.6 – 14.5 ˚C at Site B. Over the spring and summer months hourly temperature recordings at both sites show more fluctuation at the surface than at 9 m. During the summer months, the surface waters will be subject to warming on sunny days as well as inputs of freshwater and rainfall, producing natural fluctuation in temperature. Progressing into winter, the temperatures at 9 m appear to show more fluctuation while the surface records indicate a slight decrease in the degree of fluctuation. Temperature profiles for the surface loggers show a similar pattern at the two sites.

A similar seasonal pattern is seen in the hourly salinity recording for the study period (Figures 3.14 and 3.14). Surface salinity ranged from 2.6 – 27.9 at site A and 2.0 – 26.3 at Site B, while salinity at 9 m ranged from 4.1 – 27.9 at Site A and 2.7 – 27.9 at Site B. Surface salinity shows greater fluctuation over the summer months, than at 9 m. The winter period is characterised at both sites by reduced salinity at the surface and 9 m. Surface salinity at the two sites was recorded at below 15 for most of the period between November and February. At 9 m the salinity was recorded at below 20 for the majority of the same period.

Correlation analysis between hydrodynamic data and the winter and summer bacteriological data indicated that there was no correlation between tissue *E. coli* and any hydrodynamic data at either site, or any season (see Appendix II). There was a weak correlation between tissue FC levels and 9 m salinity at Site A (r=0.428, p=0.041, see Appendix II, Table I and II).

WFC and WFS levels did not correlate with hydrodynamic data during the summer, at either site. Significant negative correlations were observed between these parameters in the winter datasets for each site, indicating that during the summer levels of these parameters increased with decreasing salinity. The most significant of these was seen between the Site B WFC levels and both surface salinity parameters (24 hr mean – r= -0.673, p= 0.002; sampling time salinity – r= -0.629, p=0.005, Appendix Tables I and II)

Site A tissue FRNA bacteriophage summer data showed a weak negative correlation with surface seawater temperature (r=-0.423, p=0.044, see Appendix II, Tables I and II). No other significant correlations were observed between FRNA bacteriophage and hydrodynamic data. Site A norovirus GI displayed weak negative correlations against the winter surface and 9 m temperature parameters. The strongest correlation was against the surface temperature (r=-0.538, p=0.010). Site B norovirus GII was also negatively correlated against 9 m temperature (r=-0.494, p=0.020).

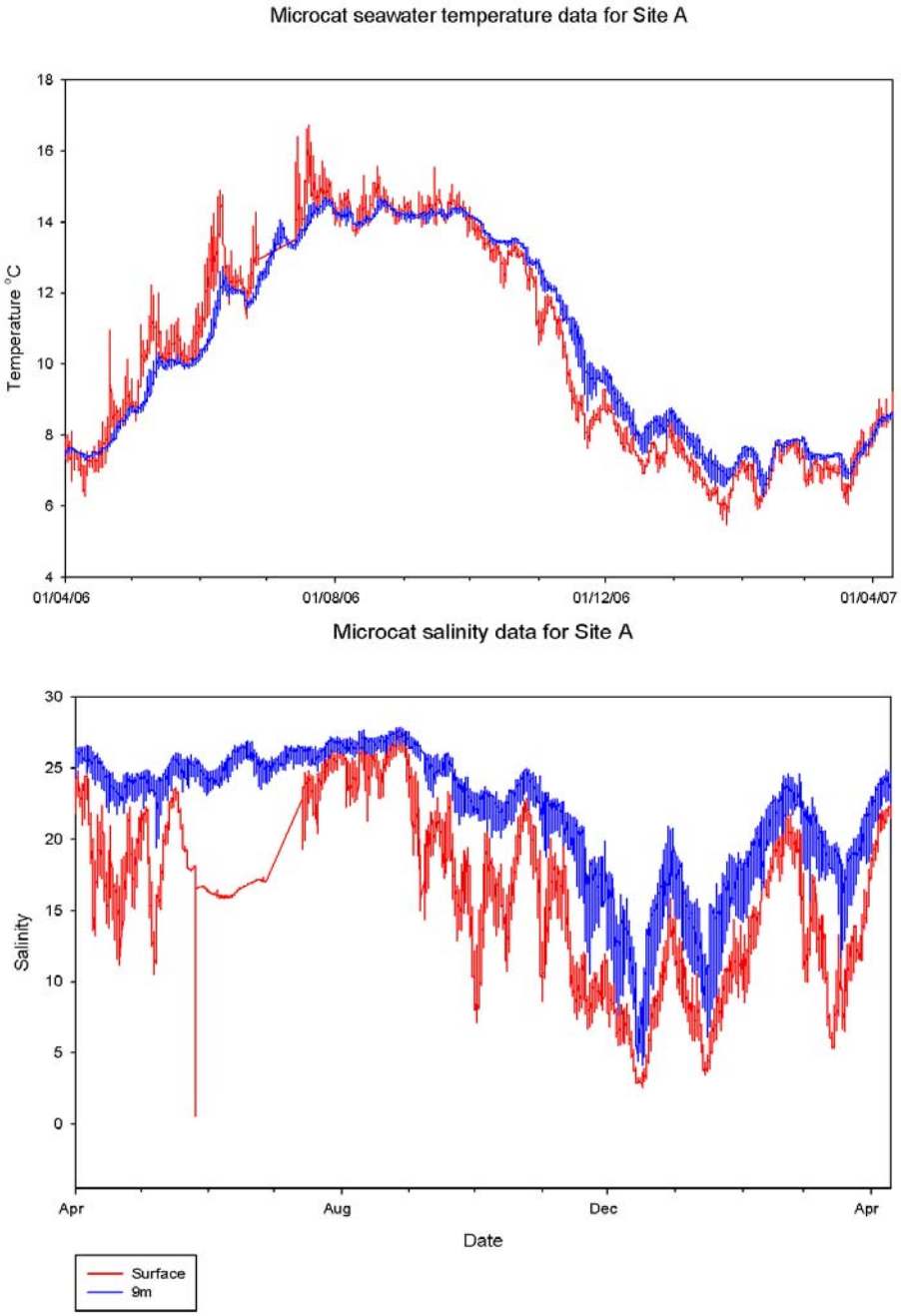


Figure 3.14. Time series plot of seawater temperature and salinity at Site A.

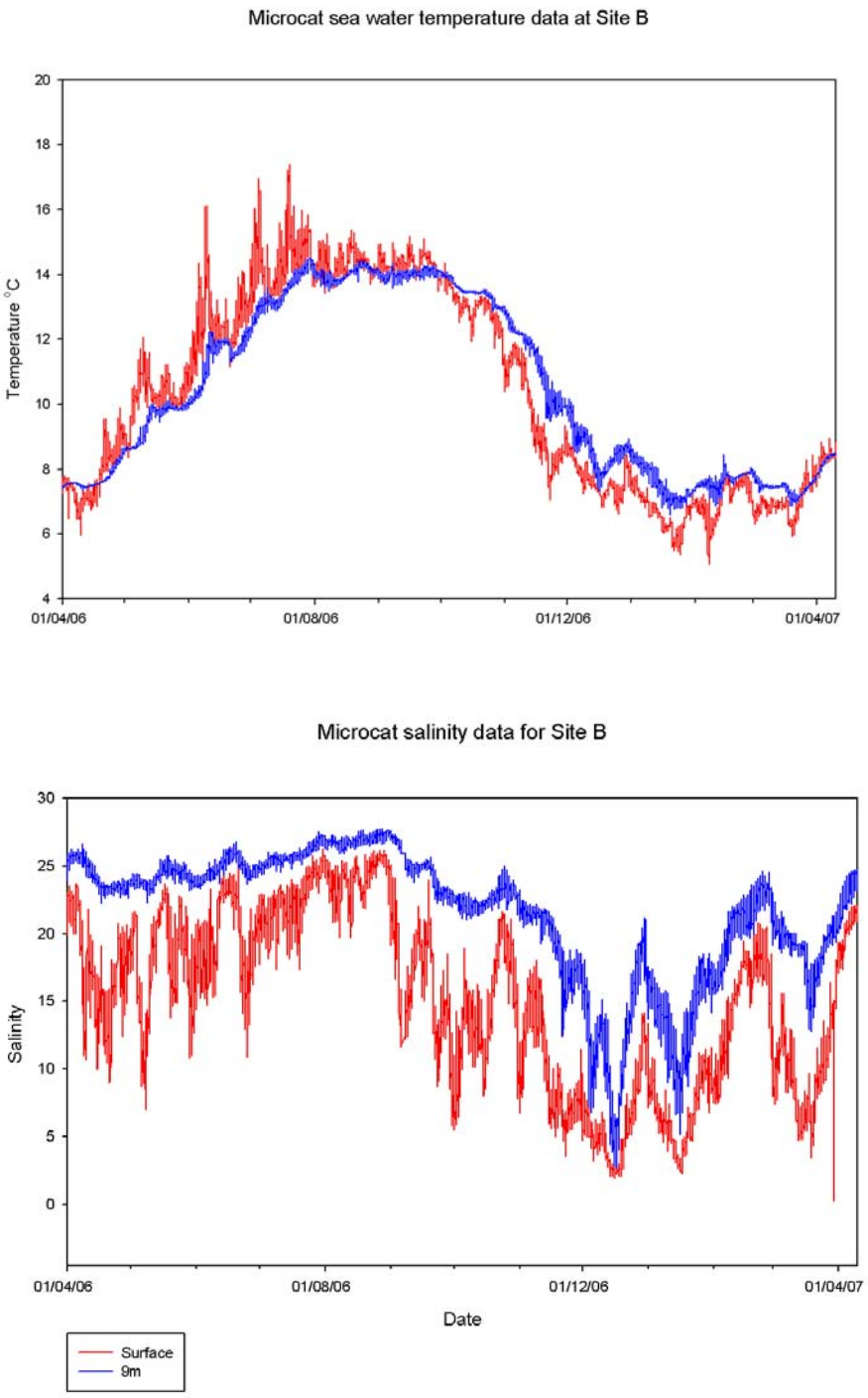


Figure 3.15. Time series plot of seawater temperature and salinity at Site B.

3.3.2.2 Meteorological data

The daily rainfall sequences (Figure 3.16) from the two monitoring sites were highly correlated (p=<0.001), as might be expected for such close locations. There was some 'altitude' effect and Glen Strae generally has a higher rainfall. The highest daily rainfall occurred at Glen Strae at 109.8 mm and there were 12 days with rainfall in excess of 40 mm (Table 3.6). Dunstaffnage had 3 days with rainfall greater than 40 mm with the highest daily value at 45.4 mm (Table 3.6). Total rainfall for the study period was greatest for Glen Strae at 3340.4 mm (*n*= 365), with total rainfall at Dunstaffnage of 2014.0 mm (*n*=354). At both sites,

rainfall was less during the summer months, as might be expected. Collation of historical rainfall data for both areas indicates that rainfall for the study period was relatively high. For Glen Strae average rainfall is estimated at 2761 mm (SEPA *pers. comm*) and the average annual rainfall for Dunstaffnage for 2002-2005 was 1509.3 mm.

Table 3.6. Daily rainfall summary.

Rainfall amount	**Glen Strae**	**Dunstaffnage**
0 mm	81	90
> 20 mm	50	22
> 40 mm	12	3
Highest daily value	109.8	45.4

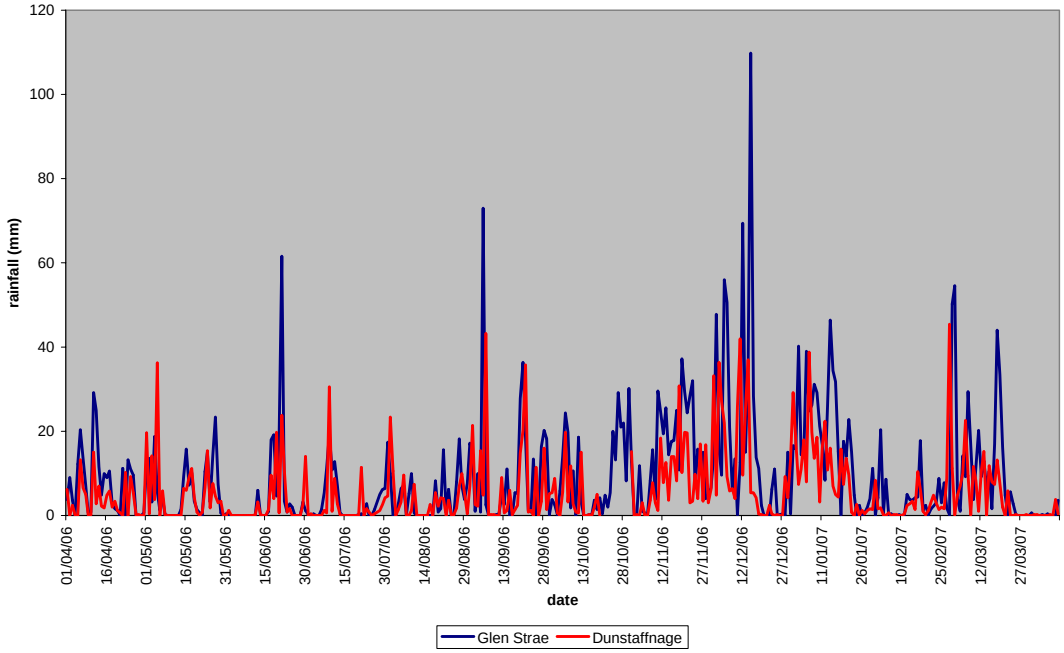


Figure 3.16. Time series plot of daily rainfall (mm) for Dunstaffnage and Glen Strae.

Correlation analysis (see Appendix II, Tables III, IV) indicated that summer tissue *E .coli* levels correlated significantly with Dunstaffnage 7 day average rainfall, with the relationship highly significant at Site B (Site A r=0.489, p=0.011; Site B r=0.655, p<0.001). At Site B there was also a significant relationship with a number of Glen Strae rainfall parameters (see Appendix II, Table III). Summer tissue FC levels at both sites were also correlated with Dunstaffnage 7 day average rainfall (see Appendix II, Table III).

WFC counts for both sites were found to correlate with a number of the rainfall parameters collated from both gauge sites (see Appendix II, Tables III, IV). The summer dataset revealed a strong correlation between the 144 hr average rainfall at Glen Strae (Site A - r=0.683, p=<0.001) and Dunstaffnage 7 day average rainfall (Site B r=0.604, p=0.001, Appendix II, Tables III, IV). There was also strong correlation between winter WFC and a number of Glen Strae and Dunstaffnage rainfall parameters, the strongest against Dunstaffnage 96 hr (Site A - r=0.768, p<0.001; Site B – r=0.722, p<0.001).

Summer WFS displayed a significant correlation with Glen Strae – 1 day rainfall (r=0.526, p=0.017) at Site A. The strongest correlation for WFS Site B occurred against Glen Strae 24 hr rainfall (r=0.571, p=0.009). During winter there was a variation in the strongest response

with Dunstaffnage 72hr rainfall displaying the strongest rainfall correlation for both sites (Site A - r=0.574, p=0.005; Site B –r=0.700, p=0.002) (see Appendix II, Tables III, IV).

Tissue FRNA bacteriophage data at Site B (summer dataset only) correlated with a number of rainfall parameters from 5-7 days prior to sampling. The most significant correlation occurred against -6 days Glen Strae rainfall (r=0.582, p=0.002). Only one negative correlation was apparent between winter Site A viral dataset and the rainfall parameters (norovirus GII/Dunstaffnage -2days – r=0.458, p=0.032). Winter Site B norovirus GI was negatively correlated with Glen Strae rainfall -5 days (r=-0.461, p=0.031), while GII was negatively correlated with Glen Strae -2day rainfall (r=-0.644, p=0.024) and Dunstaffnage -5day rainfall (r=-0.699, p=0.011) (see Appendix II, Tables III, IV).

Daily average sunshine strength shows a high degree of fluctuation (Figure. 3.17). This was particularly pronounced over the summer period. As might be expected the highest values were recorded during the summer, with a maximum daily value of 311.65 W/m^3 on 26/7/06. The lowest daily average value was recorded on 22/12/06 at 2.38 W/m^3. Mean daily average for the study period was 104.4 W/m^3 (SD 75.5 W/m^3).

There were no significant correlations between any microbiological parameters from the summer datasets and sunshine levels (see Appendix II for details). Winter Site A tissue *E. coli* levels were significantly correlated with sunshine (r=0.488, p=0.018). However, the relationship was positive, which was unexpected as increased sunlight is often associated with lower levels of *E. coli*. There was a negative correlation between sunshine and winter WFC at both sites (Site A, r=-0.611, p=0.002; Site B, r= 756, p<0.001, see Appendix II, Tables III, IV for details). No correlations were apparent between any of the winter norovirus datasets and sunshine, at either site.

A significant negative correlation was observed between FRNA bacteriophage and air temperature at the two sites during the summer (Site A – r=-0.595, p=0.002; Site B – r=-0.423, p=0.035). These relationships were not apparent during the winter. Air temperature was positively correlated with WFC levels during the winter and negatively correlated with norovirus GI during the winter (r=-0.472, p=0.027) (see Appendix II, Tables III, IV).

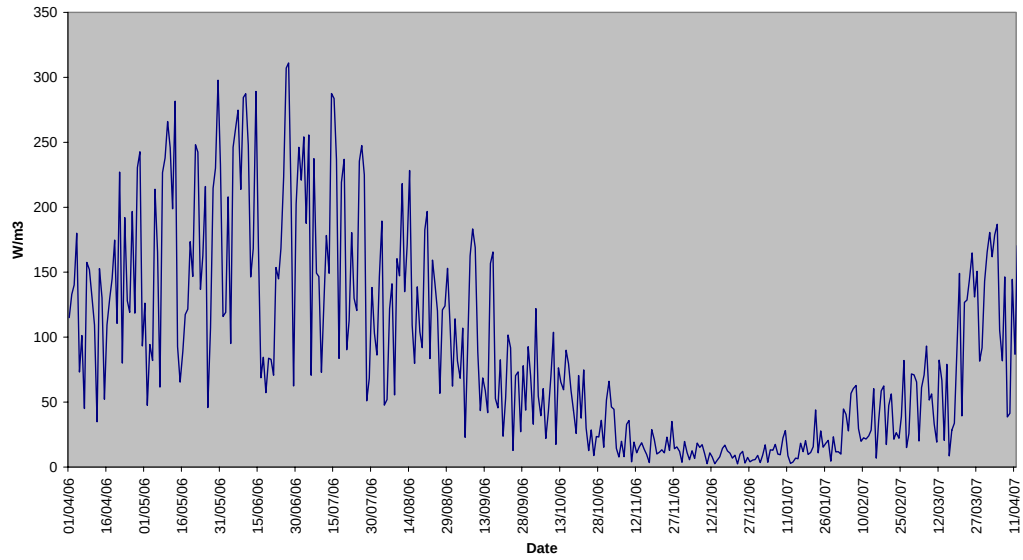


Figure 3.17. Daily average sunshine levels for Dunstaffnage

3.3.2.3 Hydrometric data

Average daily flow across the River Awe barrage is shown in Figure. 3.18. Average daily flow over the 12 month study was 71.0 m^3s^{-1}. The summer is characterised by low flow rates across the barrage (minimum flow rate was 5.2 m^3s^{-1}). Heavy rainfall throughout the winter period resulted in extended periods of high flow rates. The highest flow rate was 428.12 m^3s^{-1} on 14/12/06. Based on historical flow data for the barrage, it is estimated that the Q05 (flow rate that is exceeded 5% of the time and can be viewed as the high flow rate) for the barrage was 146.7 m^3s^{-1}. This flow was exceeded 52 times over the study period.

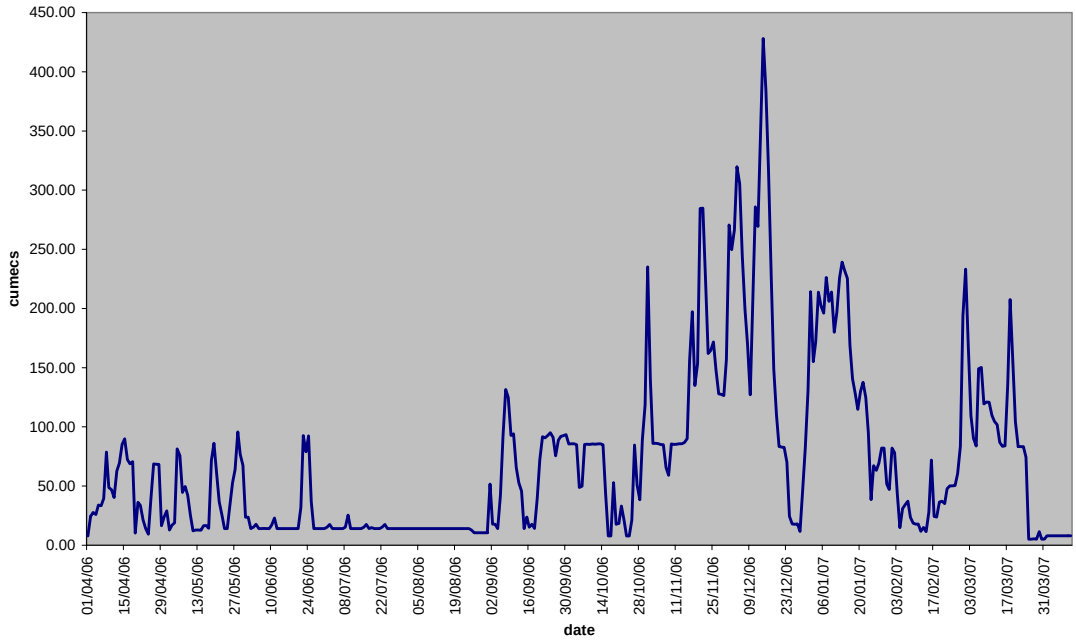


Figure 3.18. Daily average flow rate (m^3/s) for the River Awe at the barrage.

Daily average flow rate on the River Strae followed a similar pattern (Figure. 3.19) to daily rainfall pattern in Glen Strae, as might be expected. Mean daily flow rate for the study period was 3.4 m^3s^{-1} (SD 4.57 m^3s^{-1}). Highest daily flow rate was recorded on 13/12/06 at 37.54 m^3s^{-1}. Q05 for the River Strae is estimated at 10.76 m^3s^{-1} (SEPA *pers. comm.*) and was exceeded 29 times over the study period.

Correlation analysis (see Appendix II for details) indicated that *E. coli* and FC were not correlated with River Awe flow parameters, at either site. This was true for both summer and winter datasets. River Strae flow did not correlate during winter months (see Appendix II) however some relationships were displayed in the summer tissue *E. coli* and FC datasets. The most significant relationship was at Site B against the 24 hr average flow (*E. coli* – r=0.616, p=0.001, FC r=0.575, p=0.002 - Appendix II, Tables III, IV).

WFC counts for both sites showed significant positive correlation with a number of hydrometric parameters (see Appendix II, Tables III and IV for details). At Site A the most significant correlations occurred (both sites) between winter WFC and River Awe 24 hr flow, River Strae 72 hr and -2 days flow (all $p<0.001$, see Appendix II). At Site B the strongest correlations for WFC were against River Strae 72hr and 96 hr flow (both $p<0.001$ see Appendix II, Tables III, IV)

Mussel tissue FRNA bacteriophage data at Site A were not correlated with any hydrometric parameters, for either winter or summer datasets. At Site B showed no significant correlations between the winter FRNA bacteriophage dataset and hydrometric parameters. There was a correlation between River Strae -5 day and 144 hr mean flow during the summer (-5 day flow - r=0.458, p=0.021 see Appendix II). Winter norovirus GI and GII showed little relationship with any hydrometric parameters. The only significant correlation occurred between Site B norovirus GII and River Strae -4day flow (r=-0.634, p=0.027). This negative relationship suggests that the incidence of this norovirus genogroup decreases with river flow.

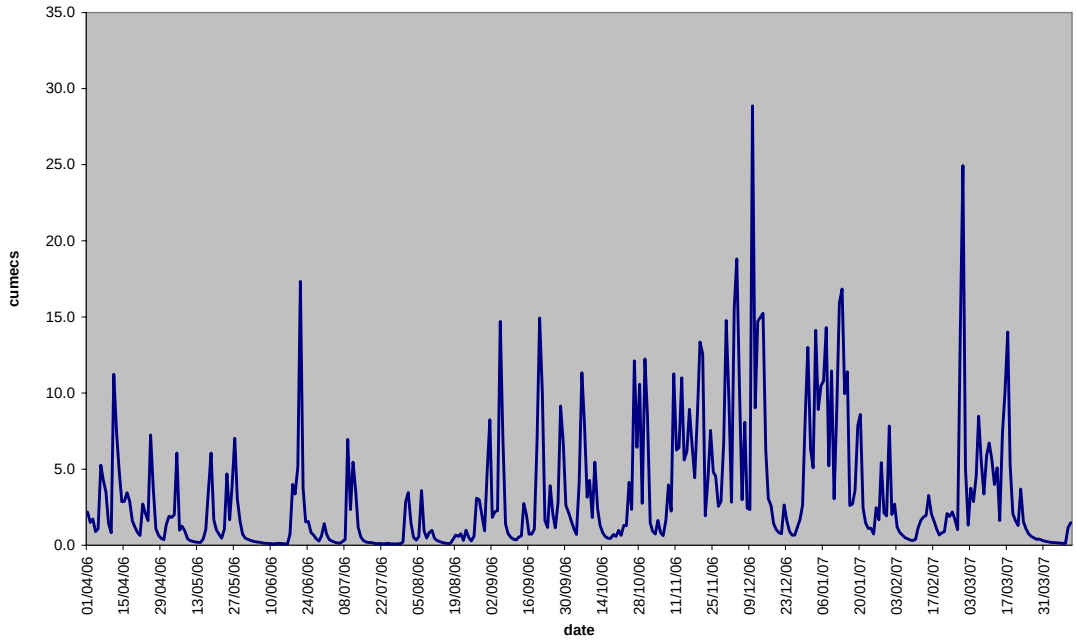


Figure 3.19. Time series plot of River Strae flow (m^3/s).

3.4 Discussion

This project is the first study to carry out a detailed assessment on the occurrence of faecal bacteria and norovirus in cultivated shellfish in Scotland.

It should be stressed that levels of enteric microbiological parameters presented here, may be regarded as a 'worse case scenario'. All shellfish flesh samples were taken from a maximum depth of 1 m. The surface waters are likely to experience maximum impact in terms of faecal contamination. The commercial mussel droppers at the study sites operate to a depth of 9 m, and contamination levels are likely to vary with depth, according to the depth of the freshwater layer. Kleinheinz *et al*., 2006 reported levels of enteric bacteria *E. coli* decreasing from the surface to 120 cm depth. In contrast, La Rosa *et al*., (2001) reported bacterial levels to be independent of depth, however in a system highly influenced by freshwater input, it is reasonable to expect the surface waters to be contain more enteric bacteria.

Relationships between microbiological parameters

The findings on the current project were based on a detailed weekly sampling regime. Sampling carried out for official classification purposes is undertaken on approximately a monthly basis and may not be capable of adequately characterising rapidly changing levels of microbiological contamination of cultivated shellfish which can be highly episodic in nature.

The correlation between microbiological parameters at the two monitoring sites may indicate that surface waters in the lower basin are well mixed. Similarity in hydrodynamic parameters between the sites supports this. The surface waters of the loch are subject to substantial tidal currents and there is substantial freshwater input. However, given that only two sampling sites could be studied it is not possible to assume that these results are representative of the entire shellfish waters of Loch Etive West. Establishment of classification sampling sites should involve a comprehensive assessment of water quality across the designated shellfish growing area. Again, for the reasons of comparability and reproducibility monitoring sites for classification should be consistent throughout the monitoring season. Under Regulation (EC) No. 854/2004, a 'sanitary survey' must be carried out by competent authorities who intend to classify shellfish production or relaying areas. Within the regulations an objective of the sanitary survey is to aid identification of representative shellfish hygiene monitoring points for that area and should include a bacterial survey of the area and surrounding waters. The results of the present study on the Loch Etive West production area also indicate that the establishment of classification sampling sites requires a comprehensive assessment of water quality across the designated area before routine monitoring points (RMP) are selected to represent the production area.

Both study sites showed a strong correlation between mussel tissue *E. coli* and FC levels, as might be expected since *E. coli* is a sub-group of FC. *E. coli* did not correlate with surface water FC, faecal streptococci concentration or norovirus parameter at Site A, in summer or winter. There was correlation at Site B between *E. coli* and surface water FC (summer dataset). FRNA bacteriophage in mussel tissue was correlated with the summer *E. coli* dataset at Site A.

The available literature reports a generally poor relationship between *E. coli* and norovirus levels, particularly in areas with an A classification and low *E. coli* levels (i.e. up to 230 MPN 100 g^{-1}) (Croci *et al.,* 2007; Formiga-Cruz *et al.,* 2002, 2003; Hernroth *et al.,* 2002). There was a weak correlation between winter *E. coli* and tissue norovirus GI and GII levels at Site B. This correlation was not observed in the winter dataset at Site A. When the 12 month monitoring data was assessed as one dataset (i.e. not split into summer and winter) there were no relationships between *E. coli* and norovirus. Significant correlations were only observed once the datasets had been divided. Results from previous studies appear to relate year round datasets (i.e. not on datasets split into summer and winter) and it is possible that analysis of winter only data in these previous studies may produce results similar to this study.

Lack of correlation between mussel tissue FRNA bacteriophage and norovirus level indicates that FRNA bacteriophage would not act as a useful indicator of norovirus presence in mussel tissue within Loch Etive Shellfish. In contrast, a number of studies have noted a significant correlation between FRNA bacteriophage and norovirus (Lowther *et al.,* in submission; Dore *et al.,* 2000). Formiga-Cruz *et al*., (2003) assessed the relationship between various bacterial and viral parameters across sites in Europe and reported FRNA bacteriophage as having a strong predictive value for norovirus, particularly at the UK site. A positive correlation between these two parameters has also been reported from shellfish sites on the Norwegian coast (Myrmel *et al.,* 2004). Data from a limited number of harvesting areas in the UK have indicated a positive relationship (CEFAS, *pers. comm.*) between the two parameters in areas more heavily influenced by sewage. It is possible that the lack of relationship between the two, as observed in the present study, is a result of the low levels detected in both parameters. FRNA bacteriophage is not always present in human faeces and may not be detected in

discharges from small private sewage systems serving a small number of individuals. Therefore this lack of correlation between norovirus levels and FRNA bacteriophage may also be evident in other west coast sealochs where the influence of domestic sewage on the production areas is small. EU Regulation 2073/2005 recognises that conventional faecal indicators are unreliable as indicators of norovirus presence in shellfish tissue. The regulation further recommends that guideline criteria needs to be established for viruses once robust detection and analytical methods are developed and validated.

A significant correlation between FRNA bacteriophage and *E. coli* levels was evident for the winter Site A dataset, but not for summer at Site A or at Site B in either summer or winter. F+ specific bacteriophage has previously been reported to occur at low levels on a number of Scottish shellfish waters (FRS, *pers. comm.*). The same study reported that two Class A shellfish production areas were found to contain high levels of both human and animal bacteriophage. This, along with the findings of the present study, suggests that FRNA bacteriophage is not an efficient indicator of *E. coli* levels in shellfish cultivated in Scotland.

Norovirus occurrence in Loch Etive shellfish

This study revealed rates of positivity (around 30 % in all cases) lower than has been observed in other British harvesting areas. Lowther *et al.,* (in submission) reported positivity rates of 52-69 % from two oyster harvesting areas, with maximum recorded levels in excess of 100 PCR units. Maximum levels detected in the present study were 50.8 PCR units at Site A and 85 PCR units at Site B.

Several studies have assessed the behaviour of viral pathogens in shellfish through depuration processes commonly utilised within the shellfish industry. These studies indicate that conventional depuration processes are inadequate in removing viral pathogens (Dore *et al.,* 1998; Le Guyader *et al.,* 2006b) and indeed prolonged depuration was ineffective in removing norovirus (Ueki *et al.,* 2007). A recent study assessed the change in viral levels in greenshell mussels (*Perna canaliculus*) following a range of heat treatments (Hewitt & Greening 2006). Norovirus levels were found to remain unchanged following conventional boiling and steaming. During cooking, mussel shells open causing a reduction in internal temperatures to a level below that required for viral removal. Full immersion in boiling water for three minutes was required to reduce viral concentration. This illustrates that there may still be a risk to human health following consumption of cooked mussels that contain viral pathogens.

It should be stressed that the actual risk presented to humans consuming shellfish contaminated with the levels of norovirus reported in this report is at present unknown. Since it is not possible to culture norovirus detection methods rely on PCR methods, which targets virus genomic material. Positive PCR results clearly indicate the previous presence of virus it remains a possibility that genomic material may persist in the environment in the absence of infectious virus. Given that numerous gastroenteritis outbreaks have been attributed to the consumption of contaminated shellfish, research to quantify and validate the relationship between such viral outbreaks in humans and the presence of norovirus genomic material in cultivated shellfish is essential. *E. coli* and FRNA bacteriophage levels in shellfish tissue were observed to be ineffective indicators of norovirus levels, therefore monitoring of shellfish beds should incorporate additional monitoring for virus contamination, once standardised and validated methods have been developed.

Seasonal fluctuation of microbiological parameters

E. coli and FC levels in mussel tissue showed strong seasonal pattern, with greater concentrations during summer months. A number of factors may contribute to increased prevalence of faecal bacteria in shellfish during summer, including

- Increased livestock numbers within the catchment area
- Increase in population during the main tourist season
- Variation in survival rate of *E. coli* and FC across seasons

The Loch Etive area is frequented by tourists over the summer months. The Sanitary Survey Report estimated that the local population is increased by approximately 12% during the summer period and it is possible this may contribute to increased flux of domestic sewage into the loch. In the local villages of Connel and North Connel approximately 53% of the population is served by an upgraded (completed July 2006) public network (Scottish Water *pers. comm.*). The sewage is pumped along to two septic systems and the outfall for both populations is now outwith the lower basin of Loch Etive (Connel outfall is 3.6 km from the Loch Etive West shellfish growing waters and North Connel is 2.8 km away). There are a number of private systems offering primary treatment of domestic sewage (septic tanks and RBC units), however, there appear to be a number of raw outfalls for domestic properties (one being a small public network in Bonawe). Some of these discharges occur within the shellfish growing waters or in close proximity to it. This issue potentially needs to be addressed. An upgrade schedule has been agreed for Bonawe, where the public network serves around 60% of a population of 140.

Other Scottish (and indeed UK-wide) catchments with a small sewage component have demonstrated extreme seasonality in FIO flux with a summer peak in both concentration and export co-efficient from rural livestock areas (Kay *et al.,* 2008a, 2008b; Kay *et al.,* 2007a, 2007b, 2007c; Kay *et al.,* 2005c; Rogers *et al.,* 2003). The catchment area of Loch Etive is largely rural. Much of the livestock grazing areas are found adjacent to the loch shore of the lower basin in close proximity to the shellfish growing waters. Livestock in the area is predominantly sheep and numbers are at a maximum during summer due to the presence of lambs. The Sanitary Survey Report for Loch Etive indicated that sheep numbers (approximately 5000 ewes) in the catchment outweighs the human population (approximately 2500 in the immediate catchment area). Grazing areas can retain substantial stores of faecal bacteria (Oliver *et al.,* 2005). Following high rainfall faecal bacteria stored on grazing areas can be readily washed into field drains, rivers and streams resulting in an increased load of faecal bacteria to coastal receiving waters (Oliver *et al.,* 2005; Davies-Colley *et al.,* 2004a; Ferguson *et al.,* 2003, 1996; Jamieson *et al.,* 2003; Rodgers *et al.,* 2003). Streams in agricultural areas have been found to store enteric bacteria with survival reported at up to 6 weeks (Wilkinson *et al.,* 2006; Jamieson *et al.,* 2003, 2005a, b). It may be necessary to assess local farming practices, including

- the access of livestock have to streams and rivers
- the proximity of feeding troughs to streams and rivers
- the location of hard standings and silage heaps in relation to streams and rivers
- the localised use of slurry application to agricultural areas

All these factors have been shown to impact faecal bacterial loads to inland waterways, which can then impact nearshore habitats, such as shellfish growing waters (Ramos *et al.,* 2006; Meals & Braun 2006; Davies-Colley *et al.,* 2004; Miller *et al.,* 2004; Rodgers *et al.,* 2003; Nunez-Delgado *et al.,* 2002). Full details are given in the Sanitary Survey Report. It is possible that management solutions may be required for practices that have an obvious

impact on the flux of enteric bacteria to rivers and streams that feed into the shellfish growing waters (Oliver *et al.,* 2007). Such issues need to be systematically assessed by the relevant regulatory authorities.

Other factors that may contribute to the seasonality observed include the bactericidal effect of UV light and survival rate of *E. coli* at lower temperatures (Troussellier *et al.,* 1998; Girones *et al.,* 1989)

In contrast to the seasonal summer pattern of *E. coli* prevalence, a greater proportion of norovirus positive samples were detected during the winter, with low or no detection in the summer. This occurrence is in agreement with the natural prevalence of the virus within the human population in northern Europe and is supported by a number of studies (Lowther *et al.,* in submission; Myrmel *et al.,* 2004; Formiga-Cruz *et al.,* 2002, 2003; Le Guyader *et al.,* 2000). In contrast Croci *et al.,* (2007) reports norovirus prevalence throughout the year, but with a slightly higher incidence during the winter. Slight increase in winter norovirus incidence is also reported by Hernroth *et al.,* (2002). Higher incidence of norovirus in winter could be a result of lower solar radiation, low temperature and higher turbidity during that time of year.

Seasonality was not obvious in FRNA bacteriophage levels in mussel tissue. A number of studies report seasonality in this parameter with higher numbers detected during the winter (Myrmel *et al.,* 2004). Dore *et al.,* (2003) also report a seasonal prevalence in FRNA bacteriophage levels, with geometric mean of 4503 pfu/100g in winter and 910 pfu/100g in summer. This compares with geometric means reported in this study of 207 (Site A) and 206 (Site B) for the present study.

The seasonal trends observed for bacterial and viral parameters highlights the need for detailed year round sampling of established shellfish waters. Monitoring sites should be fixed to allow reliable comparison of microbiological parameters throughout the year. Such a sampling strategy should also be applied during the assessment phase for new shellfish production areas. FSAS introduced a new classification system in early 2007 which specifies year round monitoring of fixed sampling points within the production areas.

Relationship between microbiological parameters and environmental parameters

The influence of wind on shellfish contamination patterns was not tested as part of the 12 Month Monitoring Report. Recent modelling studies carried on Loch Etive have indicated that, at increased wind speeds, the depth of the surface layer may increase due to increased vertical mixing (P. Gillibrand, SAMS, *pers. comm.*). It should be noted that the modelling approach described has not been validated for the lower basin of Loch Etive and does not take wind direction into consideration. However, it indicates that increased wind speeds can cause a decrease in exchange rate. Faecal bacteria are more likely to be associated with the fresher surface waters. If the depth of this surface fresh water layer increases at higher wind speeds and it is retained within the loch for longer periods, there could be an impact on cultured shellfish impacted by water in this layer. However, the true effect of wind speeds can only be quantified through further model development and validation.

Dunstaffnage 7 day average rainfall was correlated to tissue *E. coli* levels at both sites during the summer months. River Strae flow (24 and 48 hr) was also correlated with tissue *E. coli* at both sites. The resultant correlations for these parameters were stronger at Site B (i.e. highly significant relationships were observed at Site B and weaker relationships at Site A). In

general, more significant relationships against rainfall and river flow parameters were observed for Site B. This may indicate that such environmental factors have a stronger influence on faecal bacterial levels at Site B, which is in closer proximity to the major freshwater inputs to the loch. Environmental parameters appeared to show little in the way of significant relationships with winter tissue bacterial datasets. This may be related to the observed lower levels of tissue *E. coli* and FC prevalence over winter months.

Surface water FC levels at both sites did show a number of highly significant positive relationships with the rainfall and river flow data in both summer and winter. Therefore there appears to be a difference in response to environmental variables between *E. coli*/FC in mussels and surface water FC levels. This could be a result of rapid changes in surface water quality and subsequent depuration of bacteria by the mussels. It is also possible that at low salinities the mussels may cease feeding and therefore tissue bacterial concentration may not reflect water conditions when salinity is low.

Norovirus GI and GII were predominantly present during winter months, being either absent or only detected at very low levels during the summer. There was a negative correlation with winter seawater temperature data, but no correlation with salinity data at either site. A number of studies have reported a significant relationship between temperature and norovirus (Formiga-Cruz *et al.,* 2003; Girones *et al.,* 1989). Further to this, norovirus outbreaks in France in December 2002 have been linked to heavy rainfall and flooding of rivers (Le Guyader *et al.,* 2006; Miossec *et al.,* 2000). There were few significant correlations between norovirus data and environmental parameters. However, winter norovirus GI levels at Site B were correlated with Glen Strae rainfall (-5 days) and River Awe flow (-2 days). However given the low levels of norovirus detected (many of the norovirus results were at or close to the limit of detection), it would be premature to assume that these parameters have a significant influence on norovirus levels. Summer tissue FRNA bacteriophage levels showed significant negative correlation to surface seawater temperature data (Site A) and summer air temperature (both sites). Summer Site B levels were also positively correlated with -5 to -7 days rainfall in the upper catchment (Glen Strae) and -5 days River Strae flow. This indicates that FRNA bacteriophage is more prevalent at colder temperatures and in this case appears to be driven by upper catchment dynamics approximately 5-7 days before sampling. Similar relationships were reported by Hernroth et al., (2002) who reported significant relationships between river flow and temperature and FRNA bacteriophage. However, a negative relationship to river flow was reported in that case.

This 12 month monitoring report has discussed the role of potential environmental drivers in determining faecal microbial contamination in cultured shellfish. Statistical modelling of the relationship between microbial and environmental parameters was investigated but the results were inconclusive and are therefore not included here. The correlation analysis did indicate a potential predictive potential in some of the environmental parameters, however these did not always perform consistently across the two monitoring sites. Given that faecal contamination of the shellfish was evident during this study, particularly during the summer months, it was considered appropriate that a detailed quantitative study of the potential faecal pollution sources was carried out. To this effect a study of source apportionment was undertaken in order to quantify the flux of faecal bacteria from a number of sources to the lower basin of Loch Etive. The details of this study are provided in Chapter 4.

4. Source apportionment for faecal indicator fluxes into Loch Etive lower basin

Carl Stapleton[1], David Kay[1], Christopher Kay[1], Carol Francis[2] John Watkins[2] and Cheryl Davies[2]

1. **Centre for Research into** Environment and Health
   University of Wales
   Lampeter
   Ceredigion
   SA48 7ED
2. **CREH *Analytical* Ltd**.
   Hoyland House
   50 Back Lane
   Horsforth
   Leeds
   LS18 4RS

4.1 Introduction

4.1.1 Context and aims of the project

For more than 20 years major expenditure has been committed to improve the microbiological quality of bathing and shellfish waters as required by European Union Directives. This has brought about significant improvement within the UK and the European Union as a whole. However failures against the standards of these Directives still occur. Historically, within the UK, the major cause of these failures was related to effluent discharging from sewage treatment works and sewer overflows. These assets are being dealt with through investment in improved treatment and sewerage infrastructure, and attention is now focusing on remaining pollution sources such as run-off from agriculture.

Faecal indicator organism fluxes from catchment systems have received much less research attention worldwide than other water quality parameters such as nutrients. In Europe, new catchment based regulatory tools, outlined in Directive 200/60/EC establishing a framework for Community action in the field of water policy (the 'Water Framework Directive'; Anon., 2000), are raising the profile of faecal indicators and defining mechanisms for controlling their concentrations in catchment systems. These new regulations are highlighting the need for models able to predict faecal indicator organism flux from large catchments with a complex mix of land uses.

This report presents the results of an investigation into faecal indicator sources and budgets for catchments discharging to the outer basin of Loch Etive. Field sampling of selected catchments was undertaken during the summer of 2006 whilst additional, generally smaller, catchments were sampled during summer 2007. The report describes the combined budgets of faecal indicator organisms from both sewage and riverine sources discharging to the Loch. The field data collection exercise reported herein was designed provide characteristic riverine and effluent quality that could be combined with flow data to enable calculation of flux estimates. Thus, the field study phase involved routine and opportunistic sample collection from riverine and point source inputs. The sampling periods also included installation of temporary river and sewer flow monitors to augment existing flow monitoring equipment.

4.1.2 The study area and study period

Funding available to this investigation for the initial 2006 study (Stapleton *et al*., 2007a) allowed for a summer monitoring period of approximately 40% of the identified 34 potential stream and sewage inputs which could impact on the outer basin of Loch Etive. The larger volumetric inputs were generally chosen for detailed characterisation and the data reported in Stapleton *et al*. (2007a) relate only to these monitored inputs, potentially making the flux estimates reported therein somewhat optimistic. To enable a more complete characterisation of the flux estimates to the lower basin of Loch Etive additional funding was allocated to allow sampling of catchments omitted from the 2006 study together with additional sampling of key inputs identified in Stapleton *et al*. (2007a). In total, 26 catchment inputs were covered by the 2006 and 2007 sampling. The approach taken to integrating the results from both years' sampling programmes herein was to use the 2006 flow data since flows from Taynuilt WwTWs were available for part of the study period from that year. No additional sewage flow data were measured during 2007.

This study assumed that sewage discharges south of the Falls of Lora do not impact on water quality in the lower basin above the Falls. Confirmation of this assumption would require a tracer study outwith the scope of the investigation reported herein. The data reported below should not be considered characteristic of winter inputs to the Loch due to the extreme seasonality in faecal indicator flux observed in recent empirical studies sponsored by the Scottish Executive and SEPA (Kay *et al.*, 2007a, d).

The catchment areas (km^2) referred to in this report were derived from data supplied by the Scottish Environment Protection Agency (SEPA), the Scottish Association for Marine Science (SAMS) and estimated by CREH using a geographical information system (GIS). The total monitored catchment area draining to Loch Etive amounted to 957.9 km^2. The largest catchment, covering 828.6 km^2 was that of the River Awe, although flows into Loch Etive are regulated by the hydroelectric power plant at Inverawe. The River Awe forms the easternmost input to Loch Etive considered in this report. The remaining catchments included within this study were considerably smaller, the largest that of the River Nant, covering 45.0 km^2. The next largest catchment sampled was Lusragan Burn (21.8 km^2), which flows into Loch Etive at Connel and represents the westernmost input considered. Other named catchments monitored include Abhainn Achnacree, River Esragan, Blacreen Burn, River Luachragan, Allt na h-Airde, Allt Tig Dhonnchaidh, Allt Ardache, Allt Dail a Mhuilinn and several unnamed catchments draining the areas between the larger catchments (Figure 4.1).

The study period during 2006 covered a 34-day period (816 hours) commencing at 12:00 GMT on 7th July 2006 and ending at 12:00 GMT on 10th August 2006. This period is referred to as the 'study period' throughout the report since it is for this period that the most detailed flow data are available and for which budget estimates were made. The additional sampling carried out during 2007 was undertaken between 6th August 2007 and 14th September 2007.

4.2 Data sources, sampling and analysis

4.2.1 Hydrometric data

4.2.1.1 River discharge, stream level and rainfall

Flow data for the River Awe at the HEP barrage were supplied to CREH by the operators of the HEP, Scottish Southern Energy (Figure 4.1, Table 4.1).

All catchment outlet sites, with the exception of the Rivers Nant and Awe, were monitored for stage through installation of manometric level recorders (Ellenkamp) (two at each site) and 'compensation' atmospheric pressure recorders at strategic locations. Where practical, stream level staff gauges (1 m) were also installed at sampling locations for the duration of the study. At sites where manometric level recorders were installed, the staff gauges were referenced to the level recorders.

Hourly time-series of flows were derived at five sample sites (Figure 4.1, Table 4.1) through spot gauging of velocity using a calibrated electromagnetic current meter. Stage-discharge relationships were established using the velocity measurements combined with the stage records from the manometric level recorders. Open channel flow surveys followed England and Wales Environment Agency guidance using the mid-section method to establish stage-discharge relationships (Environment Agency, 2003). The 'curve fitting' routines of the SPSS statistical computer software package (SPSS, 2002) were used to derive the best-fit relationships for the data from each site. Linear, exponential, logarithmic and power models

were investigated and the model with the greatest coefficient of determination (r^2) and statistical significance (p) selected.

Rainfall input (mm) was monitored at four tipping bucket rain gauges, installed for the duration of the study by CREH, across the catchment (Figure 4.1; Table 4.1).

Table 4.1: Summary details of flow ($m^3 s^{-1}$ or $l s^{-1}$), level (m) and rain gauges (mm) used in the current study. Temporary gauges were installed for the period 7/7/06 to 10/8/06 (i.e. the study period) unless otherwise specified.

Name	NGR	Type
River Awe, Barrage	NN045287	Flow ($m^3 s^{-1}$) (Permanent)
Taynuilt WwTW	NN016317	Flow ($l s^{-1}$) (Permanent)*
		Flow ($l s^{-1}$) (Temporary)†
		Rain (mm) (Temporary)
Site 202 – Inion Farm stream	NM958752	Stage (m) (Temporary)
		Rain (mm) (Temporary)
Site 204 – Blacreen Burn	NM992351	Stage (m) (Temporary)
		Rain (mm) (Temporary)
Site 207 – Culnadalloch stream	NM949337	Rain (mm) (Temporary)
Site 208 – Allt Nathais	NM980328	Stage (m) (Temporary)
Site 209 – R. Luachragan	NM978332	Stage (m) (Temporary)
Site 210 – Allt na h-Airde	NM998318	Stage (m) (Temporary)

* Permanent flow monitors at Taynuilt WwTW appeared to be operating incorrectly. These data were therefore not used for the current study.

† Temporary flow monitors at Taynuilt WwTW installed on inlet, CSO and combined final effluent + CSO for the period 20/6/06 to 26/7/06 for purposes of another project.

4.2.1.2 Discharge from the sewerage infrastructure

Permanent flow monitoring equipment was installed at Taynuilt WwTW, where three sample sites were present (final effluent (FE), combined sewage overflow (CSO) and the mixed FE and CSO effluent) although this was not operative during the study. However, flow data were available for the period 20/6/06 to 26/7/06 from temporary monitors installed for another project undertaken by Abertay University and supplied by Scottish Water. Flow data were available for the inlet, CSO effluent and the combined FE and CSO effluent. The other sewage effluent sample site, a CSO in Connel, did not have any flow measurement equipment installed.

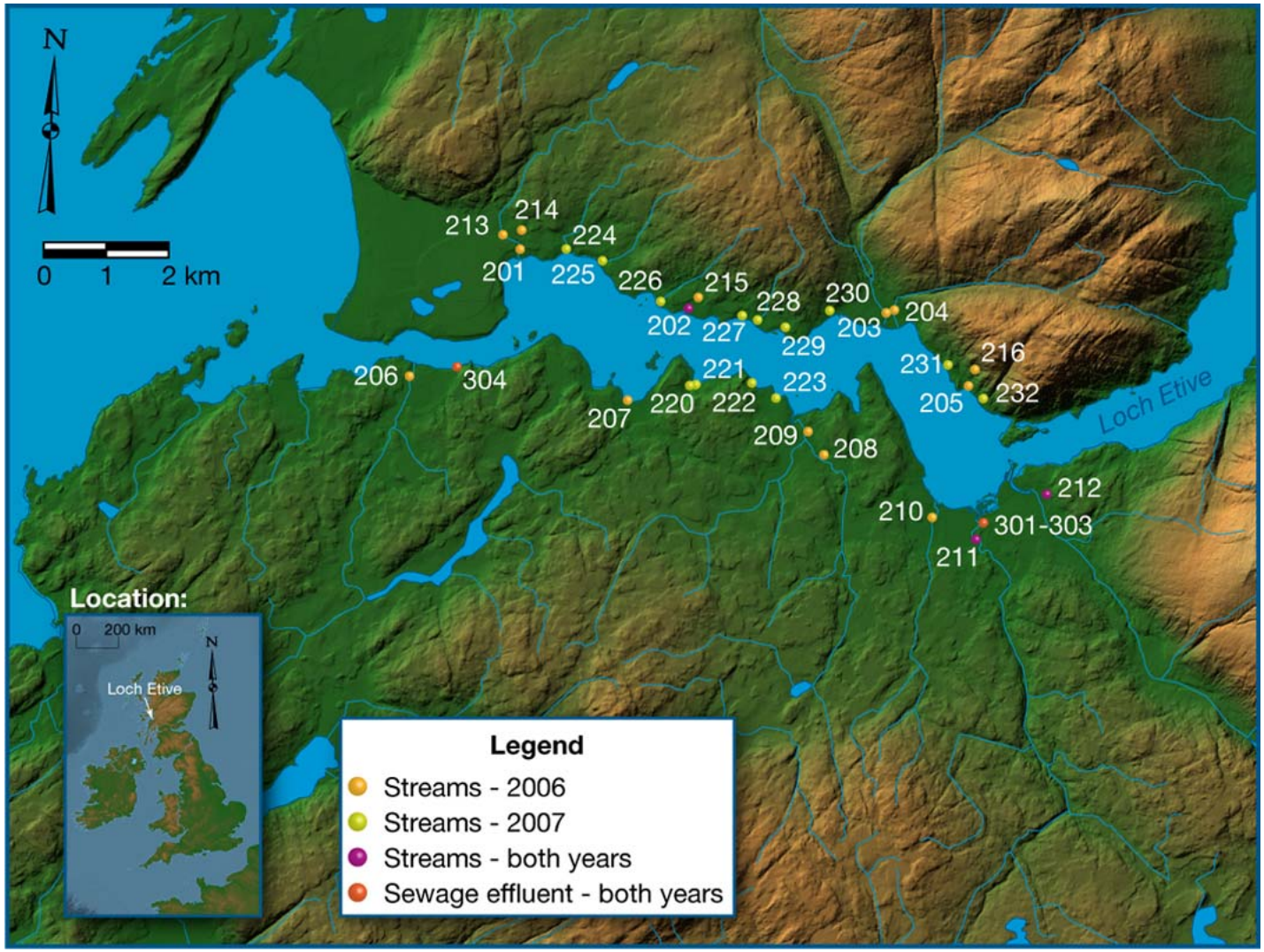


Figure 4.1: Riverine and sewage sample points monitored during the study. Rain gauges were located at sites 202, 204, 207 and 302. Stage recording monitors were located at sites 202, 204, 207, 208, 209 and 210.

4.2.2 River, stream and effluent sampling

4.2.2.1 Rivers and streams

During the 2006 survey, river and stream water samples were collected at 16 locations (Table 4.2; Figure 4.1). Twelve sites were initially selected to allow budget calculations (i.e. 11 sites close to the selected catchment outlets plus one on Allt Nathais upstream of its confluence with the River Luachragan). An additional four sites were added to the sampling regime after initial results indicated particularly high base flow FIO concentrations in Abhainn Achnacree (site 201), Inion Farm stream (site 202) and Kenmore stream (site 205). Two of these additional sites were located within the Abhainn Achnacree catchment, one on the main river (site 213) and one on Allt nam Ban (site 214), both upstream of their confluence. One site was added to Inion Farm stream, upstream of Inion Farm (site 215), whilst the final additional site was located upstream of Ardchattan school on the Kenmore stream (site 216).
The 2007 survey included three sites sampled during 2006, Inion Farm stream (site 202), the River Nant (site 211) and the River Awe (site 212) plus a further 13 new locations, all close to where the streams flow into the lower basin of Loch Etive (Table 4.3; Figure 4.1). These new streams were all relatively small, with the largest catchment area being that of Allt an t-Siomain at 2.4 km^2.

Table 4.2: River and stream water quality sites sampled during the 2006 survey**.**

Site Code	River	Location	NGR	Catchment Area (km^2)
201	Abhainn Achnacree	Tidal limit	NM931361	7.0
202	Inion Farm stream	Tidal limit	NM959351	0.7
203	R. Esragan	Tidal limit	NM990351	15.6
204	Blacreen Burn	Tidal limit	NM992035	5.4
205	Kenmore Bay stream	Tidal limit	NN003339	0.2
206	Lusragan Burn	Tidal limit	NM914341	21.8
207	Culnadalloch stream	Tidal limit	NM949534	1.8
208	Allt Nathais	ptc* R. Luachragan	NM980328	10.3
209	R. Luachragan	Tidal limit	NM978332	18.2
210	Allt na h-Airde	Tidal limit	NM998532	4.7
211	R. Nant	Tidal limit	NN005317	45.0
212	R. Awe	Tidal limit	NN016322	828.6
213	Abhainn Achnacree	ptc* Allt nam Ban	NM929364	—
214	Allt nam Ban	ptc* Abhainn Achnacree	NM932364	—
215	Inion Farm stream	upstream of Inion Farm	NM960354	—
216	Kenmore stream	upstream Ardchattan School	NN005342	—

* ptc: prior to confluence

Table 4.3: River and stream water quality sites sampled during the 2007 survey.

Site Code	River	Location	NGR	Catchment Area (km^2)
202	Inion Farm stream	Tidal limit	NM959351	0.7
211	R. Nant	Tidal limit	NN005317	45.0
212	R. Awe	Tidal limit	NN016322	828.6
220	Achnacloich Plantation Burn	Tidal limit	NM959339	0.2
221	Mussel Farm Beck	Tidal limit	NM960339	0.2
222	Stream to Rubha nan Carn	Tidal limit	NM969339	0.1
223	Stream to Rubha Ban	Tidal limit	NM972337	0.3
224	Allt Tig Dhonnchaidh	Tidal limit	NM939631	1.6
225	Allt an t-Siomain	Tidal limit	NM945359	2.4
226	Allt Ardachy	Tidal limit	NM954352	1.6
227	Allt Dail a' Mhuilinn	Tidal limit	NM967350	0.3
228	Eas Mhaodain	Tidal limit	NM969349	0.6
229	Stream passing 'Sheep Wash'	Tidal limit	NM974348	0.5
230	Un-named Stream	Tidal limit	NM981351	0.1
231	Stream north of Bonawe	Tidal limit	NN000342	0.1
232	Allt Garbh	Tidal limit	NN006337	0.8

4.2.2.2 Sewage effluent

Four sewage sample sites were sampled during both the 2006 and 2007 surveys (Table 4.4). Three sites were located at Taynuilt wastewater treatment works (WwTW) and included the continuous treated final effluent (FE) discharge (site 301), the intermittent combined sewage overflow (CSO) at the works (site 302) and the combined effluent sampled from their common outfall (site 303). The forth sample point was of the storm overflow effluent from the CSO in Connel, although this did not spill during the 2006 study period. Samples were collected during the 2007 survey although the lack of any flow data, preclude its inclusion in the budget estimates.

Table 4.4: Final effluent and storm overflow effluent sampling points at wastewater treatment works and CSOs.

Site Code	Effluent	Effluent type	NGR
301	Taynuilt WwTW FE	Activated sludge secondary treated final effluent	NN006317
302	Taynuilt WwTW CSO	Combined sewage overflow effluent	NN006317
303	Taynuilt WwTW FE+CSO	Combined FE (301) and CSO (302) effluent	NN006317
304	Connel CSO	Combined sewage overflow effluent	NM922342

4.2.2.3 Rainfall

Hourly rainfall was recorded at four sampling locations using tipping bucket rain gauges installed by CREH for the duration of the 2006 study period. The rain gauges were installed at catchment outlet sites on Inion Farm stream (site 202), Blacreen Burn (site 204) and Culnadalloch stream (site 207), and at Taynuilt WwTW.

4.2.2.4 Sampling programme

During the 2006 survey, water and effluent quality sampling runs commenced on 14th July with the final data being collected on 6th August 2006. Manometric level recorders and rain gauges installed by CREH were *in-situ* between 7th July 2006 and 12:00GMT on 10th August 2006. During the 2007 survey, water and effluent quality sampling was undertaken between 6th August and 14th September 2007.

Three sampling runs were scheduled during each week of the respective study periods. This was amended in response to rainfall, in order to target sampling during hydrograph events and obtain samples from intermittent sewage effluent discharges from storm tanks and CSOs.

4.2.2.5 Sampling techniques

Samples were obtained either directly into 150 ml sterile disposable plastic containers (Media Disposables™) using a laboratory clamp and telescopic landing rod or by lowering a clean stainless steel can into the flowing water/effluent. If the sampling can was used, this was lowered into the flow three times, on the first two occasions the can rinsed and the water/effluent discarded. After the third collection a sample was obtained by pouring into a 150 ml sterile plastic container. On return to the sampling vehicle, the inside of the sampling can was immediately dried with absorbent paper towel and then wiped clean with alcohol impregnated cloth (Azo wipe™, Vernon-Carus Ltd.), allowing time for the alcohol to evaporate on the journey to the next sampling location.

Samples were stored in the dark inside a cool box during transport to the CREH *Analytical* field laboratory located at the Scottish Association for Marine Science, Dunstaffnage Marine Laboratory in Dunbeg.

4.2.3 Laboratory analysis

Indicator organism enumerations (colony forming units (cfu) 100 ml^{-1}) followed Standing Committee of Analysts Blue Book methods based on membrane filtration (Environment Agency, 2000). Sample dilutions were determined from the initial sampling run and faecal coliforms (FC) and intestinal enterococci (EN) enumerations were performed at two or three sample dilutions. A complete duplicate analysis was carried out on at least one sample collected during each sampling run for quality control purposes. Samples were generally analyzed as soon as possible after reception at the laboratory.

A total of 469 samples were collected during the 2006 field study period. A valid FC and EN enumeration was obtained for every sample (i.e. no enumerations exceeded the upper limit of detection). Over half the samples (52.5%) were analysed within six hours, 72.9% within 12 hours and 95.5% after 18 hours of collection (mean: 7 hours 57 minutes, standard deviation: 5 hours 11 minutes). All samples were analysed within 24 hours of collection (maximum: 20 hours 40 minutes) in accordance with the Blue Book methods (Environment Agency, 2000).

During the 2007 field survey a total of 563 samples were collected. Again, no enumerations exceeded the upper limit of detection. Again over half (56.3%) were analysed within six hours, 66.3% within 12 hours and 91.5% within 18 hours of collection (mean: 8 hours, standard deviation: 6 hours 15 minutes). All samples were analysed within 24 hours of collection (maximum: 22 hours 46 minutes).

4.2.4 Statistical analysis

For the purposes of statistical analyses, samples where no organisms were detected were recorded as the detection limit value. The distribution of microbial concentrations found in stream and sewage effluent samples, taken under base flow and high flow conditions, showed a closer approximation to normality when $\log_{10}$ transformed. All microbial concentration data were, therefore, $\log_{10}$ transformed prior to statistical analysis. The SPSS statistical computer software package (SPSS, 2002) was used for statistical analyses. Descriptive statistics were used to characterize the distribution of bacterial concentrations at each sampling location. These statistics include the geometric mean (GM)), calculated as the antilog of the mean of $\log_{10}$ transformed concentrations, the standard deviation (SD) of $\log_{10}$ transformed concentrations, the 95% confidence interval for the mean and the range of values at each site. The significance of differences between GM concentrations was examined using Student's t-test to compare the means of $\log_{10}$ transformed concentrations. The methodology included Levene's test for homogeneity of variances to determine whether or not to use a t-test assuming equal or unequal variances in the two groups compared, with the hypothesis of equal variance being rejected at $p<0.05$ (Pallant, 2001).

All statistical tests were assessed at $\alpha = 0.05$ (i.e. 95% confidence level or 5% significance level) by comparing p, the calculated probability at which the null hypothesis for a particular test is accepted, to α. Rejection of the null hypothesis (e.g. that two means are not different from each other or that a regression line slope is not different from zero) and acceptance of the alternative hypothesis (e.g. that two means are different from each other or that a regression line slope is different from zero) occurs when $p < \alpha$ (i.e. $p < 0.05$).

4.3 Riverine and point source flow volumes

4.3.1 River discharge

Flow in the river Awe is regulated by the operation of the HEP plant at Inverawe and data were provided by Scottish Southern Energy. Essentially, flow throughout the 2006 study period was constant at 14.2 $m^3\ s^{-1}$ with the exception of 'freshets' of three twelve hour periods over the weekends of $8^{th}/9^{th}$, $16^{th}/17^{th}$ and $22^{nd}/23^{rd}$ July 2006, when the flow was 23.7 $m^3\ s^{-1}$. For the remaining period of the field study planned freshets were not released due to low levels in Loch Awe (M. Cruickshank, Scottish Southern Energy, *pers comm.*). An hourly flow time-series was constructed based on this information.

Stage-discharge relationships were derived for the five sites with r^2 ranging between 98.3% (site 209) and 99.2% (sites 204 and 210). The resultant functions are given below:

Site 202:	$Q = 9.9767\text{x}10^{-14}\ h^{7.8617}$	$p < 0.001$	$r^2 = 99.2\%$	(3.1)
Site 204:	$Q = 1.3628\text{x}10^{-6}\ h^{3.3418}$	$p = 0.001$	$r^2 = 98.9\%$	(3.2)
Site 208:	$Q = 7.9969\text{x}10^{-6}\ h^{3.0846}$	$p < 0.001$	$r^2 = 99.1\%$	(3.3)
Site 209:	$Q = 0.0030e^{0.1090h}$	$p = 0.008$	$r^2 = 98.3\%$	(3.4)
Site 210:	$Q = 1.1187\text{x}10^{-6}\ h^{3.2996}$	$p < 0.001$	$r^2 = 99.2\%$	(3.5)

where: Q = discharge ($m^3\ s^{-1}$) and h = stage (cm)

Discharge from 2006 catchments from which no flow data were available and from 2007 catchments were based on catchment area and rainfall input using 2006 rainfall data (Table 4.5) from the nearest available CREH rain gauge. The proportion of rainfall contributing to

total flow and the base flow index (proportion of total flow contributing to base flow volumes) was taken from the nearest most similar monitored catchment.

Table 4.5 Rainfall (mm) recorded at temporary tipping-bucket gauges installed by CREH during the period 7/7/06 to 10/8/06.

Site Code	Location	Rainfall (mm)
202	Inion Farm stream, tidal limit	88.6
204	Blacreen Burn, tidal limit	91.2
207	Culnadalloch stream, tidal limit	88.2
301	Taynuilt WwTW	106.2

4.3.1.1 Flow separation

The hourly discharge records for flow monitoring stations were split into two components: (i) base flow (Q_b) and (ii) high flow (Q_h) event response to rainfall. This was achieved using a combination of computer programs (Pascal) and visual inspection of individual events by detailed hydrograph analysis (Wyer *et al*., 1996). Given the regulated flow of the River Awe, it was considered inappropriate to separate the flow into base flow and high flow since this is unlikely to be dominated by rainfall. However, to estimate a representative flow budget for Loch Etive, discharge from the River Awe should be included within both the base flow and high flow components. To achieve this, flow in the River Awe was considered to be 'high flow' during the periods of high flow in the River Luachragan, this being the largest and most proximal of the gauged catchments.

4.3.1.2 Riverine discharge results

The River Awe was estimated to discharge a total of 4.3 x 10^7 m^3 during the 2006 study period, of which 3.5 x 10^7 m^3 (83%) was designated as base flow (i.e. flow during the base flow period for site 209) and 7.5 x 10^6 (17%) was designated as high flow. Clearly, the duration of the base and high flow periods are the same as those of site 209 (Table 4.6), on which the flow separation was based.

A summary of discharge data for the monitored rivers during the 2006 study period is shown in Table 4.6. The largest of these rivers was the River Luachragan, which discharged 5.2 x 10^5 m^3 during the 2006 study period, of which 2.8 x 10^5 m^3 (54%) was discharged during high flow events. High flow events on this river prevailed for 140 hours (17% of the study period), the longest duration of high flow events of all the monitored rivers. A greater high flow volume (55%) than base flow volume was also discharged by Blacreen Burn, which was the second largest of the rivers monitored by CREH for flow (4.6 x 10^5 m^3). The remaining three rivers discharged a greater volume during base flow conditions. The smallest of the rivers was Inion Farm stream, which discharged 2.6 x 10^4 m^3 during the 2006 study period. This was unsurprising given that this was the smallest of the flow-monitored catchments. Estimated flow volumes for each of the catchments not monitored for flows are shown in Table 4.7. Total discharge at these outlets ranged from a maximum of 6.99 x 10^5 m^3 in the River Nant (site 211) to a minimum of 3.2x10^3 m^3 in the stream to Rubha nan Carn(site 222).

Table 4.6. Summary of discharge (m^3) and duration (hours) measured at CREH temporary flow monitoring stations and the River Awe between 7/7/06 and 10/8/06 (duration 816 hours).

				Flow Volume		
Site Code	**River**	**Base Flow (Q_b) (m^3)**	**High flow (Q_h) (m^3)**	**Total flow (Q_t) (m^3)**	**Base flow (Q_b) (%)**	**High flow (Q_h) (%)**
202	Inion Farm stream	25,663	23,005	48,668	52.7	47.3
204	Blacreen Burn	206,507	250,283	456,789	45.2	54.8
208	Allt Nathais	275,885	141,996	417,881	66.0	34.0
209	R. Luachragan	241,016	283,577	524,593	45.9	54.1
210	Allt na h-Airde	137,753	106,037	243,790	56.5	43.5
212	River Awe	35,412,120	7,533,000	42,945,120	82.5	17.5
				Flow Duration		
Site Code	**River**	**Base flow (Q_b) (hours)**	**High flow (Q_h) (hours)**	**Total flow (Q_t)∫(hours)**	**Base flow (Q_b) (%)**	**High flow (Q_h) (%)**
202	Inion Farm stream	726	90	816	89.0	11.0
204	Blacreen Burn	691	125	816	84.7	15.3
208	Allt Nathais	690	126	816	84.6	15.4
209	R. Luachragan	676	140	816	82.8	17.2
210	Allt na h-Airde	707	109	816	86.6	13.4
212	River Awe	676	140	816	82.8	17.2

Table 4.7. Summary of estimated discharge (m^3) at catchment outlet riverine water quality monitoring points not monitored for flow between 7/7/06 and 10/8/06 (duration 816 hours).

Sample Site		**Area (km^2)**	**Base flow (Q_b) (m^3)**	**High flow (Q_h) (m^3)**	**Total flow (Q_t) (m^3)**	**Base flow (Q_b) (%)**	**High flow (Q_h) (%)**
201	Abhainn Achnacree[a]	7.0	262868	318591	581459	45.2	54.8
203	R. Esragan[b]	15.6	602150	729796	1331946	45.2	54.8
205	Kenmore Bay stream[c]	0.2	6724	6028	12752	52.7	47.3
206	Lusragan Burn[d]	21.8	239298	281557	520855	45.9	54.1
207	Culnadalloch stream[e]	1.8	43266	33304	76570	56.5	43.5
211	R. Nant[f]	45.0	594774	699806	1294580	45.9	54.1
220	Achnacloich Plantation Burn[e]	0.2	5863	4513	10377	56.5	43.5
221	Mussel Farm Beck[e]	0.2	5548	4271	9819	56.5	43.5
222	Stream to Rubha nan Carn[e]	0.1	3197	2461	5658	56.5	43.5
223	Stream to Rubha Ban[e]	0.3	8326	6409	14735	56.5	43.5
224	Allt Tig Dhonnchaidh[g]	1.6	61304	54954	116259	52.7	47.3
225	Allt an t-Siomain[g]	2.4	91263	81809	173072	52.7	47.3
226	Allt Ardachy[g]	1.6	60229	53990	114219	52.7	47.3
227	Allt Dail a' Mhuilinn[g]	0.3	12107	10853	22959	52.7	47.3
228	Eas Mhaodain[g]	0.6	24333	21812	46145	52.7	47.3
229	Stream passing 'Sheep Wash'[g]	0.5	17825	15978	33803	52.7	47.3
230	Un-named Stream[c]	0.1	4632	4152	8783	52.7	47.3
231	Stream north of Bonawe[c]	0.1	5652	5066	10718	52.7	47.3
232	Allt Garbh[c]	0.8	30885	27686	58571	52.7	47.3

[a] Based on Inion Farm rainfall and Blacreen Burn flows.
[b] Based on Blacreen Burn rainfall and flows.
[c] Based on Blacreen Burn rainfall and Inion Farm stream flows.
[d] Based on Culnadalloch stream rainfall and R. Luachragan flows.
[e] Based on Culnadalloch stream rainfall and Allt na h-Airde flows.
[f] Based on Taynuilt WwTW rainfall and R. Luachragan flows.
[g] Based on Inion Farm rainfall and flows.

NB the duration (hours) of base and high flow components is the same as for the flow gauge each flow estimate is based on (see Table 4.6).

4.3.2 Sewage effluent discharge

4.3.2.1 Data availability

Flow data at 2-minute intervals were available for the Inlet, CSO and combined FE+CSO streams at Taynuilt WwTW for the period 20/6/06 to 26/7/06. No flow data were available for the 2007 study period. The 2006 data were processed to provide hourly time-series of flows (m^3) for the 2006 study period. The FE flow was calculated from the difference of the combined FE+CSO and CSO data. The first eight days of data were characterised by periods of missing data for one or more of the monitors, preventing the calculation of the FE flow over this period. Consequently, data before 12:00 GMT on 28/6/06 were disregarded. The remaining data provided a continuous time-series of hourly flows for the CSO and FE for a 28 day period between 12:00 GMT 28/6/06 and 12:00 GMT 26/7/06. Hence, these data corresponded with the 2006 study period between 7/7/06 and 26/7/06, a duration of 19 days (456 hours). In the absence of a time-series of flow for the complete study period (i.e. 7/7/06 to 10/8/06; 34 days) it was felt that the most robust method for estimating the flow would be to scale the flow for the 28 day period between 28/6/06 and 26/7/06 by a factor of 1.214 (i.e. 34/28). Whilst this method would provide a reasonable estimation of the total flow over the study period, it does not provide an hourly time-series for the period of the study after the sewage flow monitors maintained by the University of Abertay were removed. Consequently, whilst overall budget estimates of flow and FIO delivery can be made, a full time-series of flow and FIO delivery can only be provided up to the end of the available flow data (see for example Figure 4.8).

4.3.2.2 High flow separation

The hourly 2006 discharge record for the FE at Taynuilt WwTW was split into base flow and high flow event components in response to rainfall. This was achieved through detailed inspection of the flow record and comparison with a typical daily dry weather flow pattern derived using data from eight dry days (14^{th}-16^{th}, 18^{th}-19^{th} and 22^{nd}-25^{th} July 2006) and rainfall records. Flows that deviated significantly from the typical daily dry weather flow pattern corresponding with rainfall were categorised as high flows. All discharges from the CSO at Taynuilt WwTW were categorised as high flows.

4.3.2.3 Sewage discharge results

The resultant base flow and high flow volumes (m^3) and duration (hours) for the FE and CSO at Taynuilt WwTW are summarized in Table 4.8. The majority of treated final effluent (3343 m^3; 74%) was discharged during base flow conditions, which prevailed for 92% of the study period (Table 4.8). The CSO spilled an estimated volume of 80 m^3 over a period of 28 hours (3.4% of the study period).

Table 4.8. Estimates of final effluent and combined sewage overflow volumes at Taynuilt WwTW between 7/7/06 and 10/8/06 (duration 816 hours).

			Flow Volume				
Effluent		**pe***	**Base flow (Q_b) (m^3)**	**High flow (Q_h) (m^3)**	**Total flow (Q_t) (m^3)**	**Base flow (Q_b) (%)**	**High flow (Q_h)(%)**
301	Final treated effluent	1400	3343	1164	4507	74.2	25.8
302	CSO	1400	—	80	80	—	100.0
303	Combined FE +CSO	1400	3343	1244	4587	72.9	27.1
			Flow Duration				
Effluent		**pe***	**Base flow (Q_b)(hours)**	**High flow (Q_h)(hours)**	**Total flow (Q_t)(hours)**	**Base flow (Q_b)(%)**	**High flow (Q_h)(%)**
301	Final treated effluent	1400	754	62	816	92.4	7.6
302	CSO	1400	—	28	816	—	3.4
303	Combined FE +CSO	1400	753	63	816	92.3	7.7

* pe: population equivalent

4.4 Results of faecal indicator organism analysis

4.4.1 Riverine sites

Results from each river and stream sampling site were classified into two categories, base flow and high flow, according to flow conditions. For temporary stations monitored during 2006 the flow separated discharge record was used as a basis for this classification. Where continuous records were unavailable the record from the nearest neighbouring site was employed. Samples collected during the 2007 field survey were separated using stage records from temporary monitors, stage board readings at each individual site and field team notes.

Since the flow of the River Awe was regulated, separation of the microbiological data into base flow and rainfall impacted high flow categories is not applicable. Therefore, all data from each survey period for site 212 was used to calculate a GM for each FIO which was used to characterise water quality during the periods assigned to both base flow and high flow.

Statistical summaries of the results of the 2006 and 2007 river and stream water quality monitoring programmes are provided in Figure 4.2 and Table 4.9 for FC and Figure 4.3 and Table 4.10 for EN. For data collected during 2006 at least twelve high flow results were available from each site during the course of the study with 15 base flow samples available for each of the catchment outlet sites (i.e. sites 201-211). At least 12 base flow and 13 high flow samples were available for each of the 2007 catchment outlet sites. The combined GM values used for the River Awe were based on a total of 27 samples for data collected during 2006 and 29 samples for data collected during 2007 (although three enterococci results were omitted due to their relatively high lower detection limit).

Table 4.9. Geometric mean and standard deviation of $\log_{10}$ transformed presumptive faecal coliform concentrations (cfu 100 ml^{-1}) at riverine sampling points in selected catchments draining to Loch Etive during the 2006 and 2007 field surveys.

Site	Base Flow			High Flow		
	Geometric mean concentration (cfu 100 ml^{-1})	S.D.[a]	n^b	Geometric mean concentration (cfu 100 ml^{-1})	S.D.[a]	n^b
2006 Study Period Data						
201 Abhainn Achnacree, tidal limit	13,262	0.711	15	7,765	0.513	13
202 Inion Farm stream, tidal limit	1,939	0.751	15	104,563*	0.516	13
203 R. Esragan, tidal limit	63	0.786	15	14,262*	0.786	13
204 Blacreen Burn, tidal limit	922	0.545	15	1,647	0.610	13
205 Kenmore Bay stream, tidal limit	8,606	0.761	15	16,818	0.470	13
206 Lusragan Burn, tidal limit	6,978	0.380	15	10,380	0.181	12
207 Culnadalloch stream, tidal limit	1,304	0.228	15	17,862*	0.375	12
208 Allt Nathais, R. Luachragan	278	0.255	15	2,611*	0.186	12
209 R. Luachragan	627	0.357	15	3,357*	0.404	12
210 Allt na h-Airde	589	0.428	15	5,464*	0.524	12
211 R. Nant, tidal limit	464	0.705	15	1,360*	0.319	12
212 R. Awe, tidal limit[c]	464[c]	0.987	27	464[c]	0.987	27
213 Abhainn Achnacree ptc Allt nam Ban	92	0.514	5	1,838*	0.561	13
214 Allt nam Ban ptc Abhainn Achnacree	62,946	0.582	5	23,207	0.399	13
215 Inion Farm stream, upstr. Inion Fm	383	1.987	3	31,933*	0.512	13
216 Kenmore stream upstr. school	23	0.548	5	174*	0.820	13
2007 Study Period Data						
202 Inion Farm stream, tidal limit	11,866	0.457	14	15,904	0.304	16
211 R. Nant, tidal limit	467	0.295	14	2,230*	0.260	15
212 R. Awe, tidal limit[c]	233[c]	0.539	29	233[c]	0.539	29
220 Achnacloich Plantation Burn	43	0.635	14	1,523*	0.362	16
221 Mussel Farm Beck	153	0.716	14	1,267*	0.376	16
222 Stream to Rubha nan Carn	26	0.610	14	1,341*	0.226	16
223 Stream to Rubha Ban	154	0.361	14	2,170*	0.223	16
224 Allt Tig Dhonnchaidh	752	1.729	16	17,206*	0.247	15
225 Allt an t-Siomain	83	0.423	15	9,528*	0.171	16
226 Allt Ardachy	1,053	0.375	14	11,565*	0.348	16
227 Allt Dail a' Mhuilinn	1,110	0.653	14	7,523*	0.171	16
228 Eas Mhaodain	1,216	0.631	14	8,444*	0.308	16
229 Stream passing 'Sheep Wash'	296	0.507	14	4,786*	0.339	16
230 Un-named Stream	85	0.588	14	8,486*	0.357	16
231 Stream north of Bonawe	19	0.450	13	533*	0.408	15
232 Allt Garbh	32	0.467	14	385*	0.525	14

[a] S.D. = standard deviation of $\log_{10}$ transformed concentrations

[b] n = number of observations

[c] Data for the River Awe was not categorised into base flow and high flow. A single GM was calculated using all data collected from this site

* Results of Student's t-test show a significant elevation in geometric mean at high flow compared to base flow at $\alpha = 0.05$ (95% confidence)

Generally, FC concentrations were around an order of magnitude higher than EN concentrations during both years' surveys. The results show an increase in concentration in response to hydrograph events with the exception of FC at Abhainn Achnacree tidal limit, which showed a decrease during high flow conditions, although the difference was not statistically significant (Table 4.9), and the 2007 EN at Inion Farm (site 202), where the decrease was statistically significant. Statistically significant increases in GM FC concentrations during high flow events were present at ten of the 2006 sites and 14 of the 2007 sites (Table 4.9; Figure 4.2). Similar increases in GM EN concentrations were observed

at 9 of the 2006 sites and 14 of the 2007 sites (Table 4.10; Figure 4.3). In most cases, the elevation in GM concentration at high flow was at least an order of magnitude over the base flow concentration. Similar elevations in GM faecal indicator concentrations during high flow conditions following rainfall events have been recorded in previous CREH studies. They are attributable to a combination of increased surface runoff, the extension of the stream network into the contributing areas enhancing 'connectivity', and entrainment of organisms from stream bed sources, all of which increase the numbers of organisms entering watercourses. During hydrograph events increased stream flow velocities and turbidity act to reduce the opportunities for die-off (through exposure to UV light) and sedimentation whilst enhancing transportation of microorganisms.

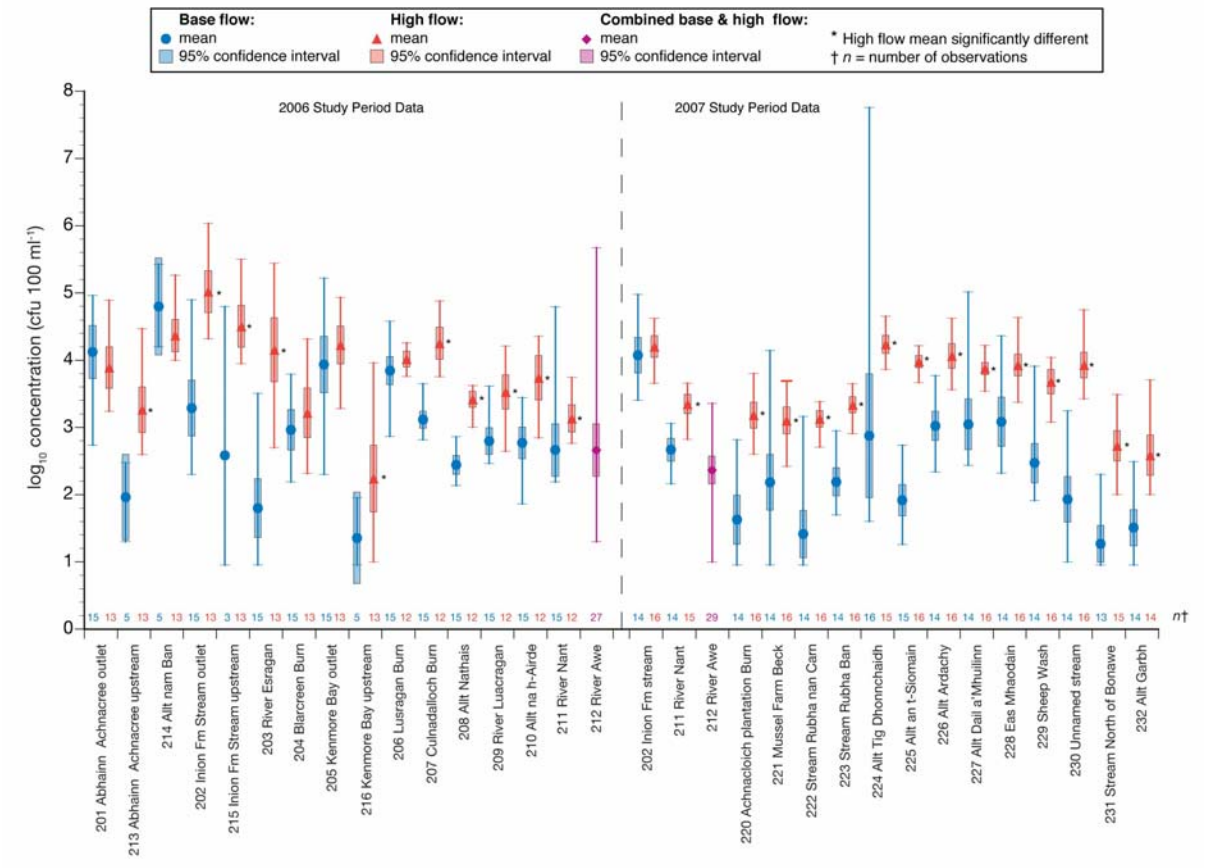


Figure 4.2. Mean, range and 95% confidence intervals of the mean for $\log_{10}$ transformed faecal coliform concentrations (cfu 100 ml^{-1}) in samples taken from river sampling sites during the 2006 and 2007 field surveys. N.B. 95% confidence interval not shown if n<5.

The lack of a statistically significant difference between base flow and high flow FC and/or EN at some sites can be attributed to unusually high base flow GM concentrations given the land cover of the respective catchments. The high base flow GM FC concentration at the Abhainn Achnacree tidal limit (1.3 x 10^4 cfu 100 ml^{-1}) can be attributed to the even greater base flow FC concentration (6.2 x 10^4 cfu 100 ml^{-1}) in one of its tributaries, Allt nam Ban (site 214) (Table 4.9). Upstream of its confluence with Allt nam Ban, Abhainn Achnacree (site 213) displayed a much lower GM concentration of 92 cfu 100 ml^{-1}, indicating that the source of contamination was located within the catchment of Allt nam Ban. Relatively high base flow GM FC concentrations were also present in the Kenmore Bay stream (site 205) and Lusragan Burn (site 206). The Kenmore Bay stream displayed the highest base flow EN concentration (6.1 x 10^4 cfu 100 ml^{-1}), much greater than would be expected for the

catchment size and land-cover and an order of magnitude greater than the next highest concentration (Table 4.10).

Table 4.10. Geometric mean and standard deviation of log_{10} transformed presumptive enterococci concentrations (cfu 100 ml^{-1}) at riverine sampling points in selected catchments draining to Loch Etive during the 2006 and 2007 field surveys.

Site	Base Flow			High Flow		
	Geometric mean concentration (cfu 100 ml^{-1})	S.D.[a]	*n*[b]	Geometric mean concentration (cfu 100 ml^{-1})	S.D.[a]	*n*[b]
2006 Study Period Data						
201 Abhainn Achnacree, tidal limit	270	0.408	15	1,803*	0.623	13
202 Inion Farm stream, tidal limit	3,043	1.663	15	65,504*	0.644	13
203 R. Esragan, tidal limit	16	0.442	15	772*	0.827	13
204 Blacreen Burn, tidal limit	278	0.995	15	462	0.573	13
205 Kenmore Bay stream, tidal limit	60,964	0.522	15	74,268	0.806	13
206 Lusragan Burn, tidal limit	696	0.484	15	1,285	0.320	12
207 Culnadalloch stream, tidal limit	455	0.235	15	3,233*	0.509	12
208 Allt Nathais, ptc R. Luachragan	37	0.412	15	490*	0.335	12
209 R. Luachragan, tidal limit	34	0.288	15	438*	0.478	12
210 Allt na h-Airde, tidal limit	176	0.365	15	1,276*	0.458	12
211 R. Nant, tidal limit	125	0.418	15	339*	0.408	12
212 R. Awe, tidal limit[c]	84[c]	0.862	27	84[c]	0.862	27
213 Abhainn Achnacree ptc Allt nam Ban	105	0.302	5	248	0.400	13
214 Allt nam Ban ptc Abhainn Achnacree	4,585	0.391	5	3,405	0.452	13
215 Inion Farm stream, upstr. Inion Fm	171	1.440	3	3,354*	0.474	13
216 Kenmore stream upstr. school	54	0.794	5	56	0.936	13
2007 Study Period Data						
202 Inion Farm stream, tidal limit	43,507	0.449	14	7,276†	0.482	16
211 R. Nant, tidal limit	107	0.518	14	419*	0.296	14
212 R. Awe, tidal limit[c]	25[c]	0.587	26	25[c]	0.587	26
220 Achnacloich Plantation Burn	12	0.278	14	408*	0.211	16
221 Mussel Farm Beck	166	0.942	14	695*	0.370	16
222 Stream to Rubha nan Carn	14	0.375	12	214*	0.331	13
223 Stream to Rubha Ban	20	0.379	14	490*	0.354	16
224 Allt Tig Dhonnchaidh	133	1.204	16	1,926*	0.242	15
225 Allt an t-Siomain	12	0.277	15	2,393*	0.416	16
226 Allt Ardachy	131	0.695	14	1,950*	0.495	16
227 Allt Dail a' Mhuilinn	98	1.031	14	978*	0.420	16
228 Eas Mhaodain	141	0.741	14	2,161*	0.745	16
229 Stream passing 'Sheep Wash'	31	0.751	14	1,094*	0.293	16
230 Un-named Stream	29	0.499	14	976*	0.192	16
231 Stream north of Bonawe	10	0.093	14	66*	0.566	11
232 Allt Garbh	13	0.376	14	220*	0.790	15

[a] S.D. = standard deviation of log_{10} transformed concentrations

[b] *n* = number of observations

[c] Data for the River Awe was not categorised into base flow and high flow. A single GM was calculated using all data collected from this site

* Results of Student's t-test show a significant elevation in geometric mean at high flow compared to base flow at $\alpha = 0.05$ (95% confidence)

† Results of Student's t-test show a significant decrease in geometric mean at high flow compared to base flow at $\alpha = 0.05$ (95% confidence).

During 2006 high flow conditions, Inion Farm stream at its tidal limit (site 202) displayed the highest FC concentration (1.0×10^5 cfu 100 ml^{-1}), an order of magnitude greater than any other site, and the second highest EN concentration (6.6×10^4 cfu 100 ml^{-1}). Concentrations of both FC and EN (3.2×10^4 cfu 100 ml^{-1} and 3.4×10^4 cfu 100 ml^{-1} respectively) were an

order of magnitude lower at the sample site upstream of Inion Farm (site 215) (Table 4.9 and Table 4.10). Despite these concentrations being lower than at the tidal limit, they were still the second highest FC concentrations observed during high flow conditions. Site 202 also displayed the highest 2007 base flow GM FC and EN concentrations (1.2 x 10^4 cfu 100 ml^{-1} and 4.4 x 10^4 cfu 100 ml^{-1} respectively), the highest high flow GM EN concentration (7.3 x 10^3 cfu 100 ml^{-1}) and the second highest high flow GM concentration (1.6 x 10^4 cfu 100 ml^{-1}) (Table 4.9 and Table 4.10). Comparison of the 2006 and 2007 data for site 202 shows that there were statistically significant differences for base flow and high flow GM FC concentrations. Base flow FC concentrations were greater during 2007 although high flow FC concentrations were lower during 2007. Both base and high flow EN concentrations were lower during 2007 when compared to 2006 although the differences were not statistically significant.

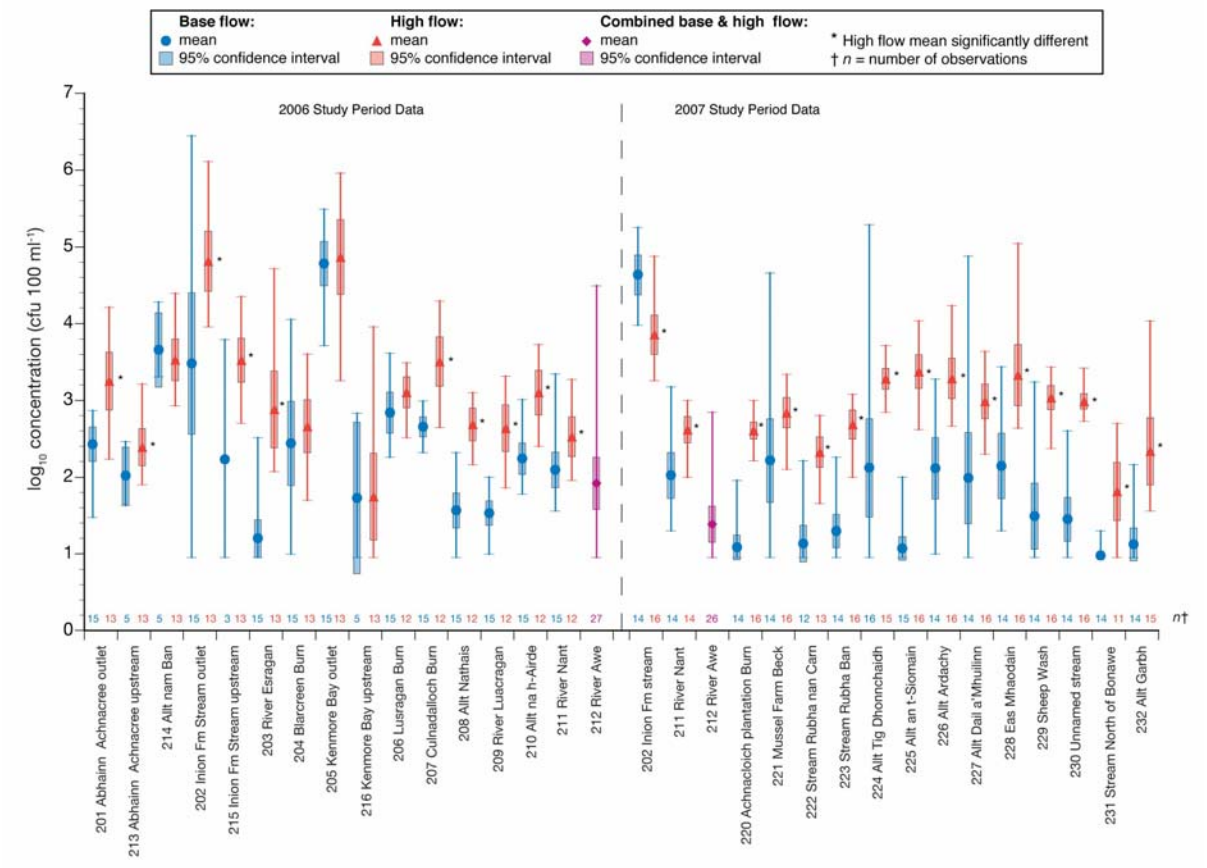


Figure 4.3. Mean, range and 95% confidence intervals of the mean for log_{10} transformed enterococci concentrations (cfu 100 ml^{-1}) in samples taken from river sampling sites during the 2006 and 2007 field surveys. N.B. 95% confidence interval not shown if $n<5$.

The greatest EN concentrations during 2006 high flow conditions (7.4 x 10^4 cfu 100 ml^{-1}) were in the Kenmore Bay stream at the tidal limit (site 205). Concentrations were 3 orders of magnitude lower at the site upstream of Ardchattan School (site 215), and were the lowest high flow concentrations observed (FC = 174 cfu 100 ml^{-1}; EN = 56 cfu 100 ml^{-1}). The greatest high flow FC concentration during the 2007 survey was in Allt Tig Dhonnchaidh (site 224) (1.7 x 10^4 cfu 100 ml^{-1}).

Concentrations in the River Awe were lower during the 2007 survey than during 2006 (2007: FC = 233 cfu 100 ml^{-1}, EN = 25 cfu 100 ml^{-1}; 2006: FC = 464 cfu 100 ml^{-1}; EN = 84 cfu 100 ml^{-1}). The difference between the 2006 and 2007 EN GMs was statistically significant,

although not for FC. These very low concentrations and year-on-year changes are probably due to management of the impoundment and its regulation producing the flow from Loch Awe (Table 4.9 and Table 4.10).

The only other site sampled during both study periods was the River Nant (site 211). The GM base flow and high flow concentrations were remarkably similar between years (Table 4.9 and Table 4.10) although t-tests between data from each year shows that statistically significant differences exist for both base flow and high flow conditions. Base flow concentrations were higher during 2007 whilst high flow concentrations were greater during 2006 for both FC and EN (Table 4.9 and Table 4.10).

4.4.2 Sewage effluents

Statistical summaries of the 2006 and 2007 GM concentrations for faecal indicators in the FE (site 301), CSO (site 302) and combined FE and CSO samples (site 303) at base and high flow are shown in Table 4.11 (FC) and Table 4.12 (EN). Figure 4.4 shows the mean, range and 95% confidence intervals of the mean for $\log_{10}$ transformed concentrations of the two faecal indicators at base and high flow. Data for the Connel CSO (site 304) is also presented in Table 4.11 and Table 4.12 although due to the lack of flow data, this input was not included in the budget calculations described in Section 4.5.

During the 2006 survey period both FC and EN concentrations in the Taynuilt WwTW FE (site 301) displayed a statistically significant decrease in GM concentration during high flow conditions, in both cases by over an order of magnitude (Table 4.11 and Table 4.12). Such a decrease in FIO concentrations in activated sludge treated effluent is not commonly observed and may reflect the fact that the works was operating at <50% capacity and a relatively high proportion of surface water was entering the sewerage system during high flow conditions compared to the foul sewage content, thus diluting the influent to the works. Both the base flow and high flow GM concentrations of FC and EN in the FE were below the 95% confidence interval for activated sludge and generic 'secondary biological' treated effluents observed in previous CREH catchment studies (Kay *et al*., 2007d) (Table 4.13). The GM concentrations of the untreated CSO effluent (site 302) were also below the 95% confidence intervals for similar effluents observed in previous studies (Table 4.13). However, the 2007 data showed notably different results with statistically significant increases in high flow GM concentrations in Taynuilt WwTW FE (site 301) and combined FE and CSO effluent (site 303) (Table 4.11 and Table 4.12). This change in the effluent quality in response to high flow events at the WwTW is more in keeping with observations at other activated sludge WwTWs. Nevertheless, the base flow FC GM concentration in the FE (site 301) remained lower than the 95% confidence interval for activated sludge and generic 'secondary biological' treated effluents observed in previous CREH catchment studies (Table 4.13), whilst that of EN was only slightly greater than the lower 95% limit for activate sludge plants, but below that of generic secondary treated effluents.

Comparison of the Taynuilt WwTW FE (site 301) data from 2006 and 2007 shows that both the base flow FC and EN concentrations during the 2007 study period were lower than the data from 2006, with the differences being statistically significant. The high flow FC and EN concentrations from 2007, however, were greater than those observed during 2006, although the difference was only statistically significant in the case of FC.

Table 4.11. Geometric mean and standard deviation of $\log_{10}$ transformed faecal coliform concentrations (cfu 100 ml^{-1}) at sewage sampling points during the 2006 and 2007 field surveys.

Site	Base Flow			High Flow		
	Geometric mean concentration (cfu 100 ml^{-1})	S.D.[a]	n^b	Geometric mean concentration (cfu 100 ml^{-1})	S.D.[a]	n^b
2006 Study Period Data						
301 Taynuilt WwTW FE	528,031	0.296	15	31,293†	0.797	12
302 Taynuilt WwTW CSO	—	—	—	3,398,222	0.141	2
303 Taynuilt WwTW FE+CSO	459,261	0.337	15	102,223†	0.600	12
2007 Study Period Data						
301 Taynuilt WwTW FE	57,706	0.702	13	342,647*	0.376	15
302 Taynuilt WwTW CSO	—	—	—	514,432	0.354	7
303 Taynuilt WwTW FE+CSO	57,256	0.728	13	416,466*	0.319	15
304 Connel CSO	—	—	—	410,856	0.548	15

[a] S.D. = standard deviation of $\log_{10}$ transformed concentrations

[b] n = number of observations

* Results of Student's t-test show a significant increase in geometric mean at high flow compared to base flow at $\alpha = 0.05$ (95% confidence)

† Results of Student's t-test show a significant decrease in geometric mean at high flow compared to base flow at $\alpha = 0.05$ (95% confidence)

Table 4.12. Geometric mean and standard deviation of $\log_{10}$ transformed enterococci concentrations (cfu 100 ml^{-1}) at sewage sampling points during the 2006 and 2007 field surveys.

Site	Base Flow			High Flow		
	Geometric mean concentration (cfu 100 ml^{-1})	S.D.[a]	n^b	Geometric mean concentration (cfu 100 ml^{-1})	S.D.[a]	n^b
301 Taynuilt WwTW FE	129,885	0.355	15	10,058†	0.680	12
302 Taynuilt WwTW CSO	—	—	—	847,261	0.049	2
303 Taynuilt WwTW FE+CSO	142,054	0.321	15	16,329†	0.488	12
2007 Study Period Data						
301 Taynuilt WwTW FE	20,238	0.447	13	56,491	0.775	15
302 Taynuilt WwTW CSO	—	—	—	48,389	0.256	7
303 Taynuilt WwTW FE+CSO	14,616	0.519	12	73,943*	0.724	14
304 Connel CSO	—	—	—	202,575	0.677	15

[a] S.D. = standard deviation of $\log_{10}$ transformed concentrations

[b] n = number of observations

* Results of Student's t-test show a significant increase in geometric mean at high flow compared to base flow at $\alpha = 0.05$ (95% confidence)

† Results of Student's t-test show a significant decrease in geometric mean at high flow compared to base flow at $\alpha = 0.05$ (95% confidence)

Table 4.13: Summary of faecal indicator organism concentrations (cfu 100 ml^{-1}) for untreated storm overflow, activated sludge and generic secondary treated sewage effluents under base and high flow conditions. Source (Kay *et al*., 2007c).

Effluent type	Flow condition	*n*	Geometric mean	Lower 95% CI	Upper 95% CI
Faecal coliforms (FC)					
Storm sewage overflows	High	203	$2.5x10^6$	$2.0x10^6$	$2.9x10^6$
Activated sludge	Base	261	$2.8x10^5$*	$2.2x10^5$	$3.5x10^5$
	High	93	$5.1x10^5$*	$3.1x10^5$	$8.5x10^5$
Secondary	Base	864	$3.3x10^5$*	$2.9x10^5$	$3.7x10^5$
	High	184	$5.0x10^5$	$3.7x10^5$	$6.8x10^5$
Enterococci (EN)					
Storm sewage overflows	High	201	$3.8x10^5$	$3.2x10^5$	$4.5x10^5$
Activated sludge	Base	262	$2.1x10^4$*	$1.8x10^4$	$2.7x10^4$
	High	91	$4.1x10^4$*	$2.7x10^4$	$6.0x10^4$
Secondary	Base	871	$2.8x10^4$*	$2.5x10^4$	$3.2x10^4$
	High	182	$4.7x10^4$	$3.6x10^4$	$6.1x10^4$

* Statistically significant (p<0.05) differences between base-flow and high-flow GM concentrations.

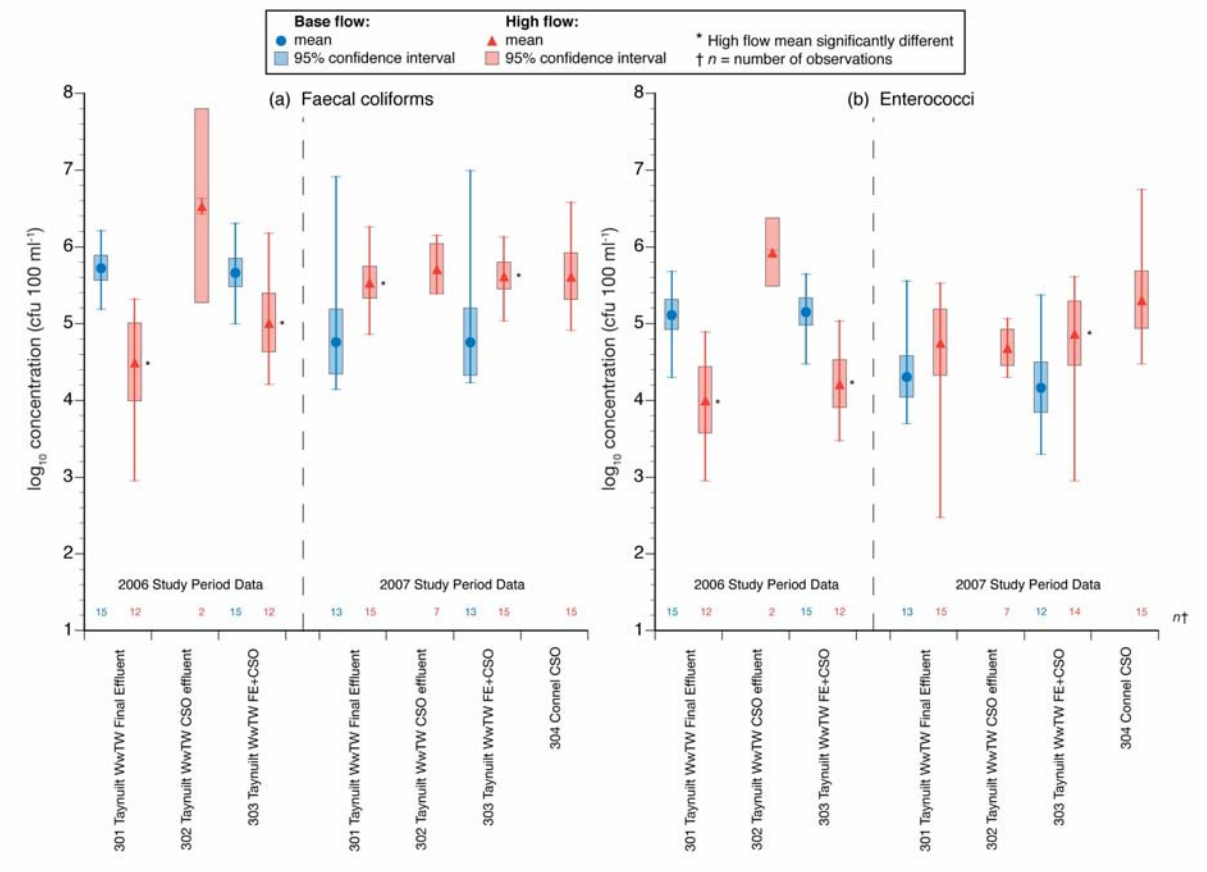


Figure 4.4. Mean, range and 95% confidence intervals of the mean for log_{10} transformed concentrations (cfu 100 ml^{-1}) in sewage effluent samples during the 2006 and 2007 field surveys: (a) faecal coliforms; (b) enterococci.

4.5 Catchment faecal indicator organism budgets

Budget calculations were made using data sets from the tidal limits of the catchments samples during 2006 and 2007 study periods to examine the faecal indicator organism inputs from riverine sources and sewage effluent to the outer basin of Loch Etive. It is important to note that the budgets for the loch describe the FIO load from each of these sources at their point of discharge (i.e. at the tidal limit of river catchments or point of sampling in the case of sewage effluent discharges) and do not include sources downstream of these points (for example, unmonitored small streams). The budgets also do not include inputs from a few unmonitored catchments draining to the outer basin of the loch, inputs from the inner basin or from inputs seaward of the Falls of Lora. Other inputs, for example from avian populations direct to the loch, were also not quantified. Additionally, these estimates in no way imply any linkage between any source, whether in close proximity or distant, to any monitoring point within the loch.

These budgets are intended to provide an indication of the relative proportional contribution of the studied sources under normal operating conditions during the period of monitoring (in this case the summer) using GM concentrations from data collected during the 2006 and 2007 study periods. Alternative budget estimates incorporating different budget scenarios are included in Section 4.5.2.

The relative proportions (%) of sources contributing to the budgets were calculated as follows:

(i) The load (L (organisms)) of each indicator organism was calculated for each source (i) for base flow (b) and high flow (h) discharge components during the study period:

$$L_{ib} = Q_{ib} \text{ x } C_{ib} \quad \textbf{5.1}$$
$$L_{ih} = Q_{ih} \text{ x } C_{ih} \quad \textbf{5.2}$$

where:
Q = flow (m^3) during the study period
C = geometric mean (GM) concentration (per m^3).

(ii) Total load (L_{it} (organisms)) from each source was calculated as:

$$L_{it} = L_{ib} + L_{ih} \quad \textbf{5.3}$$

(iii) The total load (L_S (organisms)) from all sources is given by:

$$L_s = \text{S } L_{it}. \quad \textbf{5.4}$$

(iv) Proportional contributions (PC_{ix} (%)) from each source (i) associated with each flow component (x (base flow, high flow or total flow)) for each study were finally calculated as:

$$PC_{ix} = (L_{ix} / L_s) \text{ x } 100 \quad \textbf{5.5}$$

Similar proportional contributions were calculated for base flow, high flow and total discharge estimates. The results were then plotted as a series of pie charts. Calculations were similarly performed on an hourly basis to examine the temporal pattern of faecal indicator organism loading from the various sources of interest.

4.5.1 Current Loch Etive budget

This section describes budgets comprising all the catchment outlets sampled during the 2006 and 2007 field study period: 24 riverine inputs the FE and CSO effluent from Taynuilt WwTW. A schematic diagram illustrating the relationship between these sampling sites is included as Figure 4.5. It was not possible to include an estimate of the flux from Connel CSO as no flow data were available for this input.

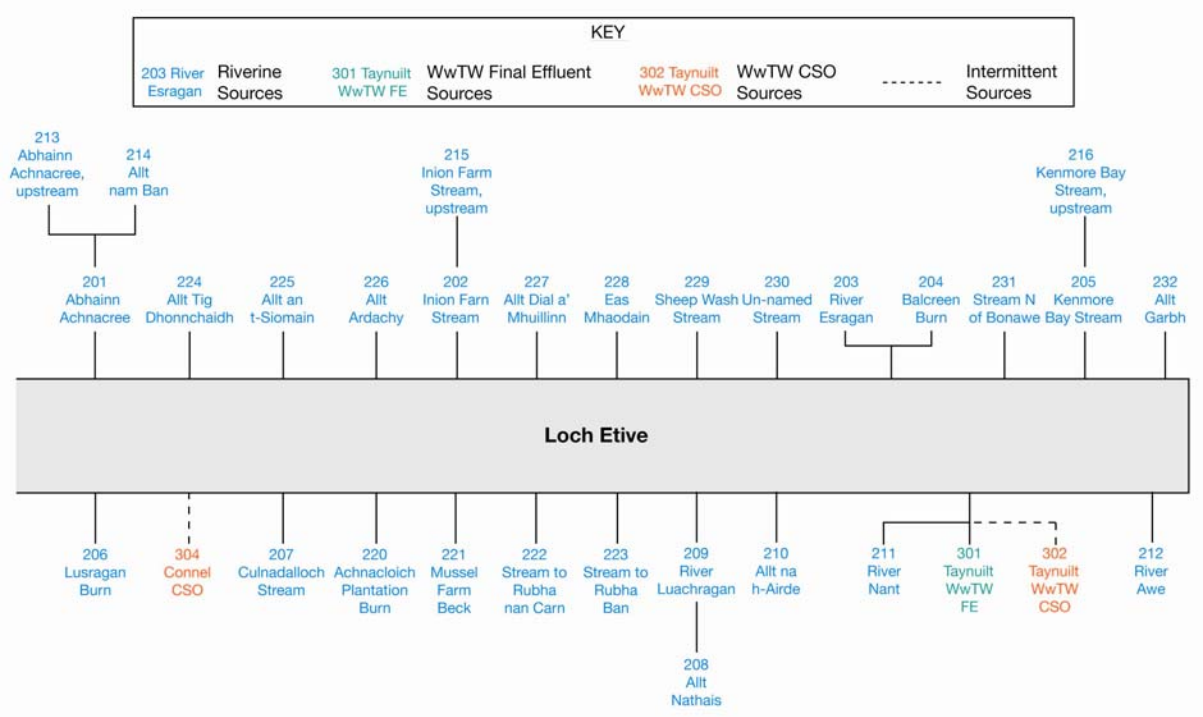


Figure 4.5: Schematic diagram showing the relationship between bacterial input sample points in the Loch Etive study area**.**

The discharge estimates for rivers (Table 4.6 and Table 4.7) and sewage discharges for the 2006 study period (Table 4.8) were used in conjunction with the FIO concentrations for the tidal limit sites and Taynuilt WwTW described in Table 4.9 to Table 4.12 to derive the budgets described below. Two estimates of the flux to the outer basin were made to accommodate the different results observed for sites sampled during both field study periods (i.e. Inion Farm stream (site 202), River Nant (site 211), River Awe (site 212) and Taynuilt WwTW Final and CSO effluents (sites 301 and 302 respectively). These budgets are referred to below as the '2006' and '2007' budgets although it should be noted that both of these flux estimates used the same flow data, which was characterised by the flows measured during the 2006 study period, and that each budget contains data from either 2006 or 2007 for the inputs sampled only during one of the two field study periods.

The estimated discharge and faecal indicator organism loads of all inputs sampled during the 2006 and 2007 field work periods discharging to the outer basin of Loch Etive are shown in Table 4.14 and Table 4.15 respectively. The percentage contribution of each source to the

faecal indicator budgets is shown in Table 4.16. Pie charts for the discharge and FIO budgets are shown in Figure 4.6a (split by major source) and Figure 4.7 (split by flow conditions). Diffuse catchment sources (i.e. the rivers) accounted for virtually the entire discharge budget (99.99%), 78% of this being discharged during base flow events (Table 4.14), although this distribution is skewed somewhat by the regulated flow from the River Awe (Table 4.14). The River Awe contributed 88% of the total flow discharged during the study period (Table 4.14; Figure 4.6a) and accounted for 93% of the base flow discharge volume (Figure 4.7). During high flow conditions, the River Awe accounted for 71% of the flow whilst the proportional contribution of all other rivers was greater (Figure 4.7) and, in the case of the larger rivers, represented a greater proportional contribution to the overall discharge budget than the same rivers during base flow (Table 4.14). The volume of sewage from Taynuilt WwTW input to Loch Etive was insignificant compared to that from the studied rivers (0.01%).

Table 4.14. Estimated discharge budget (m^3) and percentage contribution to Loch Etive from sampled catchments for the period 7/7/06 and 10/8/06.

	Discharge volume (m^3)			% contribution to discharge budget		
	Base flow	**High flow**	**Total flow**	**Base flow**	**High flow**	**Total flow**
201 Abhainn Achnacree	$2.63x10^5$	$3.19x10^5$	$5.81x10^5$	0.54	0.65	1.19
202 Inion Farm stream	$2.57x10^4$	$2.30x10^4$	$4.87x10^4$	0.05	0.05	0.10
203 River Esragan	$6.02x10^5$	$7.30x10^5$	$1.33x10^6$	1.24	1.50	2.74
204 Blacreen Burn	$2.07x10^5$	$2.50x10^5$	$4.57x10^5$	0.42	0.51	0.94
205 Kenmore Bay stream	$6.72x10^3$	$6.03x10^3$	$1.28x10^4$	0.02	0.02	0.03
206 Lusragan Burn	$2.39x10^5$	$2.82x10^5$	$5.21x10^5$	0.49	0.58	1.07
207 Culnadalloch stream	$4.33x10^4$	$3.33x10^4$	$7.66x10^4$	0.09	0.07	0.16
209 River Luachragan	$2.41x10^5$	$2.84x10^5$	$5.25x10^5$	0.50	0.58	1.08
210 Allt na h-Airde	$1.38x10^5$	$1.06x10^5$	$2.44x10^5$	0.28	0.22	0.50
211 River Nant	$5.95x10^5$	$7.00x10^5$	$1.29x10^6$	1.22	1.44	2.66
212 River Awe	$3.54x10^7$	$7.53x10^6$	$4.29x10^7$	72.76	15.48	88.24
220 Achnacloich Plant. Burn	$5.86x10^3$	$4.51x10^3$	$1.04x10^4$	0.01	0.01	0.02
221 Mussel Farm Beck	$5.55x10^3$	$4.27x10^3$	$9.82x10^3$	0.01	0.01	0.02
222 Str. to Rubha nan Carn	$3.20x10^3$	$2.46x10^3$	$5.66x10^3$	0.01	0.01	0.01
223 Stream to Rubha Ban	$8.33x10^3$	$6.41x10^3$	$1.47x10^4$	0.02	0.01	0.03
224 Allt Tig Dhonnchaidh	$6.13x10^4$	$5.50x10^4$	$1.16x10^5$	0.13	0.11	0.24
225 Allt an t-Siomain	$9.13x10^4$	$8.18x10^4$	$1.73x10^5$	0.19	0.17	0.36
226 Allt Ardachy	$6.02x10^4$	$5.40x10^4$	$1.14x10^5$	0.12	0.11	0.23
227 Allt Dail a' Mhuilinn	$1.21x10^4$	$1.09x10^4$	$2.30x10^4$	0.02	0.02	0.05
228 Eas Mhaodain	$2.43x10^4$	$2.18x10^4$	$4.61x10^4$	0.05	0.04	0.09
229 Str. passing 'Sheep Wash'	$1.78x10^4$	$1.60x10^4$	$3.38x10^4$	0.04	0.03	0.07
230 Un-named Stream	$4.63x10^3$	$4.15x10^3$	$8.78x10^3$	0.01	0.01	0.02
231 Stream north of Bonawe	$5.65x10^3$	$5.07x10^3$	$1.07x10^4$	0.01	0.01	0.02
232 Allt Garbh	$3.09x10^4$	$2.77x10^4$	$5.86x10^4$	0.06	0.06	0.12
301 Taynuilt WwTW FE	$3.34x10^3$	$1.16x10^3$	$4.51x10^3$	0.01	0.00	0.01
302 Taynuilt WwTW CSO	—	$7.99x10^1$	$7.99x10^1$	0.00	0.00	0.00
Rivers Total	$3.81x10^7$	$1.06x10^7$	$4.87x10^7$	78.29	21.70	99.99
Sewage Total	$3.34x10^3$	$1.24x10^3$	$4.59x10^3$	0.01	<0.01	0.01
Total (all sources)	$3.81x10^7$	$1.06x10^7$	$4.87x10^7$	78.30	21.70	100.00

An estimated total of $5.3x10^{14}$ FC and $9.9x10^{13}$ EN were discharged into the outer basin of Loch Etive from the 24 catchments and Taynuilt WwTW using 2006 data for sites sampled during both survey periods (Table 4.15a) whereas the estimated total was less using 2007 data for these sites at 4.0x $x10^{14}$ FC and $6.7x10^{13}$ EN (Table 4.15b). The majority of the total FIO load input into the estuary during the study period was delivered from diffuse catchment sources (i.e. the rivers), which accounted for 96-98% of the FC budget and 95-98% of the EN budget (Table 4.16a & b; Figure 4.6a & b). The '2006 budget' showed a slightly greater load

was input under high flow conditions, accounting for 53% of the FC load and 54% of the EN load (Table 4.16a), although this proportion was higher for FC in the '2007' budget, contributing 62% of the FC load, whilst the EN proportion was also 54% despite the actual flux of organisms being lower (Table 4.16b). Treated sewage effluents accounted for only 3.9% of the FC budget and 5.2% of the EN budget using the 2006 data (Table 4.16a) and only 1.1% and 2% of the respective budgets using 2007 data (Table 4.16b) for the sites which were sampled during both years. The almost equal proportions of base flow and high flow FIO delivery for most of the budget estimates was somewhat at variance with the findings of previous budget studies of other catchments (e.g. Crowther *et al*., 2002; 2003; Kay *et al*., 2005a, b; Stapleton *et al*., 2007b; Wyer *et al*., 1994; 1996; 1997a, b; 1998), which were generally dominated by high flow delivery of FIOs from riverine sources. However, none of these previous studies included the input from an impounded and regulated input such as the Awe.

Table 4.15a. Estimated faecal indicator organism loads to Loch Etive from sampled catchments for the period 7/7/06 and 10/8/06, using geometric mean concentrations calculated from 2006 data for Inion Farm stream, R. Nant, R. Awe and Taynuilt WwTW sewage effluents**.**

	Faecal coliform load (no. of organisms)			**Enterococci load (no. of organisms)**		
Source	**Base flow**	**High flow**	**Total flow**	**Base flow**	**High flow**	**Total flow**
201 Abhainn Achnacree	$3.49x10^{13}$	$2.47x10^{13}$	$5.96x10^{13}$	$7.09x10^{11}$	$5.75x10^{12}$	$6.45x10^{12}$
202 Inion Farm stream*	$4.98x10^{11}$	$2.41x10^{13}$	$2.46x10^{13}$	$7.81x10^{11}$	$1.51x10^{13}$	$1.59x10^{13}$
203 River Esragan	$3.80x10^{11}$	$1.04x10^{14}$	$1.04x10^{14}$	$9.66x10^{10}$	$5.63x10^{12}$	$5.73x10^{12}$
204 Blacreen Burn	$1.90x10^{12}$	$4.12x10^{12}$	$6.03x10^{12}$	$5.73x10^{11}$	$1.16x10^{12}$	$1.73x10^{12}$
205 Kenmore Bay stream	$5.79x10^{11}$	$1.01x10^{12}$	$1.59x10^{12}$	$4.10x10^{12}$	$4.48x10^{12}$	$8.58x10^{12}$
206 Lusragan Burn	$1.67x10^{13}$	$2.92x10^{13}$	$4.59x10^{13}$	$1.66x10^{12}$	$3.62x10^{12}$	$5.28x10^{12}$
207 Culnadalloch stream	$5.64x10^{11}$	$5.95x10^{12}$	$6.51x10^{12}$	$1.97x10^{11}$	$1.08x10^{12}$	$1.27x10^{12}$
209 River Luachragan	$1.51x10^{12}$	$9.52x10^{12}$	$1.10x10^{13}$	$8.23x10^{10}$	$1.24x10^{12}$	$1.32x10^{12}$
210 Allt na h-Airde	$8.11x10^{11}$	$5.79x10^{12}$	$6.61x10^{12}$	$2.42x10^{11}$	$1.35x10^{12}$	$1.60x10^{12}$
211 River Nant*	$2.76x10^{12}$	$9.51x10^{12}$	$1.23x10^{13}$	$7.44x10^{11}$	$2.37x10^{12}$	$3.12x10^{12}$
212 River Awe*	$1.64x10^{14}$	$3.49x10^{13}$	$1.99x10^{14}$	$2.97x10^{13}$	$6.33x10^{12}$	$3.61x10^{13}$
220 Achnacloich Plant. Bn	$2.49x10^{9}$	$6.88x10^{10}$	$7.12x10^{10}$	$7.19x1^{08}$	$1.84x10^{10}$	$1.91x10^{10}$
221 Mussel Farm Beck	$8.49x10^{9}$	$5.41x10^{10}$	$6.26x10^{10}$	$9.23x1^{09}$	$2.97x10^{10}$	$3.89x10^{10}$
222 Str. to Rubha nan Carn	$8.32x10^{8}$	$3.30x10^{10}$	$3.38x10^{10}$	$4.37x1^{08}$	$5.27x10^{9}$	$5.71x10^{9}$
223 Stream to Rubha Ban	$1.29x10^{10}$	$1.39x10^{11}$	$1.52x10^{11}$	$1.66x1^{09}$	$3.14x10^{10}$	$3.31x10^{10}$
224 Allt Tig Dhonnchaidh	$4.61x10^{11}$	$9.46x10^{12}$	$9.92x10^{12}$	$8.18x10^{10}$	$1.06x10^{12}$	$1.14x10^{12}$
225 Allt an t-Siomain	$7.55x10^{10}$	$7.79x10^{12}$	$7.87x10^{12}$	$1.08x10^{10}$	$1.96x10^{12}$	$1.97x10^{12}$
226 Allt Ardachy	$6.34x10^{11}$	$6.24x10^{12}$	$6.88x10^{12}$	$7.89x10^{10}$	$1.05x10^{12}$	$1.13x10^{12}$
227 Allt Dail a' Mhuilinn	$1.34x10^{11}$	$8.16x10^{11}$	$9.51x10^{11}$	$1.19x10^{10}$	$1.06x10^{11}$	$1.18x10^{11}$
228 Eas Mhaodain	$2.96x10^{11}$	$1.84x10^{12}$	$2.14x10^{12}$	$3.43x10^{10}$	$4.71x10^{11}$	$5.06x10^{11}$
229 Str. pass. Sheep Wash	$5.28x10^{10}$	$7.65x10^{11}$	$8.18x10^{11}$	$5.57x10^{9}$	$1.75x10^{11}$	$1.80x10^{11}$
230 Un-named Stream	$3.94x10^{9}$	$3.52x10^{11}$	$3.56x10^{11}$	$1.32x10^{9}$	$4.05x10^{10}$	$4.19x10^{10}$
231 Str. north of Bonawe	$1.05x10^{9}$	$2.70x10^{10}$	$2.80x10^{10}$	$5.43x10^{8}$	$3.33x10^{9}$	$3.87x10^{9}$
232 Allt Garbh	$9.97x10^{9}$	$1.07x10^{11}$	$1.17x10^{11}$	$4.14x10^{9}$	$6.08x10^{10}$	$6.49x10^{10}$
301 Taynuilt WwTW FE*	$1.76x10^{13}$	$3.64x10^{11}$	$1.80x10^{13}$	$4.34x10^{12}$	$1.17x10^{11}$	$4.46x10^{12}$
302 Taynuilt WwTW CSO*	—	$2.72x10^{12}$	$2.72x10^{12}$	—	$6.77x10^{11}$	$6.77x10^{11}$
Rivers Total*	$2.27x10^{14}$	$2.81x10^{14}$	$5.07x10^{14}$	$4.01x10^{13}$	$5.42x10^{13}$	$9.43x10^{13}$
Sewage Total*	$1.76x10^{13}$	$3.08x10^{12}$	$2.07x10^{13}$	$4.34x10^{12}$	$7.94x10^{11}$	$5.14x10^{12}$
Total (all sources)*	$2.44x10^{14}$	$2.84x10^{14}$	$5.28x10^{14}$	$4.45x10^{13}$	$5.49x10^{13}$	$9.94x10^{13}$

* Flux based on geometric mean concentrations using data collected during 2006 field study period wherever possible.

Table 4.15b**.** Estimated faecal indicator organism loads to Loch Etive from sampled catchments for the period 7/7/06 and 10/8/06, using geometric mean concentrations calculated from 2007 data for Inion Farm stream, R. Nant, R. Awe and Taynuilt WwTW sewage effluents. Note table only shows loads different to those in Table 4.15a.

	Faecal coliform load (no. of organisms)			Enterococci load (no. of organisms)		
Source	**Base flow**	**High flow**	**Total flow**	**Base flow**	**High flow**	**Total flow**
202 Inion Farm stream†	$3.05x10^{12}$	$3.66x10^{12}$	$6.70x10^{12}$	$1.12x10^{13}$	$1.67x10^{12}$	$1.28x10^{13}$
211 River Nant†	$2.78x10^{12}$	$1.56x10^{13}$	$1.84x10^{13}$	$6.34x10^{11}$	$2.93x10^{12}$	$3.56x10^{12}$
212 River Awe†	$8.24x10^{13}$	$1.75x10^{13}$	$9.99x10^{13}$	$8.70x10^{12}$	$1.85x10^{12}$	$1.06x10^{13}$
301 Taynuilt WwTW FE†	$1.93x10^{12}$	$3.99x10^{12}$	$5.92x10^{12}$	$6.76x10^{11}$	$6.58x10^{11}$	$1.33x10^{12}$
302 Taynuilt WwTW CSO†	—	$4.11x10^{11}$	$4.11x10^{11}$	—	$3.87x10^{10}$	$3.87x10^{10}$
Rivers Total†	$1.47x10^{14}$	$2.49x10^{14}$	$3.97x10^{14}$	$2.94x10^{13}$	$3.68x10^{13}$	$6.62x10^{13}$
Sewage Total†	$1.93x10^{12}$	$4.40x10^{12}$	$6.33x10^{12}$	$6.76x10^{11}$	$6.97x10^{11}$	$1.37x10^{12}$
Total (all sources)†	$1.49x10^{14}$	$2.54x10^{14}$	$4.03x10^{14}$	$3.01x10^{13}$	$3.75x10^{13}$	$6.76x10^{13}$

† Flux based on geometric mean concentrations using data collected during 2007 field study period wherever possible.

Table 4.16a. Percentage contribution of inputs to Loch Etive to the faecal indicator organism loads for the period 7/7/06 and 10/8/06, using geometric mean concentrations calculated from 2006 data for Inion Farm stream, R. Nant, R. Awe and Taynuilt WwTW sewage effluents.

	Faecal coliform load (%)			Enterococci load (%)		
Source	**Base flow**	**High flow**	**Total flow**	**Base flow**	**High flow**	**Total flow**
201 Abhainn Achnacree	6.60	4.68	11.28	0.71	5.78	6.49
202 Inion Farm stream*	0.09	4.55	4.65	0.79	15.15	15.94
203 River Esragan	0.07	19.71	19.78	0.10	5.67	5.76
204 Blacreen Burn	0.36	0.78	1.14	0.58	1.16	1.74
205 Kenmore Bay	0.14	0.24	0.37	5.11	5.58	10.69
206 Lusragan Burn	3.16	5.53	8.69	1.67	3.64	5.31
207 Culnadalloch stream	0.11	1.13	1.23	0.20	1.08	1.28
209 River Luachragan	0.29	1.80	2.09	0.08	1.25	1.33
210 Allt na h-Airde	0.15	1.10	1.25	0.24	1.36	1.60
211 River Nant*	0.52	1.80	2.32	0.75	2.39	3.14
212 River Awe*	31.08	6.61	37.69	29.90	6.36	36.27
220 Achnacloich Plant. Bn	<0.01	0.01	0.01	<0.01	0.02	0.02
221 Mussel Farm Beck	<0.01	0.01	0.01	<0.01	0.03	0.04
222 Str. to Rubha nan Carn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
223 Stream to Rubha Ban	<0.01	0.03	0.03	<0.01	0.03	0.03
224 Allt Tig Dhonnchaidh	0.09	1.79	1.88	0.08	1.06	1.15
225 Allt an t-Siomain	0.01	1.48	1.49	0.01	1.97	1.98
226 Allt Ardachy	0.12	1.18	1.30	0.08	1.06	1.14
227 Allt Dail a' Mhuilinn	0.03	0.15	0.18	0.01	0.11	0.12
228 Eas Mhaodain	0.06	0.35	0.40	0.03	0.47	0.51
229 Str. pass. Sheep Wash	<0.01	0.14	0.15	<0.01	0.18	0.18
230 Un-named Stream	<0.01	0.07	0.07	<0.01	0.04	0.04
231 Str. north of Bonawe	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
232 Allt Garbh	<0.01	0.02	0.02	<0.01	0.06	0.07
301 Taynuilt WwTW FE*	3.34	0.07	3.41	4.37	0.12	4.48
302 Taynuilt WwTW CSO*	—	0.51	0.51	—	0.68	0.68
Rivers Total*	42.89	53.18	96.07	40.38	54.46	94.84
Sewage Total*	3.34	0.58	3.93	4.37	0.80	5.16
Total (all sources)*	46.24	53.76	100.00	44.74	55.26	100.00

Flux based on geometric mean concentrations using data collected during 2006 field study period wherever possible.

Estimates based on the 2006 data for sites sampled during both years showed diffuse catchment sources contributed 92.8% of the base flow FC load and 90.2% of the EN load,

increasing to 98.9% and 99.0% of the high flow load respectively (Figure 4.7a). Treated sewage effluent from Taynuilt WwTW (site 301) contributed the remaining 7.2% and 9.8% of the base flow FC and EN budgets respectively, whilst during high flows the contribution decreased to less than 0.2% for each organism (Figure 4.7a). The CSO at Taynuilt accounted for 1.0% of the high flow FC and EN budgets. Estimates using the 2007 data for sites sampled during both years showed the diffuse catchment sources to contribute a greater proportion of the base flow budgets (FC = 98.7%; EN = 97.8% (Figure 4.7b)), primarily due to the much lower estimated flux from the Taynuilt WwTW treated effluent (Table 4.15b) compared to that estimated using the 2006 data for this source (Table 4.15a). However, the estimated proportional contribution of the diffuse catchment inputs to the high flow budget using 2007 data for the sites sampled during both years (FC = 98.2%; EN = 98.1%; Figure 4.7b) was slightly lower than when using the 2006 data. During base flows, Taynuilt WwTW FE was estimated to contribute 1.3% and 2.2% of the FC and EN budgets respectively, whilst during high flows the contribution was 1.6% and 1.8% respectively (Figure 4.7b).

Table 4.16b. Percentage contribution of inputs to Loch Etive to the faecal indicator organism loads for the period 7/7/06 and 10/8/06, using geometric mean concentrations calculated from 2007 data for Inion Farm stream, R. Nant, R. Awe and Taynuilt WwTW sewage effluents.

	Faecal coliform load (%)			Enterococci load (%)		
Source	**Base flow**	**High flow**	**Total flow**	**Base flow**	**High flow**	**Total flow**
201 Abhainn Achnacree	8.65	6.14	14.80	1.05	8.50	9.55
202 Inion Farm stream†	0.76	0.91	1.66	16.52	2.48	18.99
203 River Esragan	0.09	25.84	25.93	0.14	8.33	8.48
204 Blacreen Burn	0.47	1.02	1.50	0.85	1.71	2.56
205 Kenmore Bay	0.18	0.31	0.49	7.52	8.21	15.72
206 Lusragan Burn	4.14	7.25	11.40	2.46	5.35	7.81
207 Culnadalloch stream	0.14	1.48	1.62	0.29	1.59	1.88
209 River Luachragan	0.38	2.36	2.74	0.12	1.84	1.96
210 Allt na h-Airde	0.20	1.44	1.64	0.36	2.00	2.36
211 River Nant†	0.69	3.87	4.56	0.94	4.33	5.27
212 River Awe†	20.45	4.35	24.80	12.88	2.74	15.62
220 Achnacloich Plant. Bn	<0.01	0.02	0.02	<0.01	0.03	0.03
221 Mussel Farm Beck	<0.01	0.01	0.02	0.01	0.04	0.06
222 Str. to Rubha nan Carn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
223 Stream to Rubha Ban	<0.01	0.03	0.04	<0.01	0.05	0.05
224 Allt Tig Dhonnchaidh	0.11	2.35	2.46	0.12	1.57	1.69
225 Allt an t-Siomain	0.02	1.94	1.95	0.02	2.90	2.91
226 Allt Ardachy	0.16	1.55	1.71	0.12	1.56	1.67
227 Allt Dail a' Mhuilinn	0.03	0.20	0.24	0.02	0.16	0.17
228 Eas Mhaodain	0.07	0.46	0.53	0.05	0.70	0.75
229 Str. pass. Sheep Wash	0.01	0.19	0.20	<0.01	0.26	0.27
230 Un-named Stream	<0.01	0.09	0.09	<0.01	0.06	0.06
231 Str. north of Bonawe	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
232 Allt Garbh	<0.01	0.03	0.03	<0.01	0.09	0.10
301 Taynuilt WwTW FE†	0.48	0.99	1.47	1.00	0.97	1.97
302 Taynuilt WwTW CSO†		0.10	0.10		0.06	0.06
Rivers Total†	36.57	61.86	98.43	43.47	54.49	97.97
Sewage Total†	0.48	1.09	1.57	1.00	1.03	2.03
Total (all sources)†	37.05	62.95	100.00	44.48	55.52	100.00

† Flux based on geometric mean concentrations using data collected during 2007 field study period wherever possible.

The River Awe (site 212) dominated the base flow budgets for both FC and EN when using 2006 data for the sites sampled during both years, accounting for 67.2% and 66.8% respectively (Figure 4.7a). Other relatively large contributors to the base flow FC budget for estimates using 2006 data were Abhainn Achnacree (site 201; 14.3%), Taynuilt WwTW FE

(site 301; 7.2%) and Lusragan Burn (site 206; 6.8%). Relatively large contributions to the base flow EN budget were made by Kenmore Bay stream (site 205; 11.4%) and Taynuilt WwTW FE (site 301; 9.8%) (Figure 4.7a). The contribution of the Kenmore Bay stream is particularly notable due to its small catchment area (0.7km^2) and small contribution to the high flow discharge budget (0.01%). The estimates using 2007 data for the sites sampled during both survey periods showed the River Awe (site 212) and Taynuilt WwTW FE (site 301) to have a lower base flow FC contributions (55.2% and 1.3% respectively) whilst Abhainn Achnacree (site 201) and Lusragan Burn had higher FC contributions (23.4% and 11.2%) respectively ((Figure 4.7b). The base flow EN budget estimated using 2007 data for the sites sampled during both years (Figure 4.7b) was markedly different to that estimated using the 2006 data (Figure 4.7a). The proportional contribution of the River Awe was less than half the estimate made with the 2006 data at 29% whilst the largest source was Inion Farm stream (site 202) accounting for 37.1%. Again a large contributor to the base flow EN budget was Kenmore Bay stream, which accounted for 16.9% (Figure 4.7b). The primary reason for these differences between the two base flow EN fluxes was the higher base flow EN GM concentration observed during the 2007 field survey (Table 4.10).

The high flow FC budgets using data either from 2006 or 2007 field surveys for sites sampled during both periods were broadly similar. The River Awe (site 212) contributed a much lower proportion of the high flow FC budget, accounting for only 12.3% (2006 data) and 6.9 (2007 data) , despite contributing 73.4% of the high flow discharge volume (Figure 4.7a & b). The high flow FC budgets were dominated, however, by the River Esragan (site 203), which accounted for 36.7% (2006 data) and 41.0% (2007 data). Again, Abhainn Achnacree (site 201) and Lusragan Burn (site 206) contributed relatively large proportions of the high flow FC budgets whilst Inion Farm stream (site 202) contributed a relatively high proportion of the budget using 2006 data, despite its small catchment area (0.8 km^2) and small contribution to the high flow discharge budget (0.2%) (Figure 4.7a). However, the lower base flow GM concentration for Inion Farm stream observed during 2007 (Table 4.9) meant that this source was not significant in the budget estimated using 2007 data (Figure 4.7b).

The distribution of the proportional contributions of EN during high flow conditions was different to that of FC, perhaps reflecting the different survival of this organism both in sewage effluents and the environment. Again the budgets estimated using either the 2006 and 2007 data were similar, with the main difference being the contribution of Inion Farm stream (site 202). The high flow EN budget estimated using 2006 GM values was dominated by Inion Farm stream (site 202), which contributed 27.4%, more than twice the contribution of any other source (Figure 4.7a) although using the 2007 GM values reduced this streams contribution to 4.5% (Figure 4.7b). Relatively large contributions to the EN high flow budget were also made by the River Awe (site 212; 2006 data budget only), the River Esragan (site 203), Abhainn Achnacree (site 201) and Kenmore Bay stream (site 205) (Figure 4.7a & b).

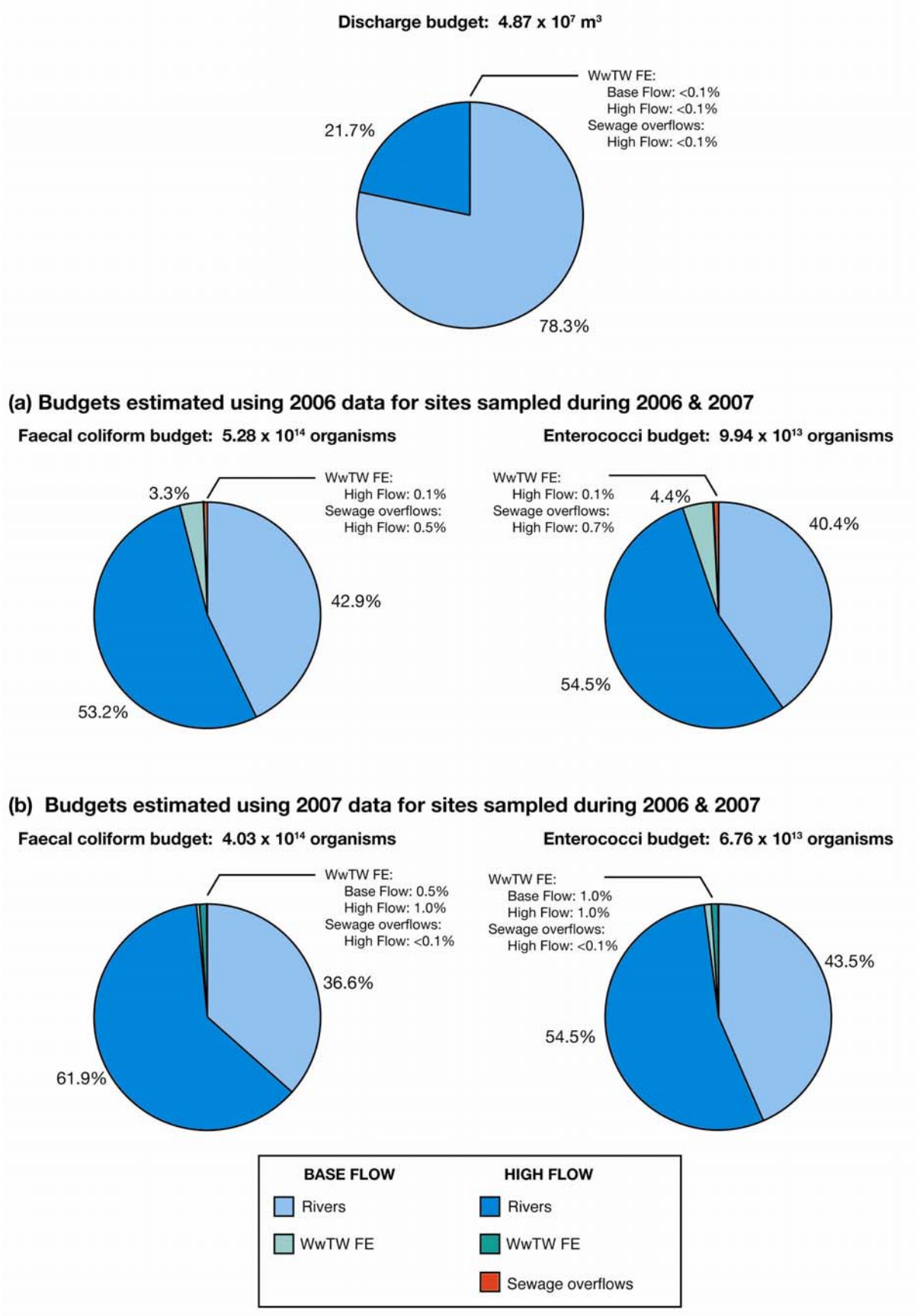


Figure 4.6. Total discharge and faecal indicator organism budgets for Loch Etive split by major source estimated using (a) 2006 data and (b) 2007 data for Inion Farm stream, R. Nant, R. Awe and Taynuilt WwTW FE and CSO.

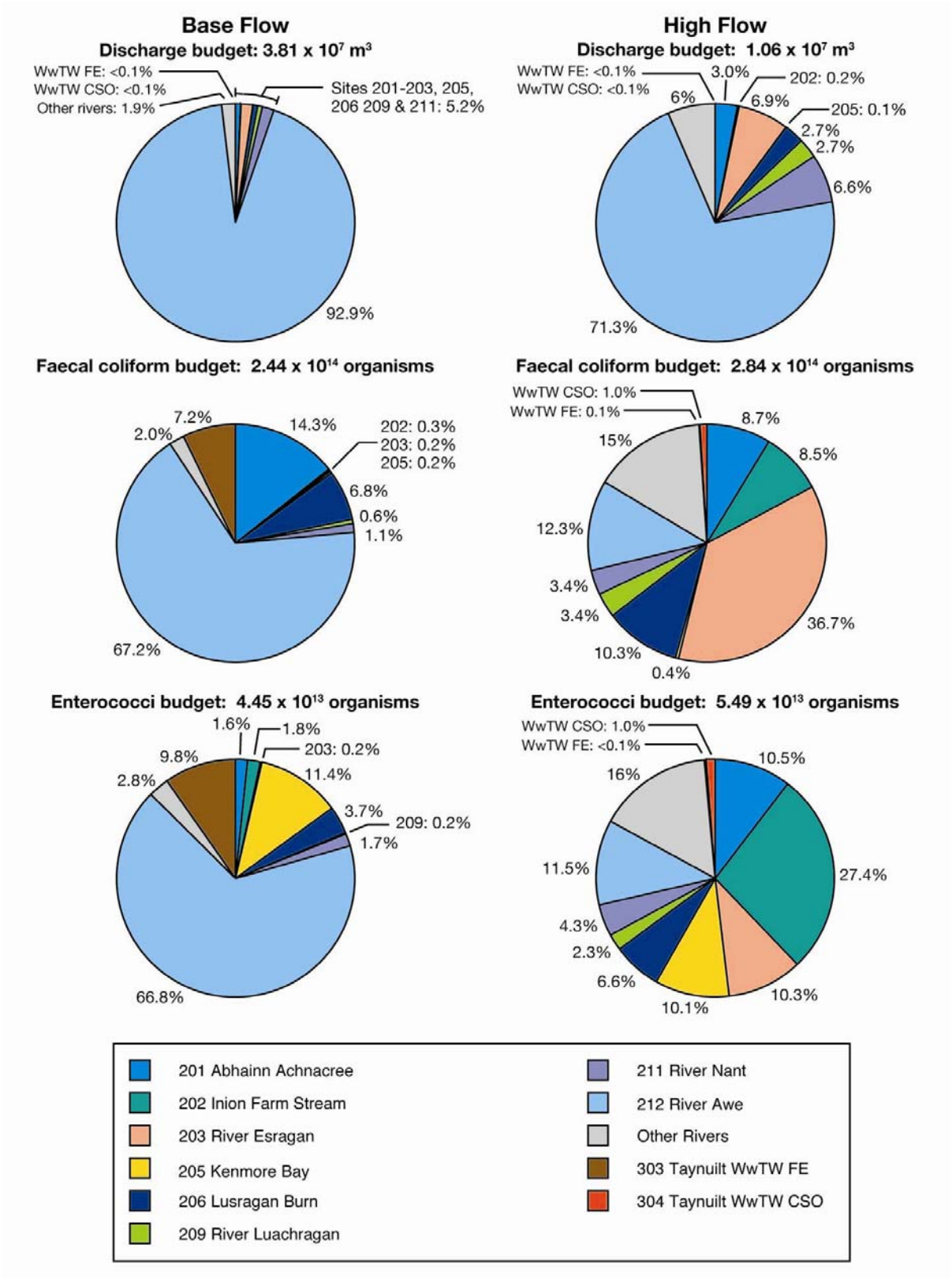


Figure 4.7a. Discharge and faecal indicator organism budgets for Loch Etive, split by flow conditions, estimated using 2006 data for Inion Farm stream, R. Nant, R. Awe and Taynuilt WwTW FE and CSO.

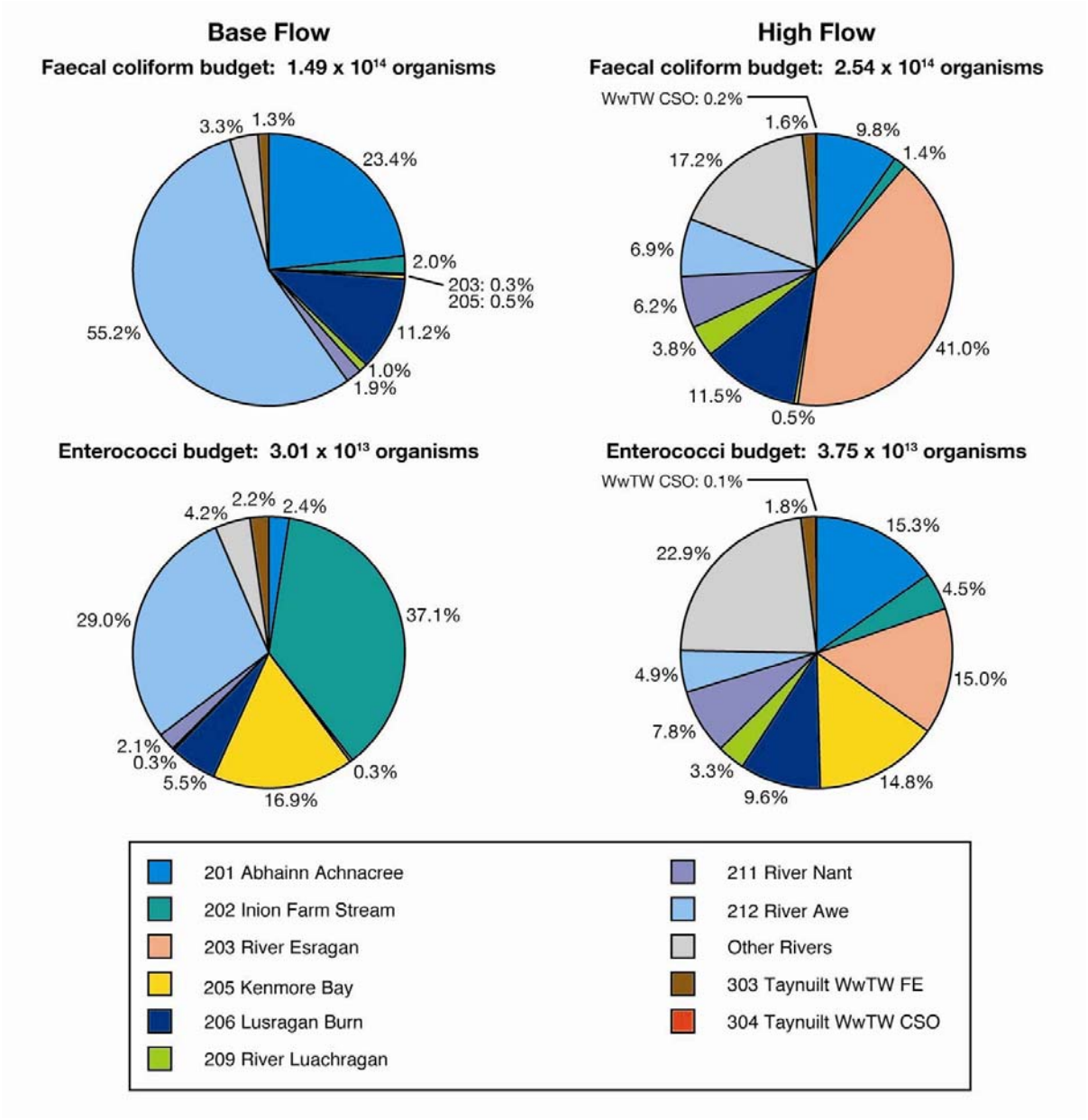


Figure 4.7b. Faecal indicator organism budgets for Loch Etive, split by flow conditions, estimated using 2007 data for Inion Farm stream, R. Nant, R. Awe and Taynuilt WwTW FE and CSO.

Figure 4.8 shows the hourly rainfall for site 207, estimated hourly FC load (organisms second^{-1}) and hourly proportional contributions (%) of the riverine and sewage inputs during base and high flow conditions. Figure 4.9 shows a similar plot for EN. Both figures contain plots for budgets estimated using 2006 and 2007 data for Inion Farm stream, the Rivers Nant and Awe and Taynuilt WwTW FE and CSO effluent (sites 202, 211, 212, 301 and 302 respectively. Note that the load from the FE and CSO at Taynuilt WwTW (sites 301 and 302) are only included on the plots up to the end of the available flow data on 26/7/06. The two temporal plots for FC (Figure 4.8a & b) are similar both in terms of FIO delivery (centre graph on Figure 4.8a & b) and proportional contribution of the various inputs, although those for EN (Figure 4.9a & b) are different. However, both these figures show the delivery of faecal indicator organisms (centre graphs on Figure 4.8a & b and Figure 4.9a & b) increased

in response to rainfall events and, for FC, but to a lesser extent, when the freshets on the River Awe are released around hours 200 and 370.

Figure 4.8a & b and Figure 4.9a shows that the River Awe dominated the delivery of FC and EN (for the budget estimated using 2006 data for the inputs sampled during both years) during base flow conditions although its proportional contribution to the greater delivery during high flow conditions was much lower. Despite instantaneous proportional delivery of Taynuilt WwTW (site 301) being lower in the '2007' budget estimates, the 'sawtooth' delivery of organisms from Taynuilt WwTW FE (site 301) was evident during base flow conditions in both budgets, accounting for between 1% and 17% of the instantaneous FC and EN loads, although during high flow conditions the input from the FE was not discernible when the rivers were also in high flow. Nevertheless, the high flow instantaneous load from the FE was shown to represent up to 41.8% of the '2007' FC budget and 43.2% of the '2007' EN budget during the period when the WwTW was in high flow but the rivers were yet to respond to rainfall (e.g. around hours 48 and 90) (Figure 4.8b and Figure 4.9b). The relatively small contribution of the CSO at the WwTW (site 302) can be seen during the first high flow event on the '2006' estimates (Figure 4.8a and Figure 4.9a), amounting to no more than 6% of the instantaneous FC delivery and 9% of the instantaneous EN delivery, although this is not discernable on the plots for the '2007' estimate, with the proportional contribution never exceeding 1%. The '2007' estimate for EN (Figure 4.9b), however, shows the proportional contribution of the River Awe (site 212) to be much lower both during base flows, with the exception of the period between hours 470 and 510 when inputs from all other rivers was very low by virtue of very low discharge. The contribution of the River Awe during high flow periods in the '2007' EN budget decreases to a very small proportion.

Some differences do exist between the hourly plots for each of the FC and EN budgets. For FC (Figure 4.8) the load from Abhainn Achnacree (site 201) was clearly evident, ranging from a minimum of 3% to a maximum of over 40% of the instantaneous load in the '2006' estimate (Figure 4.8a) and to over 52% of the instantaneous load in the '2007' budget (Figure 4.8b). The load from Lusragan Burn (site 206) remained fairly constant throughout the majority of the study period ranging between 4% and 10% during base flow conditions in the '2006' budget and 5% and 25% during base flow in the '2007' budget. However, during high flow events, the proportional contribution varied depending on the dominance of other sources, from less than 2% at the beginning of events when inputs from the smaller and more 'flashy' streams are greater, to over 30% (in both budgets) during the recession limb of events when the smaller rivers have reverted back to base flows. The increased contribution of the River Esragan (site 203) can be seen during high flow events, during which its proportional contribution was as much as 58% in the '2006' budget (64% in the '2007' budget), whilst the Inion Farm stream (site 202) also became more dominant during high flow events, contributing up to 33% of the instantaneous delivery in the'2006' budget. However, the instantaneous contribution of Inion Farm stream to the '2007' budget was low throughout the study period (<7.3%) due to the lower GM FC concentrations observed during the sampling in 2007.

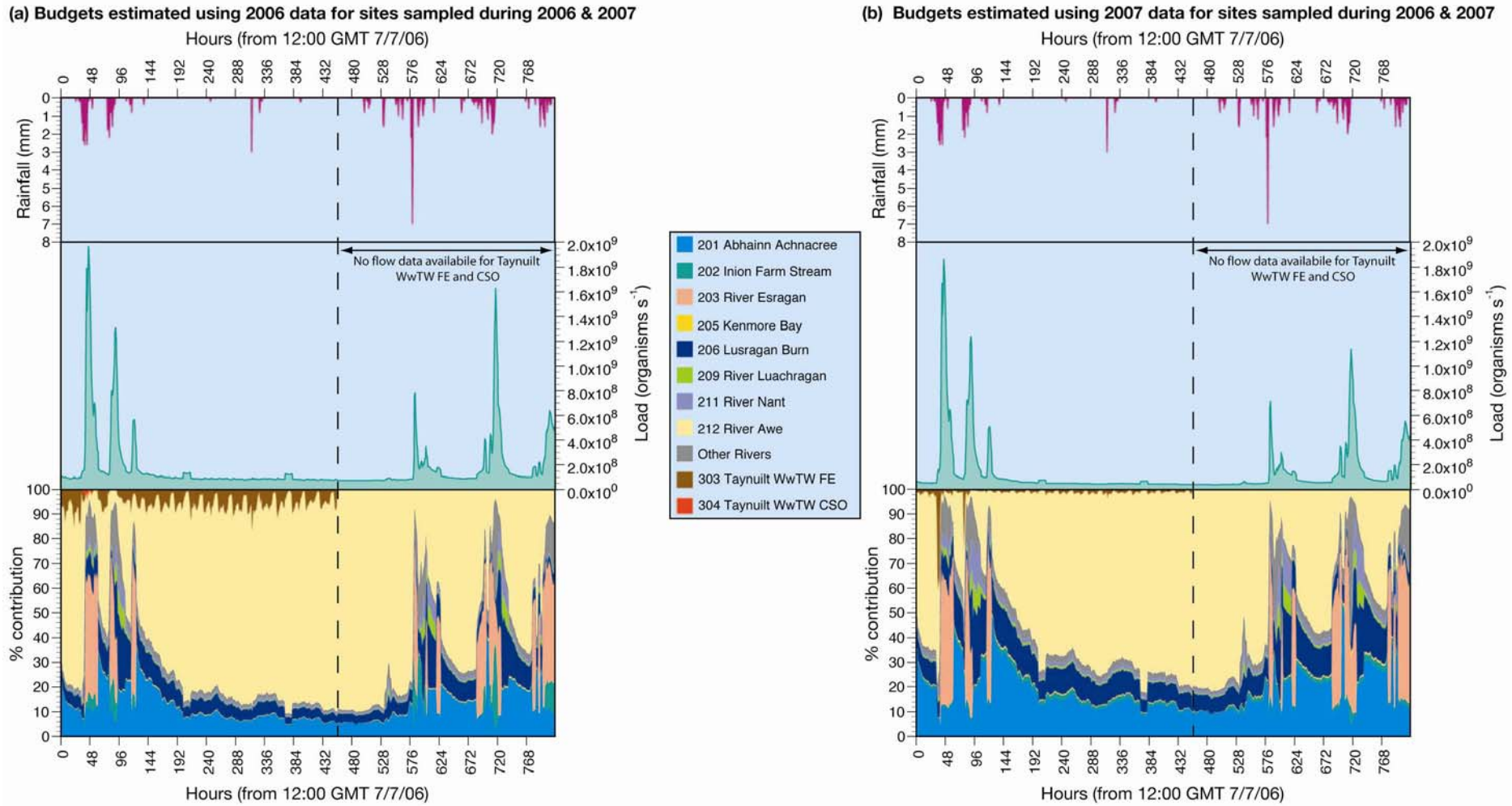


Figure 4.8. Hourly rainfall (mm) at Inion Farm (site 202), instantaneous faecal coliform load (organisms s^{-1}) and proportional contributions (%) of faecal coliforms to the hourly load input to Loch Etive estimated using (a) 2006 data and (b) 2007 data for Inion Farm stream, R. Nant, R. Awe and Taynuilt WwTW FE and CSO.

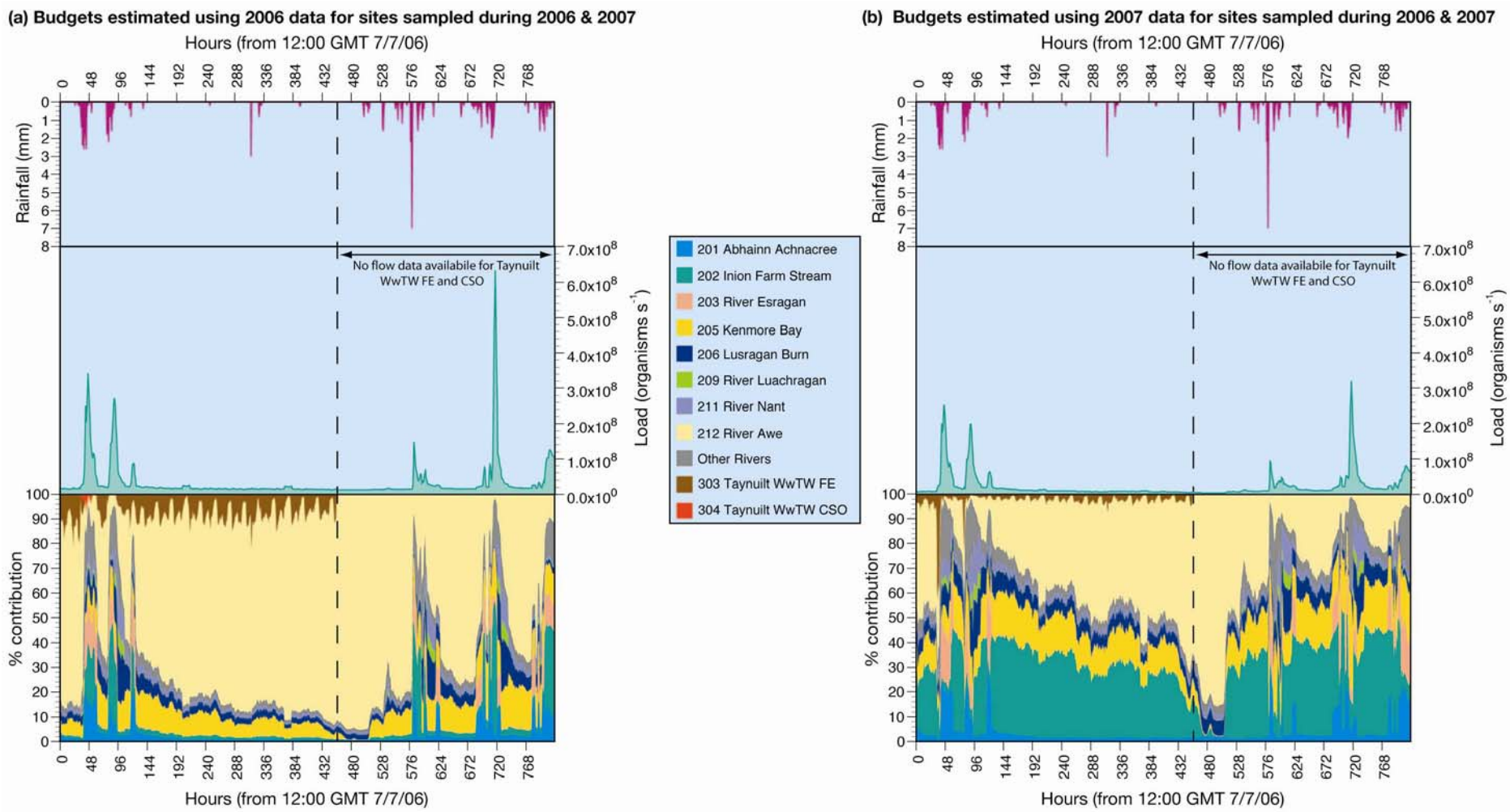


Figure 4.9. Hourly rainfall (mm) at Inion Farm (site 202), instantaneous enterococci load (organisms s^{-1}) and proportional contributions (%) of faecal coliforms to the hourly load input to Loch Etive estimated using (a) 2006 data and (b) 2007 data for Inion Farm stream, R. Nant, R. Awe and Taynuilt WwTW FE and CSO.

The EN plots in Figure 4.9 shows how Abhainn Achnacree (site 201) contributed a small proportion of the instantaneous EN load during base flow conditions (<6% for both budgets), although during high flow events its contribution increased to a maximum of 24% in the '2006' budget and 30.5% in the '2007' budget. The Kenmore Bay stream (site 205) was shown to contribute a relatively constant proportion of between 5% and 12% during base flow conditions in the '2006' budget (up to 22% for the '2007' budget), although this decreased to almost zero between hours 470 and 510 when the flow in the stream was estimated to decrease considerably due to prolonged dry weather. During high flow events, the proportional contribution to the instantaneous delivery of the Kenmore Bay stream varied more, increasing to a maximum of over 20%. The pattern of proportional contribution from Lusragan Burn (site 206) is similar to FC, although it represented a slightly lower proportion of the instantaneous load during both base flows ('2006 budget' 2% to 11%; '2007' budget 4% to 13%) and high flows (maximum: '2006' & 2007' budgets: 23%). The River Esragan (site 203) also contributed a relatively large proportion of the instantaneous load of EN during high flow conditions, accounting for up to 23% ('2006' & '2007. budgets). The main difference between the EN budgets using 2006 and 2007 data for the inputs sampled during both years, however, was the estimated contribution of Inion Farm stream (site 202). The '2006' budget showed the stream contributed between 1-2% of the instantaneous load during base flow conditions, whilst during high flow its contribution peaked at 69% during high flows (Figure 4.9a). However, the '2007' budget showed the instantaneous base flow contribution to be much higher at between 11% and 49% (with the exception of the low flow period between hours 470 and 510) whilst the high flow contribution was lower, peaking at only 12% (Figure 4.9b).

4.5.2 Impact of reducing FIO concentrations in selected catchments

Section 4.4.1 highlighted three catchments with particularly high FC and/or EN GM concentrations during either or both base flow and high flow conditions during the 2006 study period. Subsequent sampling upstream of potential sources demonstrated that each of the catchments displayed lower GM concentrations, often by an order of magnitude or more. In the case of Abhainn Achnacree (site 201), the tributary Allt nam Ban (site 214) displayed high GM concentrations whilst prior to their confluence Abhainn Achnacree had lower GM concentrations. The tidal limit site on Inion Farm stream (site 202) displayed GM concentrations an order of magnitude higher than upstream of the farm (site 215) whilst upstream of Ardchattan School Kenmore Bay stream (site 216) displayed FC and EN concentrations two to three orders of magnitude lower than the tidal limit site (site 205). Inion Farm stream (site 202) was sampled again during the 2007 study and the data confirms the input as one of poorest quality streams entering Loch Etive. The budgets presented in Section 4.5.1 have also demonstrated that these catchments contribute a significant proportion of the FC and EN loads despite their low contribution to the discharge budget. Clearly, therefore, there are sources of FIOs within these catchments that could be investigated for remediation to bring the quality of these inputs into line with others included in this study.

To investigate the potential impact of remediation measures within these three catchments, further budgets were calculated for the 2006 study period replacing the tidal limit GM concentrations of Abhainn Achnacree (site 201), Inion Farm stream (site 202) and Kenmore Bay stream (site 205) with the lower concentrations observed upstream of the potential sources (i.e. sites 213, 215 and 216). For this budget, FIO concentrations in the Rivers Nant

and Awe and Taynuilt WwTW FE and CSO effluent (sites 211, 212, 301 and 302) were characterised by data from the 2006 study period since data for the upstream sites used for the Abhainn Achnacree, Inion Farm Stream and Kenmore Bay stream were also collected during 2006. All discharge volumes were unchanged from those described in Section 4.5.1 whilst GM concentrations for the remaining sources were also left unchanged. The resultant FIO budgets are shown in Table 4.17 (absolute load of FC and EN), Table 4.18 (percentage contribution to the adjusted budgets), Figure 4.10 (total flow split by major source, base flow and high flow pie charts) and Figure 4.11 (hourly time-series of FC and EN).

Table 4.17. Estimated faecal indicator organism loads to Loch Etive from Abhainn Achnacree, Inion Farm stream and Kenmore Bay stream between 7/7/06 and 10/8/06 adjusted to model potential improvements in water quality.

	Faecal coliform load (no. of organisms)			Enterococci load (no. of organisms)		
Source	**Base flow**	**High flow**	**Total flow**	**Base flow**	**High flow**	**Total flow**
213 Abhainn Achnacree	$2.42x10^{11}$	$5.85x10^{12}$	$6.10x10^{12}$	$2.76x10^{11}$	$7.91x10^{11}$	$1.07x10^{12}$
215 Inion Farm stream	$9.84x10^{10}$	$7.35x10^{12}$	$7.44x10^{12}$	$4.40x10^{10}$	$7.72x10^{11}$	$8.16x10^{11}$
216 Kenmore Bay stream	$1.88x10^{9}$	$1.30x10^{10}$	$1.49x10^{10}$	$4.48x10^{9}$	$4.19x10^{9}$	$8.67x10^{9}$
Rivers Total	$1.91x10^{14}$	$2.44x10^{14}$	$4.35x10^{14}$	$3.39x10^{13}$	$2.94x10^{13}$	$6.33x10^{13}$
Sewage Total	$1.76x10^{13}$	$3.08x10^{12}$	$2.07x10^{13}$	$4.34x10^{12}$	$7.94x10^{11}$	$5.14x10^{12}$
Total (all sources)	$2.08x10^{14}$	$2.47x10^{14}$	$4.56x10^{14}$	$3.82x10^{13}$	$3.02x10^{13}$	$6.84x10^{13}$

Note table only shows loads different to those in Table 4.15a.

Intervention in the Abhainn Achnacree, Inion Farm stream and Kenmore Bay stream catchments could potentially decrease the estimated load input Loch Etive from the catchments to $4.6x10^{14}$ FC and $6.8x10^{13}$ EN during the study period (Table 4.17). This represents a 14% reduction in the FC load and 31% reduction in the EN load compared to the budget estimated using 2006 data wherever possible. Given the proximity of these inputs to the outer basin shellfish harvesting areas, remediation efforts centred in these catchments would be most likely to effect improvement in shellfish flesh quality. Riverine inputs still dominated the FC and EN budgets, accounting for 95.5% and 92.5% of the adjusted budgets respectively (Table 4.18), a slight decrease for the '2006' budget described in Section 4.5.1. Consequently, there was a slight increase in the proportional contribution of Taynuilt WwTW FE, which accounted for 4.6% of the FC load and 7.5% of the EN load Table 4.18). Whilst the delivery of FC in the adjusted budget was dominated by high flow riverine inputs, as was the case in the original '2006' budget, the greatest proportion of EN was delivered during base flow conditions rather than high flow conditions (Figure 4.10).

During base flow conditions, the proportional load from Abhainn Achnacree decreased to represent only 0.1% of the adjusted budget (Figure 4.10) compared to 14.3% in the original '2006' budget. The base flow EN load from Kenmore Bay stream decreased to represent <0.1% of the adjusted budget compared to 11.4% of the original budget. During high flow conditions the proportional contributions of Abhainn Achnacree, Inion Farm stream and Kenmore Bay stream decreased respectively to 2.4%, 3.0% and <0.1% of the adjusted high flow FC budget (down from 8.7%, 8.5% and 0.4% of the original '2006' high flow FC budget) and 2.6%, 2.6%, and <0.1% of the adjusted high flow EN budget (down from 10.5%, 27.4% and 10.1% of the original high flow EN budget) (Figure 4.10).

A consequence of the reduction in loads from Abhainn Achnacree, Inion Farm stream and Kenmore Bay stream was the increase in the proportional contribution of the other sources (Figure 4.10). The River Awe contributed the largest proportion to base flow FC and EN adjusted budgets and the high flow EN adjusted budget, contributing over 77% of the base

flow budgets and 47.4% of the high flow EN budget (Figure 4.10). The high flow FC adjusted budget was dominated by the River Esragan, which contributed 42.1% of the high flow load. The proportional contribution of Taynuilt WwTW FE increased to represent 8.5% of the adjusted base flow FC budget and 11.4% of the adjusted base flow EN budget. Other large contributors to the adjusted high flow FC budget were Lusragan Burn (11.8%) and the River Awe (14.1%) (Figure 4.10). Other large contributors to the adjusted high flow EN budget were the River Esragan (18.7%), Lusragan Burn (12.0%) and the River Nant (7.9%) (Figure 4.10).

Table 4.18. Percentage contribution of inputs to Loch Etive to the faecal indicator organism loads between 7/7/06 and 10/8/06, adjusted to model improvements in water quality in Abhainn Achnacree, Inion Farm stream and Kenmore Bay stream.

	Faecal coliform load (%)			Enterococci load (%)		
Source	**Base flow**	**High flow**	**Total flow**	**Base flow**	**High flow**	**Total flow**
201 Abhainn Achnacree†	0.05	1.29	1.34	0.40	1.16	1.56
202 Inion Farm stream†	0.02	1.61	1.63	0.06	1.13	1.19
203 River Esragan	0.08	22.85	22.93	0.14	8.24	8.38
204 Blacreen Burn	0.42	0.91	1.32	0.84	1.69	2.53
205 Kenmore Bay†	<0.01	<0.01	<0.01	<0.01	<0.01	0.01
206 Lusragan Burn	3.66	6.41	10.08	2.43	5.29	7.72
207 Culnadalloch stream	0.12	1.31	1.43	0.29	1.57	1.86
209 River Luachragan	0.33	2.09	2.42	0.12	1.82	1.94
210 Allt na h-Airde	0.18	1.27	1.45	0.35	1.98	2.33
211 River Nant*	0.61	2.09	2.69	1.09	3.47	4.56
212 River Awe*	36.03	7.66	43.70	43.48	9.25	52.73
220 Achnacloich Plant. Bn	<0.01	0.02	0.02	<0.01	0.03	0.03
221 Mussel Farm Beck	<0.01	0.01	0.01	0.01	0.04	0.06
222 Str. to Rubha nan Carn	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
223 Stream to Rubha Ban	<0.01	0.03	0.03	<0.01	0.05	0.05
224 Allt Tig Dhonnchaidh	0.10	2.08	2.18	0.12	1.55	1.67
225 Allt an t-Siomain	0.02	1.71	1.73	0.02	2.86	2.88
226 Allt Ardachy	0.14	1.37	1.51	0.12	1.54	1.65
227 Allt Dail a' Mhuilinn	0.03	0.18	0.21	0.02	0.16	0.17
228 Eas Mhaodain	0.06	0.40	0.47	0.05	0.69	0.74
229 Str. pass. Sheep Wash	0.01	0.17	0.18	<0.01	0.26	0.26
230 Un-named Stream	<0.01	0.08	0.08	<0.01	0.06	0.06
231 Str. north of Bonawe	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
232 Allt Garbh	<0.01	0.02	0.03	<0.01	0.09	0.09
301 Taynuilt WwTW FE*	3.87	0.08	3.95	6.35	0.17	6.52
302 Taynuilt WwTW CSO*		0.60	0.60		0.99	0.99
Rivers Total*	41.88	53.57	95.45	49.57	42.92	92.49
Sewage Total*	3.87	0.68	4.55	6.35	1.16	7.51
Total (all sources)*	45.76	54.24	100.00	55.92	44.08	100.00

* Flux based on geometric mean concentrations using data collected during 2006 field study period wherever possible.

† Flux adjusted using base flow and high flow geometric mean concentrations for sample sites upstream of the outlet.

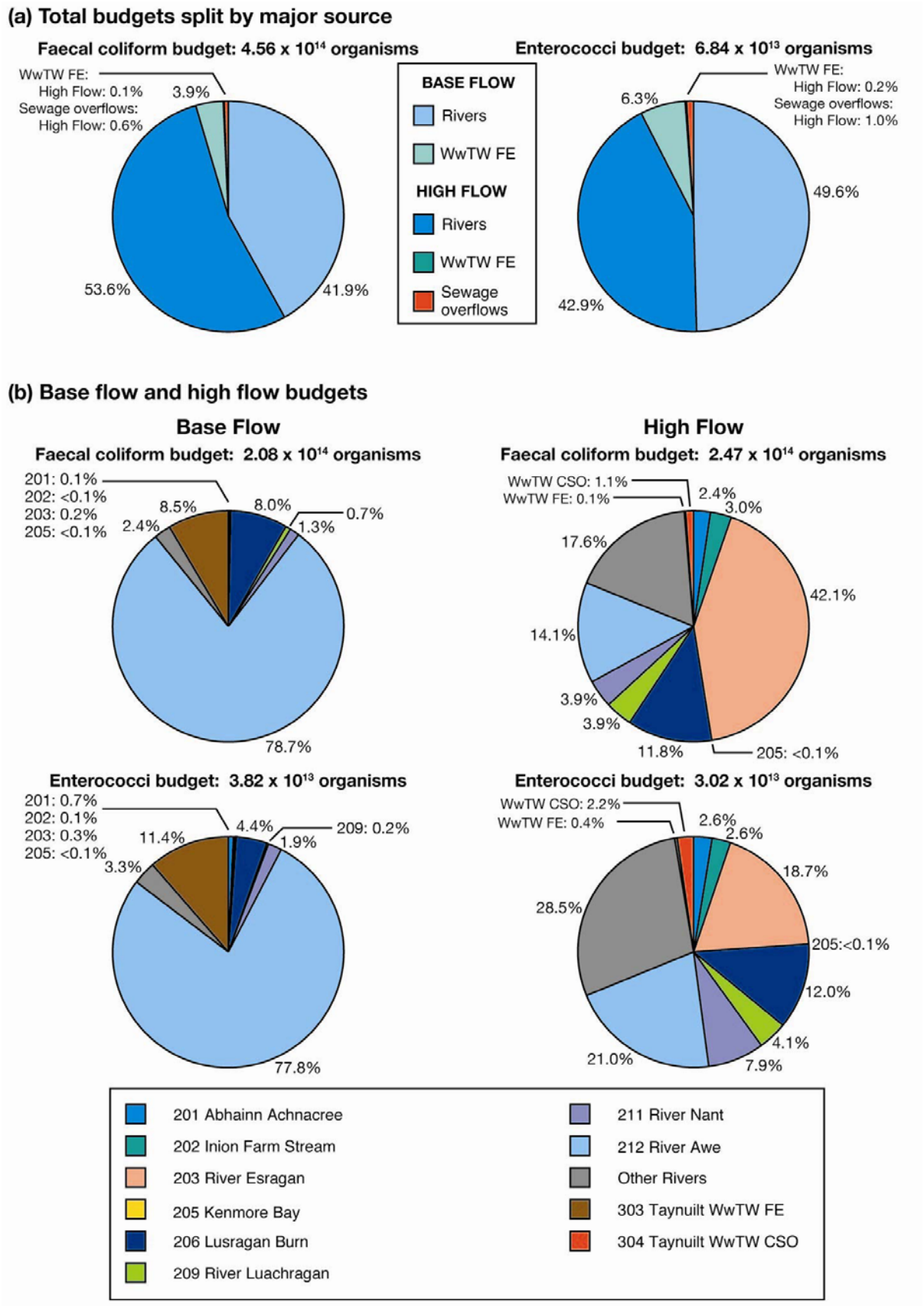


Figure 4.10. Estimated faecal indicator organism budgets for Loch Etive adjusted to reflect water quality upstream of potential FIO sources: (a) Total budgets split by major source; (b) Base flow and high flow budgets (split by flow conditions).

The adjusted FC hourly plot (Figure 4.11a) illustrates how the contribution of Abhainn Achnacree (site 201) is considerably lower, being insignificant during base flow conditions and a maximum of 4% of the instantaneous load during high flow conditions. The pattern of proportional contribution from Lusragan Burn (site 206) is similar to the unadjusted budget, although it represents a slightly greater proportion of the instantaneous load (maximum: 38%; previously 31% of the '2006' budget). The River Esragan (site 203) would also contribute a greater proportion of the instantaneous load of FC during high flow conditions, accounting for up to 68% (previously 58% of the '2006' budget). However, the high flow contribution of Inion Farm stream (site 202) is reduced to a maximum of 14% from 34% in the '2006' budget described in Section 4.5.1. The proportional contribution of the River Awe displays a maximum of 95%, up from 90% in the unadjusted '2006' budget (Figure 4.11a).

Intervention to reduce EN concentrations in Abhainn Achnacree, Inion Farm stream and Kenmore Bay stream has the effect of reducing the proportional EN inputs to less than 1% for the majority of the base flow periods (Figure 4.11b). During high flows, Abhainn Achnacree delivers a maximum proportional contribution of 5.5%, reduced from 23% in the '2006' unadjusted budget, Inion Farm stream delivers a maximum contribution of 10%, reduced from 54% in the '2006' unadjusted budget and Kenmore Bay stream delivers a maximum of 0.1%, reduced from 20% (Figure 4.11b). As a consequence of the reduction in the EN load delivered from these rivers, the proportional contribution of the remaining rivers increase. The main contributor during base flow periods was still the River Awe, which accounted for up to 96% of the adjusted instantaneous budget, although during high flow periods this contribution was much lower (Figure 4.11b). Other relatively large contributors during base flow conditions included Lusragan Burn and the River Nant. During high flow conditions, when the actual delivery of organisms per second is greater, large contributors include the River Esragan (maximum: 39%), Lusragan Burn (maximum: 27%) (Figure 4.11b) and Allt na h-Airde (maximum: 21%) (contained within the 'other rivers' category in (Figure 4.11b).

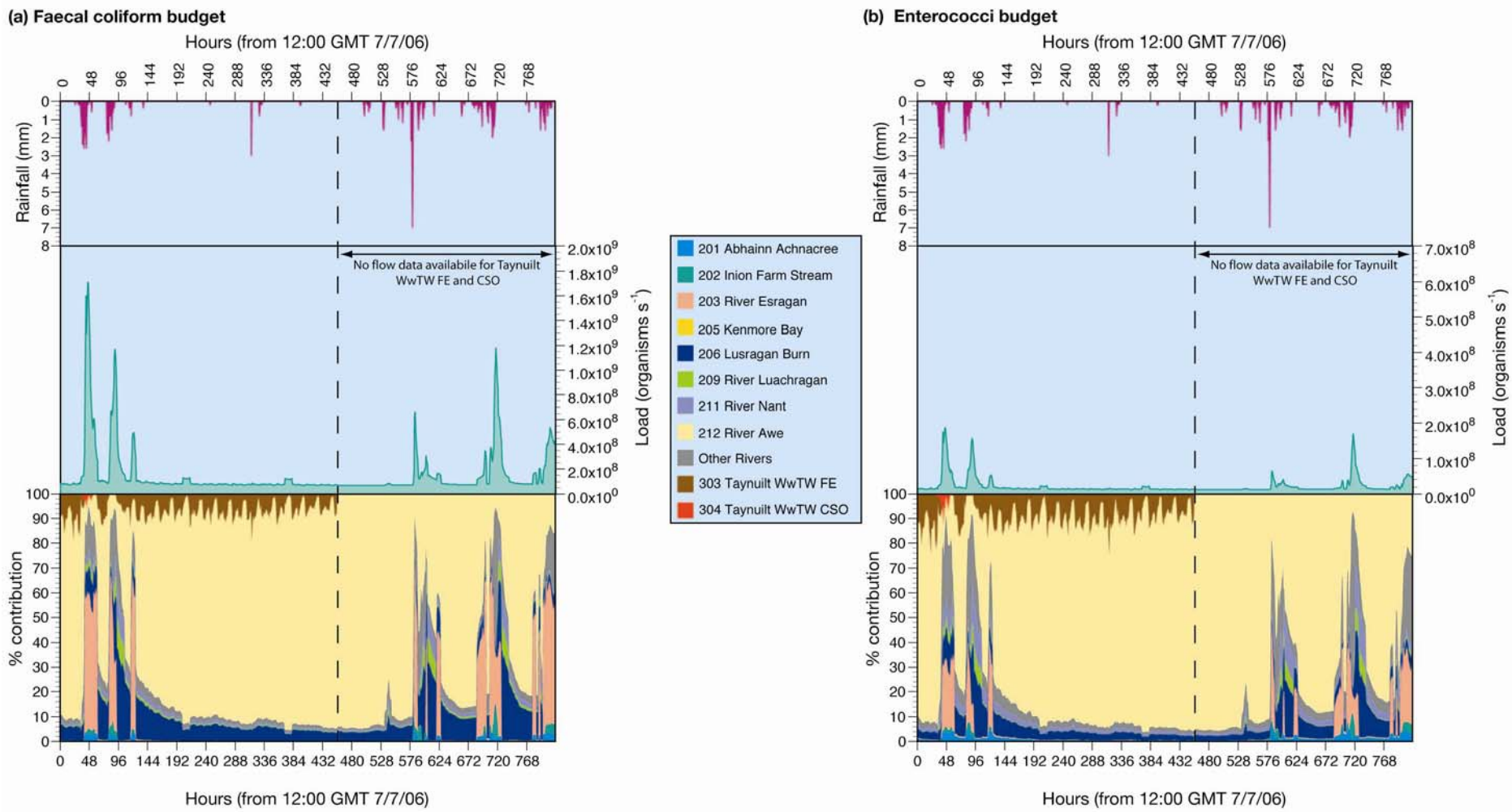


Figure 4.11. Hourly rainfall (mm) at Inion Farm (site 202), instantaneous faecal indicator load (organisms s^{-1}) and proportional contributions (%) of faecal indicator to the hourly load input to Loch Etive adjusted to reflect water quality upstream of potential FIO sources: (a) Faecal coliforms; (b) Enterococci.

4.6 Summary and conclusions

This study investigated faecal indicator organism (FIO) budgets from sewage and riverine sources draining to the outer basin of Loch Etive above the Falls of Lora.

The field study water quality monitoring program, carried out over two field survey periods, successfully generated faecal indicator organism concentrations for both base flow and rainfall induced high flow conditions within the selected study catchments. Gathering such data requires an intensive effort involving sampling teams located close to the study area and ready to respond to rainfall 24 hours a day, so that the episodic conditions, often missed in routine monitoring programs, are adequately characterised. The results of this and similar studies carried out by CREH highlights the importance of high flow events in faecal indicator organism flux estimation. Over half of the FIO flux input to the outer basin of Loch Etive was delivered during high flow periods, which accounted for only 11% to 15% of the 2006 study period upon which flow volumes were based. This presents a significant problem for the use of data derived from routine monitoring which are systematically biased to base flow conditions. This can lead to the erroneous appraisal of catchment derived faecal indicator fluxes from both diffuse and point discharges and could result in inappropriate expenditure decisions.

One question, not addressed by this investigation, is whether faecal indicators discharged from the septic tank to Dunstaffnage Bay could, under certain tidal conditions, contribute to concentrations above the Falls of Lora. This would best be addressed by a microbial tracer release with a sampling programme of locations close to shellfish harvesting areas.

The results described above have shown that very small catchments discharging only a very small proportion of the total freshwater input to the loch, for example Inion Farm stream and Kenmore Bay stream, can contribute relatively high proportions of FIOs during both base flow and high flow conditions, exceeding those input from sewage sources. Sampling of selected inputs during both survey periods also showed that, whilst the relative concentrations during base flow and high flow varied in some instances, that overall the budgets estimated using the alternative data produced, for the most part, similar results and temporal distributions; i.e. the conclusions were not changed if 2006 or 2007 data were used.

The largest contributor of faecal indicator organisms to the outer basin of Loch Etive was the River Awe, which was estimated to deliver up to 37% of the FC and EN during the 2006 study period. The majority of this contribution was delivered during base flow conditions when the instantaneous delivery of organisms to the loch was relatively low. The regulation of flows within this river means that it does not respond to rainfall events like other rivers flowing into the loch, and it was necessary, therefore, to characterise water quality within this river by a single geometric mean (GM) concentration for each organism and assign a portion of flow to the high flow budgets. The GM concentrations for the River Awe are, in fact, some of the lowest observed during the study, probably due to impoundment within Loch Awe and the relatively constant flow. The high load from this river is therefore a consequence of its substantially greater contribution to the total volume discharged to the loch than any of the other rivers considered; i.e. representing 88% of the total freshwater input

from the studied catchments. Any inputs from tributaries downstream of the Awe barrage, which would be subject to the normal dynamics of FIO delivery during high flow events, are likely to have only limited impact on the quality of the river due to the difference in flows between the main channel and the tributaries. It is recognised that planned changes to flow do occur, for example the freshets released during the study period, which may have an impact on FIO concentrations within the River Awe. However, the low FIO concentrations within the river and the fact that the high load is driven by the large discharge, means that management options for reducing the load from this source are probably limited.

The results of the water quality and budget analysis have highlighted some catchments displaying high faecal indicator organism concentrations and / or delivering relatively high loads of faecal indicator organisms to the loch. Such catchments include Abhainn Achnacree, Inion Farm stream and Kenmore Bay stream, which all displayed high FIO concentrations at their catchment outlets, and consequently, relatively high contributions to the FIO loads delivered to the loch. Additional sampling upstream of potential sources within these catchments during the 2006 study period showed that lower concentrations were indeed present, suggesting that identification and remediation of the contamination could lead to a decrease in the overall load contributed to the loch. An assessment of the impact of potential remediation, using the lower observed FIO concentrations to characterise the water quality of these catchments suggested that the FC load discharged to the loch could be reduced by 14% and the EN load reduced by 31%. Given the absence of consented point sources within these catchments measures to reduce faecal indicator organism inputs should concentrate on diffuse agricultural sources. Potential measures include the fencing of rivers to prevent livestock access, collection and treatment of runoff from agricultural farm building roofs and hardstanding areas and relocation of farmyard manure heaps away from field edges where these are close to drainage ditches, streams etc., maintenance of buffer strips along river corridors and avoidance of spreading manures and slurry during wet weather or when the soil is saturated. Additionally, attention should be given to closing potential flow pathways between sources such as farm hardstandings and watercourses provided by farm tracks etc. Studies designed to evaluate the effectiveness of such remediation measures (sometimes called best management practices) have been investigated by the present team and scientific partners in Scotland (Dickson *et al.*, 2005; Kay *et al.*, 2005b; Kay *et al.*, 2007a) and the policy issues involved have been addressed in related papers (Kay *et al.*, 2006a; Kay *et al.*, 2006b; Kay *et al.*, 2007c). It is also possible that septic tanks serving individual properties may be contributing to the load within these catchments.

Other rivers and streams discharging to the outer basin of Loch Etive have also been identified as contributing a relatively high proportion of FIOs. These include the River Esragan, particularly during high flow conditions, and Lusragan Burn. Inveresragan may be the potential source of FIOs to the River Esragan whilst inputs from Connel may be impacting on the Lusragan Burn. Further reductions in FIO flux to Loch Etive may be achieved through investigation and remediation of potential sources along these rivers.

Whilst it is possible to estimate the impact of remediation measures through use of empirical data collected upstream of potential sources, it is difficult to assess whether

these data actually represent the FIO concentrations expected given the land cover of the catchment. An alternative approach to the collection of empirical data with this objective is to predict water quality through models relating the proportion of land cover types within a catchment to FIO concentration. Such models have been developed by CREH and the methodology has been successfully applied to the River Ribble catchment, the UK's sentinel Water Framework Directive catchment (Wyer *et al.*, 2003; Kay *et al.*, 2005a) and the Leven Estuary catchment in Cumbria (Stapleton *et al*., 2006). The results from these predictions, adjusted for the runoff characteristics of the study catchments, could form benchmark concentrations against which remediation measures can be assessed. It is possible to predict such concentrations using a generic model developed by CREH, as has been applied to the catchment draining to the Severn Estuary (Stapleton *et al*., 2007c), although more accurate results could be achieved through the development of a model specific to the study catchment. Such models utilise data such as that collected during this study, although further data collection may be required to quantify inputs from strategic points within the river catchments to further enhance the model predictions.

The final effluent (FE) from Taynuilt WwTW was estimated to contribute between 2% and 5% of the FIOs delivered to Loch Etive during the study period, although it should be noted that this estimate was based on incomplete flow data. Data from the two sampling periods showed different patterns. The data from 2006 showed the majority of this load was delivered during base flow conditions, when FIO concentrations in the effluent were generally higher than during high flow periods. A decrease in FIO concentration in activated sludge-treated effluents during high flow conditions has not been observed by CREH before although this may be due to the fact that the plant is currently operating at <50% capacity and foul sewage is being diluted by a relatively high proportion of surface water runoff. However, the data from 2007 showed the more usual pattern of an increase in FIO concentrations during high flow events, with a greater proportion of the total load from the FE being discharged during the high flow events. However, the observed concentrations were relatively low compared to data from other CREH studies and this, again, may be due to the points noted above. Although the overall contribution of the FE from Taynuilt WwTW was relatively small, the two alternative budgets constructed for the current situation showed that the proportion of the instantaneous flux of FIOs represented by the FE could peak at 43% for short periods. The combined sewage overflow (CSO) at Taynuilt WwTW was estimated to contribute less than 1% of the load delivered to the outer basin of the loch, although it represented up to 9% of the instantaneous flux during high flow conditions when the flux of organisms is already elevated by increased contributions form catchment sources.

5. Acknowledgements

Mussels for the sampling programme were provided by a local commercial producer. The assistance with sample collection and advice provided by the staff there was invaluable to the success and smooth-running of the project. The authors would also like to thank John Waddells team, David Findlay and Alice Feehan from SEPA East Kilbride, and the staff at CEFAS Weymouth who provided laboratory support during weekend sampling. The CREH field team was assisted by Paula Hopkins (logistics), Daniel Bennett, Tom Chibnall and Katie Whiting. Andy Davies provided valuable help with ArcGIS and mapping for Chapters 2 and 3 of the report. Mark Wyer and Keren Smith provided GIS mapping and cartographic support for Chapter 4 of the report.

The following people were instrumental in providing the information and datasets utilised in the project;
Steve Anderton, hydrometric/hydrology data (SEPA)
Sarah Bennet (Argyll and Bute Council)
Mike Burrows, sunshine data (Scottish Association for Marine Science)
Duncan Campbell (Scottish Water)
Mike Cruikshank, hydrology data Loch Awe barrage (Scottish and Southern Energy)
Paul Daw (bird population data)
Martin Dunne (Scottish Water)
Jim Frame, information of private discharge licenses (SEPA)
General Register for Scotland, population data
Philip Gillibrand, POLCOMMs model (Scottish Association for Marine Science)
Barry Hardy, information on public network facilities (Scottish Water)
Anne Henderson (SEPA)
Shona Hogg, meteorological data (Met Office)
David Kay (CREH)
Peter Kirk, deer census data, (Deer Commission Scotland)
Mark James (SARF)
Lorna Marshall, Historical *E coli* classification data (Food Standards Agency Scotland)
Scottish Executive Environment and Rural Affairs Dept, agricultural census data
Walter Speirs (Muckairn Mussels)
Stephan Walker, information on public network facilities (Scottish Water)
Susannah White, forestry information (Forestry Commission Scotland)

6. Bibliography

Aitken MN (2003) Impact of agricultural practices and river catchment characteristics on river and bathing water quality. Water Science and Technology **48** 217-224

Al-Harbi AH (2003) Faecal coliforms in pond-water, sediments and hybrid tilapia *Oreochromis niloticus* x *Oreochromis aureus* in Saudi Arabia. Aquaculture Research **34** 517-524

Anon, (2000). Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Official Journal of the European Communities. L327: p. 1-72.

Burnes B (2003) Antibiotic resistance analysis of faecal coliforms to determine faecal pollution sources in a mixed-use watershed. Environmental Monitoring and Assessment **85** 87-98

Carroll S, Hargreaves M, Goonetilleke A (2005) Sourcing faecal pollution from onsite wastewater treatment systems in surface waters using antibiotic resistance analysis. Journal of Applied Microbiology **99** 471-482

Cheng PKC, Wong DKK, Chung TWH, Lim WWL (2005). Norovirus contamination found in oysters worldwide. Journal of Medical Virology **76** 593-597.

Chigbu P, Gordon S, Strange T (2004) Influence of inter-annual variations in climatic factors on faecal colifrm levels in Mississippi Sound. Water Research **38** 4341-4352

Commission F (2003) Forests and Water Guidelines, Forestry Commission, Edinburgh

Costantini V, Loisy F, Joens L, Le Guyader FS, Saif LJ (2006). Human and animal enteric caliciviruses in oysters from different coastal regions of the United States. Applied and Environmental Microbiology **72** 1800-1809.

Cox P, Griffith M, Angles M, Deere D, Ferguson C (2005) Concentrations of pathogens and indicators in animal feces in the Sydney watershed. Applied and Environmental Microbiology **71** 5929-5934

Croci L, Losio MN, Suffredini E, Pavoni E, Di Pasquale S, Fallacara F, Arcangeli G (2007). Assessment of human enteric viruses in shellfish from the northern Adriatic sea. International Journal of Food Microbiology **114** 252-257.

Crowther J, Kay D, Wyer M (2002) Faecal-indicator concentrations in waters training lowland pastoral catchments in the UK: relationships with land use and farming practices. Water Research **36** 1725-1734

Crowther J, Kay D, Wyer MD (2001) Relationships between microbial water quality and environmental conditions in coastal recreational waters: The Flyde coast, UK. Water Research **35** 4029-4038

Crowther J, Wyer MD, Bradford M, Kay D, Francis CA (2003) Modelling faecal indicator concentrations in large rural catchments using land use and topographic data. Journal of Applied Microbiology **94** 962-973

Davies-Colley R, Nagels J, Donnison, Muirhead R (2004a). Flood flushing of bugs in agricultural streams. Water and Atmosphere **2**(2).

Davis-Colley RJ, Nagels JW, Smith RA, Young RG, Phillips CJ (2004b) Water quality impact of a dairy cow herd crossing a stream. New Zealand Journal of Marine and Freshwater Research **38** 569-576

Davis-Colley RJ, Bell RG, Donnison AM (1994). Sunlight inactivation of enterococci and faecal coliforms in sewage effluent diluted in seawater. Applied Environmental Microbiology **60** 2049-2058.

DEFRA (2004) Water Quality: A Diffuse Pollution Review, DEFRA

Derlet RW, Carlson JR, Noponen MN (2004) Coliform and pathologic bacteria in Sierra Nevada National forest wilderness area lakes and streams. Wilderness and Environmental Medicine **15** 245-249

Dickson JW, Edwards AC, Jeffrey B, Kay, D. (2005) Catchment scale appraisal of best management practices (BMPs) for the improvement of bathing water – Brighouse Bay. Edinburgh SAC Environmental, Auchincruive
http://www.scotland.gov.uk/Topics/Environment/Water/15561/15068

Dombek P, Johnson L, Zimmerley S, Sadowsky M (2000) Use of repetitive DNA sequences and the PCR to differentiate *Escherichia coli* isolates from human and animal sources. Applied and Environmental Microbiology **66** 2572-2577

Donnison AM, Ross CM (1999) Animal and human faecal pollution in New Zealand. New Zealand Journal of Marine and Freshwater Research **33** 119-128

Dore WJ, Mackie M, Lees DN (2003). Levels of male-specific RNA bacteriophage and Escherichia coli in molluscan bivalve shellfish from commercial harvesting areas. Letters in Applied Microbiology **36** 92-96.

Dore WJ, Henshilwood K, Lees DN (1998). The development of management strategies for control of virological quality in oysters. Water Science and Technology **38** 29-35

Edwards A, Edelsten DJ (1977) Deep water renewal of Loch Etive: A three basin Scottish fjord. Estuarine and Coastal Marine Science **5** 575-595

Edwards A, Sharples F (1985) Scottish Sealochs: A catalogue, Scottish Marine Biological Association Report No 134

Ensign S, Mallin MA (2001) Stream water quality changes following timber harvest in a coastal plain swamp forest. Water Research **35** 3381-3390

Environment Agency (2000). The microbiology of recreational and environmental waters. Methods for the examination of waters and associated materials. Standing Committee of Analysts. Environment Agency, Bristol

Environment Agency (2003) Hydrometric Manual. Chapter 4: Instantaneous flow measurement. Environment Agency, Bristol.

Ferguson C, de Roda Husman AM, Altavilla N, Deere D (2003). Fate and transport of surface water pathogens in watersheds. Critical Reviews in Environmental Science and Technology **33** 299-361.

Ferguson CM, Coote BG, Ashbolt NJ, Stevenson IM (1996). Relationships between indicators, pathogens and water quality in an estuarine system. Water Research **30** 2045-2054.

Fernandez-Alvarez RM, Carballo-Cuervo S, Delarosa-Jorge MC, Rodriguez-Delecea J (1991) The influence of agricultural runoff on bacterial populations in a river. Journal of Applied Bacteriology **70** 437-442

Fischer JR, Zhao T, Doyle MP, Goldberg MR, Brown CA, Sewell CT, Kavanaugh DM, Bauman CD (2001) Experimantal and field studies of *Escherichia coli* 0157:H7 in white-tailed deer. Applied and Environmental Microbiology **67** 1218-1224

Formiga-Cruz M, Allard AK, Conden-Hansson A-C, Henshilwood K, Hernroth BE, Jofre J, Lees DN, Lucena F, Papapetropoulou M, Rangdale RE, Tsibouxi A, Vantarakis A, Girones R (2003) Evaluation of potential indicators of viral contamination in shellfish and their applicability to diverse geographic areas. Applied and Environmental Microbiology **69** 1556-1563

Formiga-Cruz M, Tofino-Quesada G, Bofill-Mas S, Lees DN, Henshilwood K, Allard AK, Conden-Hansson A-C, Hernroth BE, Vantarakis A, Tsibouxi A, Papapetropoulou M, Furones MD, Girones R (2002) Distribution of human virus contamination in shellfish from different growing areas in Greece, Spain, Sweden and the United Kingdom. Applied and Environmental Microbiology **68** 5990-5998

Gage J (1972) A preliminary survey of the benthic macrofauna and sediments in Lochs Etive and Creran, sea-lochs along the west coast of Scotland. Journal of the Marine Biological Association of the United Kingdom **52** 237-276

Gantzer C, Dubois E, Crance JM, Billaudel S, Kopecka H, Schwartzbrod L, Pommepuy M, Le Guyader F (1998) Influence of environmental factors on the survival of enteric viruses in seawater. Oceanologica Acta **21** 983-992

Geldreich EE, Clarke NA (1966) Bacterial pollution indicators in the intestinal tract of freshwater fish. Applied Microbiology **14** 429-437

Girones RJ, Jofre J, Bosch A (1989) Natural inactivation of enteric viruses in seawater. Journal of Environmental Quality **18** 34-39

Gourmelon M, Touati D, Pommepuy M, M. Cormier (1997) Survival of *Escherichia coli* exposed to visible light in seawater: analysis of rpoS-dependant effects. Canadian Journal of Microbiology **43** 1036-1043

Green J, Henshilwood K, Gallimore CI, Brown DW, Lees DN (1998). A nested reverse transcriptase PCR assay for detection of small round-structured viruses in environmentally contaminated molluscan shellfish. Applied and Environmental Microbiology **64** 858-863.

Griffin DW, Stokes R, Rose JB, Paul JH (2000) Bacterial indicator occurrence and the use of an F+ specific RNA coliphage assay to identify faecal sources in Homosassa Springs, Florida. Microbial Ecology **39** 56-64

Hagedorn C, Robinson SL, Filtz JR, Grubbs SM, Angier TA, Reneau RB Jnr (1999). Determinign sources of fecal pollution in a rural Virginian watershed with antibiotic resistance patterns in fecal strepptococci. Applied and Environmental Microbiology **65** 5522-5531.

Havelaar AH, Furuse K, Hogeboom WM (1986). Bacteriophages and indicator bacteria in human and animal faeces. Journal of Applied Bacteriology, **60** 255-262.

Hernroth BE, Condon-Hansson A-C, Rehnstam-Holm A-S, Girones R, Allard AK (2002) Environmental factors influencing human viral pathogens and their potential indicator organisms in the blue mussel, *Mytilus edulis*; the first scandanavian report. Applied and Environmental Microbiology **68** 4523-4533

Hewitt J, Greening GE (2006). Effect of heat treatment on hepatitis A virus and norovirus in New Zealand greenshell mussels (*Perna canaliculus*) by quantitative real-time reverse transcription PCR and cell culture. Journal of Food Protection 69 2217-2223.

Howell JM, Coyne MS, Cornelius P (1995) Faecal bacteria in agricultural waters of the bluegrass region of Kentucky. Journal of Environmental Quality **24** 411-419

Hunter C, Perkins J, J. Tranter, Gunn J (1999) Agricultural land-use effects on the indicator bacterial quality of an upland stream in the Derbyshire peak district in the UK. Water Research **33** 3577-3586

Husman AMDR, Lodder-Verschoor F, Van Den Berg HHJL, Le Guyader FS, Van Pelt H, Van der Poel WHM, Rutjes SA (2007). Rapid virus detection procedure for molecular tracing of shellfish associated with disease outbreaks. Journal of Food Protection **70** 967-974.

Jamieson R, Joy DM, Lee H, Kostaschuk R, Gordon RJ (2005a). Resuspension of sediment-associated Escherichia coli in a natural stream. Journal of Environmental Quality 34 581-589.

Jamieson R, Joy DM, Lee H, Kostaschuk R, Gordon RJ (2005a). Transport and deposition of sediment-associated Escherichia coli in natural streams. Water Research **39** 2665-2675.

Jones F, White WR (1984) Health and amenity aspects of surface waters. Water Pollution Control **83** 215-225

Kay D, McDonald AT (1980) Reduction of coliform bacteria in two upland reservoir feeder streams: the significance of distance decay relationships. Water Research **14**: 305-318.

Kay D, Wyer M D, Crowther J, Stapleton C, Bradford M, McDonald AT, Greaves J, Francis C, Watkins J (2005a). Predicting faecal indicator fluxes using digital land use data in the UK's sentinel Water Framework Directive catchment: The Ribble study. Water Research **39**: 655-667.

Kay D, Wyer MD, Crowther J, Wilkinson J, Stapleton C, Glass P. (2005b) Sustainable reduction in the flux of microbial compliance parameters from urban and arable land use to coastal bathing waters by a wetland ecosystem produced by a marine flood defence structure. Water Research **39**: 3320-3332

Kay D, Wilkinson J, Crowther J, Reid S, Francis C, Kay C, Hopkins M, Watkins J, Edwards A, McDonald A, Wyer M, Stapleton C (2005c). Monitoring the effectiveness of field and steading measures to reduce diffuse pollution from agriculture to bathing waters in the Ettrick, Cessnock, Nairn and Sandyhills catchments. Scottish Executive Report reference: ENV/7/4/04, Edinburgh.

Kay D, McDonald AT, Stapleton CM, Wyer MD, Fewtrell L. (2006a) The challenges of the water framework directive. Proceedings of the Institution of Civil Engineers. Water Management **159**: 58-64.

Kay D, Stapleton CM, Wyer MD, McDonald AT, Crowther J. (2006b) Total Maximum Daily Loads (TMDL). The USEPA approach to managing faecal indicator fluxes to receiving waters: Lessons for UK environmental regulation? L. Gairns, C. Crighton, and B. E. Jeffrey Agriculture and the Environment VI; Managing Rural Diffuse Pollution. Proceedings of the SAC/SEPA Biennial Conference. Edinburgh International Water Association, Scottish Agricultural College, Scottish Environmental Protection Agency, pp. 23-33.

Kay D, Aitken M, Crowther J, Dickson I, Edwards AC, Francis C, Hopkins M, Jeffrey W, Kay C, McDonald AT, McDonald D, Stapleton CM, Watkins J, Wilkinson J, Wyer MD (2007a). Reducing fluxes of faecal indicator compliance parameters to bathing waters from diffuse agricultural sources: The Brighouse Bay study, Scotland. Environmental Pollution **147**: 138-149.

Kay D, Crowther J, Fewtrell L, Francis C, Hopkins M, Kay C, McDonald AT, Stapleton CM, Watkins J, Wilkinson J, Wyer, MD, (2007b). Quantification and control of microbial pollution from agriculture: a new policy challenge? Environment Science and Policy (Theme Edition invited paper in press).

Kay D, Edwards AC, Ferrier RC, Francis C, Kay C, Rushby L, Watkins J, McDonald AT, Wyer M, Crowther J, Wilkinson J (2007c). Catchment microbial dynamics: the emergence of a research agenda. Progress in Physical Geography 31, 59-76.

Kay D, Stapleton CM, Crowther J, Wyer MD, Fewtrell L, Edwards A, McDonald AT, Watkins J, Francis CA, Wilkinson J. (2007d) Faecal indicator organism concentrations in sewage and treated effluents. Water Research (in press) (DOI:10.1016/j.watres.2007.07.036)

Kay D, Crowther J, Stapleton CM, Wyer MD, Fewtrell L, Anthony SG, Bradford M, Edwards A, Francis CA, Hopkins M, Kay C, McDonald AT, Watkins J, Wilkinson J (2008a). Faecal indicator organism concentrations and catchment export coefficients in the UK. Water Research (in submission).

Kay D, Lee R, Wyer MD, Stapleton CS (2008b). Integrated catchment studies: source identification and modelling., In Management of shellfish harvesting waters for public health protection. ed. G. Rees, p. in press. International Water Association and World Health Organization., London.

Kirschner AKT, Zechmeister TC, Kavka GG, Beiwi C, Harzig A, Mach RL, Farnleitner AH (2004) Integral strategy for evaluation of faecal indicator performance in bird-influenced saline inland waters. Applied and Environmental Microbiology **70** 7396-7403

Kleinheinz GT, McDermott CM, Leewis MC, Englebert E (2006). Influence of sampling depth on Escherichia coli concentrations in beach monitoring. Water Research Volume **40**: 3831-3837

Kohn, MR, Farley TA, Ando T, Curtis M, Wilson SA, Jin Q, Monroe SS, Baron RC, Mcfarland LM, Glass RI**.** 1995. An outbreak of Norwalk virus gastroenteritis associated with eating raw oysters—implications for maintaining safe oyster beds. JAMA **273:**466-471

La Rosa T, Mirto S, Marino A, Alonzo V, Maugeri TL, Mazzola A (2001). Heterotrophic bacteria community and pollution indicators of mussel - farm impact in the Gulf of Gaeta (Tyrrhenian Sea). Marine Environmental Research Volume **52**: 301-321

Le Guyader FS, Bon F, DeMedici D, Parnaudeau S, Bertone A, Crudeli S, Doyle A, Zidane M, Suffredini E, Kohli E, Maddalo F, Monini M, Gallay A, Pommepuy M, Pothier P, Ruggeri FM (2006a). Detection of multiple noroviruses associated with an international gastroenteritis outbreak linked to oyster consumption. Journal of Clinical Microbiology **44** 3878-3882.

Le Guyader F, Haugarreau L, Moissec L, Dubois E, Pommepuy M (2000) Three-year study the access human enteric viruses in shellfish. Applied and Environmental Microbiology **66** 3241-3248

Le Guyader FS, Loisy F, Atmar, RL, Hutson AM, Estes MK, Ruvoen-Clouet N, Pommepuy M, Le Pendu J (2006b). Norwalk virus specific binding to oyster digestive tissues. Emerging Infectious Diseases **12** 931-936.

Lee RJ, Morgan OC (2003) Environmental factors influencing the microbiological contamination of commercially harvested shellfish. Water Science and Technology **47** 65-70

Lees, D. (2000). Viruses in bivalve shellfish. Int. J. Food Microbiol. **59** 81-116.

Levesque B, Brosseau P, Simard P, Dewailly E, Meisels M, Ramsey D, J. Joly (1993) Impact of the ring-billed gull (*Laras delawarensis*) on the microbiological quality of recreational water. Applied and Environmental Microbiology **59** 1228-1230

Licence K, Oates KR, Synge BA, Reid TMS (2001) An outbreak of *E.coli* 0157 infection with evidence of spread from animals to man through contamination of a private water supply. Epidemiology and Infection **126** 135-138

Lillehaug A, Bergsjo B, Schau J, Bruheim T, Vikoren T, Handeland K (2005) *Campylobacter* spp., *Salmonella* spp., verocytotoxic *Escherichia coli* and antibiotic resistance in indicator organisms in wild cervids. Acta Veterinaria Scandinavica **46** 23-32

Lisle JT, Smith JJ, Edwards DD, McFeters GA (2004) Occurrence of microbiological indicators and *Clostridium perfingens* in wastewater, water column samples, sediments, drinking water, and Weddell seal faeces collected at McMurdo Station, Antarctica. Applied and Environmental Microbiology **70** 7269-7276

Lowther J, Henshilwood K, Lees DN (in submission). Determination of norovirus contamination in oysters from two commercial harvesting areas over an extended period using semi-quantitative real-time RT-PCR". Applied and Environmental Mocrobiology

Mallin MA, S.H. Ensign, McIver MR, G.C. Shank, Fowler PK (2001) Demographic, landscape and meteorological factors controlling the microbial pollution of coastal waters. Hydrobiologia **460** 185-193

Mallin MA, Williams KE, Esham EC, Lowe RP (2000) Effect of human development on bacteriological water quality in coastal water sheds. Ecological Applications **10** 1047-1056

Meals DW, Braun DC (2006) Demonstration of methods to reduce *E.coli* runoff from dairy manure application sites. Journal of Environmental Quality **35** 1088-1100

Merceron M, Kempf M, Bentley D, Gaffet JD, Grand JL, Lamort-Datin L (2002) Environmental impact of a salmonid farm on a well flushed marine site: I. Current and quality. Journal of Applied Ichthyology **18** 40-50

Miller JJ, Handerek BP, Beasley BW, Olson ECS, Yanke LJ, Larney FJ, McAllister TA, Olson BM, Selinger LB, Chanasyk DS, Hasselback P (2004) Quantity and quality of runoff from a beef cattle feedlot in southern Alberta. Journal of Environmental Quality **33** 1088-1097

Moissec L, Guyader FL, Haugarreau L, Pommepuy M (2000) Magnitude of rainfall on viral contamination of the marine environment during gastroenteritis. Revue D' Epidemiollogie et de Sante Publique **48** 62-71

Muirhead RW, R.J. Davis-Colley, Donnison AM, Nagels JW (2004) Faecal bacteria yields in artificial flood events: quantifying in-stream stores. Water Research **38**: 1215-1224

Myrmel M, Berg EMM, Rimstad E, Grinde B (2004). Detection of enteric viruses in shellfish from the Norwegian coast. Applied and Environmental Microbiology **70** 2678-2684.

Nagels JW, R.J. Davis-Colley, Donnison AM, Muirhead RW (2002) Faecal contamination over flood events in a pastoral agricultural stream in New Zealand. Water Science and Technology **45** 45-52

Nash CE, Iwamoto RN, Mahnken CVW (2000) Aquaculture risk management and marine mammal interactions in the Pacific Northwest. Aquaculture **183** 307-323

Niemi M (1985) Faecal indicator bacteria at freshwater rainbow trout (*Salmo gairdneri*) farms.

Nishida T, Nishio O, Kato M, Chuma T, Kato H, Iwata H, Kimura H (2007). Genotyping and quantitation of noroviruses in oysters from two distinct sea areas in Japan. Microbiology and Immunology 51, 177-184.

Nunez-Delgado A, Lopez-Periago E, Viqueira FDF (2002) Chloride, sodium, potassium and faecal bacteria levels in surface runoff and subsurface percolates from grassland plots amended with cattle slurry. Bioresource Technology **82** 261-271

Oliver DM, Clegg CD, Haygarth PM, Heathwaite AL (2005). Assessing the potential for pathogen transfer from grassland soils to surface waters. Advances in Agronomy **85**, 125-180.

Oliver DM, Heathwaite AL, Hodgson CJ, Chadwick DR, (2007). Mitigation and current management attempts to limit pathogen survival and movement within farmed grassland. Advances in Agronomy **93**, 95-152.

Orskov F, Orskov I (1981) Enterobacteriaceae. In: Broude AI (ed) Medical Microbiology and Infectious Diseases. W.B. Saunders Co., Philidelphia, p 340-352

Pal D, Gupta CD (1992) Microbial pollution in water and its effect in fish. Journal of Aquatic Animal Health **4**:29-32

Pallant J. (2001). SPSS Survival Manual. OUP: Buckingham

Parveen S, Portier KM, Robinson K, Edmiston L, Tamplin ML (1999) Discriminant analysis of ribotype profiles of *Escherichia coli* for differentiating human and nonhuman sources of fecal pollution. Applied and Environmental Microbiology **65** 3142-3147

Phan TG, Khamrin P, Akiyama M, Yagyu F, Okitsu S, Maneekarn N, Nishio O, Ushijima H, (2007). Detection and genetic characterization of norovirus in oysters from China and Japan. Clinical Laboratory **53** 405-412

Pommepuy M, Dumas F, Caprais MP, Camus P, Le Mennec C, Parnaudeau S, Haugarreau L, Sarrette B, Vilagines P, Pothier P, Kholi E, Le Guyader (2004) Sewage impact on shellfish microbial contamination. Water Science and Technology **50** 117-124.

Power U F, Collins JK (1990). Tissue distribution of a coliphage and *Esherichia coli* in mussels after contamination and depuration. Appl. Environ. Microbiol. **56** 803-807

Ramos M, Quinten J, Tyrrel S (2006) Effects of cattle manure on erosion rates and runoff water pollution by faecal coliforms. Journal of Environmental Management **78** 97-101

Ricca DM, Cooney JJ (1998) Coliphages and indicator bacteria in birds around Boston Harbor. Journal of Industrial Microbiology and Biotechnology **21** 28-30

Rio-Rodriguez RED, Inglis V, Miller SD (1997) Survival of *Escherichia coli* in the intestine of fish. Aquaculture Research **28** 257-264

Rodgers P, Soulsby C, Hunter C, Petry J (2003) Spatial and temporal bacterial quality of a lowland agricultural stream in northeast Scotland. Science of the Total Environment **314** 289-302

Rogerson PA. (2001). Statistical Methods for Geography. Sage, London

Shehane SD, Harwood VJ, Whitlock JE, Rose JB (2005) The influence of rainfall on the incidence of microbial faecal indicators and the dominant sources of faecal pollution in a Florida river. Journal of Applied Microbiology **98** 1127-1136

Sinton L, Findlay R, Hannah R (1998) Distinguishing human from animal faecal contamination in water. New Zealand Journal of Marine and Freshwater Research **32** 323-348

Solutions SW (2005) Small Coastal Villages: Total Marine Impact Assessment

SPSS (2002). SPSS 11.0 for Macintosh Brief Guide. .156pp. SPSS Inc

Stapleton CM, Wyer MD, Crowther J, Kay D, Kay C, Francis CA, Watkins J, Anthony S (2006). Assessment of Point and Diffuse Sources of Faecal Indicators and Nutrients in the Windermere and Crake Catchments. Phase II Volume I: Faecal Indicator organism budgets and land cover – water quality modelling. ICREW Pilot Action 2 Technical Report. Centre for Research into Environment and Health, University of Wales Aberystwyth and ADAS Consulting Ltd., Wolverhampton. http://www.icrew.info/documents/2/Phase_2_Vol_1_WQ_Modelling_Faecal_Indicator_Organism_Inputs.pdf.

Stapleton CM, Kay D, Kay C, Francis CA, Watkins J. (2007a) Partial source apportionment for faecal indicator fluxes into Loch Etive outer basin, summer 2006. Final report to Scottish Aquaculture Research Forum. February 2007. Centre for Research into Environment and Health, University of Wales, Aberystwyth.

Stapleton CM, Wyer MD, Crowther, J, McDonald AT, Kay D, Greaves J, Wither A, Watkins J, Francis CA, Humphrey N, Bradford M. (2007b) Quantitative catchment profiling to apportion faecal indicator organism budgets for the Ribble system, the UK's sentinel drainage basin for Water Framework Directive research., Journal of Environmental Management. In Press. DOI: 10.1016/j.jenvman.2006.11.03.

Stapleton CM, Wyer MD, Kay D, Bradford M, Humphrey N, Wilkinson J, Lin B, Yang L, Falconer RA, Watkins J, Francis CA, Crowther J, Paul ND, Jones K, McDonald AT, (2007c) Fate and Transport of Particles in Estuaries. Volume II: estimation of enterococci inputs to the Severn estuary from point and diffuse sources. Environment Agency Science Report SC000002/SR http://Publications.environment-agency.gov.uk/pdf/SCHO0307BMED-e-e.pdf

Suprihatin I, Fallowfield H, Bentham R, Cromar N (2003) Determination of faecal pollutants in Torrens and Patawalonga catchment waters in South Australia using faecal sterols. Water Science and Technology **47** 283-289

Troussellier M, Bonnefont JL, Courties C, Derrien A, Dupray E, Gauthier M, Gourmelon M, Joux F, Lebaron P, Martin Y, Pommepuy M (1998). Responses of enteric bacteria to environmental stresses in seawater **21** 965-981

Ueki, Y., Shoji, M., Suto, A., Tanabe, T., Okimura, Y., Kikuchi, Y., Saito, N., Sano, D., Omura, T., 2007. Persistence of caliciviruses in artificially contaminated oysters during depuration. Applied and Environmental Microbiology 73, 5698-5701

Vinten AJA, J.T. D, Lewis DR, Aitken MN, Fenlon DR (2004a) Simulating transport of *E. coli* derived from faeces of grazing livestock using MACRO model. Soil Use and Management **20** 195-202

Vinten AJA, Lewis DR, McGechan M, Duncan A, Aitken M, Hill C, Crawford C (2004b) Predicting the effect of livestock inputs of *E. coli* on microbiological compliance of bathing waters. Water Research **38** 3215-3224

Wakelin SC, Elefsiniotis P, Wareham DG (2003) Assessment of stormwater retention basin water quality in Winnipeg, Canada. Water Quality Research Journal of Canada **38** 433-450

Weaver RW, Entry JA, Graves A (2005) Numbers of fecal streptococci and *Escherichia coli* in fresh and dry cattle, horse and sheep manure. Canadian Journal of Microbiology **51** 847-851

Webby RJ, Carville KS, Kirk MD, Greening G, Ratcliff RM, Crerar SK, Dempsey K, Sarna, M, Stafford R, Patel M, Hall G, (2007). Internationally distributed frozen oyster meat causing multiple outbreaks of norovirus infection in Australia. Clinical Infectious Diseases **44** 1026-1031

Wiggins B, Andrews R, Conway R, Corr C, Dobratz E, Dougherty D, Eppard J, Knupp S, Limjoco M, Mettenburg J, Rinehardt J, Sonsino J, Torrijos R, Zimmerman M (1999) Use of antibiotic resistance analysis to identify non-point sources of faecal pollution. Applied and Environmental Microbiology **65** 3483-3486

Wilkinson J, Kay D, Wyer M, A. Jenkins (2006) Processes driving the episodic flux of faecal indicator organisms in streams impacting on recreational and shellfish harvesting waters. Water Research **40** 153-161

Wither A, Greaves J, Dunhill I, Wyer M, C. Stapleton, Kay D, Humphrey N, Watkins J, Francis C, McDonald A, Crowther J (2005a) Estimation of diffuse and point source microbial pollution in the Ribble catchment discharging to bathing waters in the north west of England. Water Science and Technology **51** 191-198

Wither A, Rehfisch M, Austin G (2005b) The impact of bird populations on the microbiological quality of bathing waters. Water Science and Technology **51** 199-207

WHO (2003) Guidelines for safe recreational water environments Volume 1: Coastal and freshwaters World Health Organisation, Geneva.

Wyer MD, Jackson GF, Kay D, Yeo J, Dawson H (1994). An assessment of the impact of inland surface water input to the bacteriological quality of coastal waters. Journal of the Institution of Water and Environmental Management **6**: 459-467.

Wyer MD, Kay D, Dawson HM, Jackson GF, Jones F, Yeo J, Whittle J. (1996). Delivery of microbial Indicator organisms to coastal waters from catchment sources. Water Science and Technology, **33**: 37-50.

Wyer MD, O'Neill JG, Goodwin V, Kay D, Jackson G, Tanguy L, Briggs J. (1997a). Non-sewage derived sources of faecal indicator organisms in coastal waters: case studies. pp. 120-132 in Kay D. and Fricker, C. (Eds.) Coliforms and E. coli problem or solution. Royal Society of Chemistry special publication 191.

Wyer M, O'Neill G, Kay D, Crowther J, Jackson G, Fewtrell L. (1997b) Non-outfall sources of faecal indicator organisms affecting the compliance of coastal waters with Directive 76/160/EEC. Water Science and Technology **35**: 151-156.

Wyer MD, Kay D, Crowther J, Whittle J, Spence A, Huen V, Wilson C, Carbo P, Newsome J. (1998). Faecal-indicator budgets for recreational coastal waters: a catchment approach. Journal of the Chartered Institution of Water and Environmental Management **12**: 414-424.

Zar, J (1999) *Biostatistical Analysis*. 4th ed. Upper Saddle River, New Jersey: Prentice-Hall.

Appendix I. Determination of Norovirus and FRNA Bacteriophage

Preparation of shellfish homogenate for analysis of norovirus.
A sub sample of 10 mussels were opened and the animals removed from their shells. The peripheral flesh and organs of each animal were then cut away from the hepatopancreas and discarded. The hepatopancreases were finely chopped using a razor blade before being added to an equal volume per weight of 100 µg/ml Proteinase K (30 U/mg) solution. The sample was then incubated at 37°C with shaking at 320 rpm for a duration of 1 hr, and subsequently incubated at 65°C for a duration of 15 min. Finally, the sample was centrifuged at 3000 x g for 5 min., and the soluble portion (homogenate) retained for downstream testing. Shellfish and homogenates were prepared and assayed whilst still fresh.

Purification of viral RNA and reverse transcription.
Purification of viral RNA was largely based on the method previously published by (Boom et. al. 1990). A 300 µl volume of oyster homogenate was split equally across three 1.5 ml microcentrifuge tubes each containing 10 µl of well mixed silica bead suspension ('glassmilk'; Anachem) and 5 µl of feline calicivirus (FCV) process control material (tissue culture supernatant from the F9 strain of FCV grown on Crandall-Reese feline kidney cells, frozen in single use aliquots). For each set of samples a negative control sample consisting of shellfish homogenate which had tested negative by repeated assay was tested in parallel. A 900 µl volume of lysis buffer (61% (w/v) guanidine isothiocyanate (GITC), 0.05 M Tris pH 6.4, 0.02 M EDTA, 1.3% (v/v) Triton X-100) was added to each tube, the contents of which were then mixed by inversion for 20 min. before being pelleted using a microcentrifuge (60 s at 12500 x g) and the supernatant removed by aspiration. The beads were washed with 1 ml wash buffer (61% (w/v) GITC, 0.05 M Tris pH 6.4) by resuspension of the pellet followed by microcentrifugation and the removal of the supernatant. This pellet wash cycle was repeated with another 1 ml wash buffer, followed by 1ml ice cold 70% (v/v) ethanol, and finally 1ml ice cold acetone. The pellet was then resuspended in 50 µl TE buffer (10 mM Tris HCl pH 8.0, 1 mM EDTA) and incubated at 56 ºC for 10 min. to elute viral RNA from the beads. The beads were pelleted and the supernatant added to 2.2 volumes of ice cold 100% ethanol and 0.1 volumes 3 M sodium acetate (pH 5.2), then incubated at –80 ºC for 30-120 min. to precipitate nucleic acids. The precipitant was pelleted using a refrigerated centrifuge (20 min. at 22000 x g) and all the supernatant removed by aspiration. Complementary DNA (cDNA) was then synthesised in a reverse transcription (RT) step as follows; each RNA pellet was resuspended in 8.9 µl of a reaction mix containing 20 U of Rnasin (Promega) and 500 ng random hexamers (Promega) and overlaid with a drop of mineral oil. The reaction was incubated at 70°C for 5 min. on a thermal cycler then snap-cooled on a freezer block. A 6.1 µl volume of a reaction mix producing concentrations in the final reaction volume of 10 mM Tris (pH 8.3), 50 mM KCl, 5 mM $MgCl_2$, and 1 mM each deoxynucleotide triphosphate (dNTP) plus 25 U/reaction MuLV-RT enzyme (Promega) was then added to each tube under the oil layer, and the reactions incubated for 10 min. at 23°C followed by 60 min. at 37°C to generate cDNA. The reaction was stopped by incubation for 5 min. at 95°C. The three cDNAs generated from each sample were pooled together to give a 45 µl final volume.

5' fluorogenic nuclease assay (TaqMan®) analysis. For both norovirus genogroup-specific TaqMan® primer/probe sets (Table 1), 3 aliquots of 5 µl cDNA were added to adjacent wells of a 96-well optical reaction plate and made up to 25 µl with TaqMan® reaction mix (final concentration of 1x TaqMan® Universal PCR Master Mix (Applied Biosystems), 900 nM each primer, and 25-225 nM each probe; optimal concentrations determined according to Applied Biosystems protocol). For the FCV primer/probe set two aliquots of 5 µl cDNA were used. For each assay positive and negative PCR control material were also tested. The plate was placed in an Applied Biosystems SDS 7000 real-time PCR machine with the following amplification program; 50°C for 2 min., then 95°C for 10 min., followed by 50 cycles of 95°C for 15 s and 60°C for 1 min. For analysis, threshold values were set at 0.10 fluorescence units, then C_t values were determined using the GeneAmp system software. For each norovirus assay, samples giving a positive reaction in any replicate (determined as having a sigmoid-shaped curve which rises above the threshold) were counted as positive for that genogroup. For any sample giving C_ts for the FCV assay in excess of the batch-specific action limit (set for the original batch to correspond to an extraction/amplification efficiency approximately 10% of the average obtained with multiple extractions of 5µl FCV added to 100µl water; reset for subsequent batches by direct comparison of FCV concentrations), or where the positive PCR controls indicated PCR reagent failure, or for any positive sample where the negative extraction or PCR controls showed contamination, the homogenate was retested.

Preparation of shellfish homogenate for bacteriology

Sediment was removed from the shellfish by rinsing/scrubbing under cold, running tap water of potable quality. Shellfish were then allowed to drain and were opened using a shucking knife. Shucked shellfish extracts (flesh and intravalvular fluid) were collected, weighed and diluted (1:3) with two parts of 0.1% peptone. The mixture was homogenised with a Waring-type blender for approximately one minute.

Enumeration of F+RNA bacteriophage in shellfish flesh

Enumeration of F+RNA bacteriophage was based on the International Organization for Standardization (ISO) method ISO 10705-1 (International Organization for Standardization, 1995). 50 ml of each sample were centrifuged at 3000 x g for 5 minutes. Further dilutions of the supernatant were made if necessary. The genetically modified host, *Salmonella typhimurium* WG49 (NCTC 12484), was grown to a cell density of between 7 x 10^7 and 40 x 10^7 colony forming units (cfu)/ml at 600 nm in tryptone yeast extract broth (TYGB) containing 1% calcium-glucose solution at 37˚C. A one ml aliquot of host, was then added to 2.5 ml of molten 1% tryptone yeast extract agar at 45˚C. One ml of the prepared sample was then added to the molten agar and host cells. Each vial was mixed thoroughly by inversion and poured onto petri dishes containing previously prepared 2% TYGA. Plates were inverted and incubated at 37˚C for 18±4h. Final results were expressed as plaque forming units (pfu) per 100 g.

References

Boom, R. et al.,. (1990). *Rapid and simple method for purification of nucleic acids* J. Clin. Micro. 28, 495–503.

Appendix II. Microbiological and environmental correlation results.

Table I. Results of Pearson's correlation analysis between bacterial counts and hydrodynamic data at Site A. Bold indicates significant correlations ($p<0.050$). Top values are correlation coefficients, lower figures are p values

	Summer dataset					**Winter dataset**				
	24 hour mean				Salinity at	24 hour mean				Salinity at
	Surface temp	Surface salinity	9m temp	9m salinity	sampling	Surface temp	Surface salinity	9m temp	9m salinity	sampling
Mussel tissue										
E. coli	0.291	-0.102	0.315	0.054	-0.133	0. 061	0.207	-0.026	0.283	0.196
	0.168	0.636	0.117	0.794	0.536	0.780	0.344	0.905	0.191	0.369
Faecal coliforms	0.224	0.086	0.290	0.088	0.240	0.189	0.346	0.143	0.428	0.338
	0.293	0.691	0.151	0.669	0.260	0.388	0.106	0.517	0.041	0.115
FRNA	**-0.423**	-0.037	-0.386	-0.209	-0.011	0.043	0.029	0.042	0.027	0.015
Bacteriophage	**0.044**	0.866	0.057	0.316	0.961	0.850	0.899	0.852	0.905	0.948
NV GI						**-0.538**	0.142	**-0.476**	0.078	0.099
						0.010	0.528	**0.025**	0.732	0.662
NV GII						0.237	-0.216	0.283	-0.146	-0.197
						0.287	0.334	0.201	0.517	0.381
Surface water										
WFC	-0.036	-0.230	0.077	-0.256	-0.230	0.066	**-0.480**	0.244	-0.364	**-0.459**
	0.872	0.303	0.722	0.227	0.304	0.766	**0.020**	0.261	0.088	**0.028**
WFS	-0.202	0.129	-0.155	-0.028	0.164	**0.490**	0.324	**0.460**	0.419	0.311
	0.421	0.610	0.515	0.905	0.516	**0.021**	0.141	**0.031**	0.052	0.159

Table II. Results of Pearson's correlation analysis between bacterial counts and hydrodynamic data at Site B. Bold indicates significant correlations ($p<0.050$). Top values are correlation coefficients, lower figures are p values

	Summer dataset					**Winter dataset**				
		24 hour mean			Salinity at		24 hour mean			Salinity at
	Surface temp	Surface salinity	9m temp	9m salinity	sampling	Surface temp	Surface salinity	9m temp	9m salinity	sampling
Mussel tissue										
E. coli	0.009	-0.310	0.039	-0.104	-0.266	0.187	0.092	0.234	0.186	0.165
	0.966	0.140	0.848	0.614	0.189	0.405	0.685	0.295	0.408	0.463
Faecal coliforms	0.309	-0.022	0.365	0.020	-0.158	0.080	0.158	0.174	0.275	0.150
	0.142	0.921	0.067	0.923	0.442	0.731	0.494	0.451	0.227	0.515
FRNA	-0.362	-0.006	-0.354	-0.049	-0.377	0.097	-0.060	0.060	-0.051	-0.053
Bacteriophage	0.090	0.980	0.083	0.817	0.063	0.676	0.797	0.798	0.827	0.819
NV GI						-0.384	0.239	**-0.494**	0.175	0.190
						0.077	0.283	**0.020**	0.436	0.396
NV GII						0.352	0.162	0.316	0.166	0.064
						0.262	0.614	0.317	0.607	0.843
Surface water										
WFC	0.236	-0.224	0.250	0.009	-0.098	-0.032	**-0.673**	0.343	**-0.506**	**-0.619**
	0.278	0.305	0.227	0.966	0.640	0.896	**0.002**	0.151	**0.027**	**0.005**
WFS	-0.283	0.017	-0.231	0.043	0.063	0.268	-0.108	0.248	-0.036	-0.069
	0.255	0.946	0.327	0.847	0.793	0.298	0.679	0.338	0.889	0.794

Norovirus genogroups were only assessed in the winter dataset.

Table III Significance level of correlations between **Summer** bacterial concentrations (in mussel tissue and water samples) and environmental parameters.

Parameter		Site A					Site B				
		E. coli	FC	WFC	WFS	bacteriophage	*E.coli*	FC	WFC	WFS	bacteriophage
Rainfall	GS 24 hr	0.327	0.343	0.308	0.230	0.007	**0.417**	**0.422**	**0.499**	**0.571**	0.081
		0.102	0.087	0.143	0.330	0.973	**0.034**	**0.032**	**0.011**	**0.009**	0.701
	GS 48 hr	0.258	0.270	0.382	0.404	0.019	**0.396**	0.367	**0.470**	**0.548**	0.034
		0.204	0.181	0.065	0.077	0.929	**0.045**	0.065	**0.018**	**0.012**	0.871
	GS 72 hr	0.283	0.276	0.396	0.337	-0.006	**0.396**	**0.406**	**0.485**	**0.460**	0.010
		0.161	0.172	0.055	0.147	0.979	**0.045**	**0.040**	**0.014**	**0.042**	0.963
	GS 96 hr	0.338	0.315	**0.441**	0.338	0.059	**0.472**	**0.489**	**0.488**	**0.517**	0.058
		0.091	0.117	**0.031**	0.144	0.778	**0.015**	**0.011**	**0.013**	**0.020**	0.748
	GS 120hr	0.347	0.298	**0.532**	0.157	0.070	**0.533**	**0.505**	**0.518**	0.325	0.129
		0.083	0.140	**0.007**	0.509	0.740	**0.005**	**0.008**	**0.008**	0.163	0.538
	GS 144hr	0.386	0.377	**0.559**	0.183	0.051	**0.557**	**0.514**	**0.566**	0.137	0.286
		0.051	0.058	**0.004**	0.441	0.807	**0.003**	**0.007**	**0.003**	0.566	0.166
	GS 7 day	0.374	0.374	**0.575**	0.235	0.039	**0.512**	**0.451**	**0.604**	0.161	0.277
		0.060	0.060	**0.003**	0.319	0.854	**0.008**	**0.021**	**0.001**	0.498	0.179
	GS -1day	0.137	0.163	0.355	**0.526**	-0.073	0.223	0.179	0.341	0.354	-0.099
		0.506	0.426	0.088	**0.017**	0.729	0.274	0.380	0.096	0.126	0.638
	GS -2day	0.304	0.211	0.285	0.078	0.001	0.228	0.210	0.353	0.058	-0.108

		0.131	0.302	0.177	0.745	0.998	0.262	0.303	0.083	0.807	0.607
	GS-3day	0.257	0.143	0.333	0.021	0.200	**0.411**	0.368	0.217	0.228	0.134
		0.206	0.485	0.111	0.931	0.337	**0.037**	0.064	0.297	0.333	0.522
	GS -4day	0.042	0.012	0.231	-0.347	0.180	0.179	0.217	0.178	-0.206	0.234
		0.838	0.952	0.277	0.134	0.390	0.382	0.287	0.394	0.384	0.261
	GS -5day	0.260	0.308	0.028	-0.045	0.237	0.294	0.196	0.153	-0.090	**0.437**
		0.199	0.126	0.896	0.850	0.254	0.144	0.338	0.465	0.707	**0.029**
	GS -6 day	0.132	0.142	0.263	0.030	0.417	**0.414**	0.331	0.130	0.015	**0.582**
		0.521	0.489	0.214	0.899	0.038	**0.036**	0.099	0.535	0.950	**0.002**
	GS -7 day	-0.041	0.011	0.082	-0.205	0.299	0.217	0.124	0.130	-0.200	**0.439**
		0.843	0.958	0.705	0.386	0.146	0.288	0.545	0.536	0.398	**0.028**
	Duns 24hr	0.031	0.073	0.305	0.008	0.126	0.260	0.287	0.403	0.245	0.220
		0.883	0.730	0.157	0.974	0.558	0.209	0.164	0.051	0.298	0.302
	Duns 48hr	0.243	0.245	0.385	0.244	0.170	0.380	0.306	**0.483**	**0.461**	0.207
		0.241	0.238	0.070	0.315	0.426	0.061	0.136	**0.017**	**0.041**	0.332
	Duns 72hr	0.240	0.223	**0.423**	0.253	0.125	**0.443**	0.317	**0.513**	**0.465**	0.179
		0.248	0.283	**0.044**	0.295	0.561	**0.026**	0.122	**0.010**	**0.039**	0.402
Parameter				Site A					Site B		
		E. coli	FC	WFC	WFS	bacteriophage	*E.coli*	FC	WFC	WFS	bacteriophage
	Duns 96hr	0.279	0.304	0.411	0.347	0.063	**0.439**	**0.450**	0.363	**0.518**	0.083
		0.176	0.140	0.052	0.146	0.771	**0.028**	**0.024**	0.081	**0.019**	0.700
	Duns 120hr	0.219	0.309	**0.540**	0.155	0.089	**0.505**	**0.531**	**0.395**	0.371	0.072
		0.282	0.124	**0.006**	0.513	0.671	**0.009**	**0.005**	**0.051**	0.108	0.732
	Duns 144hr	0.371	**0.487**	**0.683**	0.204	0.021	**0.584**	**0.615**	**0.479**	0.153	0.245
		0.062	**0.012**	**<0.001**	0.308	0.920	**0.002**	**0.001**	**0.015**	0.518	0.238
	Duns 7day	**0.489**	**0.527**	**0.629**	0.283	0.124	**0.655**	**0.618**	**0.501**	0.216	0.337
		0.011	**0.006**	**0.001**	0.227	0.556	**<0.001**	**0.001**	**0.011**	0.360	0.099
	Duns -1day	0.213	0.164	0.319	0.350	0.032	0.213	0.180	0.306	0.425	0.034
		0.307	0.434	0.139	0.141	0.882	0.306	0.388	0.146	0.062	0.873
	Duns -2day	0.161	0.139	0.297	0.189	-0.138	0.335	0.295	**0.435**	0.167	-0.119
		0.442	0.506	0.169	0.438	0.521	0.101	0.152	**0.033**	0.480	0.580
	Duns -3day	0.329	0.217	0.152	0.196	0.018	0.167	0.296	-0.084	0.250	-0.120
		0.109	0.296	0.488	0.421	0.934	0.424	0.151	0.696	0.287	0.577
	Duns -4day	-0.011	0.047	0.268	-0.285	0.173	0.228	0.265	0.082	-0.148	0.131
		0.957	0.823	0.217	0.237	0.418	0.273	0.201	0.702	0.535	0.542
	Duns -5day	0.269	0.389	0.064	0.008	0.065	0.111	0.229	0.083	-0.257	0.269
		0.184	0.050	0.768	0.973	0.757	0.588	0.262	0.693	0.273	0.193
	Duns -6day	0.362	0.307	-0.017	0.127	0.501	0.338	0.143	0.088	0.167	-0.082
		0.069	0.127	0.939	0.595	0.011	0.091	0.484	0.677	0.482	0.732
	Duns -7day	0.169	0.100	0.426	0.006	0.246	**0.394**	0.298	0.222	**0.466**	**0.505**
		0.410	0.629	0.038	0.981	0.235	**0.047**	0.140	0.287	**0.019**	**0.010**
Riverflow	RS 24 hr	**0.429**	**0.403**	0.393	0.306	-0.041	**0.616**	**0.575**	**0.533**	0.334	0.102
		0.029	**0.041**	0.057	0.189	0.847	**0.001**	**0.002**	**0.006**	0.149	0.626

Parameter	Site A					Site B				
	E. coli	FC	WFC	WFS	bacteriophage	*E.coli*	FC	WFC	WFS	bacteriophage
RS 48hr	**0.397**	0.297	0.404	0.324	-0.001	**0.540**	**0.505**	**0.425**	0.263	0.074
	0.045	0.141	0.050	0.163	0.996	**0.004**	**0.009**	**0.034**	0.263	0.724
RS 72 hr	0.384	0.218	**0.420**	0.205	0.047	**0.541**	**0.507**	0.369	0.204	0.102
	0.053	0.285	**0.041**	0.387	0.824	**0.004**	**0.008**	0.069	0.388	0.627
RS 96 hr	0.363	0.207	**0.438**	0.114	0.090	**0.568**	**0.518**	0.394	0.161	0.156
	0.068	0.311	**0.032**	0.634	0.669	**0.002**	**0.007**	0.051	0.497	0.455
RS 120 hr	0.392	0.308	0.405	0.151	0.125	**0.581**	**0.547**	0.311	0.063	0.342
	0.048	0.125	0.050	0.524	0.552	**0.002**	**0.004**	0.131	0.793	0.094
RS 144 hr	0.384	0.309	0.377	0.131	0.159	**0.572**	**0.509**	0.299	0.007	**0.401**
	0.053	0.125	0.069	0.581	0.448	**0.002**	**0.008**	0.146	0.975	**0.047**
RS 7day	0.377	0.288	**0.418**	0.026	0.192	**0.542**	**0.440**	0.332	-0.065	0.370
	0.057	0.154	**0.042**	0.915	0.357	**0.004**	**0.024**	0.105	0.786	0.069
RS -1day	0.197	0.043	0.278	0.191	0.096	0.253	0.272	0.101	0.020	0.045
	0.336	0.833	0.189	0.419	0.649	0.212	0.178	0.631	0.932	0.832
RS -2day	0.221	0.024	0.298	-0.005	0.149	0.032	0.338	0.109	0.022	0.117
	0.278	0.907	0.157	0.984	0.478	0.110	0.091	0.604	0.926	0.579
RS -3day	0.205	0.088	0.335	-0.165	0.195	**0.415**	0.380	0.259	-0.002	0.250
	0.315	0.670	0.110	0.486	0.350	**0.035**	0.056	0.212	0.994	0.227
RS -4day	0.272	0.297	0.225	-0.178	0.186	0.257	0.232	0.147	-0.228	0.330
	0.179	0.140	0.290	0.453	0.372	0.204	0.254	0.483	0.333	0.107
RS -5day	0.273	0.309	0.178	-0.040	0.235	0.332	0.217	0.172	-0.227	**0.458**
	0.177	0.124	0.405	0.867	0.259	0.097	0.286	0.411	0.337	**0.021**
RS -6day	0.085	0.040	0.067	-0.163	0.248	0.215	0.225	-0.103	-0.250	0.359
	0.678	0.846	0.757	0.493	0.231	0.291	0.268	0.623	0.287	0.078
RS -7day	-0.175	-0.272	-0.090	-0.365	-0.032	0.039	0.080	-0.115	-0.399	0.124
	0.393	0.178	0.677	0.113	0.878	0.850	0.699	0.585	0.082	0.554
Awe 24hr	0.160	0.103	0.348	0.010	0.088	0.233	0.105	0.264	-0.158	0.093
	0.439	0.616	0.096	0.966	0.676	0.253	0.611	0.201	0.506	0.659
Awe 48hr	0.170	0.127	0.398	-0.019	0.097	0.235	0.123	0.222	-0.193	0.113
	0.406	0.537	0.054	0.937	0.645	0.248	0.551	0.287	0.415	0.592
Awe 72hr	0.195	0.129	**0.435**	-0.075	0.144	0.279	0.159	0.230	-0.197	0.194
	0.339	0.531	**0.033**	0.754	0.491	0.168	0.438	0.270	0.405	0.353
Awe 96hr	0.149	0.121	**0.428**	-0.053	0.219	0.262	0.129	0.199	-0.172	0.284
	0.469	0.557	**0.037**	0.823	0.294	0.195	0.530	0.341	0.468	0.169
Awe 120hr	0.132	0.114	0.414	-0.001	0.273	0.245	0.114	0.162	-0.110	0.326
	0.519	0.581	0.045	0.996	0.186	0.227	0.581	0.440	0.645	0.112
Awe 144hr	0.108	0.061	0.358	0.026	0.292	0.206	0.076	0.113	-0.068	0.325
	0.599	0.766	0.086	0.914	0.157	0.314	0.713	0.589	0.775	0.113
Awe 7day	0.095	0.009	0.317	0.045	0.290	0.166	0.043	0.071	-0.033	0.304
	0.646	0.967	0.131	0.851	0.160	0.417	0.835	0.734	0.891	0.139
Awe -1day	0.161	0.139	**0.421**	-0.040	0.113	0.223	0.138	0.180	-0.216	0.135
	0.433	0.497	**0.041**	0.868	0.592	0.273	0.503	0.390	0.360	0.519

	Awe -2day	0.221	0.133	**0.453**	-0.142	0.187	0.307	0.205	0.220	-0.193	0.291
		0.278	0.517	**0.026**	0.552	0.372	0.127	0.315	0.290	0.416	0.159
	Awe -3day	0.027	0.096	0.376	0.013	0.308	0.176	0.054	0.112	-0.127	0.414
		0.898	0.642	0.071	0.956	0.134	0.390	0.792	0.593	0.595	0.040
	Awe -4day	0.039	0.091	0.344	0.156	0.398	0.131	0.066	0.036	0.064	**0.428**
		0.851	0.659	0.100	0.511	0.049	0.523	0.750	0.864	0.789	**0.033**
	Awe -5day	-0.000	-0.154	0.073	0.113	0.214	-0.015	-0.024	-0.057	0.108	0.143
		0.999	0.453	0.735	0.635	0.303	0.941	0.908	0.786	0.649	0.496
	Awe -6day	-0.006	-0.320	0.015	0.096	0.196	-0.123	-0.157	-0.185	0.206	0.014
		-0.978	0.110	0.945	0.688	0.349	0.549	0.442	0.375	0.383	0.947
	Awe -7day	-0.084	-0.318	-0.059	0.224	-0.050	-0.207	-0.167	-0.238	0.193	-0.268
		0.684	0.114	0.758	0.343	0.814	0.310	0.415	0.252	0.416	0.194
Sunshine		0.020	0.044	-0.203	-0.154	0.064	-0.030	-0.010	-0.111	0.010	0.071
		0.924	0.831	0.341	0.516	0.763	0.884	0.962	0.598	0.966	0.735
Air Temp		0.100	0.081	-0.136	-0.032	**-0.595**	0.099	0.224	0.270	-0.137	**-0.423**
		0.627	0.696	0.528	0.893	**0.002**	0.631	0.270	0.192	0.565	**0.035**

Table IV Significance level of correlations between winter bacterial concentrations (in mussel tissue and water samples) and environmental parameters. Note that all Norovirus correlations are based on rank data.

Parameter		Site A							Site B						
		E.coli	Faecal coliform	WFC	WFS	bacteriophage	NV G1	NV G2	*E.coli*	Faecal coliform	WFC	WFS	bacteriophage	NV GI	NV GII
Rainfall	GS 24 hr	0.093	0.150	**0.647**	0.102	-0.005	-0.165	0.343	-0.140	0.116	**0.681**	**0.505**	0.036	-0.161	0.078
		0.672	0.494	**0.001**	0.652	0.984	0.462	0.118	0.536	0.616	**0.001**	**0.039**	0.876	0.475	0.809
	GS 48 hr	0.129	0.100	**0.599**	0.059	0.151	-0.201	0.280	-0.092	0.128	**0.677**	0.479	0.145	-0.204	-0.028
		0.559	0.651	**0.003**	0.793	0.502	0.370	0.207	0.685	0.579	**0.001**	0.052	0.531	0.363	0.931
	GS 72 hr	0.112	0.063	**0.573**	0.068	0.147	-0.163	0.187	0.218	0.112	**0.624**	**0.562**	0.241	-0.260	-0.427
		0.611	0.775	**0.004**	0.764	0.513	0.469	0.404	0.330	0.630	**0.004**	**0.019**	0.293	0.242	0.166
	GS 96 hr	0.060	0.053	**0.594**	0.077	0.096	-0.158	0.174	0.289	0.150	**0.611**	**0.555**	0.208	-0.274	-0.488
		0.785	0.810	**0.003**	0.732	0.672	0.482	0.439	0.192	0.516	**0.005**	**0.021**	0.365	0.217	0.108
	GS 120hr	0.059	0.071	**0.620**	0.097	0.076	-0.205	0.210	0.308	0.162	**0.631**	**0.537**	0.134	-0.322	-0.447
		0.789	0.747	**0.002**	0.669	0.736	0.359	0.348	0.163	0.482	**0.004**	**0.026**	0.563	0.144	0.145
	GS 144hr	0.067	0.071	**0.600**	0.068	0.104	-0.197	0.219	0.318	0.171	**0.647**	**0.504**	0.113	-0.362	-0.426
		0.761	0.748	**0.002**	0.763	0.645	0.397	0.326	0.149	0.459	**0.003**	**0.039**	0.625	0.097	0.178
	GS 7 day	0.023	0.064	**0.662**	0.114	0.013	-0.191	0.285	0.280	0.175	**0.706**	**0.508**	0.008	-0.359	-0.399
		0.917	0.771	**0.001**	0.612	0.954	0.394	0.198	0.207	0.449	**0.001**	**0.037**	0.972	0.101	0.198
	GS -1day	0.062	0.011	**0.460**	-0.020	0.353	-0.186	0.140	-0.039	0.161	**0.511**	0.244	0.285	-0.221	-0.114
		0.779	0.960	**0.027**	0.930	0.107	0.408	0.535	0.862	0.487	**0.025**	0.346	0.210	0.323	0.725
	GS -2day	-0.017	0.074	0.406	0.105	0.035	-0.163	0.029	**0.438**	0.066	0.435	**0.534**	0.129	-0.348	**-0.644**
		0.937	0.736	0.054	0.643	0.878	0.469	0.900	**0.041**	0.778	0.062	**0.024**	0.578	0.113	**0.024**
	GS-3day	-0.153	-0.100	**0.515**	0.060	-0.090	-0.175	0.169	0.303	0.279	**0.497**	0.449	0.094	-0.229	-0.484
		0.486	0.995	**0.012**	0.790	0.692	0.435	0.452	0.171	0.221	**0.031**	0.070	0.685	0.305	0.111
	GS -4day	-0.001	0.166	**0.622**	0.111	-0.013	-0.466	0.377	0.052	0.163	**0.644**	0.271	-0.247	-0.422	0.177
		0.995	0.449	**0.002**	0.622	0.953	0.029	0.084	0.817	0.480	**0.003**	0.293	0.280	0.050	0.581
	GS -5day	-0.032	0.065	**0.470**	-0.105	0.067	-0.159	0.250	0.197	0.152	**0.637**	0.260	-0.064	**-0.461**	-0.027
		0.884	0.769	**0.023**	0.641	0.766	0.481	0.263	0.379	0.512	**0.003**	0.313	0.782	**0.031**	0.934

GS -6day	0.334	0.176	0.182	-0.079	0.311	-0.111	0.005	0.067	0.247	**0.553**	0.353	0.207	-0.196	-0.205
	0.120	0.422	0.407	0.728	0.158	0.624	0.982	0.769	0.280	**0.014**	0.265	0.368	0.383	0.523
GS -7day	0.258	0.055	0.143	-0.202	0.112	0.169	-0.102	-0.055	0.135	0.433	-0.017	0.056	0.142	0.056
	0.235	0.804	0.516	0.368	0.621	0.451	0.653	0.808	0.558	0.064	0.950	0.808	0.529	0.862
Duns 24hr	-0.008	0.152	**0.658**	**0.593**	-0.318	-0.221	0.354	0.113	0.206	**0.500**	**0.600**	-0.201	-0.111	-0.143
	0.971	0.488	**0.001**	**0.004**	0.150	0.322	0.107	0.617	0.371	**0.029**	**0.011**	0.383	0.624	0.658
Duns 48hr	-0.001	0.204	**0.693**	**0.497**	-0.264	-0.329	0.352	0.111	0.267	**0.655**	**0.617**	-0.342	-0.336	-0.106
	0.998	0.305	**<0.001**	**0.019**	0.236	0.135	0.108	0.623	0.242	**0.002**	**0.008**	0.129	0.127	0.743
Duns 72hr	0.068	0.301	**0.705**	**0.574**	-0.247	-0.349	0.364	0.142	0.249	**0.623**	**0.700**	-0.305	-0.389	-0.088
	0.758	0.163	**<0.001**	**0.005**	0.268	0.112	0.096	0.528	0.276	**0.004**	**0.002**	0.179	0.073	0.786
Duns 96hr	-0.030	0.086	**0.768**	0.385	-0.166	-0.311	0.372	0.002	0.129	**0.722**	**0.513**	-0.157	-0.304	-0.091
	0.890	0.697	**<0.001**	0.077	0.461	0.158	0.089	0.991	0.577	**<0.001**	**0.035**	0.496	0.169	0.778
Duns 120hr	-0.090	0.156	0.344	0.315	-0.056	-0.144	-0.332	0.268	0.161	0.116	0.405	0.206	-0.120	**-0.580**
	0.684	0.476	0.109	0.154	0.805	0.552	0.132	0.228	0.485	0.636	0.106	0.370	0.589	**0.048**
Duns 144hr	-0.077	0.189	**0.452**	0.355	-0.087	-0.195	-0.279	0.251	0.203	0.249	0.452	0.186	-0.152	-0.497
	0.727	0.389	**0.030**	0.105	0.699	0.384	0.208	0.260	0.377	0.305	0.069	0.419	0.499	0.100
Duns 7day	0.023	0.025	**0.713**	0.255	-0.035	-0.262	0.302	0.223	0.108	**0.701**	**0.520**	0.024	-0.346	-0.387
	0.918	0.909	**<0.001**	0.253	0.878	0.240	0.173	0.318	0.640	**0.001**	**0.032**	0.916	0.115	0.214
Duns -1day	-0.071	-0.035	**0.509**	0.110	0.114	-0.231	0.291	-0.093	0.208	**0.667**	0.251	-0.086	-0.356	-0.109
	0.748	0.873	**0.013**	0.625	0.613	0.301	0.189	0.681	0.366	**0.002**	0.332	0.712	0.104	0.739
Duns -2day	0.095	0.146	**0.693**	**0.497**	-0.264	-0.275	**0.458**	-0.041	0.050	**0.626**	**0.578**	0.017	-0.335	-0.115
	0.666	0.506	**<0.001**	**0.019**	0.236	0.216	**0.032**	0.855	0.831	**0.004**	**0.015**	0.940	0.128	0.721
Duns -3day	-0.100	-0.050	**0.707**	-0.001	-0.067	-0.228	0.115	-0.178	0.044	**0.672**	0.190	-0.016	-0.104	0.135
	0.650	0.822	**<0.001**	0.996	0.768	0.308	0.611	0.428	0.850	**0.002**	0.464	0.945	0.644	0.676
Duns -4day	0.023	-0.073	**0.463**	-0.222	0.206	-0.106	0.081	0.142	0.055	**0.606**	0.258	0.090	-0.225	-0.414
	0.919	0.740	**0.038**	0.321	0.357	0.637	0.721	0.527	0.811	**0.006**	0.317	0.698	0.314	0.181
Duns -5day	-0.269	0.042	0.410	0.202	-0.153	0.052	0.003	0.354	0.183	0.270	**0.526**	-0.066	-0.197	**-0.699**
	0.215	0.850	0.052	0.367	0.497	0.818	0.989	0.106	0.426	0.264	**0.030**	0.776	0.378	**0.011**
Duns -6day	-0.070	0.031	**0.484**	**0.455**	-0.113	-0.208	0.115	0.067	0.247	**0.553**	0.353	0.207	-0.053	-0.221
	0.750	0.887	**0.019**	**0.033**	0.617	0.354	0.610	0.769	0.280	**0.014**	0.165	0.368	0.814	0.491
Duns -7day	-0.193	0.242	**0.436**	0.268	-0.252	-0.226	0.002	-0.055	0.135	0.433	-0.017	0.056	-0.183	-0.293
	0.377	0.266	**0.037**	0.227	0.257	0.231	0.994	0.808	0.558	0.064	0.950	0.808	0.416	0.356
Riverflow RS 24 hr	-0.112	0.009	**0.635**	0.317	-0.076	-0.165	0.373	-0.241	0.194	**0.664**	0.452	-0.017	-0.113	0.056
	0.611	0.969	**0.001**	0.150	0.736	0.462	0.087	0.279	0.400	**0.002**	0.069	0.942	0.618	0.863
RS 48hr	-0.002	-0.057	**0.645**	0.211	0.019	-0.126	0.407	-0.174	0.077	**0.702**	0.390	0.021	-0.162	-0.029
	0.994	0.797	**0.001**	0.346	0.935	0.576	0.060	0.439	0.742	**0.001**	0.122	0.929	0.472	0.929
RS 72 hr	-0.028	-0.057	**0.694**	0.164	0.011	-0.131	0.384	-0.130	0.073	**0.736**	0.393	0.021	-0.185	-0.024
	0.898	0.796	**<0.001**	0.466	0.960	0.561	0.078	0.563	0.753	**<0.001**	0.119	0.929	0.411	0.941
RS 96 hr	-0.014	-0.115	**0.408**	-0.076	0.149	-0.168	0.353	-0.040	0.091	**0.749**	0.400	0.038	-0.239	-0.116
	0.948	0.602	**0.054**	0.735	0.508	0.455	0.107	0.860	0.695	**<0.001**	0.112	0.871	0.284	0.719
RS 120hr	-0.017	-0.029	**0.634**	0.093	0.049	-0.069	0.228	0.170	0.153	**0.664**	**0.490**	0.099	-0.203	-0.414
	0.937	0.894	**0.001**	0.681	0.829	0.759	0.307	0.450	0.509	**0.002**	**0.046**	0.669	0.365	0.181
RS 144hr	0.021	-0.007	**0.619**	0.104	0.083	-0.095	0.197	0.233	0.169	**0.655**	**0.506**	0.169	-0.254	-0.442

	0.923	0.975	**0.002**	0.645	0.715	0.673	0.379	0.297	0.463	**0.002**	**0.038**	0.465	0.254	0.150
RS 7day	0.001	-0.016	**0.606**	0.083	0.094	-0.175	0.227	0.216	0.149	**0.677**	0.481	0.152	-0.372	-0.408
	0.997	0.943	**0.002**	0.714	0.679	0.436	0.309	0.333	0.518	**0.001**	0.051	0.511	0.089	0.188
RS -1day	0.096	-0.087	**0.585**	0.091	0.099	-0.092	0.411	-0.090	-0.010	**0.694**	0.355	0.077	-0.207	-0.105
	0.662	0.693	**0.003**	0.687	0.663	0.682	0.057	0.692	0.965	**0.001**	0.162	0.742	0.355	0.744
RS -2day	-0.032	0.001	**0.680**	0.041	0.046	-0.105	0.256	-0.044	0.093	**0.680**	0.350	0.095	-0.155	0.026
	0.884	0.997	**<0.001**	0.855	0.839	0.643	0.252	0.847	0.689	**0.001**	0.169	0.682	0.491	0.936
RS -3day	0.074	-0.004	**0.525**	-0.042	0.196	-0.214	0.143	0.241	0.175	**0.624**	0.346	0.146	-0.339	-0.317
	0.737	0.986	**0.010**	0.852	0.381	0.338	0.526	0.281	0.448	**0.004**	0.174	0.528	0.123	0.316
RS -4day	-0.199	-0.089	0.376	0.071	-0.037	-0.084	0.093	0.399	0.064	0.318	0.431	0.076	-0.325	**-0.634**
	0.363	0.687	0.077	0.748	0.869	0.712	0.680	0.066	0.782	0.184	0.084	0.745	0.140	**0.027**
RS -5day	0.142	0.099	0.352	-0.020	0.160	-0.241	0.072	0.353	0.232	**0.549**	**0.514**	0.339	-0.358	-0.545
	0.519	0.652	0.100	0.929	0.476	0.280	0.748	0.107	0.311	**0.015**	**0.035**	0.132	0.102	0.067
RS -6day	0.189	0.230	0.351	-0.055	0.142	-0.213	0.038	0.129	0.214	**0.589**	0.399	0.096	-0.408	-0.124
	0.388	0.292	0.100	0.806	0.528	0.340	0.865	0.568	0.353	**0.008**	0.113	0.680	0.059	0.701
RS -7day	0.030	-0.211	0.391	-0.321	0.050	-0.058	0.104	0.028	-0.161	**0.643**	0.193	0.141	-0.242	-0.189
	0.893	0.334	0.065	0.145	0.824	0.797	0.646	0.900	0.487	**0.003**	0.457	0.542	0.277	0.556
Awe 24hr	-0.197	-0.105	**0.689**	0.168	-0.034	-0.062	0.183	-0.036	0.114	**0.624**	0.350	0.081	-0.1846	-0.256
	0.366	0.632	**<0.001**	0.456	0.881	0.785	0.415	0.874	0.623	**0.004**	0.168	0.727	0.408	0.416
Awe 48hr	-0.049	-0.078	**0.602**	0.058	0.122	-0.041	0.182	0.089	0.172	**0.659**	0.312	0.133	-0.212	-0.300
	0.826	0.725	**0.002**	0.796	0.589	0.856	0.418	0.695	0.455	**0.002**	0.223	0.566	0.344	0.344
Awe 72hr	-0.022	-0.044	**0.589**	0.055	0.119	-0.023	0.153	0.169	0.204	**0.658**	0.348	0.131	-0.227	-0.366
	0.919	0.841	**0.003**	0.809	0.597	0.918	0.495	0.452	0.376	**0.002**	0.171	0.570	0.310	0.243
Awe 96hr	-0.012	-0.046	**0.566**	0.024	0.126	-0.029	0.121	0.186	0.190	**0.647**	0.373	0.168	-0.211	-0.423
	0.958	0.835	**0.005**	0.915	0.576	0.897	0.591	0.408	0.410	**0.003**	0.141	0.468	0.346	0.171
Awe 120hr	0.010	-0.037	**0.551**	0.019	0.113	-0.030	0.118	0.200	0.186	**0.653**	0.373	0.160	-0.212	-0.443
	0.964	0.866	**0.009**	0.932	0.618	0.894	0.600	0.372	0.420	**0.002**	0.140	0.488	0.344	0.149
Awe 144hr	0.009	-0.049	**0.531**	0.004	0.120	-0.013	0.117	0.197	0.180	**0.648**	0.338	0.161	-0.214	-0.426
	0.967	0.823	**0.009**	0.984	0.595	0.954	0.603	0.379	0.436	**0.003**	0.185	0.485	0.339	0.168
Awe 7day	0.028	-0.045	**0.514**	-0.011	0.109	0.001	0.118	0.187	0.167	**0.651**	0.340	0.151	-0.199	-0.417
	0.898	0.839	**0.012**	0.963	0.629	0.998	0.602	0.406	0.469	**0.003**	0.181	0.513	0.375	0.178
Awe -1day	0.008	-0.046	**0.552**	0.019	0.153	-0.015	0.156	0.164	0.203	**0.651**	0.309	0.141	-0.214	-0.335
	0.971	0.836	**0.006**	0.935	0.497	0.946	0.489	0.465	0.378	**0.003**	0.227	0.542	0.338	0.287
Awe -2day	0.006	0.027	**0.555**	0.066	0.096	-0.010	0.088	0.291	0.256	**0.630**	0.406	0.114	-0.247	-0.468
	0.978	0.903	**0.006**	0.770	0.672	0.963	0.697	0.189	0.262	**0.004**	0.106	0.623	0.268	0.125
Awe -3day	0.004	-0.048	**0.461**	-0.068	0.142	-0.036	0.030	0.214	0.147	**0.590**	0.416	0.246	-0.170	-0.549
	0.986	0.829	**0.027**	0.765	0.528	0.872	0.893	0.340	0.524	**0.008**	0.096	0.282	0.449	0.064
Awe -4day	0.095	-0.010	**0.456**	-0.018	0.074	-0.038	0.112	0.249	0.151	**0.643**	0.348	0.130	-0.222	-0.516
	0.666	0.964	**0.029**	0.936	0.744	0.868	0.620	0.263	0.512	**0.003**	0.171	0.574	0.322	0.086
Awe -5day	-0.014	-0.115	0.408	-0.076	0.149	0.083	0.131	0.150	0.141	**0.586**	0.141	0.128	-0.222	-0.113
	0.948	0.602	0.054	0.735	0.508	0.714	0.561	0.505	0.543	**0.008**	0.588	0.581	0.320	0.618
Awe -6day	0.057	-0.077	0.390	-0.120	0.063	0.090	0.134	0.074	0.068	**0.614**	0.240	0.086	-0.128	-0.134
	0.797	0.726	0.066	0.594	0.781	0.690	0.552	0.745	0.769	**0.005**	0.353	0.711	0.571	0.553
Awe -7day	0.139	-0.038	**0.634**	0.093	0.049	0.066	0.172	0.111	0.079	**0.572**	0.271	0.082	-0.134	-0.184

	0.527	0.862	**0.001**	0.681	0.829	0.772	0.443	0.624	0.732	**0.011**	0.293	0.723	0.553	0.566
Sunshine	**0.488**	0.297	**-0.611**	0.092	0.364	0.214	-0.185	0.245	0.085	**-0.756**	-0.015	0.224	0.248	-0.100
	0.018	0.168	**0.002**	0.683	0.096	0.338	0.411	0.272	0.714	**<0.001**	0.955	0.330	0.266	0.758
Air Temp	-0.025	0.205	0.152	**0.517**	-0.213	**-0.472**	0.035	0.052	0.155	0.094	0.202	-0.100	-0.080	0.311
	0.911	0.347	0.489	**0.014**	0.342	**0.027**	0.877	0.819	0.502	0.703	0.436	0.665	0.722	0.325

WFC- Faecal coliform level in surface water sample, WFS – faecal strep level in surface water sample. GS – Glen Strae rainfall, Duns – Dunstaffnage rainfall, RS – River Strae flow, Awe – Awe Barrage flow

Appendix III. List of Abbreviations

CSO – combined sewer overflow
CFU – coliform forming units
CREH - Centre for Research into Environment and Health
EHO – Environmental Health Office
EN – intestinal Enterococci
EO – emergency outfall
FC – faecal coliform
FE – final effluent
FIO – faecal indicator organism
FRS – Fisheries Research Services
FSAS – Food Standards Agency Scotland
GI – norovirus GI genogroup
GII – norovirus GII genogroup
GM – geometric mean
MPN – mean probable number
NGR – National Grid reference
NSSP – (US) National Shellfish Sanitation Programme
PCR – polymerase chain reaction
RBC – rotating biological contactor
RERAD – Rural Environmental Research and Analysis Directorate
SAC – Special Area of Conservation
SEPA – Scottish Environmental Protection Agency
SGW – shellfish growing water
SSSI – Site of Special Scientific Interest
SMRU - Sea Mammal Research Unit
UKAS - United Kingdom Accreditation Service
WFC – surface water faecal coliform
WFS – surface water faecal streptococci
WWTW – waste water treatment works