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Automated event sampling for microbiological and related analytes in remote sites: a comprehensive system

D. Roser*, J. Skinner, C. LeMaitre**, L. Marshall*, J. Baldwin**, K. Billington***, S. Kotz***, K. Clarkson and N. Ashbolt***

* Centre for Water and Waste Technology, Cooperative Research Centre for Water Quality and Treatment, School of Civil and Environmental Engineering, University of New South Wales, Sydney NSW 2052, Australia

** Ecowise Environmental Ltd., PO Box 1834, Fyshwick ACT 2609, Australia

*** South Australia EPA Mount Lofty Ranges Watershed Protection Office, Suite 2, 85 Mount Barker Road, Stirling SA 5152, Australia

**** Melbourne Water, 630 Church Street, Richmond 3121, Australia

Abstract Pathogen concentrations are most often monitored during dry weather. Generally in Australia, however, loads mobilised during storms are of more concern. The filling of reservoirs commonly occurs from heavy rain events, and flood inputs may destabilise reservoir hydraulics leading to short-circuiting of contaminates to water supply off-takes. To capture storm events that can occur rapidly in remote locations at any time, automated sampling would seem appropriate. Unfortunately no commercial sampling system appears suitable for collecting multiple large volume samples along a hydrograph. We report here the development of an Event Sampling System (ESS) and associated resources, designed to address the special needs of microbiological sampling and varying event and site characteristics. The resultant ESSs consist of a standardised sampling module, enclosed in housings suited to different circumstances that is currently being field tested at six sites. Sampling module components include ISCO samplers modified to collect 24 ten litre ambient and 24 one litre refrigerated samples at remote sites along with *in situ* stream data. Essential to this hardware are sample collection and ESS management protocols covering issues such as storm warning, collection team mobilisation, laboratory coordination, ESS commissioning and maintenance. Some issues remain to be addressed, hence the resulting ESSs are seen as prototypes in the development of standardised storm-event based microbiological sampling well suited to remote locations.
Keywords Autosamplers; indicators; microorganisms; pathogens; rainfall; storm events

Introduction

A multi-state Australian project (#2.2.1 of the Cooperative Research Centre for Water Quality and Treatment) is assessing major tributary concentrations of pathogens, faecal indicators and possible surrogates in selected drinking source waters ranging from fully protected through to heavily degraded urban and agriculturally impacted catchments. A key aim is to assess the extent to which analyte loads (25 parameters in total) are elevated during storm events. Data from stormwater studies indicates that microbial loads may increase by orders of magnitude compared to during dry weather (e.g. Jagals *et al*., 1995; Ferguson *et al*., 1996). High quality event based data on the quality of catchment run-off/stormwater is needed as:

- much filling of Australian raw water reservoirs occurs during storm events rather than during dry weather when most routine monitoring has traditionally been undertaken;
- all catchments house pathogen sources such as animal scats, septic tanks and stockyard debris whose pathogen loads will most likely be mobilised into overland flow during intense storms;
- experience of the 1998 Sydney *Cryptosporidium* incident indicated that high volume or flood inputs may destabilise normal reservoir hydraulics leading simultaneously to

short circuiting and increased concentrations of pathogens, and hence a highly enhanced risk of pathogen contamination at water supply off-takes (McLellan, 1998).

Preliminary analysis of the selected catchments' hydrological behaviour indicated that collecting representative samples would not be a simple matter of utilising small commercially available automated samplers alone. Several sampling sites were remote, resulting in collection being expensive, unsafe and difficult or impossible during events. Furthermore, for the collection of protozoan pathogens and faecal sterols (Leeming *et al*., 1998), it was seen as essential to collect multiple 10 L samples rather than the normal size of <1 L. Due to the labile nature of the faecal indicator analytes reliable sample refrigeration was also needed (Ferguson, 1994; Standard Methods for the Examination of Water and Wastewater, 1995). With the markedly variable event and site hydrographs, triggering of sample collection schedules needed to be more sophisticated than those available in "off the shelf" autosamplers and the capacity to select specific samples for analysis from a larger set on the basis of on-line hydrograph data was also needed. Finally, various logistical problems had to be overcome, such as aseptic collection under all weather conditions likely to make sample handling difficult if not dangerous.

Given the variety of perceived technical and logistical issues, one of the CRC project partners, Ecowise Environmental Ltd., was approached to construct the necessary hardware, and work with resource managers to develop a generally applicable Event Sampling System (ESS) which was reliable and could be used in the selected catchments. Ecowise had previously demonstrated their technical proficiency by constructing mobile event sampling laboratories for nutrient monitoring that seemed to have some of the necessary attributes, e.g. independence of mains power, and easy installation at remote locations (Figure 1). Development work on the ESSs revealed a second complementary need – detailed, validated and standardised equipment management and use protocols.

This paper reports on the key aspects of the resulting ESSs, and the process of designing, constructing, installing, commissioning, and managing them. It is presented to stimulate discussion and work on improving and standardising event sampling, and as an example of how microbiological sampling programs might be implemented.

Methods

Monitoring sites

Six monitoring sites were selected in the water supply catchments of Canberra (Burra Creek), Adelaide (Sixth Creek, Myponga Creek and Aldgate Creek) and Melbourne (O'Shannassy River, McMahon's Creek) for event monitoring (Figure 2).

Figure 1 Ecowise mobile laboratory

Figure 2 Study site locations

ESS development

Ecowise were requested to build ESSs with the following essential features. Systems were to collect replicate and multiply 10 L samples and refrigerated 1 L samples and record in-stream data from the six sites in catchments of 10–70 km^2 in area. Systems were to be capable of operating away from mains power but have the option to connect to on-site power if available. Systems were to be readily transportable but not necessarily be trailer mounted. Control programmes were to be flexible and allow collection of samples from events of different size and duration at variable time intervals. Other needs identified included ensuring security, warning of events and protection for personnel.

In response, Ecowise provided a basic design based on their mobile systems and proposed various enhancements. Site visits were then undertaken and long-term hyetograph/hydrograph data analysed to ensure that installation of the proposed design was feasible and program parameters appropriate to each site were identified. Once the basic units were developed, consultation with end-users was undertaken followed by installation, training and commissioning mainly undertaken in Adelaide by the South Australia EPA.

ESS design

Sample collection components. Each sampling system consisted of a standardised internal set of components for sample collection, enclosed in a housing whose specifications suited local site and client needs (Figures 3–5). The sample collection components were as follows. ESSs operation was controlled using a DataTaker 50 (DataTaker Pty. Ltd., www.datataker.com) with a 1 Mb data storage card. This unit recorded hydrograph and other data from in line meters (turbidity, temperature etc.), monitored autosampler status and water depth changes and initiated refrigeration and sample collection.

The sample collectors consisted of two modified ISCO 3700 control units and peristaltic pumps (ISCO, Lincoln, NE, USA). The ISCO units extracted samples from the stream and control sample volume. Large volume sample collection was achieved by suspending an ISCO unit 1 m off the ground and replacing the normal sample bottle section with a manifold that distributed samples to a set of 24 × 15-L PVC containers. The maximum sampling volume possible was 10 L per sample. The second sampler comprised an ISCO pumping and control unit attached to the top of a 12 V refrigerator supplying up to 24 × 1 L sample bottles. Five deep cycle lead acid batteries provided power. Ancillary equipment included Druk pressure transducers (Keller, Winterthur, Switzerland), turbidity tranducers (Mindata Australia Pty. Ltd., Seaford, Victoria), supplied by Baron (Bundaberg, Queensland). The

12 V batteries were capable of supplying sufficient power to collect a full set of samples and provide refrigeration for 24 hours. Refrigeration was turned on simultaneously with the time zero sample collection.

Housing and ancillary equipment. Site specific components included protective housing (ecological hut, steel container or trailer with alarm), secondary power sources (replacement batteries, solar panels, mains connection), and event sample collection team alert system (mobile or land line telephone, secondary monitoring site telemetry). Housings were all large enough to protect equipment from inclement weather and provide sampling teams with space for manipulating samples.

Results and discussion

Catchment characterisation

Hydrograph behaviour. From daily rainfall data, the largest ten events in each year over a period of 3–20 years were identified in each catchment. High-resolution hydrograph and hyetograph data were then plotted for 10–20 randomly selected events. Run-off events corresponding to rainfall events of a few hours were found to last from 4 to 24 hours with peak flows occurring between 30 minutes and 12 hours after rainfall commencement. In some catchments a minimum of 20 mm of rainfall was required to increase stream-flow. In others <5 mm triggered run-off. Hydrographs could rise and fall gradually or rapidly and show single or multiple peaks (Figures 6–8). Where rainfall duration and intensity were similar so were hydrograph patterns for any given catchment.

Catchment and event features. The first step in developing a sampling strategy was to identify key features of "run-off" events of interest in the systems under study. In practice this

Figure 3 "Enviroshed" housing

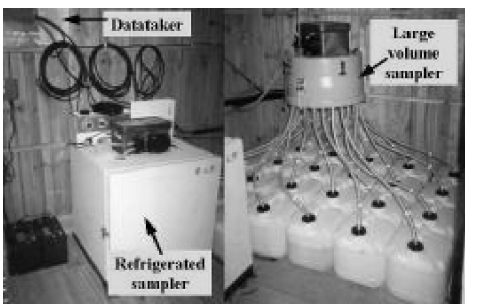

Figure 4 Sample collection systems, control unit (on wall) and spare bottles

Figure 5 Sample extraction and in-line monitoring point

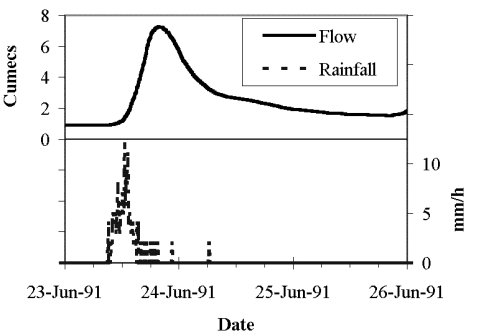

Figure 6 O'Shannassy River Hydrograph/ Hyetograph – short intense event leading to gradual rise in hydrograph

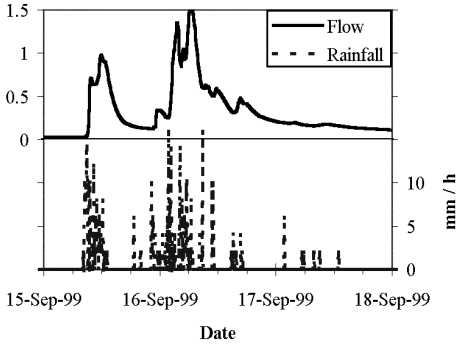

Figure 7 Aldgate Creek. Hydrograph/Hyetograph – complex prolonged event leading to multiple rapidly rise hydrograph peaks

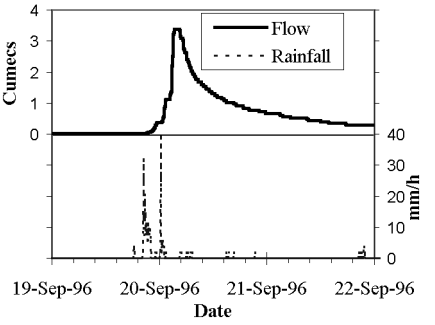

Figure 8 Burra Creek. Hydrograph/Hyetograph – short intense event leading to rapid response

meant using hydrographs and hydrographs of rainfall events occurring at a frequency of between one in a month and one in a year as models for those events which would be sampled in practice. Concurrent measurements of rainfall intensity and run-off showed the six model catchments could be divided between three types where rainfall occurred over a few hours. At one extreme were slowly responding catchments where flow mainly increased in response to high rainfall (>20 mm) and peaked in 6–12 hours and tailed off over 24 hours. Unless rainfall persisted over more than a day, typically only a single peak was observed. Dry weather baseflow, especially in winter, was >10% of peak event flow (O'Shannassy River, McMahon's Creek and Myponga Creek). At the other extreme was a rapidly responding catchment (Aldgate Creek) that flowed even after very low rainfall (<2 mm), peaked rapidly (<1 hour), and tailed off over a period of 3–6 hours. Hydrographs recorded over a prolonged rainy period showed multiple saw-tooth peaks reflecting variations in rainfall intensity. Preceding dry weather baseflow was <10% of the peak event. Between these extremes were intermediately responsive catchments which rose rapidly but had an elongated tail (Burra Creek and Sixth Creek).

Most event data generally showed an initial distinct peak, where it was postulated that microbiological contamination is mobilised, typical rates of stream depth changed, the delay in runoff reaching the monitoring station following rain, and the delay before peak flows occurred. Using this information the following principles were developed for programming the computer control system for sample collection:

- initial triggering of ESS sampling to be based on simultaneous rapid increases in water depth and a larger long duration change compared to dry weather flow;
- sampling is then triggered on a time-wise basis targeted at the duration of the initial stages of an event (typically 6–18 hours) rather than variations in flow volume;
- height change trigger and sampling interval values set on a site by site basis;
- time intervals between the collection of samples to be individually programmed for flexibility in when samples are collected, e.g. after short intervals during the rapid initial rise of the hydrograph and longer intervals during the hydrograph tail;
- paired sampling intervals to allow for collection of duplicates;
- a capacity to download, and examine on-site or upon collection, a site's hydrograph with a view to selecting a subset of samples for analysis.

Sampling logistics

At least several hours was required for warning and planning in order to activate sampling systems, organise collectors and alert laboratories to the likely arrival of a large number of water samples. In South Australia this warning was provided by a combination of 4–5 day

weather forecasting, flood alert services, regional rainfall radar monitoring (see for example http://www.bom.gov.au/weather/), and local real-time rainfall and stream gauge telemetry and will be the subject of a separate paper (Billington *et al*. preparation).

Many laboratories do not operate on a 24 hour, 7 day week schedule or impose a cost surcharge. Consequently in developing analytical arrangements it is essential to recognise resource and laboratory constraints. The general issue of coordination was raised with the South Australian analytical services provider (Australian Water Quality Centre) during construction of the ESSs. They noted that they were busiest during wet weather when pollution control systems fail more frequently, when regulation compliance directed samples would take priority over research ones, staff might not be available out of hours and the receipt of large sample numbers at once could tax their ability to process microbiological samples rapidly enough.

Preliminary results

ESS commissioning is still in progress so experimental results to date are limited. Two initial findings are noted here.

Managing cross contamination. Traditionally, good microbiological practice involves collection of water samples into clean sterile containers. This cannot yet be done with automatic sampling devices and some carryover via sampling lines between samples can be expected. Normal autosampler functions include a flushing cycle for the sample line between the sample source and the peristaltic pump, however, it was not clear how much this would prevent adherence of analytes to collection tubing. The extent of potential carryover was measured by simulating an overnight water collection run from a highly polluted stream using the refrigerated sampler. Eight samples of a 20% v/v primary settled sewage suspension were collected at 2 hour intervals and a 6 m head and suffuse the pumping system with pollutants. The extraction hose was transferred to 10 L of filtered sterilised water and two further sample collections then undertaken.

The initial trial showed that counts of bacterial indicators (*E. coli*, total coliforms, *Clostridium perfringens*) carried over into the first clean water sample averaged 7% of the contaminated water numbers and that a similar degree of dilution took place in the next sampling (e.g. for *C. perfringens* 230 cfu/100 mL v. 2800 cfu/100 mL initially). Examination of sampling lines indicated that a low point in the sampling line had probably increased carryover. When this dead zone was removed by shortening the sample dispensing tube, the carryover was reduced to 2% per sampling. Similar levels of carryover were measured with total *P* and turbidity. Hence it appears that while carryover is measurable, it is of minor significance and can be allowed for and minimised if equipment is clean.

The first flush. Various reports in the literature discuss a "first flush" phenomenon occurring in urban stormwater streams and drains whereby pollutants are disproportionately mobilised in the initial phases of an event (Faulkner *et al*., 2000; Shinya *et al*., 2000). A key question for our study was whether it occurred in protected water supply catchments. Data recently obtained from our completely forested catchments repeatedly demonstrated a first flush in respect to turbidity. Figures 9 and 10 illustrate how turbidity (which is proposed to be associated with pathogens) is enhanced during a storm event. Residual analysis of the data appears to offer a means for identifying where abnormal particle loads are located along a hydrograph and where pathogen concentrations should be analysed.

ESS management issues and sampling protocols

In the process of developing the ESSs it became clear that both a descriptive overview and

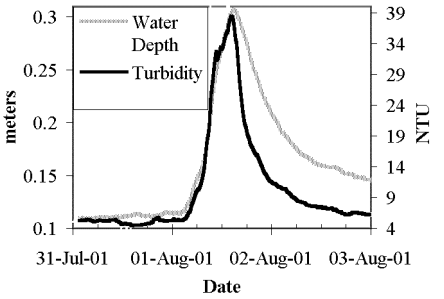

Figure 9 First flush of turbidity in O'Shannassy River – hydrograph and turbidity changes with time

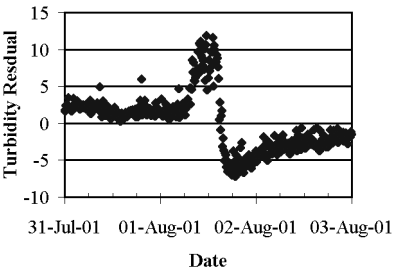

Figure 10 First flush of turbidity in O'Shannassy River – regression residuals of turbidity v. water depth

detailed procedures for ESS operations along with several days training were required to capture and transmit the experience obtained in ESS development. Installation and management were also found to be complex tasks in need of complementary documentation and training protocols. The need for such an overview and usage protocol is illustrated by some of the unexpected hindrances encountered.

Sample extraction. The auto-sampler peristaltic pumps had a nominal water lifting capacity of 8 m but in practice were best installed closer to the stream. The effect of pump height on the time to pump each sample from a stream was especially critical with the large volume samples. With an extraction height of 4 m *ca* 10 minutes was required to collect 10 L of water. In practice a minimum time between sampling of 15 minutes was necessary. In slowly responding catchments this was not a problem but in responsive catchments, whose flow peaked in <1 hour, pumping rate markedly restricted the number of samples which could be collected on the rising hydrograph and the comparability of "replicate" samples. A related problem was submergence of ESSs during floods. In many localities (e.g. floodplains) a rise of more than 4 m during storm events would not be unusual. One way to manage this was to relocate the pump near the water body as the 8 m limitation is governed by air pressure rather than by peristaltic pump power.

Siting and security. Vandalism was seen as a major problem, and was actually experienced in the most protected Victoria catchment during ESS commissioning. The most reliable option for ensuring security appears to be to site ESSs on private property. Unfortunately this can mean excessive disturbance to landholder privacy to collect samples.

Sample preservation. In the case of microbiological samples significant changes in analyte concentration during storage is a concern. *Standard Methods for the Examination of Water and Wastewater* (1995) recommend that analysis of microbial indicator samples should commence within 6 hours of collection. This was impractical for some of our sites and a more achievable sampling delay of 24 hours was settled on. Work by Ferguson (1994) indicated that the impacts of this delay on bacterial indicator counts are likely to be small.

Preservation of 10 L samples is still a problem as cooling was not practical in a battery powered site, but it was not considered to be a serious problem for the analytes of concern here. Two types of sample were scheduled for collection, protozoan cysts and oocysts and faecal sterols. (Oo)cysts are noted for their persistence and do not multiply outside their host so that marked changes in their concentration are unlikely provided samples are collected within a day and the weather is not hot. Faecal sterol losses at 12°C and 17°C (Leeming *et al*., 1998) are significant, however, the estimated decline after 1 day was

expected to be <15% which is relatively small compared with the range of concentrations detected in the environment.

Conclusions

Event sampling is not a novel activity, nor is the sampling of microbial analytes in events (e.g. Jagals *et al*., 1995; Ferguson *et al*., 1996; Faulkner *et al*., 2000; Shinya *et al*., 2000). Automated monitoring of is also well developed (AWQMFM, 1999). Our efforts to develop event sampling for quantifying storm loads of pathogens, however, indicated that further standardisation, validation and refinement of event sampling techniques is still needed and that good application of event sampling technology requires the following at a minimum.

- Before committing resources to event sampling identify and collect catchment data to demonstrate that a proposed study is practical and affordable. In our study high resolution hydrography and rainfall and basic catchment data were essential. This may not always be available.
- Sampling equipment must be suited to the task at hand. A basic sampler was not enough to sample the hydrograph and component options for Event Sampling Systems, should be further explored.
- Detailed and documented sampling protocols and training must complement hardware. We are currently developing such a document using the AWQMFM (1999) document as a model. Discussions with other groups who have undertaken stormwater sampling indicates that much experience has been gained in the past but not well documented.
- Such protocols must equally document ancillary issues such as laboratory coordination, sampler cleanliness, and event warning.

Our ESSs still have limitations. Asepsis and sample preservation are not fully resolved. Anyone embarking on such a sampling programme will also need to be constantly aware of simple management pitfalls such as power failure, vandalism, dead zones in sampling lines. Nevertheless automated event sampling technologies show sufficient promise to warrant fuller development and calibration with potential pathogen related surrogates for catchment management.

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