Residual indicator bacteria in autosampler tubing: a field and laboratory assessment

J. M. Hathaway, W. F. Hunt, R. M. Guest and D. T. McCarthy

ABSTRACT

Microbial contamination in surface waters has become a worldwide cause for concern. As efforts are made to reduce this contamination, monitoring is integral to documenting and evaluating water quality improvements. Autosamplers are beneficial in such monitoring efforts, as large data sets can be generated with minimized effort. The extent to which autosamplers can be utilized for microbial monitoring is largely unknown due to concerns over contamination. Strict sterilization regimes for components contacting the water being sampled are difficult, and sometimes logistically implausible, when utilizing autosamplers. Field experimentation showed contamination of fecal coliform in autosamplers to be more of a concern than that of *Escherichia coli*. Further study in a controlled laboratory environment suggested that tubing configuration has a significant effect on residual *E. coli* concentrations in sampler tubing. The amount of time that passed since the last sample was collected from a given sampler (antecedent dry weather period – DWP) tubing was also a significant factor. At a DWP of 7 days, little to no contamination was found. Thus, simple protocols such as providing positive drainage of tubing between sample events and programming samplers to include rinses will reduce concerns of contamination in autosamplers.

Key words | autosamplers, contamination, *E. coli*, fecal coliform, field monitoring, indicator bacteria

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INTRODUCTION

Water quality sampling for pathogens and indicator bacteria is a tedious process dominated by the need for sterility of sampling equipment, proper storage of samples, and prompt transport to a laboratory for analysis. As pathogens are biological pollutants prone to growth and decay, sample concentrations may vary should the proper conditions not be provided during this process. The standard and most recognized monitoring techniques for pathogens rely on grab sampling of the water being analyzed followed by immediate analysis (Burton & Pitt 2002). Grab samples are generally considered to be a discrete 'snapshot' of water quality conditions, and may not accurately depict the variability present in a given water source (McCarthy *et al.* 2012). However, contamination is most easily avoided when using this methodology.

Under a grab sample methodology, bottles can remain under sterile, sealed conditions until just prior to a sample being collected, minimizing the potential for contamination. Pre-sterilized bottles can be purchased, or reusable bottles can be autoclaved at 121 °C for 15 min and capped (USEPA 2012). Care must be taken not to touch the inside of the bottle or bottle cap to avoid contamination.

Automatic samplers can provide more robust data sets than grab samples, and minimize the labor associated with water quality sampling (relative to grab samples) (Ma *et al.* 2009; Harmel *et al.* 2010). The obvious challenge when utilizing automatic samples is the need to maintain sterility in sampling equipment. Studies such as Hathaway *et al.* (2010), have attempted to minimize the risk of contamination when employing automatic samples by utilizing autoclaved, pie-shaped bottles in a refrigerated sampler and removing, autoclaving, and replacing sampler, pump, and distribution tubing in between each captured storm events. This can obviously be a time consuming, expensive, and risky exercise, especially when confined space entry is required to replace the tubing.

Other studies have attempted to analyze the influence of automatic samplers on pathogen and indicator bacteria concentrations. In an analysis by Line *et al.* (2008), traditional automatic samplers were utilized to collect samples from

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multiple watersheds. Line *et al*. (2008) explored potential contamination of fecal coliform at one of the sampling stations by running distilled (sterile) water through the sampler on two occasions. Samples of the water collected after passing through the sampler averaged 12 most probable number (MPN)/100 ml of fecal coliform. A study quantifying the quality of water from an agriculturally influenced groundwater source by Boyer & Kuczynska (2003) showed similar results. Distilled water run through the sampling equipment showed no *Cryptosporidium parvum* oocysts and average fecal coliform concentrations of 9.7 colony-forming units (CFU)/100 ml. The tubing in the Boyer & Kuczynska (2003) study was periodically replaced with new, sterile tubing. Boyer & Kuczynska (2003) also collected grab samples and automatically collected samples simultaneously during site visits. These simultaneously collected samples showed no significant difference. Despite these studies suggesting minimal bacterial contamination in automatic samplers, more robust studies may be required to determine to what degree contamination exists, and which sampling protocols and monitoring configurations minimize contamination.

Further analysis of indicator bacteria contamination in automatic sampler equipment will allow a greater understanding of the influence of residual microbes on sample concentrations and help determine if autosamplers can be effectively utilized for bacteria sample collection. Previous studies related to this issue have not involved systematic investigation, numerous monitoring locations, or multiple sampler tubing configurations. The purpose of this study was to: (1) perform a field study to characterize residual concentrations of indicator bacteria in active monitoring stations and (2) perform laboratory analyses to confirm the results of the field study and determine if various monitoring configurations influence residual indicator bacteria concentrations.

MATERIALS AND METHODS

Field study

Active research sites in Knightdale, NC and Wilson, NC, USA, were selected for the analysis of residual indicator bacteria in autosamplers. The Knightdale site was utilized by Luell *et al*. (2011) to evaluate the performance of two bioretention areas and a vegetated swale receiving highway runoff. The Wilson site was utilized by Knight *et al*. (2013) to evaluate the performance of four vegetated filter strips and a vegetated swale receiving parking lot runoff. Overall, residual indicator bacteria analyses were performed at 11 of the monitoring stations across the two research sites, with one sample being collected for each station. Details for each location are provided in Table 1. It should be noted that no looped or dipped tubing was seen at any of the field sites. However, it is not uncommon to have looped or dipped tubing at a given sampling station. Thus, these configurations were analyzed in the laboratory study described below.

For each analysis, the sampler tubing installed at the monitoring station was removed and the outside was rinsed with sterile water. The sampler tubing was adjusted as necessary to ensure gravity drainage throughout the analysis. The autosampler was directed to collect a

Table 1 | Summary observations for field sites

Location	Number	Description	Normally submerged?	Presence of straight section?	Sloped	Looped or dipped?
Knightdale	1	Bioretention inlet – pipe	Partially	No	Mostly	No
Knightdale	2	Bioretention outlet – concrete junction box	Yes	Yes – 2 m	No	No
Knightdale	3	Bioretention outlet – concrete junction box	Yes	Yes – 2 m	No	No
Knightdale	4	Swale outlet – weir box	Yes	No	Yes	No
Knightdale	5	Swale inlet – pipe	Partially	No	Mostly	No
Wilson	1	Level spreader inlet – forebay pool	Yes	No	Yes	No
Wilson	2	Swale outlet – grassed conveyance	No	No	Yes	No
Wilson	3	Level spreader outlet – weir box	Yes	Yes – 0.5 m	Mostly	No
Wilson	4	Level spreader outlet – weir box	Yes	Yes – 0.5 m	Mostly	No
Wilson	5	Level spreader outlet – weir box	Yes	Yes – 0.5 m	Mostly	No
Wilson	6	Level spreader outlet – weir box	Yes	Yes – 0.5 m	Mostly	No

sample, initiating a purge cycle that removed any residual stormwater from antecedent storms. After the purge, the tubing was placed into a bottle of sterile water as the autosampler began to take a sample. The sample was collected in a sterilized bottle, capped, and placed on ice for transport. Samples were analyzed for fecal coliform (thermotolerant coliform) and *Escherichia coli* using a modified Colilert® analysis (Yakub *et al.* 2002). The modified analysis involved incubation at a higher temperature (44.5 °C) to differentiate between total and fecal coliforms.

Laboratory study

Autosamplers were set up in the laboratory to replicate field conditions. This laboratory study was undertaken in Australia, while the field studies described above were undertaken in the USA. A reservoir of semi-synthetic stormwater was utilized for sampler uptake. The stormwater was created using a technique similar to that reported by others in the literature (e.g. Chandrasena *et al.* 2012; Guest *et al.* 2012; Li *et al.* 2012). In short, sediments from the inlet of a nearby stormwater wetland were taken and passed through a 1 mm sieve. Sediments were then added to de-chlorinated tap water, together with nitrogen, phosphorus and various heavy metals to reach target concentrations. This process resulted in fairly consistent stormwater quality, comparable to worldwide reviews of stormwater literature (i.e. Makepeace *et al.* 1995; Duncan 1999): suspended sediment (mean 70 mg/L, range 28–120 mg/L), total nitrogen (mean 2.24 mg/L, range 1.70–2.50 mg/L) and total phosphorus concentrations (mean 0.34 mg/L, range 0.29–0.39 mg/L). Pure cultures of *E. coli* (strain ATCC11775) were then spiked into the solution at different concentrations, also typical of stormwater (McCarthy *et al.* 2012; geometric mean 5,600 MPN/100 mL, range 400–20,000 MPN/100 mL). It is acknowledged that there is a limitation in this method, since the artificially inoculated *E. coli* strain may not be fully representative of the wide range of *E. coli* strains present in stormwater. However, the effective use of this particular strain has been demonstrated in a wide range of stormwater experiments (Bratieres *et al.* 2012; Li *et al.* 2012; Chandrasena *et al.* 2013). Furthermore, the die-off of this particular *E. coli* strain shows similar kinetics in water treatment systems to an environmental *E. coli* strain isolated from a stormwater system in Melbourne (Chandrasena *et al.* in press).

Four tubing configurations were chosen for the laboratory study to reflect common autosampler installations. The configurations from the sampler to the semi-natural stormwater reservoir were: (1) straight tubing (Straight), (2) straight tubing with one loop (Loop), (3) tubing sloped at 5% toward source (Sloped), and (4) straight tubing with one dip (Dip) where water is allowed to pool between sampling events (Figure 1).

On the morning of each sampling event, the outside ends of the four tubes that were inundated in the stormwater reservoir were first washed with sterile water. To simulate various rinsing/purging setups, samples were either taken

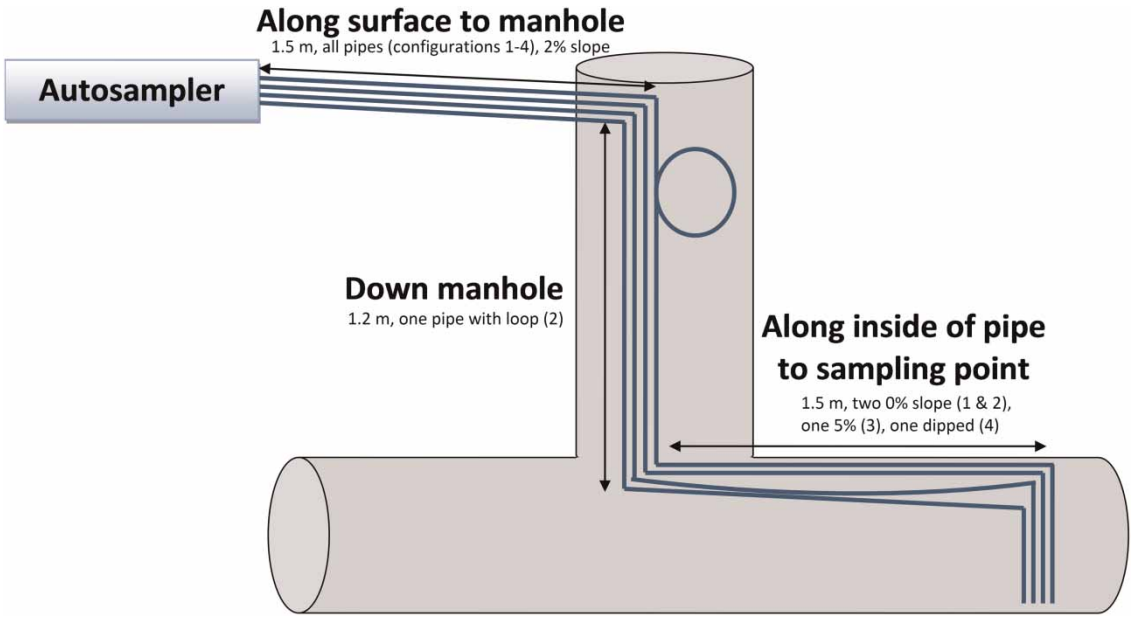

Figure 1 | Laboratory setup of sampling tubes experiment.

with zero rinses, one or two rinse cycles. Rinse cycles were always conducted using sterile deionized (DI) water, whereby one rinse cycle involved pumping DI water through each tube, followed by purging.

The first sample taken on the day of each event was done so using sterile DI water (i.e. a 1 L sterile DI water sample was taken through each tube after either zero, one or two rinse cycles; purging always followed). This process allowed the residual water remaining in the sampling tubes from the previous event to be mobilized and collected; these samples are henceforth known as 'between-event residual' samples. Directly after this, 1 L of semi-natural stormwater (prepared fresh on each sampling day) was pumped through each tube, and again purged. This mimicked the typical sampling that occurs during actual stormwater events. Again following either zero, one or two rinsing cycles, 1 L of sterile DI water was pumped through the four tubes and collected. These samples are henceforth known as 'within-event residual' samples and represent the carry-over contamination that may occur between samples within a single event. Semi-natural stormwater was then passed through the tubes (5 L) to provide a source of potential pollution to the tubing for the next sampling event. After purging, the tubes were typically left for 7 days, but up to 23 days, to simulate the inter-event timespan of field monitoring stations.

On each day, the eight tube samples (four between-event residual and four within-event residual), a semi-synthetic stormwater sample and a sterile DI control were transported on ice and analyzed for *E. coli* using the Colilert© technique at ALS Environmental, Scoresby, VIC, Australia. All samples were analyzed within 8 hours of collection, and usually within 2–3 hours of collection.

RESULTS AND DISCUSSION

Field study

For the 11 sites evaluated, fecal coliform concentrations varied from 15 to >2,400 MPN/100 ml. *E. coli* concentrations ranged from <1 to 62.7 MPN/100 ml, with nearly all samples having concentrations lower than 23 MPN/100 ml (Table 2). When using the maximum and minimum reporting limit values without adjustment, the geometric mean concentrations of fecal coliform and *E. coli* were 403 and 4 MPN/100 ml respectively. The fecal coliform concentrations noted in this study are of much greater concern, representing relatively substantial contamination when these values are compared to typical recreational water quality guidelines (USEPA 1986). Fecal coliforms are generally regarded as less fecal specific than *E. coli*, are found in higher concentrations, and have some natural, non-fecal sources in the environment.

Table 2 | Results of field study

Location	Sample #	Approximate days since sampling	Fecal coliform MPN/100 ml	*E. coli* MPN/100 ml
Raleigh	1	6–7	15	< 1
Raleigh	2	6–7	44	< 1
Raleigh	3	6–7	52	< 1
Raleigh	4	6–7	83	< 1
Raleigh	5	6–7	252	< 1
Wilson	1	7	> 2,400	10
Wilson	2	20	> 2,400	62.7
Wilson	3	7	1,337	11
Wilson	4	7	2,083	23
Wilson	5	7	> 2,400	4
Wilson	6	7	1,710	9

Contamination of sampler tubing by fecal coliform appears high enough that sample concentrations could be influenced. In particular, the Wilson sites showed high fecal coliform contamination. It should be noted that the sampler tubing at the Raleigh site was periodically flushed with DI water as part of a metals monitoring protocol. This may have resulted in relatively cleaner sampler tubes.

Laboratory study

The results of the field assessment suggest sampler tubing can remain contaminated between storm events; however, the influence of tubing configuration and antecedent dry weather period (DWP) could not be elucidated from these data. Laboratory analysis of various sample collection scenarios was performed to support and expand upon the findings of the field study (Table 3).

Tubing setup

Geometric mean concentrations of *E. coli* for tubing configurations 1, 2, 3, and 4 were 33.0, 27.1, 15.2, and 18.8 MPN/100 ml respectively. A Wilcoxon signed rank analysis was performed on the tubing configurations paired by the date of collection. Significant differences were observed between tubing configurations (Table 4). Per this analysis, the statistical differences are as follows: configuration 3 < configuration 4 < configuration 2 = configuration 1 (Table 4). These results

Table 3 | Laboratory study results

			E. coli concentration by configuration (based on one sample per date)				
Date	**Antecedent DWP**	**Number of DI rinses before sample taken**	**Inflow stormwater**	**Straight tubing (1)**	**Tubing with loop (2)**	**Sloped tubing (3)**	**Tubing with dip (4)**
22 June 2010	7 days	0	–	< 1	< 1	< 1	< 1
23 June 2010	30 min	0	20,830	1,600	830	220	550
1 July 2010	7 days	0	12,000	< 10	< 10	< 10	< 10
	30 min			320	380	98	280
13 July 2010	12 days	1	14,000	< 10	< 10	< 10	< 10
	30 min			310	260	130	160
5 August 2010	23 days	1	16,000	63	< 10	< 10	10
	30 min			240	260	52	130
12 August 2010	7 days	1	18,000	11	20	20	5
	30 min			350	270	170	260
19 August 2010	7 days	1	4,100	10	12	3	3
	30 min			280	190	140	120
26 August 2010	7 days	1	6,100	5	2	2	2
	30 min			250	330	77	140
16 September 2010	21 days	2	5,700	< 10	< 10	< 10	< 10
	30 min			230	85	31	52
23 September 2010	7 days	2	8,200	< 1	< 1	< 1	< 1
	30 min			140	120	46	68
30 September 2010	7 days	2	310	< 1	< 1	< 1	< 1
	30 min			50	43	17	22
7 October 2010	7 days	2	410	< 1	< 1	< 1	< 1
	30 min			49	44	12	32

suggest that the most promising setup is to ensure that the tubing is always sloped at least at a 5% gradient towards the source water body.

Between-event and within-event carry-over/residual/contamination

The effect of antecedent DWP was evaluated by performing a Wilcoxon signed rank analysis. Data were paired based on collection date and tubing configuration. The results of this analysis showed that a DWP of 30 min resulted in significantly higher *E. coli* concentrations than a DWP of 7 or more days ($p = <0.0001$). This suggests that residual microbes in sampler tubing will die off and/or desiccate in between storm events. Indeed, even though the semi-synthetic stormwater had a geometric mean of 5,600 MPN/100 mL, almost no residual contamination remained in any of the tubes after 7 days. In fact, with two rinse cycles, *E. coli* was never detected in the residual water between events, while when using one rinse cycle, the geometric mean residual was just 6 MPN/100 mL. These results demonstrate that for most stormwater or river-water sampling campaigns where sampling occurs once per week, one or two rinse cycles are sufficient to ensure residual contamination between events is minimal. It is noted that a short duration DWP (i.e. <7days) could cause some residual contamination of subsequent samples.

Table 4 | Results of statistical analysis on tubing configuration (*p* values)

Configuration	**2**	**3**	**4**
1	0.147	0.0003	< 0.001
2	–	0.0005	0.0002
3	–	–	0.0054

1 = Straight, 2 = Loop, 3 = Sloped, and 4 = Dip.

While the between-event contamination was minimal, the within-event residual contamination from previously collected samples was more substantial, with an average within-event residual of 130 MPN/100 mL (geometric mean). Even under a sloped tubing configuration and two rinse cycles, samples contained residual, with *E. coli* concentrations of 12–31 MPN/100 ml (see sampling dates 16 September 2010, 23 September 2010, 30 September 2010, and 7 October 2010 in Table 3). In fact, there was a

significant (Spearman $R = 0.78$, $p < 0.005$) positive relationship between the within-event residual level and the concentration of the stormwater in the previous sample. While the relative percentage of residual contamination was usually rather low (i.e. mean 4%, range 1–11% of the previous sample's concentration), this still has implications when taking samples with a great difference of contamination between subsequent samples.

CONCLUSIONS

As fecal indicator bacteria have been identified as a leading cause of water body impairments in the United States, Australia, and elsewhere, microbial monitoring regimes to support watershed pollution control efforts are becoming widespread. To accomplish such time-consuming sampling and gather large amounts of data, automatic samplers are being utilized to collect water quality samples. In the existing scientific literature, monitoring installations have been shown to have limited contamination of indicator bacteria; however, the field study performed herein suggested that contamination is possible, especially if no precautions are taken, and in particular for fecal coliform.

Laboratory testing showed the influence of tubing configuration on residual microbe concentrations, with sloped tubing (which drains between samples) being the most effective installation for reducing residual microbe concentrations. The DWP was also identified as a significant factor, with samples showing little to no contamination of *E. coli* after 7 days without a sample being collected. Shorter duration DWPs (i.e. <7 days) could introduce some contamination, but this remains to be tested. Thus, contamination of a given sample by the preceding sample collected during a storm event appears to be the primary concern regarding residual microbes in sampler tubing. Programming samplers to perform one or two rinses prior to a sample being collected will likely alleviate nearly all residual microbe contamination.

Common quality assurance/quality controls should be considered when utilizing automatic samplers. These controls include utilizing autoclaved sample bottles, making sure all tubing can drain via gravity flow between sample collection, ensuring tubing never dips or is looped from the sampler to the source, and setting programming to include rinses prior to a sample being collected. As some contamination may occur in autosamplers, all pathogen/indicator bacteria samples that are collected to inform short-term or immediate regulatory decisions directly applicable to public health (beach closures for instance) should be made using grab samples.

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