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Effects of field storage method on *E. coli* concentrations measured in storm water runoff

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Abstract Storm water runoff is increasingly assessed for fecal indicator organisms (e.g., *Escherichia coli*, *E. coli*) and its impact on contact recreation. Concurrently, use of autosamplers along with logistic, economic, technical, and personnel barriers is challenging conventional protocols for sample holding times and storage conditions in the field. A common holding time limit for *E. coli* is 8 h with a 10 °C storage temperature, but several research studies support longer hold time thresholds. The use of autosamplers to collect *E. coli* water samples has received little field research attention; thus, this study was implemented to compare refrigerated and unrefrigerated autosamplers and evaluate potential *E. coli* concentration differences due to field storage temperature (storms with holding times ≤24 h) and due to field storage time and temperature (storms >24 h). Data from 85 runoff events on four diverse watersheds showed that field storage times and temperatures had minor effects on mean and median *E. coli* concentrations. Graphs and error values did, however, indicate a weak tendency for higher concentrations in the refrigerated samplers, but it is unknown to what extent differing die-off and/or regrowth rates, heterogeneity in concentrations within samples, and laboratory analysis uncertainty contributed to the results. The minimal differences in measured *E. coli* concentrations cast doubt on the need for utilizing the rigid conventional protocols for field holding time and storage temperature. This is not to say that proper quality assurance and quality control is not important but to emphasize the need to consider the balance between data quality and practical constraints related to logistics, funding, travel time, and autosampler use in storm water studies.

Keywords Fecal indicator bacteria · Water quality · Nonpoint source pollution

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Introduction

The presence of pathogens in surface waters is increasingly a concern in the USA and worldwide with fecal indicator bacteria typically being used to indicate the presence of pathogen contamination in surface waters. According to the Clean Water Act §305(b) and §303(d) lists (USEPA 2014), more stream and river miles remain

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impaired due to pathogens than any other pollutant; therefore, the issue is receiving increased attention. As such, the effects of field storage conditions on measured *E. coli* concentrations have been a topic of scientific debate in recent years. In published studies of *E. coli* flux in storm water, unrefrigerated autosamplers have been used to collect water quality samples to determine concentrations (e.g., Harmel et al. 2010, 2013; Wagner et al. 2012, 2013) under EPA approved quality assurance project plans and/or sampling protocols. In these studies, samples were retrieved from the field *within 24 h of the conclusion of storm events when possible*. Desai and Rifai (2013) also utilized unrefrigerated samples but placed ice in the samplers to maintain storage temperature ≤6 °C. However, re-examination of *E. coli* sampling protocols by EPA Region 6 in 2011 resulted in a determination that samples collected for regulatory purposes under the Clean Water Act must adhere to methods described in 40 CFR 136.3 (6 h, <10 °C), but those collected for non-regulatory purposes may be held up to 24 h at <10 °C (in the case of automated sampling this means *within 24 h of collection of the first subsample*). As a result, EPA made a significant investment in refrigerated samplers and related equipment in 2012 to support EPA-funded projects in Texas. More recently, EPA Region 6 refined its determination by granting an extension of the 6 h holding time for samples collected for water quality standards and assessment purposes under CWA §305(b), allowing up to a 30-h holding time in cases "when transport conditions necessitate delays in delivery longer than 6 h" (TCEQ 2014).

For non-potable water compliance analyses of *Escherichia coli* (*E. coli*), standard storage time between collection and processing is ≤8 h with the sample held below 10 °C during this period (APHA, AWWA, and WEF 1998). For non-compliance sampling, the standard hold time given by APHA, AWWA, and WEF (1998) is 24 h with the sample held below 10 °C during storage. However, as logistical, economic, technical, and personnel barriers frequently prevent short hold times, studies have been performed to determine *E. coli* concentration changes associated with various hold time durations and storage conditions, but these studies have yielded variable results. Utilizing hold times longer than 8 h for fecal indicator bacteria is supported by several studies (e.g., Pope et al. 2003; Selvakumar and Borst 2004; 2006). For example, in an EPA-funded study, Pope et al. (2003) reported that a majority of sites showed no significant differences in *E. coli* densities between the 0 and 48 h holding times and that a majority of *E. coli* samples held at 20 and 35 °C showed no significant difference between the 0 and 8 h holding times. The authors concluded that *E. coli* samples analyzed beyond 8 h after sample collection still generate comparable concentration data, provided that samples are held below 10 °C and not allowed to freeze. A Texas Commission on Environmental Quality (TCEQ) study (2008) showed that samples held at <4 °C had small decreases in *E. coli* concentrations after 24 and 48 h relative to those after 8 h, but concentrations increased slightly after 30 h. In a comparative study of *E. coli* concentrations determined after hold times of 6 and 24 h, changes after 6 h ranged from −21 to +30 % (avg. = 1 %); however, decreases occurred in most of the samples by 24 h (avg. = −20 %, although one sample increased by 61 %) (USEPA 2006b). Thus, numerous research studies have utilized 24 h as a hold time threshold (e.g., Solo-Gabriele et al. 2000; Characklis et al. 2005; Hathaway et al. 2010).

McCarthy et al. (2008) concluded that holding time in the field is not a significant factor for the *E. coli* level of stored samples up to 24 h. The uncertainty due to storage varied between ±9 and ±44 % with an average of ±25 % for six experiments in Australia. For these samples stored in environmental conditions, *E. coli* concentrations increased initially (4 and 8 h) but decreased after 24 h. In a literature review, McCarthy et al. (2009) found that using refrigerated autosamplers (compared with unrefrigerated samplers) slightly reduced *E. coli* die-off in water samples stored for 24 h but that there was no difference if the samples were stored for less than 8 h. The bi-directional response noted by USEPA (2006b), TCEQ (2008), and McCarthy et al. (2008) indicates various degrees of die-off and/or regrowth, which are not well understood but that certainly affect conclusions related to field holding time and storage requirements.

Since the effects of holding time and storage temperature have not been adequately evaluated *with field research*, this study was implemented to evaluate potential differences in measured *E. coli* concentrations in storm water samples collected with refrigerated and unrefrigerated autosamplers. Specifically, *E. coli* concentrations were compared for events with (1) ≤24 h holding times in which only field storage temperature differed between samplers, and (2) holding times >24 h to evaluate the potential effects of field storage temperature and holding times. The practical question at hand

is whether the requirements for refrigerated autosamplers and 24 h holding time (from the time of the first subsample) are justified based on available data and knowledge.

Materials and methods

Site description

Four watersheds at the USDA-ARS Grassland, Soil and Water Research Laboratory Riesel Watersheds near Riesel, TX (Table 1) were selected for this study. The four watersheds were selected to evaluate the effect of environmental storage conditions across a range of land use and management conditions (Table 1). The watersheds vary in scale from edge-of-field to farm, in land use from cultivated to remnant native prairie, and fertilizer applied (poultry litter and/or inorganic fertilizer or none).

Runoff water quality samples have been collected at the Riesel Watersheds and analyzed for *E. coli* since 2008. The Riesel Watersheds are dominated by Houston Black clay soil (fine, smectitic, thermic, udic Haplustert), which is recognized throughout the world as the classic Vertisol. These highly expansive clays, which shrink and swell with changes in moisture content, have a typical particle size distribution of 17 % sand, 28 % silt, and 55 % clay. These soils are very slowly permeable when wet (saturated hydraulic conductivity ≈ 1.5 mm/h); however, preferential flow associated with soil cracks contributes to high infiltration rates when the soil is dry (Arnold et al. 2005; Allen et al. 2005; Harmel et al. 2006c).

Data collection

Water quality sample collection

Data collection began in August 2012 and encompassed three study years from August through July (2012–13, 2013–14, 2014–15). For each site, runoff data and water quality samples were collected from a flow control structure (v-notch weir or a flume) located at the watershed outlet. For each runoff event, water quality samples were collected with an ISCO 6700 autosampler and an ISCO Avalanche refrigerated autosampler (Teledyne-ISCO, Inc., Lincoln, NE); these samplers differ only in the refrigerated storage capacity of the Avalanches. Each sampler was programmed to collect frequent flow-interval (1.32 mm volumetric depth) samples and composite them into a single bottle as discussed in Harmel et al. (2006a, b, 2013). Prior to collection of each sample, each sampler executed a rinse of the sample tubing with ambient runoff water. Samples were collected on equal flow intervals and composited into a single bottle; thus, the resulting concentrations represent *E. coli* event mean concentrations (EMCs).

Because of the EPA waiver of the 6 h requirement, which allowed a 24-h holding time if samples were refrigerated, runoff events in this study were divided into two groups based on storm duration/field holding time (≤24 h, >24 h). For runoff events with holding times ≤24 h, the entire sample was retrieved at the

Table 1 Watersheds descriptions and previously measured *E. coli* concentrations (CFU/100 ml)

	Y2	Y6	Y10	SW12
Area (ha)	53	6.6	7.5	1.2
Cultivated (%)	56	100	100	0
Land use	Mixed	Crop	Crop	Native remnant prairie
E. coli source	Grazing cattle, poultry litter, background, wildlife	Background, wildlife	Poultry litter, background, wildlife	Background, wildlife
E. coli concentration (CFU/100 mL)[a]	25th% = 2200	25th% = 193	25th% = 140	25th% = 430
	Median = 5050	Median = 475	Median = 340	Median = 2000
	75th% = 8450	75th% = 1675	75th% = 1500	75th% = 6650
	IQR = 6250	IQR = 1482	IQR = 1360	IQR = 6220
	IQR/median = 1.2	IQR/median = 3.1	IQR/median = 4.0	IQR/median = 3.1

IQR interquartile range

[a] Measured from these sites from August 2008 through July 2011 (Harmel et al. 2013)

conclusion of the runoff event (when the flow rate dropped below the storm threshold) and replaced with a clean sample bottle for the Avalanche (refrigerated) and 6700 (unrefrigerated) samplers. For runoff events with holding times >24 h, each Avalanche refrigerated sample was retrieved within the first 24 h and during each subsequent 24 h period until the conclusion of the runoff event; a clean sample bottle was replaced at each change. For runoff events with holding times >24 h, the *E. coli* concentration at the end of the runoff event was reported for the unrefrigerated sampler.

This sample retrieval methodology was designed to evaluate the effects of temperature and field holding time. For each storm event, the runoff volume, the time from the first sample until retrieval, and the storage temperature were recorded. For runoff events with holding times ≤24 h, the effect of storage temperature in the field was evaluated by comparing *E. coli* concentrations collected with the refrigerated and unrefrigerated samplers. For storm events with holding times >24 h, the combined effects of holding time and storage temperature in the field were evaluated by comparing *E. coli* concentrations determined as the EMC from multiple bottles from the refrigerated sampler and the refrigerated sampler containing the entire event's sample.

Sample retrieval and transport

For each sample bottle, a thoroughly mixed subsample was poured into a 0.71 L (24 oz.) sterile, polyethylene Whirl-Pak (NASCO Inc., Fort Atkinson, WI) bag. Once the sample bag was approximately ¾ full, it was twirled, securely closed, and checked for leaks by gently squeezing. Samples were stored in a cooler on ice during transport to the laboratory.

E. coli enumeration and sediment concentrations

The *E. coli* concentration in each water sample was determined with EPA method 1603 (USEPA 2006a). Five to six dilutions (25, 10, 1, 0.1, 0.01, 0.001 mL) were filtered using 0.45 μm membrane filters. The filters were then placed in petri dishes containing modified mTEC agar and incubated at 35 ± 0.5 °C for 2 ± 0.5 h to resuscitate injured or stressed bacteria and then incubated at 44.5 ± 0.2 °C for 22 ± 2 h. Finally, the number of red or magenta colonies were counted and recorded. For a vast majority of samples, only one dilution produced between 20 and 80 colonies on the petri dishes. In this case, that dilution served as the count. In the few cases in which only the highest dilution produced countable colonies, that dilution served as the count. For quality control, 100 mL of phosphate buffered saline was processed as a blank with each batch of samples and a laboratory duplicate was evaluated with each batch. The sediment (total settleable solids) concentration was determined by mass after settling for 3–5 days, decanting off a majority of the solution, and drying at 116 °C for 18–24 h.

Data analysis

Data analysis focused on determining whether statistically significant and/or practical differences occurred in *E. coli* concentrations as determined from samples collected and stored in the field in refrigerated and unrefrigerated samplers. Since the majority of the data were not normally distributed based on Kolmogorov-Smirnov test results, the Mann-Whitney test was used to determine significant differences in median values. In addition, error values (R^2, % bias, relative error, and absolute error) and graphical analyses (box-and-whisker plots and log-log plots) were used to explore practical differences. Stepwise multiple linear regression analyses were also used to examine possible between *E. coli* concentrations (measured concentrations and concentration differences between the refrigerated and unrefrigerated sampler at each site) and independent variables (storage temperatures and temperature differences, sediment concentrations, runoff volume, and field storage time). In the multiple linear regression analyses, only *E. coli* concentrations were log transformed (not absolute and relative concentration differences). All statistical analyses were conducted with Minitab (Minitab 2015) and JMP 2012) according to procedures described by Helsel and Hirsch (1993) and Haan (2002).

Results and discussion

Runoff sampling events

Although this study began in August 2012, the first event with sufficient runoff to sample occurred in October 2013. At least 18 events were measured at each site and included small events (runoff <10 mm) and large events (runoff >50 mm). Similarly, a wide range of sediment concentrations and storage temperature and

times occurred in these events, producing a robust data set (Table 2). Events occurred throughout the year, but a majority occurred in the spring (61 %) and fall (18 %). As shown in Table 2, the maximum temperature of the samples during storage in the field was 25–27 °C even in the hot summer months, which is attributed to cooler, cloudy days associated with runoff events. Seventy-one runoff events had holding times ≤24 h, and 14 events exceeded 24 h.

Comparison of *E. coli* concentrations between refrigerated and unrefrigerated samplers

Summary statistics and error values for measured *E. coli* concentrations appear in Table 3.

E. coli concentrations from refrigerated samplers trended higher in some events, and in others unrefrigerated samples trended higher. The magnitude of differences also varied substantially. Whether the bi-directional responses, also noted by USEPA (2006b), TCEQ (2008), and McCarthy et al. (2008), indicate differing die-off and/or regrowth rates, heterogeneity in concentrations within the water sample, and/or laboratory analysis uncertainty is unknown.

When data for short events (≤24 h holding time) and long events (>24 h) were separated, Mann-Whitney tests revealed no significant difference in *E. coli* concentrations between the refrigerated and unrefrigerated samplers at any site or when data were grouped across sites. The lack of differences for long runoff events was unexpected because refrigerated subsamples were each

Table 2 Summary statistics for storage temperatures, runoff volumes, and sediment concentrations for *E. coli* sampling events

		Y2	Y2A[a,b]	Y6	Y6A	Y10	Y10A	SW12	SW12A
Storage temperature (°C)	25th%	16.5	0.6	21.7	1.1	21.3	1.0	14.1	0.6
	Median	21.7	1.1	23.3	1.1	23.1	1.1	21.7	1.1
	75th%	22.8	1.1	24.4	2.2	24.0	2.8	23.3	1.7
	IQR	6.3	0.5	2.7	1.1	3.7	1.8	9.2	1.1
	IQR/median	0.3	0.5	0.1	1.0	0.2	1.6	0.4	1.0
Storage temp. difference (°C)	25th%	13.2		17.8		17.8		13.3	
	Median	20.6		21.7		21.7		18.3	
	75th%	21.5		22.8		23.3		21.5	
	IQR	8.3		5.0		5.5		8.2	
	IQR/median	0.4		0.2		0.3		0.4	
Field storage time (h)	25th%	7.3		7.4		7.1		7.8	
	Median	11.0		10.9		10.8		12.4	
	75th%	21.7		20.4		23.3		21.7	
	IQR	14.4		13.0		16.2		13.9	
	IQR/median	1.3		1.2		1.5		1.1	
Runoff volume (mm)	25th%	5.3		5.2		12.7		3.1	
	Median	11.9		9.5		19.7		8.0	
	75th%	22.1		27.9		43.0		22.7	
	IQR	16.8		22.7		30.3		19.0	
	IQR/median	1.4		2.4		1.5		2.4	
Sediment concentration (mg/L)	25th%	40.9		190.0		85.4		31.0	
	Median	62.0		273.3		164.9		53.0	
	75th%	117.8		408.0		290.7		121.2	
	IQR	76.9		218		205.3		90.2	
	IQR/median	1.2		0.8		1.2		1.7	

[a] Refrigerated samplers are indicated with "A"

[b] For the refrigerated samplers, a few events were quite rapid and a higher proportion of samples were held at >4 °C before sampler could reduce temperatures to 4 °C

Table 3 Summary statistics and error values for event mean *E. coli* concentrations (CFU/100 mL)

	Y2	Y2A[a]	Y6	Y6A	Y10	Y10A	SW12	SW12A
CFU/100 mL								
25th%	1950	2575	3000	2900	1255	1525	388	473
Median	3600	4702	4400	5200	5650	5950	910	1161
75th%	12,250	10,635	16,000	16,000	6725	8425	6100	4650
IQR	10,300	8060	13,000	13,100	5470	6900	5712	5123
IQR/median	2.9	1.7	3.0	2.5	1.0	1.2	6.3	4.4
Number	24		19		18		24	
R^2	0.978		0.861		0.734		0.994	
Slope	1.476		1.578		1.339		0.880	
Bias (%)	−25		−30		−33		13	
Absolute error (CFU/100 mL)								
Mean	−4780		−3540		−1608		1363	
Minimum	−140,000		−74,000		−8000		−3000	
Maximum	14,000		16,000		2000		20,000	
Relative error (%)								
Mean	−2		1		−9		−9	
Minimum	−75		−59		−83		−98	
Maximum	77		70		77		100	

The random and systematic uncertainty in measured *E. coli* concentrations were estimated to be ±35 % and +6 %, respectively, based on Harmel et al. (2015)

[a] Refrigerated samplers are indicated with "A"

held at 4 °C and collected retrieved from the field in <24 h, whereas unrefrigerated samples were held at ambient temperatures for 24 to 40 h. When relative and absolute differences in *E. coli* concentrations were grouped for all sites, Mann-Whitney tests again revealed no significant differences between long and short runoff events. As a result, data from short and long runoff events were grouped for all subsequent analyses. With this grouping, Mann-Whitney tests again revealed no significant differences between refrigerated and unrefrigerated samplers (Table 3).

Thus, refrigerated storage had minor effects on the central tendencies of *E. coli* concentrations, although mean and median *E. coli* concentrations for the refrigerated samples tended to be larger at three of four sites (Table 3). The lack of significant differences may be partly attributed to less than expected temperature differences because of cloudy conditions and cooler temperatures on rainy days. Alternatively, the influence of holding time and temperature may be weaker than typically assumed.

The variability of concentrations was also greater for the refrigerated samplers at three of the sites in spite of much smaller ranges in storage temperatures, which was unexpected. The R^2 values indicate strong linear relationships between *E. coli* concentrations between the refrigerated and unrefrigerated samplers (Table 3), but the slopes (>1.34 at three sites, and 0.88 at one site) indicate differing concentrations. Similarly, a number of points were outside the 95 % confidence intervals when graphed on a log-log scale (Fig. 1).

Error values in Table 3 provide further insight into potential differences in *E. coli* concentrations collected and stored in refrigerated and unrefrigerated samplers. Percent bias, which is an indicator of the average tendency of over- or under-estimation according to Gupta et al. (1999) and Moriasi et al. (2007), indicated that concentrations tended to be higher for the refrigerated samplers at the three of the four sites. At the same three sites, average absolute errors also indicated higher concentrations for the refrigerated samplers. In addition, 53 % of the runoff events had higher concentrations for the refrigerated sampler. In contrast, average relative errors indicated the opposite effect for two sites (Y6 and SW12), but the average values were all quite low (< ±10 %). Together these quantitative measures indicate a

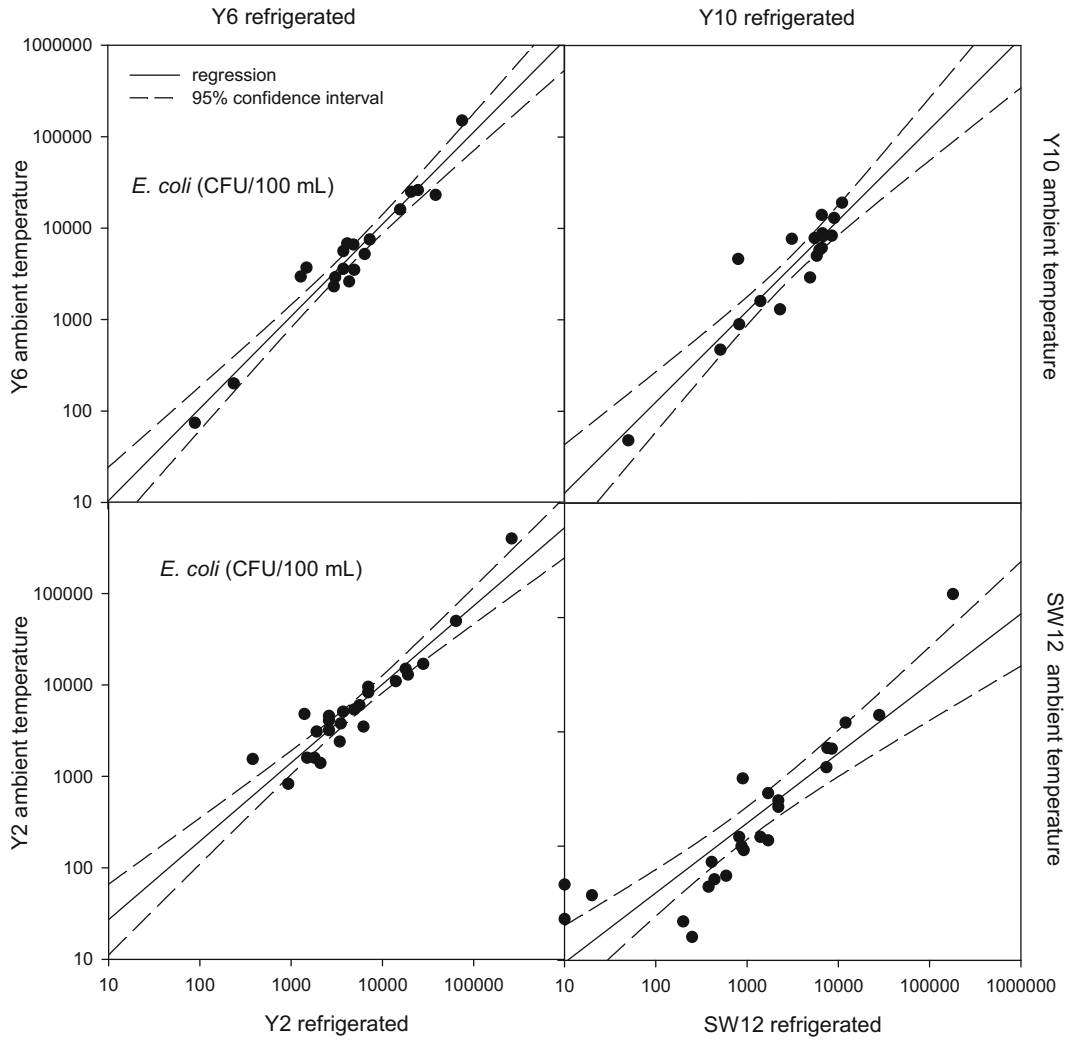

Fig. 1 Regression line and 95 % confidence intervals for *E. coli* concentrations collected and stored in unrefrigerated and refrigerated samplers

weak tendency for the refrigerated samplers to produce higher *E. coli* concentrations suggesting that refrigerated samplers (lower temperatures) suppress post-collection *E. coli* die-off as noted in previous research (e.g., Pope et al. 2003; Pachepsky et al. 2014). A similar conclusion can be drawn by examining *E. coli* concentrations in Figs. 1, 2, and 3; however, the tendency is weak and certainly not consistent for all sites or all storm events.

Factors affecting *E. coli* concentrations and differences between refrigerated and unrefrigerated samplers

Examination of storage temperature differences, sediment concentration, runoff volumes, and field storage time revealed that all of these factors were related to differences in *E. coli* concentrations between the refrigerated and unrefrigerated samplers in certain instances. The significant relationships determined with stepwise multiple linear regression all had low R^2 values (<0.36) indicating considerable variability in the relationships (Table 4). Similarly, no single factor was consistently related to *E. coli* concentration differences, which is likely due to interactions between the factors and natural variability. Time, temperature difference, and sediment concentration were shown to have significant influence in 3–4 instances, whereas runoff was significant in only one instance. Increasing temperature difference reduced relative differences at Y2 but increased absolute differences. Similarly,

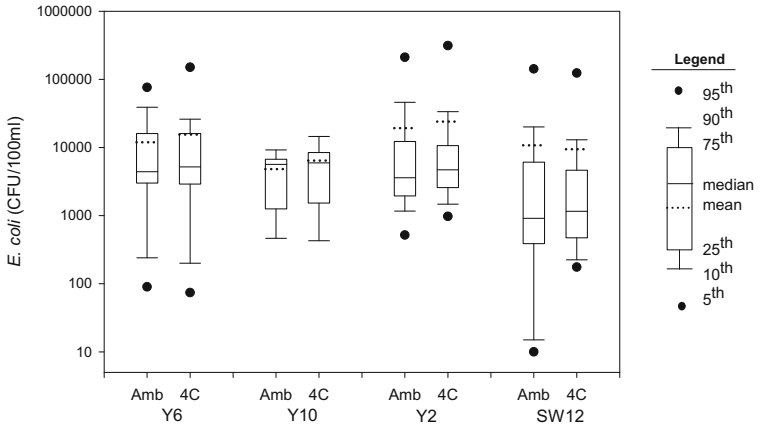

Fig. 2 Box and whisker plots of *E. coli* concentrations for samples collected with an unrefrigerated sampler (ambient temperature, *Amb*) and a refrigerated sampler (held at 4 °C, *4C*)

increased sediment concentrations increased absolute differences at SW12 but decreased absolute differences at Y10.

Stepwise multiple linear regression also revealed several significant relationships between measured *E. coli* concentrations and storage temperature, sediment concentration, runoff volume, and field storage time (Table 4). Again, these relationships contained substantial variability as indicated by low R^2 values (<0.26). In contrast to concentration differences, runoff volume and sediment concentration were much more frequently related to measured *E. coli* concentrations than storage temperature or field storage time. In every instance with a significant relationship, *E. coli* concentrations decreased as runoff decreased, and concentrations increased as sediment concentrations increased. The relationship between sediment and *E. coli* was not surprising as previous research (e.g., Jamieson et al. 2005; Characklis et al. 2005) noted the presence of substantial amounts of *E. coli* adhered to suspended sediment particles in water samples.

The IQR/median values shown in Tables 2 and 3 show that variability in storage temperature, storage temperature difference, field storage time, runoff volume, and sediment concentrations (range 0.2 to 2.4) were typically smaller than for *E. coli* concentrations. The IQR/median values for *E. coli* concentrations were 1.7 and 2.9 at Y2, 2.5 and 3.0 at Y6, and 4.4 and 6.3 at SW12. In contrast, *E. coli* concentration variability was lower at Y10 than the other sites (1.0 and 1.2). Harmel et al. (2010, 2013) also reported substantial variability in measured *E. coli* concentrations with Cv values ranging from 1.2 to 4.0. These examples highlight the potential for extreme variability in measured *E. coli* concentrations to obscure the effects of land management, storage conditions, and runoff characteristics.

Conclusion

Several noteworthy findings resulted from this study. These are relevant in addressing the practical issue at

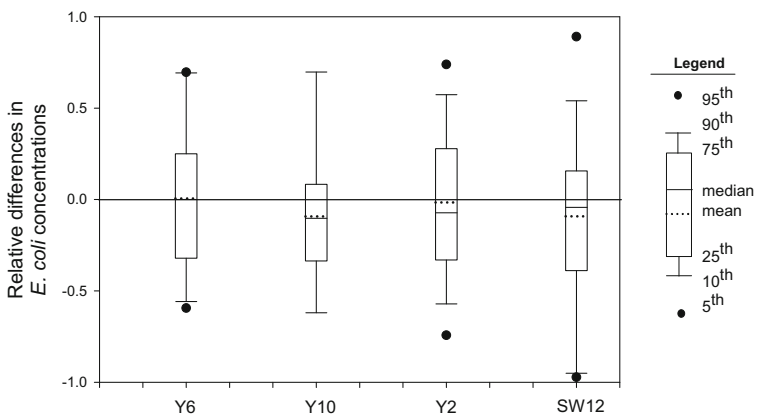

Fig. 3 Box and whisker plots of relative differences in *E. coli* concentrations between samples stored in an unrefrigerated samplers (ambient temperature) and a refrigerated sampler at 4 °C (*s* indicate higher concentrations for the refrigerated sampler)

Table 4 Significant multiple linear regression relationships between *E. coli* concentrations and selected factors (storage temperature, sediment concentrations, runoff volume, and field storage time)

Site(s)	Relative differences in *E. coli* concentrations (rel diff, %)	Absolute differences in *E. coli* concentrations (abs diff, CFU/100 mL)
All	Rel diff = 0.147 − 0.014 (time[c]) $p = 0.01$, $R^2 = 0.07$	–
Y2	Rel diff = 0.605 − 0.023 (time) − 0.017 (temp diff[d]) $p = 0.07$, $R^2 = 0.22$	Abs diff = −35,338 + 1737.9 (temp diff) $p = 0.08$, $R^2 = 0.14$
Y6	–	–
Y10	Rel diff = 0.247 − 0.022 (time) $p = 0.02$, $R^2 = 0.30$	Abs diff = 4710 − 110.9 (time) − 167.9 (temp diff) − 6.35 (sed[e]) $p = 0.09$, $R^2 = 0.36$
SW12	Rel diff = −0.431 + 0.009 (runoff[f]) + 0.002 (sed) $p = 0.02$, $R^2 = 0.33$	Abs diff = −1011 + 28.0 (sed) $p = 0.01$, $R^2 = 0.27$
Site(s)	*E. coli* concentrations (CFU/100 mL)	log *E. coli* concentrations (CFU/100 mL)
All	*E. coli* = 18,397 − 258.2 (runoff) $p = 0.09$, $R^2 = 0.02$	*log E. coli* = 3.45 − 0.005 (runoff) + 0.0008 (sed) $p = 0.01$, $R^2 = 0.06$
Unref.[a]	–	*log E. coli* = 2.89 − 0.007 (runoff) + 0.0007 (sed) + 0.028 (temp[g]) $p = 0.03$, $R^2 = 0.10$
Ref.[b]	–	*log E. coli* = 3.51 − 0.005 (runoff) + 0.0007 (sed) $p = 0.09$, $R^2 = 0.06$
Y2	–	–
Y6	–	*log E. coli* = 4.04 − 0.007 (runoff) − 0.017 (time) $p = 0.10$, $R^2 = 0.12$
Y10	*E. coli* = 2499 + 15.3 (sed) $p < 0.01$, $R^2 = 0.26$	*log E. coli* = 3.44 − 0.007 (runoff) + 0.001 (sed) $p = 0.02$, $R^2 = 0.21$
SW12	–	*log E. coli* = 2.67 + 0.005 (sed) $p < 0.01$, $R^2 = 0.24$

[a] Unrefrigerated sample (Ambient temperature)

[b] Refrigerated sampler (cools to 4 °C)

[c] Field storage time (*time*, h)

[d] Difference in storage temperatures between samplers (*temp diff*, 4 °C)

[e] Sediment concentration (*sed*, mg/L)

[f] Runoff volume (*runoff*, mm)

[g] Storage temperature (*temp*, 4 °C)

hand and answering the question: Are the requirements for refrigerated autosamplers and 24 h holding time justified for *E. coli* data collection in storm water runoff?

- The magnitude and direction (±) of differences in *E. coli* concentrations between refrigerated and unrefrigerated autosamplers varied substantially from storm to storm and across sites.
- *E. coli* concentrations from refrigerated samplers exceeded those from unrefrigerated samplers in 53 % of runoff events, but the opposite occurred in 42 % of events. Concentrations were equal in 5 % of samples.
- Unrefrigerated storage and longer field holding times had little statistically significant impact on central tendencies of *E. coli* concentrations; however, *E. coli* concentrations for the refrigerated samples trended higher at three of four sites.
- There were no statistically significant *E. coli* concentration differences between refrigerated and unrefrigerated samplers for long runoff events (>24 h) even though unrefrigerated samples were held at warmer temperatures for longer times.
- Graphs and error values also indicated the tendency for higher concentrations at the refrigerated samplers; however, the tendency was quite weak and not consistent for all sites or all storm events.
- Although results indicated a weak tendency for higher concentrations at the refrigerated samplers, it is unknown which better represents actual concentrations in the water sample at the time of collection.
- It is also unknown how much differences in die-off and/or regrowth rates, heterogeneity in concentrations within the water sample, and laboratory analysis uncertainty contributed to the results.
- Storage temperature differences between the refrigerated and unrefrigerated samplers were less than expected, which can be attributed to cooler, cloudy days associated with rainfall and runoff even during hot summer months.

- When examining *E. coli* data, it is important to consider that the extreme variability in measured concentrations reported by the present study and others can obscure the effects of land management, storage conditions, and runoff characteristics.

The minimal differences in measured *E. coli* concentrations in this study cast doubt on the necessity of conventional protocols with rigid requirements for field holding time and storage temperature. Proper quality control and quality assurance are certainly important, but balancing data quality while appreciating the practical constraints related to logistics, funding, travel time, and autosampler use is recommended for storm water studies.

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