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The concentration of *Cryptosporidium* and *Giardia* in water – The role and importance of recovery efficiency

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ABSTRACT

The concentration of *Cryptosporidium* and of *Giardia* in surface water is a subject of importance to public health and public water supply. The term concentration is a fundamental property of any water quality parameter having a classical definition as used in chemistry and biology. Analytical methods for measuring the occurrence of *Cryptosporidium* and *Giardia* in water find only a fraction of the organisms actually present. This paper collects recently available data to examine the role and importance of recovery efficiency measurement to description of the concentrations of these organisms. Data from Australian sources graphically illustrate the variability of recovery efficiency at individual sites over relatively short time scales. Additional data on replicated recovery measurements establish their reproducibility. The recently released USEPA LT2 data along with those from Australia illustrate the independent variation of *Cryptosporidium* and *Giardia* occurrence and recovery efficiency at individual sampling locations. Calculation of concentration from paired raw numbers and recovery efficiency measurements clearly shows the magnitude and importance of taking recovery into account in expressing the concentration of these organisms.

1. Introduction

Cryptosporidium and *Giardia* are protozoan pathogens having world-wide distribution. They both have numerous species and subtypes identifiable by molecular means, Xiao et al. (2001); Monis and Thompson (2003); Bakheit et al. (2008), many of which have been established as infectious to the human population, Chalmers (2011); Fayer (2011). They are excreted in large numbers as oocysts (*Cryptosporidium*) and cysts (*Giardia*) that are immediately infectious at minimal exposure doses, hardy in the environment, and resistant to oxidizing disinfection, Monis et al. (2003); Smith et al. (2006); Ramirez et al. (2004). These characteristics make them of concern to public health and public water supply and a recent review has summarized more than 500 instances of community scale waterborne transmission of protozoan illness predominantly cryptosporidiosis and giardiasis, Baldursson and Karanis (2011). Understanding and control of waterborne transmission of these organisms requires measuring their presence in representative samples of sources of public water supply.

Measuring the presence of *Cryptosporidium* oocysts and *Giardia* cysts quantitatively is important to any quantitative use of the information in relation to managing water quality for public water supply. Important applications include: evaluation of risk; comparison of water quality between sources, sampling sites, and sampling times; evaluation of watershed management effectiveness; and measurement of treatment process performance. All such applications can produce accurate estimates only if measurements of oocyst and cyst occurrence are made quantitatively by appropriate

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measurement of recovery efficiency and by taking recovery efficiency into account to calculate organism concentrations. Yet, the most common practice, largely driven by the most widely accepted procedure for detection of *Cryptosporidium* and *Giardia* in water, USEPA Method 1622/1623, USEPA, (2005), omits the routine measurement of recovery efficiency and does not apply recovery in expressing analytical results.

To date, with few exceptions noted below, existing references in the literature to *Cryptosporidium* and *Giardia* occurrence in water, often termed "density", are incomplete having ignored recovery efficiency and are thus misleading. This is a subject of technical controversy and has been influenced heavily by "practical" issues related to the effort and expense of adding recovery measurement to monitoring requirements. Although common practice has neglected the importance of recovery efficiency, prominent examples of its measurement and application have been reported Ongerth and Stibbs (1987), Ongerth (1989, 1990); Hansen and Ongerth (1991), Nieminski and Ongerth (1995), Nieminski et al. (1995), Ongerth and Pecoraro (1995). In this previous work key features of recovery efficiency that were apparent were that it varied roughly inversely related to water quality (turbidity), from site to site, and at individual sampling sites between sampling times. Work by others investigating recovery characteristics of the IMS process and of Method 1623 overall have found replicated recoveries in good agreement, Reynolds et al. (1999); McCuin et al. (2001), but wide variation has been reported between recoveries measured at different sampling sites and for differing water qualities, DiGiorgio et al. (2002); Francy et al. (2004).

Further description of the importance of measuring concentration requires careful definition and use of the terms concentration and closely related, limit of detection. Definitions are as follows. The term "limit of detection" is the minimum number of the target organism, i.e. one (1) oocyst or one (1) cyst, recoverable in a defined sample volume processed to completion, reduced by the recovery efficiency expressed as a decimal fraction (e.g. 'Matrix Spike' recovery), Eq. (1).

$$\text{Limit of Detection} = \frac{\text{One Organism}}{\text{Sample Volume} \times \text{Recovery Efficiency}} \quad (1)$$

The term concentration is used in the classical sense, the number of target organisms, oocysts or cysts, present per unit of volume of representative sample. Accordingly, the organism concentration is defined as the number of organisms (oocysts or cysts) found on completion of microscopy according to Method 1623 or 1623.1, divided by the sample volume processed to completion, adjusted by the recovery efficiency expressed as a decimal fraction, Eq. (2)

$$\text{Organism Concentration} = \frac{\text{Number of Organisms Found}}{\text{Sample Volume(L)} \times \text{Recovery Efficiency}} \quad (2)$$

Where ever the term concentration is used in the remainder of this paper it conforms to this definition. Where data on occurrence of *Cryptosporidium* or *Giardia* in terms of raw numbers not adjusted for recovery efficiency are used they will be referred to simply as occurrence data.

Analyzing water samples for *Cryptosporidium* oocysts and *Giardia* cysts has several unusual features that are important to understand and appreciate when approaching any task involving the need for data on these organisms. First, they are microorganisms but unlike other microbes monitored in water they are non-reproducing as existing in the environment outside the host, occurring in water as discrete and essentially inert particles. They are small; *Cryptosporidium* oocysts are spherical 3–5 μm in diameter, and *Giardia* cysts are ovoid 5–7 × 10–15 μm. They have been found in surface waters at levels at or near the limit of detection of available analytical procedures, i.e. 0.01–0.1/L but in a background of other native particles in the target organism size range (i.e., 2–15 μm) that number ca. 1–5 × 10^6/L. Accordingly, the analytical procedure must find a "needle in the haystack" by a combination of physical and immunochemical means (USEPA Method 1622/1623, USEPA Method 1623.1). This best available method cannot find all of the target organisms present in a sample, but typically only 20–50%, Messner (2011), and the effectiveness of recovery depends unpredictably on water quality in ways that will be described further below.

Furthermore, the low surface water concentrations, e.g. 0.1/L or 1 in 10 L dictate that large sample volumes are required and that the small number of organisms cannot be distributed uniformly (Ongerth and Saaed, 2012). If the cyst or oocyst concentration at a sampling point in a stream was 1 in 10 L, many 10 L volumes passing the sampling point would not contain a single organism and some would contain more than one. The theoretical distribution of discrete objects at low concentration is not normal but skewed and described by the Poisson distribution, Rosner (1990).

The purpose of this paper is to examine features of *Cryptosporidium* and *Giardia* concentrations in water samples focusing on the importance of recovery efficiency including features that affect the need for its routine measurement with virtually every water sample analysed. Data from a variety of sources are presented to illuminate essential questions regarding the extent and degree of variation in matrix-driven recoverability of *Cryptosporidium* oocysts and *Giardia* cysts from environmental water samples.

2. Methods and procedure

Data on the occurrence and concentration of *Cryptosporidium* and *Giardia* in water have been assembled from previously published and some publicly available but yet unpublished data to illustrate the significance of measuring recovery efficiency and taking it into account to calculate concentrations of *Cryptosporidium* oocysts and *Giardia* cysts. All data used here have been generated using the USEPA Method 1622/1623 procedure, USEPA, (2005), USEPA (2012a) from three independent sources: 1) Data presented by the Sydney Catchment

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Authority (SCA) at a Water Quality Research Australia (WQRA) Workshop on *Cryptosporidium* Research Priorities, Melbourne, Vic., December, 2009, Whiffen (2009); 2) Saaed (2012). Doctoral Thesis, University of Wollongong, NSW; and 3) The *Cryptosporidium* raw occurrence data from monitoring under the USEPA LT2ESWTR, posted on the EPA LT2 website, July 13, 2012, USEPA, (2012b).

The LT2 data are in the form of an Excel ".csv" file, consisting of 45,033 lines of data, one for each field and matrix spike sample analysed under LT2 from 2006 to 2010. The file includes about 50 columns providing identifying information for each field sample and matrix spike. The data are organized in groups according to the sampling location. The master file was downloaded, then sorted using Excel functions to form sub files of data to be examined for indications of consistency or variation in Matrix Spike (MS) recovery efficiency. The first file was formed including all MS samples; the second was sorted to eliminate all sources of the same sample site code at which no *Cryptosporidium* were found in any of the field samples. A total of 1831 sample sites recorded a total of 3370 MS sample measurements. The purpose of the second file was to use the MS measurements paired with concurrent field sample results to calculate concentration for comparison to raw numbers/L to assess the magnitude of the difference. The data for sampling sites having non zero field samples, grouped by sampling site, were searched to find non zero samples having a MS analysis recorded on or about the same date. The resulting subset was used to compare raw oocyst numbers and concentrations calculated by Eq. (2), above.

Data from four coastal streams in mid south eastern coastal Australia were derived from samples collected in 2010 and processed to measure *Cryptosporidium* and *Giardia* concentrations using USEPA Method 1623 Saaed (2012). A total of 31 samples ranging from 30 to 50 L were analysed. Recovery efficiency was measured for both *Cryptosporidium* and *Giardia* for each sample using Method 1623 Matrix Spike procedure. For 9 of the 31 samples, recovery efficiency was measured in triplicate.

Data from the above sources were processed for statistical analysis and graphical presentation using Microsoft Excel 2007 analysis and plotting tools. Processing for probability plot preparation and analysis used Origin 8.6 Pro (OriginLab, Northhampton, MA).

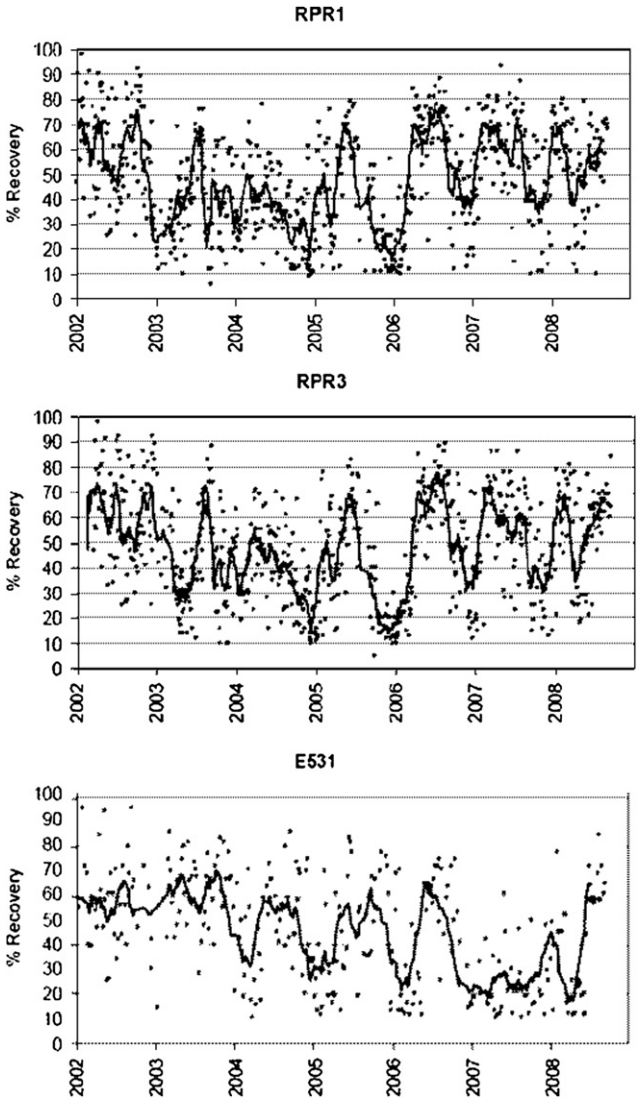

Fig. 1 – a, b, c. *Cryptosporidium* recovery efficiency, by method 1623 and moving averages at three sampling stations on a single water source in east-central New South Wales. From Whiffen, (2009). (a.RPR1–Prospect Res. Center; b.RPR3–Prospect Res, Nr Raw Water Pump Sta.; c. E531–Werriberri Ck. at Werombi).

3. Results

3.1. *Variation in matrix spike recovery efficiency*

Matrix spike recovery efficiency was measured and recorded weekly at three sampling locations in the same public water supply raw water system in coastal south eastern Australia over the period from 2002 to 2009, (Fig. 1a, b, c, Whiffen (2009)). The sampling points RPR1 and RPR3 (Fig. 1a, b) are in a balancing reservoir between the major storage, Lake Burragorang, and water treatment. Sampling point E531 is in a tributary to Lake Burragorrang near Warragamba Dam, Sydney Catchment Authority (2009).

At each station the recovery efficiency can be seen to vary over approximately annual cycles that have some similarities but with obvious differences from year to year. Similarities are greatest between stations RPR1 and RPR3, both in the same ca 50,000 ac ft reservoir. Similar cycles are apparent in data from station E531 although varying in a somewhat narrower range. While the MS recoveries at each station varied between 10 and 90%, week to week variation was more modest indicated by the moving averages suggesting reproducibility of measurements conducted within a single laboratory applying QA/QC procedures required by Method 1623 but affected by the combination of chemical and biological water quality specific to individual samples.

The USEPA LT2 data were examined to assess variations in recovery efficiency. The application of Method 1623 procedures during LT2 monitoring resulted in matrix spike (MS) recovery measurements for most of the 1831 sampling sites

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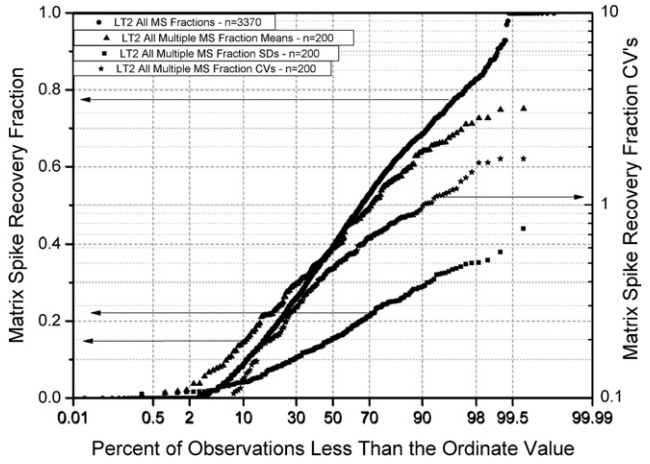

Fig. 2 – Normal probability distributions of (O) all 3370 LT2 MS fractions; (△) all LT2 multiple MS means; (□); all LT2 multiple mean standard deviations; and an exponential distribution of all LT2 multiple MS means CV's (✳).

represented in the LT2 data set. Each MS sample entry includes the number of *Cryptosporidium* oocysts added (spiked) and the number of oocysts recovered. An MS recovery fraction was calculated (number recovered ÷ number spiked) for each of 3370 entries and used to derive descriptive statistics including mean, standard deviation (SD) and coefficient of variations (CV). The overall mean recovery fraction was 0.397 (SD = 0.223; CV = 56.2%). Although the MS values from disparate sample sites cannot be considered representative of a single population, the distribution of the 3370 MS values was approximately normally distributed, Fig. 2 (o).

To examine MS recovery characteristics at individual sampling sites, MS data from sites for which multiple (>3) MS measurements were compiled. Among the 3370 MS values were 201 sample sites for which three or more MS measurements were included: 162 3's; 30 4's; 4 5's; one each included 6, 7 and 8 MS measurements; and 2 sites included 9 MS measurements. Characteristics of these multiple MS measurements reported at individual sites were examined with a major interest in how consistent they might be and in factors likely to contribute to either consistency or lack of it. Examination of tabulated data grouped by sampling site code indicated wide variation within MS fractions reported at individual sites with individual values ranging from nil to over 0.9 i.e. >90% recovery. Mean values of MS recovery fractions measured at individual sites were calculated for each of the 201 groups reporting three or more MS values. The means were also approximately normally distributed with slightly less variation than the 3370 parent values, Fig. 2 (O & △) and had a mean value of 0.396 identical to that of the total population.

The fact that 3370 MS fraction measurements were made at 1831 sampling sites on over 1000 different surface water systems in every part of the USA derived from watersheds as different as the Mississippi River and Lake Michigan should emphasize that the measurements are not derived from a single population. The population from which individual MS measurements are derived is defined by the watershed-driven water quality conditions existing at the individual sampling sites at the times of sampling. Analysis of these data showing any particular statistical distribution, e.g. Fig. 2, must be considered with care regarding possible inferences suggesting cause and effect.

With the above population principle in mind, the LT2 data from sites at which multiple MS measurements were made were examined in more detail for indications of consistency or variability. The degree of variation within the 201 groups of multiple MS measurements is indicated by standard deviations (SD's) for the individual sites. The SD's ranged between nil and 0.44. Distribution of the SD's was independent of the corresponding mean values regardless of whether recoveries were low or high, Fig. 3. Regression of SD's against MS means for all 201 sites reporting more than three, Fig. 3, shows the relatively uniform distribution of SD's across the range of mean MS values of 0 to the highest values of >0.7. The degree of variation in MS values indicated by the SD's was only poorly related to the mean values on a site by site basis as indicated by the relatively insignificant linear regression correlation coefficient ($R^2 = 0.13$, $P = 0.143$). In other words MS recoveries varied widely whether the average recovery was low, high, or in between. One must consider that for each sample site the three or more MS measurements were spaced over a 12–24 month period so that the water represented in the "matrix" being evaluated in the individual MS measurements had no relation apart from being collected at the same site.

The degree of variation among the independent groups of multiple values is portrayed more objectively by the coefficient of variation (CV) i.e. the standard deviation ÷ the mean value.

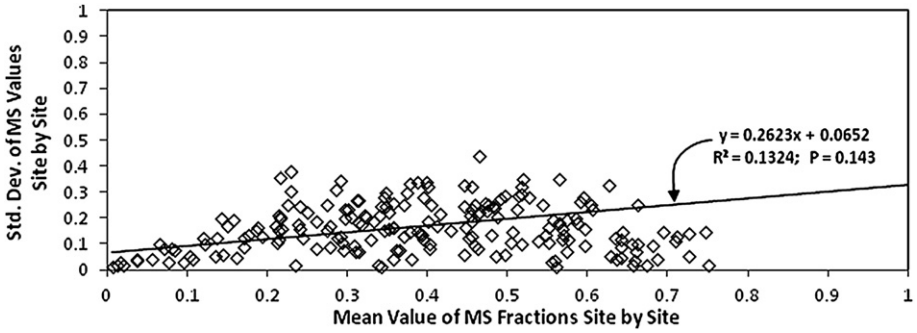

Fig. 3 – Least squares regression of the standard deviations calculated for Matrix Spike (MS) measurements from the 201 of 1831 LT2 sample sites reporting > 3 MS measurements against mean values of the multiple MS measurements for the 201 sites.

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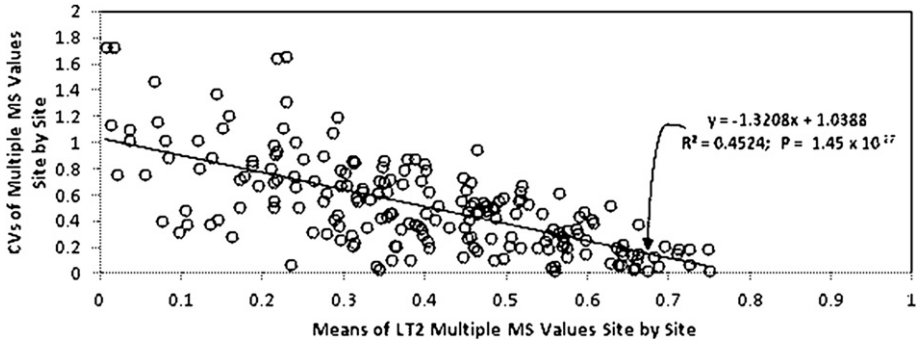

Fig. 4 – Least squares regression of the coefficients of variation calculated for matrix spike (MS) measurements from the 201 of 1831 LT2 sample sites reporting > 3 MS measurements against mean values of the multiple MS measurements for the 201 sites.

The values of CV for the 201 multiple MS fraction groups ranged from virtually nil to 2 i.e. from 0 to more than 100%. The CV values were scattered broadly across the range of means among the groups reporting multiple MS measurements, Fig. 4. Regression of the CV values against the means shows a modest but significant trend ($R^2 = 0.45$, $P = 1.45 \times 10^{-27}$) toward lower variation at higher MS recovery. The degree of variability between MS measurements at an individual site was more likely to be high at low average recovery than at high average recovery. Perhaps the most impressive feature of the CV's for these 201 sample sites is the magnitude of variability for the vast majority of sites. More than half of these sites had relative variations of greater than 50% (avg. CV = 0.52). If higher MS recovery is associated with "better" water quality, e.g. lower turbidity, then less variability may well be associated with such conditions. Less variation in water quality factors affecting *Cryptosporidium* recovery by Method 1623 would be expected in a small pristine watershed or Lake Michigan for example compared to downstream locations on large river systems such as the Ohio, Missouri, or Mississippi.

Table 1 – Recovery efficiencies of *Cryptosporidium* oocysts and *Giardia* cysts measured in nine sets of triplicate matrix spikes for water samples from coastal streams in mid Southern New South Wales, Australia.

MS Rep's	Cryptosporidium				Giardia			
	C-Rec %	Avg %	Std dev	C.V.	G-rec %	Avg %	Std dev	C.V.
1-1	8.1				76.7			
1-2	10				53.5			
1-3	9.5	9.2	1	0.11	67.7	65.9	11.7	0.18
2-1	3.8				69.7			
2-2	4.3				47.7			
2-3	1.9	3.3	1.3	0.39	65.1	60.8	11.6	0.19
3-1	4.7				39.4			
3-2	7.1				49.7			
3-3	4.3	5.4	1.5	0.28	52.3	47.1	6.8	0.14
4-1	15.1				54.5			
4-2	18.5				48.2			
4-3	18.5	17.4	2	0.11	50.3	51	3.2	0.06
5-1	29.2				46.6			
5-2	39				46.1			
5-3	27.8	32	6.1	0.19	47.1	46.6	0.5	0.01
6-1	7.3				75.9			
6-2	7.8				75.4			
6-3	6.3	7.1	0.7	0.10	61.3	70.9	8.3	0.12
7-1	10				9.7			
7-2	22.9				10.1			
7-3	9.1	14	7.7	0.55	4.8	8.2	2.9	0.35
8-1	22.9				62.1			
8-2	14.8				30.1			
8-3	23.9	20.5	4.9	0.24	58.3	50.2	17.5	0.35
9-1	8.6				58.7			
9-2	14.4				47.1			
9-3	17.2	13.4	4.4	0.33	60.2	55.3	7.2	0.13
Avg.	13.6			0.26	50.7			0.17
Std dev	9.2				18.9			

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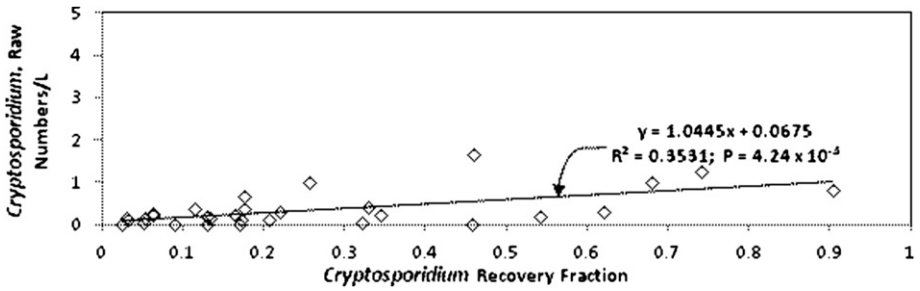

Fig. 5 – Least squares regression of 31 pairs of NSW coastal stream *Cryptosporidium* raw No's/L measured by method 1623 against the corresponding recovery fractions measured for each oocyst/L sample.

3.2. *Reproducibility of matrix spike recovery measurements*

In 2010 water samples for *Cryptosporidium* and *Giardia* concentration measurement were collected from nine sample sites on four coastal streams in mid south eastern Australia Saaed (2012). To permit measuring concentrations, the recovery efficiencies for both *Giardia* and *Cryptosporidium* were measured by matrix spiking of 10 L portions of 60 L samples collected from each site. Analysis of initial samples had shown appreciable differences in recovery efficiencies for both organisms between longitudinal sampling sites along the same streams within distances of a few miles. In subsequent sampling additional water was collected to permit 3× replication of recovery measurements to determine their reproducibility. The resulting data, Table 1, show that in three identical 10 L volumes of water collected at individual sampling sites, recovery efficiencies were closely replicated. Typically, *Giardia* recoveries were significantly greater than those for *Cryptosporidium*, averaging 50.7% and 13.6% respectively ($P = 1.9 \times 10^{-11}$). As indicated by the coefficients of variation, variability for *Cryptosporidium* averaging 26% was greater than observed for *Giardia*, averaging 17% ($P = 0.099$).

3.3. *Independence of organism occurrence and recoverability*

It is conceivable that numbers of organisms found in water samples might vary in direct proportion to recovery efficiency. Such a relationship could influence the need for recovery measurement. To examine this possibility the relation between numbers of organisms found and the corresponding recovery efficiency measurement was examined. Regression of raw *Cryptosporidium* numbers/L against the paired recovery fractions (Saaed, 2012), Fig. 5, shows only a modest but significant relation ($R^2 = 0.35$, $P = 4.24 \times 10^{-4}$) between them. In other words, occurrence of *Cryptosporidium* expressed in terms of raw numbers/L depended only moderately on whether recovery efficiency was high or low.

Regression of raw *Giardia* numbers against the paired recovery fractions (Saaed, 2012), Fig. 6, shows virtually no relation ($R^2 = 0.07$, $P = 0.149$) between them. Compared to the observation for *Cryptosporidium*, *Giardia* occurrence expressed in terms of raw numbers/L was virtually independent of whether recovery efficiency was high or low.

The USEPA LT2 data were examined similarly. The sorted LT2 data including the matrix spike data for the sites having positive findings consisted of 5234 lines of which 1577 were for MS data. These sorted data included 2894 field samples in which at least one *Cryptosporidium* was found. These data included a subset of 319 positive *Cryptosporidium* field sample findings for which an MS measurement was made on or about the same day. These pairs of raw *Cryptosporidium* numbers/L and corresponding MS values were plotted chronologically, Fig. 7. The raw *Cryptosporidium* numbers/L were predominantly 0.1–0.2/L but ranged from about 0.1 to 2.0/L. The matrix spike values averaged 0.68 and appear to be distributed randomly in the range from <0.1 to >0.9, Fig. 7.

Regression of this subset of 319 LT2 raw *Cryptosporidium* numbers/L against the paired matrix spike recovery fractions, Fig. 8, shows virtually no relation between them ($R^2 = 0.006$, $P = 0.17$). As observed above, the lack of a relationship between *Cryptosporidium* oocyst occurrence and recovery efficiency indicated specifically by the LT2 data should not be

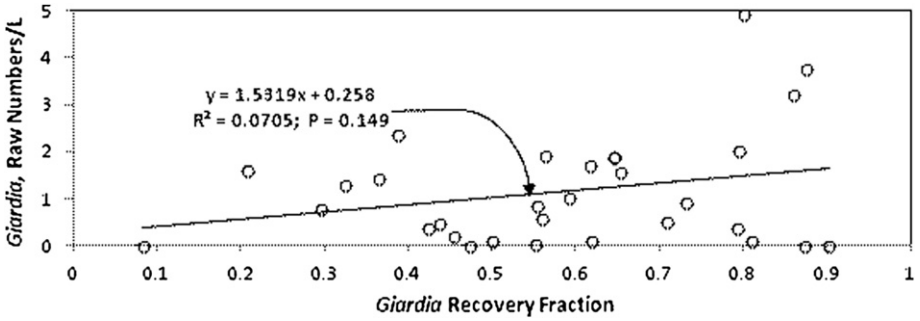

Fig. 6 – Least squares regression of 31 pairs of NSW coastal stream *Giardia* raw No's/L measured by method 1623 against the corresponding recovery fractions measured for each cyst/L sample.

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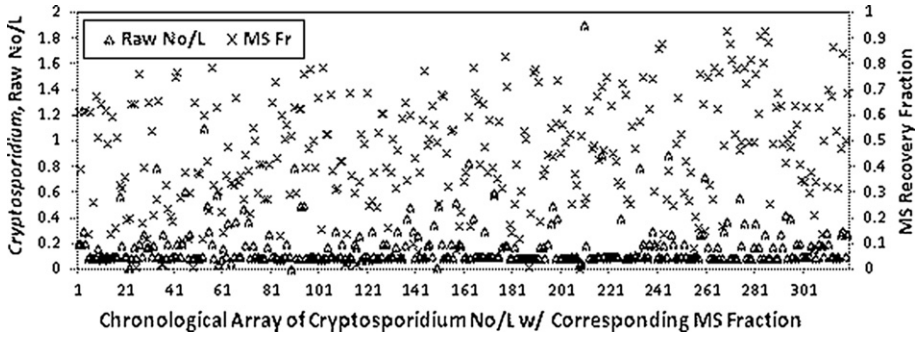

Fig. 7 – Comparison of the Chronological occurrence of the 319 paired values of LT2 *Cryptosporidium* raw No's/L (△) and method 1623 matrix spike (MS) recovery fractions (×).

surprising. The more than 300 observations were made at nearly as many different sampling sites bearing no relation to one another in terms of the water being analysed. Inferences from such data completely lacking population homogeneity should not be made.

3.4. *Magnitude of the difference between raw numbers and concentration*

Probability distributions of the 319 data pairs of LT2field samples and concurrent MS measurements were examined. *Cryptosporidium* occurrence was expressed in terms of raw numbers/L(△) and in terms of concentrations, oocysts/L(o), Fig. 9. Although the data are not of a single homogenous population and the distributions of No's/L and concentration (oocysts/L) do not conform well to the log-normal distribution, the distributions effectively illustrate the differences. First, the overall distribution of concentrations has a median value of ~0.38/L compared to the median of the raw no's/L of ~0.13/L. The ratio of 0.38/0.13 = 2.92 corresponds closely to the overall ratio of concentration to numbers/L derived from the overall average MS of 0.39, i.e. 1/0.39 = 2.56. Second, the slope of the concentration distribution is greater than that of the raw numbers due to the independent variation of the MS values and the corresponding field sample measurements.

Similar analysis was applied to the Australian data for both *Cryptosporidium* and *Giardia* Saaed (2012). The NSW coastal stream matrix spike recovery efficiency data including the average of the triplicate measurements (Table 1) and single measurements were accompanied by the raw numbers of both *Cryptosporidium* and *Giardia* and by *Cryptosporidium* and *Giardia* concentrations calculated from organism numbers, sample volumes, and recovery efficiencies, Table 2. Overall, recovery efficiencies for *Cryptosporidium* averaged 25.8% (range: 2.5–92.8%, std. dev. 24.2%). Recovery efficiency for *Giardia* averaged 62.3% (range: 8.2–97.1%, std. dev. 21.2%).

The average of *Cryptosporidium* expressed as concentration was 7 times (701%) the raw numbers expressed as the number of oocysts/L (range, 1.1–30.3 times). The average of *Giardia* expressed as concentration was 1.65 times (165%) the raw numbers (range, 1.1–3.7 times), Table 2.

When the raw numbers per litre and concentration data for *Cryptosporidium* and for *Giardia* were presented in terms of log probability distributions, Figs. 10 and 11, respectively, the magnitude of the effect of taking recovery efficiency into account is illustrated graphically as the difference between 50 percentile values and differences in the slope (standard deviation) of the distributions. The average *Cryptosporidium* recovery efficiency was 25.8% (range 9–90%, SD = 23.2%). The average *Giardia* recovery efficiency was 58.4% (range, 20.8–90.3, SD = 20.8%). Clearly, the importance of taking recovery efficiency into account is magnified for lower average recoveries.

The distribution of *Cryptosporidium* oocyst concentrations, Fig. 10, was nearly an order of magnitude greater than the distribution of raw oocyst/L numbers. On the average

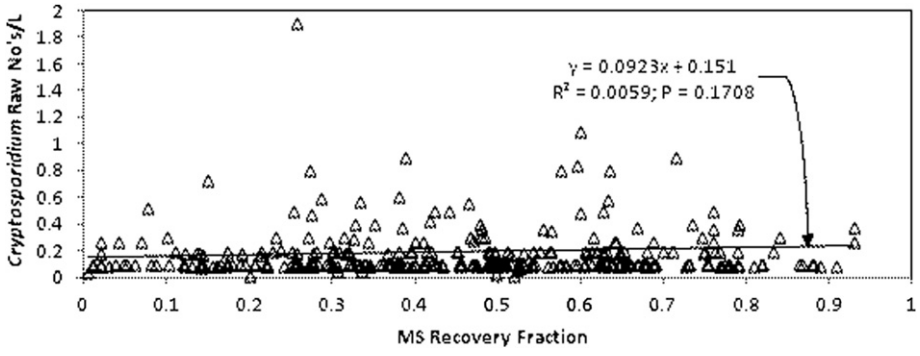

Fig. 8 – Least squares regression of 319 paired values of LT2 *Cryptosporidium* raw No's/L against the corresponding reported measurement of method 1623 matrix spike (MS) recovery fraction.

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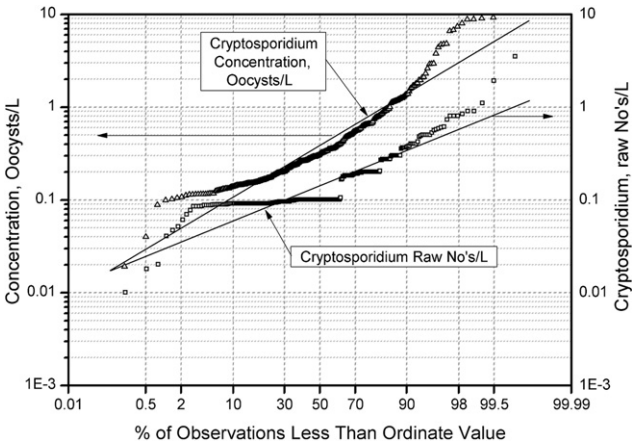

Fig. 9 – Log probability distributions of LT2 *Cryptosporidium* oocyst raw No's/L (△) and *Cryptosporidium* oocyst concentrations calculated from paired raw No's/L and corresponding MS fractions (○) for the 319 pairs of LT2 field sample measurements having concurrent MS measurements.

Cryptosporidium concentrations averaged 6.8 times (680%) raw oocyst/L neglecting recovery.

The distribution of *Giardia* cyst concentrations, Fig. 11, was also higher than that of raw cyst/L numbers. The average *Giardia* cyst concentration was ~80% greater than raw cyst/L numbers neglecting recovery.

4. Discussion

Two major questions are addressed in presentation and analysis of the preceding data: 1) Are raw numbers or concentration taking recovery efficiency into account essential to provide accurate and useful information on occurrence of *Cryptosporidium* and *Giardia* in water samples; and 2) Can recovery efficiency be effectively generalized between sampling times and possibly sampling sites?

As observed in the introduction, numbers of *Cryptosporidium* oocysts or *Giardia* cysts are not equal to or in any way equivalent to concentration. The critical difference is the recovery efficiency that measures the inefficiency of available

Table 2 – Summary of raw numbers, recovery efficiencies, and calculated concentrations for *Cryptosporidium* oocysts and *Giardia* cysts found using USEPA method 1623 in 30 surface water samples from four coastal streams in SE New South Wales, Australia.

No.	*Cryptosporidium*				*Giardia*		
	Vol, L	No.	Rec. Fr.	Conc.	No.	Rec. Fr.	Conc.
1	50	63	0.741	1.70	100	0.795	2.52
2	50	15	0.62	0.48	5	0.619	0.16
3	50	50	0.68	1.47	29	0.559	1.04
4	50	10	0.542	0.37	18	0.793	0.45
5	50	0	0.17	0.00	45	0.732	1.23
6	50	1	0.09	0.22	51	0.593	1.72
7	20	33	0.46	3.59	75	0.875	4.29
8	50	13	0.063	4.13	25	0.709	0.71
9	50	5	0.033	3.03	42	0.554	1.52
10	30	0	0.458	0.00	0	0.874	0.00
11	50	7	0.054	2.59	64	0.324	3.95
12	50	2	0.052	0.77	24	0.438	1.10
13	32	12	0.176	2.13	50	0.653	2.39
14	50	18	0.115	3.13	71	0.364	3.90
15	50	12	0.164	1.46	117	0.388	6.03
16	32	4	0.172	0.73	51	0.208	7.66
17	50	21	0.329	1.28	96	0.563	3.41
18	50	2	0.321	0.12	9	0.455	0.40
19	50	12	0.344	0.70	246	0.801	6.14
20	50	1	0.13	0.15	93	0.646	2.88
21	50	11	0.063	3.49	86	0.617	2.79
22	50	50	0.257	3.89	160	0.86	3.72
23	32	10	0.219	1.43	0	0.903	0.00
24	50	1	0.13	0.15	93	0.646	2.88
25	50	9	0.129	1.40	0	0.083	0.00
26	50	8	0.031	5.16	39	0.297	2.63
27	50	0	0.025	0.00	0	0.473	0.00
28	50	5	0.206	0.49	4	0.5	0.16
29	32	26	0.903	0.90	3	0.81	0.12
30	50	34	0.176	3.86	18	0.425	0.85
31	50	8	0.134	1.19	2	0.553	0.07
Mean			0.258			0.584	
Std dev			0.232			0.208	

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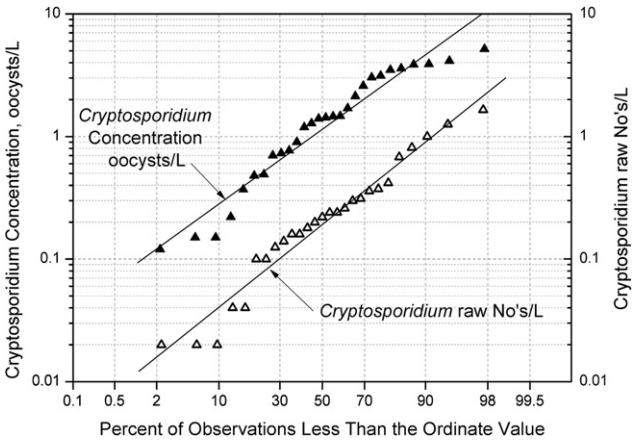

Fig. 10 – Log probability distributions of raw *Cryptosporidium* oocyst No's/L (△) measured by method 1623 and *Cryptosporidium* oocyst concentrations (▲) calculated from the raw numbers/L and accompanying recovery efficiency measurements for 31 NSW coastal stream samples, 0.09 < recovery fraction < 0.90, avg. = 0.26.

analytical methods for recovering these organisms from representative water samples.

Reasoning of investigators having published previously on occurrence of *Cryptosporidium* and *Giardia* in water for not measuring and taking recovery into account has simply not been articulated. Significant research effort and numerous technical articles have addressed the need to improve methodology for measuring the occurrence of these organisms in water. The literature includes work specifically directed to measuring and/or improving the recovery efficiency. Authors often have observed that recovery efficiency is both inefficient (e.g. Reynolds et al. (1999)) and variable (e.g. Francy et al. (2004)). But, with exceptions noted in the introduction, authors have consistently ignored/neglected/avoided/or not even suggested application of recovery efficiency to calculating concentration from raw numbers.

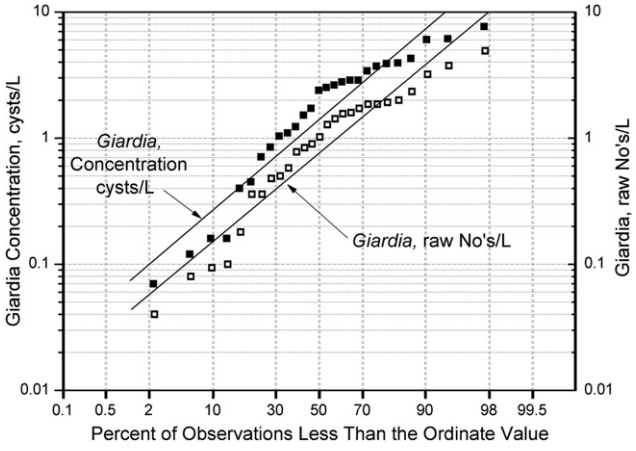

Fig. 11 – Log probability distributions of raw *Giardia* cyst No's/L (△) measured by method 1623 and *Giardia* cyst concentration, cysts/L (■) calculated from the raw numbers/L and accompanying recovery efficiency measurements for 31 NSW coastal stream samples, 0.21 < recovery fraction < 0.90, avg. = 0.58.

Informally this issue has been a common feature of discussion among investigators working on the development and application of methods for measuring *Cryptosporidium* and *Giardia* occurrence. Reasons for not measuring and taking recovery into account have varied principally dealing with practical issues. Significant time and effort is required. Effort increases inversely proportion to water quality, generally with increasing turbidity. Sufficient effort is required that virtually no data on the reproducibility of individual measurements (prior to data presented here) exist. Investigators have believed that their own lab procedures were sufficiently consistent to assume that recovery was effectively a constant, suggesting that numbers could be considered roughly equivalent to concentration.

5. Conclusions

The data assembled in this paper show clearly that:

1. Recovery efficiency measured at a single site varies widely with time;
2. Recovery efficiency measured at closely related sites may differ significantly;
3. Recovery efficiency measurements are reproducible;
4. Recovery efficiency and occurrence of both *Cryptosporidium* and *Giardia* vary independently at any given location; and
5. The difference between raw numbers and concentration is typically a factor of from 2 to 10 depending on water quality.

Accordingly, accurate quantification of *Cryptosporidium* and *Giardia* concentrations requires the routine measurement of recovery efficiency relevant to every water sample analysed. Simply put, numbers are not equivalent to concentration and inferences drawn from raw numbers as if they were concentration are most likely to be misleading.

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