Understanding the fate of *Cryptosporidium* and *Giardia* in storage reservoirs: a legacy of Sydney's water contamination incident

Peter R. Hawkins, Peter Swanson, Malcolm Warnecke, Siva Raj Shanker and Colin Nicholson

ABSTRACT

High levels of *Cryptosporidium* oocysts and *Giardia* cysts (C&G) were detected in the raw and filtered water supply in Sydney, Australia, in July and August 1998. This paper describes the results of daily monitoring in the Sydney water supply catchments between December 1998 and May 1999, as one response to that incident.

C&G were most abundant in the largest and most developed catchment. The levels of contamination in Sydney's raw water supply were of similar magnitude to raw waters in the USA and Europe, but the frequency of contamination was much lower.

Physical variables of increased stream flow and turbidity were associated with the presence of C&G. The abundance of *Giardia* cysts was highly correlated with the number of *Cryptosporidium* oocysts. Other microbial indicators of faecal contamination (faecal coliforms and *C. perfringens*) were not useful indicators of either C or G.

Both organisms occurred episodically in brief events, which spread rapidly through the water supply system, following stream rises in the catchment. Lake Burragorang was a poor barrier to the transfer of C&G into the bulk supply. C&G disappeared from Lake Burragorang within 4 weeks after each event. This loss rate was more rapid than predicted from settling theory. We observed settling rate of 5–10 m day^{-1}, and postulate that sedimentation was accelerated by agglomeration of oo/cysts with other suspended particles.

A design for monitoring programmes and management action in lakes to prevent C&G entering the supply to water treatment plants is suggested.

Key words | catchments, *Cryptosporidium*, *Giardia*, management, reservoirs, oo/cyst sedimentation rates

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INTRODUCTION

The improvement in detection methods for *Cryptosporidium* and *Giardia* (C&G) over the past decade has confirmed their ubiquitous distribution in the aquatic environment, even in pristine watersheds (Mager *et al.* 1998). As a consequence of these analytical developments and the recognition of the public health risk associated with the presence of these pathogenic organisms, estimation of the abundance of the resistant stages of these organisms in catchments and surface waters has become an issue of priority for water authorities.

Several species of *Cryptosporidium* and *Giardia* infect vertebrates. Native and domesticated fauna including birds and reptiles as well as humans can harbour these parasites (Atwill 1995; Rose 1997). Extensive environmental contamination of the encysted life stage of C&G can occur. The human pathogen, *Cryptosporidium*

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parvum, is of particular concern because of the resistance of the oocyst to chlorine disinfection.

Several extensive surveys of C&G abundance in water have provided 'snapshots' of their abundance across wide geographic areas in the USA (Le Chevallier *et al*. 1991; Le Chevallier & Norton 1995). However, the complexity and expense of analysis has meant that few intensive monitoring studies have been undertaken of raw waters.

Storm water is a likely vector for the transport of C&G into surface water supplies, because of the known association between storm water and other bacteriological indicators of faecal contamination. Nevertheless, surveys specifically designed to resolve variation in C&G concentrations due to rainfall have been inconclusive (Stewart *et al*. 1997; Abbaszadegan *et al*. 1998; Atherholt *et al*. 1998; States *et al*. 1998*a*; Young & Komisar 1998). This is due in part to limitations of the analytical method (the Information Collection Rule; Clancy *et al*. 1994). These limitations are exacerbated by the high turbidity usually associated with storm water (Young & Komisar 1998).

High levels of *Cryptosporidium* oocysts and *Giardia* cysts (hereafter collectively termed oo/cysts) were detected in the raw and filtered water supply in Sydney, Australia, in July and August 1998. Three city-wide 'boil water' notices were issued, affecting more than 2 million consumers. The extent of the social and economic impact of the protozoan contamination incident prompted a judicial inquiry (McClellan 1998). One outcome from this inquiry was a recommendation for daily monitoring of the city's raw water sources for *Cryptosporidium* and *Giardia*.

The initial results from that programme are presented here, and provide a rare opportunity for a detailed assessment of the occurrence of C&G in natural waters using detection methods with high recovery efficiency even for turbid water samples (Hoffman *et al*. 1997; Champion 1998).

MATERIALS AND METHODS

Water for Sydney's population of 3.7 million people comes entirely from surface supplies, drawn from six main catchments to the south and west of the metropolis. Water samples were collected regularly from sites throughout the watershed between 1 December 1998 and 24 May 1999. All sites have been categorised by location in the water supply system as catchment, lake or bulk supply sites (Figure 1). The frequency of sampling at each site is listed in Table 1.

The Hawkesbury River was the most intensively monitored catchment site. A single location near North Richmond was monitored three times each week. The discharge from all the storage lakes in the Warragamba and Nepean catchments eventually reaches the Hawkesbury River. This flow is supplemented by some inflow from streams in the urbanised Hawkesbury catchment. All other catchment sites were streams in the Warragamba catchment, the watershed of Lake Burragorang. This is the largest water supply catchment with the densest rural and urban development. The nine sewage treatment plants (STPs) located within this catchment were expected to increase the likelihood of C&G contamination compared with other catchments (McClellan 1998).

The streams in the Warragamba catchment were sampled at gauging stations near their point of entry into Lake Burragorang (Figure 1). Grab samples were collected for faecal coliforms (Fc) and *Clostridium perfringens* (Cp) and physical/chemical analysis, as well as C&G analysis (20 l grab). A refrigerated autosampler at Kelpie Point sampled two flow events in the Cox's River in April. The sampler was triggered by a predetermined rise in river height and each flood event yielded a composite floodwater sample, collected as up to 24 × 1 litre samples over 72 hours.

Storage Lakes

Lake Burragorang was the focus of the reservoir monitoring programme. This 2 million megalitre reservoir provides 85% of the stored water capacity for Sydney. A site 500 m upstream from the water supply offtakes in the dam wall was sampled daily. Nine other sites in the upper reaches of Lake Burragorang were sampled irregularly, after contaminated water was detected in the catchment. Other storage lakes were sampled at weekly or fortnightly

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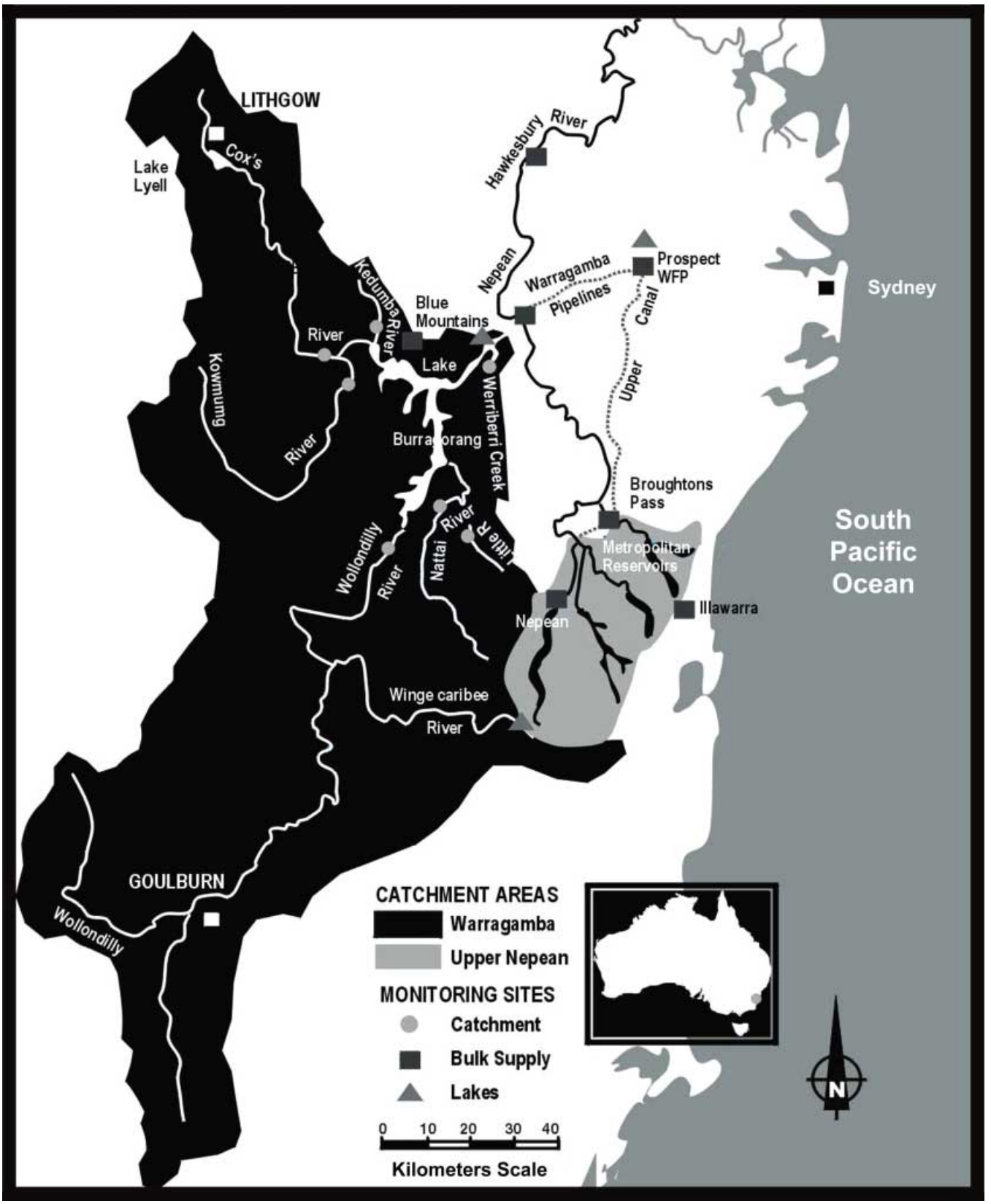

Figure 1 | Sampling sites and major water supply catchment boundaries. Canal and pipe elements of the bulk supply are dotted lines.

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Table 1 | Sampling frequency and determinands for all sites, grouped by sample type

Sample type	Frequency	Number of samples	Microbiology	Chemistry	Physical
Catchment					
Wollondilly River	Monthly	7	Fc and Cp	pH, turbidity, EC	Temperature, flow
Cox's River	Monthly	11	Fc and Cp	pH, turbidity, EC	Temperature, flow
Kowmung River	Monthly	4	Fc and Cp	pH, turbidity, EC	Temperature, flow
Kedumba Ck	Flows	1	Fc and Cp	pH, turbidity, EC	Temperature, flow
Little River	Flows	2	Fc and Cp	pH, turbidity, EC	Temperature, flow
Nattai River	Monthly	5	Fc and Cp	pH, turbidity, EC	Temperature, flow
Werriberri Ck	Weekly	30	Fc and Cp	pH, turbidity, EC	Temperature, flow
Hawkesbury River	3 per week	69	Fc	pH, turbidity	Temperature
Storage Lakes					
#Burragorang (1 site at 2 depths)	Daily	389	Fc and Cp*	pH, turbidity, EC	Temperature, depth
Wingecaribee (1 site)	Weekly	25	Fc	pH, turbidity, EC	Temperature, depth
Prospect (1 or 2 sites)	Fortnightly	36	Fc	pH, turbidity, EC	Temperature, depth
Others	Before use	25	Fc	pH, turbidity, EC	Temperature, depth
Bulk Supply					
Upper Canal at Broughtons Pass	Daily	161	Fc	pH, turbidity, chlorine	Temperature
Prospect WFP inlet	Daily	162	Fc	pH, turbidity, chlorine	Temperature
Warragamba pipeline at dam	**Daily	52	Fc	pH, turbidity	Temperature
#Blue Mountains (Greaves, Woodford and Cascade reservoirs)	3 per week	70	Fc	pH, turbidity	Temperature
#Illawarra (Nepean, Woronora and Avon reservoirs)	3 per week	84	Fc	pH, turbidity	Temperature

Fc, faecal coliforms; Cp, *Clostridium perfringens* (*Lake Burragorang only measured in December).
**Warragamba pipeline was collected daily but only analysed during events.
#Routine samples were supplemented with additional collection and analysis during events.

intervals, or prior to a reservoir entering supply. All samples from these lakes were collected near the water supply offtakes.

Thermistor measurement of water temperature

In addition to the daily profiles in Lake Burragorang, the water temperature at the dam wall was measured by a

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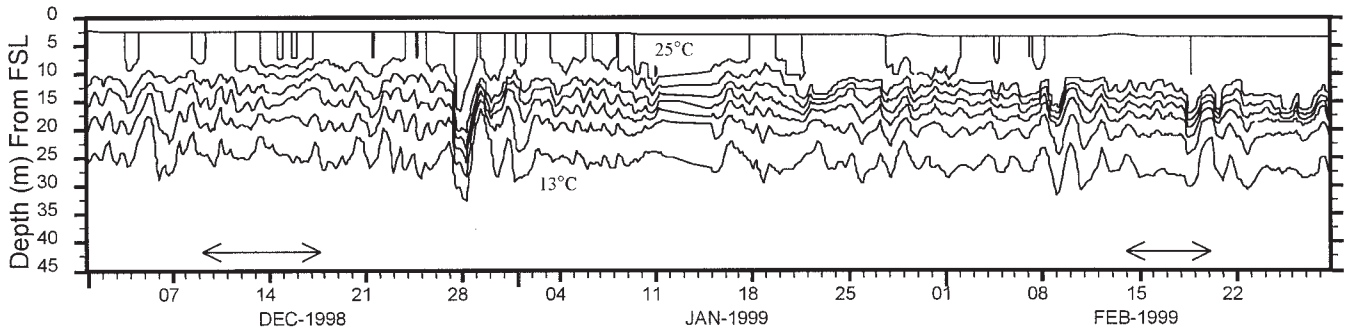

Figure 2 | Isotherms measured by thermistor chain at the dam wall in Lake Burragorang. Data averaged over 6 h periods and plotted at 2°C intervals. The duration of each C&G event in the lake is indicated by horizontal arrows.

chain of 20 thermistors (precision of ± 0.2°C), spaced at 2–3 m intervals down to 60 m below the full supply level (FSL). These data were logged at 30 or 60 min intervals and used to plot the stratification and mixing behaviour in the lake (Figure 2).

Lake sampling methods

Turbidity, conductivity and temperature were measured *in situ* at 1 m vertical intervals using submersible probes (Yeokal 611™ or Hydrolab™ minisonde). The profile was used to locate the position of the metalimnion and any floodwater intrusions within the water column prior to C&G sampling each day.

The C&G samples from lakes were integrated water samples collected through specific vertical intervals in the water column. The sample intervals were selected either to intersect a region where floodwater was likely to intrude (e.g. the upper metalimnion in Lake Burragorang in summer), or to monitor the abstraction zone in operating reservoirs.

Lake water samples were collected from a moored boat, by raising a reinforced plastic suction hose through the predetermined interval at a constant rate. As the suction head was raised through the sample interval, the pump discharge was collected on board. The correct starting depth was confirmed before sampling commenced, by matching the turbidity and conductivity of the water from the pump against the vertical profile determined earlier. Several void volumes of the pump system were discarded before collecting an integrated sample and a duplicate was reserved for other analyses by splitting the pump discharge during collection.

Samples from at least two intervals were always collected near the dam wall in Lake Burragorang. The interval from the top of the metalimnion to the 14°C isotherm (usually between 10 and 20 m) was the target of the greatest sampling effort in the upper water column during December and February. The lower sampling intervals were positioned to match the depth of the offtakes. These was generally positioned between the 14°C isotherm and 60 m below full supply level, although they were moved higher during contamination events.

Assignment of integrated depth samples to specific layers

All C&G results from integrated depth samples collected at the dam wall site during December and February were assigned to either a 'top' or a 'bottom' layer to simplify the description of the vertical distribution of C&G, in terms of the thermal strata of the lake. The top layer, above the 14°C isotherm, had a maximum depth of 22 m in December or 20 m in February, and represented the epilimnion and the upper metalimnion. This layer was usually represented by a single integrated sample. In summer, epilimnetic temperatures exceeded 25°C and the epilimnion was often less than 10 m deep (Figure 2). Between December and March, when thermal stratification was pronounced, floodwater typically inserts into the upper metalimnion (Ferris & Tyler 1992). Therefore the 'top' layer was expected to include floodwater intrusions. The 'bottom' layer extended from below the 14°C isotherm (usually at 20–22 m) down to 60 m. This layer represented the lower metalimnion and the hypolimnion (water

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temperature of 12°C). The 'bottom' layer was often represented by two sample intervals, matching different abstraction depths within the hypolimnion.

The daily arithmetic mean concentration of all presumptive (FITC) oocysts and cysts from all samples collected within the top or bottom layer each day was used to represent the C&G concentrations within each layer. The concentration of presumptive counts of both species (C + G) was pooled to increase the sensitivity of the analysis. This was justified because both protozoans appeared and disappeared in synchrony. Samples in which neither C or G were detected (i.e. <5 oocysts/cysts $100\,l^{-1}$) were counted as zero.

The bulk supply is a system of rivers, canals and pipes used to transport raw water from storage lakes to water filtration plants (WFPs). The two elements which transport most water in the Sydney Bulk Supply system are the Upper Canal and the Warragamba pipeline (Figure 1). Water in both bulk supplies was sampled at sites close to their inlets and analysed daily from January 1999.

The Upper Canal system transports water from three storage lakes in the Upper Nepean Catchment. The water travels down river, then through a 50 km open canal (Upper Canal) to the Prospect WFP. The Warragamba pipeline transports the bulk supply from Lake Burragorang to Prospect WFP. Both supplies mix before entering the Prospect WFP. This mixture was also sampled daily after December 1998.

All bulk supply samples were collected as 20 l grabs. The bulk supply from storage lakes in the Blue Mountains and Illawarra regions was sampled three times weekly. Individual samples from the system elements within each region were combined to provide a single 20 l composite sample representative of the bulk supply for that region (Table 1 lists the lakes by region). All samples were stored in new 20 l polyethylene drums at 4°C until analysis, usually within 48 hours.

Analysis of C&G in water

All C&G enumerations were performed at the same laboratory (AWT, West Ryde, NSW). A measured volume (usually 20 l) of each raw water sample was filtered by the flat bed method (Ongerth & Stibbs 1987).

Immunomagnetic separation (Ausflow®) and flow cytometry (modified Becton-Dickinson FACSCalibur) procedures were used to concentrate and sort oo/cysts from other detritus in all water samples. C&G were labelled using specific antibodies conjugated to fluorescein isothiocyanate (FITC) for immunofluorescent assay (IFA). The specificity of the antibody stain has been reported by Ferrari *et al.* (1999). Entire samples were always counted to avoid the substantial error associated with partial counting methods (Nahrstedt & Gimbel 1996; Young & Komisar 1998).

The antibody FITC stained pellet was enumerated microscopically to give a presumptive (FITC) count. A second confirmatory staining procedure used DAPI (4′6-diamidino-phenylindole) to better identify intact and potentially viable oocysts (Campbell *et al.* 1992). All results presented are for concentrations of DAPI stained oo/cysts per 100 l unless otherwise indicated.

A positive control sample, followed by a negative control sample was processed after every eight environmental samples, as a quality assurance check and to allow ongoing assessment of recovery efficiency. The oo/cysts used in the positive control samples were purified by density gradient centrifugation, suspended in an antibiotic solution or in sterile water and maintained at 4°C. The enumeration of these oo/cyst suspensions was performed by filtering an aliquot through a 13 mm membrane mounted on a vacuum sort stage, followed by staining with the AusFlow® preparation and examining by microscopy. *Cryptosporidium* oocysts used in the positive controls were supplied by Sterling Laboratory, University of Arizona. Waterborne Inc. or AusFlow® supplied *Giardia* cysts.

Reported data are not corrected for recovery. Recovery was calculated by seeding a representative water sample with a known number (200–500) of oo/cysts. The acceptance criteria for positive controls were within the range 25–100%. This range was based on a data set accumulated from previous recoveries measured on this water type. The analytical method (IFA with flow cytometry concentration) coupled with the complete enumeration of the sample pellet, gave a consistent detection limit for C&G of less than 5–20 oo/cysts $100\,l^{-1}$ (recovery corrected).

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Table 2 | Summary statistics for all microbiological indicators monitored in the Sydney water supply system; grouped by sample type

Site description	C&G samples (n)	Crypto max (100 l^{-1})	Crypto present (%)	Giardia max (100 l^{-1})	Giardia present (%)	Fc samples (n)	Fc max (cfu 100 ml^{-1})	Fc present (%)	Cp samples (n)	Cp max (cfu 100 ml^{-1})	Cp present (%)
All sites	1,133	7,980	9	5,240	8	512	9,500	70	75	220	44
All catchment sites	129	7,980	10	5,240	9	126	9,500	99	54	220	61
Warragamba catchment	60	7,980	20	5,240	20	58	9,500	100	54	220	61
Hawkesbury catchment	69	7	1	0	0	68	320	99			
All lakes	475	2,320	12	2,940	12	133	72	34	21	0	0
Lake Burragorang	389	2,320	14	2,940	13	98	2	11	21	0	0
All other lakes	86	225	3	110	5	35	72	99			
All bulk supply	529	4,305	6	4,935	5	253	710	74			
Warragamba pipeline	52	4,305	27	4,935	23	11	1	9			
Prospect WFP	162	265	5	750	4	42	300	31			
Upper Canal	161	280	3	1.190	3	160	710	99			
Blue Mts	70	480	6	260	6	6	20	83			
Illawarra	84	250	2	360	2	34	3	29			

C&G, *Cryptosporidium* and *Giardia*; Fc, faecal coliforms; Cp, *Clostridium perfringens*.

Methods of statistical analyses

Indicators of *Cryptosporidium* and *Giardia*

Stream flow

The dependence of C&G occurrence near the dam wall on antecedent rainfall and stream flow in the Warragamba catchments was tested by a Chi-squared test (SAS 1987). The frequencies of occurrence that would be expected if there was no effect were compared with actual frequencies reported in Table 2.

Logistic regression

Logistic regression analysis (Hosmer & Lemeshow 1989) was used to test for association between water quality indicators (turbidity, conductivity, pH, Fc and Cp) and the presence of C or G. The logistic model was chosen because of the high number of non-detections of both C and G. The model is similar to linear regression, except that the value of the dependent variable (concentration of C or G) was assigned as either zero or one (i.e. either present or absent).

RESULTS

Microbiological indicators of faecal contamination

C&G were monitored at all sites and on all occasions. The other indicators of faecal contamination (Fc and Cp) were not monitored at all sites. The frequency of incidence of all microbiological indicators was grouped by sample type, namely catchments, lakes and bulk supply (Table 2).

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Catchments

Microbial indicators of faecal contamination were common in the catchment streams. Fc were present in 99% of samples from the streams in the Warragamba catchment and from the Hawkesbury River. Only samples from the Warragamba catchment were tested for Cp and there was a 61% incidence (Table 2).

The highest level of Fc at any site was 9,500 (cfu 100 ml^{-1}), in the Warragamba catchment at Werriberri Creek in mid December. No C&G were detected in that sample. Fc levels in the Hawkesbury River were lower than in the Warragamba catchment streams. The maximum Fc concentration in the Hawkesbury River was 320 cfu 100 ml^{-1}, in mid February. No C&G were present in that sample and only a single sample from the Hawkesbury River site contained C&G (7 oocysts 100 l^{-1}). C&G were more prevalent and at higher concentrations in the Warragamba catchment. They occurred in 20% of all samples collected, and at five of the seven sites. The only sites where C&G were not detected were Little River (0/2 samples) and Kowmung River (0/4 samples).

Incidence of C&G in catchments in relation to flow

Gauges at sampling sites on the major inflows to Lake Burragorang record approximately 80% of all flows into the lake. The 6 month study period was relatively dry and inflows were low in comparison to flood records for the reservoir. There were three small flow events when the cumulative daily inflow exceeded 1,000 Ml. The peak daily inflow, on 10 February 1999, was 3,850 Ml (Figure 3) and the gauged inflow for the entire 6 month period was 80,000 Ml. The flood prior to the Sydney contamination incident (a one in five year flood event) yielded more than 800,000 Ml with a peak daily inflow of 173,000 Ml.

Stream rises in the Warragamba catchment were associated with the occurrence of C&G. Forty-eight samples were collected in 'dry' or baseflow conditions in the routine programme. Seven of these samples (15%) contained C&G. In addition, 12 'flow event' samples were collected by grab or autosampler, during or immediately after river rises. Five of these 'flow event' samples (42%) contained C&G. Five of the six highest C&G concentrations in all catchment samples were 'wet weather' samples from the Warragamba catchment. High C&G levels were also found in other catchment samples collected soon after flow events in the Warragamba catchment (Figure 3).

There was a lower incidence of C&G at catchment sites where samples were collected more frequently on a routine basis (Werriberri Creek and Hawkesbury River). This may have been a result of a time based rather than event based sampling design. The 'low flow' period of this study provided many samples of the base flow condition. The number of samples containing C&G collected from the Werriberri Creek was greater than at any other site (4/30 or 4 positives in 30 samples).

Alternatively, the low incidence of C&G in the Hawkesbury River (1/69) could indicate that the Hawkesbury catchment above at North Richmond was 'cleaner' than the Warragamba catchment. This seems unlikely as two sewage treatment plants are situated above North Richmond and other catchment land uses include extensive urban development and intensive farming. As the three samples were collected each week, no additional flow event samples were collected from this site.

Lakes

Fc and Cp were not monitored continuously in the storage lakes. The incidence of Fc was very high in all lakes except Lake Burragorang, but concentrations were modest. Of the 35 samples from the other reservoirs 34 contained Fc and the highest level was 72 cfu 100 ml^{-1} (Wingecaribee Reservoir).

In Lake Burragorang microbial testing was not continuous. It was concentrated during the C&G contamination events in December (Fc and Cp) and February (Fc only). Fc and Cp were rare during these events. There were 11/98 detections of Fc, with a maximum of 2 cfu 100 ml^{-1}. *Clostridium perfringens* was not found in any sample (0/21).

Incidence of C&G in lakes

The streams of the Warragamba catchment discharge to Lake Burragorang and this lake had the highest incidence of C&G of all storage lakes (14% of all samples tested).

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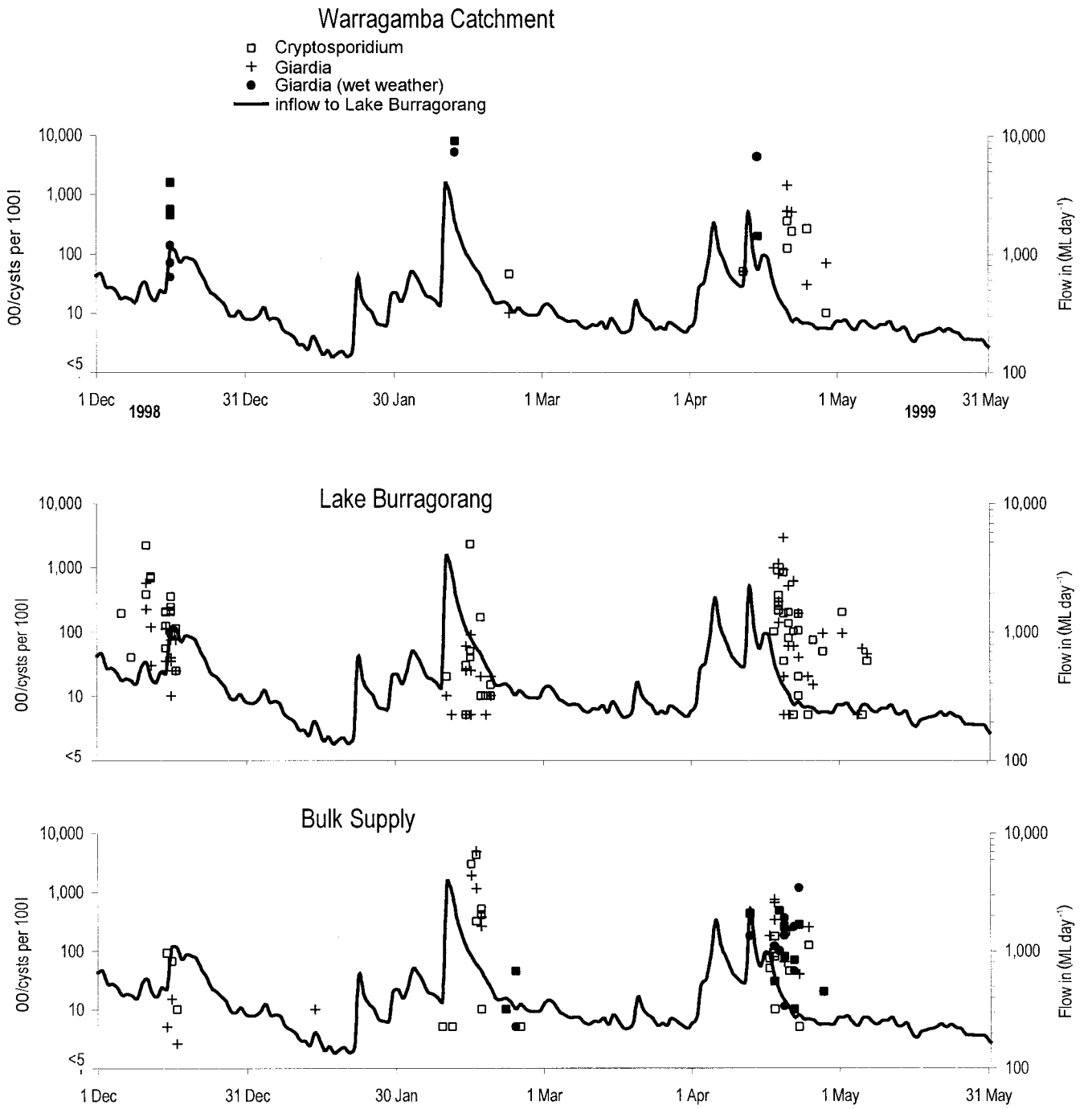

Figure 3 | Incidence of *Cryptosporidium* and *Giardia* in the Warragamba catchment, Lake Burragorang and all bulk supply sites, in relation to flow into Lake Burragorang. 'Flood event' samples from catchment sites are indicated by solid symbols in the top panel. The sampling frequencies are listed in Table 1. Non-detections in daily monitoring of Lake Burragorang (middle panel) and the bulk supply after December (lower panel) are not shown. C&G in Warragamba elements of the bulk supply are open symbols in the bottom panel.

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The apparent pattern of incidence of C&G after flow events that was observed at sites in the Warragamba catchment, was even clearer in Lake Burragorang (middle panel of Figure 3). Three contamination 'events' are clearly evident, in December 1998, February 1999 and April 1999. Sampling was daily but samples where C&G were not detected (<5 oo/cysts 100 l^{-1}) have not been shown. The peak oo/cyst concentrations exceeded 1,000 100 l^{-1}. Events lasted a minimum of 11 days (December and February) and a maximum of three weeks (May 1999).

Stream flow in relation to C&G in Lake Burragorang

The occurrence of both C and G near the dam wall was significantly associated with flow events in Werriberri Creek ($P = 0.001$ for both C and G) and with the total inflow to Lake Burragorang ($P = 0.02$ and $P = 0.001$ for C and G, respectively).

Werriberri Creek is the only waterway close to the dam wall that is gauged (Figure 1). Flow in this creek would reflect the runoff from the environs of the dam wall. Flow events during the period were relatively small. There were seven runoff peaks from the Werriberri catchment, ranging between 3 and 28 Ml day^{-1}. C&G were detected in the creek after two of these events. The flow peaks were not sampled.

When C&G were detected in the Warragamba catchment following a flow event, the sampling effort in Lake Burragorang was increased. In addition to the daily samples adjacent to the offtakes in the dam wall, samples were collected from sites in the two main arms of the reservoir. Exclusion of the results from the upstream sites from the data presented in Table 2 reduced the incidence of C&G in Lake Burragorang from 14% to 11%. This compares with 5% incidence (4/86) in all other reservoirs. The incidence of C&G in other lakes coincided with C&G events in Lake Burragorang (i.e. one detection in Prospect reservoir in mid-February; two detections in Wingecaribee Reservoir in April; and one detection in the Blue Mountains in April).

Magnitude of C&G concentrations in lakes

During each C&G contamination event, the maximum level of both C and G near the dam wall in Lake Burragorang was in the range 1,000–2,000 oo/cysts 100 l^{-1}, an order of magnitude higher than the maximum concentration recorded in the other lakes.

Bulk supply

There was a very high incidence of Fc in the Upper Canal Bulk Supply at Broughtons Pass (Table 2). The Upper Canal was chloraminated immediately below Broughtons Pass, 50 km upstream of Prospect WFP. The Warragamba pipelines were rarely tested for Fc. The incidence was the same as in Lake Burragorang (11%). The mixed bulk supply at Prospect WFP is typically one part Upper Canal and four parts Warragamba pipeline. The Prospect WFP samples were only tested for Fc during C&G events in Lake Burragorang. The frequency of occurrence of Fc was 31%. The source of this Fc contamination was most likely to be the Upper Canal. The detection of Fc despite chloramination in the Upper Canal probably indicates a loss of chlorine residual or ingress of surface water into the canal.

Fc were relatively common in the few samples from the Blue Mountains. The samples from Illawarra that were tested for Fc were drawn either from the bulk supply from Nepean or Woronora reservoirs. There was an intermediate incidence of Fc (29%) in these samples, but the level of contamination was very low. The maximum was only 3 cfu 100 ml^{-1}. The bulk supply was not tested for Cp.

Incidence of C&G in the bulk supply

In general the incidence of C&G in the bulk supply network (Figure 3 lower panel) coincided with the contamination events in Lake Burragorang. Non-detections (<5 oo/cysts 100 l^{-1}) have not been shown, but the entire system was monitored at least daily at two or more sites after December. The highest frequency of occurrence was in the elements of the bulk supply fed from Lake Burragorang.

The December event in Lake Burragorang (6–17 Dec) was poorly represented in the bulk supply monitoring programme. During this 12 day period, the Upper Canal was not sampled; there were 0/4 detections at the WFP and 3/6 detections in the Warragamba pipeline. Daily monitoring in the canal and at the WFP commenced after December. During subsequent events the sampling frequency occasionally rose to two or three per day at the WFP.

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The highest incidence of C&G in the bulk supply was in the Warragamba pipeline (27%). Two pipes convey water drawn from offtakes in the dam wall to Prospect WFP and both pipes were sampled every day, immediately downstream of the offtake. However, these samples were only analysed if C&G were detected either downstream (at the WFP) or upstream in Lake Burragorang. In the February and April contamination events there were eight incidences of C&G at Prospect WFP over 7 days and 11 incidences in the pipelines, over 8 days.

The incidence of C&G in other elements of the bulk supply (solid symbols in Figure 3 lowest panel) reflected the low detection frequencies in the storage lakes that fed them (Table 2). The Upper Canal was not sampled in December, but the incidence of C&G in the Upper Canal in February (1) and April (4) coincided with the contamination events in Lake Burragorang. The incidence of C&G in the other two systems also coincided with the event in Lake Burragorang in April.

Association between C and G

The presumptive (FITC) and confirmed (DAPI) abundance data for C&G in all sample types was log transformed and tested for correlation between C&G (MS Excel). Correlation coefficients for both data sets were extremely high (FITC = 0.94; DAPI = 0.90). This finding agreed with the co-occurrence of C&G (FITC only) reported during the Sydney contamination incident (Hawkins unpublished).

The consistently high correlation between the abundance of *Cryptosporidium* and *Giardia* throughout the Sydney water system agrees with the conclusions of the government inquiry into the Sydney water contamination incident (McClellan 1998) in dismissing suggestions that C&G reported in August 1998 were actually misidentified algal cells. Both *Cryptosporidium* and *Giardia* can be mistaken for phytoplankton cells (US EPA 1999). However, *Cryptosporidium* is quite different in appearance from *Giardia*. In order to misidentify both protozoans in the same sample, one phytoplankton species of the size, shape and autofluorescence of FITC stained *Cryptosporidium* must be present simultaneously with a phytoplankton species that mimics *Giardia*. We contend that the probability of the occurrence together of algal cells that resembled both *Cryptosporidium* and *Giardia* would be less likely than the appearance of one algal 'double' for either *Cryptosporidium* or *Giardia*. Yet in this and previous data sets collected from the Sydney water supply, *Giardia* was present in more than 85% of samples in which *Cryptosporidium* was detected.

If we accept that the laboratory identifications were accurate, there are at least two possible explanations for this pattern of co-occurrence. First, separate *Cryptosporidium* and *Giardia* infections occurred at different times and locations but the environmental stages of both protozoans were transported simultaneously from all sources by floodwater. A second interpretation is that both organisms are widespread and endemic throughout the catchment perhaps in a number of hosts. The concurrent isolation of C&G has been reported in a range of animals with and without diarrhoel disease (Olson *et al*. 1997; Hoar *et al*. 1999). These explanations are not mutually exclusive.

Indicators of C&G

There was no significant association between faecal coliforms or *Clostridium perfringens* spores and the presence of C&G. The presence of both C and G was associated with turbidity ($P = 0.09$) and conductivity ($P = 0.05$) in Warragamba catchment streams based on the logistic regression analysis. The association of C&G with higher turbidity was consistent with transport at higher flows, when turbidity was also elevated. The observed association with conductivity has less predictive value, as conductivity varies between Warragamba sub-catchments as well as with flow.

In Lake Burragorang, at the 90% significance level, only C was associated with turbidity ($P = 0.09$) and only G was associated with Fc ($P = 0.07$). C&G were both significantly associated with pH ($P = 0.01$ and 0.08). The association with conductivity was not tested. The significance of pH and turbidity as indicators of C&G in Lake Burragorang was an important finding because these signals can be measured in real time and used to identify water layers such as flood inflows for sampling. Their usefulness as indicators diminished over time, probably

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Table 3 | Geometric mean of C&G in each element of the Warragamba water supply system for each contamination event

	Crypto positive (n)			*Cryptosporidium* 100 l^{-1}			*Giardia* 100 l^{-1}		
Period	**Ca**	**L**	**BS**	**Ca**	**L**	**BS**	**Ca**	**L**	**BS**
All events	12	39	22	243	79	69	199	46	146
December	3	15	4	742	197	40	73	62	6
February	2	10	8	600	32	102	230	16	1,023
April	7	14	10	117	57	57	294	74	195

Ca, all Warragamba catchment streams.
L, Lake Burragorang site at the dam wall.
BS, bulk supply (Warragamba Pipeline and Prospect WFP).
Geometric mean of all DAPI positive cysts and oocysts per 100 l.

due to the separation of the protozoans from the inflow water mass by sedimentation.

Barriers to transport of C&G within the water system

Timing

C&G were detected in Lake Burragorang two or three days after their appearance in streams in the Warragamba catchment in February and in April. The daily sampling frequency failed to distinguish between the arrival of C&G in Lake Burragorang and in the bulk supply. In April C&G were detected in the bulk supply one day before their detection in Lake Burragorang. Therefore C&G travelled rapidly through the system and selective withdrawal at the dam wall was not a barrier to the entry of these contaminants into the bulk supply.

Losses of C&G within the raw water system

Losses of C&G at different stages of the water system were assessed by comparing concentrations of C&G at catchment, lake and bulk supply sites in the Warragamba watershed (Table 3). The mean and maximum concentrations of both C and G were almost always lowered after passage from the catchment to the Lake Burragorang in each event (Table 3).

However, the concentrations in Lake Burragorang and the bulk supply were little different, for each event or overall. In February and April the mean concentration of C and G in the bulk supply was greater than or equal to concentrations in the lake (Table 3). In the December event the highest levels of C&G were in the lake, but sampling in the bulk supply was infrequent and only four positive samples were detected. These results demonstrate that selective withdrawal was not effective in preventing the transfer of C&G from the lake to the bulk supply.

Vertical distribution of oo/cysts in Lake Burragorang

Internal waves commonly cause vertical displacement of the hypolimnion–metalimnion boundary in Lake Burragorang. This phenomenon was an agent for the contamination of the bulk supply by floodwater during the Sydney contamination incident (McClellan 1998). During the December and February contamination events, excursions of the base of the metalimnion of up to 8 m were recorded (Figure 2, 7 December and 10 February). The amplitude of this oscillation was damped in the upper metalimnion by the steep thermal (density) gradient.

To ensure that excursions of the metalimnion did not confound the C&G sampling, the boundaries of the sample intervals were defined by isotherms, not by depth below the surface. During both the December and February 'events' in Lake Burragorang the thermocline was sharply delineated (Figure 2). The 13°C isotherm was at 25 ± 4 m below the surface in December.

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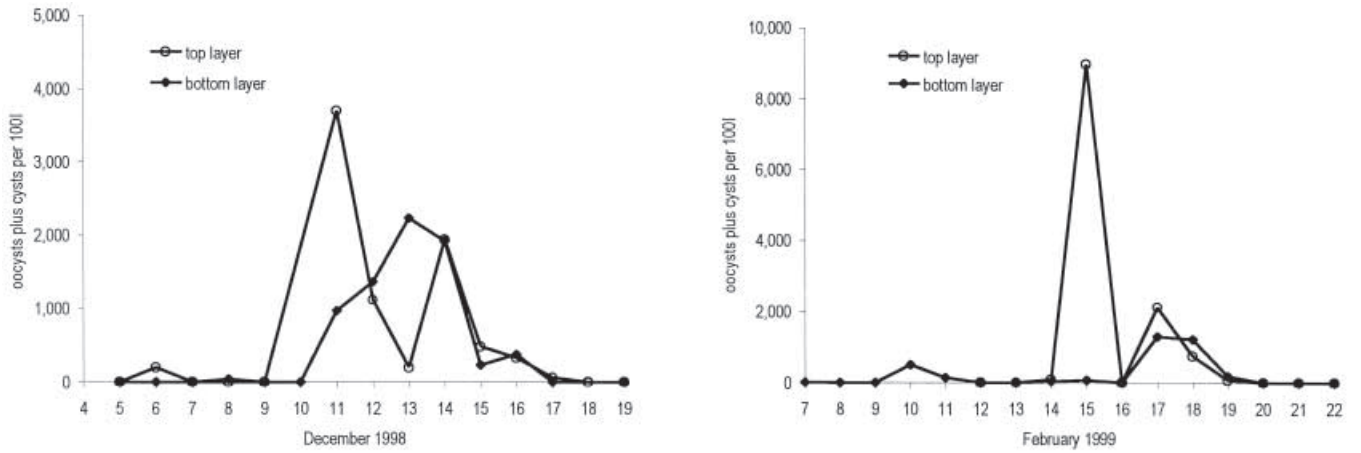

Figure 4 | Daily change in the distribution of protozoans (C+G), near the dam wall in Lake Burragorang during December and February. The C+G data are the arithmetic mean of all *Cryptosporidium* and *Giardia* each day, integrated within the appropriate layer.

Sample intervals that spanned the epilimnion and the upper metalimnion were combined as the 'top' layer. This was anticipated to be the locus for C&G entering the lake in storm water, because inflows in summer are colder than the epilimnion but much warmer than the hypolimnion. On 12 and 13 February the peak flow from Werriberri Creek would have inserted at the 19°C isotherm, about 15 m below the surface.

Observations of the February event

A pulse of oocysts and cysts was detected in the 'top' layer on 15 February with trace contamination in the hypolimnion (Figure 4). On the second day, no oocysts or cysts were detected in the water column, but a high concentration was found in the pipeline, which was drawing water from both layers between 22 and 44 m. On the third day (17 February), oocysts and cysts were again found in both layers. On the fourth day, the oocyst + cyst maximum was below 35 m, in the bottom layer. Six days after the initial peak, oocysts and cysts were no longer detectable in the upper 50 m of the water column. This pattern of distribution suggested rapid sedimentation of C&G from the top to the bottom layer.

Observations of the December event

The oocyst + cyst distribution in the December event (left panel in Figure 4) showed a similar pattern to that of February. There was a small flood event in Werriberri Creek with a peak flow on 10 December (Day 1). No sample was collected from the top layer on that day and C&G were not present in the bottom layer. A pulse of C&G was detected in the top layer on day 2 (11 December) and there was evidence of a second pulse of C&G in the top layer on day 5 (14 December). The bimodal pattern of C&G in the top layer was matched by a similar distribution in the bottom layer, lagged by two days.

DISCUSSION

Transport of C&G within the watershed

The transport of particulate material by streams is a cyclic process of sedimentation and accumulation, then re-suspension and down river movement. Within Sydney's water supply catchments, brief storm events export most of the particulate load accumulated over weeks or months in a single spate. In the Werriberri catchment in 1990, 61% of the annual phosphorus load was exported by storm flows in only three days (Cullen 1991). Automated sampling, triggered by increased stream height, is the most reliable method to monitor the transport of particulate loads accurately through these streams.

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If C&G behave like other particles in streams, then similar sampling methods are required. The sample with the highest concentration of C&G in the Warragamba catchment was collected by an autosampler on the Cox's River during a flood flow. Grab samples, collected from catchment streams at the peak of the hydrograph in December, February and April, all showed that C&G were transported during flood events, but did not represent the entire event load. Further evidence for the transport of C&G by storm flows is available from 'within-study' comparisons elsewhere (Hansen & Ongerth 1991; Stewart *et al*. 1997).

Sources of C&G

Sewer overflow in storms is a common cause of C&G contamination of storm water (States *et al*. 1998*b*). However, there were no reported sewer overflows within the Warragamba catchment during this study. The relatively low levels of Fc and Cp in the catchment streams supported this anecdotal evidence.

Diffuse sources appear to be more likely contributors to the C&G observed in the Warragamba catchment. C&G from grazing or native animals would be transported into waterways by flow events. The levels of C&G in streams in pristine Californian watersheds increased during storm flows (Mager *et al*. 1998). In rural Australian streams, modest rainfall of more than 9 mm, within 48 hours before sampling, was sufficient to increase *Cryptosporidium* oocyst concentration (Thurman *et al*. 1998).

An unusual flow event occurred in the Cox's River after the partial breach of the dam wall in Lake Lyell in April 1999. C&G could only have derived from river banks or bed sediments, because there was no flood runoff from the catchment at the time. The C&G transported by this event and collected in the autosampler at Kelpie Point was identified as type II or bovine genotype of *C. parvum* (Hawkins *et al*. in prep.). This material must have been transported from grazing lands below Lake Lyell. The type II genotype is not conclusive evidence of a non-human source. Nevertheless, there are no towns below Lake Lyell, so we speculate that the source(s) was most likely domesticated or native herbivores. The relatively low levels of *Clostridium perfringens* in the catchment streams support herbivores as a source of C&G. Cp commonly occur in human and dog faeces but are relatively uncommon in the faeces of herbivores.

Sources and transport of C&G in Lake Burragorang

The Sydney water contamination incident in August 1998 was preceded by a 700,000 Ml inflow to Lake Burragorang. The high concentrations of C&G near the dam wall during the incident were probably transported into the lake by this flood, then advected to the dam wall within the floodwater intrusion (McClellan 1998). The Cox's and Wollondilly Rivers enter the lake more than 40 km upstream from the dam wall. The first detections of C&G in Lake Burragorang after the August 1998 flood coincided with the arrival of floodwater from these tributaries at the dam wall (McClellan 1998).

The three contamination events recorded during this study were also preceded by stream rises throughout the Warragamba catchment. However the timing and the volume of these inflows suggest the C&G contamination at the dam wall could not have arisen from the major tributaries. The largest inflow was only 4,000 Ml, from the Cox's River in February 1999. Inflows of this volume were unlikely to penetrate to the dam wall. In all three events, C&G were detected at the dam wall within one day of the flood peak entering the lake. There was no evidence of a lag in the arrival of oo/cysts at the dam wall consistent with transport across the lake in water masses emanating from the major tributaries.

Type II *Cryptosporidium* oocysts were also found near the dam wall in each event (Hawkins *et al.* in press). Human sources or domesticated or native animals could potentially have contaminated Werriberri Creek or the smaller streams in the environs of the dam wall. The low Fc levels and the absence of *C. perfringens* was suggestive of a herbivore source.

Loss processes

A sedimentation rate for purified *Cryptosporidium parvum* oocysts of 0.03 m day^{-1} has been reported by Medema

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et al. (1998) and confirmed by our calculations. This rate is too slow to account for the disappearance of oocysts from Lake Burragorang after the *Cryptosporidium* crisis and in the subsequent events reported here. Free oocysts, sedimenting at 0.03 m day^{-1}, would persist in the water column in Lake Burragorang for 5 years (assuming no turbulence). In fact, C&G disappeared completely from the water column 3 weeks after the first detection in every event.

The disappearance of C&G could be due to decay, predation or accelerated sedimentation. The decay of all oocysts and cysts within 3 weeks seems unlikely. Oocysts are robust and long-lived in the environment with die-off rate (loss of viability) estimated at 0.001–0.004 h^{-1} (Medema *et al*. 1997). Non viable or excysted oo/cysts would still be detected by the flow cytometry technique, as presumptive (FITC) counts.

Predation by zooplankton could account for the disappearance of oo/cysts. Copepod and cladoceran zooplankters can selectively sieve particles of this size from water, as they graze on algal cells of similar dimensions. However, these efficient filter feeders do not usually populate the metalimnion or hypolimnion, as their algal prey grow in the epilimnion. The most likely explanation for the disappearance of C&G was accelerated sedimentation of the oo/cysts through the water column. But how could C&G sediment so rapidly?

Measurements of C&G sedimentation rates are rare. One experimental study showed that *Cryptosporidium* oocysts and *Giardia* cysts added to secondary sewage effluent settled at between 2 and 5 m day^{-1}, the same rate as larger, low density, bio-floc particles (Medema *et al*. 1998). The authors reported that 75% of the oocysts and cysts were bound to the bio-floc.

Agglomeration of C&G with other particles accelerates sedimentation

The adhesive properties of C&G have been widely reported. Attachment of seed oocysts to sample containers was identified as a common source of systematic error in *Cryptosporidium* enumeration (Klonicki *et al*. 1997). An important source of imprecision in the ICR (Information Collection Rule) method for *Cryptosporidium* was variable recovery efficiency in the density gradient purification step (Klonicki *et al*. 1997; Bukhari *et al*. 1998; Champion 1998). The apparent density of *Cryptosporidium* seed oocysts would alter if they were incorporated in an agglomeration of silt particles formed in a floodwater sample. The more dense agglomerate (including the oocysts) would sink through sucrose or Percoll gradients designed to float off single oo/cysts; reducing recovery efficiency.

Variation in recovery efficiency has been shown to depend on the contact time between the oocyst spike and the raw water (Fricker 1995). In that study, adsorption kinetics between the seed and other particles in raw water was suggested as the basis for variable recovery.

The extensive evidence for the adhesive nature of *Cryptosporidium* oocysts from the literature on *Cryptosporidium* detection methods suggests a mechanism that may explain the simultaneous disappearance of both C and G from the water column: accelerated sedimentation of oo/cysts by binding to large floc particles in storm water. Storm water is highly turbid and flocs with median size of 70 μm have been measured in storm water intrusions in Lake Burragorang (Hawkins unpublished).

The clumping behaviour of *Cryptosporidium* is recognised in the statistical models developed to predict the occurrence of oocysts in water samples. These models use non-Poisson distributions such as negative binomial (Teunis *et al*. 1997) or the beta distribution (Nahrstedt & Gimbel 1996), specifically designed to deal with clumped populations.

A simple numerical model of the sedimentation of oo/cysts through a layered medium was developed. This two-layer model was fitted to the observed data from the December and February events. The parameter values to obtain best fit were very similar for both sets of experimental data. These were sedimentation rate of 10 m day^{-1} by C&G attached to 17 μm particles with a matrix density of 1.8. The decay coefficient was 0.01–0.02 h^{-1}.

Surveys elsewhere

The methods used to measure C&G in water in the past decade are not renowned for high levels of reproducibility or accuracy (Clancy *et al*. 1994; Klonicki *et al*. 1997), so

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Table 4 | The incidence of *Cryptosporidium* and *Giardia* contamination in surface waters

Description	Sample number	% Crypto occurrence	Mean* Crypto 100 l^{-1}	% *Giardia* occurrence	Mean* *Giardia* 100 l^{-1}	Ref.
General surface waters US	257	55	43	16	3	Rose *et al.* 1991
Surface raw water for 72 US WFP	262	52	240	45	200	LeChevallier and Norton 1995
Surface raw water—6 German WFP	105	47	116	64	88	Karanis *et al.* 1998
Surface water in Warragamba catchment	60	20	243	20	199	This study
Lake Burragorang	389	14	88	10	53	This study
Bulk supply from Lake Burragorang	214	10	69	8	146	This study

*Geometric mean.

comparisons between studies are difficult. Nevertheless, the sensitivity of the IFA method used in the Sydney study was at least equal to methods used elsewhere (Hoffman *et al*. 1997). Therefore we can state with some confidence that the concentrations of C&G that challenge Sydney's WFPs were of the same order as in waters supplying WFPs in USA and Europe, but their occurrence in Sydney was much less frequent (Table 4).

CONCLUSIONS

The study monitored the sources and the transport of C&G during a period of low flows in the catchment and intense thermal stratification in the reservoir. C&G were mobilised by increased stream flow within the catchment. The concentrations of C&G were highest in the catchment and diminished after entry into the lake. However, reservoir storage and selective withdrawal failed to reduce the concentration of C&G entering the bulk supply. There were several reasons for the ineffectiveness of the lake as a barrier:

1. The rapid arrival of pulses of C&G at the offtake zone in Lake Burragorang suggested the sources were in nearby catchments and retention time in the lake was minimal, so sedimentation and dilution processes had limited effect.
2. Thermal stratification of the lake in summer created a sharp density gradient, which inhibited mixing and dilution of storm water.
3. The time lag between sample collection and receipt of C&G analytical results delayed recognition of the onset of a contamination event until after contaminants had already entered the bulk supply.

Loss processes

The rapid disappearance of C&G from the lake water within 10–20 days was incompatible with the slow sedimentation rate of free oo/cysts. We propose that the accelerated sedimentation of C&G observed in Lake Burragorang was due to incorporation of C&G into large flocs produced in storm water intrusions.

Management to improve the effectiveness of the lake as a barrier

Effective reservoir management requires an understanding of the system and recognition of the kind of events that will mobilise C&G from catchment sources and transport these particles through the water system. Strategies to avoid contamination of the supply by C&G during events include real time monitoring of catchment and lake water

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quality and positioning of offtakes to avoid abstraction of floodwater and prolong the detention of storm water in lakes.

- Thermistors can be used to monitor the activity of internal waves in real time and permit optimisation of offtake depths to minimise the risk of C&G being abstracted.
- Abstraction from the epilimnion of warm monomictic lakes should minimise the risk of C&G contamination, as floodwater will underflow or interflow through these waterbodies.
- Detention of storm water for short periods (days) should effectively remove C&G transported by floods, through sedimentation.

Monitoring design

Daily monitoring clearly showed that contamination in the Sydney water supply system was episodic. These brief pulses of C&G contamination would be better sampled by automated integrating methods than instantaneous grabs. In future surveillance of C&G in the system, the sampling effort should be focused on hazard events that resuspend and transport particulate material.

Indicators of C&G contamination

Each contamination event was preceded by stream rises in the Warragamba catchment, but not all hydrographic events transported C&G. Physico-chemical identifiers of floodwater were also associated with C&G in Lake Burragorang. However, the association diminished over time, as oocysts and cysts apparently sank. Faecal coliforms and *Clostridium perfringens* were poor predictors of the presence of C or G.

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