Association of *Cryptosporidium* with bovine faecal particles and implications for risk reduction by settling within water supply reservoirs

Justin D. Brookes, Cheryl M. Davies, Matthew R. Hipsey and Jason P. Antenucci

ABSTRACT

Artificial cow pats were seeded with *Cryptosporidium* oocysts and subjected to a simulated rainfall event. The runoff from the faecal pat was collected and different particle size fractions were collected within settling columns by exploiting the size-dependent settling velocities.

Particle size and *Cryptosporidium* concentration distribution at 10 cm below the surface was measured at regular intervals over 24 h. Initially a large proportion of the total volume of particles belonged to the larger size classes (>17 μm). However, throughout the course of the experiment, there was a sequential loss of the larger size classes from the sampling depth and a predominance of smaller particles (<17 μm). The *Cryptosporidium* concentration at 10 cm depth did not change throughout the experiment.

In the second experiment samples were taken from different depths within the settling column. Initially 26% of particles were in the size range 124–492 μm. However, as these large particles settled there was an enrichment at 30 cm after one hour (36.5–49.3%). There was a concomitant enrichment of smaller particles near the surface after 1 h and 24 h. For Pat 1 there was no difference in *Cryptosporidium* concentration with depth after 1 h and 24 h. In Pat 2 there was a difference in concentration between the surface and 30 cm after 24 h. However, this could be explained by the settling velocity of a single oocyst. The results suggested that oocysts are not associated with large particles, but exist in faecal runoff as single oocysts and hence have a low (0.1 m d^{-1}) settling velocity. The implications of this low settling velocity on *Cryptosporidium* risk reduction within water supply reservoirs was investigated through the application of a three-dimensional model of oocyst fate and transport to a moderately sized reservoir (26 GL). The model indicated that the role of settling on oocyst concentration reduction within the water column is between one and three orders of magnitude less than that caused by advection and dilution, depending on the strength of hydrodynamic forcing.

Key words | *Cryptosporidium*, cow pats, pathogens, oocyst, risk assessment

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INTRODUCTION

The pathogenic protozoan *Cryptosporidium* is of particular concern to the water industry because of its longevity (Medema *et al.* 1997; Olson *et al.* 1999) and the resistance of its oocysts to treatment processes (Robertson *et al.* 1992). Oocysts can enter surface waters via sewage discharges, septic seepage, agricultural runoff and by direct deposition of animal faeces.

Cattle can be a major source of *Cryptosporidium* in watersheds, with infected animals shedding faecal concentrations of up 10^7 oocysts per g (Medema *et al.* 2001)

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while asymptomatic animals may still shed up to 10^4 per g (Fayer *et al.* 2000). The mastication of plant material within the cattle gut and the subsequent scouring of the stomach wall dislodges oocysts, and hence has a significant impact on *Cryptosporidium* – particle interactions.

The size of particles with which *Cryptosporidium* is associated is a major factor influencing the transport of these pathogens across a landscape, along a river or through a reservoir. *Cryptosporidium* associated with large particles has a greater chance of interception or settling, and so potentially there is a greater reduction in risk than if they were associated with small particles or transported as single unattached oocysts.

In contrast to bacteria and viruses (LaBelle & Gerba 1979; Gantzer *et al.* 2001), there is a growing body of evidence suggesting that the tendency of *Cryptosporidium* oocysts to attach to particles is relatively low (Dai & Boll 2003). Electrophoretic mobility measurements and calculated zeta potentials for *Cryptosporidium* oocysts indicate that these particles are strongly negatively charged at neutral pH (Ongerth & Pecoraro 1996). Consequently they may be adequately aggregated and flocculated during conventional water treatment under alkaline conditions but may not adsorb well to natural clays in the environment. This hypothesis is supported by Dai & Boll (2003) who determined that oocysts tended not to attach to natural soil particles but would travel freely in the water. Novel work by Considine *et al.* (2000, 2001) using atomic force microscopy to examine the surface properties of oocysts generally supported this hypothesis but concluded that protein-linked tethering between silica and oocysts can occur, which may facilitate adhesion. However, this interaction relies on contact and so there must be adequate turbulence in the system to increase the probability of collision between particles and oocysts.

Individual oocysts have been found to have a settling velocity in water of approximately $0.03\,\mathrm{m\,d^{-1}}$ (Medema *et al.* 1998). This was found to increase to approximately $2.5\,\mathrm{m\,d^{-1}}$ when associated with particles from biologically treated sewage effluent, in which the sediment dynamics of the particles dominated over the smaller oocysts (Medema *et al.* 1998). These sedimentation values were found to be well described by theoretical sedimentation kinetics, i.e. Stokes' law:

$$V_s = \frac{g d^2}{18\,\mu}(\rho_p - \rho_w) \quad (1)$$

where V_s is the settling velocity ($\mathrm{m\,s^{-1}}$), g is the acceleration due to gravity ($\mathrm{m\,s^{-2}}$), d is the particle diameter (m) (of either the single oocyst or aggregated particle), μ is the dynamic viscosity of water ($\mathrm{N\,s\,m^{-2}}$), ρ_p is the density of the particle ($\mathrm{kg\,m^{-3}}$) and ρ_w is the density of water ($\mathrm{kg\,m^{-3}}$). Stokes' settling velocity calculation assumes that the displaced water movement around the particle is laminar (Reynolds 1984). McNown & Malaika (1950) demonstrated that, for calculated Reynolds numbers (*Re* values) of less than 0.1, there is little departure from the Stokes' equation and for $Re < 0.5$ the error is less than 10%. However, larger particles (radius $>300\,\mu\mathrm{m}$) move at higher velocities, experience proportionately more drag and consequently the laminar flow condition of the Stokes' equation is violated as the critical particle–Reynolds number exceeds 0.5 (Reynolds 1984).

Based on field measurements from Lake Burragorang, NSW, Australia, Hawkins *et al.* (2000) estimated sedimentation rates of oocysts of $5-10\,\mathrm{m\,d^{-1}}$. Whilst the settling of individual oocysts is extremely slow, the ability for the oocysts to attach to particles and potentially increase their settling velocity by two orders of magnitude is an important issue for the modelling of pathogen transport and managing risk in drinking water reservoirs.

There appear to be two conflicting arguments as to whether *Cryptosporidium* is associated with particles. The surface properties of oocysts suggest they would not adsorb readily to particles but the very high settling velocities recorded by Hawkins *et al.* (2000) and Medema *et al.* (1998) would suggest the oocysts must be associated with large, heavy particles. An alternative is that the oocysts may be physically encased within an organic matrix of cattle faeces, given that it may be a major source of *Cryptosporidium* in grazed catchments. The aim of this study was to determine the particle size distribution of cattle faeces and determine which size particles, if any, *Cryptosporidium* is associated with. The determination of the particle associations of *Cryptosporidium* will enable more accurate modelling of transport through a reservoir and the potential for risk reduction due to pathogen settling and implications for reservoir management.

METHODS

Rainfall simulation

The rainfall simulator was designed by Dr Paul Hackney and co-workers, University of Western Sydney, NSW, Australia and consisted of a frame 4.3 m in length, 2.65 m wide and 2.9 m high. The distance from the tip of the nozzles to the surface of the cow pat was approximately 2.5 m. A single downward facing nozzle delivered the spray, which was regulated with a pressure gauge to control rainfall intensity at a rate of 55 mm h^{-1} delivered for 30 min. A 12 V motor controlled the sweeping of the nozzles at a frequency of approximately 0.5 Hz. The base of the simulator consisted of a tray on which the faecal pats were placed. The slope of the base of the simulator was set at 5°. Water that did not fall on the tray was collected by gutters on the side of the frame and recycled.

Artificial rainwater was pumped from a 1000 L container and consisted of deionised water to which was added 4.07 mg L^{-1} $NaNO_3$, 3.24 mg L^{-1} NaCl, 0.35 mg L^{-1} KCl, 1.65 mg L^{-1} $CaCl_2.2H_2O$, 2.98 mg L^{-1} $MgSO_4.7H_2O$, 3.41 mg L^{-1} $(NH_4)_2SO4$ (after Laegdsmand 1999).

Artificial cow pat preparation

Artificial cow pats were prepared using the procedure described by Davies *et al.* (2005). Briefly, 1 kg of gamma-irradiated (120 kGy) fresh cattle faeces was seeded with 10^6 *Cryptosporidium* oocysts. A springform tin with a diameter of 19 cm was used to mould the 1 kg of seeded faeces into a cow pat shape. The tin was removed and the pat was allowed to spread and dry in an incubator at 20°C for 22–24 h to mimic normal drying and crusting.

Cryptosporidium detection and enumeration

Runoff samples (10 mL portions) were assayed by immunomagnetic separation (IMS) (Dynal Biotech, Oslo, Norway) followed by immunofluorescent antibody (IFA) staining with FITC-labelled monoclonal antibody (EasyStain™, BTF Decisive Microbiology, North Ryde, NSW, Australia) according to the manufacturer's recommended procedure. An internal control in the form of 100 ColorSeed™ oocysts (BTF Decisive Microbiology) was added to four samples to determine the percent recovery of *Cryptosporidium* from different fractions of the runoff (at 0, 5 and 15 min, and 24 h).

Settling column set-up

Duplicate artificial cow pats which had been seeded with 10^6 *Cryptosporidium* oocysts were simultaneously disintegrated under a simulated rainfall event and the generated runoff was collected and settled to enable different size fractions to be collected. The particle size distribution and *Cryptosporidium* concentration of each fraction was determined.

Experiment 1

Duplicate faecel pats were subjected to a simulated rainfall event. The runoff slurry generated from each pat was mixed thoroughly and poured into six measuring cylinders to a height of 30 cm. Each duplicate treatment consisted of three 1 L measuring cylinders. Samples were withdrawn from each cylinder from 10 cm below the surface in a time series: initial, 1 min, 2 min, 5 min, 15 min, 60 min, 240 min, 390 min and 1440 min. At 1440 min a sample was also taken from the very surface of the column. The three columns in each duplicate were sampled sacrificially to minimise impacts from reduction in volume due to over-sampling. Each measuring cylinder had four samples taken from it sequentially.

At each sampling time 10 mL was withdrawn for *Cryptosporidium* concentration determination and 10 mL for particle size determination. The particle size distribution was determined using a Malvern Mastersizer.

Experiment 2

Duplicate faecel pats were subjected to rainfall as above and the runoff generated from each pat was divided between two measuring cylinders. Initial samples were withdrawn for the determination of particle size distribution and *Cryptosporidium* concentration. In this experiment single cylinders were used to create a gradient and each was

sampled at either 1 h or 24 h from the surface, 10 cm, 20 cm and 30 cm below the surface.

Data analysis

Data analysis was performed with JMP software from SAS Institute Inc. ANOVA were performed following a test for homogeneity of variance. Where variance was not homogeneous, a Welch ANOVA was used. Treatments with significant differences were identified using a Tukey–Kramer *post hoc* test.

RESULTS

The particle size distribution of a portion of unmixed cow faeces was determined to confirm that the procedure for preparing the cow pat and seeding with *Cryptosporidium* did not affect the size of faecal particles (not shown). There was no significant difference in size distribution of faecal particles in untreated faeces and artificially prepared cow pats ($p = 0.999\,81$). It is apparent that cattle are efficient at masticating and digesting grass into very small particles. Consequently these particles will settle slowly in a water column.

Experiment 1

The particle size distribution at 10 cm below the surface was measured at regular intervals over 24 h. Initially a large proportion of the total volume of particles belonged to the larger size classes (Figure 1). However, throughout the course of the experiment, there was a sequential loss of the larger size classes from the sampling depth and a predominance of smaller particles. Initially 30% of the total particle volume at 10 cm was in size classes less than 17.2 μm. However, following 24 h in a settling chamber this increased to 83% (Figure 1).

If the *Cryptosporidium* oocysts were attached to larger particles a decrease in *Cryptosporidium* concentration at the depth sampled from would be expected over time. Figure 2 shows that this was not the case and that there was no significant difference in the concentration of oocysts in runoff from Pat 1 at any time ($p = 0.6053$). The concentration of oocysts in runoff from Pat 2 after 15 min was different from the concentration after 1440 min but no other samples differed significantly ($p = 0.0310$). Recoveries of ColorSeed™ oocysts from different fractions were similar at 63% ± 4.4% ($n = 4$).

Experiment 2

The volume of runoff collected was 6.8 L and 6.2 L from Pat 1 and Pat 2, respectively. There was no significant difference in the size distribution of particles in runoff from Pat 1 and Pat 2 at any time or depth ($p < 0.0001$) and only Pat 1 results are shown in Figure 3. There was a significant difference in the size distribution of particles at 0 cm and at 30 cm in the column after 1 h ($p = 0.2659$) and 24 h ($p = 0.1043$). The settling column effectively fractionated the particles into different size classes over time. Initially 24.5% of particles were in the size range 124–492 μm. However, as these large particles settled there was an enrichment at 30 cm after 1 h (36.5% for Pat 1, Table 1, 49.3% in Pat 2, Table 2). There was a concomitant enrichment of smaller particles near the surface after 1 h and 24 h (Figure 3, Table 1, Table 2).

There was no significant difference in *Cryptosporidium* oocyst concentration between depths after 1 h ($p = 0.399$) or 24 h ($p = 0.2421$) for Pat 1 (Table 3). For Pat 2 there was no difference in *Cryptosporidium* oocyst concentration with depth after 1 h ($p = 0.5580$, Table 3), but after 24 h there was a significant difference ($p = 0.0493$). There was no significant difference between 0 cm and 30 cm (Table 3). There was no significant difference in *Cryptosporidium* concentration at any depth between 1 h and 24 h except for Pat 2 at 0 cm which was significantly less at 24 h than at 1 h ($p = 0.0249$).

DISCUSSION

Settling columns were used to size-fractionate particles from cow pat slurry. The two experiments used slightly different approaches to sampling. The first experiment had a sampling point 10 cm below the surface so the sampling over time showed a sequential loss of large particles and an enrichment of smaller particles. There was no significant

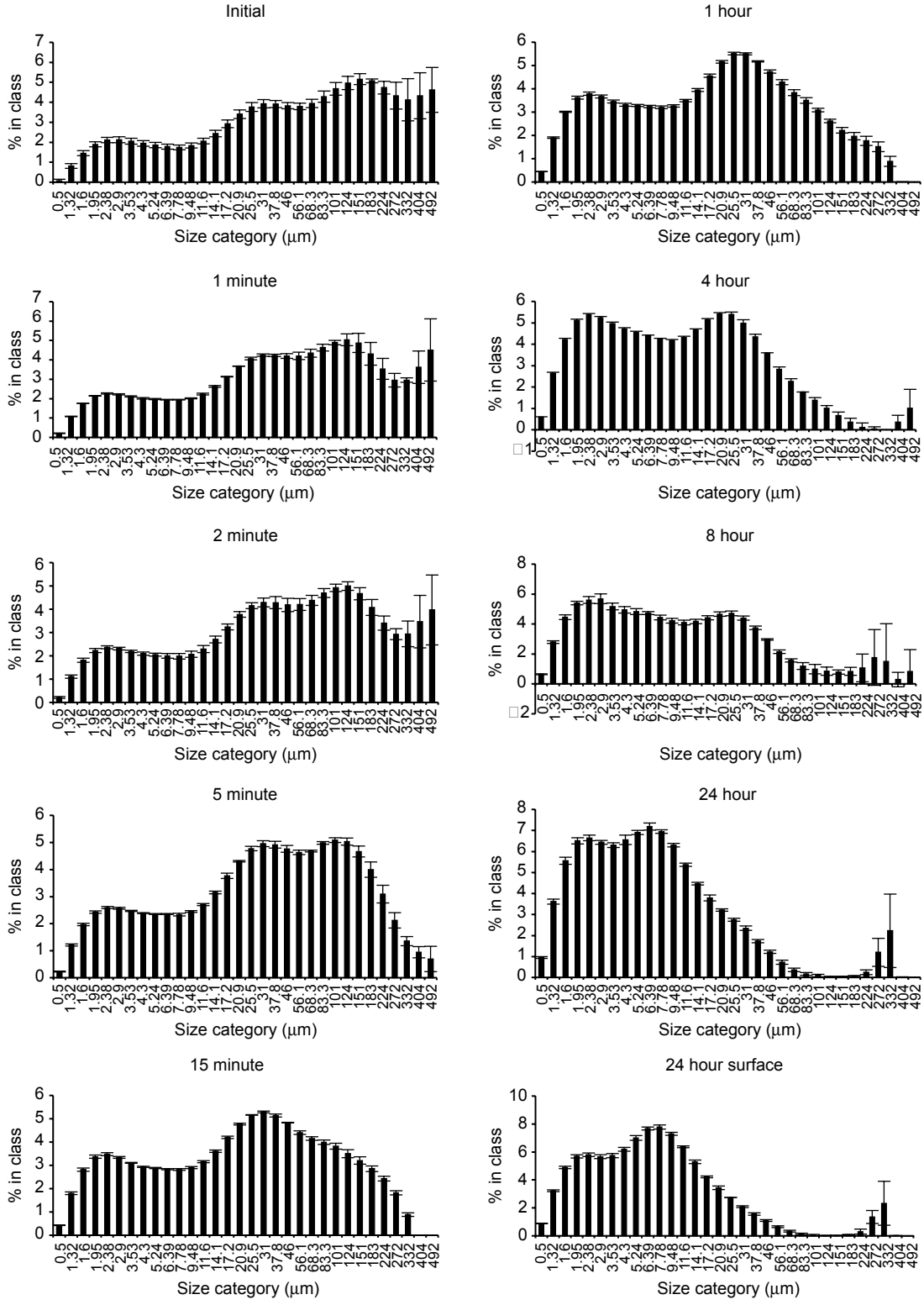

Figure 1 | Size particle distribution of faecel particles in runoff at a depth of 10 cm after different periods of settlement.

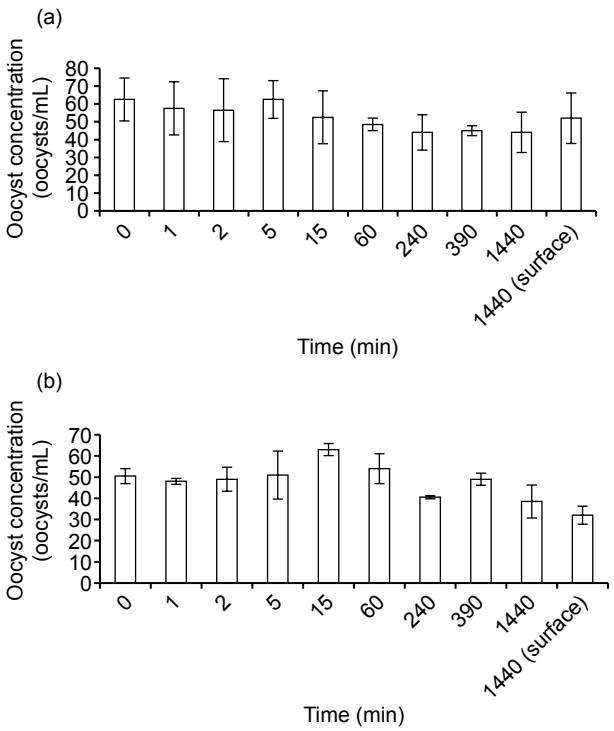

Figure 2 | *Cryptosporidium* oocyst concentration from Pat 1 (a) and Pat 2 (b) sampled at 10 cm below the surface of a settling column for a range of times. Values are means of two replicate columns, error bars are ±1 SD.

change in *Cryptosporidium* oocyst concentration with time, suggesting that oocysts were not attached to large particles but remained suspended throughout the course of the experiment.

In the second experiment, in which samples were withdrawn from various depths of the settling column, there was a time-dependent enrichment of larger particles towards the bottom of the settling column. However, an enrichment of oocysts was only observed in one replicate cow pat. The apparent enrichment could be explained by the settling velocity of a single oocyst or, at most, the settling velocity of a particle with the equivalent size of two aggregated oocysts.

The change in oocyst concentration observed after 24 h in Pat 2 (Table 3) suggests the oocysts must be settling at least 10 cm and possibly up to 30 cm in 24 h. This equates to a settling rate of between $1.16 \times 10^{-6}\,\mathrm{m\,s^{-1}}$ and $3.47 \times 10^{-6}\,\mathrm{m\,s^{-1}}$. Medema *et al.* (1998) measured the geometric mean density of oocysts using Percoll density gradients to be $1045.4\,\mathrm{kg\,m^{-3}}$, and given an oocyst diameter of 5 μm, the theoretical settling velocity of a single oocyst is $6.45 \times 10^{-7}\,\mathrm{m\,s^{-1}}$. To accommodate the observed settling velocity the diameter of oocyst-associated particles would only be 6.7–11.6 μm: either single oocysts, or at most two oocysts aggregated together.

In conclusion, oocysts were not associated with large particles, but existed in cow pat slurry from rainfall runoff as single oocysts. This has significant implications for *Cryptosporidium* transport in a watershed and the risk reduction that could be expected due to settling in water supply reservoirs.

MODELLING *CRYPTOSPORIDIUM* DISTRIBUTION: THE ROLE OF SETTLING IN RISK REDUCTION

Model description

To investigate the significance of particle settling in drinking water reservoirs and its implications for reservoir management, a *Cryptosporidium* module was developed within the three-dimensional (3D) hydrodynamic–ecological model ELCOM–CAEDYM (Hipsey *et al.* 2004). In this model, oocyst viability is governed by temperature (T) and exposure to ultraviolet light (UV), and transport is governed by advection, settling and resuspension. The expression for oocyst fate and transport, without the 3D advective terms, is summarized as

$$\frac{\mathrm{d}C}{\mathrm{d}t} = \left[-\mathrm{f}(T) - \mathrm{f}(UV) - \frac{V_\mathrm{s}}{\Delta z}\right]C + \mathrm{f}(\tau) \qquad (2)$$

where C is the oocyst concentration (oocysts/m^3), V_s is the settling velocity ($\mathrm{m\,s^{-1}}$), Δz is the vertical grid size (m), τ is the shear stress above the sediments ($\mathrm{N\,m^{-2}}$) and t is time (s). The function f(T) describes the oocyst response to temperature and was adopted from Walker & Stedinger (1999) and the function f(UV) is based on data taken from Craik *et al.* (2001); for a detailed description see Antenucci *et al.* (2005). The oocyst resuspension function f(τ) applies only in the grid cell immediately above the sediment and depends on the critical shear stress required for oocyst resuspension.

Case study: Myponga Reservoir, South Australia

The coupled model was applied to Myponga Reservoir (26 GL), South Australia, during an inflow event in May

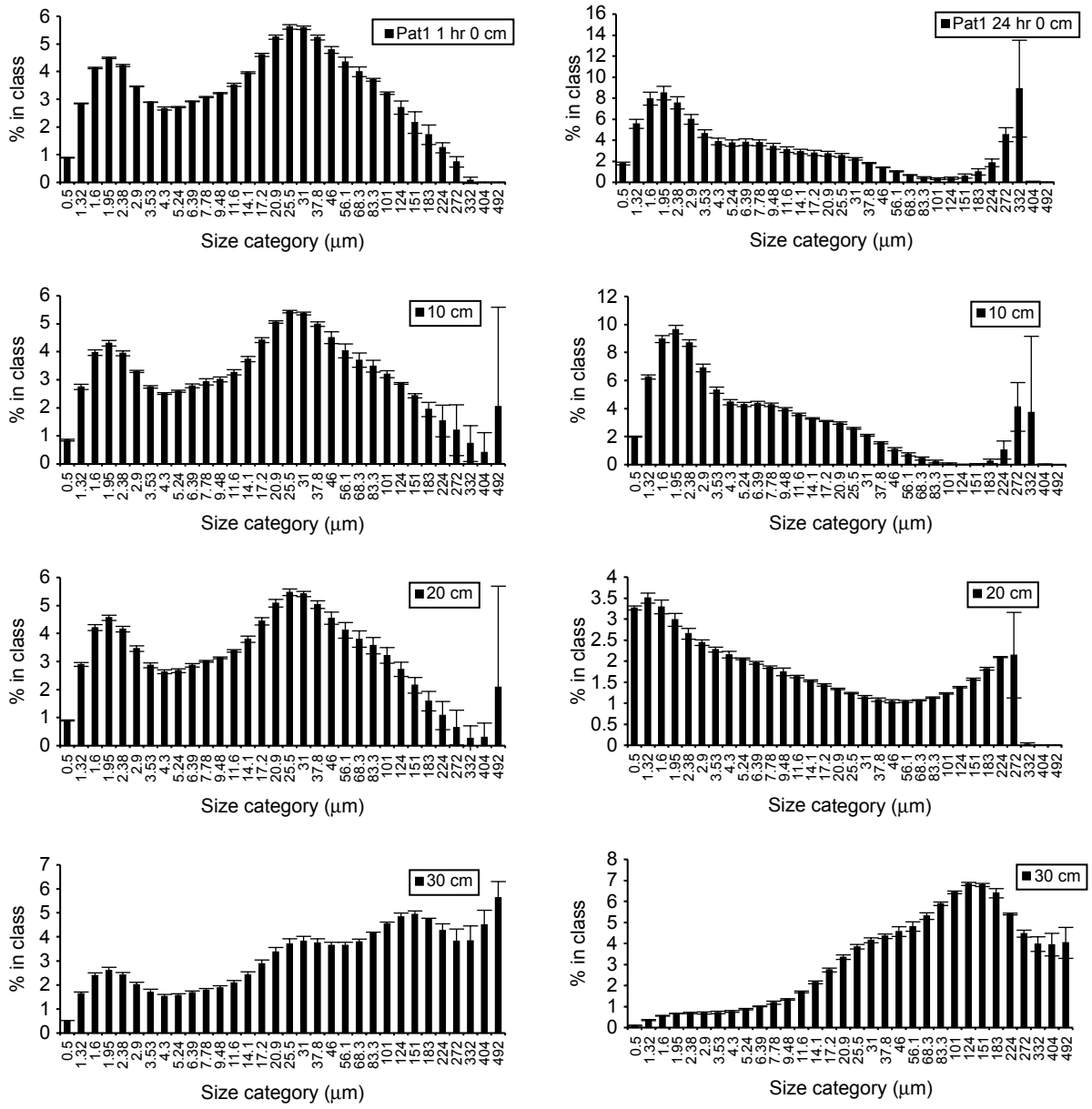

Figure 3 | Particle size distribution of cow pat slurry from 0 cm, 10 cm, 20 cm and 30 cm in a settling column after 1 h (first column) and after 24 h (second column). Values are means of three replicates, error bars are ±1 SD.

2001 that was known to introduce a considerable concentration of *Cryptosporidium* oocysts to the reservoir sidearm. During this event, the inflowing water (~10°C) was significantly cooler than the reservoir water (12–15°C) and formed an underflowing density current that moved along the reservoir base to the dam wall. Hipsey *et al.* (2004) showed that the hydrodynamics (particularly the inflow dynamics and surface thermodynamics) for this 12-day event were in excellent agreement (to within 0.5°C) with the thermistor chain data collected at two locations within the reservoir.

Measured oocyst loads in the river water were specified as the inflow boundary condition to the model (Figure 4), of which 60% were considered viable. The inflow scenario was

Table 1 | Particle size distribution of faecal pat slurry at different depths in a settling column after 1 h or 24 h – Pat 1 (summarising Figure 3)

		% in size range							
		Pat 1 1 h				Pat 1 24 h			
Size range (μm)	Initial	0 cm	10 cm	20 cm	30 cm	0 cm	10 cm	20 cm	30 cm
0.5–2.38	10.4	16.6	15.8	16.7	9.5	31.3	35.5	15.7	2.4
2.9–14.1	19.5	28.4	26.9	27.8	16.6	35.2	40.4	17.6	10.4
17.2–101	44.0	46.3	44.2	44.7	37.3	16.1	14.9	11.8	45.4
124–492	26.2	8.7	13.2	10.8	36.5	17.3	9.2	9.0	41.8

Table 2 | Particle size distribution of faecal pat slurry at different depths in a settling column after 1 h or 24 h – Pat 2

		% in size range							
		Pat 2 1 h				Pat 2 24 h			
Size range (μm)	Initial	0 cm	10 cm	20 cm	30 cm	0 cm	10 cm	20 cm	30 cm
0.5–2.38	12.1	19.9	19.8	19.1	7.7	38.9	36.6	14.7	2.9
2.9–14.1	19.7	30.2	29.7	29.2	12.6	40.3	38.5	15.0	12.1
17.2–101	43.6	43.0	42.8	44.0	30.4	11.7	10.0	8.6	47.4
124–492	24.5	7.0	7.7	7.7	49.3	9.0	14.9	6.8	37.7

run using a representative particle settling velocity of $1.16 \times 10^{-6}\,\mathrm{m\,s^{-1}}$ ($0.1\,\mathrm{m\,d^{-1}}$) as estimated from the cow faeces fractionation experiments.

A cross section through Myponga Reservoir showing oocyst concentration at various times throughout the inflow is presented in Figure 5. The inflow is denser than the reservoir water and so the oocyst-laden river water inserts at the bottom of the water column. The inflow water reaches the dam wall within 24 h and, as the

Table 3 | *Cryptosporidium* oocyst concentration of runoff from two artificial cow pats (Pat 1 and Pat 2) collected from four depths in a settling column after 1 h and 24 h Values are means of triplicate samples, 1 SD in parentheses

	Pat 1		Pat 2	
Depth in column	1 h	24 h	1 h	24 h
0 cm	35 (11)	36 (9)	46 (6)	30 (5)
10 cm	50 (12)	38 (3)	46 (10)	40 (5)
20 cm	40 (6)	33 (2)	38 (8)	41 (6)
30 cm	30 (11)	41 (5)	49 (8)	49 (5)

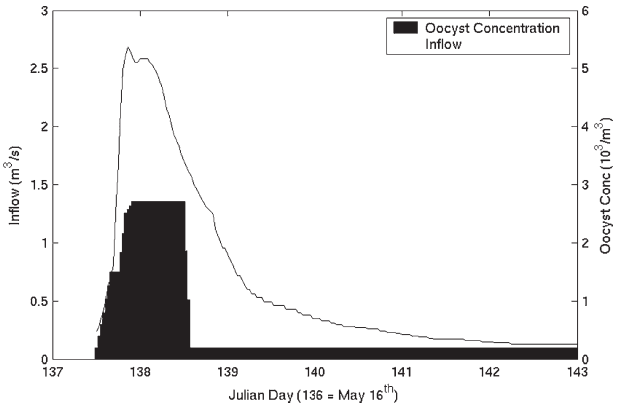

Figure 4 | Inflow hydrograph entering Myponga Reservoir during May 2001 showing the inflowing oocyst loading.

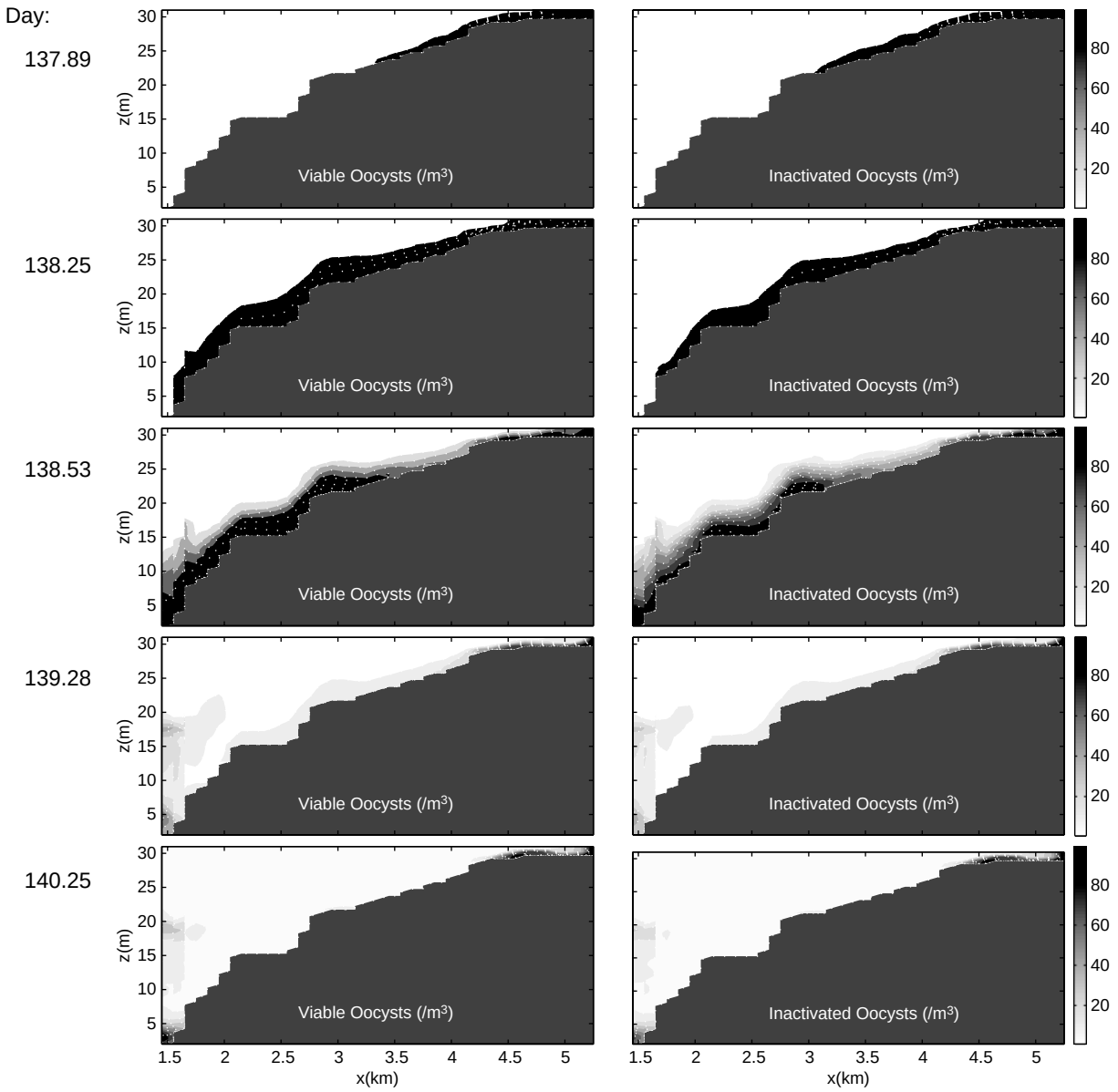

Figure 5 | Cross sections of Myponga Reservoir during the May inflow event showing the evolving spatial distribution of viable and inactivated oocysts during the inflow. The simulations used an oocyst settling velocity of 0.1 m d^{-1}, as suggested by the laboratory investigation.

inflow continues to flood the deepest part of the basin, the parcel of water with the highest oocyst concentrations is forced up past the 15 m offtake where it begins a return surface current. Between the inflow and the dam wall, the inflowing concentrations are diluted by a factor of 10.

The relative importance of each of the processes impacting on the concentration of oocysts is shown in Figure 6, indicating that concentration reduction due to settling is three orders of magnitude less than that due to dilution/advection during inflow forcing. After the pass of the hydrograph peak, however, dilution due to advective forcing is less pronounced, but still maintains dominance over settling by a factor of 10.

These results clearly indicate that oocyst settling from the water column has little role in risk reduction for small to

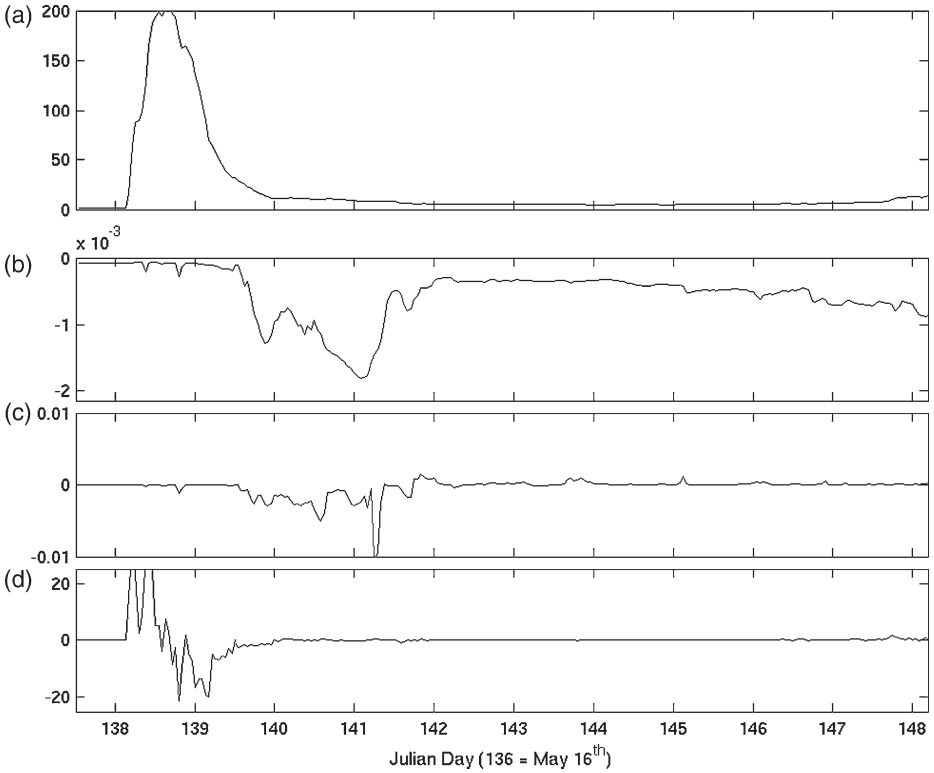

Figure 6 | Simulated time series data taken from a point in the reservoir 2 m from the bottom during the May 2001 inflow event, illustrating (a) viable oocyst concentration (oocysts m^{-3}), (b) concentration change due to inactivation (oocysts m^{-3}), (c) concentration change due to settling (oocysts m^{-3}) and (d) concentration change due to the effects of advection (oocysts m^{-3}). Note that these concentration changes are based on a model time-step of 6 min.

moderate sized reservoirs where oocysts enter via a river inflow. For the case of non-point-source oocyst contamination and weak hydrodynamic forcing, however, it is worth noting that settling is an order of magnitude more important than risk reduction due to inactivation alone.

Anticipated risk reduction from catchment to tap: a back of the envelope calculation

To calculate the scale of risk reduction that is achievable within the catchment, reservoir and treatment plant, thereby putting the role of oocyst settling into perspective, a simple calculation was performed based on catchment characteristics, modelled particle movement and a knowledge of treatment plant efficiency. There are a number of assumptions in the estimates, including the assumption that the entire cattle herd in the catchment is infected with *Cryptosporidium* and the concentration of oocysts is conserved throughout the hydrograph. The model workings are shown in Table 4 and is again applied to Myponga Reservoir. There is a one-log reduction of *Cryptosporidium* in the Myponga catchment, a one-log reduction in concentration in the reservoir and a three-log reduction in the treatment plant. Once past the water treatment there are no more physical barriers capable of removing oocysts even though a chlorine residual is generally maintained which should control most other pathogens.

CONCLUSIONS

The results from the laboratory component of this work suggest that, providing there were no major changes to the surface properties of the oocysts during purification from calf faeces and during preparation of the artificial cow pats, the oocysts were not associated with large particles but existed in faecel runoff predominantly as single oocysts.

Table 4 | Catchment features and anticipated risk reduction of *Cryptosporidium* from catchment to tap

Catchment area	124 km^2
Number of cattle in catchment	4500 head
Number of stools produced per head per day	12 stools d^{-1}
Average weight of stool	2 kg
Possible number of oocysts per gram	295 oocysts g^{-1} (Heitman *et al.* 2002)
Possible number of oocysts per stool	5.9×10^5 oocysts/stool
Number of days stool persists on landscape (number of days to decay to 1% given a decay rate ~0.2 per d)	23 d
Number of active oocysts on .landscape	7.33×10^{11}
Maximum recorded concentration of oocysts in influent	65 oocysts per 10 L (presumptive)
	25 oocysts per 10 L (confirmed)
Maximum flood magnitude in an "event" (Sept 2001 Peak flow 1045 ML d^{-1} – total flow in four day flood 3370 ML)	3370 ML
Maximum possible oocyst load	2.19×10^{10} oocysts
Proportion of oocysts which transported from landscape to creek at sampling point	0.299 (30% of oocysts in catchment)
Reduction due to dilution during entrainment	8 times dilution (Hipsey *et al.* 2004)
Reduction due to settling at 0.1 m d^{-1}	10 times reduction (including dilution)
Reduction due to treatment process	2.5–3 log reduction in conventional treatment based upon particle counting data. Turbidity in treated water consistently <0.1 NTU

This has significant implications for *Cryptosporidium* transport in a watershed and the risk reduction that can be expected due to settling in water supply reservoirs.

In drinking water reservoirs there is the opportunity for significant risk reduction due to dilution and settling if the *Cryptosporidium* oocysts were associated with large particles. However, in smaller reservoirs the travel time-scale of inflowing water is too rapid for a significant risk reduction due to inactivation. If pathogens aggregate within a large matrix of faecel material then there is a much greater chance they will settle. By incorporating the laboratory settling estimates into a 3D model of *Cryptosporidium* fate and transport, it is shown that risk reduction due to settling of oocysts in drinking water reservoirs of small to moderate size under inflow forcing is considerably smaller than concentration reduction due to entrainment and dilution. This suggests that an understanding of the inflow entrainment and dilution process is the most important factor in determining the risk reduction capability of a reservoir.

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