

Review article

## Fate and transport of pathogens in lakes and reservoirs

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### Abstract

Outbreaks of water-borne disease via public water supplies continue to be reported in developed countries even though there is increased awareness of, and treatment for, pathogen contamination. Pathogen episodes in lakes and reservoirs are often associated with rain events, and the riverine inflow is considered to be major source of pathogens. Consequently, the behaviour of these inflows is of particular importance in determining pathogen transport and distribution. Inflows are controlled by their density relative to that of the lake, such that warm inflows will flow over the surface of the lake as a buoyant surface flow and cold, dense inflows will sink beneath the lake water where they will flow along the bathymetry towards the deepest point.

The fate of pathogens is determined by loss processes including settling and inactivation by temperature, UV and grazing. The general trend is for the insertion timescale to be shortest, followed by sedimentation losses and temperature inactivity. The fate of *Cryptosporidium* due to UV light inactivation can occur at opposite ends of the scale, depending on the location of the oocysts in the water column and the extinction coefficient for UV light. For this reason, the extinction coefficient for UV light appears to be a vitally important parameter for determining the risk of *Cryptosporidium* contamination.

For risk assessment of pathogens in supply reservoirs, it is important to understand the role of hydrodynamics in determining the timescale of transport to the off-take relative to the timescale of inactivation. The characteristics of the riverine intrusion must also be considered when designing a sampling program for pathogens. A risk management framework is presented that accounts for pathogen fate and transport for reservoirs.

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### 1. Introduction

Outbreaks of waterborne disease via public water supplies continue to be reported in developed countries even though there is increased awareness of, and treatment for pathogen contamination (Herwaldt et al., 1992; Moore et al., 1994; MacKenzie et al., 1994; Lisle and Rose, 1995; Payment et al., 1997; Gibson et al., 1998; Howe et al., 2002). However, the presence or absence of pathogens within a reservoir does not provide a satisfactory indication

of risk to human health. This data needs to be considered in a management framework that encompasses the whole water supply system from catchment to customer tap. An important component of this framework are data describing the inactivation and transport of pathogens of public health significance. For example, *Cryptosporidium* and *Giardia* have been identified in Lake Kinneret at levels that may present a health hazard; however, no major outbreaks have been reported in Israel (Zuckerman et al., 1997). Conversely, several outbreaks of cryptosporidiosis have now been documented where the water quality met microbiological standards based on bacterial indicators (MacKenzie et al., 1994; Lisle and Rose, 1995). To obtain a more realistic assessment of the overall pathogen risk, it is necessary to understand the critical variables controlling pathogen fate

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and distribution in each part of the water supply system, including lakes and reservoirs.

## 2. Water storage as a barrier to pathogen transport

The first control point for pathogen risk management is minimising the concentrations entering rivers from the catchment (Venter et al., 1997). A study by Hansen and Ongerth (1991) reported oocyst concentrations in water draining a controlled water supply catchment that were 10 times lower than an adjacent uncontrolled catchment. Within the reservoir, pathogen distribution and transport is a function of the pathogen load in the inflowing water, the settling or entrainment characteristics of the particles (Walker and Stedinger, 1999), and resuspension of pathogens from sediment by turbulence in the benthic boundary layer. The inactivation of pathogens will also be impacted by predation and decay through light and/or temperature exposure. Knowledge of the fate of pathogens is critical to link the timescales of hydrodynamic events with the timescales of pathogen inactivation and the predicted risk to water treatment.

Viability of pathogens is primarily affected by temperature and ultraviolet light, and if inactivation leads to decomposition, distribution will also be limited. Chemical parameters that influence pathogen decay in reservoirs are probably limited to pH, which would be relevant in only a few reservoirs. Biological parameters that may be important are predation by protozoa and/or invertebrates (Simek et al., 2001).

It is important to determine how well water supply reservoirs are operating as a barrier to pathogen transport as it is the second control point within most water supply systems. Of primary concern is *Cryptosporidium* because of its longevity (Medema et al., 1997) and the resistance of oocysts to treatment processes (Robertson et al., 1992).

Both Van Breemen et al. (1998) and Bertolucci et al. (1998) report significant decreases in *Giardia* and *Cryptosporidium* concentrations during water storage in reservoirs. In the Dutch reservoirs examined by Van Breemen et al. (1998), the elimination rates for pathogenic microorganisms ranged between 1.7 and 3.1 log<sub>10</sub> units. Retention time and contamination by water flow were considered to be the major factors influencing the elimination rate.

The water body examined by Bertolucci et al. (1998) was an off-line storage with water pumped from the Turin River, Italy, at a rate of 1000 l/s into a disused gravel quarry. Water was returned to the river at the same flow rate so the theoretical detention time was about 18 days. The average concentration of protozoans in the inflowing water was 137 *Giardia* cysts per 100 l (range 10–561) and 70 *Cryptosporidium* oocysts per 100 l (range 3–536). Concentrations in the outflowing water were 46 *Giardia* cysts per 100 l and 7 *Cryptosporidium* oocysts per 100 l. This equates to a removal rate, as water passed through the reservoir, of 55.8% of *Giardia* and 78.1% of *Cryptosporidium* oocysts.

## 3. Use of surrogates to estimate pathogen risks

A fundamental principle of drinking water supply is to use high-quality, protected source waters as a means of reducing the potential load of drinking water contaminants and thus reducing treatment costs and subsequent health risks to consumers. A study by Edzwald and Kelley (1998) of the mean concentration of *Cryptosporidium* oocysts in protected reservoirs (0.52 oocysts 100 l<sup>-1</sup>) and pristine lakes (0.3–9.3 oocysts 100 l<sup>-1</sup>) compared with polluted rivers (43–60 oocysts 100 l<sup>-1</sup>) and polluted lakes (58 oocysts 100 l<sup>-1</sup>) highlights the merit of this strategy. However, the complexity, cost and time constraints associated with the direct enumeration of pathogens and their distribution in large water bodies frequently limits the ability of water utilities to detect the intrusion of poorer quality water inflows into storages. While particle counting and turbidity measurements are suitable tests for the integrity and efficiency of treatment processes, there is considerable discussion in regulatory organisations and water utilities as to their applicability for detecting the presence of poor water quality intrusions and the distribution of pathogens in reservoirs.

Traditionally, bacterial indicators of faecal contamination, notably faecal coliforms and enterococci, have been used to assess the microbial quality of water sources (Thurman et al., 1998). However, it is now apparent that these bacterial indicators are not suitable for assessing the risk posed by protozoan pathogens and some enteric viruses (Ashbolt et al., 2001). Several outbreaks of cryptosporidiosis have now been documented where the water quality met microbiological standards based on bacterial indicators (MacKenzie et al., 1994; Lisle and Rose, 1995).

Various bacteriophages have been proposed as index organisms for enteric viruses in freshwater (Havelaar et al., 1993; Armon and Kott, 1995; Tartera et al., 1989). Faecal bacteriophages, however, are not suitable index organisms, as they are always present in a range of animal as well as human faeces, whereas human enteric viruses are only excreted by infected humans (Grabow, 2001). Furthermore, human enteric viruses have been detected in 1–500 ml water samples in the absence of bacteriophages (Grabow et al., 2001).

Hence, bacteriophages should only be used as models for human enteric virus behaviour. The rationale for their use as model organisms is based upon their similar size and morphology, along with the low cost, ease and speed of detection compared to human enteric virus assays. Most work has focused on coliphage systems, including double- and single-stranded DNA and RNA-containing phages and a range of bacterial hosts. The ideal host bacteria would be of human faecal origin only, consistently present in sewage in sufficient numbers for detection, and lysed by phages that do not replicate in another host or the environment. While bacteriophages to *Bacteroides fragilis* strain HSB40 appear to be human specific and do not replicate in the environment

(Tartera et al., 1989), their phage numbers are too low for general use. Hence, the single-stranded F-RNA coliphages (f2, MS-2 and Q beta) that attach to the sides of the pili that only occur on exponentially growing specific (F+) strains of *Escherichia coli* or an engineered *Salmonella typhimurium* (strain WG49) are currently the models of choice (Havelaar et al., 1993; Grabow, 2001).

It has also been suggested that spores of *Clostridium perfringens* are good indicators of human faecal contamination and may correlate with human parasitic protozoa and enteric viruses (Payment and Franco, 1993; Ferguson et al., 1996). Yet two confounders need to be addressed when considering this surrogate, firstly, that spores are very persistent (Davies et al., 1995) and, secondly, various animals may also excrete *C. perfringens* (Leeming et al., 1998). Hence, spores of *C. perfringens* show no relationship with parasitic protozoa in animal-impacted raw waters, and could be misleading about the likely presence of infective human viruses.

Other potential surrogates for pathogens include particle counting and turbidity. However, Edzwald and Kelley (1998) regarded these surrogates as unreliable indicators for parasitic protozoa in raw waters, due to the low concentrations of pathogens compared to the concentration of other particles. This is notwithstanding the weak epidemiological evidence that suggests waterborne illness may be associated with raw water turbidity (Juraneck and Mackenzie, 1998). Studies of *Cryptosporidium* and *Giardia* in rivers and at water treatment plants often show that these pathogens are detected during periods of high turbidity (Hawkins et al., 2000; Hsu et al., 2000; Mallin et al., 2001), and are often associated with rainfall and elevated river levels (Atherholt et al., 1998; Curriero et al., 2001).

The use of turbidity alone to predict pathogen presence is difficult because turbidity is dependent on a range of processes that are independent of pathogen presence. For example, it is well established that many young calves are infected with *Cryptosporidium* (Ongerth and Stibbs, 1989); however, calving is timed to coincide with the period when feed is abundant and cows are on a rising plane of nutrition. Consequently calving occurs at the time catchments are well vegetated and hence turbidity may be low, whereas *Cryptosporidium* excretion rates may be high. Alternatively, animal husbandry in some climates dictates that livestock are housed during winter and put out to pasture in spring increasing both turbidity and *Cryptosporidium* concentration. Consequently, it is critical to have system knowledge for each site not just rely on universal rules. In addition, surrogates such as turbidity are influenced by catchment soil type and non-grazing land-use such as horticulture, which do not correlate with pathogen inputs. However, turbidity is a readily measurable parameter which warrants investigation as a surrogate or at least as an early warning mechanism.

While no single water quality indicator can reliably assess the bacterial, protozoan and viral contamination of

aquatic environments in all circumstances, it is feasible that a suite of surrogates may be identified that will estimate levels of microbial contamination within defined circumstances, such as within a storage reservoir with well characterised inputs. The strategy for risk assessment of any given pathogen in water supply reservoirs is to sample and analyse for the target organism in a systematic way. By this, we mean that the sampling must be coupled with a sound knowledge of the biological, physical and chemical factors, which control transport, distribution and inactivation in natural source waters.

#### 4. Factors controlling pathogen transport and distribution

The processes of dispersion, dilution horizontal and vertical transport determines the distribution of pathogens in lakes and reservoirs. Settling of pathogen particles operates in conjunction with these hydrodynamic processes.

Dispersion describes both the turbulent dispersion of particles (for example, in the surface mixed layer) and shear dispersion due to the presence of a horizontal or vertical velocity shear. Both processes are important in determining the distribution of particles, not only the changes in concentration of an initial distribution or cloud but also the differential advection of particles within an initial cloud leading to some particles travelling significantly further than the centre of the initial cloud.

The horizontal transport is predominantly driven by inflows and basin-scale circulation patterns including wind-driven currents and internal waves. Although wind-driven currents only influence the surface layer, inflows can occur at any depth in a stratified reservoir and internal waves can generate significant internal currents that can act in different directions at different depths. Internal waves also generate significant vertical movements in lakes and reservoirs of the order of tens of metres. Internal waves have been shown to be responsible for the vertical advection of pathogen particles past off-take structures resulting in periodic variations in water quality (Deen et al., 2000).

While it is acknowledged that recreational activities can contribute to pathogen concentrations in reservoirs (Anderson et al., 1998), the riverine inflow is considered to be the major source of pathogens, and consequently the behaviour of these inflows is of particular importance. Inflows are controlled by their density relative to that of the lake, such that warm inflows will flow over the surface of the lake as a buoyant surface flow and cold, dense inflows will sink beneath the lake water where they will flow along the bathymetry towards the deepest point. In either case, the inflow will entrain water from the lake, increasing its volume, changing its density and diluting the concentration of pathogens and other properties.

In the case of a dense underflow, when the density of the underflow matches that of the adjacent lake the underflow

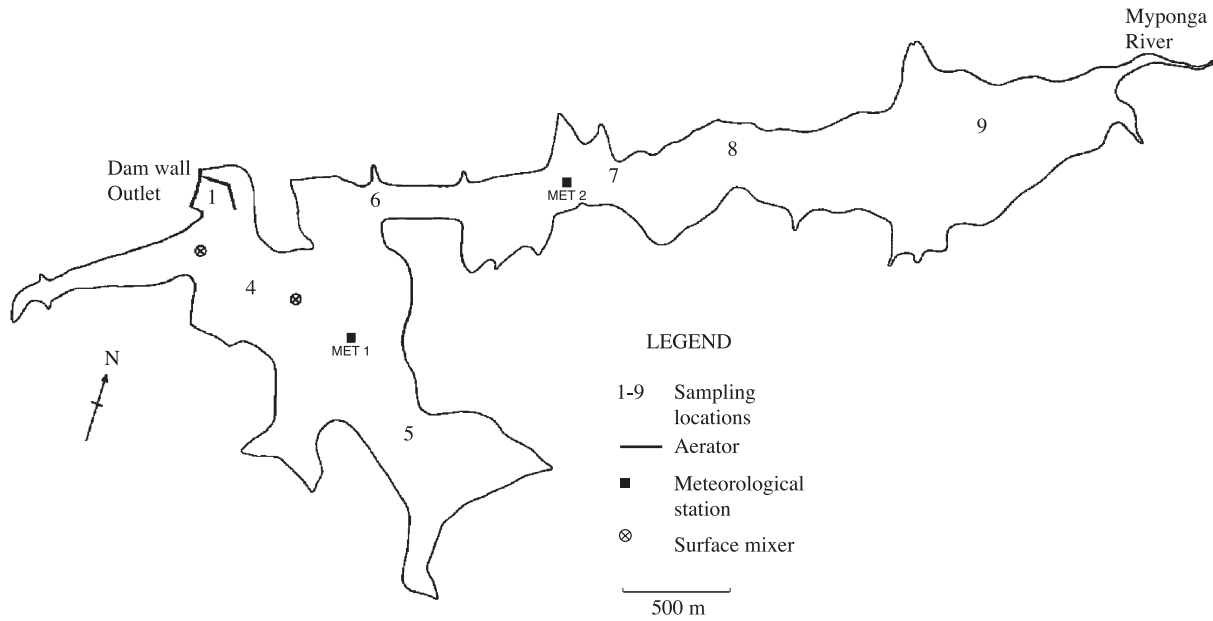


Fig. 1. Sampling locations and meteorological station including thermistor chains at Myponga Reservoir.

will become an intrusion. In some cases, the underflow is denser than any water in the lake and it will flow all the way to the deepest point. This is of particular interest for most drinking water reservoirs because the deepest point is often where the off-take is located (i.e. at the dam wall). As oocysts will survive longest in cold and dark water, this mechanism can potentially produce the greatest risk. A further complication is introduced where the density difference is derived from particulate matter (turbidity current), in which case the settling of these particles will influence the density and propagation of the inflow.

The speed at which an inflow travels through a lake, its entrainment of lake water and resulting dilution of its

characteristics and its insertion depth are all of critical importance in determining the hydrodynamic distribution of pathogens in lakes and reservoirs. For example, a small flood with a peak daily total flow of 240 ML was recorded at Myponga Creek (Fig. 1) on May 18, 2001 (Brookes et al., 2002). The temperature of the intruding creek water during the elevated flow ranged between 9.5 and 10.6 °C. The intruding flow was detected as a departure from isothermal conditions and cooling at depth. The intrusion was detected at meteorological station 2, in the side arm, (Fig. 2) at 17:10 on May 17 and at meteorological station 1, in the main basin 1.3 km away, at 04:00 on May 18. This equates to a velocity of 0.041 m s<sup>-1</sup> and therefore a

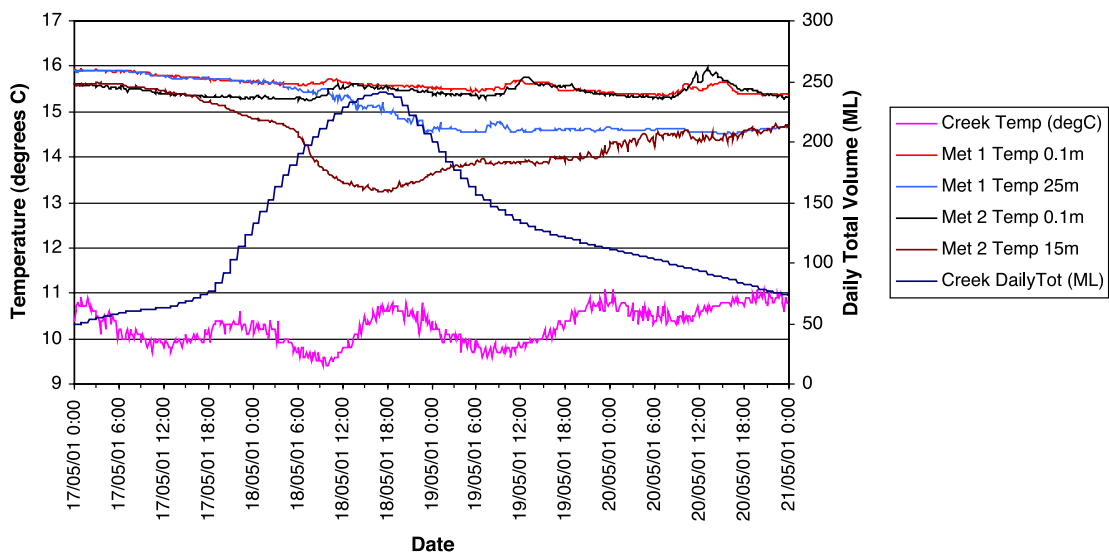


Fig. 2. Temperature at the surface and bottom at Met stations 1 and 2 in Myponga Reservoir and daily total creek inflow and temperature recorded in Myponga Creek.

potential travel time from inflow to off-take of 30 h, whereas the retention of the reservoir computed on a whole-of-volume basis is 3.1 years.

4.1. Settling

The accurate prediction of the vertical distribution of pathogens and/or surrogates in stratified lakes and reservoirs is central to their transport and detection. The settling rate of particles is affected by size and density according to Stokes law (Reynolds, 1984). Consequently, the aggregation of pathogens to particulate material, or the integration of pathogens within a matrix of organic material, will influence the rate of pathogen settling. It is possible that predation of pathogens and the subsequent incorporation of pathogens into faecal pellets will also influence the settling of pathogens. However, the significance of this ecological process is unknown. In general, the theoretically calculated sedimentation kinetics of freely suspended oocysts show good agreement with experimentally observed kinetics (Medema et al., 1998). However, because cysts and oocysts readily attach to other particles in effluent, it is necessary to consider a range of particle sizes when determining settling and resuspension characteristics of pathogens in reservoirs and in modelling scenarios. Although particle distribution in turbulent flows is generally assumed to be homogeneous, recent research has shown that the vertical distribution of small particles in turbulent flow is dependent on the Peclet number *Pe*, the ratio of the mixing time to sedimentation time (Condie and Bormans, 1997).

Individual oocysts have been found to have a settling velocity in water of approximately 0.03 m day<sup>-1</sup> (Medema et al., 1998). This was found to increase to approximately 2.5 m day<sup>-1</sup> when attached to particles from biologically treated sewage effluent, in which the sedimentation dynamics of the particles dominated over the smaller oocysts (Medema et al., 1998). These sedimentation values were shown to fit theoretical sedimentation kinetics, i.e. Stokes law:

$$V_s = \frac{gd^2}{18\mu}(\rho_p - \rho_w) \tag{1}$$

where *V<sub>s</sub>* is the settling velocity [m s<sup>-1</sup>], *g* is the acceleration due to gravity [m s<sup>-2</sup>], *d* is the particle diameter [m] (of either the single oocyst or aggregated particle), *μ* is the dynamic viscosity of water [Ns m<sup>-2</sup>], *ρ<sub>p</sub>* is the density of the particle [kg m<sup>-3</sup>] and *ρ<sub>w</sub>* is the density of water [kg m<sup>-3</sup>]. Stokes settling velocity calculation assumes that the displaced water movement around the particle is laminar (Reynolds, 1984). McNown and Malaika (1950) demonstrated that, for calculated Reynolds number (*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 μm) 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, 1987).

Hawkins et al. (2000) estimated sedimentation rates of oocysts of 5–10 m day<sup>-1</sup>, based on field measurements from Lake Burragorang in Sydney. While 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 (Table 1).

4.2. Aggregation

The aggregation of particles on organic matter or clay minerals (Schiffenbauer and Stotzky, 1982; Loveland et al., 1996) will play a major role in pathogen transport, settling and survival (LaBelle and Gerba, 1979). The surface charge of the particles plays a key role in particle–particle interactions in water (Ongerth and Pecoraro, 1996). There is some evidence that aggregation of *Cryptosporidium* oocysts to particles and to each other is pH-dependent (Drozd and Schwartzbrod, 1996). This is essentially a result of pH modifying the hydrophobic and electrostatic nature of the oocyst surface. These studies highlighted the dependence over a wide range of pH values (LaBelle and Gerba, 1979; Loveland et al., 1996); however, there seems to be little variation over the small range of pH values applicable to drinking water reservoirs. In any event, Drozd and Schwartzbrod (1996) were unable to provide a relationship between pH and the size of the aggregated particles. This raises the question of how the aggregation of *Cryptosporidium* oocysts

Table 1  
Size and settling velocity of pathogens and surrogates

Organism	Size (μm)	Density (kg m <sup>-3</sup> )	Sinking velocity	Reference
<i>Cryptosporidium</i> (purified oocysts)		1945.4	0.03 m day <sup>-1</sup>	Medema et al., 1998
	4–5 μm	>1000 to <1060		Fayer and Ungar, 1986; Arrowood and Sterling, 1987
	In effluent bio-floc		2–5 m day <sup>-1</sup>	Medema et al., 1998
	In a matrix 17 μm	1800	10 m day <sup>-1</sup>	Hawkins et al., 2000 (modelled data)
<i>Hepatitis A</i>	27	1340		Siegel et al., 1981
Coliphages	Associated with particles >8.0 μm, >0.65 μm <0.25 μm			Gerba et al., 1978
Viruses	Median 1.8 μm diameter			Payment et al., 1987
<i>V. anguillarum</i>	<7 μm aggregates			Hejkal et al., 1981 Heise and Gust, 1999

will be handled in any proposed models, particularly because of the sensitivity of settling velocities to aggregation state (Hawkins et al., 2000; Medema et al., 1998). This represents a significant knowledge gap with only limited data available on the size of particles that pathogens are associated with in natural water (Payment et al., 1987).

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. If *Cryptosporidium* is associated with large particles, there is greater chance of interception, or settling, and so potentially greater risk reduction than if these pathogens were associated with small particles or were transported as single unattached oocysts. Animal faeces are a major source of *Cryptosporidium* in watersheds. *Cryptosporidium* concentrations in faeces vary with animal host, age (Davies et al., 2003) and season. However, it is likely that the mastication of plant material within the cattle gut and the subsequent scouring of the stomach wall, which dislodges oocysts, will have a significant impact on *Cryptosporidium*–particle interactions. Few studies have been published on the dispersion, survival and viability of pathogens excreted in faecal matrices (Jenkins et al., 1999b; Bradford and Schijven, 2002) although Ferguson et al. (2003) reviewed the factors which determine their subsequent fate and transport in surface waters.

The other factor affecting the size of *Cryptosporidium*-associated particles is aggregation. The electrophoretic mobility measurements and calculated zeta potentials for *Cryptosporidium* oocysts, reported by Ongerth and Pecoraro (1996), indicate that these particles are strongly negatively charged at neutral pH. Consequently, they may be adequately aggregated and flocculated during conventional water treatment but may not adsorb well to natural clays in the environment. A study by Dai and Boll (2003) determined that oocysts do not attach to natural soil particles and would travel freely in the water. Similarly, Considine et al. (2000, 2001) supported this hypothesis but concluded that protein-linked tethering between silica and oocysts can occur and 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.

In contrast the viruses, poliovirus type 1, coxsackievirus type B3, echovirus type 7 and rotavirus (SA-11) showed greater than 99% adsorption to sediment (La Belle and Gerba, 1979). Gantzer et al. (2001) also observed significant adsorption to soil of somatic coliphages, F-specific RNA phages and faecal coliforms from wastewater (61%, 78% and 86%, respectively). Lipson and Stotzky (1983) concluded that the cation exchange capacity is the major characteristic of clays involved in the adsorption of reovirus.

There appears to be two conflicting arguments as to whether *Cryptosporidium* is associated with particles. The surface charge 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 that, at least in certain situations, oocysts must be associated with larger particles. An alternative is that the oocysts may be physically enmeshed within an organic matrix of faecal material and/or soil particles during entrainment in surface water runoff. A recent study by Feng et al. (2003) demonstrated that suspended particles present in reservoir water contributed to enhanced recovery of *Cryptosporidium parvum* oocysts and that particle size and concentration could affect oocyst recovery. The optimal particle size was determined to be in the range of 5–40  $\mu\text{m}$ , and the corresponding optimal concentration of suspended particles was 1.42 g for 10 l of tap water.

It is highly likely that the aggregation of oocysts to particles in water will be primarily controlled by turbulence. Turbulence in lakes is typically  $1 \times 10^{-8} \text{ m}^{-2} \text{ s}^{-3}$ , only rarely reaching values of  $1 \times 10^{-6} \text{ m}^{-2} \text{ s}^{-3}$  (Saggio and Imberger, 2001). In a saline underflow, Dallimore et al. (2001) measured turbulent dissipation averaging greater than  $1 \times 10^{-6} \text{ m}^{-2} \text{ s}^{-3}$ , whereas turbulent dissipation in rivers can reach  $1 \times 10^{-2}$  to  $1 \times 10^{-1} \text{ m}^{-2} \text{ s}^{-3}$  (Nikora and Smart, 1997). Consequently, if aggregation is going to occur, it is much more likely to occur in the inflowing rivers than within the lake or reservoir.

#### 4.3. Resuspension

Since pathogens may remain viable for variable periods in the sediments of a lake or reservoir, it is important to consider the relative importance of their resuspension and subsequent re-distribution. Sediment resuspension occurs when turbulent velocity fluctuations reach a critical level. In a lake this occurs within the turbulent benthic boundary layer, where the turbulence is driven by a combination of currents and internal wave breaking (Lemckert and Imberger, 1998). Currents capable of generating critical bed-shear can be caused by large underflow events and by basin-scale internal waves. Smaller-scale internal waves breaking on the sloping boundaries of a lake will also generate turbulence that can result in the resuspension of particulate material (Michallet and Ivey, 1999).

The energy transfer is thus from the basin scale waves to groups of nonlinear high frequency free internal waves, which finally lose their energy by breaking at the perimeter of the metalimnion. This, in combination with bottom velocities induced by the basin-scale waves, sets up a turbulent benthic boundary layer that provides a conduit for material to move from the lower to the upper water mass (Imberger and Ivey, 1993; Lemckert and Imberger, 1998).

Recent measurements have confirmed that in large lakes such as Lake Kinneret, Israel, the turbulent benthic boundary layer plays an important role in resuspending sediment and therefore could even potentially resuspend viable oocysts (although the occurrence of these oocysts in the sediments has not been confirmed). The turbulent zone of the benthic boundary layer may indeed coincide with zones

of substantial sediment accretion where particulates in inflows would normally settle out, as it is noted that many of the inflow insertions occur around the depth of the metalimnion. Thus there may be large amounts of potentially readily resuspendable material in this zone.

## 5. Fate of pathogens in reservoirs

Detailed information regarding the persistence of pathogens in reservoirs is necessary to estimate their contribution to health risks. Furthermore, knowledge of the relative persistence of surrogates and pathogens is critical to determine which surrogates may be appropriate indicators for the estimation of human health risks from pathogens. For instance, if a particular indicator species (surrogate) is less persistent than the pathogens of concern, then by only monitoring for the surrogate the true risk may be underestimated.

### 5.1. Inactivation

The persistence of pathogens in the aquatic environment is a function of both survival and transport. Different pathogens persist for different amounts of time and the major mode of inactivation or mortality may vary significantly. Factors that control inactivation include temperature, salinity, pressure, solar radiation (visible and UV) and predation by organisms higher in the food chain. However, light and temperature are the major inactivation mechanisms, although predation may be significant for some organisms. Table 2 shows some examples of pathogen survival rates in natural waters.

### 5.2. Temperature

Several studies have examined the effect of temperature on *Cryptosporidium* infectivity and/or viability (Jenkins et al., 1997; Robertson et al., 1992; Walker et al., 2001). Walker and Stedinger (1999) summarised the work of Jenkins et al. (1997) and Robertson et al. (1992), to derive a first-order decay function:

$$C(t) = C_0 e^{-kt}, \quad (2)$$

$$k = 10^{-2.68} 10^{0.058T}, \quad (3)$$

where  $t$  is the time in days,  $C$  is the concentration of viable oocysts, and  $T$  is the temperature in degrees Celsius (Fig. 3a). A comprehensive study by Fayer et al. (1998a) derived relationships over a wide range of time and temperature scales. The trends seen in this model are supported by laboratory data on amylopectin (a storage polysaccharide associated with cell viability and longevity), whose concentrations vary as a function of temperature and time (Fig. 3b) similar to viability. However, little information exists that

Table 2  
Persistence of pathogens and surrogates in natural waters

Pathogen	Context	Persistence	Major loss mechanism	Reference
<i>C. parvum</i>	St. Lawrence river	Several weeks	Biological antagonism	Chauret et al., 1998
	Artificial seawater: 10, 20, 30 ppt	12 weeks		
<i>E. coli</i>		30 days		Wcislo and Chrost, 2000
	Decay experiments	Decay rates: 1 m, 0.32/day; 10 m, 0.8/day; 20 m, 0.7/day	Light intensity	Garvey et al., 1998
Viruses		Decay rate (log 10)		
Poliovirus type 1	Estuarine water	1.0		LaBelle and Gerba, 1980
Echovirus type 1	Estuarine water	2.8		LaBelle and Gerba, 1980
Poliovirus type 1	River water	0.77		O'Brien and Newman, 1977
Poliovirus type 3	River water	1.0		O'Brien and Newman, 1977
Coxsackievirus B3	River water	0.83		O'Brien and Newman, 1977

quantifies the relationship between total counts and viability. The data presented in Jenkins et al. (1997) suggests that the rate of *C. parvum* inactivation is controlled by the effect of temperature, and is relatively independent of the suspension matrix (Table 3). The inactivation rates of *C. parvum* in water and in faecal slurries had decay rates within the same order of magnitude at the same temperature (Table 3). This finding means that in temperature inactivation models, the rate of temperature-induced inactivation can have a constant decay rate ( $k$ ) regardless of whether the oocyst is freely suspended or is bound within a matrix of faecal or organic material. However, this result is contradicted, by the study of Olson et al. (1999) who found that cyst and oocyst degradation was more rapid in faeces and soil, presumably as a function of microbial antagonism and/or predation (Table 4). However, the low number of replicates used in the study means that these results should be used with caution.

The effect of low temperature on viability and infectivity of *C. parvum* was investigated by freezing purified oocysts to  $-10$ ,  $-20$  and  $-70$  °C for between 1 and 168 h (Fayer and Nerad, 1996). Oocysts frozen to  $-10$ ,  $-15$  and  $-20$  °C were not morphologically distinguishable from unfrozen control samples, as determined by microscopy. Freezing to  $-70$  °C appeared to crack the walls of some oocysts. Freeze-thawed oocysts were administered to young mice and samples of the ileum, colon and cecum were taken for histological analysis 72–96 h after dosing. Infectivity was determined by the developmental stage *C. parvum* present in epithelial cells. The

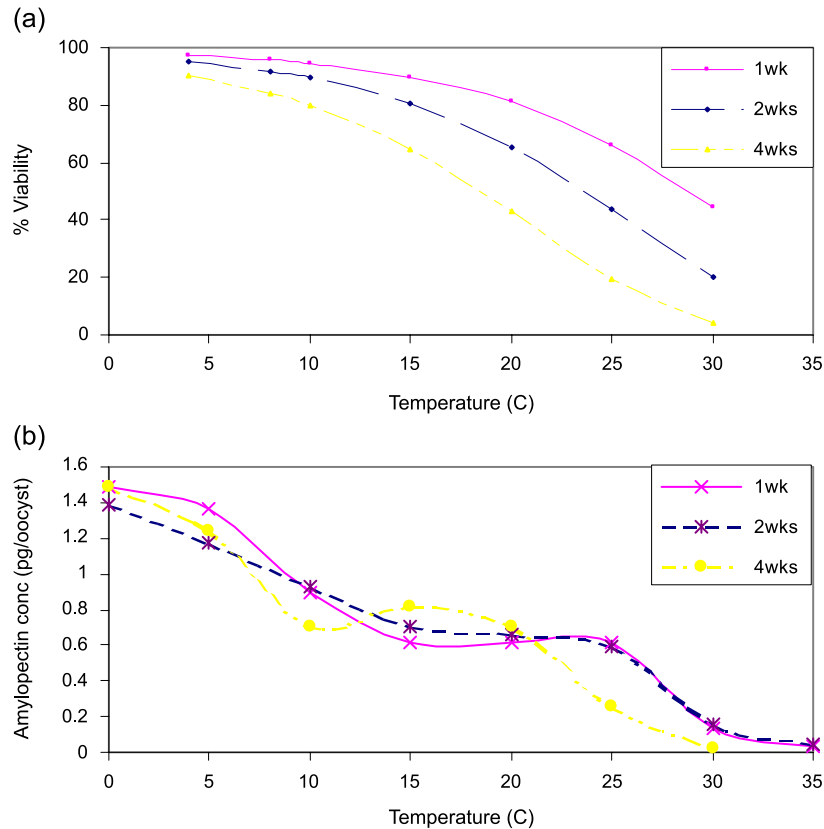


Fig. 3. (a) First-order decay relationship presented in Walker and Stedinger (1999) showing oocysts viability as a function of temperature and storage time; (b) oocyst amylopectin concentration data presented in Fayer et al. (1998a) as a function of temperature and storage time.

general relationship between temperature, freezing time and infectivity is that *C. parvum* oocysts can retain viability and infectivity after freezing and the oocysts can survive longer at higher freezing temperatures (Fayer and Nerad, 1996). There was evidence that freezing, for even the shortest period rendered a portion of the oocysts non-infectious relative to the control (Fayer and Nerad, 1996). In a similar study, Robertson et al. (1992) found that after 21 h at  $-22^{\circ}\text{C}$ , 67% of oocysts had become nonviable, as determined by 6-diamidino-2-phenylindole (DAPI) and propidium iodide staining (Campbell et al., 1992). With prolonged exposure to freezing (152 h), 90% were not viable; however, a small proportion remained viable even after 750 h at  $-22^{\circ}\text{C}$ .

Pathogen inactivation by freezing in reservoirs is only relevant at latitudes and altitudes where ice cover persists. Inactivation by freezing would be restricted to the upper boundary where ice cover forms and only a portion of pathogens in a reservoir would be contained within the ice layer. Furthermore, the formation of ice cover contributes to stratification and so riverine intrusions would still move rapidly through the storage introducing fresh pathogens to the system and potentially resuspending previously settled pathogens from sediments. Consequently, freezing of the reservoir does not necessarily negate the pathogen risks in reservoirs. Additionally, the low water temperatures may

actually prolong pathogen survival. If the ice cover forms nocturnally and thaws through the day, then the actual volume of frozen water is generally negligible compared with the total volume of the reservoir. Consequently, inactivation of the total *Cryptosporidium* content would be negligible and the inactivation can be described by Eq. (2) for free water. The inactivation of *Cryptosporidium* in the ice cover of lakes will be most relevant when there is prolonged ice cover that can be described by a simple loss equation. The temperature of the ice will range from  $0^{\circ}\text{C}$  and the temperature of the adjacent air. A recent study by Sattar et al. (1999) provides data on *Cryptosporidium* viability at  $-20^{\circ}\text{C}$ , which could be used to determine a rate of inactivation in the ice cover of lakes (Fig. 4). The inactivation of *Giardia* at  $-20^{\circ}\text{C}$  is more rapid than inactivation of *Cryptosporidium* at the same temperature (Sattar et al., 1999), with a  $1 \log_{10}$  reduction in viability in the first 12 h and most *Giardia* cysts not viable after 24 h (Table 5).

A difficulty with using *E. coli* as an indicator species (surrogate) is that it may grow in natural waters outside of the animal host (Springthorpe et al., 1993, 1997). Freezing may not necessarily have a negative impact on bacteria such as *E. coli*. In fact freezing at  $-70$  to  $-80^{\circ}\text{C}$  may be used as a means of storing bacterial strains in the laboratory prior to experimental use. Other bacterial species such as *Cam-*

Table 3  
Inactivation of *C. parvum* oocysts stored in faecal slurries or water at various temperatures

Oocysts storage medium	Viability test used	Incubation time (days)	Incubation temperature (°C)	K (day <sup>-1</sup> )	95% confidence interval or S.D.	Reference
Distilled water with antibiotics	Dye permeability assay	667	4	0.0025	0.0020–0.0030	Jenkins et al. (1997).
Phosphate buffer saline	Dye permeability assay	6	22	0.0320	0.0214–0.0426	Jenkins et al. (1997).
	Dye permeability assay	1	37	0.918	0.780–1.056	Jenkins et al. (1997).
Calf faecal slurry	Dye permeability assay	410	4	0.0040	0.0023–0.0059	Jenkins et al. (1997).
	In vitro excystation	410	4	0.0046	0.0032–0.0061	Jenkins et al. (1997).
Calf faecal slurry	Dye permeability assay	259	4	0.0056	0.0038–0.0074	Jenkins et al. (1997).
	In vitro excystation	259	4	0.0079	0.0047–0.0113	Jenkins et al. (1997).
Sterile RO water	Dye permeability assay	176	4	0.0042	0.0034–0.0048	Robertson et al. (1992)
	Animal infectivity	147	15	0.0355	0.0315–0.0395	Korich et al. (1993)
Sterile distilled water	In vitro excystation	147	15	0.0211	0.0180–0.0242	Korich et al. (1993)
	Animal infectivity	28	15	0.1555	0.1071–0.2039	Korich et al. (1993)
	In vitro excystation	28	15	0.0907	0.0553–0.1261	Korich et al. (1993)
	In vitro excystation	48	0.0–0.5	0.003	SD 0.000	Chauret et al., 1998
River water, St. Lawrence River	In vitro excystation and total counts	48	0.0–0.5	0.013	SD 0.004	Chauret et al., 1998
	In vitro excystation	32	0.5–2.0	0.002	SD 0.000	Chauret et al., 1998
River water, St. Lawrence River	In vitro excystation and total counts	32	0.5–2.0	0.039	0.005	Chauret et al., 1998
	In vitro excystation	26	11.2–20.8	0.003	0.002	Chauret et al., 1998
River water, St. Lawrence River	In vitro excystation and total counts	26	11.2–20.8	0.039	0.000	Chauret et al., 1998
	Excystation	5	35	0.01	0.003–0.016	Medema et al., 1997
Natural surface water	Dye exclusion	5	35	0.01	0.003–0.017	Medema et al., 1997
	Excystation	15	35	0.024	0.021–0.026	Medema et al., 1997
Natural surface water	Dye exclusion	15	35	0.018	0.000–0.037	Medema et al., 1997

Modified from Jenkins et al. (1997) with additional references.

Table 4  
Maximum duration of *Giardia* cyst and *Cryptosporidium* oocyst survival in different matrices based on mice infectivity (from Olson et al., 1999)

Material	Duration of survival (weeks)		
	Temperature (°C)	<i>Giardia</i> cysts	<i>Cryptosporidium</i> oocysts
Water	–4	<1	>12
	4	11	>12
	24	2	10
Normal soil	–4	<1	10
	4	7	8
	25	1	4
Autoclaved soil	–4	<1	>12
	4	6	>12
	25	1	6
Cattle faeces	–4	<1	>12
	4	1	8
	25	1	4

*pylobacter* also exhibit temperature-dependent inactivation. Comparison of the survival times of different *Campylobacter* strains in water microcosms over a range of temperatures under aerobic conditions showed mean survival times of 201.6, 175.6, 42.6 and 21.8 h at 4, 10, 22 and 37 °C, respectively (Buswell et al., 1998).

### 5.3. Salinity

Salinity has been found to have an effect on *Cryptosporidium* viability only at concentrations in excess of 20 ppt (Fayer et al., 1998b; Robertson et al., 1992). The World Health Organisation recommends drinking water salinity should be below 800 EC units, which corresponds to approximately 0.48 g l<sup>-1</sup> or 0.48 ppt. Since potable

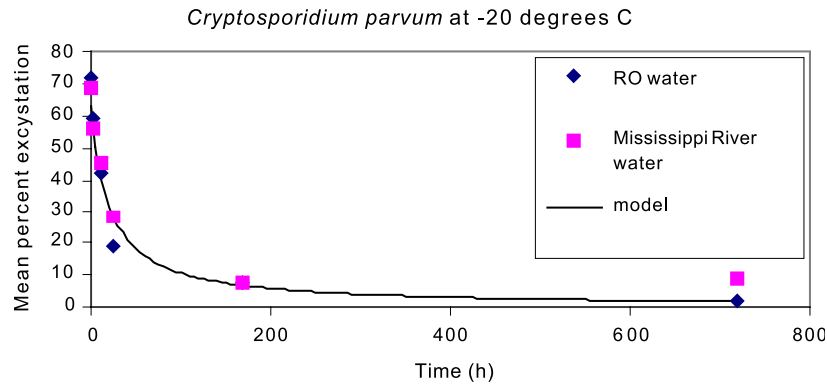


Fig. 4. *C. parvum* oocyst inactivation at  $-20^{\circ}\text{C}$  in reverse osmosis water and Mississippi River water. Data taken from Sattar et al. (1999). The model which describes the inactivation with time in RO water is  $y=1/(c+mt)$  where  $c=0.01583$ ,  $m=0.0079$  and  $t$  is in hours.

water sources will generally be lower than this guideline, the impact of salinity on *C. parvum* inactivation will be negligible. However, variations in the salinity of surface water runoff may impact on pathogen mobilisation. Bradford and Schijven (2002) calculated release efficiencies of *Cryptosporidium* oocysts, relative to manure release, and found that dispersion decreased with increasing solution salinity.

#### 5.4. Pressure

Slifko et al. (2000) studied the effects of high hydrostatic pressure on *Cryptosporidium*, and found that pressures in excess of  $5.5 \times 10^8$  Pa were required to render oocysts nonviable. This is equivalent to a water depth of 55,000 m, beyond the depth of the deepest ocean, thus the effects of pressure on *Cryptosporidium* inactivation will be negligible even in deep lakes and reservoirs.

#### 5.5. Solar radiation and inactivation of pathogens

A study by Garvey et al. (1998) identified light intensity as the most influential factor causing coliform die-off in a freshwater lake. The study also investigated the impact of other environmental factors, such as light intensity, predation, temperature and bacterial source, to determine

their contribution to coliform decay (Garvey et al., 1998). Sinton et al. (2002) examined sunlight inactivation of a range of bacteria and bacteriophages and found that sunlight inactivation rates, as a function of cumulative solar radiation, were more than 10 times higher than the corresponding dark inactivation rates. The relative susceptibility to light of a range of microbes, determined by Ks ranking from greatest to least inactivation, was as follows: enterococci > faecal coliforms  $\geq$  *E. coli* > somatic coliphages > F-RNA phages.

It appears that the most lethal wavelengths are within the ultraviolet range. The greatest germicidal effect on *Cryptosporidium* oocyst inactivation occurs at wavelengths between 250 and 270 nm (Linden et al., 2001). Sinton et al. (2002) examined inactivation of four faecal indicators at a range of different wavelengths. Inactivation was more acute at shorter wavelengths (Fig. 5); however, the greatest inactivation occurred at full sunlight. Because the enterococci and F-RNA phages were inactivated by a wide range of wavelengths, this suggests photooxidative damage. On the other hand, the inactivation of faecal coliforms and somatic coliphages were inactivated mainly by UV-B wavelengths, which suggests photobiological damage (Sinton et al., 2002) (Table 6).

#### 5.6. Ultraviolet radiation

Ultraviolet (UV) radiation dosing is a well-documented technique for the inactivation of *Cryptosporidium* oocysts (Clancy et al., 2000). UV radiation represents approximately 3–4% of incoming solar (<2800 nm) radiation (Kirk, 1994; Ziegler and Benner, 2000). For summer conditions at mid-latitude, a typical measurement for an hourly average incoming shortwave radiation around midday is  $1000 \text{ W m}^{-2}$ , which corresponds to approximately  $30 \text{ W m}^{-2}$  of UV light. For a 1-h exposure, this corresponds to a cumulative UV light dose of  $360,000 \text{ mJ cm}^{-2}$ . This is extremely high compared to water treatment dosages (Craik et al., 2001), which are typically of the range  $20\text{--}120 \text{ mJ cm}^{-2}$  (Fig. 6), and so it is highly likely that UV dosing under

Table 5  
Effect of freezing ( $-20^{\circ}\text{C}$ ) on viability of *G. muris* cysts in Mississippi River water (taken from Sattar et al., 1999)

Time (h)	Mean percent excystation	
	Based on numbers of free-swimming trophozoites	Based on numbers of empty shells and partially excysted trophozoites
0	79.2	80.2
1.5	45.8	49.8
12	4.6	11.3
24	0.6	2.5
168	0.0	0.0
720	0.0	0.0

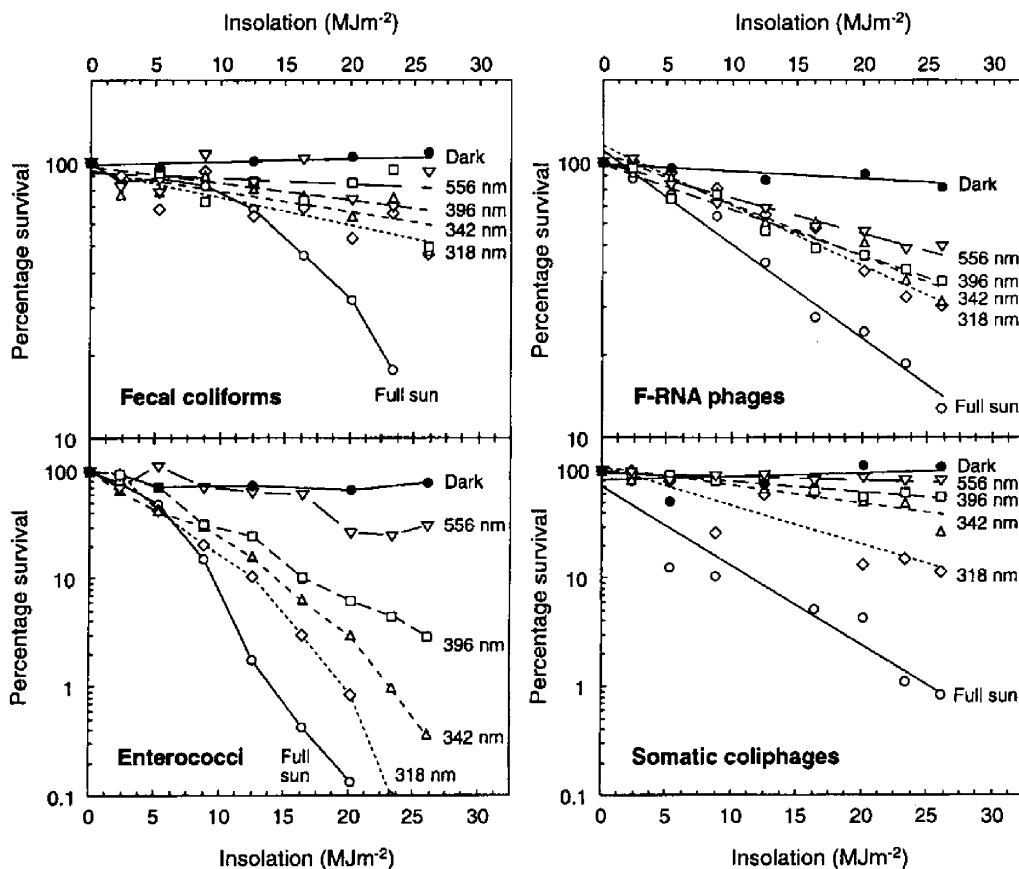


Fig. 5. Inactivation in river water of faecal coliforms, enterococci, F-RNA phages, and somatic coliphages from Waste stabilization pond effluent in the dark (●), under full sun (○), and under 556 nm (orange, ▽), 396 nm (polycarbonate, □), 342 nm (acrylic, △), and 318 nm (polyester, ◇) optical filters. Note the difference in y-axis scales. Figures taken from Sinton et al. (2002).

Table 6  
Mean sunlight inactivation parameters from Waste stabilisation pond effluent and raw sewage in fresh (river) water

Indicator	Effluent	Ks (m <sup>2</sup> MJ <sup>-1</sup> ) (n; CV%)		T90 (h)	
		Summer	Winter	Summer	Winter
Faecal coliforms	WSP	0.0086 (42; 30)	0.084 (20; 15)	8.8	16.8
	RS	0.275 (22; 23)	0.216 (7; 20)	3.3	7.7
<i>E. coli</i>	WSP	0.078 (41; 44)	0.073 (22; 14)	9.2	20.3
	RS	0.287 (22; 38)	0.237 (7; 19)	3.3	6.9
Enterococci	WSP	0.276 (39;30)	0.110 (21; 19)	3.0	14.5
	RS	0.137 (21;35)	0.138 (7; 13)	6.4	12.6
Somatic coliphages	WSP	0.077 (50; 19)	0.049 (25; 20)	11.6	30.6
	RS	0.098 (27; 29)	0.087 (9; 20)	8.3	15.9
F-RNA phages	WSP	0.070 (43; 25)	0.050 (18; 11)	13.3	27.5
	RS	0.075 (26; 18)	0.074 (9; 10)	12.5	19.1

Modified from Sinton et al. (2002).

environmental conditions in lakes and reservoirs has the potential to inactivate *Cryptosporidium*, at least during summer. Two log inactivation of *Cryptosporidium* oocysts was observed with a dose of just 2 mJ cm<sup>-2</sup> at wavelengths between 250 and 270 nm (Linden et al., 2001).

Using the data of Craik et al. (2001), oocyst inactivation due to UV exposure follows an exponential decay function similar to temperature (Fig. 7):

$$C(t) = C_0 e^{-k_{UV}H} \tag{4}$$

where  $H$  is UV dosage (i.e. intensity integrated over time) estimated following:

$$H = I_{UV}t. \tag{5}$$

Here,  $I_{UV}$  is UV intensity,  $k_{UV}$  is the decay coefficient of UV light and  $C(t)$  is the concentration of viable oocysts at time  $t$ . Based on the laboratory data of Craik et al. (2001), the value of  $k_{UV}$  is approximately 1.2.

The vertical distribution of UV light is described by an exponential function equivalent to Beer's law:

$$I_{UV}(z) = I_{UV(\text{surface})} e^{-\mu z} \tag{6}$$

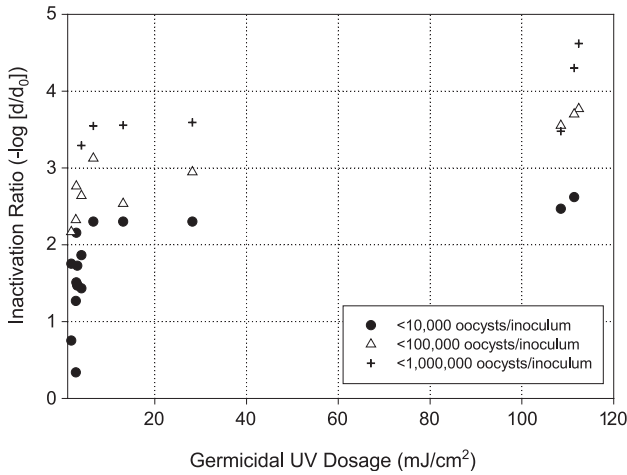


Fig. 6. Variation of oocysts viability with UV dosage for three different concentrations of oocysts samples (data taken from Craik et al., 2001).

where  $\mu$  is the attenuation coefficient of UV light. This attenuation coefficient is closely related to dissolved organic carbon (DOC), i.e.  $\mu=f(\text{DOC})$  (Kirk, 1994; Morris et al., 1995). Attenuation coefficients for UV light are different to those for photosynthetically active radiation (PAR), and have been measured at values between 0.1 and 40  $\text{m}^{-1}$  (Morris et al., 1995). Given the possible range of UV light attenuation coefficients, the penetration depth of UV light (defined as the depth for reduction to 1% of surface irradiation levels) for lakes can be anywhere between 0.1 and 46 m. This means that UV light inactivation of *Cryptosporidium* can potentially be important for lakes of significant depth, provided the DOC concentrations are low. In any event, the impact of UV light on oocyst viability is governed by what is essentially a double exponential (Eqs. (4) and (5)), implying that there is a sharp transition in the water column between UV light having a dramatic effect, and UV light having an insignificant effect. Because of the large variability of DOC between water bodies, this ‘tran-

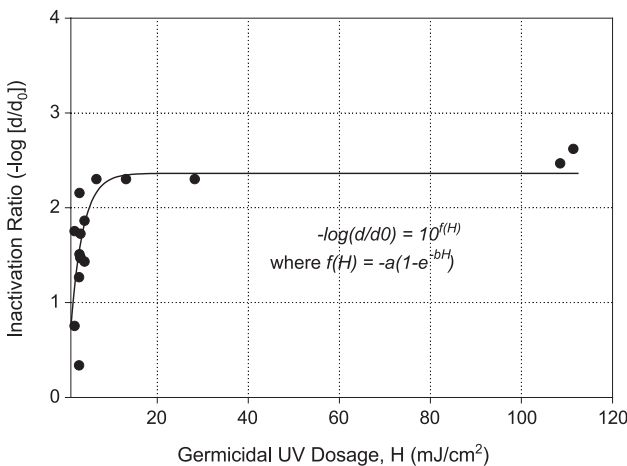


Fig. 7. Variation of oocysts viability with UV dosage for the least concentrated data samples, showing the expression (solid line) of best fit— $R^2=0.52$  (data taken from Craik et al., 2001).

sitional’ depth may vary from the surface layer to deep into the water column.

Complete inactivation of *Giardia lamblia* measured using mice infectivity assays has been observed at UV doses of 2–3  $\text{mJ cm}^{-2}$  (Mofidi et al., 2002). However, other studies have found that if the UV dose received is relatively low ( $<25 \text{ mJ cm}^{-2}$ ), *Giardia muris* and *C. parvum* are still capable of causing infection using mice assays (Belosevic et al., 2001). However, at doses above 60  $\text{mJ cm}^{-2}$ , there was no reactivation of either *G. muris* cysts or *C. parvum* oocysts, which suggests that this is the minimum dose required for permanent inactivation. A study by Meng and Gerba (1996) found significant variability in the resistance of viruses to UV light exposure. Relative resistance to UV dose from least to greatest was poliovirus type 1, the coliphages PRD-1 and MS-2, and enteric adenoviruses EAd41 and EAd40, respectively (Table 7). MS-2 is considered a good model for enteric virus disinfection since it is more resistant to UV than most enteroviruses and enteric bacteria. PRD-1 is a double-stranded DNA phage and is similar in size to rotaviruses and adenoviruses (Meng and Gerba, 1996). Because of the resistance of adenovirus EAd40 to UV inactivation, it may be a useful indicator virus for conservative estimates of virus inactivation in natural waters.

5.7. Ammonia

Free ammonia ( $\text{NH}_3$ ) has been found to cause high levels of inactivation of *Cryptosporidium* oocysts at concentrations above 0.007 M (Jenkins et al., 1998, 1999a). For a pH of 8, this is equivalent to an ammonium ( $\text{NH}_4^+$ ) concentration of 1780  $\text{mg l}^{-1}$  (this increases to 17,800  $\text{mg l}^{-1}$  for a pH of 7). Increased levels of ammonium ( $\text{NH}_4^+$ ) are often observed in the hypolimnion of lakes towards the end of the stratified period, as low dissolved oxygen concentrations result in sediment release of  $\text{NH}_4^+$ . These values, however, are typically much less than 1  $\text{mg l}^{-1}$ , and so the impact of free ammonia on *Cryptosporidium* oocyst viability will be negligible in drinking water reservoirs.

Table 7  
Log<sub>10</sub> survival ratios of viruses after UV exposure (taken from Meng and Gerba, 1996)

UV dose ( $\text{mW s cm}^{-2}$ )	Log <sub>10</sub> $N_t$ /log <sub>10</sub> $N_0$				
	Polio-1	PRD-1	MS-2	EAd 41	EAd 40
0	0	0	0	0	0
9	-2	-0.9	-7.8	-0.5	-0.2
18	-3.43	-2.1	-1.33	-0.9	-0.68
24	-4.75	-3.1	-1.69	-1.07	-0.87
35	-5.7	-4.6	-2.36	-1.39	-1.21
80	ND	ND	-5	-3.0	-2.6
90	ND	ND	ND	-3.3	-3
120	ND	ND	ND	ND	-4

1  $\text{mW s cm}^{-2}=1 \text{ mJ cm}^{-2}$ ; ND: not determined.

### 5.8. Predation of pathogens

The grazing of pathogens by aquatic invertebrates has several implications for transport through the reservoir, settling characteristics and possible transmission to human consumers. Grazing of pathogens by free-living protozoa or small zooplankton may change the settling behaviour of the pathogen if it is excreted intact in a faecal pellet. However, passage through the gut of a predator may render the pathogen nonviable. Weislo and Chrost (2000) detected *E. coli* up to 30 days after input to Zegrzynski Reservoir. They deduced that the major factor responsible for the mortality of *E. coli* was microflagellate grazing and exposure to the aquatic environment. The selective grazing of pathogens may affect the choice of microorganisms for use as indicators or surrogates for pathogen contamination. *C. perfringens* spores have been reported as a potential conservative indicator of sewage presence as they accumulate in sediments and appear to be resistant to predation (Davies et al., 1995).

If pathogens are ingested by grazing microflagellates and subsequently excreted as a faecal pellet, this will affect the settling characteristics of the pathogens. A recent review of the sedimentary flux of organic particles in marine and freshwater ecosystems has identified that smaller faecal pellets of microzooplankton and small mesozooplankton are mostly recycled or repackaged in the water column by microbial decomposition and coprophagy and contribute more to processes in the water column than flux to the benthos (Turner, 2002).

Research on predation of *Cryptosporidium* oocysts is limited. Stott et al. (2001), investigated oocyst grazing by ciliated protozoa where they were exposed to prey densities of  $10^4$ – $10^6$  oocysts  $\text{ml}^{-1}$ . Typical water supply reservoir concentrations of oocysts range between 0 and 100 oocysts per 100 l, that is 0–0.001 oocysts  $\text{ml}^{-1}$ , which represents an extremely low prey density for grazing. Phytoplankton densities are typically 1000–100,000 cells  $\text{ml}^{-1}$ , and so the effect of grazing is likely to be more obvious on the phytoplankton populations. Fayer et al. (2000) examined the ingestion of oocysts by zooplankton, and found that oocysts accumulated in the stomachs of various rotifer species. Oocysts were found to be excreted in boluses with up to eight oocysts aggregated together; however, it is not clear whether the oocysts were still viable.

### 5.9. Accumulation of pathogens in filter-feeding shellfish

Filter-feeding molluscan shellfish can accumulate waterborne pathogens in their tissue, which may have significant human health implications if the shell fishery is harvested for human consumption. Important issues are the magnitude and type of pathogens and species and whether these organisms remain viable and infective in the mollusc tissue. *Giardia* spp. has been identified in

*Macoma balthica* and *Macoma mitchelli* clams in Rhode River, a Chesapeake Bay sub-estuary (Graczyk et al., 1999a). *C. parvum* contaminated Bent mussels (*Ischadium recurvum*) have been recovered from Chesapeake Bay (Graczyk et al., 1999b) and infectious *C. parvum* oocysts have also been detected in oysters from Chesapeake Bay tributaries (Fayer et al., 1998b). While these shellfish do not represent an economic fishery, the presence of pathogens highlights a possible route for transmission to humans. In the St. Lawrence River, Quebec, zebra mussels (*Dreissena polymorpha*) were found to have concentrations of 67 oocysts  $\text{ml}^{-1}$  of hemolymph, and 129 oocysts  $\text{g}^{-1}$  of soft tissue (Graczyk et al., 2001). This translates to a concentration of 440 oocysts per mussel. This is a concern since it demonstrates that molluscs can serve as a mechanical vector for this pathogen.

### 5.10. Survival in sediments

The ability of microorganisms, to survive in aquatic sediments implies that faecal coliforms detected in the water column of lakes and reservoirs may not always indicate recent contamination but may be the result of sediment resuspension (LaLiberte and Grimes, 1982). Since microbial activity in sediments is greatly encouraged by the presence of organic matter (Millis, 1988; Ferguson, 1994), it is possible that in nutrient-rich environments, microorganisms may survive in sediments for extended periods of time (Davies et al., 1995). When assessing pathogen risks within a reservoir, it is important to determine whether pathogen resuspension may occur within the pathogen survival timeframe. The resuspension of pathogens from sediment due to turbulence at the benthic boundary, attributable to internal waves or wave action of leeward shores, may present a pathogen risk not anticipated from river inflow data alone. The prolonged survival and accumulation of microorganisms in sediments, and the likelihood of their being desorbed by dilution or water turbulence indicates that sediments, as well as surface waters, should be assessed when estimating potential

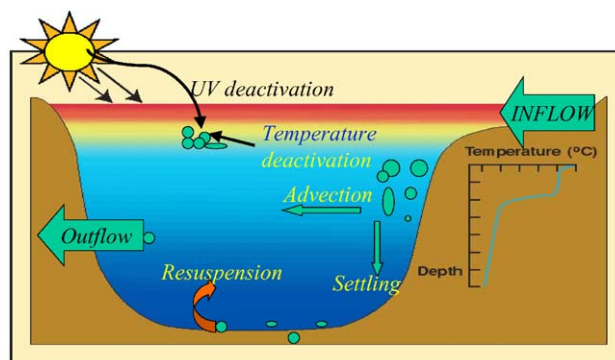


Fig. 8. Conceptual model of the major processes affecting pathogen fate and transport through a reservoir.

Table 8

Timescales for Burragorang and Myponga (in days), for inflow insertion and several different settling velocities

Timescale	Burragorang	Myponga
Insertion	7	1.25
Settling (0.03 m day <sup>-1</sup> )	2800	1100
Settling (0.5 m day <sup>-1</sup> )	170	66
Settling (2 m day <sup>-1</sup> )	43	17
Settling (5 m day <sup>-1</sup> )	17	7

health risks (Geldreich, 1970; LaLiberte and Grimes, 1982; Ferguson, 1994; Davies et al., 1995).

## 6. Conceptual model

It is apparent that the major processes affecting pathogen fate and transport in reservoirs are the riverine intrusion and inactivation by UV light and temperature. These processes are conceptualised in Fig. 8.

## 7. Importance of timescales for pathogen inactivation and transport

The timescales of the major processes for pathogen inactivation and temperature determine the magnitude of possible risk reduction in reservoirs. These are:

- the insertion (or advection) timescale, the time taken for the inflow to reach the off-take point after entering the reservoir (Table 8),
- the settling (or sedimentation) timescale, the time taken for particles in the surface layer to sink to the bottom at the deepest point in the reservoir (Table 8),
- the temperature inactivation timescale, the time taken for pathogens, e.g. oocysts to become inactive after exposure to water of a certain temperature (~ 84 days for 25 °C, >170 days for 15 °C),
- the UV inactivation timescale, the time taken for pathogens, e.g. oocysts to receive a fatal dose of UV radiation (Tables 9 and 10).

The general trend is for the insertion timescale to be shortest, followed by sedimentation losses and temperature

Table 9

UV light dose (mJ/cm<sup>2</sup>) for various depths and UV attenuation coefficients, for a theoretical day of 12 h sunlight duration with maximum incoming shortwave radiation of 1000 W/m<sup>2</sup> at midday and 3% of incoming shortwave as UV

z (m)	$k_{d(UV)}=0.1$	$k_{d(UV)}=1.0$	$k_{d(UV)}=5.0$	$k_{d(UV)}=10.0$
2	67,500	11,200	3.75	0.00
5	50,000	556	0.00	0.00
10	30,400	3.75	0.00	0.00
20	11,200	0.00	0.00	0.00
40	1510	0.00	0.00	0.00

Table 10

Timescale for inactivation by UV irradiation (in days), for a fatal dose of 50 mJ cm<sup>-2</sup> (from Clancy et al., 2000; Craik et al., 2001), based on Table 9

z (m)	$k_{d(UV)}=0.1$	$k_{d(UV)}=1.0$	$k_{d(UV)}=5.0$	$k_{d(UV)}=10.0$
2	<1	<1	13	>1×10 <sup>5</sup>
5	<1	<1	>1×10 <sup>5</sup>	>1×10 <sup>5</sup>
10	<1	13	>1×10 <sup>5</sup>	>1×10 <sup>5</sup>
20	<1	>1×10 <sup>5</sup>	>1×10 <sup>5</sup>	>1×10 <sup>5</sup>
40	<1	>1×10 <sup>5</sup>	>1×10 <sup>5</sup>	>1×10 <sup>5</sup>

z is depth.

inactivity. The fate of *Cryptosporidium* due to UV light inactivation can occur at opposite ends of the scale, depending on the location of the oocysts in the water column and the extinction coefficient for UV light. For this reason, the extinction coefficient for UV light appears to be a vitally important parameter for determining the risk of *Cryptosporidium* contamination. Several authors have found a relationship between the UV light extinction coefficient and dissolved organic carbon (DOC) concentration (Scully and Lean, 1994; Morris et al., 1995). An approximate DOC concentration was determined for each of the UV light extinction coefficients presented in Table 8, and are presented in Table 9. It appears that for lakes in which the extinction coefficient for UV light exceeds 10 (corresponding to a DOC concentration of approximately 4–10 mg l<sup>-1</sup>), the inactivation of *Cryptosporidium* oocysts by UV light can be ignored as the timescales are so long (Tables 10 and 11).

## 8. Risk management framework for pathogens: how does the reservoir “barrier” fit into the big picture of risk management?

Risk management frameworks aim to satisfy three objectives:

- maximise the quality of the product,
- minimise the risk to consumers, and
- maximise security to the business.

Water supply managers are embracing a range of risk-based approaches for water supply management. Within this context a storage reservoir becomes a barrier to minimise the pathogen densities reaching the water supply off-take points. The types of risk-based systems being adopted by water suppliers draw from the generic systems developed for quality assurance, process control and risk

Table 11

Dissolved organic carbon concentration (mg l<sup>-1</sup>) corresponding to light extinction coefficients for UV-A (380 nm) and UV-B (320 nm) based on Morris et al. (1995)

	$k_{d(UV)}=0.1$	$k_{d(UV)}=1.0$	$k_{d(UV)}=5.0$	$k_{d(UV)}=10.0$
320 nm	0.067	0.52	2.18	4.05
380 nm	0.16	1.17	4.70	8.54

management. These include the Hazard Analysis and Critical Control Points (HACCP) principles as well as the expansion of the Partnerships for Safe Water approach to include storages.

An Australian example of the application of a risk management approach is the draft NHMRC “Framework for Management of Drinking Water Quality” released in May 2001 (McRae et al., 2001). The implicit intention of the framework is to shift the focus for water quality management away from “end product testing” or compliance monitoring to overall quality assurance management of the system. The framework approach extends from the catchment to the customer tap and requires system knowledge and understanding. There is a similar philosophy within the “Hazard Analysis and Critical Control Points” (HACCP) management system developed for the food industry. Aspects of HACCP are also being considered for incorporation into water quality management systems (Deere and Davison, 1998; Gray and Morain, 2000).

These frameworks incorporate the principles of both multiple barriers for hazard interception and management, and “Critical Control Points” (CCPs) to enhance security in the supply system. Within the context of the discussion of the barrier approach, there has been debate about the role of reservoirs. In some cases, the reservoir provides a detention-time barrier for the progress of contaminants such as pathogens, turbidity or pesticides from the catchment to the off-take or treatment plant. However, it is possible for contaminants to be carried by intruding flows rapidly and directly from inlet to outlet through the reservoir, i.e. short-circuiting. This transfer can be significantly faster than expected, based on a nominal retention time for turnover of the whole volume. Also, in the case of other water quality problems, such as those caused by cyanobacterial growth and iron and manganese, the reservoir may be a source of hazards rather than a barrier.

Within the HACCP system, the “Critical Control Points”, by definition, require both continuous monitoring

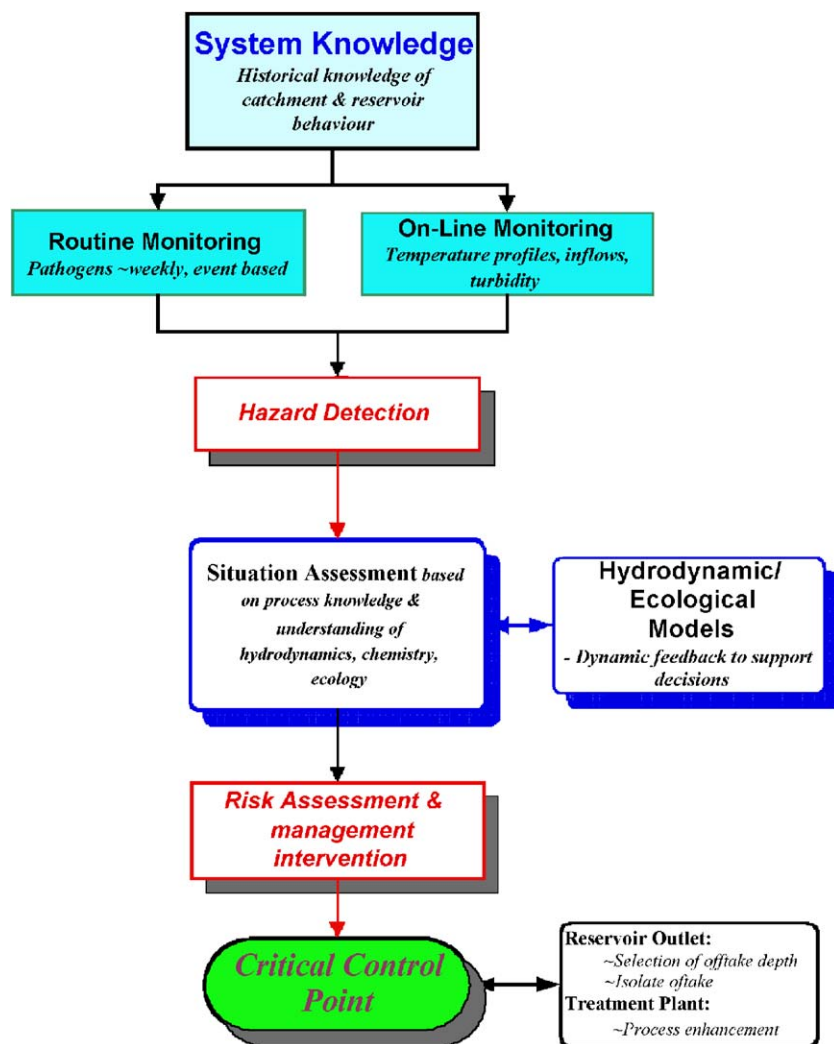


Fig. 9. Conceptual framework for pathogen monitoring and risk assessment in reservoirs.

and are also process steps that are amenable to intervention or corrective action to prevent a downstream problem. The aim is to control hazards as close as possible to their source (Deere and Davison, 1998). In the case of a reservoir as a component of the water supply system, a potential CCP is the off-take, where intervention could consist of selection of different depths to take water, or choosing not to take water if the quality is compromised. The usefulness of a CCP at the reservoir outlet depends upon the speed with which the hazard can be detected and assessed for pro-active intervention.

### 9. Reservoir processes, hydrodynamics and timescales of hazard development

An essential component of a risk-based management framework for water quality is the requirement for a high level of knowledge and understanding of the particular system in question (Teunis et al., 1997). To develop this requires the assessment of historical information to identify hazards, and to understand how they could evolve into risks in that system. In reservoirs, it is important to understand both the processes that control the hazards, and the timescales over which they occur.

There is also a need to optimise the performance of the storage as a barrier to provide the best possible water quality to customers as well as a need to have early warning when this barrier fails. One objective will be to provide a tool that enables pathogen concentrations to be included when optimising water harvesting and storage management regimes. This will enable water suppliers to optimise the performance of their storage as a barrier to pathogen transport. The second objective will be to provide tools to assist water suppliers to predict and quantify pathogen events. This will facilitate modelling of the reservoir and enable prediction of times when raw water quality may be unacceptable.

A conventional routine monitoring program usually provides weekly or monthly data on the physical, chemical and biological status of the reservoir. However, as indicated, some of the hazards can develop at timescales that are shorter than the sampling interval. The keys to developing an enhanced monitoring system for real-time hazard detection are, firstly, an understanding of the reservoir hydrodynamics and, secondly, integrating the required on-line physical and chemical data and routine data with an understanding of the ecological, chemical and physical processes. A process flow chart of these steps is given in Fig. 9. Examples of background historical system knowledge would include an understanding of seasonal hydrology, knowledge of sources of pathogens in the catchment, as well as knowledge of stratification and riverine inflow behaviour of the reservoir.

The on-line monitoring considered here consists of automatic flow gauging on inflow streams, high-resolution thermistor chains in the reservoir, and turbidity probes

suspended at depth. The monitoring is combined with detailed knowledge of hazard and reservoir processes (Fig. 9) to carry out the situation and risk assessment. In its most advanced form, this monitoring could be linked to ‘state of the art’ hydrodynamic and ecological models to provide extra decision support by predictive modelling of stratification, riverine inflow and pathogen transport. An example of such a sophisticated model is ELCOM-CAEDYM developed by the Centre for Water Research in Western Australia (<http://www.cwr.uwa.edu.au>).

On-line monitoring of reservoir water temperature, creek inflow and turbidity can readily be integrated into the water treatment process to improve the efficiency of treatment for potable water. The conceptual models illustrated in this paper are preliminary and represent a framework for developing integrated reservoir and treatment plant operating strategies.

To generate the best risk management strategy incorporating on-line monitoring, the following conceptual framework should be applied:

- Review historical data,
- Identify hazards to water quality and threats to treatment plant,
- Deploy on-line monitoring,
- Continue routine analysis of pathogens,
- Determine hydrodynamic features which lead to hazard generation,
- Develop conceptual model for predicting risk and treatment response, given the prevailing and predicted hydrodynamics.

### 10. Conclusion

Variability in pathogen fate and transport introduces many levels of complexity in determining appropriate surrogates for risk analysis. Regardless of whether the actual pathogen or surrogate is analysed, the sampling program must be optimised to maximise the information gathered from a small number of samples. For risk assessment of pathogens in supply reservoirs, it is important to understand the role of hydrodynamics in determining the timescale of transport to the off-take relative to the timescale of inactivation. The characteristics of the riverine intrusion must also be considered when designing a sampling program for pathogens. This will provide guidance for sample site, timing and frequency of sampling and allow a better prediction of pathogen risk.

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