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Modelling of sedimentation and transport of *Cryptosporidium* in a drinking water source

Master of Science Thesis in the Master's Programme Geo and Water Engineering

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ABSTRACT

The parasite *Cryptosporidium* has been implicated as the causative agent of many waterborne disease outbreaks, e.g. in recent outbreaks in Östersund and Skellefteå, Sweden. In order to perform a risk assessment of health hazards in a drinking water source it is essential to understand the processes that affect the fate and transport of pathogenic microorganisms (e.g. *Cryptosporidium*) in the aquatic environment. The settling mechanism is a central part in this context. The aim of this study was to develop a modelling approach that takes into account the settling kinetics of pathogens, particularly *Cryptosporidium*. The developed modelling approach was applied to simulate the transport and settling of *Cryptosporidium* in Lake Rådasjön, which is the main drinking water source of Mölndal, Sweden. The settling modelling approach was implemented in the ecological module, ECO Lab, which was coupled to the three-dimensional hydrodynamic model MIKE 3 FM. The modelling was carried out for spring conditions, taking into account different faecal contamination sources around the lake. The result of the simulations was presented as time series of *Cryptosporidium* concentrations at the raw water intakes. It can be concluded that the magnitude of the settling velocity has great influence on the transport characteristics of *Cryptosporidium*. The magnitude of the settling velocity is to a great extent governed by the ability of the parasite to attach to particles. Since no settlement experiments have been performed for Lake Rådasjön, the ability of *Cryptosporidium* to attach to particles in these conditions forms a great uncertainty. The river Mölndalsån was determined to be the source that contributes most to the *Cryptosporidium* concentration at the raw water intakes.

Key words: *Cryptosporidium*, ECO Lab, MIKE 3 FM, hydrodynamic modelling, settling, sedimentation

Sedimentation och transport modellering av *Cryptosporidium* i en råvattentäkt

Examensarbete inom Geo and Water Engineering

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SAMMANFATTNING

Parasiten *Cryptosporidium* har faställts vara den orsakande källan till många sjukdomsutbrott som sprids med vatten, t.ex. nyligen inträffade utbrott i Östersund och Skellefteå. För att utföra en riskbedömning av hälsoriskerna i en råvattentäkt är det viktigt att förstå de processer som påverkar nedbrytning och transport av patogena mikroorganismer (t.ex. *Cryptosporidium*). Mikroorganismens förmåga att sedimentera i vattenmassan är en central del i detta sammanhang. Syftet med denna studie var att utveckla ett tillvägagångssätt att modellera patogeners sedimentationskinetik, speciellt *Cryptosporidium*. Det utvecklade modellerings- tillvägagångssättet tillämpades för att simulera transport och sedimentation av *Cryptosporidium* i Rådasjön, huvudråvattentäkt för Mölndal kommun. Modellerings- tillämpningsmetoden implementerades i den ekologiska modulen, ECO Lab, som var kopplad till den tredimensionella hydrodynamiska modellen MIKE 3 FM. Modelleringen utfördes under vårförhållanden, med hänsyn till olika fekala föroreningskällor runt omkring sjön. Resultatet av simuleringarna presenterades som tidsserier av *Cryptosporidium* koncentrationer vid råvattenintagen i sjön. Slutsatsen som kan dras är att sedimentationshastighetens storlek påverkar i hög grad parasitens transportegenskaper. Sedimentationshastighetens storlek styrs till en stor del av parasitens förmåga att fästa på partiklar. Eftersom inga sedimentationsförsök har utförts med vatten från Rådasjön så är parasitens förmåga att fästa på partiklar under dessa förhållanden okänd, vilket bidrar till en stor osäkerhet i studien. Mölndalsån bedömdes vara den källa som bidrar mest till *Cryptosporidium* koncentrationen vid råvattenintagen.

Nyckelord: *Cryptosporidium*, ECO Lab, MIKE 3 FM, hydrodynamisk modellering, sedimentation

Contents

Preface

In this study, a modelling approach that takes into account the settling kinetics of pathogens has been developed. The study was performed from January 2011 to June 2011. The project is a part of a present work that deals with fate and transport modelling of microbial pollution in Lake Rådasjön. The project was carried out at the Department of Civil and Environmental engineering, Division of Water Environmental Technology at Chalmers University of Technology, Sweden.

The study has been conducted by Emil Ahlbom, student at the Master's Programme Geo and Water Engineering, with Assistant Professor Thomas Pettersson and PhD student Ekaterina Sokolova as supervisors. The modelling has been carried out using the hydrodynamic model MIKE 3 and the ecological module ECO Lab, produced by DHI.

First, I would like to thank Ekaterina Sokolova for excellent supervision and invaluable support and teaching. Furthermore, I would like to thank Thomas Pettersson and Johan Åström for great assistance and interesting feedback.

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Emil Ahlbom

1 Introduction

1.1 Problem description

Outbreaks of waterborne diseases caused by pathogenic organisms pose a significant threat to the public health (Antenucci et al., 2005). In order to produce safe drinking water a risk assessment of the water reservoir supply is required. The risk assessment should include an identification of the hazards to the water quality (i.e. faecal contamination sources) and an understanding of the pathogen transport and fate processes in the water reservoir system (Brookes et al., 2004). Hydrodynamic and ecological models are convenient tools to simulate these processes. Modelling together with regularly monitoring compose an appropriate decision support for assessment of the risks regarding microbial contamination in water reservoir supplies.

The settling mechanism needs to be accounted for in the modelling since it is one of the major processes that affect the fate and transport of pathogens in aquatic systems. It is a complex mechanism, which is largely influenced by the ability of pathogens to attach to particles. The settling kinetics is of particular importance for the pathogens in the greater size range including the pathogenic protozoan *Cryptosporidium*. *Cryptosporidium* pose a particular threat to the water industry due to its resistance to environmental factors and resistance to chlorine disinfection in drinking water treatment plants (Searcy et al., 2005, Brookes et al., 2006). *Cryptosporidium* has been implicated as the cause of many waterborne disease outbreaks, e.g. recently outbreaks in Sweden in Östersund and Skellefteå.

1.2 Aim and objectives

This study is a part of a present project that works with fate and transport modelling of microbial pollution in Lake Rådasjön. The aim of the study was to develop a modelling approach that takes into account the sedimentation process for different pathogenic organisms, particularly *Cryptosporidium.* Furthermore, the objective was to implement the developed sedimentation model and examine the output result.

In order to acquire a necessary knowledge base a literature review was performed. The literature review covers sedimentation theory and general fact about pathogens, particularly *Cryptosporidium.* The most important parts discuss the ability of the pathogens to attach to particles and settle in the suspension column being aggregated to particles. Furthermore, the review focuses on compiling and comparing different settling velocities for *Cryptosporidium,* measured under various experimental conditions. The literature review includes also an evaluation of the studies that apply sedimentation modelling.

Based on the literature review the sedimentation modelling approach was developed and implemented for *Cryptosporidium.* The sedimentation was formulated using ECO Lab model, which was coupled to the three-dimensional hydrodynamic model MIKE 3 FM. The created model was applied to simulate the transport and settling of *Cryptosporidium* oocysts discharged from faecal contamination sources in Lake Rådasjön. The simulations were performed for different meteorological conditions and settling velocities. The *Cryptosporidium* concentrations at the raw water intakes were analyzed. These results were also compared with the results of the model that

takes into account only advective transport and of the model that takes into account the temperature decay.

1.3 Delimitations and assumptions

The settling and transport modelling have been performed for *Cryptosporidium.* The main objective was to show that the developed sedimentation modelling approach is valid. The assumptions and simplifications involved in the performed modelling are:

- the *Cryptosporidium* concentrations in the discharges from the faecal contamination sources are constant;
- the discharges into the lake are constant;
- the wind velocity and direction are set constant for the simulation period;
- resuspension is not taken into consideration.

2 Theoretical background

2.1 Pathogens

2.1.1 General information

There are three different groups of microorganisms that are concerned as a health risk regarding drinking water: viruses, bacteria and protozoa (WHO, 2008). The pathogenic organisms derive from excrete shed by infected hosts (animals and humans). In a drinking water reservoir the contamination sources are sewage and animals wastes (Gray, 2008).

Outbreaks of waterborne diseases caused by pathogens may affect large numbers of people (WHO, 2008). Some of the pathogens (e.g. *Salmonella*, *Vibrio cholera* and Hepatitis A virus) that are known to be transmitted by drinking water, are associated with severe diseases, such as typhoid, cholera and infectious hepatitis, while other (e.g. Norovirus, *Cryptosporidium*) normally cause less severe outcomes, such as self-limiting diarrhoeal disease.

In order to estimate the potential health risk associated with microorganisms in drinking water supplies measurements of indicator bacteria are used (Fries et al., 2006). This is performed due to the difficulties and expenses associated with testing a specific pathogen (Drozd and Schwartzbrod, 1996). The most commonly used indicator organisms belong to the coliform group and *Escherichia Coli* is the most used coliform.

Viruses consist of a core of nucleic acid (RNA or DNA) enclosed by a protein coat (Gray, 2008). The size range of viruses is about 20 – 400 nm. They are not productive without a host cell, but are able to persist in the environment for long durations. Enteric viruses are released in very high numbers from infected hosts: normally between 10^5 and 10^{11} per gram of feces (Fong and Lipp, 2005). More than 114 different enteric viruses are known (Meinick and Gerba, 1982). Viruses that pose a health risk in drinking water are: Adenoviruses, Enteroviruses, Astroviruses, Hepatitis A virus, Hepatitis E virus, Noroviruses, Sapoviruses and Rotavirus (WHO, 2008).

Among the microorganisms the bacteria group has the highest frequency of isolation and reported outbreaks of disease (Gray, 2008). The bacteria are typically rod shaped with a length between 2 and 6 µm and a width from 0.5 to 2 µm (Pachepsky et al., 2006). The most important bacterial pathogens in the temperate zone include *Salmonella, Campylobacter, Shigella, Vibrio cholrea* and *Escherichia coli* O157:H7 (Gray, 2008). *Campylobacter* is the major cause of gastroenteritis in Europe, while *Shigella* is the most frequently diagnosed cause of diarrhea in the USA.

There are mainly two types of protozoa that are frequently discovered in drinking water: *Cryptosporidium* (see Section 2.2.1) and *Giardia lamblia* (Gray, 2008). *Giardia lamblia* produces a nonproductive cyst which is shed with the excretion from infected hosts. The cyst has an oval shape of 8-14 µm long and 7-10 µm wide. The cysts have high persistence, i.e. they have the ability to persist in water reservoirs for long time periods.

2.1.2 Settling theory and Stokes' law

A number of studies have verified that the theoretical sedimentation kinetics, calculated by Stokes' law, of *Cryptosporidium parvum* oocysts correspond to the sedimentation kinetics determined in assays. (Dai and Boll, 2006, Medema et al., 1998, Pachepsky et al., 2006). The settling velocity is, according to Stokes' equation, determined as:

$$V_s = \frac{gd^2}{18\mu}\left(\rho_p - \rho_l\right) \quad (1)$$

The equation has been shown to be valid for freely suspended oocysts and for oocysts attached to particles. V_s is the sedimentation velocity (m s^{-1}), g is the gravitational acceleration (m s^{-2}), d is the particle diameter (m), μ is the dynamic viscosity of the liquid (Ns m^{-2}), ρ_p is the density of the particle (kg m^{-3}) and ρ_l is the density of the liquid (kg m^{-3}) (Medema et al., 1998).

Stokes' law is applied for low flow environment (laminar conditions) such as a reservoir (Dai and Boll, 2003). McNown and Malaika (1950) performed theoretical and experimental studies in order to evaluate the applicability of Stokes' law for different Reynolds number. The result exhibited that for a Reynolds number less than 0.1, there is little divergence from the Stokes' equation and for Re<0.5 the error is less than 10%. Reynolds (1987) determined that Stokes' law is not applicable to flow environments that are characterized by a Reynolds number exceeding 0.5.

2.1.3 Aggregation to particles

The attachment of pathogens to organic matter and clay influence largely their settling, survival and transport characteristics (Jamieson et al., 2004, LaBelle and Gerba, 1979). Because of that, an estimate of fractions of pathogens attached to particles is useful for modelers aiming to model microbial transport (Jamieson et al., 2004). The complexity and heterogeneity of soil ecosystem makes it complicated to study the adhesion mechanism between pathogens and soil particles (Stenström, 1989). The surface charge, also referred as electrophoretic mobility or zeta-potential, has a great influence on the mechanism. Particles with opposite surface charges tend to attach, while particles with like charges repel (Dai and Boll, 2003). Other parameters of importance in this context are pathogen surface characteristics like: hydrophobicity, wettability, and surface texture (Stenström, 1989). Furthermore the diversity of different types of soil particles and the ionic composition of the solution affect the process.

Two types of bacterial adsorption forces have been identified (Jamieson et al., 2004). The first type, Van der Waals, is a weak, reversible bonding that can adsorb bacteria particles in the suspension. This may occur in solutions containing high electrolyte concentrations where the repulsive zeta-potential forces are suppressed. The second adhesion type is a strong, irreversible force, which occurs when extracellular polymer in the soil coats the bacterial surface.

Schiffenbauer and Stotzky (1982) investigated the adsorption of coliphages (bacterial viruses infecting Escherichia coli) T1 and T7 to the clay minerals kaolinite and montmirollinite. The assay was performed at pH 6.8, in these conditions both the clay minerals and coliphages T1 and T7 generally have negative electrostatic charge. The

result showed that the coliphages adsorbed readily to the clay minerals, which confirm the results from (Bitton et al., 1978, Gerba et al., 1975) that soil with high clay content has high adsorption capacity of viruses. A study performed by Lipson and Stotzky (1983) aimed to examine the adsorption between reovirus and clay minerals. Their assays showed that the reovirus adsorbed essentially instant to the clay minerals. The adsorption was correlated with the cation- exchange capacity of the different, suggesting that the adsorption was primarily to the negative zeta-potential of the clays. LaBelle and Gerba (1979) showed that a variety of enteric viruses exhibited greater than 99% adsorption to estuarine sediments.

In order to estimate the proportion of the faecal contamination indicators: *Enterococcus* sp. and *E. coli* that attached to particles and settled out of the water column observations were made in Neuse River Estuary, Northern Carolina by Fries, Characklis et al. (2006) during the summer. The tests were made during both dry- and wet conditions. The result showed little variations for the proportion of faecal contamination indicator attached to particles with one exception: the *Enterococcus* sp. attached in a larger fraction for the dry condition. For the other groups, approximately 38% of the indicator organisms attached to particles and settled out of the water column. The authors speculated that the deviating result might be a consequence of different surface characteristics. Assays performed by Guber, Shelton el al. (2005) showed that the presence of manure affected negatively the *E. coli* attachment to soil particles. The maximum attachment between *E. coli* and soil particles occurred in the absence of manure, an interesting observation since *E. coli* exclusively derives from feces or manure.

Stenström (1989) concluded that the adhesion process between bacteria like: *Salmonella typhimurium, E. coli* and *Streptococcus faecalis* and mineral particles (quartz, albite, feldspar and magnetite) was correlated to the hydrophobicity of the cell surface. Bacteria cell surfaces with high hydrophobicity values always showed enhanced adsorption to the mineral particles. In the performed assays the negative charged cell surface did not affect the adsorption event while positive charges on the cells enhanced it. Variations of the pH between 4 and 9 did not considerably affect the adsorption event.

2.1.4 Survival in sediments

Many studies have reported a higher concentration of indicator and pathogenic bacteria occurring in the sediments than in the overlying water column (Burton et al., 1987). Sediments may contain 100 to 1000 times as many faecal indicator bacteria as the water column (Ashbolt et al., 1993). A literature review made by Burton et al. (1987) showed that higher concentrations of indicator and pathogenic bacteria in sediments than in the suspension are due to a combination of sedimentation, adsorption (which provides defense from bacteriophage and microbial toxicants) and the phenomenon of slower die off rates in the sediments. In addition faecal bacteria are capable to obtain nutrients associated with the sediment particles (Davies et al., 1995) Resuspension of sediments with high numbers of pathogens creates a health hazard risk in recreational water areas and in raw water sources (Burton et al., 1987). It is of great importance that this health hazard risk is taken to account when a risk assessment of a raw water source is undertaken.

Concentrations of faecal coliforms in the surface water may not always indicate a recent faecal contamination, it is probable that resuspension of viable sediment-bound bacteria has occurred (LaLiberte and Grimes, 1982). Davies et al. (1995) performed laboratory studies in order to investigate how culturable faecal coliforms and faecal streptococci may be capable to grow in freshwater and marine sediments. The study showed that the bacteria were capable to grow in the sediments, in the absence of predators, for the marine and freshwater aquatic conditions. Under natural conditions, in the presence of predators, a bacteria net die- off occurred. The studies showed that 10% of the initial number of faecal coliforms and faecal streptococci, for marine- and freshwater sediment, remained after 85 days.

LaLiberte and Grimes (1982) carried out a in situ study in a shallow lake (Lake Onalaska, U.S.) in order to investigate the survivability of *E. coli* in sediments. The study was performed by using in situ dialysis culture of sterile (autoclaved) and unsterile sediment samples. The result of the analysis indicated that the *E. coli* populations which were inoculated into unsterile sediments decreased only slightly over a 5- day period while the *E. coli* populations in the sterilized sediments increased in number.

Burton et al. (1987) investigated the survival ability of four human- associated bacteria, among them *E. coli*, in five different freshwater sediments ranging from organically rich high- clay fractions to organically poor sandy fractions. The study showed that *E. coli* survived longer in sediments containing at least 25% clay. The authors suggested that it might be due to higher concentrations of organic matter and nutrients.

2.1.5 Resuspension

Since many studies have indicated that pathogens have the ability to be viable for longer time periods in the bottom sediments it is important to consider the resuspension of pathogens as a potential health hazard in water supply reservoirs. Analyses in Lake Okeechobee, US performed by (Kang-Ren and Detong, 2007) showed that waves are the predominant factors that cause sediment resuspension in shallow lakes, while wind-induced currents are the major factors that cause transport of sediments in the suspension.

Sediment resuspension occurs when the bottom shear force exceeds a critical shear stress (Evans, 1994). The critical shear stress depends on material parameters of the bottom sediments such as grain size and water content (Håkanson and Jansson, 1983). A laboratory research carried out by de Jonge and van den Bergs (1987) showed that resuspension of bottom sediment in an estuary begins at current velocities as low as 10 cm s^{-1}.

2.2 *Cryptosporidium*

2.2.1 General information

The protozoa *Cryptosporidium* was discovered in the early 1900s and the first reported infection in human occurred in 1976 (Sterling and Adam, 2004). Waterborne outbreaks of Cryptosporidiosis have occurred with increasing frequency since the 1980s (Drozd and Schwartzbrod, 1996). The largest waterborne disease outbreak in

the history caused by *Cryptosporidium* occurred in 1993, in Milwaukee, US. Approximately 403 000 persons got affected and several immune- compromised people died (Dai and Boll, 2003).

Cryptosporidium is part of the subphylum *Apicomplexa* and the family *Cryptosporidiidae* (Medema et al., 2006). At present, 19 species of *Cryptosporidium* have been confirmed by morphological, biological and molecular data (Fayer, 2010). Human infections are normally caused by two *Cryptosporidium* species: *Cryptosporidium parvum* and *Cryptosporidium hominis*. *C. hominis* is transmitted between humans and *C. parvum* is transmitted between humans and from other animals to humans (Medema et al., 2006).

The parasite produces a nonproductive oocyst which is excreted by infected hosts (Medema and Schijven, 2001). The oocyst stage is of primary importance for the dispersal, survival and infectivity of the parasite (Fayer et al., 2000). The oocysts of *C. parvum* are immediately infective and can remain viable for many months in the biosphere due to a robust encystment that protects them from environmental stresses (Medema et al. 1998; Dai and Boll 2003). Compared to other protozoan parasites the *C. parvum* oocyst is smaller with a diameter of 4-6 µm (spherical form) and a density of approximately 1.05 g cm^{-3} (Medema et al., 2006, Searcy et al., 2005)

Infected dairy cattle have been recognized in a number of studies as a major source of *C. parvum* oocysts (Ortega-Pierres, 2008). Especially new born calves are reported to shed high number of oocysts (Medema et al., 2006). The oocysts enter in general surface water through (Medema et al., 1998):

- direct faecal input;
- discharge of treated and untreated sewage;
- runoff from agricultural lands.

Measurements in surface waters have exhibited that the concentration of *Cryptosporidium* oocysts often increases after rainfall even due to the fact that oocysts are mobilized from catchments and transported by overland flow and channel flow into the reservoir (Dai and Boll, 2006).

2.2.2 Aggregation to particles

Attachment of *Cryptosporidium* oocysts to particles is an important part of its settling kinetics. Assays performed by Medema et al. (1998) showed that the adsorbing particle influenced the sedimentation velocity of the oocyst extensively already at size fractions of 1 µm. The adhesion mechanism between the *C. parvum* oocyst and small soil particles, typically the clay sized fraction (<2µm), depend largely on the electrostatic surface charge of the particle. The hydrophobic property of the oocyst has also been shown to have a significant influence on the complex adhesion mechanism (Drozd and Schwartzbrod, 1996).

Drozd and Schwartzbrod (1996) investigated the hydrophobic and surface electrostatic charge of *Cryptosporidium* sp. at various pH values, ionic strength and conductivities. The *Cryptosporidium* sp. showed a zeta-potential close to -25 mV at neutral water, in deionized water and in river water. The assay shows that the surface charge of the protozoa increases slowly with decreasing pH in the suspension; from -35 mV at alkaline pH to 0 for acidic pH 2.5. This indicates that the zeta-potential might be completely reversed by altering the pH**.** Furthermore the performed assays

show that *Cryptosporidium* oocysts, for the pH range normally seen in surface water, do not exhibit a distinct hydrophobic property.

Particle attachment experiments performed by Dai and Boll (2003) at pH 7.1 indicated that the negatively charged oocysts do not attach to negatively charged natural soil particles. Most soil and sediment surfaces in the environment are negatively charged (Jamieson et al., 2004). Moreover, their assays showed that the negatively charged oocysts attached to positively charged beads, suggesting that the zeta-potential plays a major role in the attachment mechanism between oocysts and particles. The finding about the oocysts readiness to attach to particles, concluded by Dai and Boll (2003), differs to an experimental analysis performed by Medema et al.(1998). They mixed oocysts with secondary effluent from a biological wastewater treatment plant in order to determine the kinetics of attachment. The result showed that approximately 1/3 of the oocysts was attached to particles from the secondary effluent directly after mixing and that a maximum of 70% was achieved after 24h. It is likely that the differences in the findings of Dai and Boll (2003) and Medema et al. (1998) depend on the different characteristics of the adsorbing media (Dai and Boll, 2003). The biological particles from the secondary effluent may have different charge characteristics and behave differently compared to the soil particles.

Although the performed experiments provide useful information about the attachment kinetics of *Cryptosporidium* oocysts, an extensive obscurity still exists. Medema et al. (1998) reported that the surface charge characteristics of the oocyst may alter in the reservoirs due to purification and storage procedures and that therefore their results were only valid for experimental studies. Dai and Boll (2003) stated that there is a lack of understanding of the oocyst wall chemistry, which prevents a more comprehensive study of the physical and biochemical property of the organism. In addition there are uncertainties about the adsorbing media, e.g. that soil may contain elements that cause an overall positive zeta-potential of the soil particle.

2.2.3 Sedimentation of free oocysts

Sedimentation velocities of unattached *Cryptosporidium* oocysts are very low and are not likely to lead to any significant sedimentation in natural aquatic habitats (Dai and Boll, 2006, Medema et al., 1998, Searcy et al., 2005). The small size of the oocyst (diameter of 4-6 µm) results in that a small-scale gravitational force influences the settlement. Several studies have verified that the theoretical sedimentation velocity, calculated by Stokes' law, corresponds to the sedimentation velocity determined in assays (Dai and Boll, 2006, Medema et al., 1998, Pachepsky et al., 2006).

Medema et al. (1998) performed experimental analysis in order to determine the sedimentation velocity of *Cryptosporidium* oocysts. The result exhibited that the initial apparent sedimentation velocity in a balanced salt solution at 23 C° was 0.35 µm s^{-1}, see Table 1. Moreover, the experimental sedimentation velocity corresponded to the theoretical sedimentation velocity determined by Stokes' law. Both sedimentation velocities declined as time progressed. This is explained by the fact that oocysts of various densities were used in the assay; the denser oocysts settled out earlier than the oocysts with lower density.

A similar settling experiment was carried out by Dai and Boll (2006). The result of their assays presented an experimental sedimentation velocity of 0.27 µm s^{-1} and a theoretical sedimentation velocity, calculated by Stokes' law, of 0.36 µm s^{-1}. The

assay was performed with slightly lower oocysts densities compared to the test made by Medema et al. (1998), which may explain the lower experimental sedimentation velocity. Settling column experiments made by Searcy et al. (2005) exhibited an experimental settling velocity of 0.76 μm s^{-1} for free *Cryptosporidium* oocysts in a weak salt solution; the theoretical settling velocity was estimated to be 0.81 μm s^{-1}.

The settling velocity of free oocysts in natural aquatic environments may differ extensively from the results of the mentioned experiments due to the different conditions. The laboratory assays were performed with no particles present, which may have great influence on the sedimentation kinetics of the oocyst. Moreover, the settling velocities from the experimental assays apply to slow or non- moving water.

In surface water environments a wide range of oocyst densities is expected, due to variations in age (depletion of internal) and exposure to stressors (Medema et al., 1998). However, the oocysts utilized in the experimental tests were of the same age and had experienced similar conditions in the laboratory (Medema et al., 1998, Dai and Boll, 2006). A study made by Searcy et al. (2005) emphasized that to investigate the viability of the oocyst plays a major role in the oocysts sedimentation kinetics since it affects the density and attachment to particles.

Table 1. Settling experiments with free Cryptosporidium oocysts.

Reference	Media	Method	Oocyst properties	Settling velocity
Medema et al. (1998)	10 ml of Hanks' balanced salt solution*, filtered through 0.2 µm pore size filters.	The tests were conducted in glass tubes of 10 by 1.5 cm. At t=0 10 pieces of 1-ml samples were taken from the column in order to determine the initial oocyst concentration. The suspensions were allowed to settle, and after predetermined time intervals, 9-ml samples were taken from the settling columns.	Mean diameter: 4.9 µm, standard deviation: 0.3 µm, geometric mean density: 1045.4 kg m^{-3}.	0.35 µm s^{-1} (initial)
Dai and Boll (2006)	The oocyst working solution was diluted successive (1:1 ratio) with distilled water until the total volume reached 10 ml.	The tests were performed in completely mixed 10 ml settling tubes. One tube was sampled at t=0, while the others were sampled at different time intervals up to 47 hours.	Mean diameter: 6.6 µm, standard deviation 1.1 µm, average density: 1009 kg m^{-3}.	0.27 µm s^{-1} (average)
Searcy et al. (2005)	Oocysts (1000 oocysts/ml) were mixed in a 3 mM NaCl solution, pH 7.0.	Settling column experiments performed in an incubator at a fixed temperature of 25 °C.	-	0.76 µm s^{-1} (average)

*=A balanced salt solution including principally: sodium chloride, potassium chloride and glucose.

2.2.4 Sedimentation of oocysts attached to particles

A number of studies have noted that *Cryptosporidium* oocysts may not occur as discrete particles in natural aquatic habitats, but may have a tendency to stick to other particles or occur in clumps (Young and Komisar, 2005). The attachment mechanism affects not only the sedimentation kinetics and the hydrodynamic behavior of the oocysts, but most likely also their survival and removal during drinking water treatment by filtration, soil passage, and disinfection processes (Medema et al., 1998). Several experimental analyses have been performed in order to evaluate the ability of the oocysts to attach to different materials.

Medema et al. (1998) investigated in assays the settling velocity of oocysts that were mixed and attached to particles that derived from effluent of a biological wastewater treatment plant. Discharge of treated sewage is one of the main sources of *Cryptosporidium* to surface water. The result showed that sedimentation velocity of the oocysts attached to particles from treated sewage was up to 29 μm s^{-1}, see Table 2.

A study performed by Searcy et al. (2005) aimed to examine how the interaction between oocysts and suspended sediment would influence the sedimentation behavior of oocysts in surface water. The assays were made in settling columns using three different types of sediments: kaolinite, iron oxide particles and river bottom sediment. The average experimental sedimentation velocity increased (from 0.76 μm s^{-1} for freely suspended oocysts) to 12.6, 53.3 and 7.9 μm s^{-1} when mixed with kaolinite, iron oxide particles and river bottom sediment respectively. Moreover, the study showed that the sedimentation rate of the oocysts was faster than the average sedimentation rate of the inorganic particles. The authors argue that it is due to that the oocysts are more likely to attach to and settle out with larger particles.

A research carried out by Young and Komisar (2005) was designed to evaluate the settling behavior of fresh and aged oocysts in the presence of faecal material. The result of the study exhibited settling velocities for whole population of oocysts in a range from 0.22 to 1.06 μm s^{-1} in nearly neutral pH suspensions. The much lower calculated settling velocities compared to Medema et al. (1998) suggest less attachment between oocysts and faecal particles than between the oocysts and the secondary effluent. The fastest settling velocities occurred with aged viable oocysts in aqueous suspensions with high ionic strength and presence of Ca^{2+} and Mg^{2+}. This is due to that the aged viable oocysts, compared to the fresh oocysts, have greater densities and may also have a greater ability to agglomerate to other particles.

Table 2. Settling experiments with Cryptosporidium oocysts attached to particles.

Reference	Media	Method	Attachment to particles	Oocyst properties	Settling velocity
Medema et al. (1998)	Oocysts (3000 to 6000 per 10 ml) were added to secondary effluent*.	The oocysts were allowed to attach to the secondary effluent for 24h at a rotary shaker table. The settling experiment followed the procedure way as for the free oocysts.	The oocysts attached readily to the biological particles; 30% almost instantly and 75% after 24h.	Mean diameter: 4.9 µm, standard deviation: 0.3 µm, geometric mean density: 1045.4 kg m^{-3}.	29 µm s^{-1} (maximal)
Young and Komisar (2005)	Oocyst-containing faecal material was mixed with distilled water and fluorescent latex microsphere	Settling column experiments performed at a fixed temperature of 26 °C.	Yes, the result suggests that a part of the oocysts attached to the faecal material.	Mean diameter: 4.3µm, Mean density 1073kg m^{-3}.	0.67 µm s^{-1} (average)
Searcy et al. (2005)	A 20 ml suspension of kaolinite (50mg/L) was mixed with oocysts (1000 oocysts/ml) in a 3mM NaCl, pH 7.0 solution.	The solution was mixed for 24 h on a rotating shaker. Settling column experiments.	A great amount of the oocysts attached to the particles in the suspension.	-	12.6 µm s^{-1} (average)

*= Effluent of the sedimentation basin after activated sludge treatment.

2.3 Studies that apply sedimentation modelling

2.3.1 Brookes et al. (2006)

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

In this report the authors evaluated the significance of settling of *Cryptosporidium* oocysts in a water supply reservoir and how settling reduces the risk of *Cryptosporidium* oocysts reaching the drinking water consumers. A *Cryptosporidium* module was created and coupled to the three-dimensional hydrodynamic-ecological model ELCOM-CAEDYM (Hipsey et al., 2004). In this model the decay of *Cryptosporidium* depends on the temperature and UV-insolation, and the transport depends on advection, settling and resuspension. The formulation that describes the oocyst fate and transport, not including the 3D advective terms, is shown below:

$$\frac{dC}{dt} = \left[-f(T) - f(UV) - \frac{v_s}{\Delta z}\right] C + f(\tau) \quad (2)$$

where C is the oocyst concentration (oocysts m^{-3}) and t is the time (s). The function $f(T)$ describes the decay of oocysts due to temperature and was developed by Walker Jr and Stedinger (1999) and the function $f(UV)$ is derived from a study performed by Craik et al. (2001). v_s is the settling velocity (m s^{-1}), Δz is the vertical grid size, τ is the shear stress above the sediments (N m^{-2}).

The coupled model was employed in the Myponga Reservoir, South Australia. During an inflow event the oocyst load in a connected river was measured and specified in the model as an inflow boundary condition. In order to estimate a representative oocyst sedimentation velocity, the authors performed a settling experiment, where free oocysts were mixed with bovine faecal particles. The result exhibited a low settling velocity, 1.16×10^{-6} m s^{-1} (0.1 m d^{-1}), since the oocysts attached poorly to the particles.

The model demonstrated that the reduction of the oocyst concentration in the water column due to sedimentation kinetics is between one and three magnitudes less than the influence of advection and dilution, depending on the influence of the hydrodynamic forcing.

2.3.2 Liu et al. (2006)

Modeling the Transport and Inactivation of *E. coli* and Enterococci in the Near- Shore Region of Lake Michigan

The aim of the study was to investigate whether human faecal pollution was affecting the water quality of the beaches in the southern parts of Lake Michigan and to understand the relative importance of different processes that influence inactivation of pathogens and transport in the area.

A hydrodynamic model based on finite elements was chosen to describe the circulation conditions in Lake Michigan. Water quality model for *E. coli* and Enterococci (the used indicator organisms) was coupled to the hydrodynamic model. The modelling was performed for one month during July/August.

Two different expressions for inactivation of the indicator organisms were used: a general first-order inactivation rate that did not take the temperature, light or

sedimentation in to account; and a time- dependent inactivation rate that takes temperature, sedimentation and solar insolation in to account. The latter one is of great interest for this study, it is shown below:

$$k(I,T,v_s) = \left(f_p \frac{v_s}{H} + k_I I(t)\right) \theta^{(T-20)} \quad (3)$$

where $k(I,T,v_s)$ is the total inactivation rate, k_I is the inactivation rate for light ($W^{-1}\ m^2\ d^{-1}$), I(t) is the observed solar insolation ($W\ m^{-2}$) as a function of time, θ is a temperature correction factor, f_p is the portion of pathogens attached to the sediments in the suspension, v_s is the sedimentation velocity ($m\ s^{-1}$) and H is the water depth (m).

The result demonstrated that inactivation of the indicator organisms was primarily governed by the solar insolation and the effects of temperature and sedimentation were of minor magnitudes. Moreover, the result suggests that Enterococci survive longer in Lake Michigan than *E. coli*.

2.3.3 Connolly et al. (1999)

Modeling fate of pathogenic organisms in coastal waters of Oahu, Hawaii

The authors of this study examined the transport and decay of indicator organisms, pathogens and total suspended solids in Mamala bay, on the southern coast of the island of Oahu, Hawaii. The modelling program consisted of a three-dimensional hydrodynamic model, aimed to simulate the advection and dispersion in the bay, coupled with a pathogen fate model.

Modelling of particulate matter (total suspended solids) was performed due to its effect on light attenuation in the coastal parts during storms. The modelling of the particulate matter was performed with the same approach as for the pathogens except that settlement was taken in to account and the decay was set to zero. The following expression describes the settlement flux for total suspended particles:

$$\frac{dm}{dt} = -\frac{w_s}{h} m \quad (4)$$

where m is the total suspended solids concentration ($mg\ l^{-1}$), t is the time (s), w_s is the settling velocity ($cm\ s^{-1}$) and h is the depth of the segment. The model did not consider resuspension and the particulate matter was assumed to consist of flocculent, fine grained particles with an average settling speed of 0.008 $cm\ s^{-1}$.

3 Materials and methods

3.1 Study area

3.1.1 Site description

Lake Rådasjön is located on the border of the municipalities of Mölndal and Härryda, southeast of Gothenburg (Storkull et al., 2006). The lake is used as a raw water supply for the municipality of Mölndal (supplying about 60 000 people) and as a reserve water supply for the municipality of Gothenburg. The drinking water treatment plant (WTP) of Mölndal withdraws approx. 13 000 m^3 water from the lake per day, which is equivalent to approx. 5% of the total water flow of the lake (Olofsson, 2008).

The surface area of Lake Rådasjön is 1.92 km^2, the average depth is 8.8 m, the maximum depth is 23 m (Storkull et al., 2006) and it is located approximately 50 m above sea level (Olofsson, 2008). The renewal time in the lake is about two months (Storkull et al., 2006). The lake is situated on clay bottom but the dominating superficial soil types in the surroundings are till and glacial fluvial deposits. The catchment area is about 199 km^2 and is covered mainly with forest (53%).

Lake Rådasjön is a part of the river Mölndalsån water system, which has its source area in the surroundings of lakes Nedsjöarna in the municipality of Bollebygd, located 120 m above sea level (Storkull et al., 2006). The river falls into the river Göta älv in the lower part of its catchment area. The inlet of the river Mölndalsån to Lake Rådasjön is located in the eastern part of the lake and the outlet is located in the western part of the lake, i.e. in the narrow strait called Stålloppet that connects Lake Rådasjön with Lake Stensjön, see Figure 1. This probably results in low water exchange in the northern parts of the lake (Olofsson, 2008). The raw water intakes of Mölndal (15 m depth) and Gothenburg (8 m depth) are located in the north-western part of the lake 150 respectively 100 meter from the shore (Åström et al., 2010).

Highway 40 and the parallel Boråsvägen run adjacently the northern parts of the lake. In order to collect the runoff from the roads, three storm water dams are located between the roads and lake Rådasjön (Olofsson, 2008). There are mainly three housing areas adjacent to the lake: Helenevik, Pixbo and Mölnlycke. Furthermore, in 2006 Lake Rådasjön was classified as a nature reserve (see Figure 1); in addition the peninsula Labbera is classified as a Natura2000-area.

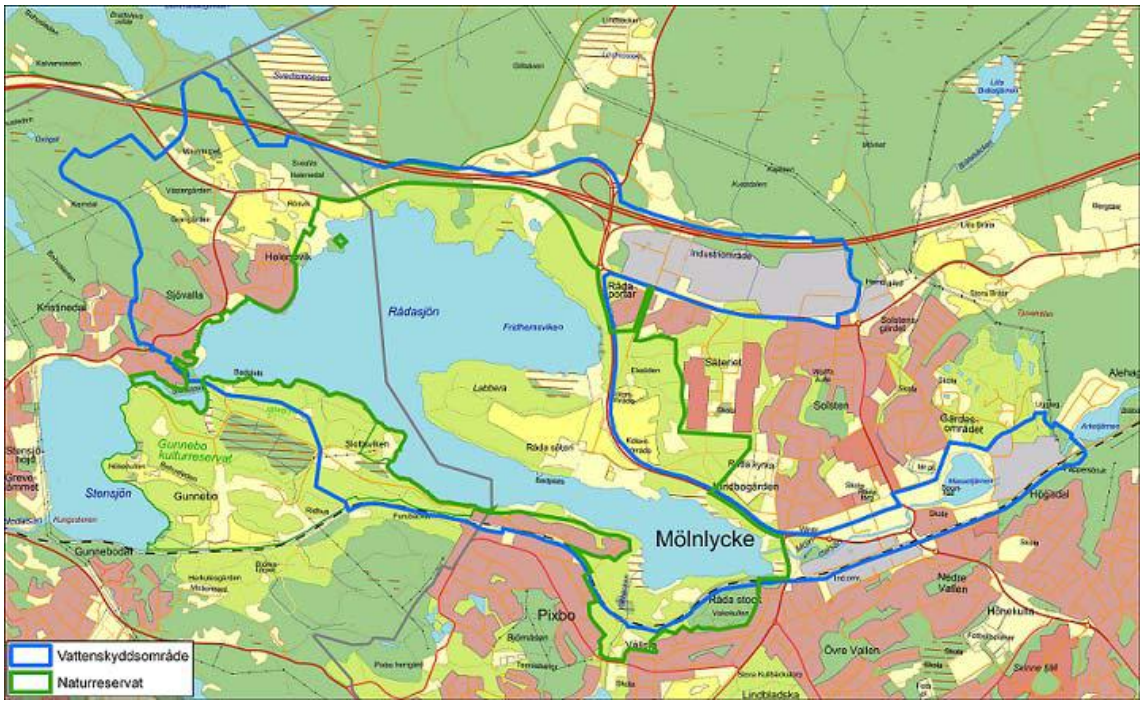

Figure 1. Lake Rådasjön and its surroundings (Olofsson, 2008). The blue line shows the delimitations for the water protection area and the green line shows the delimitations for the nature reserve.

3.1.2 Faecal contamination sources

The presence of animals and sewer system outlets in the area around Lake Rådasjön imply that faecal material is released into the lake (Åström et al., 2010). Horses and livestock graze around the lake in order to improve the ecological values in the nature reserve (Åström et al., 2011). Approximately 90 horses and a few cattle were grazing in the area in 2008, primary in the pastures northwest of the lake (Åström et al., 2010, Åström et al., 2011). Naturally occurring animals around the lake include Canada goose, Elk and Roe Deer.

The wastewater deriving from the households in the area is transported in a separate sewer system to the wastewater treatment plant in Gothenburg (Åström et al., 2011). A number of on-site sewers with inferior effluent removal exists in the area, mainly northwest of the lake in Helenevik and in the area above Lake Vällsjön (Olofsson, 2008, Åström et al., 2010). During heavy rain events the stormwater intrudes into the sewer system in the housing area south of the lake and upstream in the river Mölndalsån (Åström et al., 2010, Åström et al., 2011). This leads to infrequent discharges of untreated wastewater into the lake.

3.2 MIKE 3 Flow Model FM

MIKE 3 Flow Model FM developed by DHI is a three-dimensional numerical modelling system, applicable to areas such as oceans, coastal regions, estuaries and lakes (DHI, 2007). It is based on a finite volume method on a flexible mesh (an unstructured mesh in the horizontal plane and a structured mesh in the vertical domain), see Figure 2. It is composed of following series of modules, which can be

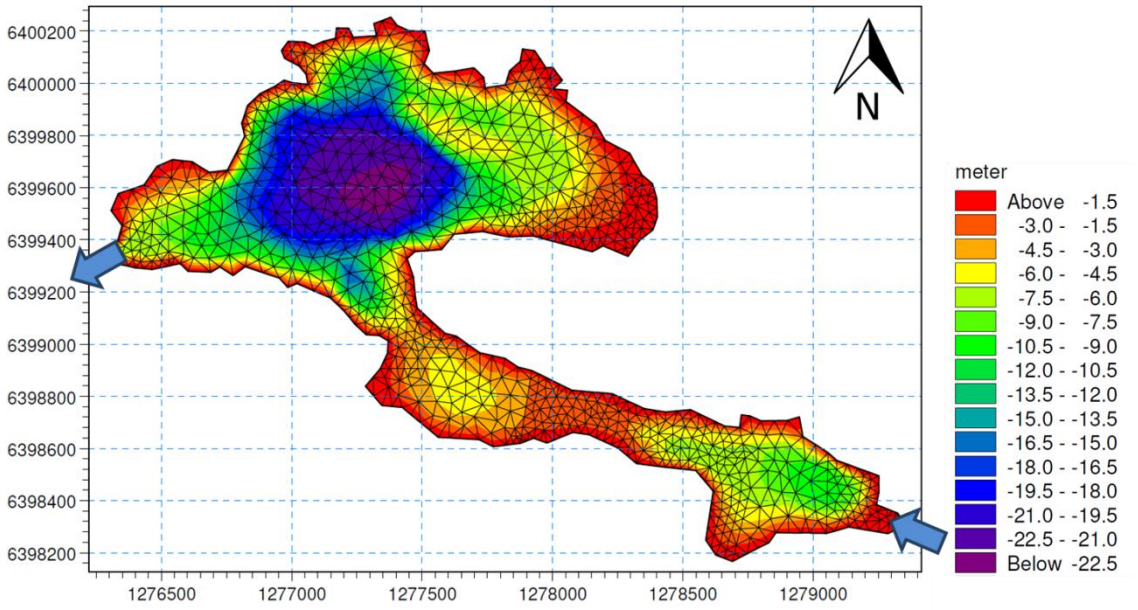

Figure 2. Mesh and bathymetry map of Lake Rådasjön (Sokolova et al., 2011a).

interconnected: Hydrodynamic module, Transport Module, Ecology and water quality Module (ECO Lab), Sand Transport Module and Mud Transport Module (DHI, 2009).

The Hydrodynamic Module is the basic component in the MIKE 3 Flow Model FM and it provides the other components with a hydrodynamic support, as well it can be used alone (DHI, 2009). The hydrodynamic module simulates unsteady flow considering external forcings, hydrographic boundary conditions, density, bathymetry etc. (DHI, 2007). The parameters are added in the user interface, see Figure 3. The system is based on the numerical solution of the three-dimensional incompressible Reynolds averaged Navier-Stokes equations, furthermore using the assumption of Boussinesq and hydrostatic pressure.

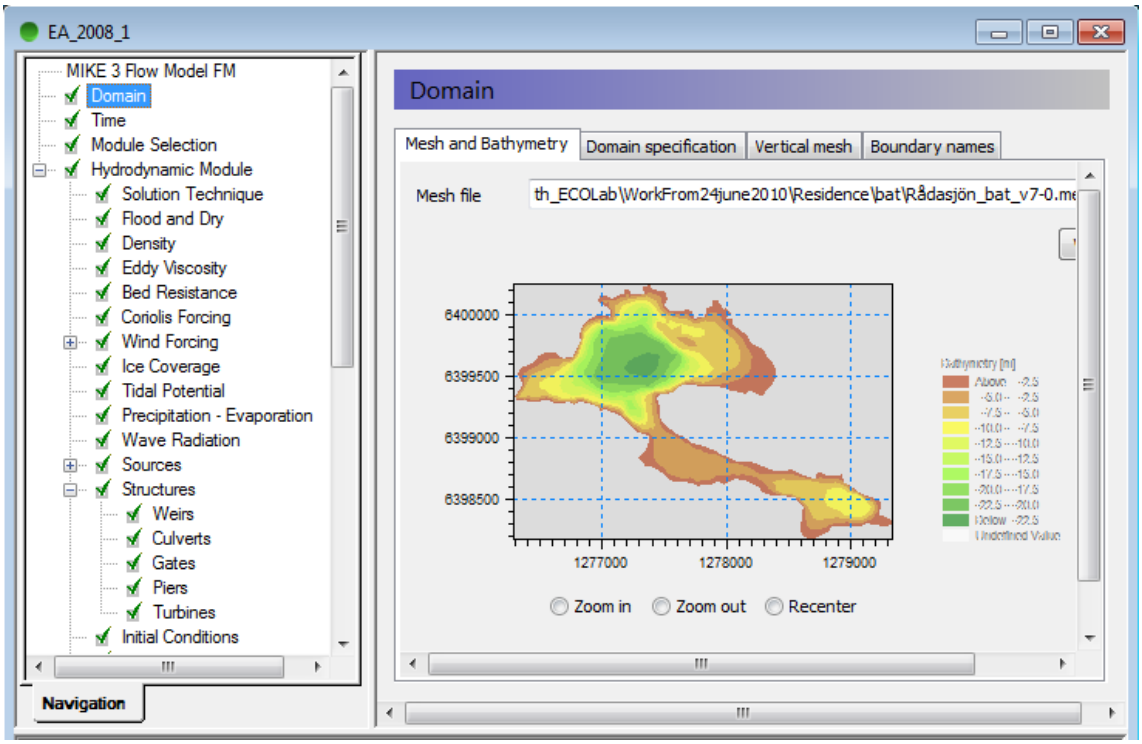

Figure 3. The user interface of MIKE3 Flow Model FM (hydrodynamic module).

The input parameters are categorized into subsequent groups: domain and time parameters, calibration factors (e.g. bed resistance, wind friction factors), initial conditions, boundary conditions and other driving forces (e.g. wind speed and direction, source/sink discharge) (DHI, 2007). The output result is computed for each grid element and for each time step. The output variables include water depth, flux densities in main directions, velocities in main directions, density, temperature and salinity.

3.3 ECO Lab Module

In order to perform environmental water quality studies, the hydrodynamic module can be coupled to the DHI developed ECO Lab Module (DHI, 2004). ECO Lab is able to simulate the spatial distribution of input parameters by subsequent processes:

- advective transport;
- biological, physical and chemical transformation processes;
- settling.

Implementation of the hypothesis/theory is performed in an ASCII file called an ECO Lab template (DHI, 2004). The ECO Lab template, defined in the ECO Lab template editor (see Figure 4) consists of: state variables, constants, forcings and processes. The state variables describe the state of the ecosystem and its spatial distribution is simulated. Constants are applied as arguments in the deterministic formulations of processes. They are constant in time, but can vary in the spatial distribution. Forcings have a similar function but vary in time as well. The processes are based on

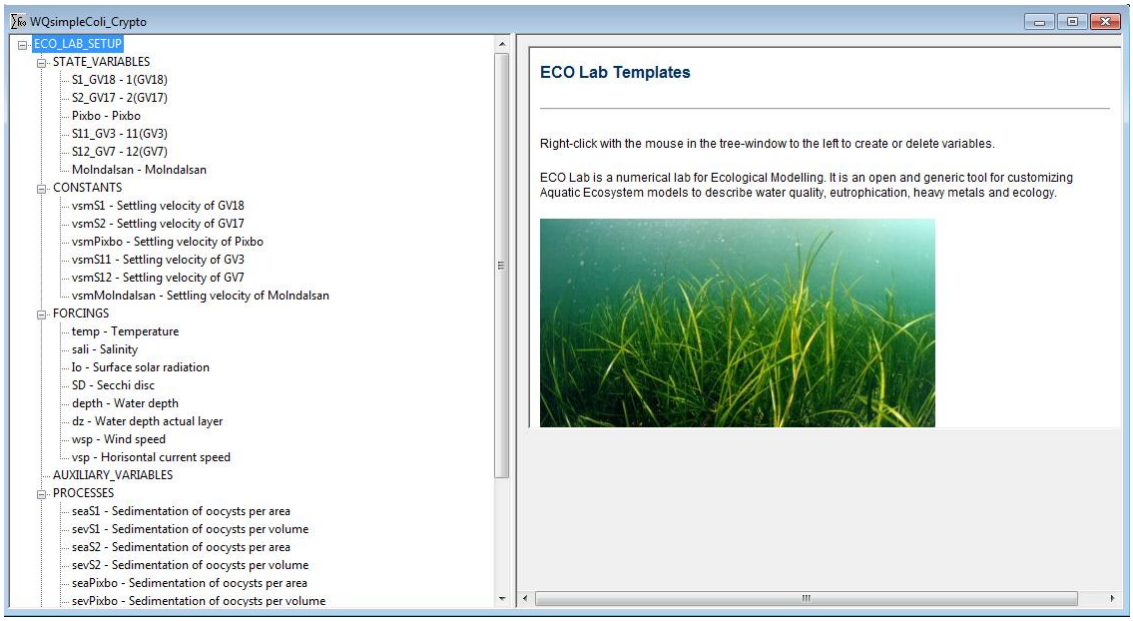

Figure 4. The user interface of the ECO Lab template editor.

arguments such as numbers, constants, forcings, state variables, mathematical functions and built-in functions.

The output result is deterministic data of the defined state variables (DHI, 2004). Furthermore, additional output data can be generated, such as processes and auxiliary variables. The output result can be presented as points, lines and volumes.

The ECO Lab template, named WQsimple_Crypto, was created to take into account the gravitational settling kinetics of *Cryptosporidium* oocysts, when coupled to the hydrodynamic module. To evaluate the source-specific transport, *Cryptosporidium* oocysts released from different sources were simulated as separate variables. The ECO Lab template is based on the following equation:

$$\frac{dc}{dt} = -\frac{v_s}{z}c \tag{5}$$

where c is the pathogen concentration (pathogens m^{-3}), v_s is the sedimentation velocity ($m\ s^{-1}$), z is the vertical grid size (m) and t is the time (s).

The state variables represent the oocyst concentration released from a respective source, see Table 3. The constants represent the settling velocity for the oocysts released from a respective source. The only forcing used, dz represents the vertical grid size for the actual layer. Two different processes are calculated for each source: sedimentation of oocysts per area (sea) and volume (sev).

A scenario that takes into account the temperature effect on the *Cryptosporidium* oocyst viability was simulated. The temperature-decay function was created by Walker Jr and Stedinger 1999:

$$\nu(T) = C\ 10^{0.058T} \tag{6}$$

where C is a constant of value $10^{-2.68}$, T is temperature in °C and ν is the decay function. Furthermore, a passive case that takes into account advective transport was simulated.

Table 3. The ECO Lab template input parameters and mathematical expressions.

Source	State variables	Constants	Forcings	Processes
GV18	S1_GV18	vsmS1	dz	seaS1: vsmS1*MAX(0,S1_GV18) sevS1: seaS1/dz
GV17	S2_GV17	vsmS2	dz	seaS2: vsmS2*MAX(0,S2_GV17) sevS2: seaS2/dz
Pixbo	Pixbo	vsmPixbo	dz	seaPixbo: vsmPixbo*MAX(0,Pixbo) sevPixbo: seaPixbo/dz
GV3	S11_GV3	vsmS11	dz	seaS11: vsmS11*MAX(0,S11_GV3) sevS11: seaS11/dz
GV7	S12_GV7	vsmS12	dz	seaS12: vsmS12*MAX(0,S12_GV7) sevS12: seaS12/dz
Mölndalslsån	Molndalsan	vsmMolndalsan	dz	seaMolndalsan: vsmMolndalsan* MAX(0,Molndalsan) sevMolndalsan: seaMolndalsan/dz

3.4 Input data

A predefined mesh and bathymetry file for Lake Rådasjön was used, see Figure 2. The simulations were performed for March conditions (between 2008-03-01, 00:00:00 and 2008-03-16, 00:00:00). The number of time steps was set to 2160 and each time step was set to 600 seconds. March conditions were chosen due to the fact that the concentrations of *Cryptosporidium* are expected to be the highest during this month. In March there is no clear density stratification in the lake and a high water circulation is expected. The temperature in the water is low, which implies that the temperature decay is of a minor size. The snow melting, which transports faecal contamination with the surface runoff into the lake, contributes with high levels of *Cryptosporidium*.

It was chosen to set a constant wind speed of 3 m s^{-1}. Two scenarios were simulated for two wind directions: southwest and southeast.

Six sources that are expected to pose the highest risk related to the presence of *Cryptosporidium* concentrations at the raw water intakes were taken into account in the model, see Figure 5. Source GV18 (Figure 5, 18) is a storm water culvert

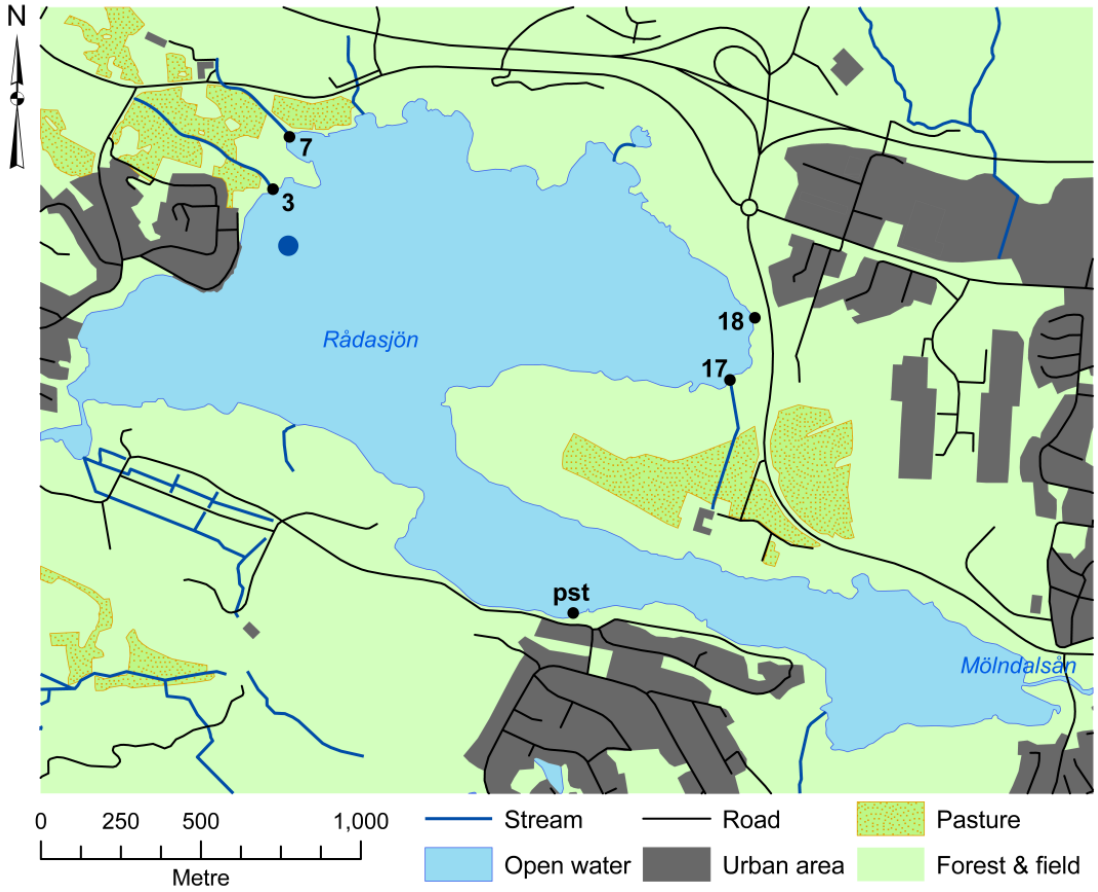

Figure 5. The map shows Lake Rådasjön and the faecal contamination sources that were used in the model. The blue circle shows the location of the raw water intakes.

(Åström et al., 2011). The faecal material that derives from GV18 may come from humans or sheep. Source GV17 (Figure 5, 17) is a stream located closely to a manor farm. Faecal hosts determined in the area are cattle and horses. Pixbo (Figure 5, pst) is a sewer pumping station that during heavy rain events discharges untreated wastewater into the lake. Source GV3 (Figure 5, 3) is a stream that enters the lake from the northwest. On-site sewers from nearby houses are connected to the stream. Source GV7 (Figure 5, 7) is a stream that flows through a horse pasture area. Faecal sources reported in the area are horses and humans (on-site sewers). River Mölndalsån enters the lake in the southeastern part, see Figure 5. During heavy rain events untreated wastewater is released to the river from sewer systems upstream.

The discharges from the sources located around the lake were defined in the hydrodynamic module, see Table 4. The used discharges are the average discharges for March 2008, which were calculated based on meteorological data. The discharges are assumed to be constant, although in reality the contribution from Pixbo is discharged infrequently.

Table 4. Discharges from the sources connected to Lake Rådasjön.

Source	Discharge (m^3/s)
GV18	0.0150
GV17	0.0079
Pixbo	0.0025
GV3	0.0085
GV7	0.0035
Mölndalsån	10.5600

The model was simulated for three different oocyst settling velocities in order to illustrate the influence of the settling kinetics on the *Cryptosporidium* transport. Two of the selected settling velocities were derived from laboratory experiments performed by Medema et al. (1998). The settling velocities determined by Medema et al. (1998) have been selected due to that:

- the study have received great recognition and the result are often used and referred to in similar studies;
- the properties of the oocysts, materials and method used in this study are accurately described;
- the results are in agreement with similar studies.

First, the model was simulated for the settling velocity, v_s=0.35 μm s^{-1}, which represents settling velocity of the free oocysts in the water column, i.e. no attachment to particles. Secondly, the model was simulated for v_s=10 μm s^{-1}. This settling velocity derives not from the study by Medema et al. (1998). It was chosen in order to illustrate the result of a settling velocity case between the minimum and maximum values. Thirdly, the model was simulated for v_s=29 μm s^{-1}, which represents the maximum settling velocity, observed for the oocysts mixed with secondary effluent (Medema et al., 1998).

The concentration of *Cryptosporidium* oocysts in the discharges from different sources, were calculated by Sokolova et al. (2011b), see Table 5. The calculated values represent epidemic conditions.

Table 5. Cryptosporidium oocyst concentrations from the sources connected to Lake Rådasjön, calculated for epidemic conditions.

Source	Concentration (oocysts/10L)
GV18	24.78495
GV17	4.201386
Pixbo	1296898
GV3	2880.989
GV7	1158.348
Mölndalsån	2299.352

4 Results

The primary result of the study and prerequisite for the performed simulations is the created ECO Lab template that is developed to take into account the sedimentation process for pathogens, particularly *Cryptosporidium* (see the Material and methods part for detailed description).

The results of the simulations made for March conditions during 15 days are presented as time series of the computed *Cryptosporidium* oocyst concentration at the raw water intakes of Mölndal (15 m depth) and Gothenburg (8 m depth), see Figures 6-8. Each figure shows the contribution from a specific source to the oocyst concentration at the raw water intakes plotted for five different *Cryptosporidium* oocyst sedimentation and decay settings:

1. Passive condition: no sedimentation or decay
2. No attachment: settling velocity for unattached oocysts according to (Medema et al., 1998)
3. Settling velocity of 10 μm s^{-1}
4. Maximum settling velocity: the oocysts have the maximal settling velocity determined by Medema et al. (1998) when oocysts were mixed with secondary effluent
5. Decay: temperature-decay function according to Walker Jr and Stedinger (1999)

For all simulated sources the five different sedimentation/decay cases demonstrate the same relative *Cryptosporidium* contribution to the oocyst concentration at the raw water intakes. The passive condition resulted in the highest registered oocyst concentration, followed closely by no attachment and decay which represent similar results. The time series of high and maximum settling velocity represent significantly lower oocyst concentrations.

The result of the simulations shows that the *Cryptosporidium* oocyst contribution from sources GV18, GV17 and GV7 to the concentrations at the raw water intakes is of a negligible magnitude. Table 6 presents the maximal registered contribution from sources GV18, GV17 and GV7 to the oocyst concentration at the raw water intakes (simulated for settling velocity of unattached oocysts). A specific detail of interest is that for all simulations the maximal oocyst concentrations are higher at the 8 m intake compared to the 15 m intake. Moreover, GV 7 is the only source that contributes with an oocyst concentration above 1 oocysts/10L (8 m raw water intake, southeastern wind). Since the oocyst contribution from these sources is of a negligible size, the effects of the sedimentation on the result are of minor interest.

Table 6. Maximal contribution from the sources GV18, GV17 and GV7 to the oocyst concentrations at the raw water intakes under conditions of southwest and southeast winds, simulated for settling velocity of unattached oocysts.

Source	Southwest wind		Southeast wind	
	Maximal oocyst concentration (oocysts/10L)		Maximal oocyst concentration (oocysts/10L)	
	Intake 8 m	Intake 15 m	Intake 8 m	Intake 15 m
GV18	0.008187	0.002488	0.019054	0.008655
GV17	0.000678	0.000203	0.001790	0.001560
GV7	0.759748	0.184051	1.067320	0.513733

The output result of the simulations indicates that river Mölndalsån is the source that poses the highest risks related to the presence of *Cryptosporidium* oocysts at the raw water intakes. Figure 6(A-D) illustrates that a higher concentration of oocysts is expected at the 15 m raw water intake compared to the 8 m raw water intake (maximal 1618 compared to 925 oocysts/10L for unattached oocysts). Figure 6(A-D) shows also that the oocyst concentrations at the raw water intakes are higher under conditions of southwestern wind. The results show further that the different simulated settling velocities have great influence on the *Cryptosporidium* transport characteristics. For example, for the conditions of southwestern wind the maximum registered *Cryptosporidium* concentration at the 15 m raw water intake are 1618, 609 and 142 oocysts/10L for the cases of no attachment, high settling velocity and maximum settling velocity. Thus, the degree of attachment between *Cryptosporidium* oocysts and particles significantly affects the oocyst concentration at the raw water intake.

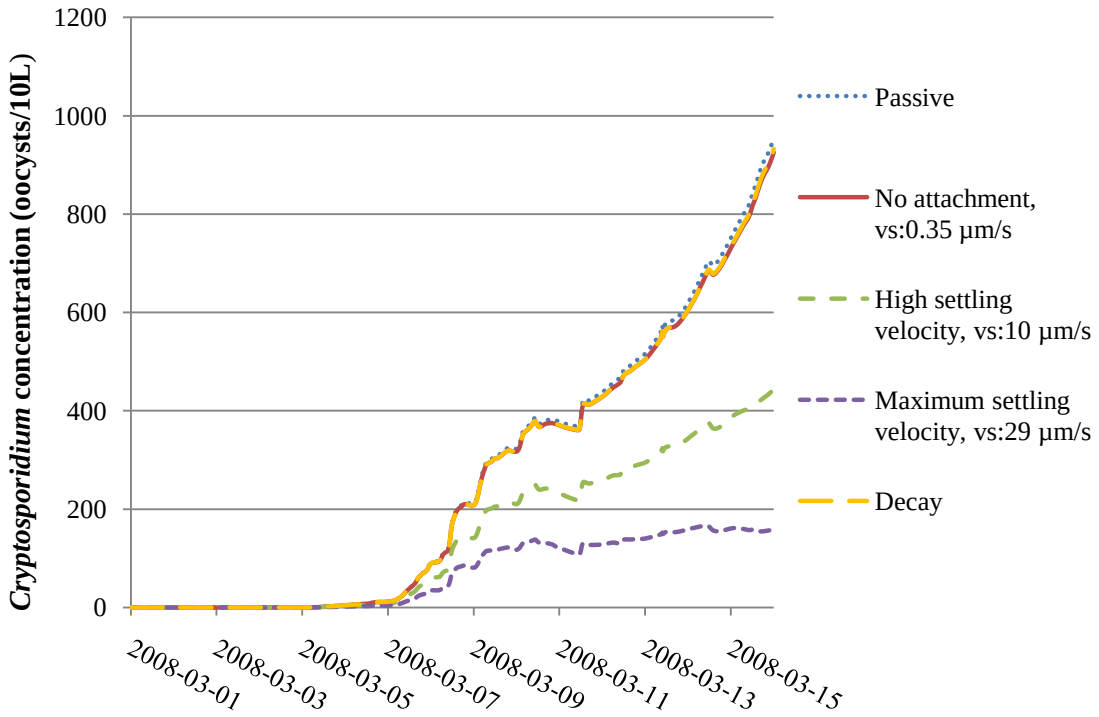

Figure 6 (A). Contribution from the river Mölndalsån to the Cryptosporidium concentration at the 8 m raw water intake under conditions of southwestern wind.

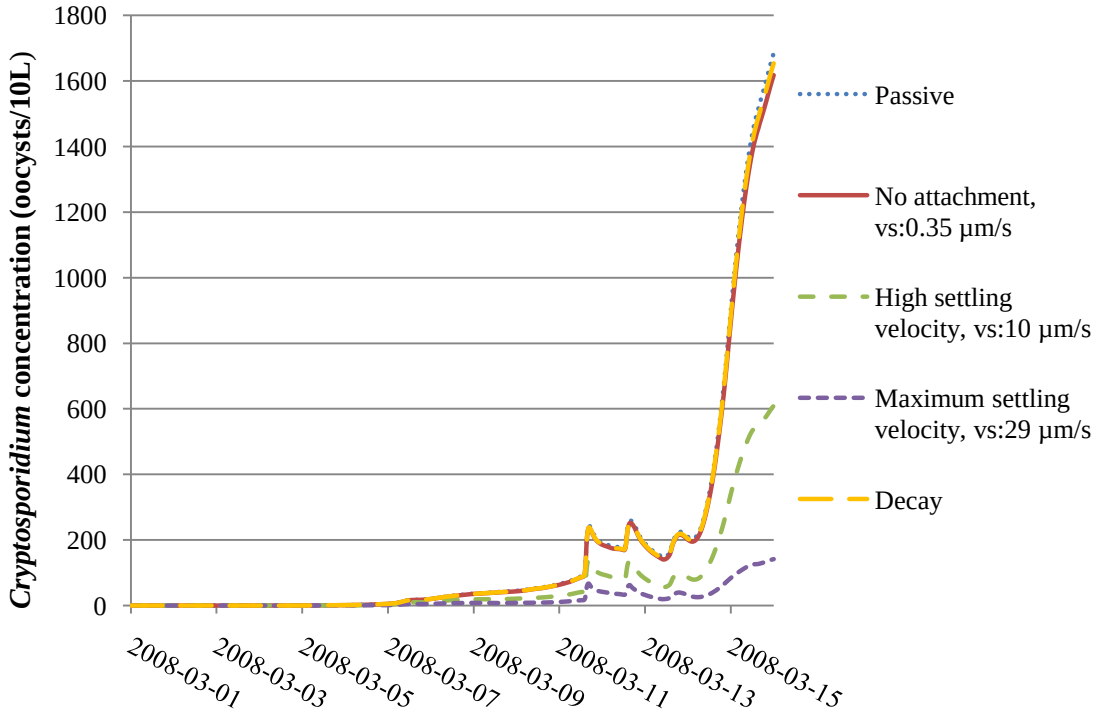

Figure 6(B). Contribution from the river Mölndalsån to the Cryptosporidium concentration at the 15 m raw water intake under conditions of southwestern wind.

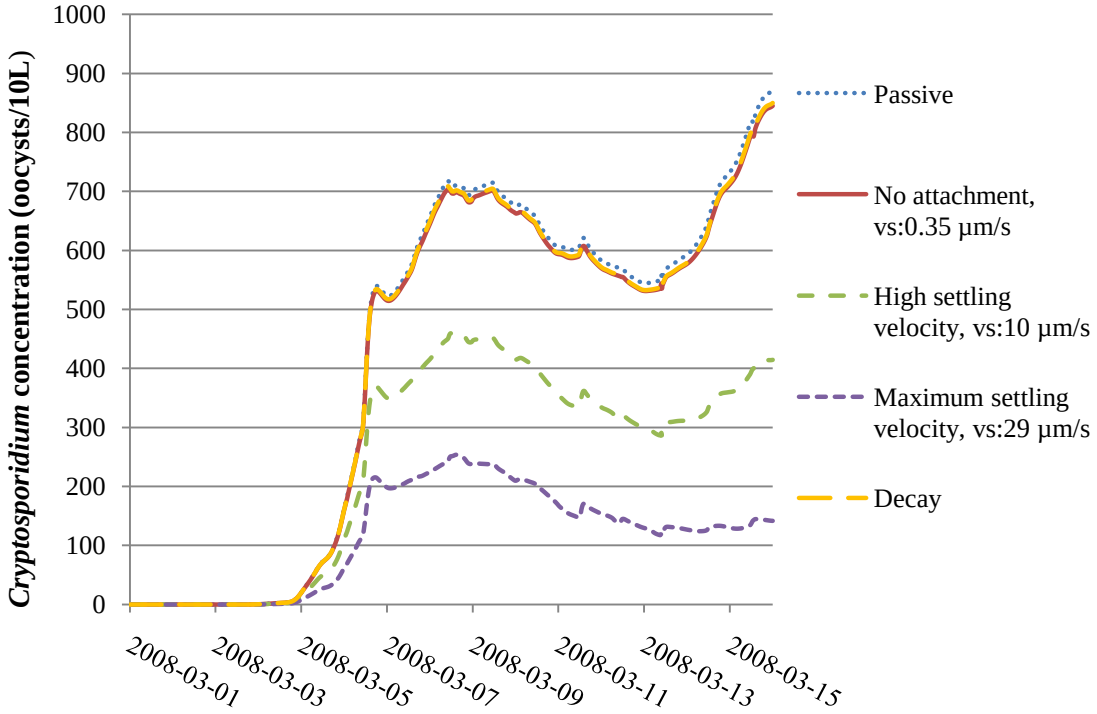

Figure 6(C). Contribution from the river Mölndalsån to the Cryptosporidium concentration at the 8 m raw water intake under conditions of southeastern wind.

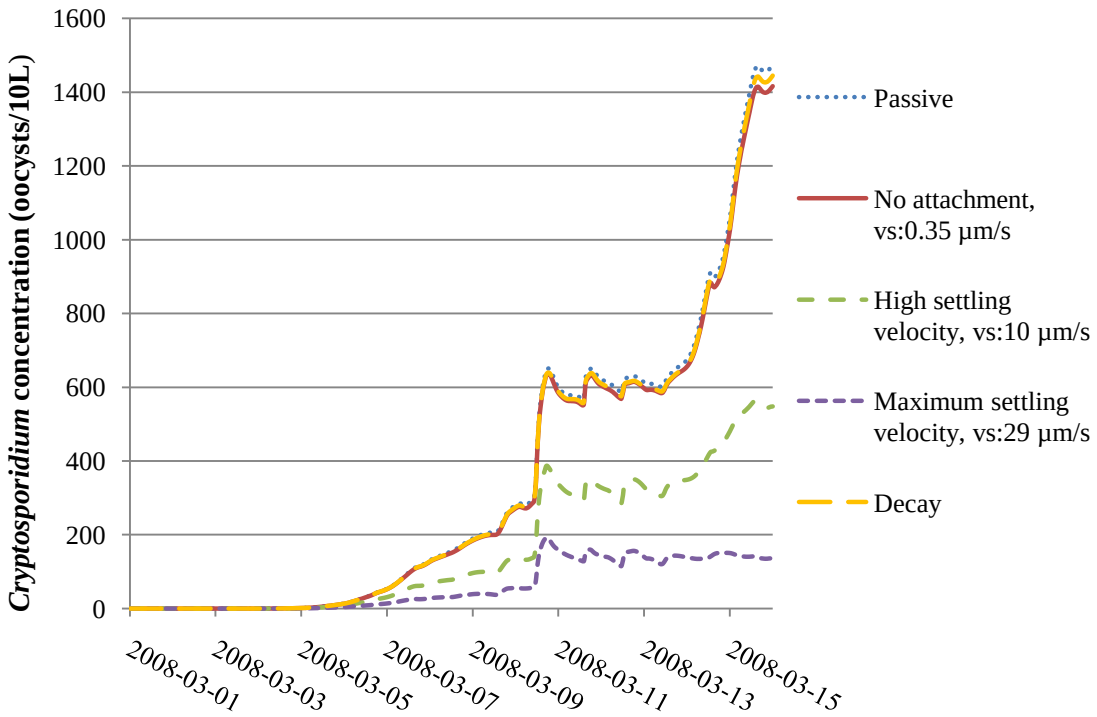

Figure 6(D). Contribution from the river Mölndalsån to the Cryptosporidium concentration at the 15 m raw water intake under conditions of southeastern wind.

According to the result of the simulations, Pixbo is the source that contributes the second highest oocyst concentration at the raw water intakes. However, the maximal registered contribution from Pixbo to the concentration at the raw water intake is about 1/7 of the maximal registered contribution from the river Mölndalsån. Similar to the results for the river Mölndalsån, results for Pixbo (Figure 7, A-D) illustrate that the highest *Cryptosporidium* concentration can be expected at the 15 m intake during southwestern conditions. For the unattached oocyst case, the highest computed concentration is 241 oocysts/10L. As presented in Figure 7(A-D) the different sedimentation velocities of the oocysts affect the results significantly, e.g. the maximum registered oocyst concentration for maximum attachment is just about 10% compared to the maximum registered oocyst concentration for no attachment for the 15 m raw water intake during southwestern wind conditions.

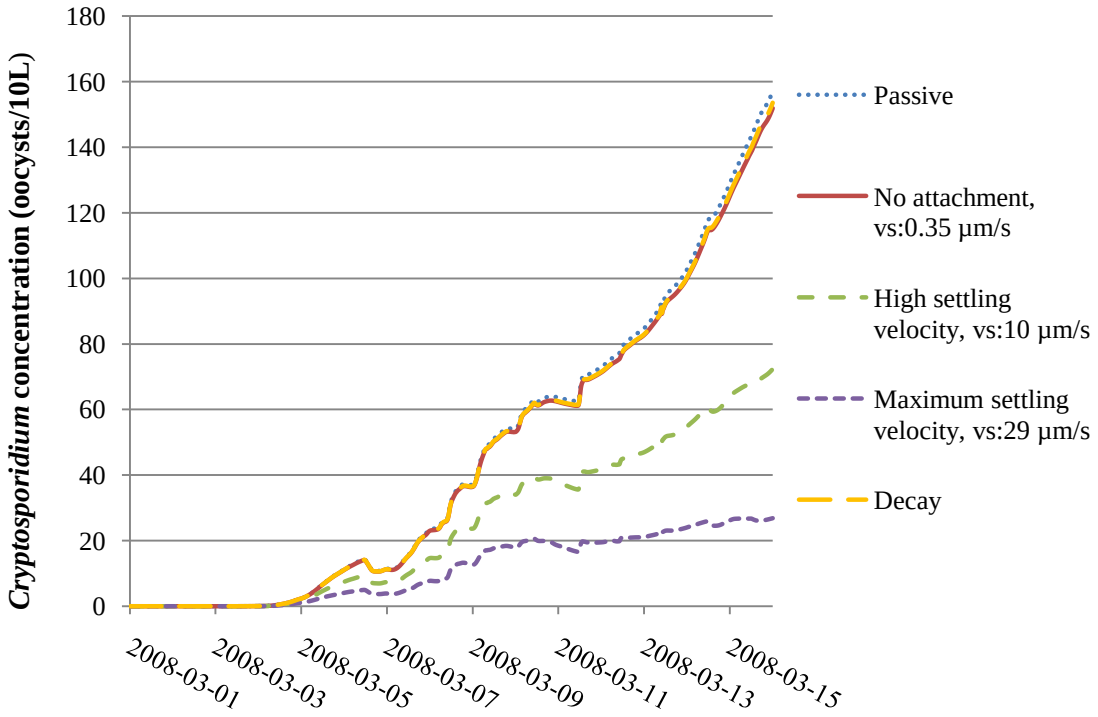

Figure 7(A). Contribution from Pixbo to the Cryptosporidium concentration at the 8 m raw water intake under conditions of southwestern wind.

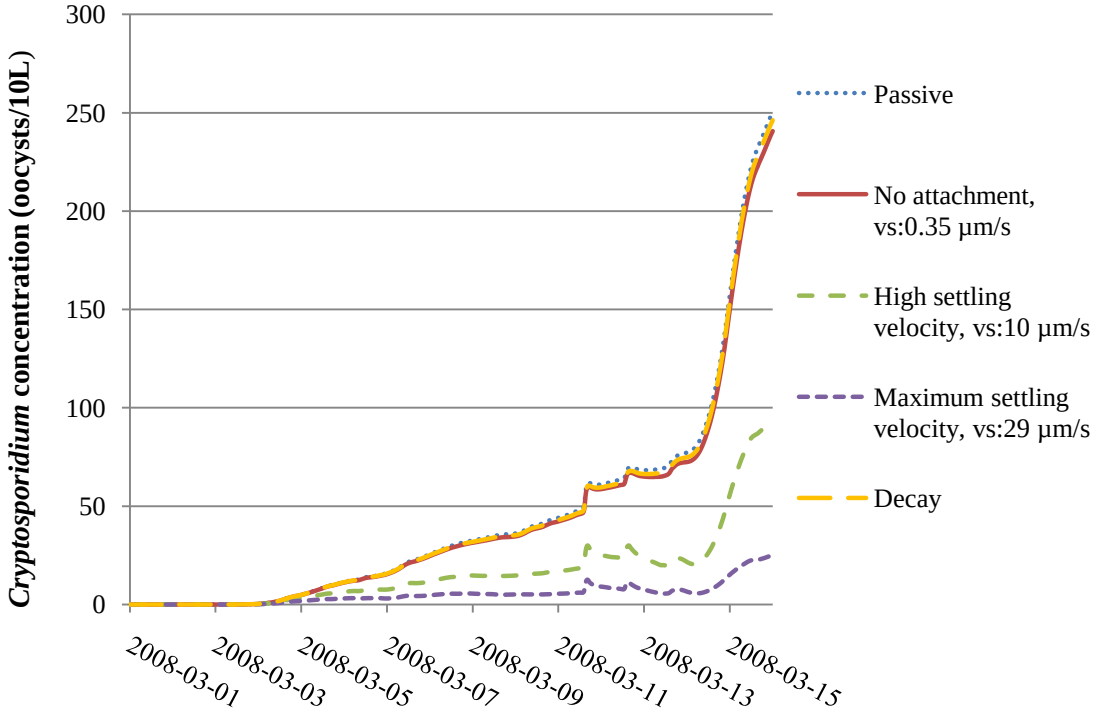

Figure 7(B). Contribution from Pixbo to the Cryptosporidium concentration at the 15 m raw water intake under conditions of southwestern wind.

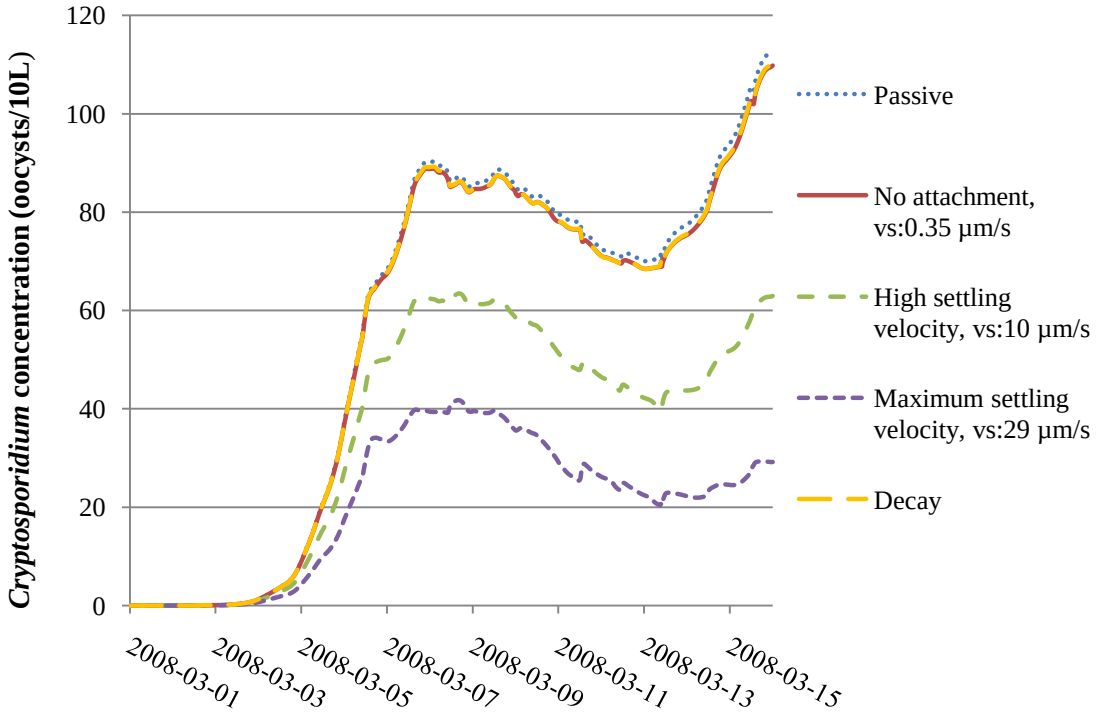

Figure 7(C). Contribution from Pixbo to the Cryptosporidium concentration at the 8 m raw water intake under conditions of southeastern wind.

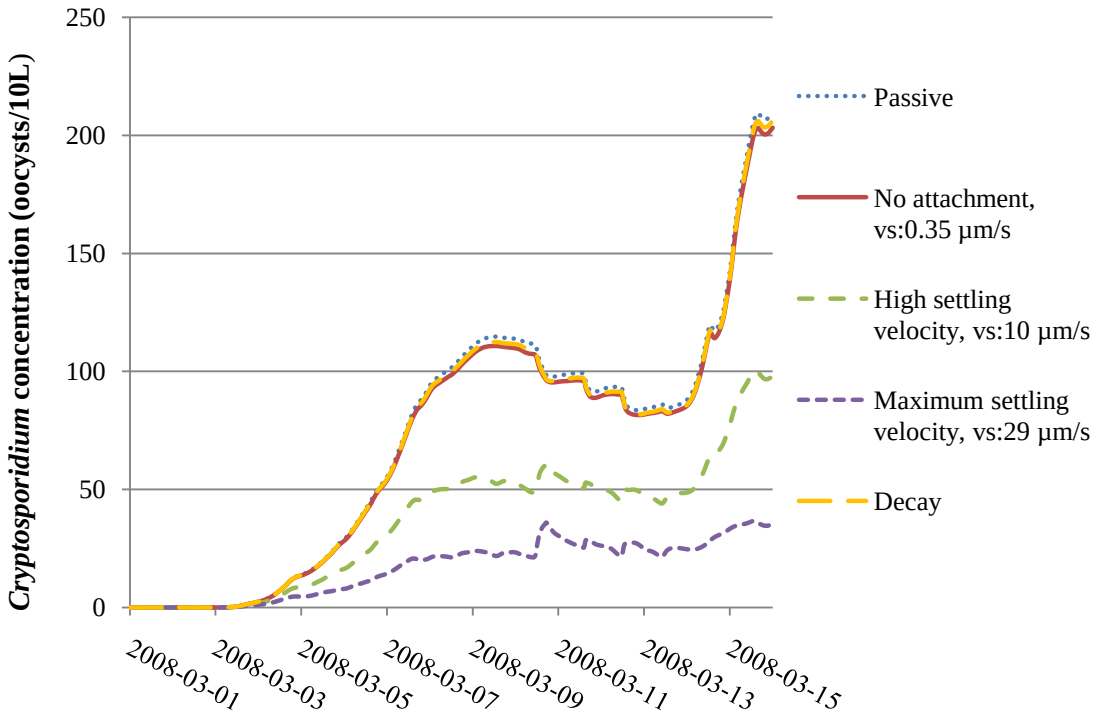

Figure 7(D). Contribution from Pixbo to the Cryptosporidium concentration at the 15 m raw water intake under conditions of southeastern wind.

The modelling results indicate that GV3 is the third most important source regarding its contribution to *Cryptosporidium* concentration at the raw water intakes. As illustrated in Figure 8(A-D) GV3 contributes with maximum 10 oocysts/10L (at raw water intake 8 m under southeastern wind conditions). The magnitude of the contribution is although minor compared with the river Mölndalsån and Pixbo (the maximum value make up only approximately 0.5% of the maximum value of the river Mölndalsån).

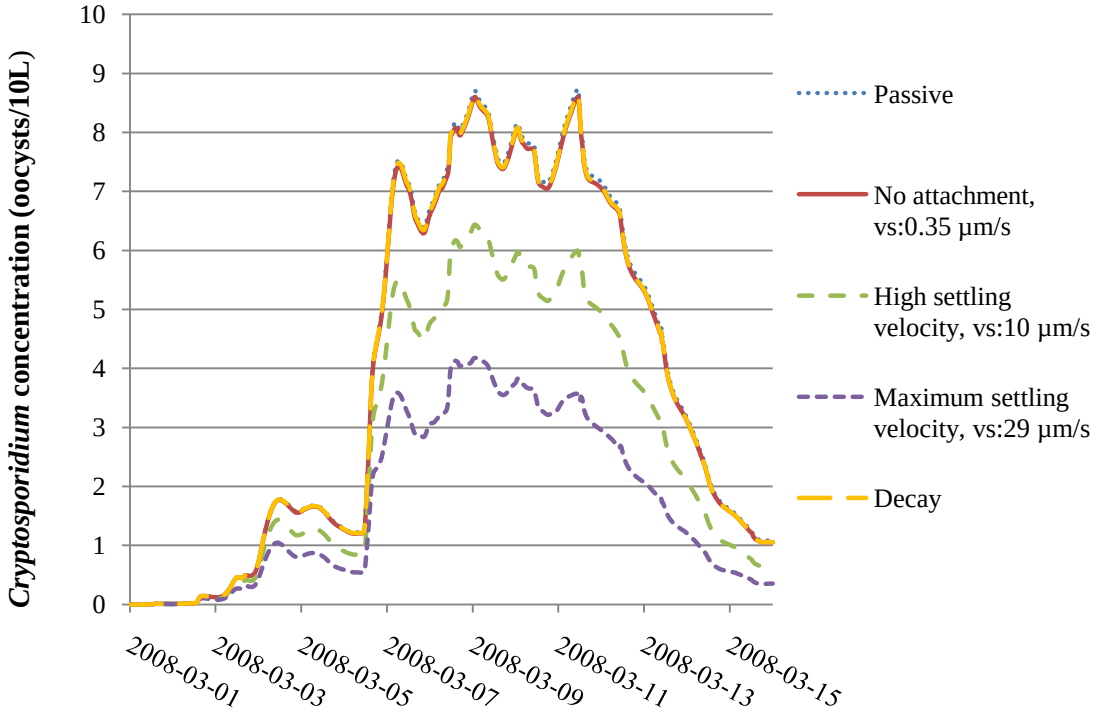

Figure 8(A). Contribution from GV3 to the Cryptosporidium concentration at the 8 m raw water intake under conditions of southwestern wind.

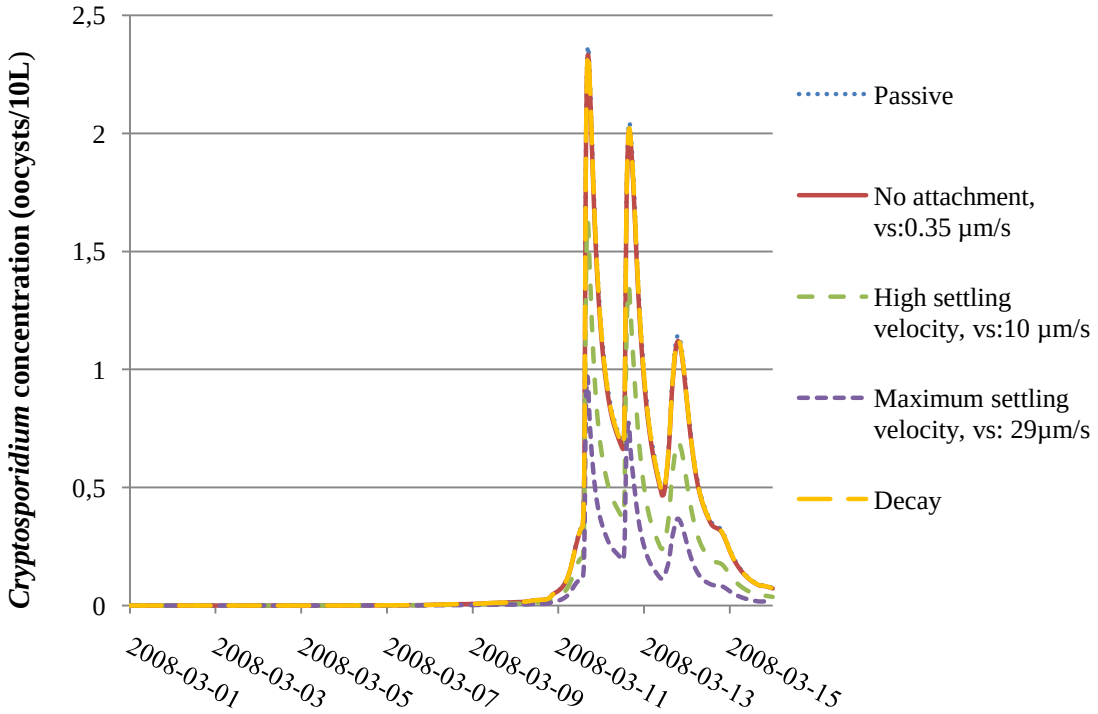

Figure 8(B). Contribution from GV3 to the Cryptosporidium concentration at the 15 m raw water intake under conditions of southwestern wind.

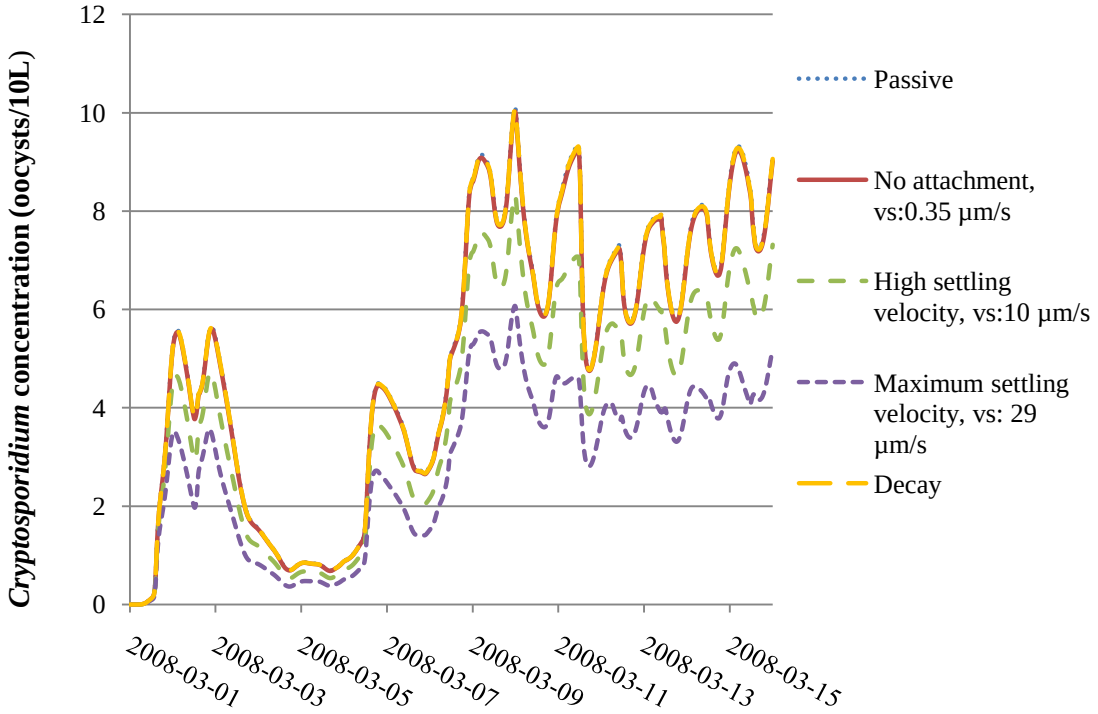

Figure 8(C). Contribution from GV3 to the Cryptosporidium concentration at the 8 m raw water intake under conditions of southeastern wind.

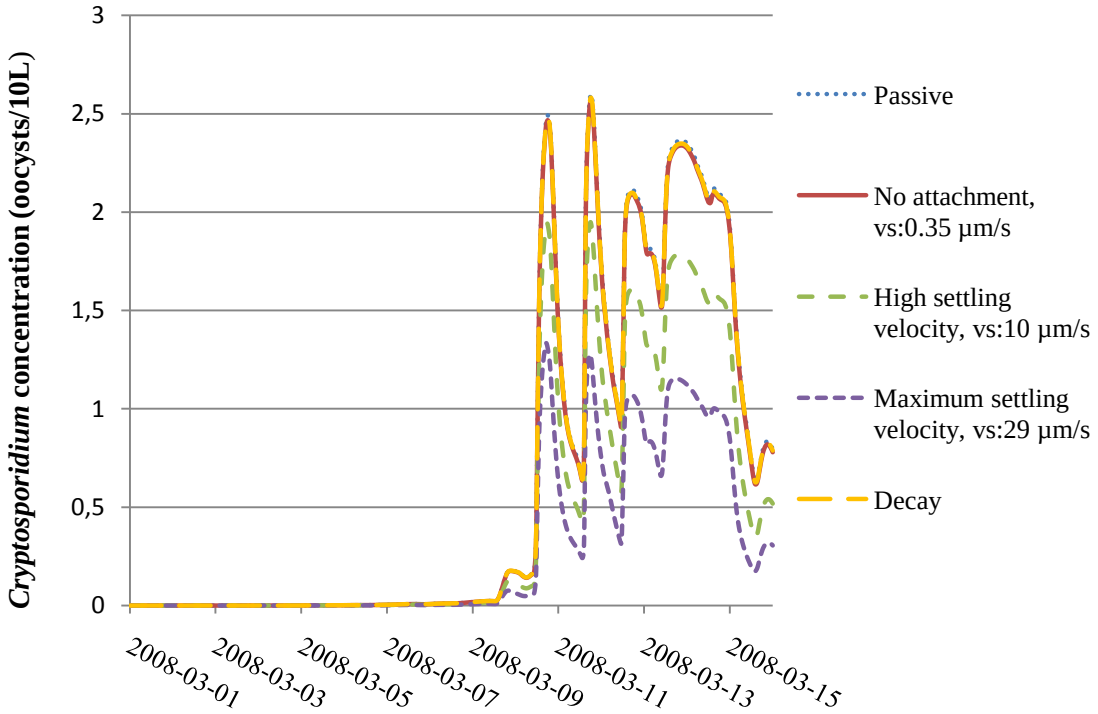

Figure 8(D). Contribution from GV3 to the Cryptosporidium concentration at the 15 m raw water intake under conditions of southeastern wind.

5 Discussion

Based on an extensive literature review a modelling approach was developed using the ecological module ECO Lab, to take into account the settling kinetics of *Cryptosporidium*. ECO Lab was coupled with the hydrodynamic model MIKE 3 and simulations of settling and transport of *Cryptosporidium* in Lake Rådasjön were performed. The results of the simulations aim to give an estimation of the contribution from the different sources to the *Cryptosporidium* concentrations at the raw water intakes and most importantly to verify that the developed sedimentation modelling approach generates reliable results.

The performed settling and transport modelling involves a number of assumptions that introduce uncertainty. The input *Cryptosporidium* concentrations were estimated with high uncertainty and represented epidemic conditions; this means that the performed modelling represents a worst case scenario. Moreover, the discharges from the sources to the lake were set constant with a constant *Cryptosporidium* concentration, which is a simplification that probably affects the results significantly. For instance, the combined sewer overflow at Pixbo in reality releases storm water limited times per year, i.e. it is a pulse discharge. This probably leads to overestimation of the magnitude of the released discharges. The wind conditions are also set constant which affect the hydrodynamics in the lake.

The modelling results indicated that the different simulated settling velocities affect the results considerably. However, there is a great uncertainty regarding the settling velocity, since no research has been performed in Lake Rådasjön regarding the ability of *Cryptosporidium* oocysts to attach to particles. It is probable that the oocysts in the lake are attached to particles at varying degree depending on the source from which contamination derives. For instance, it has been shown in laboratory experiments that the oocysts attach more readily to secondary effluent (similar to the conditions of sever overflow in Pixbo) than to faecal material (Medema et al., 1998, Young and Komisar, 2005). Moreover, laboratory assays have shown that *Cryptosporidium* oocysts attach extensively to the clay mineral kaolinite (Searcy et al., 2005) and since the lake is located on clay bottom it can be assumed that a part of the oocysts are aggregated in the water column to such particles.

The settling velocity of unattached *Cryptosporidium* oocysts used in the model was determined in laboratory experiments (Medema et al., 1998). The oocysts used in the laboratory experiments were of the same age and have experienced similar conditions. However, in the surface water environments properties of the oocysts, such as density, are likely to differ due to different ages of oocysts and different exposure to stressors. The age of the oocysts may also affect their ability to attach to particles.

The results of the simulations show that the *Cryptosporidium* contribution from the four less important sources GV18, GV17, GV3 and GV7 to the concentrations at the raw water intakes is registered higher for the 8 m intake compared to the 15 m intake, while the most significant sources sewer overflow in Pixbo and Mölndalsån contribute with a higher *Cryptosporidium* concentration to the 15 m raw water intake than to the 8 m. This is of interest since the 8 m intake is normally closed (the Gothenburg intake) and deeper located raw water intakes show normally a higher water quality (lower concentration of harmful microorganisms). The explanation is probably related to the distance between the sources and the raw water intakes. Mölndalsån and Pixbo

are located relative far from the raw water intakes; this means that the discharges from these sources are mixed in a greater extent. Since the modelling is made for March conditions, there is no clear stratification in the lake, which facilitates the mixing process.

The results of the simulations show that the time series of Mölndalsån and Pixbo are of continuously increasing characters. Since the modelling was performed for a limited period of 15 days it is obvious that a simulation for a longer time period would give a clearer illustration of the variation of the *Cryptosporidium* concentrations at the raw water intakes.

The influence of different settling velocities on the *Cryptosporidium* concentrations at the raw water intakes was compared with the influence of temperature-decay. Since the simulations were performed for March conditions, when the water temperature was low, the influence of temperature decay on the concentrations at the raw water intakes was not significant. It would be of interest to perform simulations for summer conditions, when the water temperatures are higher and greater temperature decay can be expected.

6 Recommendations

This study suggests that ecological and hydrodynamic models are suitable tools for describing the transport and fate processes of *Cryptosporidium* in water systems. These models provide a useful decision support for risk assessment regarding microbial contamination of drinking water sources, particularly combined with regular on-site monitoring.

Since it has been concluded in this study that the settling velocity of *Cryptosporidium* affects greatly its transport characteristics, an experimental analysis of the *Cryptosporidium* settling velocities for the conditions of Lake Rådasjön is recommended. It is probable that the parasite is attached to particles in varying degree, depending on the source of faecal contamination.

The modelling results show that the river Mölndalsån is the source that poses the greatest threats to the water quality at the raw water intakes regarding contamination with *Cryptosporidium*. The highest *Cryptosporidium* concentration was registered at the deeper, 15 m raw water intake. Consequently an evaluation of the sources that contributes with faecal contamination to Mölndalsån should be performed. In addition, the reasons behind the fact that higher *Cryptosporidium* concentrations were registered at the 15 m raw water intake have to be clarified.

7 Conclusions

In this study, a modelling approach that takes into account the settling kinetics of *Cryptosporidium* has been developed. The modelling approach was implemented in the ecological module ECO Lab.

The result of the modelling of *Cryptosporidium* in Lake Rådasjön shows that the magnitude of the settling velocity affects the transport characteristics greatly. The magnitude of the settling velocity is to a great extent governed by the ability of the protozoa to aggregate to particles. The aggregation mechanism depends largely on the electrostatic surface charge of the particle (positive charged particles attract the negative charged *Cryptosporidium* oocysts). The non-hydrophobic property of the oocyst has also shown to have a significant influence. Since no settling experiments have been carried out for Lake Rådasjön, the magnitudes of the *Cryptosporidium* settling velocities in the lake are uncertain.

It can be concluded that the river Mölndalsån is the source that poses the highest risk regarding the presence of *Cryptosporidium* at the raw water intakes. Unexpectedly, the highest *Cryptosporidium* concentration was registered at the 15 m raw water intake.

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9 Appendix

9.1 Results from GV18, GV17, GV7

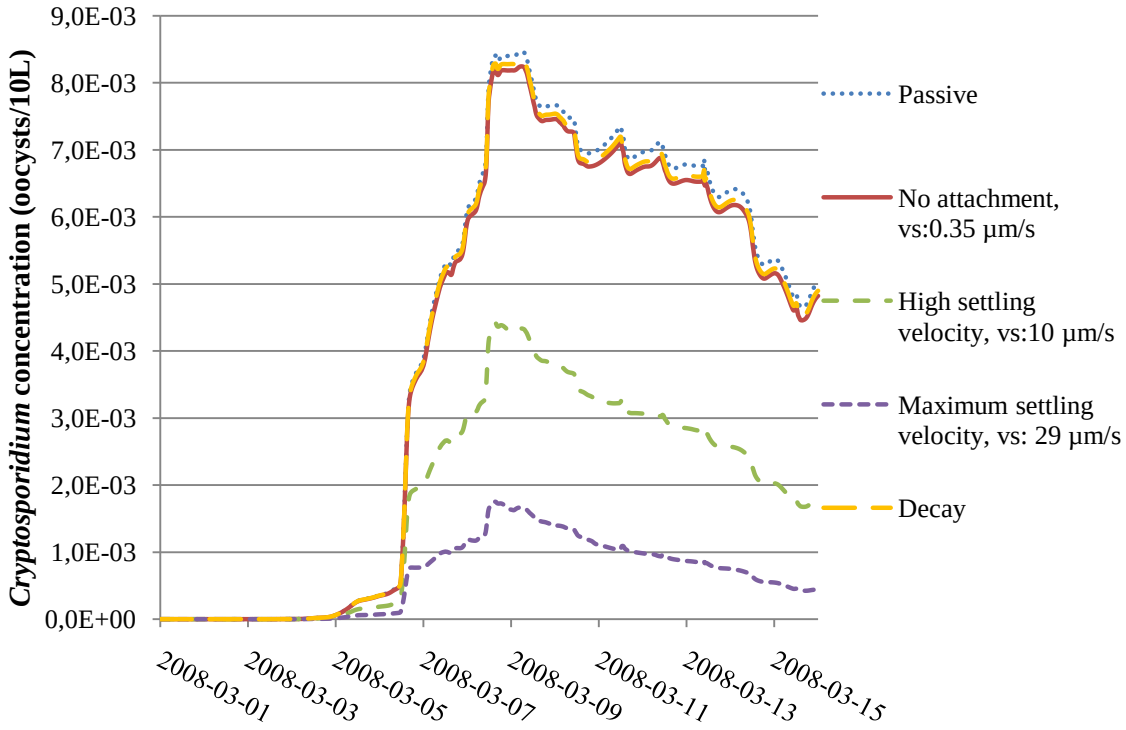

Figure 9(A). Contribution from GV18 to the Cryptosporidium concentration at the 8 m raw water intake under conditions of southwestern wind.

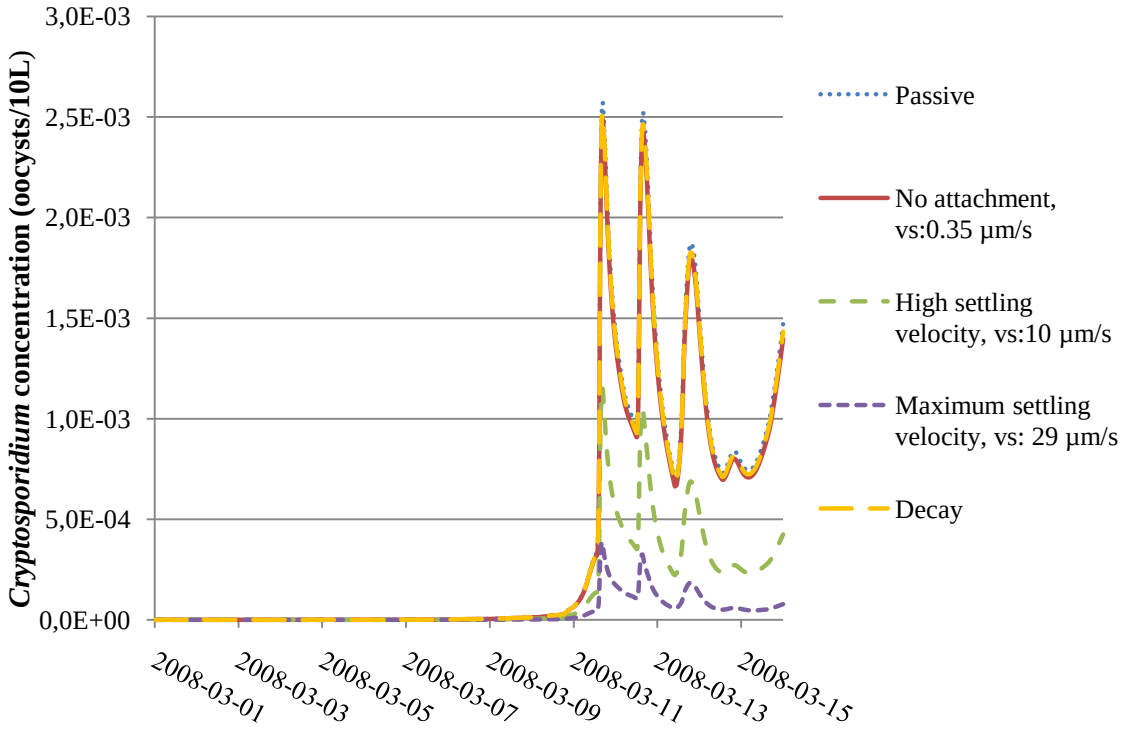

Figure 9(B). Contribution from GV18 to the Cryptosporidium concentration at the 15 m raw water intake under conditions of southwestern wind.

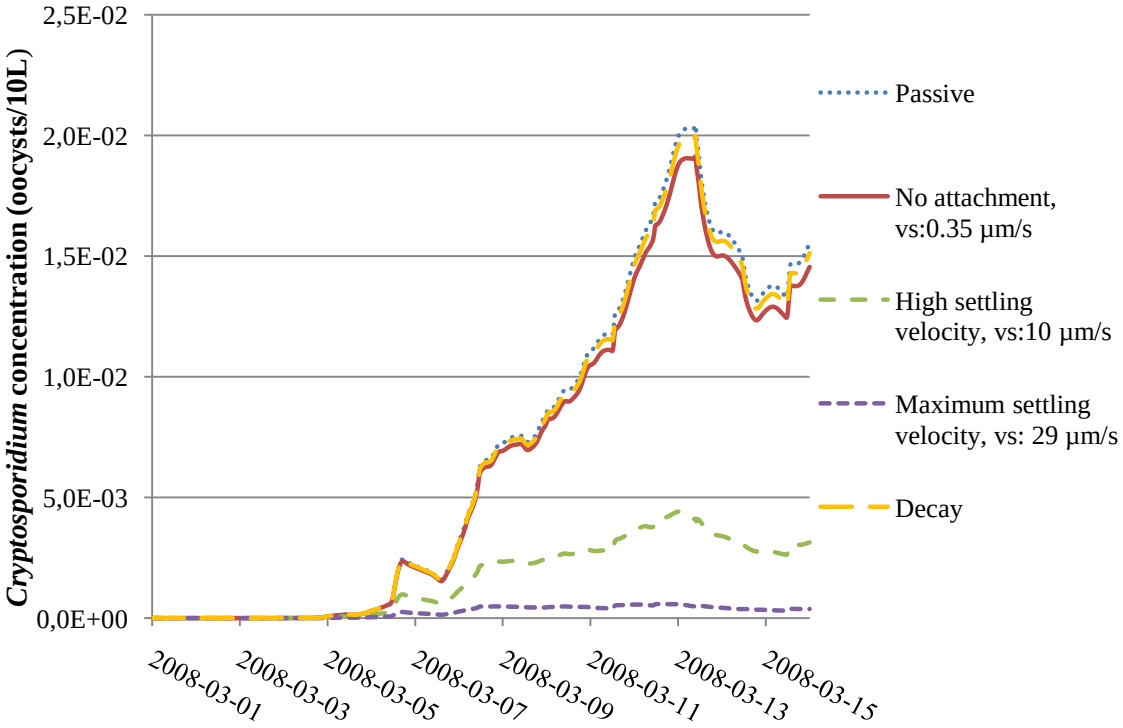

Figure 9(C). Contribution from GV18 to the Cryptosporidium concentration at the 8 m raw water intake under conditions of southeastern wind.

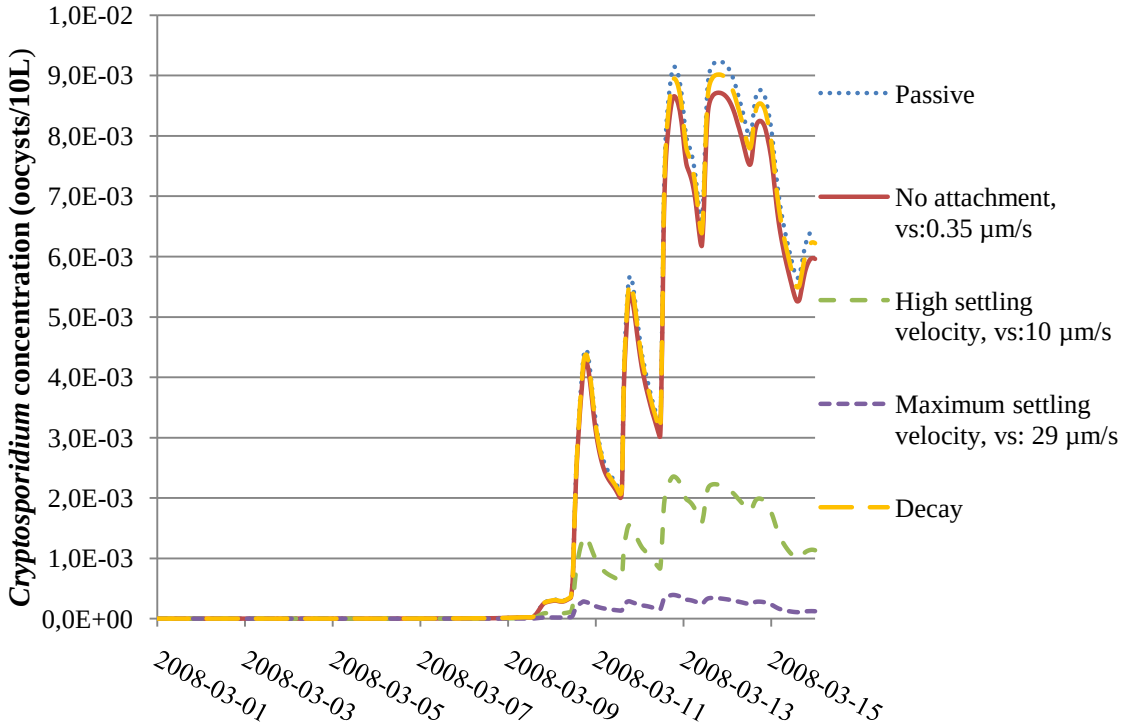

Figure 9(D). Contribution from GV18 to the Cryptosporidium concentration at the 15 m raw water intake under conditions of southeastern wind.

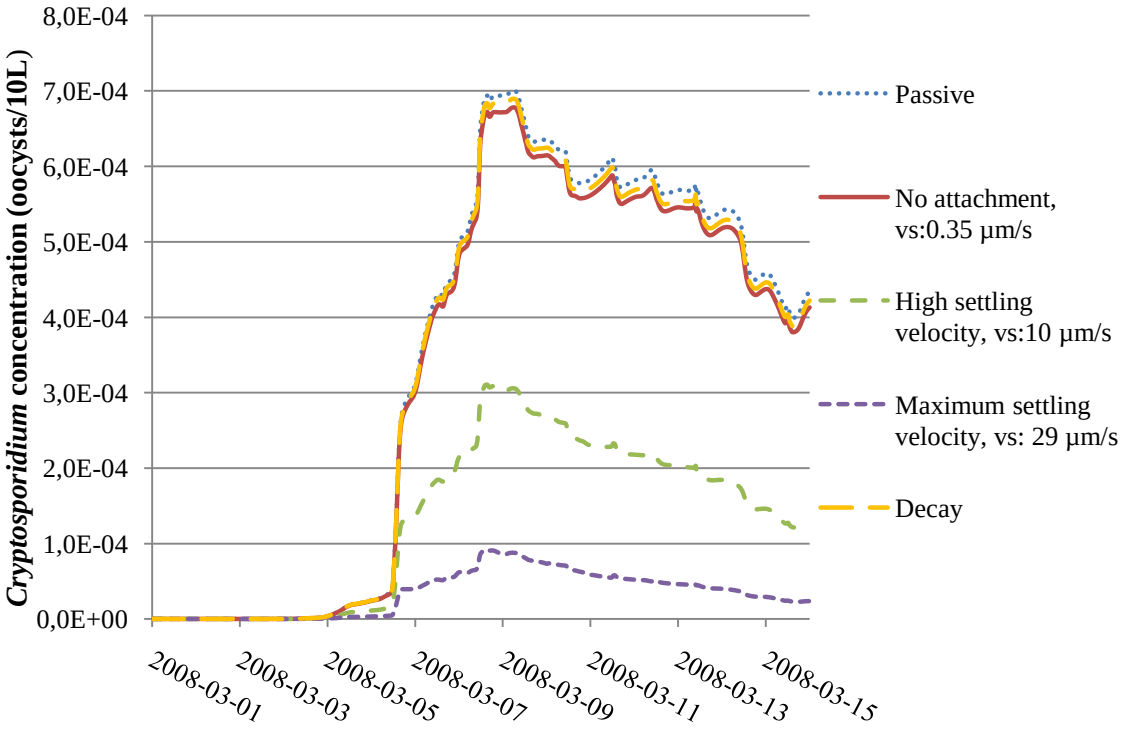

Figure 10(A). Contribution from GV17 to the Cryptosporidium concentration at the 8 m raw water intake under conditions of southwestern wind.

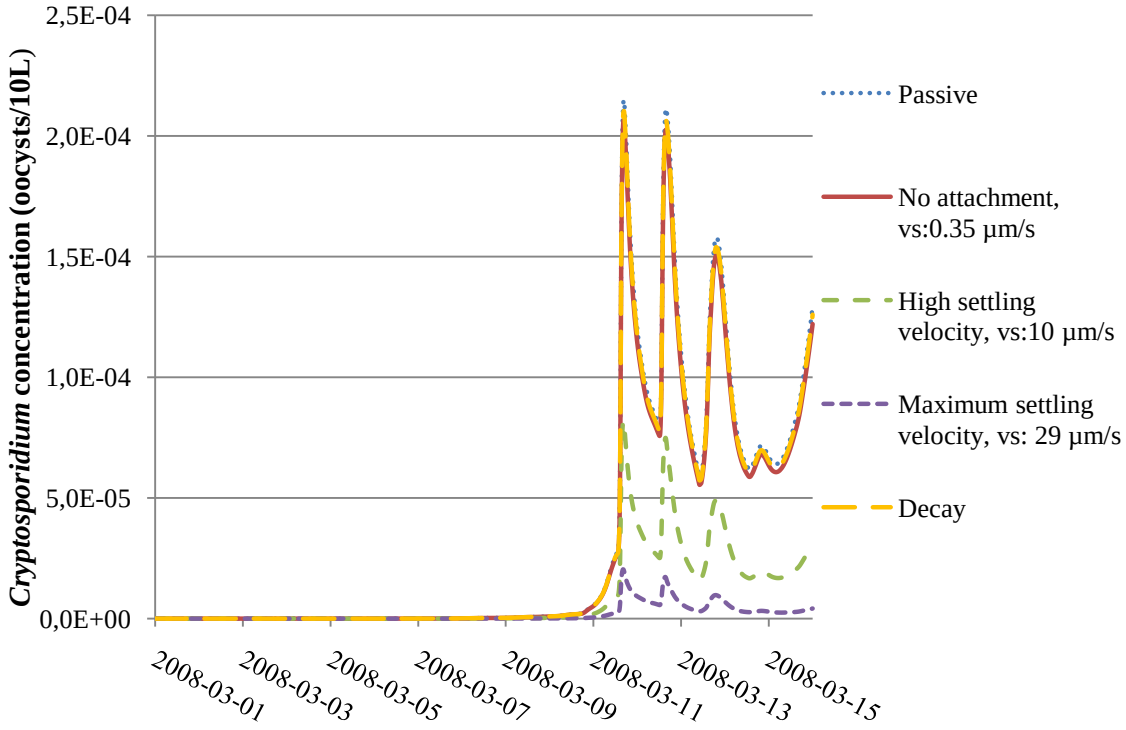

Figure 10(B). Contribution from GV17 to the Cryptosporidium concentration at the 15 m raw water intake under conditions of southwestern wind.

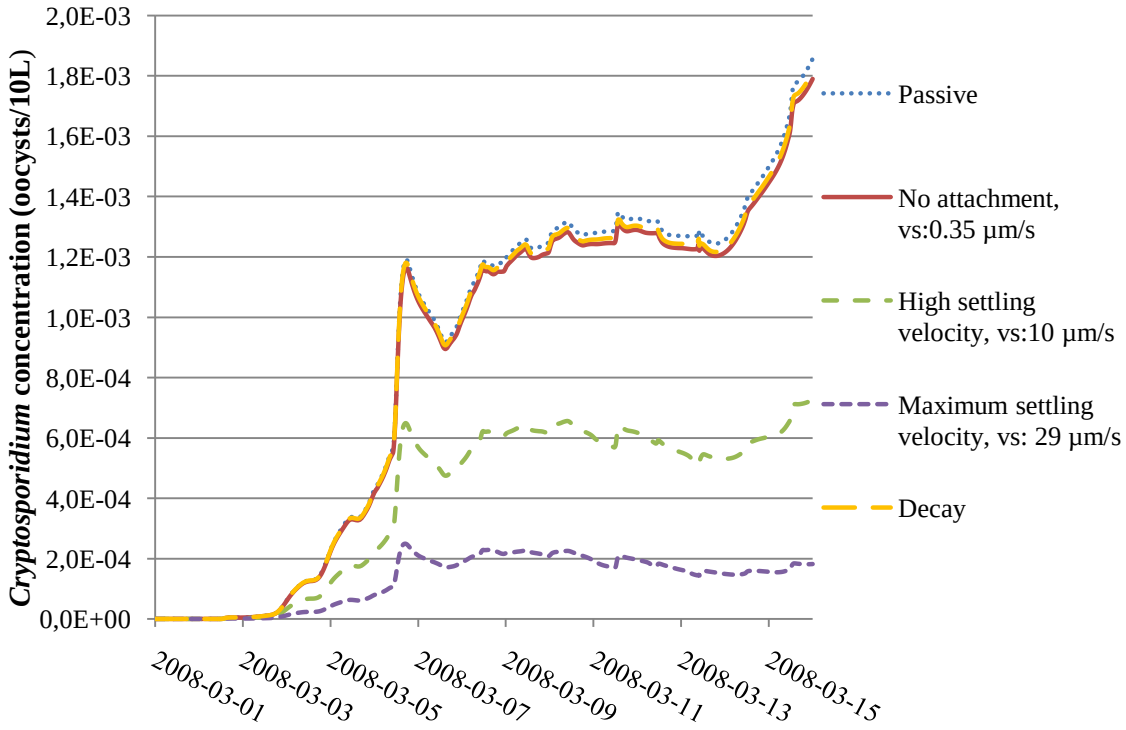

Figure 10(C). Contribution from GV17 to the Cryptosporidium concentration at the 8 m raw water intake under conditions of southeastern wind.

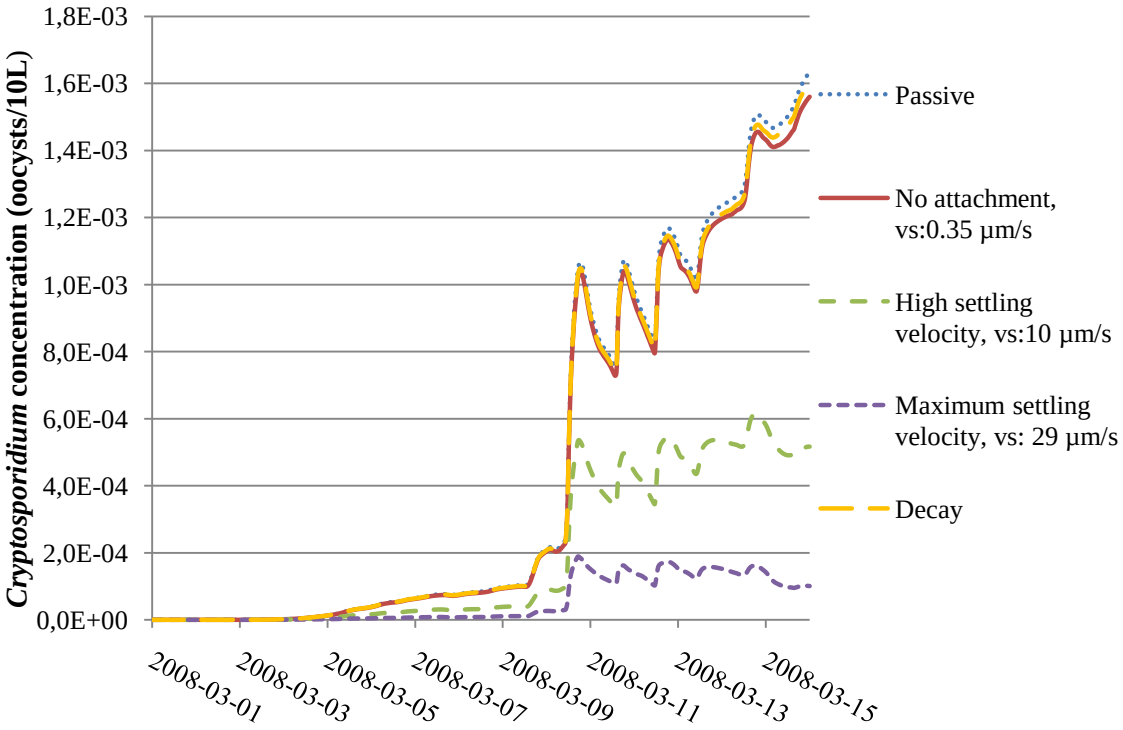

Figure 10(D). Contribution from GV17 to the Cryptosporidium concentration at the 15 m raw water intake under conditions of southeastern wind.

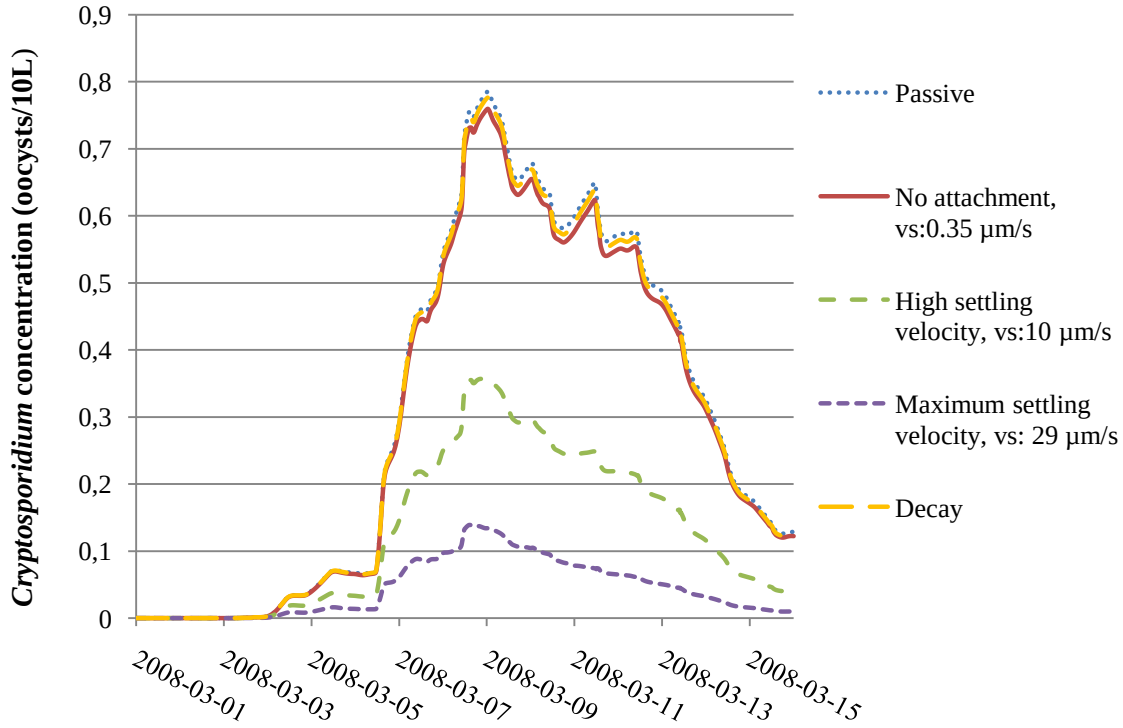

Figure 11(A). Contribution from GV7 to the Cryptosporidium concentration at the 8 m raw water intake under conditions of southwestern wind.

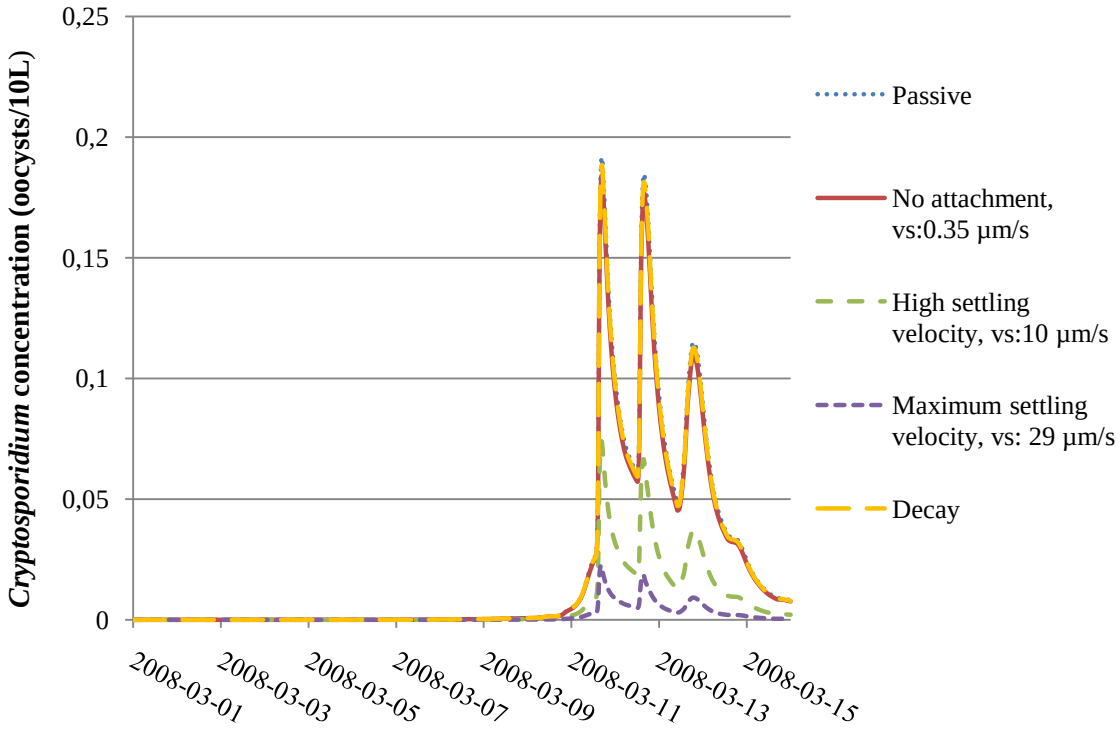

Figure 11(B). Contribution from GV7 to the Cryptosporidium concentration at the 15 m raw water intake under conditions of southwestern wind.

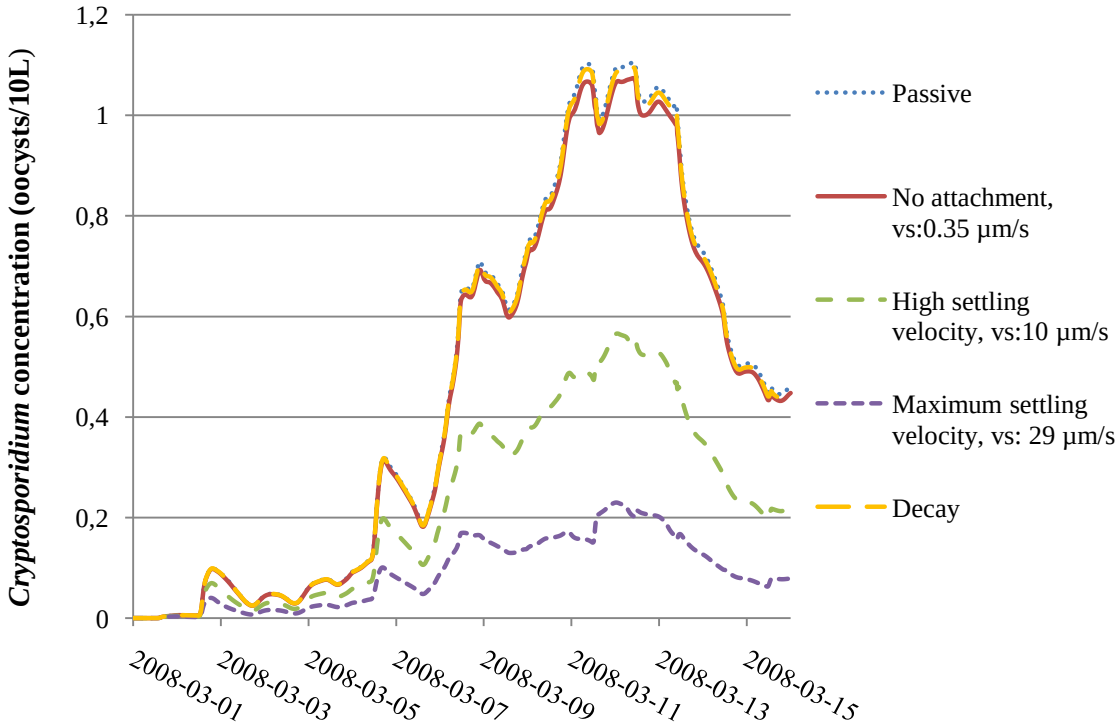

Figure 11(C). Contribution from GV7 to the Cryptosporidium concentration at the 8 m raw water intake under conditions of southeastern wind.

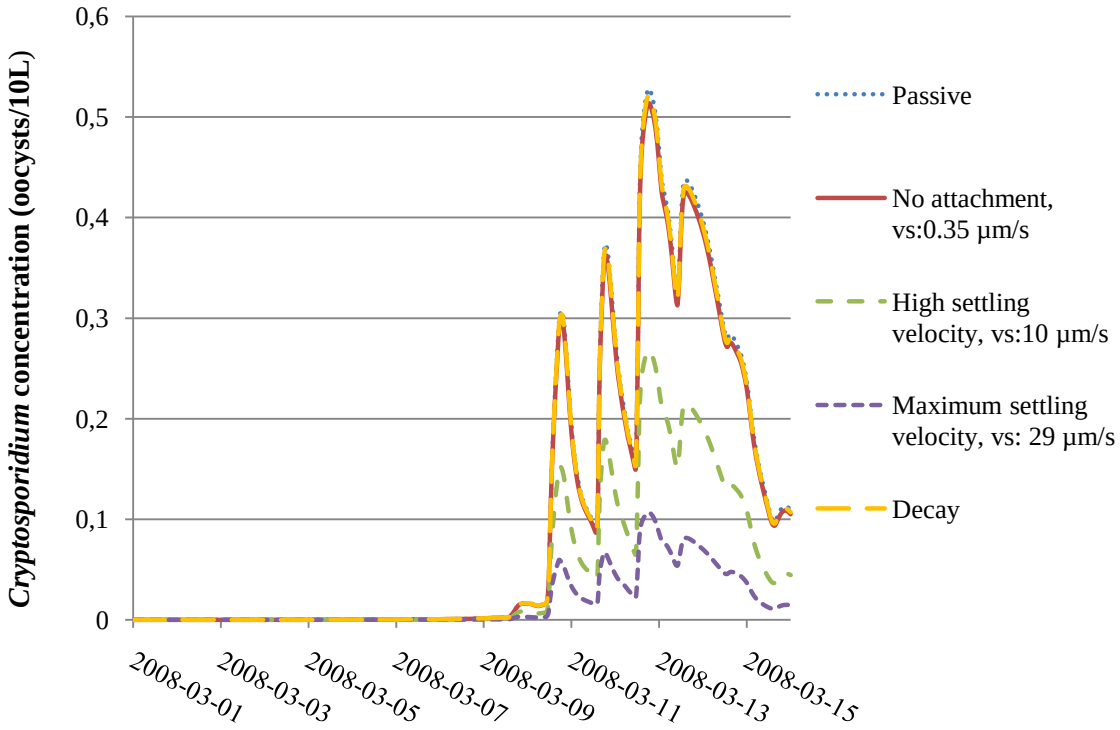

Figure 11(D). Contribution from GV7 to the Cryptosporidium concentration at the 15 m raw water intake under conditions of southeastern wind.

9.2 Terminology

Adhesion- The condition, in which two surfaces are connected together by interfacial forces, which may be valence forces or interlocking action, or both (Hawley and Lewis, 2007).

Adsorption- The establishment of a layer of gas, liquid or solid on the surface of a solid or less commonly of a liquid (Daintith, 2008).

Autoclave- A chamber, normally of cylindrical form, used to carry out chemical reactions under conditions of high temperature and pressure (Hawley and Lewis, 2007).

Conductivity- The property of a substance or mixture that defines its capacity to transport heat or electricity (Hawley and Lewis, 2007).

Cation exchange- The chemical exchange of cations, the positive charged ion (Martin, 2007).

Cryptosporidiosis- An intestinal infection of mammals and birds caused by *Cryptosporidium*. Most affected humans recover in 7-14 days, but the infection can persist in the immunocompromised, patients of old age, and young children (Martin, 2007).

Dialysis- A procedure by which small and large molecules in a solution are removed by selective diffusion through a semipermeable membrane (Daintith, 2008).

Extracellular- Situated or occurring outside cells (Martin, 2007).

Hydrophobic- Property that describes that a substance is unable to be dissolved in water (Hawley and Lewis, 2007).

Polymer- A substance having large molecules composed of repeated units (Daintith, 2008).

RNA- A complex organic compound in living cells which is connected to the protein synthesis (the process in which cells build protein). In several viruses, RNA is also the hereditary material (Martin, 2007).

Subphylum- A taxonomic classification that is ranked below phylum and above class (Martin, 2007).

Van der Waals force- An attractive force between atoms and molecules. Van der Waals forces are much weaker than those arising from valence bonds (Daintith, 2008).

Wettability- The tendency of a fluid to spread on and particularly adhere to a solid surface in the presence of other immiscible fluids (Hawley and Lewis, 2007).

9.3 References Terminology

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