THESIS FOR THE DEGREE OF LICENTIATE OF ENGINEERING

Hydrodynamic and Microbiological Modelling of Water Quality in Drinking Water Sources

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CHALMERS UNIVERSITY OF TECHNOLOGY

Gothenburg, Sweden 2011

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Abstract

Faecal contamination of drinking water sources poses risks for waterborne disease outbreaks. To manage these risks the fate and transport of faecal contamination in a drinking water source need to be understood and quantitatively described. In this study the fate and transport of faecal contamination in a drinking water source, Lake Rådasjön in Sweden, was simulated using a coupled hydrodynamic and microbiological modelling approach. To calibrate the microbiological model that describes the inactivation of faecal indicators as a function of temperature and sunlight, a microcosm experiment was performed. The experiment consisted of three outdoor microcosm trials performed in March, August and November 2010 to capture seasonal variations in the inactivation of faecal indicators. The indicators studied in the microcosm experiment included traditional faecal indicators (total coliforms, *E. coli*, enterococci, somatic coliphages) and *Bacteroidales* genetic markers (BacH and BacR) that can be used in microbial source tracking to determine the human or ruminant origin of faecal contamination. The spread of faecal contamination in the lake was simulated using *E. coli* and *Bacteroidales* genetic markers. The results indicated that hydrometeorological conditions such as wind, inflow to the lake and temperature stratification of the lake have a major impact on the spread of faecal contamination. The simulations showed that faecal contamination from the river Mölndalsån, emergency sewer overflow and on-site sewers can pose threats to the drinking water supply of the cities of Gothenburg and Mölndal. Moreover, modelling the fate and transport of *Bacteroidales* markers in a water body provided information about the contribution of different sources to the total concentration of these markers at the water intake. This can substantially improve the usefulness of *Bacteroidales* markers in microbial source tracking.

Keywords: fate and transport modelling, inactivation, pathogens, faecal indicators, *Bacteroidales*, microbial source tracking, microcosm experiment, ECO Lab, MIKE 3.

List of papers

This thesis includes the following papers:

Paper I

Sokolova, E., C. Borell Lövstedt and T.J.R. Pettersson (2011) Fate and transport modelling of microbial pollution in a lake used as a drinking water source. *Proceedings of the 34th IAHR World Congress 2011 in Brisbane, Australia (26 June – 1 July 2011).*

Paper II

Sokolova E., J. Åström, T.J.R. Pettersson, O. Bergstedt and M. Hermansson (2011) Inactivation of *Bacteroidales* genetic markers for microbial water quality modeling of drinking water sources. *Submitted to Environmental Science and Technology.*

Acknowledgements

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1. Introduction

The first chapter states the aim and objectives of this thesis and provides an overview of the attached papers.

Gastrointestinal diseases are a major cause of mortality in the world (WHO 2008a). These diseases are caused by microbial pathogens that are often present in faecal matter from animals and humans (Rusin et al. 2000). In developing countries gastrointestinal diseases are transmitted due to poor sanitation, through food sources and unsafe drinking water (Ashbolt 2004). Even in the developed world unsafe drinking water is a major source of microbial pathogens that can cause outbreaks of gastrointestinal diseases (Hrudey and Hrudey 2004).

Microbial pathogens in drinking water can originate from the water source (surface or groundwater) as well as from the distribution network (WHO 2008b). Faecal contamination of the water source used for the production of drinking water, in combination with insufficient treatment of raw water, can result in microbial contamination of drinking water (WHO 2008b). Alternatively, safe drinking water can be contaminated while being transported to the consumer in the distribution system (Payment and Robertson 2004). This research focuses on the problem of faecal contamination of surface water sources as a reason for waterborne disease outbreaks in the developed world.

1.1. Aim and objectives

Faecal contamination of the water source is a common cause of waterborne disease outbreaks (Hrudey and Hrudey 2004). Therefore, a sufficient quality of raw water is essential for the production of safe drinking water. To ensure the quality of the raw water, a risk assessment of the water source that involves the identification of faecal sources and the understanding of faecal contamination transport within the water source is required. The fate and transport of faecal contamination from the point of discharge to the raw water intake can be described using a coupled hydrodynamic and microbiological modelling approach (e.g. Hipsey et al. 2008).

The aim of this research is to describe the spread of faecal contamination in the water source using a coupled hydrodynamic and microbiological modelling approach.

To fulfil this aim several specific objectives are addressed in the papers that are attached to this thesis (Paper I and Paper II).

The specific objectives are:

- to experimentally determine the inactivation of faecal indicators in the water source (Paper II);
- to calibrate the microbiological model based on the experimentally obtained inactivation data (Paper I, Paper II);
- to apply the coupled hydrodynamic and microbiological model to simulate the spread of faecal contamination in the water source (Paper I, Paper II).

1.2. Summary of attached papers

Two papers are attached to this thesis. Short descriptions of these papers are provided below.

In Paper I the microbial contamination dynamics in a drinking water source Lake Rådasjön in Sweden were investigated using a coupled hydrodynamic and microbiological modelling approach. The spread of faecal contamination from different sources (on-site sewers, emergency sewer overflow and the river Mölndalsån) was simulated in order to examine the risk of water contamination at the intake of the water treatment plant. The fate and transport of the faecal indicators *E. coli* and somatic coliphages in the lake were simulated using MIKE 3 and ECO Lab models under different hydrometeorological conditions (inflow to the lake, wind direction and speed) in the spring season. The microbiological module was calibrated using data from an outdoor microcosm experiment performed to determine the inactivation rates of faecal indicators in lake water.

Paper I illustrated that the coupled hydrodynamic and microbiological modelling approach proved to be useful to investigate the threats for the drinking water supply Lake Rådasjön. The modelling study of the lake showed that the investigated sources of microbial pollution pose threats to the drinking water supply of the cities of Gothenburg and Mölndal. In addition, this study illustrated that the risks for the drinking water supply depend on hydrometeorological conditions: wind has a major impact on the spread of microbial contamination in Lake Rådasjön.

In Paper II we determined the inactivation of human and ruminant-specific faecal *Bacteroidales* genetic markers in lake water in relation to traditional faecal indicators (total coliforms, *E. coli*, intestinal enterococci and somatic coliphages) based on outdoor microcosm trials performed in different seasons to capture seasonal variations. The microcosms were created by inoculating water from a drinking water source (Lake Rådasjön, Sweden) with bovine faecal slurry and untreated wastewater. Inactivation data were used to calibrate a microbiological model (ECO Lab) that describes inactivation as a function of temperature and sunlight. The microbiological model was coupled with a three-dimensional hydrodynamic model (MIKE 3) to simulate the transport of *Bacteroidales* markers within Lake Rådasjön.

The modelling results in Paper II indicated that *Bacteroidales* markers discharged from human (on-site sewers, emergency discharge) and ruminant (cattle grazing area) faecal sources can reach the raw water intakes in the lake. Paper II is the first report where source-specific transport of *Bacteroidales* markers to the raw water intakes in a drinking water source is described using a coupled hydrodynamic and microbiological modelling approach that takes inactivation processes into account. This novel modelling approach improves the use of *Bacteroidales* genetic markers in tracking contamination sources, especially when several sources of faecal contamination from the same host are present in the catchment.

2. Background

In this chapter the problem of faecal contamination of drinking water sources is presented. This chapter also describes the drinking water supply system, microbial pathogens in drinking water systems and indicators of faecal contamination. Examples of several coupled hydrodynamic and microbiological models and their applications are also provided.

2.1. Problem description using the DPSIR approach

The DPSIR (*driving forces, pressures, state, impact, responses*) framework (EEA 1995) has been developed to describe environmental problems from the system analysis point of view: the *driving forces*, such as socio-economic development, exert *pressures* on the environment and the *state* of the environment changes as a consequence of these *pressures*. This leads to an *impact* on the human health and the ecosystems. Finally, the societal *responses* on *driving forces*, *pressures*, *state* or *impact* are needed to mitigate the problem (EEA 2001). Although it has been argued that the DPSIR framework is biased (Svarstad et al. 2008), the framework is useful for describing the relationships between the origins and the consequences of environmental problems. The DRSIR framework is commonly used for interdisciplinary indicator development, system conceptualisation as well as structuring research programmes and assessments (e.g. EEA 2005, Larson and Stone-Jovicich 2011, Lundin and Morrison 2002, Walmsley 2002). In this thesis, this framework is used to present the background for the problem of waterborne disease outbreaks caused by the faecal contamination of surface water sources.

2.1.1. Driving forces

The *driving forces* behind the waterborne disease outbreaks are the activities in the catchment area that can potentially cause the release of microbial contamination into the water body that is used for drinking water supply. These activities can be referred to as sources of microbial or faecal contamination. Sources of faecal contamination can generally be divided into two groups: point and nonpoint (or diffuse) sources. Point sources have a definite position but no extension in space. On the contrary, for nonpoint sources the discharge of pollution occurs due to the runoff from larger land areas. The discharge of pollution from point sources can be prevented by end-of-the-pipe solutions, while in the case of diffuse sources, land management practices are required. The review of several cases of waterborne disease outbreaks in the US shows that wastewater from point sources was implicated as the source of contamination for roughly half of the cases, while nonpoint sources, such as agricultural runoff, were suspected sources of contamination in the remaining outbreaks (Solo-Gabriele and Neumeister 1996).

Several point sources of microbial contamination can be present in the catchment, such as combined sewer overflows (CSOs), wastewater treatment plants (WWTPs) and on-site sewer systems (Arnone and Walling 2007, Rose et al. 2001). CSO is a structure that is designed to allow emergency discharges to the receiving water body from the pipe system that carries both storm and wastewater. CSOs prevent overload and flooding of the pipe system and of the WWTP during periods of heavy rainfall. During CSO events untreated wastewater is discharged into the water source, which permits the spread of microbial contamination (Patz et al. 2008). The WWTP is another source of faecal contamination, since not all of the pathogens from the wastewater are removed at the WWTP (Caccio et al. 2003, Da Silva et al.

2007). Treated wastewater from the WWTP that often contains pathogens is discharged into the receiving water body. On-site sewer systems are used to treat wastewater from households not connected to the WWTP; wastewater from on-site sewers is discharged into the receiving water body. Since the treatment provided by the on-site sewer systems has often limited efficiency, pathogens can enter the water source (Lipp et al. 2001). Microbiological pollution from point sources is mainly associated with wastewater.

The major nonpoint sources of microbial contamination are agriculture and farming in the catchment area (Jamieson et al. 2004). Other potential nonpoint sources are wildlife and the runoff from urban areas (Simpson et al. 2002). Agricultural areas can pose risks to water quality, since manure that is applied as fertilizer can contain pathogens. During rain events the manure is washed off the agricultural areas and transported by the surface runoff into the water body. In the same manner, faecal matter and pathogens from livestock can be transported by surface runoff. Excreta from wild animals and birds, as well as from cats and dogs in urban areas, can also reach the water body and contribute to faecal contamination.

2.1.2. Pressures on the environment

The *pressure* behind the problem of waterborne disease outbreaks is the release of pathogens into the water source, i.e. the fact that microbial contamination from the catchment enters the lake or river. The release of microbial contamination can be continuous, like for instance the discharges of treated wastewater from WWTPs and on-site sewer systems into the receiving water body. The load of pollution from these sources is relatively constant over time. On the other hand, the release of pathogens into the water body from sources such as agricultural areas, farming and other diffuse sources, as well as from CSOs, varies strongly over time. This is due to the fact that the release from these sources is caused by surface runoff, which is dependent on precipitation (Signor et al. 2005, Signor et al. 2007). Runoff caused by heavy rainfall or snow melt often transports faecal contamination from the catchment into the water body (Auld et al. 2004, Curriero et al. 2001).

2.1.3. State of the water source

Due to the release of microbial contamination, the *state* of the water source changes, i.e. the concentration of pathogens in the raw water increases. In other words, the concentration of pathogens in the raw water used for the production of drinking water may be higher than the design concentrations of the treatment plant. This ultimately leads to insufficient treatment that does not remove/inactivate all of the pathogens from the raw water. Consequently, pathogens enter the drinking water supply system and reach the consumer. The presence of high concentrations of pathogens in the raw water that is used for drinking water production endangers the quality of the produced drinking water and can lead to a waterborne disease outbreak (Medema et al. 2003).

2.1.4. Impact on human health

Contamination of drinking water with pathogens can have an *impact* on human health, i.e. lead to a waterborne disease outbreak. The disease is "a physiological malfunction: infection, mechanical breakdown, or degeneration resulting in reduced capacity and/or life expectancy" (Twaddle 1996). For an event to be defined as a waterborne disease outbreak, two or more

persons must have experienced a similar illness with water as the probable source of the contamination (Craun et al. 2006, Schuster et al. 2005). Waterborne disease outbreaks cause damage to human health and can even result in death. Waterborne disease outbreaks also cause economic loss to society, since many people simultaneously become sick and cannot work; besides, financial resources are needed for their medical treatment (Kourenti et al. 2007). Furthermore, waterborne disease outbreaks often lead to the loss of consumer trust in water producers (Geldreich 2005).

Many cases of waterborne disease outbreaks are reported yearly in Europe, the United States and Canada (Bartram et al. 2002, Craun et al. 2006, Geldreich 2005). Some examples of waterborne disease outbreaks, in which thousands of people suffered from a disease, are: a massive outbreak that occurred in Milwaukee, Wisconsin, USA in 1993 (Mac Kenzie et al. 1994); an outbreak in Walkerton, Ontario, Canada in 2000 (Hrudey et al. 2003); a recent outbreak in Östersund, Sweden in 2010 (SMI 2010).

2.1.5. Responses
The DPSIR approach gives an overview of the problem and facilitates a choice of appropriate *responses*. The problem of waterborne disease outbreaks caused by faecal contamination of the raw water can be mitigated by the responses directed towards the mentioned categories (*driving forces*, *pressures*, *state*, and *impact*). The best results in terms of raw water safety can be achieved by removing the *driving forces* from the catchment. However, this is often not possible or implies high costs. Alternatively, the release of pathogens into the water source (*pressures*) can be prevented by land management practices that catch the surface runoff from agricultural and farming areas (Dosskey 2001), and technical solutions regarding wastewater treatment in the case of releases from CSOs, WWTPs and on-site sewers. The mitigation of pathogens in the water source lowers the risk for disease outbreaks associated with suboptimal drinking water treatment (WHO 2008b). Furthermore, the presence of pathogens in the drinking water (*state*) can be prevented by more efficient treatment of the raw water at the drinking water treatment plant. Moreover, if the dangerous concentrations in the water source have been detected, the water provider can choose to use a reserve water source. If the measures regarding *driving forces*, *pressures* and *state* have failed, it is of great importance to detect early quality deterioration of the produced drinking water and to ensure awareness of the consumers about the situation. Risk-communication and instructions to consumers to boil their water can facilitate the prevention of a waterborne disease outbreak (Byleveld et al. 2008).

2.2. Drinking water supply system
The drinking water supply system in the developed world usually consists of a water source, a drinking water treatment plant and a piped distribution system (Fig. 1). Raw water is abstracted from a water source, treated at the drinking water treatment plant and then transported in the distribution system to the consumer. Raw water can be abstracted from surface (rivers, streams, lakes) and groundwater (underground aquifers) sources. The selection of the best protected water source is a key step in providing safe drinking water since the most

protected source waters will be the easiest and the cheapest to transform into safe drinking water (Medema et al. 2003).

The treatment of raw water usually consists of a number of microbial barriers that are designed to remove or inactivate microbial pathogens. The number of microbial barriers depends primarily on the quality of the raw water (Stanfield et al. 2003). Processes for the removal of microbes from water include: pre-treatment (any process to modify microbial water quality before, or at the entry to, a treatment plant); coagulation, flocculation and sedimentation/flotation; granular filtration; slow sand filtration (LeChevallier and Au 2004). Disinfection processes used in drinking water treatment to inactivate microbes are: pre-treatment oxidation, in which oxidants are added to water early in the treatment process; primary disinfection, which is important because granular filter media do not remove all microbial pathogens from water; secondary disinfection used to maintain the water quality achieved at the treatment plant throughout the distribution system up to the tap (LeChevallier and Au 2004).

The distribution system must provide a secure barrier to post-treatment contamination of drinking water during its transport to the consumer. Recontamination of microbiologically safe drinking water in the distribution system may occur due to damage of the integrity of the distribution system and the resulting penetration of faecal contamination. Penetration of faecal contamination can occur through: infiltration of contaminated sub-surface water; backflow of contaminated surface water; contamination of open drinking water storage reservoirs; line construction and repair (Robertson et al. 2003). In addition, microbial quality of the water in the distribution system can deteriorate when the bacteria remaining after treatment grow on residual nutrients and form biofilms (Robertson et al. 2003).
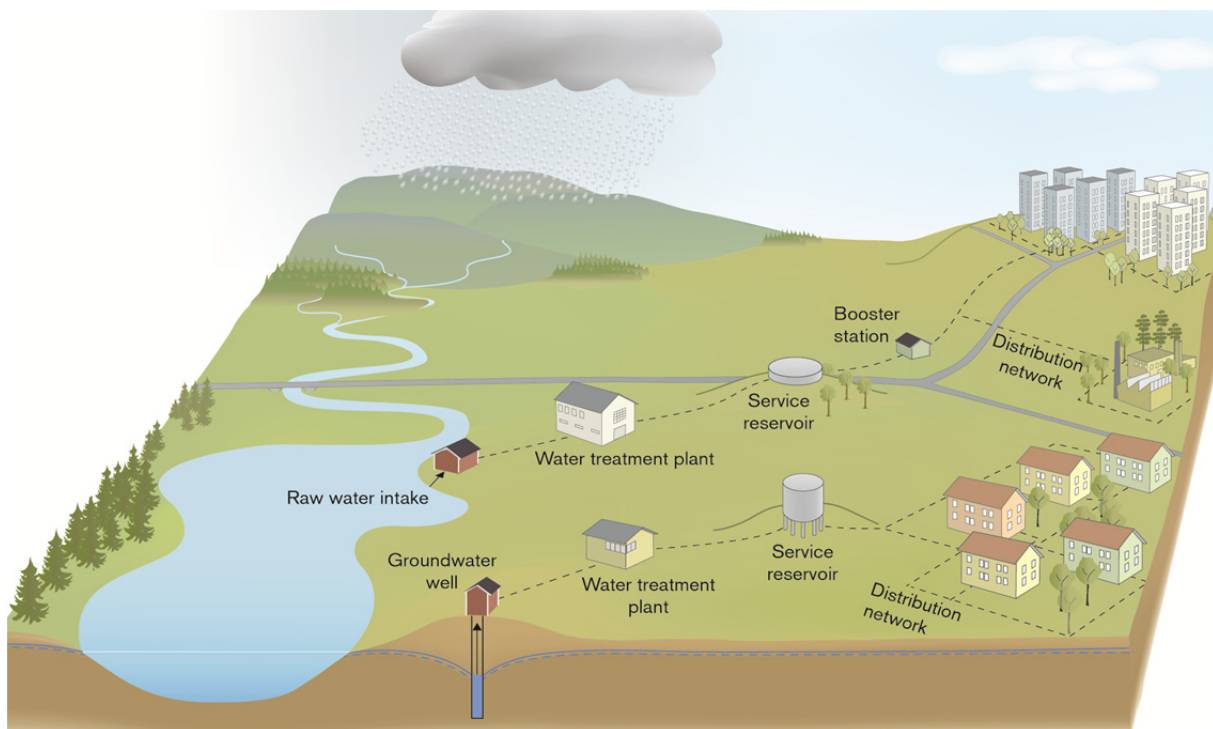

Figure 1 Schematic illustration of drinking water supply system (Lindhe 2010).

2.3. Pathogens and faecal indicators

Potential pathogens in drinking water systems include bacteria, viruses, protozoa and helminths. The effects of these pathogens on human health vary in severity from mild gastroenteritis to severe and sometimes fatal diarrhoea, dysentery, hepatitis and typhoid fever (WHO 2008b). The main known causative agents of waterborne disease outbreaks in the developed world are: the bacterial pathogens *Shigella*, *Legionella*, *E. coli* O157:H7, *Campylobacter*, *Salmonella*; the viral pathogens *Norovirus* (Norwalk-like viruses) and hepatitis A virus; protozoan pathogens *Giardia* and *Cryptosporidium* (Blasi et al. 2008, Craun et al. 2006, Schuster et al. 2005). However, despite thorough investigations the agent is often not identified, even though laboratory analyses are available (Craun et al. 2006). Most of the pathogens that can cause waterborne disease outbreaks are introduced into the drinking water systems by human or animal faeces (WHO 2008b).

Since the testing of water for pathogens is limited due to its complexity and cost, faecal indicators are usually used to detect the presence of faecal contamination in water. The criteria for faecal indicators are that they should not be pathogens themselves and should: be universally present in faeces of humans and animals in large numbers; not multiply in natural waters; persist in water in a similar manner to faecal pathogens; be present in higher numbers than faecal pathogens; respond to treatment processes in a similar way to faecal pathogens; and be readily detected by simple, inexpensive methods (WHO 2008b).

Faecal indicators that are used in drinking water systems include total coliform bacteria, *Escherichia coli* and thermotolerant coliform bacteria, intestinal enterococci, *Clostridium perfringens,* coliphages (somatic and F-RNA) (WHO 2008b). The total coliform group includes both faecal and environmental species as well as organisms that can survive and grow in water. Therefore, they are not useful as an indicator of faecal contamination in natural waters, but they can be used to assess the effectiveness of treatment and the state of distribution systems. *Escherichia coli* and thermotolerant coliforms are a subset of the total coliform group. *E. coli* is considered to be the most suitable indicator of faecal contamination, since it is present in human and animal faeces in high numbers and is rarely found in the absence of faecal pollution. Thermotolerant coliform species other than *E. coli* can include environmental organisms. However, in most circumstances, thermotolerant coliforms are composed predominantly of *E. coli*. Therefore, thermotolerant coliforms are regarded as acceptable but a less reliable indicator of faecal contamination. Intestinal enterococci can also be used for indication of faecal pollution, since they are typically excreted in the faeces of humans and other warm blooded animals and most species do not multiply in water environments. *C. perfringens* has been proposed as an indicator of enteric viruses and protozoa, since it produces spores that are exceptionally resistant in water environments. Coliphages have been reported as a useful indicator to assess the behaviour of enteric viruses in water environments, since they share many properties, such as composition, morphology, structure and mode of replication, with human viruses (WHO 2008b).

While traditional faecal indicators, such as total coliforms, *E. coli* and intestinal enterococci, give a general indication of fresh faecal contamination, there are also faecal indicators that can be used for microbial source tracking, i.e. to indicate the human or ruminant origin of

faecal contamination (Field and Samadpour 2007). For the identification of faecal matter from humans and cattle several human and ruminant-specific *Bacteroidales* assays have been proposed (e.g. Converse et al. 2009, Kildare et al. 2007, Layton et al. 2006, Reischer et al. 2007, Stricker et al. 2008). These assays are based on the detection of *Bacteroidales* 16S rRNA host-specific genetic markers.

As indicated by the criteria for faecal indicators, an understanding of the inactivation/decay processes in the water environment is required for the proper use of faecal indicators, including the *Bacteroidales* markers (Field and Samadpour 2007). The inactivation of traditional faecal indicators has been extensively studied during the past decades (e.g. Crane and Moore 1986, Davies and Evison 1991, Kim and Hur 2010, Noble et al. 2004), while microbial source tracking based on faecal *Bacteroidales* markers is a fairly new approach and relatively few experiments have been carried out to determine their inactivation (e.g. Bell et al. 2009, Dick et al. 2010, Okabe and Shimazu 2007, Walters and Field 2009). The fact that the inactivation of these markers in relation to standard indicators is poorly known is a major limitation in the interpretation of microbial source tracking data from field studies (Field and Samadpour 2007).

The inactivation of *Bacteroidales* markers, as well as of faecal indicators in general, is expected to be site-specific and depends strongly on environmental conditions, such as temperature, salinity, exposure to sunlight, predation, and physical and chemical water properties (Bell et al. 2009, Dick et al. 2010, Okabe and Shimazu 2007). In this study the term inactivation refers to the loss of culturability of faecal indicators or the degradation of their genetic matter depending on the measurement technique.

2.4. Coupled hydrodynamic and microbiological modelling

There are two major types of modelling approaches in the field of water quality: empirical models, which tend to be site-specific, since they rely on the statistical relationship between observed parameters and pathogen concentration; and complex numerical models with high data requirements, that link a hydrodynamic model with a mathematical model describing microbial dynamics, such models are often adaptable for different locations and changing environmental factors (Dyble et al. 2008). Several models of the latter type are discussed below.

One example of a coupled hydrodynamic and water quality model is the ECOMSED-RCA model. ECOMSED (HydroQual 2011a) is a three-dimensional hydrodynamic and sediment transport computer code developed for application to marine and freshwater systems by HydroQual, Inc. The development of ECOMSED has its origin in the mid-1980's with the creation of the Princeton Ocean Model (POM 2011) followed by an upgraded version called ECOM. RCA (HydroQual 2011b) is a three-dimensional generalized water quality modelling computer code developed by HydroQual, Inc. for application to marine and freshwater systems, which can be coupled to ECOMSED, the description can be found in the technical documentation (HydroQual 2011c). The applications of the models POM, ECOM and ECOMSED in combination with different fate models are summarized in Table 1 (numbers 1, 3, 5, 12, 15).

Another three-dimensional coupled fate and transport hydrodynamic model is ELCOM-CAEDYM developed at the Centre for Water Research (CWR) at the University of Western Australia. ELCOM (CWR 2011a) was developed for lakes and reservoirs, and is used to predict the variation of water temperature and salinity in space and time, detailed description of the model can be found in their scientific manual (Hodges and Dallimore 2006). CAEDYM (CWR 2011b) is an aquatic ecological model, which includes a module for the fate and transport of pathogens and microbial indicators (Hipsey et al. 2008). This module is being used in a range of organisations for a variety of applications (Hipsey 2007). The module is based on a generic set of parameterisations that describe most protozoan, bacterial and viral organisms of interest and is validated against observed data from three freshwater systems that differ in their climatic zone and trophic status (Hipsey et al. 2008). The examples of ELCOM-CAEDYM applications for microbial water quality modelling are mentioned in Table 1 (numbers 4 and 13).

Apart from ECOMSED-RCA and ELCOM-CAEDYM many other hydrodynamic models have been created during the last decade to assess the impact of microbial discharges on the water quality of the recipient water body. In Table 1 (numbers 2, 6 – 11) several other applications of coupled hydrodynamic fate and transport modelling approaches to simulate microbial water quality are mentioned.

Table 1 Examples of applications of a coupled hydrodynamic and microbiological modelling approach to simulate microbial water quality.

No	Model	Microbial indicators [a]	Study area	Reference
1	POM & fate	*E. coli*	Southern Lake Michigan beach, US	Thupaki et al. (2010)
2	2D & fate	FC	Malad Creek, Mumbai, India	Vijay et al. (2010)
3	ECOMSED-RCA	*E. coli*	Charles River, US	Hellweger and Masopust (2008)
4	ELCOM-CAEDYM	*Cryptosporidium*, TC, FC, *E. coli*, ENT, F-RNA coliphages, somatic coliphages	Myponga reservoir and Sugarloaf reservoir, Australia; Billings reservoir, Brazil	Hipsey et al. (2008)
5	ECOMSED-RCA & empirical model	*E. coli*	Charles River, USA	Hellweger (2007)
6	2D & fate	*E. coli*, F-RNA coliphages	Cotentin peninsula, France	Riou et al. (2007)
7	3D & fate	FC	Seine estuary, France	Garcia-Armisen et al. (2006)
8	2D & fate	FC, TC	Irvine Bay, UK	Kashefipour et al. (2006)
9	2D & fate	*E. coli*, ENT	Indiana shoreline of Lake Michigan, US	Liu et al. (2006)
10	2D & fate	TC, *E. coli*, ENT	Talbert Marsh, Huntington Beach, US	Sanders et al. (2005)
11	2D, 3D & fate	FC	South Wales coast, UK	Harris et al. (2004)
12	POM & fate	FC, ENT, *E. coli*	South shore of Lake Pontchartrain, US	McCorquodale et al. (2004)
13	ELCOM-CAEDYM	*Cryptosporidium*	Myponga Reservoir, Australia	Brookes et al. (2006) Hipsey et al. (2004)
14	3D & fate	FC	Quincy Bay, US	Li et al. (2003)
15	ECOM & fate	FC, ENT, *C. perfringens*, *Salmonella*, *Cryptosporidium*, *Giardia lamblia*, enterovirus	Island of Oahu, US	Connolly et al. (1999)

[a] abbreviations used in the table: FC – faecal coliforms, TC – total coliforms, ENT – intestinal enterococci

3. Materials and methods

The third chapter includes the description of the study area and of the methods used in this thesis: a microcosm experiment and a coupled hydrodynamic and microbiological model.

3.1. Study area

Lake Rådasjön is located on the west coast of Sweden and constitutes the main water source for the city of Mölndal (60 000 consumers), being a reserve water supply for the city of Gothenburg (500 000 consumers). The surface area of the lake is approximately 2.0 km^2 and the catchment area of the lake is 268 km^2. The maximum water depth in the lake is 23 m and the main inflow is the river Mölndalsån (Fig. 2) with a water flow in the range 1 to 20 m^3/s. The raw water intakes for the city of Mölndal and the city of Gothenburg are located in the northwestern part of the lake (Fig. 2) at 15 m and 8 m depths, respectively.

Lake Rådasjön is potentially subjected to microbial contamination from various faecal sources. The main inflow to the lake, the river Mölndalsån, is the major source of human faecal contamination since it is influenced by emergency discharges from sewer overflows and on-site sewers located upstream. Human faecal contamination in Lake Rådasjön can also originate from on-site sewers located close to the lake. These on-site sewers release partly treated effluents into streams that enter the lake close to the raw water intakes (Fig. 2, sites 3 and 7). Another source of human faecal contamination is an emergency discharge outlet of a pumping station in a separate sewer system (Fig. 2, site P). Discharges of untreated wastewater from this source to the lake occur several times a year during periods of heavy rainfall after intrusion of stormwater into the sewer network. In addition, human faecal contamination can enter the lake with untreated stormwater runoff from an urban area located close to the lake (Fig. 2, site 18). Furthermore, animal faecal matter can be released from a cattle grazing area and can reach the lake after a short transport in a small stream (Fig. 2, site 17).

3.2. Microcosm experiment

In order to simulate the fate and transport of microbial contamination in a water source, data about inactivation of faecal indicators are necessary. Since the inactivation of faecal indicators is expected to be site-specific and depends strongly on environmental conditions (Bell et al. 2009, Dick et al. 2010, Okabe and Shimazu 2007), local data about inactivation are beneficial for the fate and transport modelling of microbial contamination. Therefore, a microcosm experiment (described in Paper II) was performed to study the inactivation of total coliforms, *E. coli*, intestinal enterococci, somatic coliphages and *Bacteroidales* host-specific genetic markers (BacH and BacR) in water from Lake Rådasjön.

The microcosm experiment consisted of three microcosm trials that were conducted outdoors during two-week periods in March, August and November 2010 in order to capture the varying light and temperature conditions during the early spring, summer and winter in Sweden. Two microcosms were set up, one exposed to natural light (light microcosm) and another protected from light (dark microcosm) (Fig. 3).

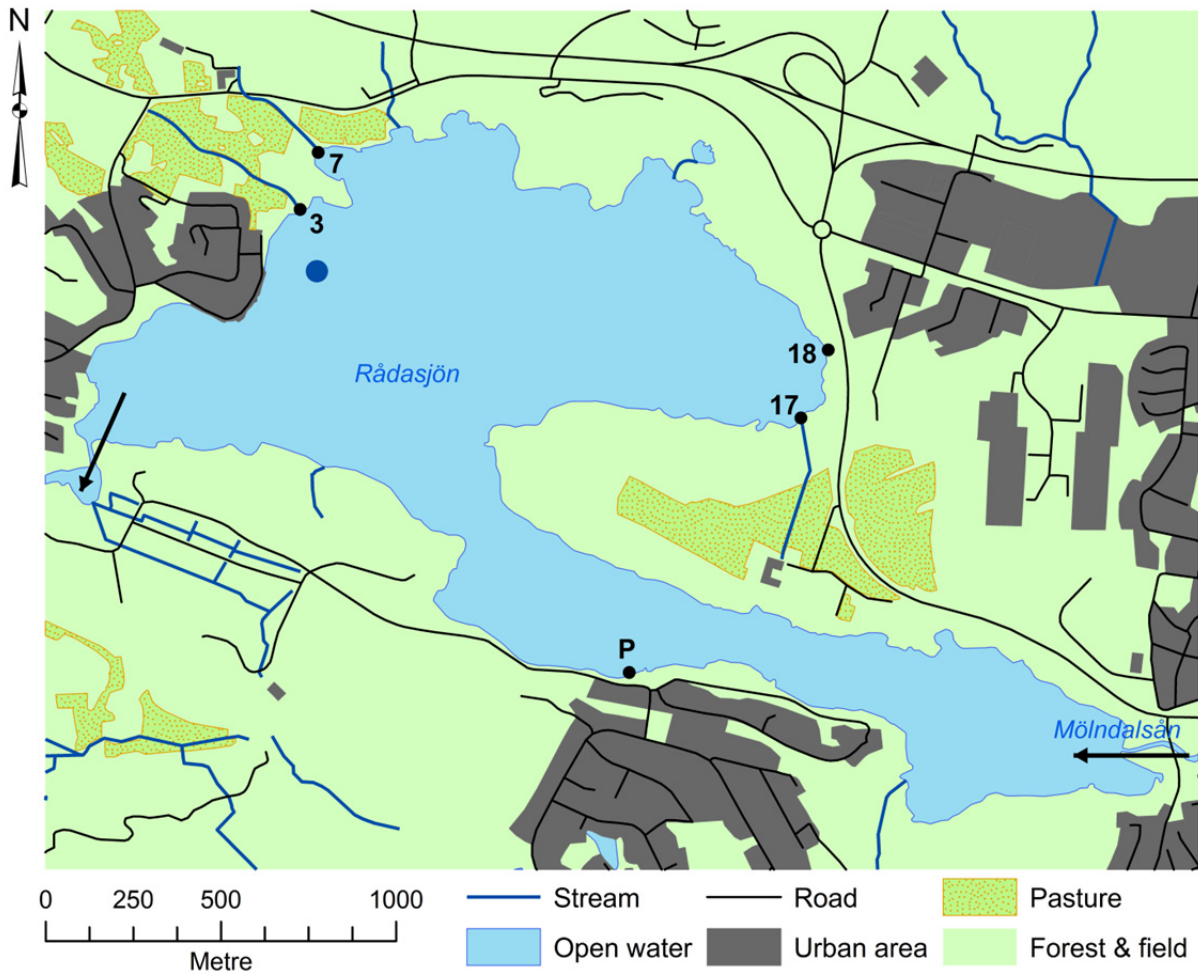*Figure 2 Map of Lake Rådasjön. Numbers show the locations of microbial contamination sources and the blue dot indicates the location of the raw water intakes. Arrows represent the inflow to the lake from the river Mölndalsån and the outflow from the lake to Lake Stensjön.*

Figure 3 Microcosm experiment arrangement, left – microcosms without lids, right – the same microcosms covered with lids.

Microcosms were constructed in aquaria filled with water from Lake Rådasjön and inoculated with untreated wastewater and bovine faecal matter. Water from Lake Rådasjön was collected from a landing stage (20 m distance from the shore) at 1.5 m water depth. Untreated wastewater was collected from a pumping station in a sewer system, from which emergency overflows to the lake can occur. Ten samples of bovine faecal matter were collected from ten animals of different age and sex. Bovine faecal slurry was prepared by mixing 60 g of faecal matter (6 g from every sample) in 300 mL sterile deionised water. Each microcosm was constructed by adding 2.5 L of untreated wastewater and 100 mL of faecal slurry to the aquarium, followed by filling up with lake water (approximately 20 L). The volumes of inocula for the microcosms were chosen to provide high initial concentrations of all indicators (Walters and Field 2009), including somatic coliphages.

The experimental arrangement was placed in the vicinity of Lake Rådasjön, next to one of the drinking water treatment plants of Gothenburg. The experimental site was partly shadowed, but exposed to sunlight during several hours in the middle of the day. The temperature, oxygen content and circulation in the microcosms were regulated.

The three inactivation trials commenced on 15 March, 16 August and 15 November 2010 and lasted 14 days each. One sample was taken from each microcosm at around noon on days 0, 1, 2, 3, 4, 7, 10 and 14. The samples were analysed for total coliforms, *E. coli* bacteria, intestinal enterococci, somatic coliphages, human and ruminant *Bacteroidales* genetic markers.

Furthermore, the water temperature in the microcosms was monitored at 10 minute intervals during each experimental period. Time series of total solar radiation in Gothenburg during each experimental period were obtained from the official environmental measurements performed by the city of Gothenburg.

The detailed description of the microcosm arrangement, performed microbial analyses, as well as data analysis, can be found in Paper II.

3.3.   Coupled MIKE 3 FM and ECO Lab modelling

3.3.1. Hydrodynamic model
To simulate water flows in Lake Rådasjön, a three-dimensional time-dependent hydrodynamic model MIKE 3 FM (Flexible Mesh) developed by DHI (DHI 2009) was used. The MIKE 3 FM model is based on the numerical solution of three-dimensional incompressible Reynolds averaged Navier-Stokes equations using Boussinesq and hydrostatic assumptions. The model consists of continuity, momentum, temperature, salinity and density equations and is closed by a turbulent closure scheme (DHI 2009).

The modelling domain was approximated with prisms (triangles in the horizontal plane) using a flexible mesh approach. The length of the triangles' sides varied from approximately 40 to 80 m, and was adjusted to describe the coastline and bathymetry (Fig. 4). The mesh resolution is finer in the narrow parts of the lake and where large velocity gradients are expected, as well as in the vicinity of the emission points to provide a better description of the contamination

spread. Vertically, the lake was divided into 27 layers. The thickness of the two uppermost layers can vary depending on the water level in the lake (sigma-layers), while the other layers have a fixed thickness (z-layers). In an undisturbed state the thickness of the two uppermost layers is 0.5 m each; these layers are followed by eight layers each of 1 m thickness down to a depth of 9 m. The range of depth from 9 to 16 m, where the thermocline is usually located, is divided into fourteen layers each of 0.5 m thickness and below there are three layers with thicknesses of 1, 2 and 5 m respectively.

The model was initially set up to simulate the hydrodynamic situation in Lake Rådasjön in the year 2008. The simulations of particular scenarios reported in Papers I and II were modifications of the model for the year 2008. The set-up of the model for the year 2008 is described below.

The initial conditions in the lake were defined by the constant surface elevation (0.12 m) and the flow velocity was set to zero. The initial temperature field was described as homogeneous in the domain (2.1 ˚C) based on the assumption that in January the lake is well mixed and horizontal and vertical gradients of temperature at this time of a year can be neglected.

The open boundary conditions (Fig. 4, arrows) were defined using the time-series of data about the discharge in the river Mölndalsån and the water level in Lake Stensjön. The land boundary was defined by zero normal velocity. The temperature on the open boundaries was described as zero gradients.
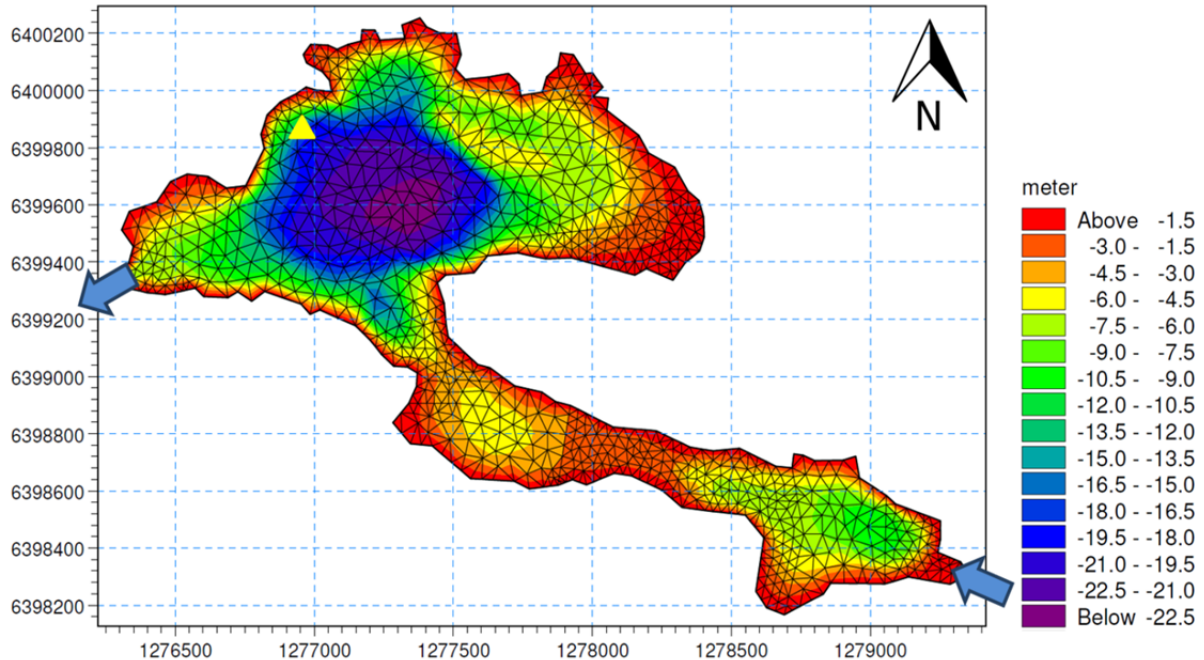

Figure 4 Lake Rådasjön: bathymetry and computational mesh. Arrows represent inflow to and outflow from the lake. The location of the raw water intakes is indicated with a triangle.

The wind forcing was defined using the data regarding wind speed and direction that varied in time and were assumed to be constant in the domain. The precipitation on the lake surface was accounted for in the model; the precipitation varied in time and was assumed to be constant in space. The withdrawal of water from the lake by the drinking water treatment plants was accounted for in the model. The inflow to the lake from small streams was estimated using precipitation data on the catchment area of each stream and surface runoff coefficients, which were assigned based on the rate of exploitation and the slope. For the calculation of the heat exchange between the atmosphere and the lake, data about time variations of air temperature, relative air humidity and clearness coefficient were used in the model.

The water density was formulated as a function of temperature. The horizontal and vertical eddy viscosities were simulated using Smagorinsky and k-epsilon formulations, respectively. The bed resistance was described by constant roughness height of 0.05 m.

3.3.2. Microbiological model

In order to simulate the fate and transport of microbial contamination in Lake Rådasjön, the hydrodynamic model was coupled with a microbiological water quality model ECO Lab (DHI 2009), which is a part of the MIKE software package. ECO Lab uses flow fields from the hydrodynamic model to calculate the concentrations of pathogens or faecal indicators in the lake.

In the ECO Lab model the inactivation of faecal indicators was described according to Eq. 1:

$$\frac{dC}{dt} = -k \cdot C \tag{1}$$

where k is the decay rate of a faecal indicator, t is time and C is a faecal indicator concentration.

The decay coefficient for inactivation of faecal indicators in the lake due to temperature and sunlight was described by Eq. 2 (Mancini 1978):

$$k = k_0 \cdot \theta_I^{Int} \cdot \theta_T^{(Temp-20)} \tag{2}$$

where k_0 (1/day) is the decay rate at 20 ˚C for a salinity of 0 ‰ and darkness; θ_I is the light coefficient; Int (kW/m^2) is the light intensity integrated over depth; θ_T is the temperature coefficient; $Temp$ (˚C) is the water temperature.

The light and temperature coefficients in Eq. 2 for different faecal indicators were estimated using the data obtained during the outdoor microcosm trials performed in different seasons (Paper II).

To evaluate the contribution of each contamination source to the total concentrations of faecal indicators at the raw water intakes, the faecal indicators released from different sources were simulated as different variables.

3.3.3. Input and validation data

To simulate the spread of faecal contamination in Lake Rådasjön during the year 2008 and to compare the modelling results with the observed concentrations the data described in Table 2 were used.

Table 2 Input and validation data used for modelling of microbial water quality in Lake Rådasjön in the year 2008.

Type of data	Source
E. coli concentrations in the discharges from contamination sources [a]	Gothenburg Water
E. coli concentrations at the 8 m raw water intake [b]	Gothenburg Water
E. coli concentrations at the 15 m raw water intake [b]	City of Mölndal
Discharge in the river Mölndalsån	City of Mölndal
Water level in Lake Stensjön	City of Mölndal
Wind speed and direction	SMHI [c]
Precipitation	Härryda municipality
Withdrawal of water by treatment plants	Gothenburg Water
Air temperature	SMHI [c]
Relative air humidity	SMHI [c]
Clearness coefficient	SMHI [c]

[a] Median values of observed concentrations during the period 2008 – 2011
[b] Concentrations observed during the year 2008
[c] Swedish Meteorological and Hydrological Institute

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4. Results

In this chapter the results regarding the inactivation of faecal indicators and the spread of faecal contamination in Lake Rådasjön are summarized based on the results described in the attached papers.

4.1. Inactivation of faecal indicators

The persistence of faecal indicators was described by the T_{90}-values (time for a 90 % reduction) calculated using Eq. 3:

$$T_{90} = \frac{\ln(10)}{k} \tag{3}$$

where k is the decay rate of a faecal indicator.

The results of the microcosm experiment indicated that, in general, the highest persistence was observed for total coliforms, followed by somatic coliphages, BacH and BacR, intestinal enterococci and *E. coli* (Paper II: Table 2). The inactivation of BacH and BacR in the microcosms was approximately in the same range as the inactivation of the traditional faecal indicators. (Paper II: Table 2, Fig. 2). The highest initial concentrations in the microcosms were observed for BacH and BacR, followed by total coliforms, *E. coli*, intestinal enterococci and somatic coliphages (Paper II: Table 2). The initial concentrations of BacH and BacR exceeded the initial concentrations of the other faecal indicators by at least 3 $\log_{10}$-units, this supports the use of BacH and BacR markers for tracking the sources of highly diluted faecal matter in water bodies.

The effect of temperature on the persistence of faecal indicators was clearly observed under dark conditions: the persistence of all indicators, with exception for somatic coliphages (the persistence of somatic coliphages was the lowest in November trial most probably due to a very low initial concentration), was the lowest in the August trial, when the highest water temperature was registered. A relation between high temperature and high decay rates is commonly found for faecal indicators and pathogens (Crane and Moore 1986, Mancini 1978). In a study on faecal indicators and *Cryptosporidium parvum*, Medema et al. (1997) reported a 1.7 to 3 times more rapid decay in river water microcosms at 15 °C than at 5 °C for all organisms. Earlier studies on *Bacteroidales* inactivation in water confirm the temperature dependency observed in the present study (Bell et al. 2009, Dick et al. 2010, Okabe and Shimazu 2007).

The persistence of faecal indicators was higher in dark conditions than under sunlight exposure in 13 out of 17 cases. This is in agreement with a microcosm study on river water, where exposure to artificial sunlight resulted in a more rapid decay of human associated *Bacteroidales* markers (i.e. HF183, BacHum) relative to cultivable *E. coli* (Dick et al. 2010). Sunlight effects are likely to be system or even site-specific, as illustrated in a previous field study, where no reduction of human-specific *Bacteroidales* markers (BacHum) was registered in marine water during exposure to sunlight, while decreasing concentrations of *E. coli*, intestinal enterococci, and somatic coliphages were observed (Boehm et al. 2009).

Moreover, microcosm trials demonstrated that the inactivation of BacH and BacR genetic markers during different seasons under different light and temperature conditions resembles the inactivation of traditional faecal indicators and corresponds with the inactivation of bacterial and viral pathogens reported in the literature (Paper II: Discussion section).

Finally, the inactivation of faecal indicators was successfully described as a function of temperature and sunlight using the inactivation data obtained from the microcosm experiment. The coefficients in Eq. 2 were estimated for each faecal indicator (Paper II: Table 3 and S1).

4.2.   Spread of microbial contamination in Lake Rådasjön

In Paper I the fate and transport of *E. coli* in Lake Rådasjön under spring conditions were described. The risks for the drinking water supply were evaluated based on the concentration of *E. coli* (MPN/100mL) in the raw water at the intake to the drinking water treatment plant using the following classification (Bergstedt 2010): 0 – low, 1-10 – moderate, 11-100 – medium, 101-500 – high, >500 – very high risks. The modelling study showed that the investigated sources of microbial contamination, i.e. the river Mölndalsån, emergency sewer overflow (Fig. 2, site P) and on-site sewers (Fig. 2, site 3), can pose threats to the drinking water supply of the cities of Gothenburg and Mölndal. The tested scenarios illustrated that the risks for the drinking water supply depend on the hydrometeorological conditions and vary from medium to high in the case of faecal contamination from the river Mölndalsån, from moderate to medium in the case of the emergency sewer overflow, and from moderate to high in the case of discharges from the on-site sewers (Paper I: Fig. 3).

In Paper I it was shown that wind is a major factor that determines the water circulation and the spread of microbial contamination in Lake Rådasjön. In the absence of wind the transport of contamination is very slow, which ensures that the concentration of microorganisms decreases significantly due to inactivation processes before the microbial contamination reaches the raw water intakes. Under the conditions of high wind speed (15 m/s) the contamination is transported faster from the discharge point to the raw water intakes. The contamination is transported either in the surface layer when the wind is blowing in the direction of the flow from the river Mölndalsån (southeast wind, Fig. 5 A and C), or with subsurface compensational flows when the wind is blowing against the flow from Mölndalsån (northwest wind, Fig. 5 B and D). In both cases the contamination reaches the raw water intakes after a shorter period of time under the conditions of high wind speed in comparison with light wind (3 m/s), i.e. fewer microorganisms are inactivated before the contamination reaches the raw water intakes. Therefore, microbial contamination from the emergency overflow and the river Mölndalsån reaches the raw water intakes at higher concentrations under the conditions of high wind speed. However, for the on-site sewers the situation is different due to their close location to the raw water intakes. Under the conditions of high wind speed the surface plume of contaminated water from the on-site sewers is transported away from the raw water intakes before it reaches the deeper layers of the lake where the water intakes are located. Hence, discharges from the on-site sewers pose lower risks under the conditions of high wind speed.

The model also illustrated the influence of the inflow to the lake from the river Mölndalsån on the circulation of the lake and consequently the spread of microbial contamination (Paper I: Table 2). The microbial contamination from Mölndalsån and from the emergency overflow was spread differently depending on the discharge into the lake (Fig. 5). When the wind blows in the direction of the flow from Mölndalsån (southeast wind), the increased discharge of the river causes a faster decrease of the pollutant concentration due to mixing processes (Fig. 5, compare 2 A and C with 1 A and C). However, when the wind blows against the flow from Mölndalsån (northwest wind), compensational subsurface flows are formed. The microbial contamination is transported with these subsurface flows faster than under the conditions of average discharge of Mölndalsån (Fig. 5, compare 2 B and D with 1 B and D), which results in higher concentrations at the raw water intakes.

The simulations of microbial water quality in Lake Rådasjön during the year 2008 (i) illustrated the contribution of different faecal contamination sources to the *E. coli* concentrations at the water intakes (Fig. 6 A, B) and (ii) demonstrated seasonal variations in *E. coli* concentrations at the water intakes (Fig. 6, 7) . According to the modelling results, the main contributors to the concentrations at the water intakes are the river Mölndalsån, the emergency sewer overflow and the on-site sewers (site 7) (Fig. 6). The highest concentrations at the water intakes occur during the spring period followed by the autumn period (Fig. 6, 7). This is partly due to mixing processes in the lake, i.e. the contamination discharged in the surface layer of the lake can be transported to the deeper layers. Moreover, low water temperatures in the spring and autumn periods, as well as low amounts of solar radiation in the autumn, result in a slower inactivation of *E. coli*. Modelling results indicated that the lowest concentrations at the water intakes occur during the summer (Fig. 6, 7). This can be explained by increased water temperature and sunlight during the summer as well as strong temperature (density) stratification of the lake. The formation of the thermocline (pycnocline) prevents the spread of faecal contamination from the surface to the deeper layers of the lake. The simulations demonstrated that at the water intake located below the thermocline (at 15 m depth) no influence of contamination sources can be seen during the summer months (Fig. 6 B), while at the raw water intake located above the thermocline (at 8 m depth) the influence of several contamination sources can be seen (Fig. 6 A).

The modelling results were compared with the observed *E. coli* concentrations at the water intakes (Fig. 7). This comparison indicated that the modelling results are generally in agreement with the observed data. The model failed to predict the increased concentrations at the 15 m water intake that were observed during the autumn period (Fig. 7). The reason is probably that as input data for the simulations the median values of the observed *E. coli* concentrations were used. These median values were used since the number of performed measurements was not sufficient for constructing the time-series of data. However, the median values cannot represent the temporal variability of the microbial concentrations in the discharges from the contamination sources.

In Paper II the fate and transport of human (BacH) and ruminant (BacR) specific *Bacteroidales* genetic markers within Lake Rådasjön were described. Fate and transport modelling of *Bacteroidales* markers can provide information about the contribution of

different sources to the total *Bacteroidales* concentration at the raw water intake. This type of modelling improves the use of *Bacteroidales* markers in microbial source tracking, especially when several sources of faecal matter from the same host are present in the catchment.

The spread of BacH and BacR markers in the lake was simulated for conditions of early spring (March), summer (August) and winter (November) and for southeast and southwest winds. The modelling results indicated that the *Bacteroidales* markers released from the faecal sources around the lake, i.e. cattle grazing area (Fig. 2, site 17), on-site sewers (Fig. 2, sites 3 and 7) and emergency sewer overflow (Fig. 2, site P), can be expected to reach the raw water intakes at varying concentrations depending on the season and wind (Paper II: Fig. 3). The simulated scenarios indicated that the emergency overflow provided the largest contribution to the *Bacteroidales* concentrations at the raw water intakes, followed by on-site sewers (site 3 and site 7 respectively) and the cattle grazing area.

For all sources, the highest concentrations at the raw water intakes were found in the simulations of March conditions (Paper II: Fig. 3). The lowest levels of genetic markers at the raw water intakes were found in the simulations of August conditions (Paper II: Fig. 3), which can be explained by the higher water temperature and solar radiation and, therefore, a relatively rapid inactivation. Furthermore, the temperature stratification of the lake in August prevents the transport of genetic markers to the deeper levels of the lake and protects the raw water intakes (Fig. 8). In general, southeast winds caused higher concentrations at the raw water intakes than southwest winds (Fig. 8; Paper II: Fig. 3).
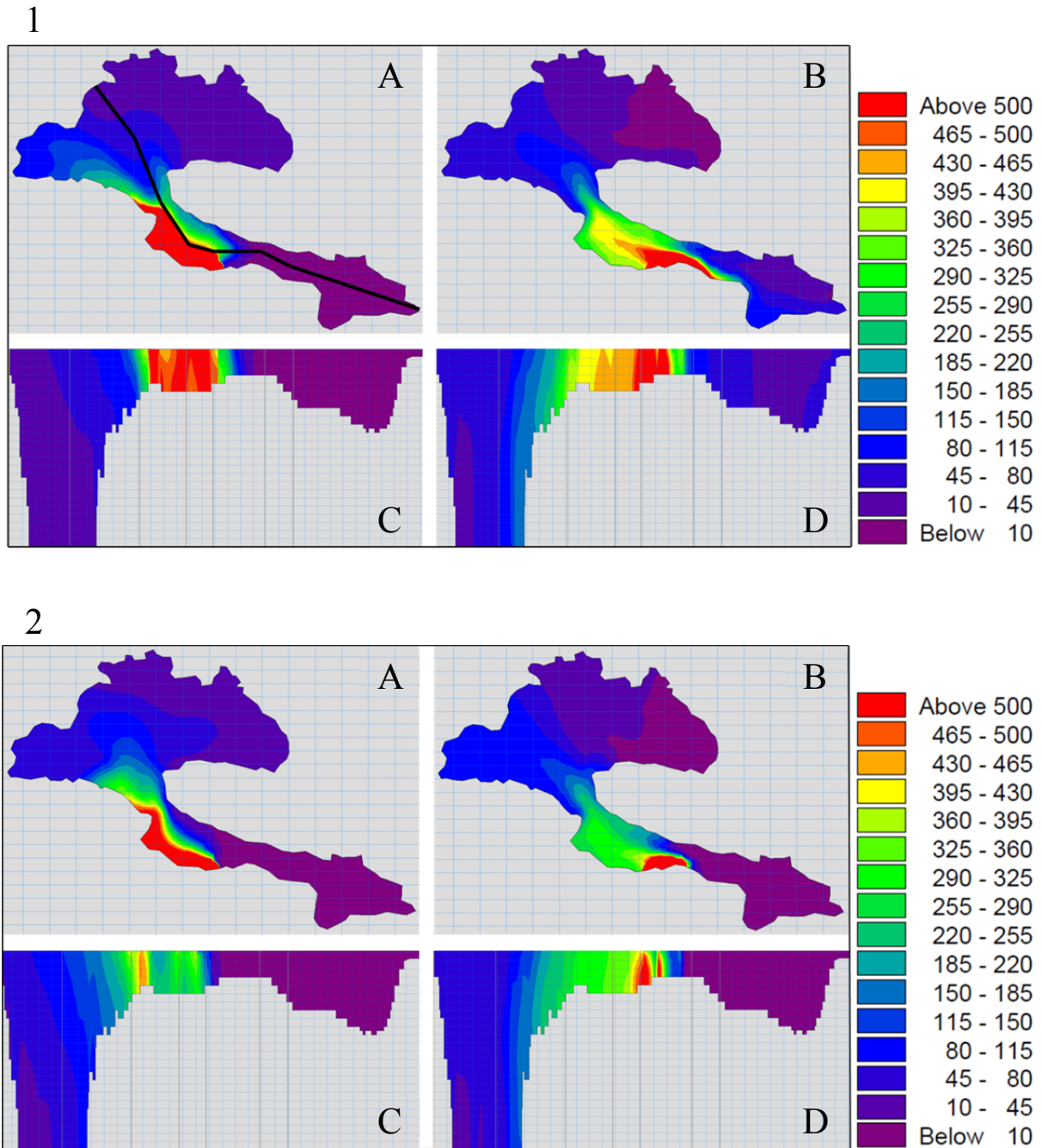
Figure 5 Spread of microbial contamination from the emergency overflow in spring (March) under conditions of (1) average (10 m^3/s) and (2) high (20 m^3/s) inflow from the river Mölndalsån and strong (15 m/s) southeast (A, C) and northwest (B, D) winds. A and B show the surface layer of the lake; C and D represent cross section of the lake as shown on 1 A. Colours represent the concentration of E. coli bacteria (MPN/100 mL).
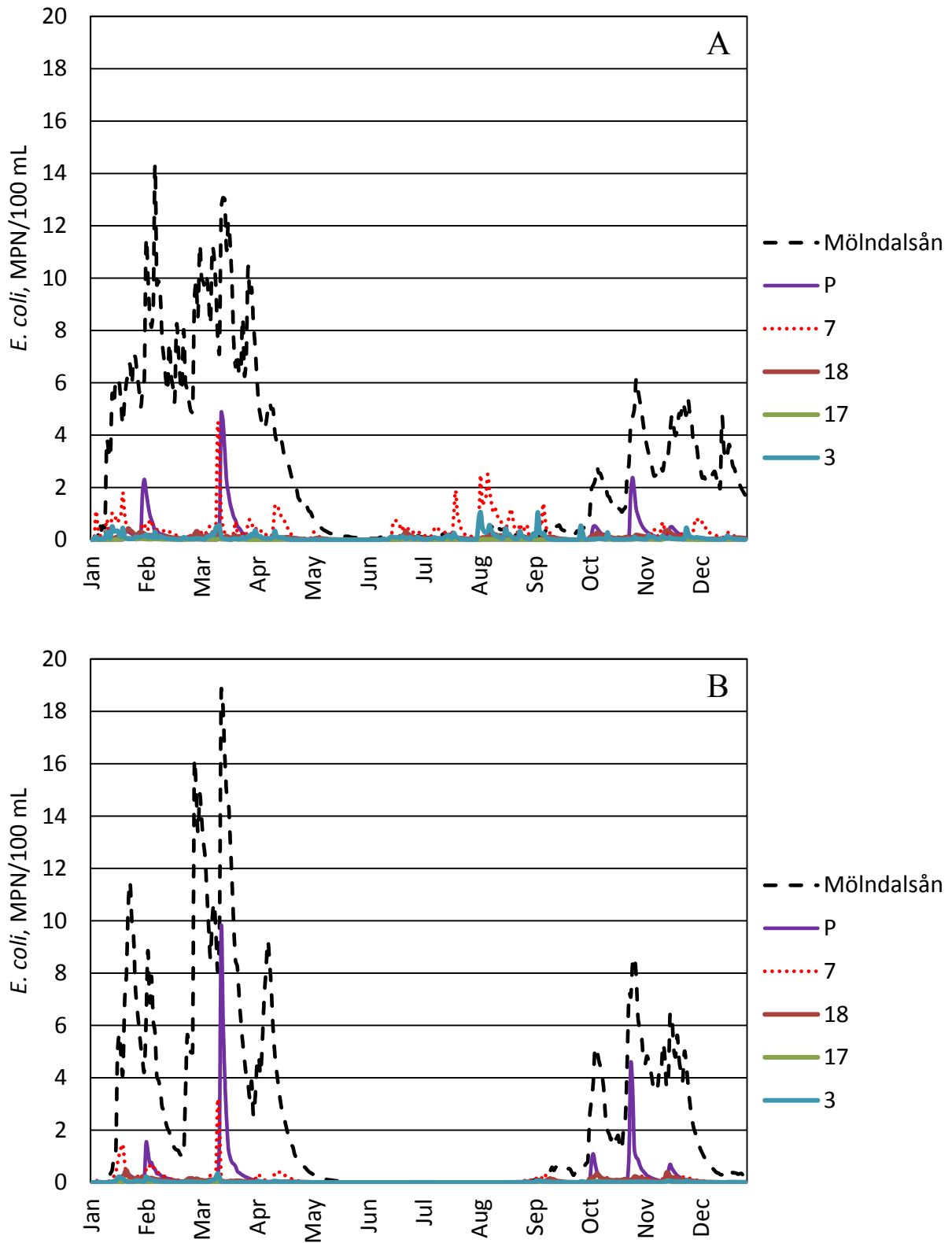
Figure 6 Contribution of different faecal contamination sources to the E. coli concentration at the raw water intakes located at 8 m (A) and 15 m (B) depths in the year 2008.
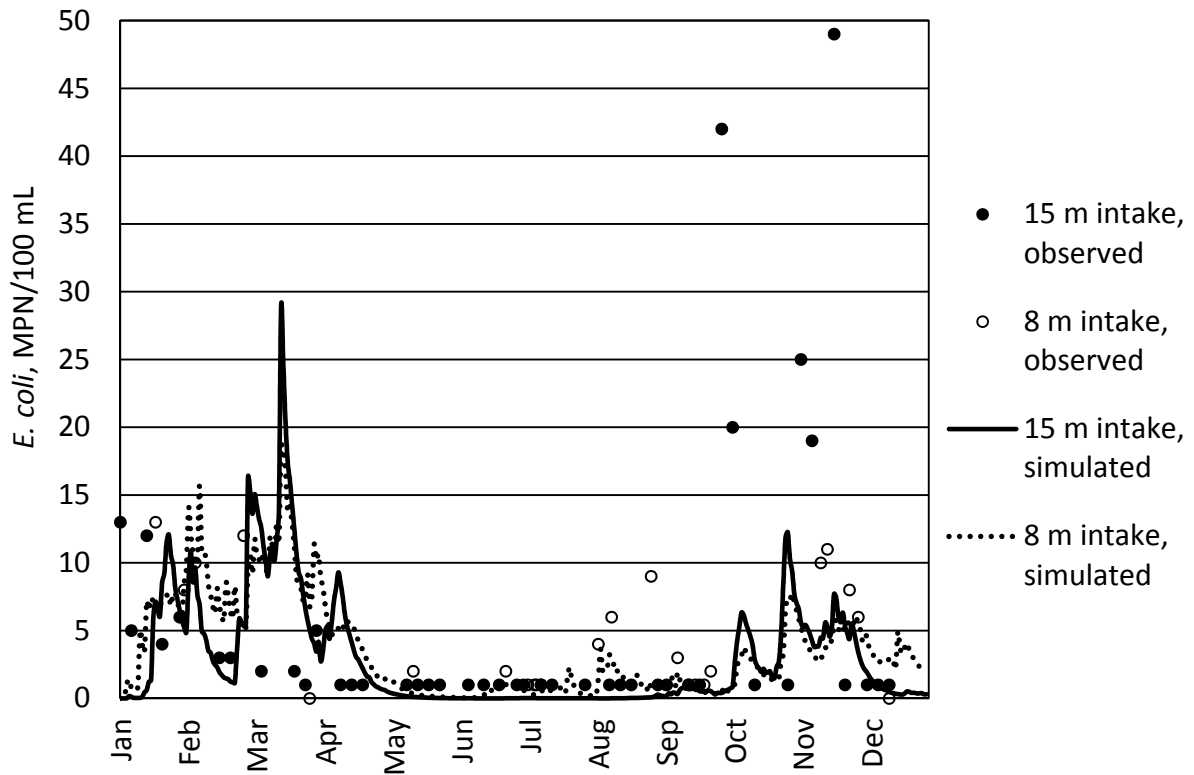
Figure 7 Comparison of modelling results with observed E. coli concentrations at the raw water intakes located at 8 m and 15 m depths.
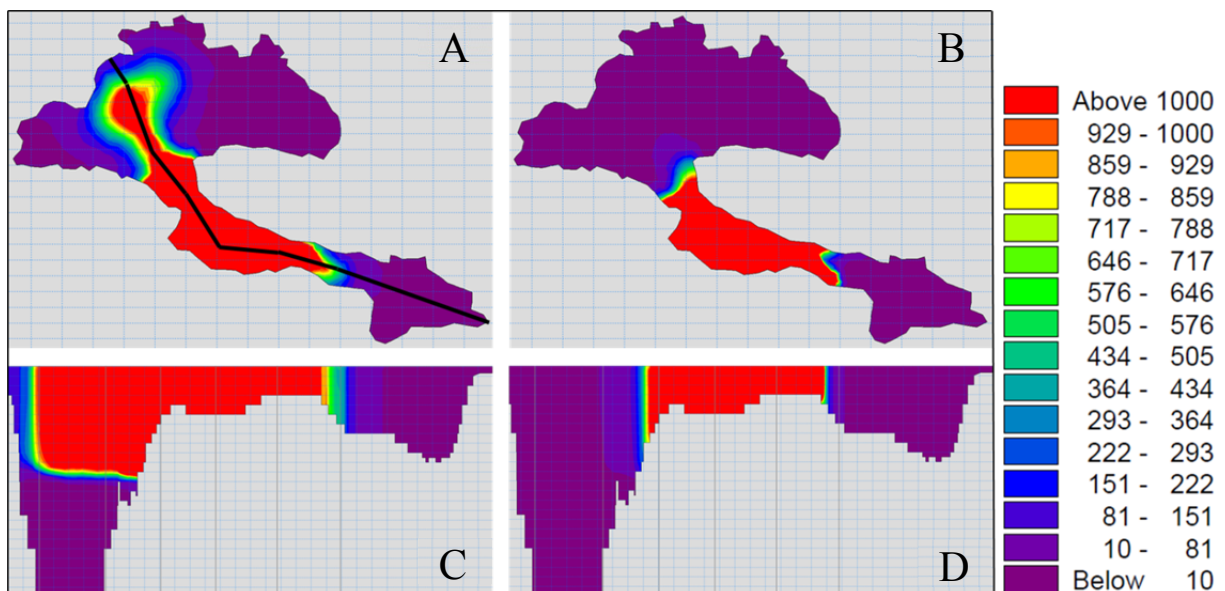
Figure 8 Spread of microbial contamination from the emergency overflow in August under conditions of summer stratification and southeast (A, C) and southwest (B, D) winds of 3 m/s. A and B represent plan view of surface layer, C and D represent cross section of the lake as shown on A. Colours represent concentration of BacH markers (ME/100 mL).

5. Discussion

In this chapter the main results of the thesis are discussed in relation to the aim and objectives of the research, the limitations and uncertainties involved in the presented modelling approach and the possible implementation of the model.

5.1. Fulfilment of the aim and objectives

The aim of this research was to describe the spread of faecal contamination in the water source using a coupled hydrodynamic and microbiological modelling approach. This aim was fulfilled by addressing the specific objectives listed in section 1.1. The inactivation of traditional faecal indicators and *Bacteroidales* genetic markers in Lake Rådasjön was experimentally determined in a microcosm experiment. The obtained inactivation data were then used to calibrate the microbiological model that describes the inactivation of faecal indicators as a function of temperature and sunlight, i.e. the indicator-specific coefficients for this model were estimated. The microbiological model was then coupled with a hydrodynamic model of Lake Rådasjön and applied to simulate the spread of faecal contamination in the lake using *E. coli* bacteria and *Bacteroidales* genetic markers.

Since the inactivation of faecal indicators resembles the inactivation of pathogens, the calibrated microbiological model can be used to simulate the fate and transport of pathogens in a water source. Somatic coliphages and *E. coli* have been reported as useful indicators to assess the behaviour of enteric viruses and bacterial pathogens in water environments, respectively (WHO 2008b). Therefore, the inactivation of viral and bacterial pathogens in Lake Rådasjön can be described using the estimated coefficients for somatic coliphages and *E. coli*, respectively.

Moreover, the results of the microcosm experiment, as well as the fate and transport modelling, help to expand the knowledge about *Bacteroidales* genetic markers and their use in microbial source tracking. The detection of *Bacteroidales* markers in a water source can indicate the human or ruminant origin of faecal contamination, but cannot provide information about the contribution of a specific contamination source, since several sources of faecal matter from the same host can be present in a catchment. However, the source-specific transport of *Bacteroidales* markers within a water source can be simulated using coupled hydrodynamic and microbiological modelling. Therefore, this modelling approach improves the usefulness of *Bacteroidales* genetic markers in tracking contamination sources, especially when several sources of faecal contamination from the same host are present in the catchment.

5.2. Limitations and uncertainties

Although the coupled hydrodynamic and microbiological modelling reported in this thesis proved to be useful to describe the spread of faecal contamination in a water source, the limitations and uncertainties need to be acknowledged.

In the present study the risks for the drinking water supply were evaluated based on the concentration of faecal indicators in the raw water at the intake to the drinking water treatment plant. The detection of faecal indicators can provide information about the presence of faecal contamination in the water source. However, the use of faecal indicators for

quantitative microbial risk assessment is limited by their different fate and transport characteristics relative to pathogens (Brookes et al. 2005).

Another major limitation of this modelling approach is the lack of input and validation data. This type of modelling requires time series of data about microbial concentrations in the contamination sources as input data for the model. Furthermore, time series of data about the microbial concentrations at some points in the water source are needed for the validation of the model. These data are often difficult to obtain due to reasons such as the lack of monitoring and/or knowledge about contamination sources. In addition, microbial concentrations are highly variable in time and difficult to monitor. Moreover, concentrations of microbial pathogens are rarely measured in water sources due to the complexity and cost of these analyses (Brookes et al. 2005), and furthermore, microbial analyses involve measurement uncertainty (Köster et al. 2003).

Sedimentation kinetics of pathogens and faecal indicators can influence the transport of microbial contamination in a water source. The sedimentation of pathogens and indicators is determined by settling velocity, which is governed by the ability of microorganisms to attach to particles and form aggregates (e.g. Ahlbom 2011). The attachment mechanism is complex and depends on factors such as the surface charge of the particle and the pathogen / faecal indicator (Dai and Boll 2003), hydrophobicity (Stenström 1989) and surface texture of a pathogen / faecal indicator, as well as ionic composition of water (LaBelle and Gerba 1979). Moreover, pathogens / faecal indicators may be incorporated within an agglomerate of faecal or effluent organic matter. Since no experiments have been performed to study the attachment to particles and settling of pathogens / faecal indicators in the conditions of Lake Rådasjön, the settling velocities are uncertain. In the model used in this study the sedimentation of faecal indicators in the lake was not accounted for, which might cause overestimation of the concentration at the water intakes.

5.3. Implementation of the model

The modelling approach presented here can be used to provide data for decision-making regarding risk reduction measures. Coupled hydrodynamic and microbiological modelling can be used in the DPSIR framework to describe the *state* of a water source, i.e. to estimate the concentrations of faecal indicators / pathogens at the water intakes, to evaluate variations in microbial load during a year and to identify the periods of high risks. This information is necessary for the proper choice of sampling locations and frequency during monitoring programs. This modelling approach can also be used to test the efficiency of different mitigation measures and to facilitate the choice of proper *responses* directed towards the *driving forces* in the catchment and the *pressures* on the water sources. Moreover, the presented model can be used for the investigation of climate change effects, such as an increased surface runoff, as well as the higher frequency of sewer overflows (Patz et al. 2008, Rose et al. 2001), on microbial water quality.

The coupled hydrodynamic and microbiological modelling can be used to estimate the concentration of pathogens at the raw water intake. These data are essential for quantitative

microbial risk assessment that is performed to evaluate the effectiveness of microbial barriers at a drinking water treatment plant and to estimate health risks.

The coupled hydrodynamic and microbiological modelling can be combined with hydrological catchment-scale modelling to address the limitations regarding the lack of input data. A hydrological catchment-scale model can be used to estimate the surface runoff and the load of faecal indicators or pathogens from the catchment area based on historical precipitation data or forecasts of future precipitation. These results can be used as input to the coupled hydrodynamic and microbiological model in order to simulate past/present hydrometeorological situation or the effects of climate change.

6. Conclusions

In this chapter the conclusions regarding the inactivation of faecal indicators and the coupled hydrodynamic and microbiological modelling are stated. Furthermore, some recommendations concerning the presented modelling approach are provided.

In this study the inactivation of faecal indicators in the water source Lake Rådasjön was experimentally investigated; the outcomes of this study are summarised below.

- The microcosm experiment performed during different seasons can be used to obtain the data about microbial inactivation.
- The inactivation of faecal indicators is dependent on temperature and sunlight.
- The inactivation data obtained from a relevant microcosm experiment can be successfully incorporated into a microbiological model that describes inactivation of faecal indicators / pathogens as a function of temperature and sunlight.
- The inactivation of *Bacteroidales* genetic markers during different seasons under different light and temperature conditions resembles the inactivation of traditional faecal indicators and is in agreement with the reported in the literature inactivation of bacterial and viral pathogens.

The spread of faecal contamination in the water source Lake Rådasjön was simulated using the coupled hydrodynamic and microbiological model, which was calibrated using the experimentally obtained data on faecal indicator inactivation. Conclusions based on the modelling results are stated below.

- This modelling approach proved to be useful to investigate the threats for the drinking water source Lake Rådasjön.
- Faecal contamination from the river Mölndalsån, emergency sewer overflow and on-site sewers can reach the raw water intakes in Lake Rådasjön and pose threats to the drinking water supply of the cities of Gothenburg and Mölndal.
- This modelling approach can be used to describe the transport of the *Bacteroidales* markers, released from various faecal sources at different sites, from the point of the release to the raw water intake of a drinking water treatment plant.
- *Bacteroidales* markers released from the cattle grazing area, on-site sewers and emergency sewer overflow can be expected to reach the raw water intakes in Lake Rådasjön.
- This modelling approach substantially improves the information that can be gained by microbial source tracking with *Bacteroidales* genetic markers.
- Hydrometeorological conditions, such as wind, inflow to the lake and temperature stratification, have a major impact on the spread of microbial contamination in Lake Rådasjön.
- The presented model can be used to provide information for risk reduction management, i.e. to provide data for quantitative microbial risk assessment, to evaluate the efficiency of mitigation measures, to estimate the possible effects of

climate change on the spread of microbial contamination in a water source, to plan water quality monitoring.

The results of this thesis lead to the following recommendations.

- The fate and transport of pathogens in a water source need to be simulated to provide data for quantitative microbial risk assessment and estimations of health risks.
- The uncertainties regarding the input and validation data for the model need to be addressed.
- The sedimentation kinetics of faecal indicators and pathogens need to be accounted for in the model.

7. Areas of further investigation

In this chapter the areas of future research are considered. This chapter also provides a short description of the methodology behind the modelling of pathogen fate and transport in Lake Rådasjön.

7.1. Modelling pathogen fate and transport in Lake Rådasjön

Modelling fate and transport of pathogens in a water source can provide data for quantitative microbial risk assessment and health risk estimations (section 5.3). The methodology behind the modelling of pathogen fate and transport within Lake Rådasjön during a year is described in this section. This work has been started and the first results have been obtained and described in the Rådasjön project report (SVU-project 29-122). However, more investigations will be performed in this area.

In order to obtain input data for pathogen fate and transport modelling, pathogen concentrations in the discharges from faecal contamination sources can be estimated. This estimation was based on the assumption that the ratio of pathogens to faecal indicators in fresh faecal matter is the same as in the microbial discharges to the drinking water source. This estimation can compensate for the lack of measured pathogen concentrations in the discharges from contamination sources.

To estimate the pathogen concentrations in the discharges from faecal contamination sources that enter Lake Rådasjön, the ratio of pathogens to faecal indicators was used according to Eq. 4:

$$\frac{P_d}{I_d} = p \cdot \frac{P_f}{I_f} \Rightarrow P_d = p \cdot \frac{P_f \cdot I_d}{I_f} \tag{4}$$

where P_d is the pathogen concentration in the discharge to the lake; I_d is the faecal indicator concentration in the discharge to the lake; p is the prevalence of a disease; P_f is the pathogen concentration in freshly released faecal matter; I_f is the faecal indicator concentration in freshly released faecal matter. The prevalence of a disease was defined according to Eq. 5:

$$p = \frac{Inc \cdot t}{365} \tag{5}$$

where Inc is the incidence of a disease and t is the duration of excretion (days).

The concentrations of *Norovirus*, *Cryptosporidium* and *E. coli* O157/H7 in the contaminated discharges to the lake were calculated according to Eq. 4 using Monte Carlo simulations and presented as percentiles (5, 50 and 95 %). In the calculations the human and ruminant *Bacteroidales* genetic markers, as well as *E. coli*, were used as faecal indicators. The faecal indicator concentrations in the discharges to the lake were measured through a monitoring campaign performed in 2008 (Åström et al. 2011) and described as lognormal distributions (location, 50, 95 %). The faecal indicator and pathogen concentrations in faecal matter, as well as data on the incidence and duration of excretion, were obtained from the literature and described as probability distributions. The estimation of the pathogen concentrations in the

discharges was performed for endemic and epidemic conditions. For epidemic conditions it was assumed that the prevalence of a disease can be described by a probability distribution of the beta type (min = 1; 5 % = 0.3; 50 % = 0.5; max = 1).

The coupled hydrodynamic and microbiological model (see section 3.3) was used to estimate the concentrations of *Norovirus*, *E. coli* O157/H7 and *Cryptosporidium* at the raw water intakes in Lake Rådasjön under endemic and epidemic conditions. For this purpose the estimated pathogen concentrations in the discharges to the lake were used. For every pathogen the simulations were performed using 5, 50 and 95 percentiles of pathogen concentrations in the discharges to the lake for endemic and epidemic conditions as input data.

It was assumed that the pathogens *Norovirus* and *E. coli* O157/H7 inactivate in the same way as the faecal indicators somatic coliphages and *E. coli*, respectively (section 5.1). Therefore, in the model light and temperature coefficients in Eq. 2 for *Norovirus* were set the same as for somatic coliphages and for *E. coli* O157/H7 the coefficients were set the same as for the *E. coli* indicator bacteria according to the results of the microcosm experiment.

The decay coefficient for temperature inactivation of *Cryptosporidium* in the lake was described by Eq. 6 (Walker Jr and Stedinger 1999):

$$k = 10^{(0.058 \cdot Temp - 2.68)}$$

(6)

The sedimentation of *Cryptosporidium* was simulated according to Eq. 7:

$$\frac{dC}{dt} = -\frac{v_s}{dz} \cdot C$$

(7)

where dz is the thickness of the layer and v_s is the settling velocity. The settling velocity was specified as 0.03 m/day, which is the settling velocity of free oocysts (Medema et al. 1998). It was assumed that *Cryptosporidium* oocysts released into the lake are not attached to particles (Brookes et al. 2006), since no other data was available (section 5.2).

7.2. Fate and transport modelling of microbial water quality in the river Göta älv

Based on the results of the sampling campaign the microbial water quality of the river Göta älv will be simulated using the coupled hydrodynamic and microbiological modelling. The river Göta älv is a main drinking water source for the city of Gothenburg and other upstream municipalities. To monitor the microbial water quality in the river Göta älv and to provide data for the fate and transport modelling of microbial contamination in the river, the sampling campaign has been started in May 2011 within the European Union project VISK (Interreg IV A program). The sampling campaign includes analyses for *Norovirus* and faecal indicators in: raw water from the river, raw water in several tributary flows; untreated wastewater that is received at the wastewater treatment plants located along the river; wastewater effluents discharged from the wastewater treatment plants into the river; water samples collected during CSO events. The sampling campaign will last for at least a year to capture seasonal variations.

The hydrodynamic model of the river Göta älv will be set up and the results of the sampling campaign will be utilised as input and validation data for the modelling of the fate and transport of *Norovirus* and faecal indicators in the river.

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