MASTER THESIS

Recreational water quality in **the** *Breda canals*

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Recreational water quality in the Breda canals

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Summary

The growing attendance at recreational water activities, in addition to an increased frequency and severity of heat waves in cities, gradually gives the urban surface water of the Netherlands, as a water-rich country, a new function in supporting and facilitating recreational water activities. In general, the urban canals in the Netherlands are not designated as official bathing sites for recreation, and their water quality is not monitored routinely. Exposure to microbiological contaminated surface water may pose health risks to humans such as gastroenteritis (GE); fever, eye, ear, and skin complaints; or more serious illnesses, such as hepatitis and meningitis. The municipality of Breda has ambitious plans to ensure the recreational water quality in the Breda canals during the bathing season. Recreational water quality is not a water quality that meets the strict rules for bathing water, but rather a sufficient quality for short-term and low-frequency use. This study was carried out in cooperation with municipality of Breda and Brabantse Delta Waterboard.

The purpose of this study was investigation of microbial water quality of the Breda canals intended for recreational purposes and simulation of water quality in the SOBEK model during the bathing seasons.
The bathing season is defined in the Netherlands as running from May 1 until October 1. In this thesis, the “Beslisnotitie’’ guideline was selected as a framework for the water quality criteria. The criteria in this guideline are defined as 1,800 [CFU/100 ml] and 400 [CFU/ 100 ml], respectively, for *E. coli* and IE.

The first part of this study focused on analysis of microbial water quality of the study area by water sampling.

Two main streams of the Aa of Weerijs and the Mark feed the Breda canals. Five sampling points were selected, with the consideration that the locations should cover whole designated area and provide a sufficient overview of the spatial variation of water quality. The measuring points were as follows: the Mark upstream site (Location 5), and the Weerijs upstream site (Location 4). The Breda Harbour, a popular location for water recreational events (Location 3), a site adjacent to confluence of two streams (Location 2), and a downstream site opposite the Belcrum artificial beach indicated how Breda affects the water quality (Location 1).
The sampling scheme was defined weekly during dry weather, 3rd day after light rainfall without a CSO discharge (day 1 being the day of the rainfall) and 3rd and 5th days after rainfall event with a CSO discharge. The sampling period was spread over 48 weeks from July 25, 2018, to June 13, 2019. Due to insufficiency of the existing data on the water quality in the Breda canals, the sampling campaign of Brabantse Delta Waterboard was also used, in addition to this study’s sampling results, to allow for a comprehensive analysis.
With the assumption of 7 [mm/h] of sewer capacity, 0.7 [m/h] of pump capacity, 5 mm of pavement storage capacity and the filtration of permeable surfaces, as a rough estimation, it was assumed that rainfall events under 12 mm do not lead to a CSO discharge. However, the first results of water sampling after rainfall events indicated that determining of whether a CSO discharge had occurred was complicated because the water level was only measured at detention tanks. To delete the uncertainty, five sensors were installed in October 2018 at five CSOs identified as having a high frequency of discharge in earlier research by ARCADIS in 2015. Since October 2018, the samples were taken when a CSO discharge occurred at one of these five CSOs.

At the end phase of research, DNA concentration of markers for four groups of organisms; humans, ruminants, dogs and birds were monitored by a DNA source trace analysis. The samples were collected from five sampling locations in June 2019 on three occasions: one in dry weather

(without sewer overflows) as reference situation and two after rainfall events with a CSO discharge.

During dry weather, the sampling results of this study and of Brabantse Delta Waterboard indicated low FIB concentrations. In the context of the "Beslisnotitie" norm, the water was safe for recreational activities.
Temporal and spatial variations of *E. coli* were observed in the Breda canals after rainfall events in the winter and summer seasons, indicating that water quality can be affected by rainfall events. The most significant peak of observed EI values was seen after rainfall events of more than 25 mm.
E. coli peaks were detected at Locations 2 and 5 more than at other measuring points. Based on the Waterboard Brabantse Delta results, the Valkenberg Park location can also be listed as critical spot.

Based on available data, it is recommended to Breda municipality to wait two days after light rainfall events of up to 12 mm with low intensity, to give permission for recreational events, for certainty's sake. After moderate rainfalls between 12 mm and 25 mm, it is advised to wait a minimum of two days, depending on an event's location. After highly intense rainfall events of 25 mm or more, it is recommended that events be postponed for a minimum of four days.
The results of the DNA resource trace of the Breda canals suggested that, in the reference situation (dry weather), humans and ruminants have always been a source of contamination. Depending on circumstances and locations, dogs and birds may also act as contaminants. After rainfall events with a CSO discharge, human faecal material was a source of contamination for most locations.

The second part of this thesis involves testing the sewer model of Breda in the context of discharged overflow volumes.

To run the water quality model, overflows volume simulated by the sewer model during the sampling period are used as input; thus, the discrepancy between the simulated and actual overflows volume should be negligible if one wishes to reach a significant correspondence between the *E. coli* concentration simulated by the water quality model and the measured *E. coli* concentration. To make the modification of the sewer model feasible, the discrepancy between the simulated and actual discharged CSO's volume was confined to Factor 2.

Rainfall data associated with sampling dates, per catchment pumping station, were simulated in the InfoWorks model. Five catchment pump stations were selected which, had a significant impact on the water quality of the study area. The behaviour of the sewer system during rainfall events in the context of sewer overflow discharges was clarified by selected pumping stations data analysis.

In contrast to the assumption that there was no CSO discharge for rainfall events of less than 12 mm, on 6-6-2019 (at 9.6 mm cumulative rainfall until moment of CSO discharge), and 10 mm on 2-8-2019 and 12-8-2019 a discharge was detected at CSO I and CSO V, followed by the *E. coli* peak at Location 2. It indicated that the sewer system of Breda, especially near Location 2, can be vulnerable to rainfall events under 12 mm.
The comparison of simulated and measured water levels at pumping stations indicated that, generally, the sewer model is valid, and the simulated water levels at pumping stations corresponded to the measured values. However, the model overestimated the water level due to inaccurate modelling of the pumps' operations and capacities. In practice, the pumps' capacities varied frequently, mainly due to maintenance work, so the operating conditions of available pumps in a catchment pumping station were not clear.

The last part of this study presents the water quality simulation in SOBEK model. Because of insufficient data on rural area, in the model developed for this thesis, the urban and rural runoff were not simulated and only two modules of 1Dflow and 1DWAQ in SOBEK were used.
Water quality analysis of Breda canals indicated that *E. coli* provides more certainty to evaluate the water quality of the Breda canals; thereby, only *E. coli* dynamics was simulated by SOBEK. The *E. coli* source at this model was defined as sewer overflows (CSOs and SSOs) and the initial *E. coli* of upstream flows; the rural runoff and WWTP effluent were neglected. All overflows that had a direct influence on the water quality of upstream and the study area within 1,000 meters distance were simulated in this model. Based on suggested *E. coli* concentration of sewer overflows of various studies, *E. coli* concentration of 1* E10 [CFU/m3] and 1*E8 [CFU/m3] were selected for CSO and SSO, respectively. Due to the absence of sampling points at the Mark and Weerijs streams' boundaries, it was assumed that the initial *E. coli* concentration at the boundaries of both the Mark and Weerijs were one log higher than the measured *E. coli* concentrations at Locations 4 and 5.

To simplify the model and decrease the simulation time, the Brabantse Delta Waterboard flow model was cut off, and five boundaries were defined. To develop an accurate model, downstream boundaries were placed where the discharges of the Mark and Weerijs streams are measured hourly by Brabantse Delta Waterboard. Based on measured temperature of the water samples; 21°C and 5°C in summer and winter period, the mortality rate of 0,8 and 0,14 was applied in the model for summer and winter period, respectively.

To calculate the water fraction, the discharge of boundaries, and calculated discharged sewer overflow volumes by InfoWorks for two periods in the summer from July 10, 2018, to September 30, 2018, and in the winter from January 1, 2019, to February 17, 2019, were imported to SOBEK. A fraction calculation allowed one to label the origin of the water in the water system. The results indicated that the maximum fractional contribution of sewer overflows was 2% and associated with SSOs outside the study area at Location 4 in the summer period. For the rest of locations, the fractional contribution of sewer overflows was less than 1% during the summer and winter. The simulation indicated no fractional contribution of overflows at Location 4 or Location 3 in the winter period. In general, the fractional contribution of sewer overflows in the summer and winter were extremely low. An underestimation of discharged overflow volumes by InfoWorks is a possible explanation for this result.

Furthermore, the calculated *E. coli* concentrations were underestimated in the summer period and overestimated in the winter period. The explanation could be underestimation of discharged sewer overflow volumes, neglecting of urban and rural runoff, higher initial *E. coli* concentrations at the Mark and Weerijs streams' boundaries, and inappropriate decay rate.

The model demonstrated sensitivity to initial *E. coli* concentration and decay rate in the summer and winter, and to *E. coli* concentration of sewer overflows in the winter period. Based on the sensitivity analysis of this model, it can be concluded that there is uncertainty surrounding some essential inputs of the model, including discharged sewer overflow volumes and initial *E. coli* concentration. To develop a more accurate water quality model, the quality of input data should be improved.

The results of this study indicated that the municipality should adopt a strategy to minimise the discrepancy between simulated and actual discharged sewer overflow volumes. In addition, a better rain–runoff model (RR module) for conducting simulations in SOBEK can be developed by the Water board. However, further research is needed to accurately simulate intense rainfall events in the summer and to compare these findings with sampling results.
Taking into account the complicated simulation of pumps' operational systems in InfoWorks and high expenses of sensor installation for collecting reliable data on all sewer overflows, water quality analysis based on collecting more sample data in different rainfall events sounds more feasible.

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List of Abbreviations

Abbreviations	Meaning
Ave.	Average
CFU	Colony forming unit
CSDT	Combined sewer detention tank
CSO	Combined sewer overflow
EMOS	Ensemble Model Output Statistics
EU	European Union
FIB	Fecal indicator bacteria
GGD	Gemeenschappelijke Gezondheidsdienst (Municipal Health Services)
GI	Gastrointestinal
IE	Intestinal Enterococci
KNMI	Koninklijk Nederlands Meteorologisch Instituut (Royal Netherlands Meteorological Institute)
SSO	Storm sewer overflow
SSRC	Spores of sulphite-reducing clostridia
STOWA	Stichting toegepast onderzoek waterbeheer (Applied Research Water Management Foundation
VFD	Variable frequency drive
WWTP	Wastewater treatment plant

1 Introduction

These days, more activities are being organized in and around water, and these are an important part of the leisure and tourism sector, which is among the fastest growing sectors in the economy (WHO, 2003; de Man H. H., 2009). The growing attendance at recreational water activities (WHO, 2003), in addition to an increased frequency and severity of heat waves in cities, gradually gives the urban surface water of the Netherlands, as a water-rich country, a new function in supporting and facilitating recreational water activities.

Today, the number of municipalities motivated to provide recreational water activities in the city is growing. These activities can be placed in official or non-official bathing waters. In practice, in the Netherlands during the summer season, almost all official bathing waters are sampled once every two weeks and controlled for faecal contamination according to European Directive 2006 (The European Parliament, 2006), whereas the water quality in non-official bathing sites is not monitored (RIVM, 2018). In 2017, at least 110 swimming events were planned in the Netherlands, including 70 triathlons. The number of participants varied from 50 to 200 participants at small-scale events to more than 2,500 at large-scale events, such as the Amsterdam City Swim 2017. Half of the City Swim events were organised in non-official bathing waters (Joosten, et al., 2018).

Consequently, the Municipality of Breda also has ambitious plans to ensure the recreational water quality in the Breda canals during the bathing season. Recreational water quality is not a water quality that meets the strict rules for bathing water, but rather a sufficient quality for short-term and low-frequency use.
Figure 1 shows the recreational water activity of "Breda Drijft'' in 2019, which has been one of the most popular events in the Breda Harbour for the last five years.

Figure 1 - "Breda Drijft'' has been one of the most popular events in the harbour of Breda for the last five years.

1.1 Problem statement

In general, the urban canals in the Netherlands are not designated as official bathing sites for recreation, and their water quality is not monitored routinely; therefore, officials have not been able to confirm the presence or absence of increased concentrations of faecal indicator bacteria (FIB).

The faeces of dogs, cats and birds, sewer overflows, illicit connections, wastewater treatment plants discharge, agricultural and urban runoff can be named as possible microbial contamination sources of urban surface water.
However, many years of investment in sewage systems and better wastewater treatment have led to significant improvements in surface water quality in the Netherlands (Goverment of the Netherlands, 2018; RIWA, 2017; European Environment Agency, 2018).
But, studies conducted in the Netherlands indicated that exposure to urban surface water that meets the water quality standards can still involve health risks (Medema, et al., 1998; Schets, et al., 2008; Schets, et al., 2010; Joosten, et al., 2017); moreover, this risk may increase following intense rainfall events. These events affect the concentration of pathogens and faecal indicators through the pollution (including dog faeces and bird droppings) washed into canals from the streets, sewer overflows, and runoff from agricultural land (Schets, et al., 2008; Sales-Ortells & Medema, 2014; de Man, et al., 2014).

Exposure to microbiological contaminated surface water may pose health risks to humans such as gastroenteritis (GE); fever, eye, ear, and skin complaints; or more serious illnesses, such as hepatitis and meningitis (WHO, 2003; Schoen & Ashbolt, 2010; Turgeon, 2012). In the Netherlands, 742 waterborne disease outbreaks correlated with untreated recreational water during the 1991–2001 period were reported, of which 3% were associated with exposure to non-official bathing water (Schets, et al, 2010). In a recent Gemeenschappelijke Gezondheidsdienst-Municipal Health Services (GGD) study on participants at five City Swim events, researchers found an increased risk of gastrointestinal (GI) among swimmers; even without an outbreak, an average of 1:14 swimmers had gastrointestinal complaints after swimming (Joosten, et al., 2018).

The ambition of the Breda Municipality is to meet the standards for recreational water quality by the middle of 2030 in the city canals; however, the water quality may be major obstacle to achieving this goal. No data are available on the current microbial water quality, possible contaminant sources and health risks associated with exposure to the Breda canals. The lack of these data makes the assessment of the (im)possibility of water recreation in the canals infeasible. Identifying either the water quality of city canals or dominant microbiological pollutants will enable authorities to investigate strategies for recreation in the Breda canals.

1.2 Research aim

The purpose of this study is to investigate the microbial water quality of the Breda canals intended for recreational purposes during bathing seasons in cooperation of Municipality of Breda and Brabantse Delta Waterboard.
Each urban body of water has unique characteristics; therefore, a specific analysis should be carried out to identify possible sources and routes that threaten human health. This analysis assists officials determining further preventive measures to limit the health risks associated with exposure to urban water. To evaluate the microbial water quality of the Breda canals and impact of rainfall events on the water quality, this thesis uses the water sampling during dry and wet weather, and a DNA source detection analysis to track the water contamination sources of the Breda canals.

Further, to interpret the water sampling results a water quality model will be built to simulate the Breda canals' water quality. This developed model might enable to predict the *E. coli* concentration of the Breda canals.
A prediction model may facilitate the investigation of water quality since high expenses and the time required for the laboratory analysis of water testing (Reder, et al., 2015) make it difficult for municipalities to monitor the water quality of canals. Hence, a water quality model may enable officials to determine the temporal and spatial variability of FIB concentrations. Furthermore, when the model predicts high FIB levels public warnings can help to prevent exposure to contaminated water.

The base of the water quality model is the hydrological transport model. The contamination loads can be used as inputs in the hydrodynamic model of the waterboard to calculate the associated FIB levels. The developed model performance can be evaluated by a comparison of measurements and model output (model validation).

1.3 Research questions

Based on the purpose of this study, the following research question was formulated:

"*Do Breda canals meet the current water quality standards of recreational activities*?"

To answer this question, 4 sub-questions were formulated:

1. What is the microbiological water quality of the Breda canals?
2. What is the impact of rainfall events on the water quality of the Breda canals?
3. What are the plausible microbial contamination sources in the Breda canals?
4. What is the contribution of contamination sources in the Breda water system?

1.4 Scope of this study

The following section defines the framework applied in this project.

- **Research area**

Since the areas under investigation only include non-official bathing waters, only the water quality of canals located in the centre of Breda with the potential of serving a recreational function are analysed. The designated canals start from where two rivers, the Aa of Weerijs and the Mark, flow in the city centre, and after converging of these two streams, the water continues to the Belcrum artificial beach downstream.
These canals are depicted in Figure 2 with red border lines.

Figure 2 - Designated canals in the context of recreational water activities marked with red border line.

- **Research time period**

It is assumed that water recreational activities period is same as bathing season. The bathing season, a period during which water quality should meet the bathing water guidelines, is defined in the Netherlands as running from May 1 until October 1 (RIVM, 2014; Schets et al., 2010). The period in which bathers are expected at bathing sites depends on weather conditions and local circumstances (European Environment Agency, 2012).
The sampling period was spread over 48 weeks from July 25, 2018 to June 13, 2019.

- **Water quality indicators**

This research focuses solely upon microbial hazards; chemical and biological water quality are beyond the scope of this analysis.
Although three parameters — *E. coli*, intestinal enterococci (IE), and cyanobacteria — should be analysed according to current implementation guidelines, in this research, only *E. coli* and IE as microbial water quality indicators were monitored during sampling campaign.

In this thesis, the general term ''FIB'' (Faecal Indicator Bacteria) applies for faecal indicators of *E. coli* and IE.

- **Water quality criteria**

The guideline, which is advised currently by GGD to the municipality to give permission for recreational activities in the Breda canals, was derived from " Beslisnotitie werkwijze individuele metingen en meetfrequentie microbiologische parameters zwemwaterrichtlijn'' - Decision memorandum individual measurements method and measurement frequency microbiological parameters bathing water directive (GGD, 2018; Rijksoverheid, 2013). In this thesis, this guideline named "Beslisnotitie'' guideline. The criteria in this guideline are defined for *E. coli* and IE, respectively, 1,800 [CFU/100 ml] and 400 [CFU/ 100 ml]. However, this water quality standard have been implemented for official bathing waters and can be overly strict for recreational purposes.
In the early phase of this research, a concept of handbook over recreational water activities in and around water was released by Stichting Toegepast Onderzoek Waterbeheer - Applied Research Water Management Foundation (STOWA) (STOWA, 2018), also same as "Beslisnotitie" guideline.

This study uses the current implemented GGD guideline (''Beslisnotitie'' guideline) to analyse the water quality of Breda canals. However, it should be taken into account that a new guideline may be released after this research (regulated by international or national authorities based on their health risk-management policies).

1.5 Outline

Figure 3 visualizes the outline of this thesis and indicates how the different parts are related. Since this study is comprised of three analyses of water quality, the sewer model, and the water quality model, most chapters consist of three separate parts.

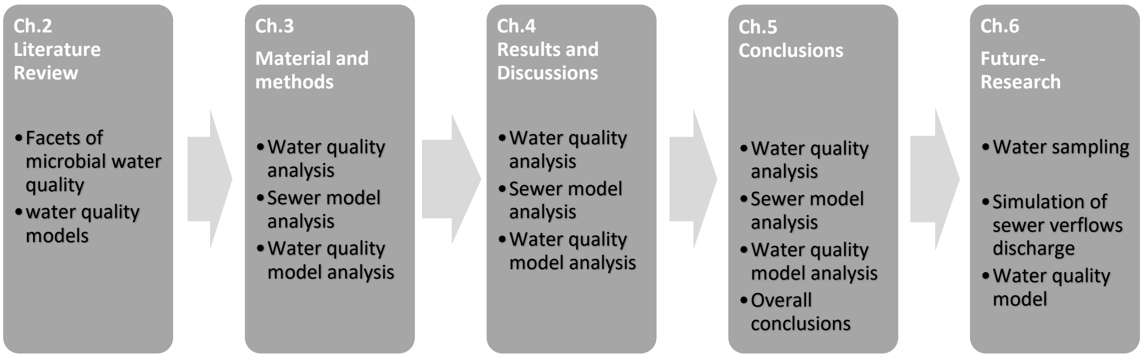

Figure 3 - Thesis outline

Chapter 2 mainly contains a literature review on microbiological water quality aspects, associated guidelines, and water quality models.

The applied methods are presented in Chapter 3. The section on the water quality analysis comprises the water quality monitoring process. In the subsequent section that includes the sewer model analysis, the method of sewer model calibration to confine the discrepancy between simulated and actual overflow volumes to factor 2 is presented. The model analysis consists of a discussion of how the data were processed and how the model was developed and calibrated.

The results and discussion sections are combined into one section, Chapter 4. The results of all analyses are presented separately, and the reliability of the results and assumptions are evaluated.

Chapter 5 presents the conclusions of three sections severally and discusses to what extent the results answer the sub-questions. In addition, an overall conclusion covers the answer to the research question.

Finally, based on the results and conclusions, recommendations for future research are presented in Chapter 6.

2 Literature Review
This chapter provides a review of studies in the literature on recreational water quality aspects and water quality modelling.

2.1 Facets of microbial water quality
To investigate the microbial water quality, various factors should be considered. The following subsections include information about major factors.

2.1.1 Relationship between water quality and diseases
The microbial risk of urban water can originate from sewage system overflows containing human enteric pathogens (Campylobacter, Cryptosporidium, norovirus, rotavirus, etc.), animal faeces containing zoonotic pathogens, or the growth of microorganisms such as toxic cyanobacteria (Sales-Ortells & Medema, 2014; de Man, et al., 2014). The quantity and composition of pathogens present in urban water are variable in time and season, and they can be affected by the health status of the inhabitants of each area and the removal efficiency of the wastewater treatment plants (WWTPs) (de Man, et al., 2014; STOWA, 2018).

Recent studies suggest that the exposure to faecal contaminated surface water can lead to GI symptoms, fever, skin, and ear complaints, among which the most frequent complaints include (GI) and skin conditions (WHO, 2003; Schets, et al., 2008; Schets, et al., 2010; RIVM, 2018). The development of an illness after infection mainly depends on an individual's immunity (ten Veldhuis, et al., 2010). Nevertheless, the detection and attribution of disease due to recreational water contact is difficult because the symptoms are mild, and people may not visit their general practitioners to cure such illness (Schets, et al., 2008; Schets, et al., 2010).

Researchers in the Netherlands have also confirmed the higher rates of GI symptoms developed by swimming in recreational water compared to those among non-swimmers (Medema, et al., 1998; Sales-Ortells & Medema, 2014; Joosten, et al., 2018; Schets, et al., 2010; Joosten, et al., 2017). Figure 4 contains a brief overview of pathogens and index organisms present in recreational water contaminated by raw sewage. Low concentrations of viruses can cause disease, while often, higher concentrations are required for pathogens in the group of bacteria and protozoa (STOWA, 2018).

Pathogen/index organism	Disease/role
Bacteria	
Campylobacter spp.	Gastroenteritis
Clostridium perfringens spores	Index organism
Escherichia coli	Index organism (except specific strains)
Faecal streptococci/intestinal enterococci	Index organism
Salmonella spp.	Gastroenteritis
Shigella spp.	Bacillary dysentery
Viruses	
Polioviruses	Index organism (vaccine strains), poliomyelitis
Rotaviruses	Diarrhoea, vomiting
Adenoviruses	Respiratory disease, gastroenteritis
Norwalk viruses	Diarrhoea, vomiting
Hepatitis A	Hepatitis
Parasitic protozoa[c]	
Cryptosporidium parvum oocysts	Diarrhoea
Entamoeba histolytica	Amoebic dysentery
Giardia lamblia cysts	Diarrhoea
Helminths[c] **(ova)**	

Figure 4 - Examples of pathogens and index organisms present in faecally contaminated water associated with raw sewage (WHO, 2003).

- **Incubation period of illnesses**

To monitor waterborne illnesses following recreational water events, the incubation period of illness should be taken into account.
Some pathogens have longer incubation times. For example, Cryptosporidium spp. has an incubation period of up to 10 days (EPA, 2012), whereas Wiedenmann et al. (2006) identified an incubation period of 1 week for GI conditions.

- **Exposure assessment**

There is a direct correlation between health risks from contaminated recreational water and the exposure and duration of contact with water (Schets, et al., 2008; Sales-Ortells & Medema, 2014). Therefore, the type of recreational activity plays a significant role (WHO, 2003; STOWA, 2018). Activities with minimal exposure include, for example, walking near water or fishing. Sailing, rowing and playing in water parks can be categorised as intermediate exposure activities, and swimming is an example of a high contact activity (Sales-Ortells & Medema, 2014; STOWA, 2018).

Routes of exposure include ingestion, inhalation and direct surface contact via skin and eyes (WHO, 2003; Waterschap De Dommel , 2016; STOWA, 2018; Deltares, 2010). The different routes were described below.

- **Ingestion**

The probability of infection due to contact with contaminated water is most sensitive to the volume of water ingestion and the concentration of a specific pathogen in the ingested water (Sales-Ortells & Medema, 2014; Joosten, et al., 2017; Hofstra, et al., 2019).
The estimated volumes of water ingestion in recent studies vary. In general, in whole-body contact activities, the ingestion probability is higher.
The WHO (2003) assumed that a 60-kg adult ingests 100–200 ml of water in a whole-body contact recreational activity in one session, whereas the water ingestion of a15-kg child can be expected equal to 250 ml. However, Schets et al. (2010) estimated water ingestion levels of 27–34 ml, 18–23 ml and 31–51 ml, respectively, by men, women and children per swimming event.

- **Inhalation**

Inhalation can be critical in activities where there is a significant amount of spray, such as in water skiing or white-water canoeing (WHO, 2003). The only harmful pathogen via inhalation is *Legionella*. In inhalation, a smaller dose of pathogenic organisms is needed to cause an infection compared to ingestion due to low PH levels in the stomach. Limited information is available about the dose–response relationship via inhalation (STOWA, 2009).

- **Direct surface contact**

Skin and mucous membrane exposure is most frequent (WHO, 2003). Aeromonas, for example, is a bacterium that can cause aggressive inflammation all around scratch or bite injuries. Little or no knowledge is available about this exposure route (STOWA, 2009).

- **Vulnerable participants**

Typically, recreational events are organised for a specific target group. Some of these groups are more vulnerable to potential contaminants in water, namely children under 8 years old, elderly people, pregnant women, and people with weak immune systems (STOWA, 2018).
Among the groups mentioned, children are most vulnerable due to their immature immune systems and higher levels of water ingestion (EPA, 2012). Some researchers (Sales-Ortells & Medema, 2014) have suggested that triathlon participants may be more vulnerable to infections due to intense exposure to water or a temporal decrease in immune response following exercise.

2.1.2 Microbial water quality indicators

The measurement of all pathogens present in water is very time consuming and expensive (Deltares, 2010). Hence, measuring the indicators of each group of pathogen can provide insights into presence of pathogens within these groups (STOWA, 2018). An indicator should have similar intrinsic characteristics and responses to environmental variations as the pathogen, which it is assumed to represent (Reder, et al., 2015).

- **Faecal indicator bacteria (FIB)**

Recent epidemiological studies suggest that guidelines based on FIB criteria can protect humans against most types of recreational water diseases, and FIB are easier to detect compared to other pathogens (Dorevitch, et al., 2010; Deltares, 2010; EPA, 2012). However, the compliance of water with FIB criteria does not guarantee the absence of other pathogens and harmful bacteria (Schets, et al., 2010; Sokolova, et al., 2013; Hofstra, et al., 2019; Cho, et al., 2016; EPA, 2012; European Environment Agency, 2012). For example, enteric viruses such as a norovirus (which are more resistant to wastewater treatment compared to bacteria) can be present in water despite absence of the FIB (Majedul Islam, et al., 2018; Joosten, B, 2018). This phenomenon is due to the fact that faecal indicators have shorter persistence compared to the unique survival characteristics of pathogens (Schoen & Ashbolt, 2010; Sokolova, et al., 2013; Reder, et al., 2015; Vermeulen, et al., 2015).

- ***E. coli* and IE**

E. coli and IE are the most commonly used faecal indicators to analyse water quality. *E. coli* and IE are not human pathogens that cause disease; rather, they behave similar to actual faecal pathogens and confirm the presence of faecal pollution (EPA, 2012; WHO, 2003). Numerous studies indicate that *E. coli* is a suitable predicator for GI- producing substances in fresh water, as well as IE for fresh and marine water (EPA, 2012; WHO, 2003; Medema, et al., 1998; Joosten, et al., 2017; Hachich, et al., 2012). However, the presence of *E. coli* and IE does not provide particular information about the contributing contamination sources (Ahmed, et al., 2006).

- **Additional indicators of water quality**

The WHO (2003) mentioned that due to the circumstances of small and shallow water bodies, two pathogenic bacteria, *E. coli* and Shigella sonnei, and two pathogenic protozoa, Giardia lamblia and Cryptosporidium parvum, can play a role in the occurrence of outbreaks.

Coliphages can be applied to detect human viral pathogens (Joosten, et al., 2017; STOWA, 2018). The eggs of Ascaris survive for a long time and are also good indicator of protozoa, and the spores of sulfite-reducing clostridia (SSRC) can be determined as an indicator for protozoa or worm eggs (STOWA, 2018).

Some researchers (Banyai et al. 2009; Matthijnssens et al. 2009) have suggested that the probability of zoonotic waterborne viral infections should be investigated since recent studies confirm that zoonotic pathogens can be reason behind some human rotaviruses (as cited in Dorevitch et al., 2010; EPA, 2012).

2.1.3 Microbial contaminant sources

Various contamination sources can affect the microbial quality of water. Since the health risks related to human versus non-human faecal sources differ, the identification of the contamination sources of the water system is an important factor (EPA, 2012; Schoen & Ashbolt, 2010; Liu, et al., 2006). In the case of the existence of multiple contamination sources in a waterbody, the dominant source should be identified to determine the potential faecal load introduced to the water (WHO, 2003; Deltares, 2010). However, the dominant source of contamination is not necessarily the dominant source of health risks (Schoen & Ashbolt, 2010). The following subsections provide information about possible faecal contamination sources in surface water.

- **Animals**

The faeces of dogs, cats, and birds contain faecal pathogens such as Campylobacter, Giardia, Salmonella, and Cryptosporidium, mainly introduced to bodies of water by rainfall runoff (Turgeon, 2012; Schets, et al., 2008). Therefore, animal-impacted water has potential human health risks (EPA, 2012; Turgeon, 2012). Nevertheless, Turgeon (2012) and (Sinton & Finlay, 1998) described that animal faecal sources have lower health risks than human sources.

Field observations have revealed that ducks mostly defecate in the water, whereas others birds, such as geese, coots, and water fowl, defecate on the shore (Hermsen, et al., 2011) and can affect the water quality during runoff.

- **Sewer overflows**

Variations in surface water quality can be expected based on the response of sewer systems to each rainfall event. In addition, WWTPs are generally overwhelmed after intense rainfall events, causing combined sewer overflow (CSO) and stormwater overflow (SSO) discharges, as well as surface runoff to urban water, thereby increasing the number of faecal indicators (WHO, 2003; Schets, et al., 2008; Joosten, et al., 2018). The rainfall intensity and prior precipitation have a direct correlation with the duration of the peak pollution period (WHO, 2003; Pongmala, et al., 2015).
It should be noticed that CSO and SSO discharges, as well as deterioration of water quality, will increase due to the higher frequency and intensity of rainfall events predicted due to climate change (Sales-Ortells & Medema, 2014; de Man, et al., 2014; ten Veldhuis, et al., 2010; Coffey, et al., 2015).

- **Combined sewer overflow**

Both Curriero et al. (2001) (as cited in ten Veldhuis et al, 2010) and Pongmala et al. (2015), based on their model approach, mentioned the CSO as a dominant contaminant source. The risk of GI conditions from accidental ingestion near a CSO can vary from 0.14 to 0.7 (ten Veldhuis, et al., 2010). Some researchers (Reinthaler, et al., 2003; Elmarghani, 2013) have demonstrated that raw sewage that enters bodies of water via CSO has extra hazards due to the antibiotic resistance of *E. coli* strains.

- **Stormwater overflow and illicit connections**

Illicit connections between sanitary and storm sewer water, as well as dirt from paved surfaces, can contaminate SSO (de Man, et al., 2014; European Environment Agency, 2018).

- **Wastewater treatment plants (WWTPs)**

The role of WWTPs is mainly to remove oxygen-binding substances and nutrients, such as nitrogen and phosphate, and not the removal of microorganisms. As a result, WWTPs discharge large quantities of microorganisms and pathogens into the receiving surface water (Deltares, 2010; Pachepsky, et al., 2018; Heijnen, et al., 2014).

- **Agricultural activities**

Faecal contamination from animals, farms manure, and animal production sites can be introduced to bodies of water by runoff. The pollution risk of manure is the highest in a short period after spreading (Turgeon, 2012). Biofilms from hydraulic equipment in agricultural irrigation can also enter the watercourse (Pachepsky, et al., 2018).

- **Urban runoff**

E. coli attached to particles from cat and dog faeces can enter urban water during rainfall events (Cho, et al., 2016; Pongmala, et al., 2015; STOWA, 2019; Heijnen, et al., 2014).

- **Sediments**

Information on the microbial contamination of bottom sediment is limited; 17%–21% or possibly 36% of stream *E. coli* can originate from bottom sediment (Cho, et al., 2016). In general, the *E. coli* concentration of bottom sediment is higher than in the water column, which can be released during heavy rainfalls due to sediment resuspension (Pachepsky, et al., 2018; Vermeulen, et al., 2015; Cho, et al., 2010; ten Veldhuis, et al., 2010). Some scholars (Pachepsky, et al., 2018; Dorevitch, et al., 2010) have stated that *E. coli* in sedimentation can grow during rainstorms.
In addition, sediment and suspended solids from sewer systems can affect the transport of faecal contamination in the CSOs (Pongmala, et al., 2015).

- **Bathers**

Bathers can affect the microbial water quality by stimulating bottom sediment resuspension and faecal accidents specifically when the number of children among the swimmers is high (Turgeon, 2012).
In addition, large numbers of bathers can have negative effects on water quality, especially in small bodies of water (Schets, et al., 2010; RIVM, 2018; Dorevitch, et al., 2010).

- **Pleasure boats**

Since 1974, in the Netherlands, pleasure boats have been exempt from discharging domestic waste water to surface water (RIZA, 2005) and assumed to have a negligible effect on water quality.

2.1.4 FIB concentration of contamination sources

The FIB load of each contamination incident can be calculated according to FIB intensity and the mass of contamination introduced to surface water. The sections below present the FIB concentration of main sources; insufficient data are available for other sources.

- **Animals**

Few studies were available regarding the faecal contamination load of animals. According to one report (EPA, 2015), *E. coli* concentrations in cows, sheep, dogs are $2.3.10^5$[CFU/gr], $1.6.10^7$[CFU/gr], and 4.10^7 [CFU/gr], respectively. Wright, Solo-Gabriele, Elmir, & Fleming (2009) estimated IE levels of $7.4.10^6$[CFU/gr] and $3,3.10^5$[CFU/g] in dog and bird faeces, respectively.

- **Agricultural activities**

Few studies including calculations of the FIB loads of farms and pastures were found. Alterra (2005) suggested variable *E. coli* and IE concentrations based on the type of soil and the type of animals grazed in pasture. Coffey et al. (2010) estimated an *E. coli* concentration of $4.2 . 10^5$ [CFU/g] in manure (as cited in Majedul Islam et al, 2018).

- **Sewer overflows and WWTPs**

Various studies have investigated the *E. coli* and IE concentrations of sewer overflows and WWTP effluent. Table 1 displays the range of FIB concentrations based on recent studies.

Table 1 - The range of FIB concentrations associated with sewer overflows according to recent studies.

Reference	CSO [CFU/100ml]	SSO [CFU/100ml]	WWTP [CFU/100ml]	Runoff [CFU/100ml]
(Turgeon, 2012)	*E. coli*=1.10^6			
(Majedul Islam, et al., 2018)	*E. coli*=$1.5.10^6$ EI=1.10^6			
(Pettersson et al., 2013)	*E. coli*=2.10^6			
(Reinthaler, et al., 2003)			*E. coli*=1.10^4	
(Pongmala, et al., 2015)		*E. coli*=$1.33.10^6$		
(RIZA, 2005)	*E. coli*=4.10^5–7.10^6			
(Koffi Ouattara et al., 2013)	*E. coli*=5.10^6			
(Waterschap De Dommel , 2016)	*E. coli*=1.10^5		*E. coli*=1.10^5	
(Deltares, Waterrecreatie in de stad: tips en trucs voor het vergroten van de kansrijkheid van projecten (in Dutch), 2010)	*E. coli*=1.10^6 EI=1.10^5	*E. coli*=10^3-10^5 IE=10^3–10^5		
(ten Veldhuis, 2010)	*E. coli*=10^5–$7*10^7$			
(STOWA, 2009)	*E. coli*=$1.83.10^5$	*E. coli*=1.10^4		
(Meals & Braun, 2006)				*E. coli*=7.10^4 (with manure) *E. coli*=300 (unmanured)
(Harmel, et al., 2010)				*E. coli*=10^2–10^3 (cultivated field) *E. coli*=10^3–10^4 (grazed pasture)

To investigate the environmental impact of illicit connections, the ensemble model output statistics (EMOS) model of STOWA was used by Arcadis company (STOWA, 2009) to calculate the emissions at different percentages of illicit connections. Table 2 provides data from Arcadis investigation of the *E. coli* concentration [CFU/100 ml] per illicit connection percentage, in which CS means ''combined sewer'', ICS "improved combined sewer", SS "separated sewer" and ISS "improved separate sewer system".

Table 2 - *E. coli* concentration [CFU/100 ml] per illicit connection percentage which CS means combined sewer, ICS improved combined sewer, SS separated sewer and ISS improved separate sewer system.

Ave. Pollution concentration	CS	ICS	SS				ISS			
Illicit percentage [%]			0	2	5	10	0	2	5	10
E-coli [CFU/100 ml]	183,000	86,000	11,600	304,000	711,000	1,319,000	11,600	22,000	37,000	63,000

- **Determination of the FIB loads of sewer overflows**

The FIB loads of sewer overflows [CFU] follow from equation (1):

$$FIB_{Load} = FIB_{conc} \cdot Q \quad (1)$$

where FIB_{conc} denotes the FIB concentration derived from Table 2, and Q is overflow discharge [m^3/s].
The Q value is calculated from the equation (2) (RIONED, 2003):

$$Q = m \,.\, 1.7 .\, b \,.\, (h)^{1,5} \quad (2)$$

where m = 0.8 runoff coefficient (depends on type of crest)[-], b represents the width of weir [m], h stands for the water height above the crest [m], and Q is overflow discharge [m^3/s].

2.1.5 Decay rate of *E. coli* and intestinal enterococci (IE)

The decay rates of *E. coli* and IE are important factors applied in the fate and transport modelling of microbial contaminations (Pongmala, et al., 2015). Koffi Ouattara et al. (2013) asserted that FIB tends to decline rapidly after release to a natural water system due to biological and physiochemical processes. Survival strategies of FIB can be activated, leading to the persistence of FIB in critical environmental situations such as, low temperatures, lack of nutrients, direct light, toxic metals, and salinity (del Mar Lleo, et al., 2005; van Elsas, et al., 2011; Liu, et al., 2006; Cho, et al., 2016).

Some authors (Ahmed, Neller, & Katouli, 2006; van Elsas, et al., 2011; Liu, et al., 2006) have stated that IE can survive longer than *E. coli*. Under stress circumstances, one part of IE lose their culturability quickly, but they can survive for a long period and the rest can keep their culturability for 6–8 weeks, and then they can hardly survive (del Mar Lleo, et al., 2005).

E. coli can survive for variable periods of time (van Elsas, et al., 2011; Cho, et al., 2016). Moreover, *E. coli* bacteria survive longer either in freshwater compared to saline water (Liu, et al., 2006) or in sediment compared to the water column (Pongmala, et al., 2015; Koffi Ouattara, et al., 2013). Among the stress conditions, sunlight is the major factor for the inactivation of *E. coli* (Liu, et al., 2006; Majedul Islam, et al., 2018).

FC. Falconer and Chen have estimated that the *E. coli* decay rate is in the range of 0.05-4.0 per day (as cited in Kashefipour et al, 2002). Table 3 displays the range of *E. coli* decay rate of different freshwater temperature based on various studies.

Table 3 - The range of *E. coli* decay rate of different freshwater temperature

Water source	Temperature [°C]	Decay rate(k) [1/day]	Reference
Fresh water	20	0.8	Mancini(1978)
Fresh water	10-12	0.29	(McFeters, et al., 1974)
Fresh water	15	0,33	(Dick, et al., 2010)
	25	0,49	
Fresh water	5	0,14	(Hijnen, et al., 2007)
	15	0,25	
	25	0,45	

- **Calculation of the *E. coli* and IE decay rate**

Mancini (1978) described the decay rate of FIB as a function of salinity, temperature, and solar radiation following from this equation:

$$\frac{dC}{dt} = -k_0 \cdot \theta_S^{Sal} \cdot \theta_T^{Int} \cdot \theta_T^{(Temp-20)} \cdot C \quad (3)$$

where C is the concentration of bacteria [CFU/100 ml], t is the time, k_0 denotes the decay rate [1/day] at 20 °C for a salinity of 0 and in a dark condition, θ_S is the salinity coefficient for the decay rate, Sal is the salinity [%0], θ_I represents the light coefficient for the decay rate, Int is the light intensity [kW/m^2], θ_T is the temperature coefficient for the decay rate, and Temp [°C] stands for the water temperature (Majedul Islam, Sokolova, & Hofstra, 2018).

2.1.6 Recreational water quality guidelines

The guideline values should be modified regarding local conditions such as site characteristics, population behaviours, as well as economic, environmental and technical aspects (WHO, 2003).

- **Recreational water guidelines in different countries**

Table 4 provides an overview of the recreational water guidelines in different countries. It should be noted that the guidelines are set up for official bathing water, but they are implemented for other recreational activities and non-official bathing water as well.

Table 4 - Recreational water quality guidelines in different countries in the context of swimming

Country/ Organization	Type of water	Indicator	Guideline values [CFU/100ml]	GI risk [%]	Reference
U.S.EPA	Fresh	*E. coli* IE	≤ 126 ≤ 35		(EPA, 2012)
Australia	Fresh/Marine	IE	95th percentile A: ≤ 40 B: 41–200 C: 201–500 D: > 500	 < 1 % 1–5 % 5–10 % > 10 %	(NHMRC, 2008)
WHO	Marine	IE	95th percentile A: ≤ 40 B: 41–200 C: 201–500 D: > 500		(WHO, 2003)
	Fresh		Not derived yet		
European Union	Fresh	*E. coli* IE	95th percentile Excellent: 500 Good quality: 1,000 90th percentile Sufficient: 900 95th percentile Excellent: 200 Good quality: 400 90th percentile Sufficient: 300	 5% < 8%	(The European Parliament, 2006) (Rijksoverheid, 2013) (Coffey et al., 2015)
"Beslisnotitie"		*E. coli* IE	Signal value: 1,800 Signal value:400		(Rijksoverheid, 2013)

- **Recreational water guideline in the Netherlands**

In the Netherlands, the signal values of 1,800 [CFU/100 ml] and 400 [CFU/100 ml] for *E. coli* and IE are, respectively, implemented for recreational water activities in urban water, based on a decision memo derived from the European Bathing Water Directive (2006/7/EC).

In this memo the signal value for *E. coli*, however, differs from the "good" class water, which is 1,000 [CFU/100 ml], because the probability of increasing health risks is nil, while *E. coli* concentrations of single measurements increase approximately by a factor of 2. The choice of value of 1,800 [CFU/100 ml] makes the guideline implementation practically feasible for responsible authorities (Rijksoverheid, 2013).

In April 2019, STOWA released a handbook for recreational water activities in and around water derived from '' Beslisnotitie'' guideline and case study of De Dommel Waterboard (STOWA, 2019). Instructions for two cases are suggested in following sections.

- **Swimming event in surface water (non-official swimming location)**
  In this case, no water quality data are known in advance. For this reason, it is advised to select two points in the route and to make weekly measurements for a minimum of six weeks prior to the event. The contribution of the pollution sources and how long it takes for pollution to enter the event trajectory should be determined. This event concerns swimming; therefore, it was decided to test the water quality based on ''Beslisnotitie'' guideline.
  Due to rainfall, FIB concentrations can exceed the guideline values. The advice is therefore as follows: The event can continue if no or little (<10 mm) rainfall has occurred in the four days prior to the event.

- **Play and splash festivals in and on surface water (non-official swimming location)**
  In these situations, water quality is also unknown, and a first step is the determination of potential sources of pollution and the measurements of the water quality. In this case, fewer people will have direct contact with the water only via splashing water or falling into the water– namely, vulnerable group of young children. Thus, it was decided to implement ''Beslisnotitie'' guideline again.
  Dog and bird faeces present near the location should be removed daily starting a week prior to the activity.
  The advice is to control water quality again on the morning of the event by a quick test. In case of poor water quality, the water activity should be cancelled.

- **Guidelines criteria and associated health risks**

Pachepsky et al. (2018) mentioned a fact ignored in most of microbial water quality standards: the absence of a correlation between pathogens and indicators. Most standards are derived from epidemiological studies on the risk of enteric diseases as a function of FIB concentration. Furthermore, the probability of GI conditions in most guidelines is solely based on water ingestion and three times head immersions in water and excludes the other exposure routes, such as skin and inhalation (Deltares, 2010).

Research suggests that, with the values of the "good" class, the risk of health problems in swimmers increases with a percentage between 40% and 70% compared to people who do not swim. This does not mean that a swimmer has a 70% chance of becoming ill. Rather, it means that the chance of illness for a swimmer compared to someone who has not been swimming is 70% higher (Rijksoverheid, 2013).

Medema et al. (1998) demonstrated that GI risk increased significantly when *E. coli* ≥ 355 [CFU/100 ml] among triathlon participants. Other researchers (ten Veldhuis, 2010; Deltares, 2010) have also stated that the GI risk for the ''excellent'' class derived from ''Beslisnotitie'' guideline can be above 3 %. Even other scholars (Deltares, 2010; STOWA, 2009) have pointed out that the health risks of the ''sufficient'' class according to ''Beslisnotitie'' guideline can be 11%.

A number of researchers believe that current guidelines that are especially based on FIB levels can-not protect the user of recreational water (Medema, et al., 1998; Schets, et al., 2010).

2.1.7 Sample processing and analysis

The main challenges in sampling are integrity and representativity (Madrid & Zayad, 2007). The sampling program should cover all conditions in the recreational water environment during the bathing period (WHO, 2003; Pachepsky, et al., 2018). A data set of five years is needed to assess microbial water quality (WHO, 2003).

- **Sampling location**

The water quality at the place where the event is held should be determined. In case of a favourite recreational activity route, several places are desirable, depending on the number of pollution sources affecting the water quality. To create a reliable picture of spatial variation of the water quality in water system and hot spots, samples at several locations should be taken (The European Parliament, 2006; STOWA, 2019).

- **Sampling interval**

Sufficient samples should be collected to make an appropriate estimate of indicators' concentrations in recreational waters (WHO, 2003). Regular measurement programs should always comprise at least 16 samples. One sample close to the starting date of each bathing season should be taken. In cases of short-term pollution, one additional sample should be taken to confirm that the incident has ended. Rijksoverheid (2013) made it compulsory to take a minimum of one sample per month in the case of single measurements.

A few researchers have found that the bathing water quality in the time between sampling and analysis results sufficiently improves, and in most cases, does not exceed the signal values (Rijksoverheid, 2013).

- **Sampling method**

The sample bottle should be placed upside down in the water between 20 cm and 30 cm below the surface and avoid entering the water from the top layer, which contains significantly more bacteria (AQUON, 2018; The European Parliament, 2006; Technical university of Crete, 2009).

- **Sampling limitations**

The samples reflect the water quality at the moment of sampling and may not represent the daily value. The *E. coli* concentration can meet the water quality standards in the morning, whereas it exceeds them in the afternoon (Joosten, et al., 2018). In addition, concentrations of FIB cannot be accurately measured. Aquon mentioned 30–35 [%] measured uncertainties (Overzicht methoden Aquon, 2019).

- **DNA source detection analysis**

Heijnen et al. (2014) stated that, with the specific DNA markers, it is possible to identify faecal contaminants originating from humans, birds, dogs, pigs, ruminants (such as a group with sheep, deer, and cows), and specific cattle. In this analysis, bacterial markers for each animal group, which are only present in the intestinal flora of these specific animal group are identified. These markers are useful to trace the source of faecal pollution.

A quick analysis of *E. coli* and IE in the laboratory takes 24 hours, whereas DNA analysis only requires a few hours. Therefore, DNA analysis can serve as a handy measurement to obtain more certainty prior to a recreational event by controlling whether human or animal faecal contamination impacts have increased or decreased compared to reference values.

2.2 Water quality models

Various studies demonstrate that FIB concentration and the contribution of potential pollution sources to FIB in water can be identified using coupled hydrodynamic and water quality models combined with measurements (Majedul Islam, et al., 2018; Pachepsky, et al., 2018; Coffey, et al., 2015; Cho, et al., 2016; Dorevitch, et al., 2010). These models can be utilised for different scenario analysis (Sokolova, et al., 2013; Pachepsky, et al., 2018).

However, Pongmala et al. (2015) mentioned that simulating the water quality of urban water affected by sewage is complicated because wastewater sources (such as CSOs) are the most variable point sources. Approaching an accurate prediction model requires an understanding of all processes affecting microbial concentrations (Pongmala, et al., 2015). Another challenge is that only a few parameters are used to simulate complex water processes depending on the hydrology and morphology of the stream. Another significant challenge is the lack of sufficient data for model calibration and testing (Cho, et al., 2016; Koffi Ouattara, et al., 2013).

3 Materials and methods

To evaluate the microbial water quality of Breda canals and impact of rainfall events on the water quality, *E. coli* and IE as microbial water quality indicators were monitored during the sampling campaign in dry and wet weather. A DNA source detection analysis was carried out to identify the microbial contamination sources of Breda canals. The section on the water quality analysis comprises the water quality monitoring process.

In the subsequent section that includes the sewer model analysis, the method of sewer model calibration to confine the discrepancy between simulated and actual overflow volumes to factor 2 is presented.

The water quality model analysis section consists of a discussion of how the data were processed and how the water quality simulation model of Breda canals was developed.

3.1 Water quality analysis

The microbial water quality of Breda canals is not monitored by Brabantse Delta Waterboard (regional organization which has function of water resources management in Breda). Nevertheless, since 2017, Brabantse Delta Waterboard has begun monthly monitoring of *E. coli* at two locations: Julianalaan (Aa of Weerijs, upstream of the study area) and Duivelsberglaan (Mark, upstream of study area). These locations displayed in Figure 5.
The water quality of Breda canals is merely controlled before each recreational water event by an event organizer to obtain a permit from the Municipality of Breda. Thus, there is a lack of information on the microbial water quality history of the canals and potential contamination sources. Sampling analysis was used to gain insights into the temporal and spatial variations of the microbial water quality of Breda canals and the calibration of the water quality model. In addition, a DNA source detection analysis was performed to trace the source of faecal pollution of Breda canals.

3.1.1 Water source of Breda canals

Two main streams of the Aa of Weerijs and the Mark feed the Breda canals. The ''Boven Mark'' originates in Flanders and flows from Galder in the Netherlands into the canals of Breda and is 11 km long. The Dutch ''Boven Mark'' is characterised as a slow stream on sand/clay (Waterschap Brabantse Delta, Watersysteemanalyse Boven Mark, 2017). The Aa of Weerijs also originates from Flanders near Brecht and flows along the east side of Zundert and Rijsbergen to Breda, where the stream flows into the canals. The Aa of Weerijs type is a slow stream on sand (Waterschap Brabantse Delta, 2018).

The Molenleij discharge from the industrial area to Breda canals (see Figure 5) in the bathing season amount to 0.

3.1.2 Selection of measuring points

Water sampling is expensive, and the limited budget only allowed for selection of only five sampling locations. The approximate locations of these measuring points were chosen in consultation with supervisors, with the consideration that the locations should cover whole designated areas and provide a sufficient overview of the spatial variation of water quality. After a visual inspection to investigate the safety and feasibility of sampling, the final measuring points were selected. These locations are displayed in Figure 5.

The measuring points were as follows: the Mark upstream site received contamination mostly from six WWTPs (two located in the Netherlands and four in Belgium) and sewer overflows

(Location 5), and the Weerijs upstream site received contamination mostly from two WWTPs (both located in Belgium) and agricultural runoff (Location 4). The Breda Harbour, a popular location for water recreational events (Location 3), a site adjacent to confluence of two streams (Location 2), and a downstream site opposite the Belcrum artificial beach indicated how Breda affects the water quality (Location 1). It should be mentioned that Location 3 is a dead-end canal. The harbour section can only be fed by sewer overflows of the harbour. The geographical locations of the five measuring sites are displayed in Table 5.
Brabantse Delta Waterboard began to measure *E. coli* monthly starting in 2017 at locations a and b, Location a (Julianalaan) located at a distance of 500 m from location 4 and Location b (Duivelsberglaan) at a distance of 1.7 km from Location 5.

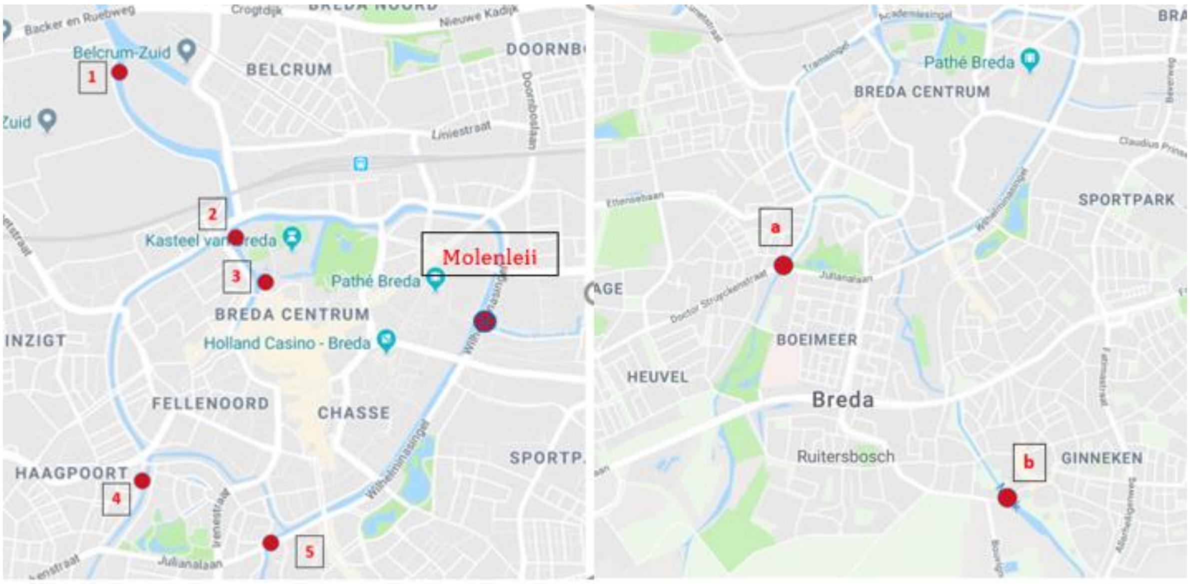

Figure 5 - The figure on the right contains a depiction of two monthly measuring points carried out by Brabantse Delta Waterboard. Location a (Julianalaan) located at a distance of 500 m from Location 4 and Location b (Duivelsberglaan) at a distance of 1.7 km from Location 5. The figure on the left displays the five selected measuring points during this thesis. The Mark upstream site received contamination mostly from six WWTPs and sewer overflows (Location 5), and the Weerijs upstream site received contamination mostly from two WWTPs (Location 4). The Breda harbour, a popular location for water recreational events (Location 3), a site adjacent to converging of two streams (Location 2) and a downstream site opposite the Belcrum artificial beach indicated how Breda affects the water quality (Location 1).

Extra sampling locations

To investigate variations of FIB across the length of the canal, once two extra samples from two points within 20 m and 40 m distance from location 3 were taken.
As with location 3, once two extra samples from two points across the width of canal within distances of 7 m and 14 m, from location 5 were taken from the bridge to investigate variations of FIB in terms of the width of the canal.
An extra sample at location 6 at the border of the Netherlands and Belgium within a distance of 10 km from Location 5 was taken on 17-8-2018 to determine the *E. coli* concentration entering the Netherlands.

Table 5 - Geographical location of the five measuring points

Name	Sampling Location	Geographical location Latitude	Longitude
Mark canal-Belcrum beach	1	51°35' 58"N	004°45'48" O
Nieuwe Mark canal	2	51°35'33" N	004°46'17" O
Mark canal- harbor	3	51°35'22" N	004°46'21" O
Weerijs canal (Bernhard brug)	4	51°34'57" N	004°45'56" O
Mark canal (Juliana brug)	5	51°34'47" N	004°46'24" O

3.1.3 *E. coli*, IE analysis

To study the microbial water quality of Breda canals, two faecal indicators of *E. coli* and IE were selected to monitor during the sampling campaign.
The selection of these indicators was based on two factors: recent epidemiological studies, which suggest that guidelines in context of FIB criteria can protect humans against most types of recreational water diseases (Dorevitch, et al., 2010; Deltares, 2010; EPA, 2012), and the GGD guideline (based on “Beslisnotitie”) for recreational water activities in the Breda canals (GGD, 2018).
In the Netherlands, the signal values of 1,800 [CFU/100 ml] and 400 [CFU/100 ml] for *E. coli* and IE are, respectively, implemented for recreational water activities in urban water. These values are based on a decision memo, derived from the European Bathing Water Directive (2006/7/EC). In this memo the signal value for *E. coli*, however, differs from the "good" class water, which is 1,000 [CFU/100 ml], because the probability of increasing health risks is nil, while *E. coli* concentrations of single measurements increase approximately by a factor of 2. The choice of value of 1,800 [CFU/100 ml] makes the guideline implementation practically feasible for responsible authorities (Rijksoverheid, 2013).

The water samples of *E. coli* and IE analysis were collected on 15 occasions beginning on July 25, 2018. The budget allowed for a total of 40 samples. Based on a rough estimate of decay rate of FIB, the sampling scheme was defined weekly during dry weather, 3rd day after light rainfall without a CSO discharge (day 1 being the day of the rainfall) and 3rd and 5th days after intense rainfall with a CSO discharge. If these dates fell within the weekend, sampling could be postponed.
By comparison of results of dry weather and rainfall events can determine if the weather conditions can affect the water quality. The 3rd day sample served as a control group. By sampling at the 3rd day after light rain can indicate whether the peak value decreased after 3 days. The rainfall events with a CSO discharge, following a higher concentration of FIB, may require more than 3 days. Therefore, an extra sample on the 5th day was taken to determine whether 5 days was sufficient for water quality to be safe for recreational activities.

During the first months, there was a challenge due to the absence of sensors at CSOs to recognise whether sewer overflow discharge had occurred during the rainfalls. With the assumption of 7 [mm/h] of sewer capacity, 0.7 [m/h]of pump capacity, and 5 mm of pavement storage capacity and the filtration of permeable surfaces, as a rough estimation, it was assumed that rainfalls under 12 mm do not lead to a CSO discharge.
Five sensors were installed in October 2018 at five CSOs to delete the uncertainty over CSO discharges and providing data for the calibration of sewer model. These five CSO’s were identified as having a high frequency of discharge in earlier research of Arcadis company in 2015. Since October 2018, the samples were taken when a CSO discharge occurred at one of these five CSOs. The locations of these CSOs are displayed in Figure 8.

Taking into account the insufficiency of the existing data on the water quality in the Breda canals, the sampling campaign of Brabantse Delta Waterboard was also used, in addition to this study’s sampling results, to allow for a comprehensive analysis. (see Appendix F, G, and H).

The *E. coli* and IE analyses were performed at Aqualab Zuid at Werkendam, using NEN-EN-ISO 9308-3 and NEN-EN-ISO 7899-1 over 2 days.

3.1.4 DNA analysis

The possible faecal contamination sources in surface water can be (water) birds, recreational activities, WWTP effluent, agricultural runoff, CSOs, SSOs, illicit connections, and runoff from dog faeces. Identification of faecal contamination sources only by analysis of bacteria indicators (*E.coli* and EI) is impossible. By using DNA techniques (qPCR), specific bacteria of animal group with faecal origin can be detected and quantified (KWR, 2019).

DNA concentration of markers for four groups of organisms; humans, ruminants, dogs and birds were monitored by a DNA source trace analysis. The samples were collected from five sampling locations (see Figure 5) in June 2019 on three occasions: one in dry weather (without sewer overflows) as reference situation and two after rainfalls with a CSO discharge (see Appendix M).

The DNA source trace analysis was performed at research institute of KWR at Delft.

3.1.5 Sample collection

To keep the expenses low, sampling was conducted solely by the researcher. Unfortunately, access to surface water sampling protocols (NEN-EN-ISO 5667-15:2009 or ISO 18400-102:2017) was not possible. Therefore, based on a few studies, the first series of sampling was taken by using a bucket. To become acquainted with professional sampling methods, the researcher participated in one water sampling session carried out by water research company of Aquon. In this way, a swing sampler was made to facilitate collecting water from the desired depth and location, as shown in Figure 6. In both methods, the researcher tried to collect the water from depth of 30–50 cm and a minimum distance of 1m from the canal edge, since ducks often swim and stay close to the water's edge. Furthermore, runoff including bird and dog droppings also come into contact first with the water edge. The water at the canal edge can be also stagnant, leading to a distortion of results.

The PH and water temperature (to estimate the decay rate of FIB) for most samples were measured on the spot. The researcher attempted to take the samples at the same time for each set. All samples were stored in an ice box and delivered to the Aqualab Zuid laboratory within 2 hours.

Figure 6- The figure on the left displays the sampling method with a bucket, and the figure on the right, the sampling method using a swing sampler. In both methods, samples were taken from depths of 30 cm–50 cm and a minimum distance of 1 m from the canal edge.

3.1.6 Data collection

To access the precipitation and water level at sewer pump stations and at detention tanks the “HydroNET’’ website was used.

The water levels at five selected CSOs were monitored through the Koenders online database. The sensors sent data every 12 hours to the online database.

3.2 Sewer model analysis

The sewer overflows and illicit connections were indicated as one the leading causes of faecal pollution of Breda canals, thereby increasing concentrations of *E. coli* and IE after rainfall events. The associated *E. coli* and IE loads released into the canals is correlated with the overflows volume per event.

To run the water quality model, overflows volume simulated by the sewer model during the sampling period are used as input; thus, the discrepancy between the simulated and actual overflows volume should be negligible if one wishes to reach a significant correspondence between the *E. coli* concentration simulated by the water quality model and the measured *E. coli* concentration. To make the modification of the sewer model feasible, the discrepancy between the simulated and actual discharged CSO's volume was confined to Factor 2.

3.2.1 Sewer system of Breda

The Breda sewer system comprises 14 catchment areas (see Appendix A), which four catchment areas of 12, 14, 10 and 7 connected to Pressure line directly.

40 % of sewer catchment areas are connected to separated system, in which storm water enters directly into the surface water. The area of each catchment based on the type of sewer system is presented in Appendix B.

3.2.2 Sewer model of Breda

The Breda sewer system was analysed by InfoWorks ICM 9.0 to calculate the discharged sewer overflows volume. The Breda sewer system was redesigned by Arcadis company in 2013.

3.2.3 Illicit connections

An illicit connection is the discharge of sanitary sewer into a storm sewer system via a pipe or other direct connection. The advisor of municipality of Breda estimated maximum percentage of 2% of illicit connections.

3.2.4 Simulated rainfalls selection

Rainfall data were available on the HydroNET database, which was based on Koninklijk Nederlands Meteorologisch Instituut–Royal Netherlands Meteorological Institute (KNMI) radar data. The collected data within 3 days were corrected, and within six weeks, they were validated as final data (HydroNET Neerslagradar, 2019). Rainfall data associated with sampling dates, per catchment pumping station, were imported to the InfoWorks model from HydroNET.

3.2.5 Catchment pumping station

Since the topography of the Netherlands does not allow for much natural gradient, the sewer system utilises pumps to collect sewage per catchment and transport it to a WWTP.

Catchment pumping stations selection

The Breda sewer system comprises 14 catchments (see Appendix A). Five catchment pump stations located in catchments 0, 1, 2, 3, and 4 were selected which, have a significant and direct impact on the water quality of the study area. The behaviour of the sewer system during rainfall events in the context of CSOs discharge was clarified by pumping station data analysis. The locations of the selected pump stations are illustrated in Figure 7.

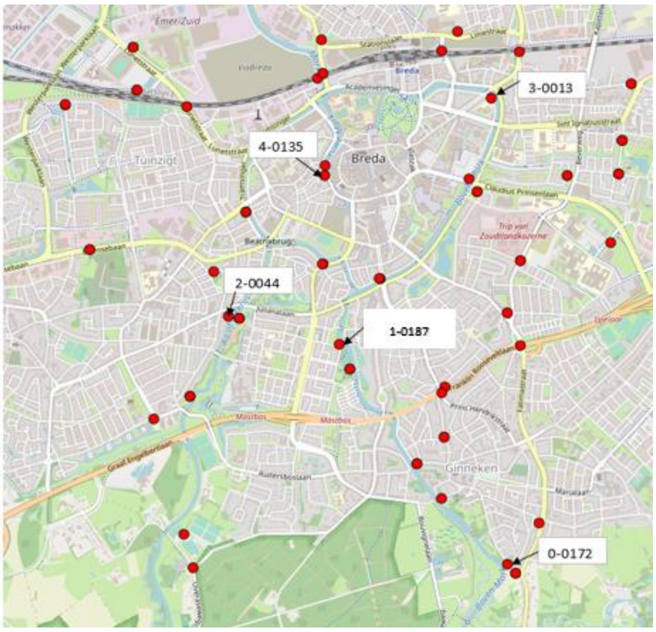

Figure 7- Selected catchment pump stations, which analysed to clarify the behaviour of the sewer system during rainfall events in context of CSO discharge.

The accurate water levels at the catchment pumping stations were monitored by sensors, and data were available at HydroNET.

Catchment pump capacities

A pump's capacity depends on size of the contributing catchment. The researcher attempted to determine the pump capacities by reading pump construction drawings, but the accurate capacity of all pumps was neither measured nor properly stored. Table 6 lists the pump capacities according to municipality and InfoWorks data.

Table 6 - Pump capacities of the selected pump catchments according to municipality and InfoWorks data. At 3-0013, due to sewer renovation, the pump capacity was temporality decreased to 50%.

Municipality data			InfoWorks data		
Pump station	Type	Capacity [m^3/h]	Pump station	Type	Capacity [m^3/h]
0-0172	VFD	1*320 1*1,180	0-0172	Screw pump	1*320 1*480
1-0187	Screw pump	1*432 2*864	1-0187	Screw pump	1*432 2*864
2-0044	Screw pump	2*430	2-0044	Screw pump	2*430
3-0013	VFD	2,000 1,000*	3-0013	Screw pump	2,000 1,000*
4-0325	Screw pump	1*432 3*814	4-0325	Screw pump	1*432 3*814

3.2.6 Combined sewer detention tanks

In Breda's sewer system, several combined sewer detention tanks (CSDTs) were constructed. The CSDTs, by increasing sewer storage capacity, can prevent flooding. After rainfall events, when the water level decreases to dry weather conditions in pipes, the storage water in CSDT is subsequently pumped back to the sewer system to transfer to a WWTP. When the tank becomes full, the overflow diverts to the canals. In addition, CSDTs reduces the contamination load introduced to receiving bodies of water (Dufresne, et al., 2009). However, some researchers

(Sales-Ortells & Medema, 2015) have indicated that pathogens may not settle efficiently in CSDTs during rainfall events; thus, high FIB concentrations during overflow periods are still to be expected.

Water levels at all the CSDTs of Breda sewer system are measured to indicate whether an overflow at these locations has occurred. However, there is uncertainty regarding the location of sensors and whether they were installed at an external weir, an internal weir, or in a sewer manhole. The locations of CSDTs in sewer catchments 0, 1, 2, 3, 4, and 13, which affect the water quality of the study area, are illustrated with black square in Appendix J.

3.2.7 CSO sensor installation

The first results of water sampling after rainfall events indicated that determining of whether a CSO discharge had occurred was complicated because the water levels of CSOs were only measured at detention tanks. The peak of *E. coli* concentration could be due to CSO discharges, but the sensors at the detention tanks did not identify any discharge.
Therefore, sensors were installed in October 2018 at five CSOs that had experienced their discharges in the study area, and had a high frequency of discharge based on Arcadis company research in 2015. Consequently, the samples could be taken with certainty after a CSO discharge occurred at one of these CSos. In addition, the model could be modified based on sensor data to approach Factor 2 regarding the discrepancy between simulated and measured discharged overflows volume. Figure 8 displays the location of monitored CSOs.

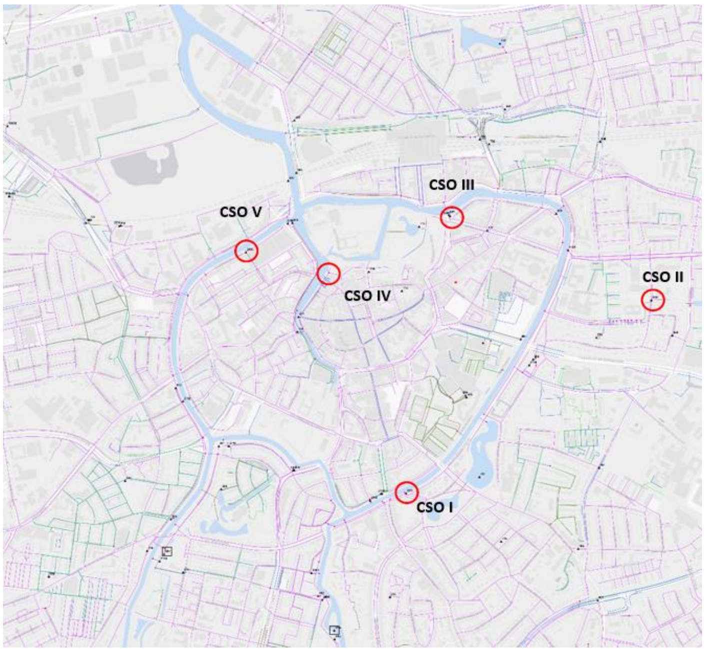

Figure 8- Location of five CSOs with sensors. All five CSOs discharge to the study area, and they had a high frequency of discharge based on Arcadis research in 2015.

A visual inspection on September 5, 2018, was performed before the installation of sensors to investigate the status of these five CSOs. The inspection indicated a clogging at CSO V where most of the clogging components were wet wipes. After intense maintenance, the sensors were installed by Koenders company in October 2018. Data on the CSOs structures, such as width and the crests' distance from ground level, were measured accurately by Koenders. In some CSOs, the measured values did not correspond to the registered database of the municipality. Table 7 presents more detaisl on the selected CSOs.

Since a negative discharge was detected by Arcadis at CSO II, two sensors at both sides of the weir were installed to identify whether a negative discharge had happened. The sensors sent data every 12 hours to an online database.

Table 7 - Details about the selected CSOs' structures measured by Koenders and comparison with registered data in InfoWorks.

	InfoWorks				**Koenders**			
	ID municipality	Crest width [m]	Manhole depth [m]	Crest height [m]	ID Koenders	Crest width [m]	Manhole depth [m]	Crest height [m]
CSO I	1-0049	2.0	3.16	1.09	S6258	2.0	3.137	1.047
CSO II	3-0643	2.0	2.4	1.25	S6259	2.1	2.428	1.303
CSO III	4-0135	3.18	2.53	1.05	S6260	3.18	2.553	1.06
CSO IV	4-0046	1.5	2.48	0.79	S6261	2.0	2.747	1.059
CSO V	13-0724	1.2	2.33	1.01	S6262	1.4	2.356	1.056

3.2.8 Sewer model modification

As a first step, the measured and simulated water levels at the pumping stations were compared to identify whether the parameters should be corrected in the model to approach a precise simulation. Each rainfall event associated with the water sampling dates was simulated in InfoWorks separately. The duration of each simulation was selected so that the behavior of each catchment pump station to make the system empty could be indicated.
First simulation indicated that a few parameters should be changed according to the actual situation because the model was designed based on data of 2013. The pump construction drawings were read to control pump capacities and pump chamber depth. Controlling the database led to some conclusions.

- The pump capacity at 0-0172 was increased from 800 [m^3/h] to 1,500 [m^3/h].
- The pump capacity and sewer pipe diameter at 3-0013, due to sewer renovation at "Nieuwe Veste" was temporally decreased to 50%.
- The chamber depth of 2-0044 in the municipality data (HydroNET) was changed from -4.75 m to -1.8 m.
- The Koenders data were adjusted to winter time.

The next step was modification of sewer model to confine the discrepancy between simulated and actual discharged CSOs volume. Regarding complexity of sewer model, the Factor 2 was selected. The discrepancy between simulated and actual discharged CSOs volumes should be negligible to approach a significant correspondence between simulated *E. coli* concentration and the measured *E. coli* concentration.

The discharge volumes of the sensors' data were calculated according to equation (2), whereas the crests' widths in the model were the same as those measured by Koenders.

- Based on the Horton equation, the infiltration parameters of InfoWorks, were employed. According to the Horton equation, the infiltration rate is calculated as follows:

$$f_t = f_c + (f_0 - f_c).\, e^{-kt} \tag{4}$$

where f_t is the infiltration rate at time t; f_0 denotes the initial infiltration rate or maximum infiltration rate; f_c represents the constant or equilibrium infiltration rate after the soil has been saturated or the minimum infiltration rate, and k stands for the decay constant specific to the soil.

Akan (DHI, 2015) suggested Horton initial (HI) values for different types of soil varing from 7.6 to 152 [mm/h]. In InfoWorks, the default values of 2[mm/h] and 4 [1/h] were selected as Horton initial and Horton decay (HD) values, respectively. To decrease the simulated values of discharged CSOs volume, the Horton initial value was increased to 10-35 [mm/h] and Horton decay was decreased to 0.5 –1 [1/h] in each catchment separately.

- The initial loss value in the rainfall–runoff model of InfoWorks was also used. The initial loss is amount of rainfall needed to make a catchment wet before the beginning of runoff. The size of the flood peak is affected by the initial loss. The initial loss of permeable paved areas, impermeable paved areas, and flat roofs was changed to 2–7 mm in InfoWorks.

- The pump capacity was the final factor. Since the accurate pump capacities were unknown, the pump capacities of five selected pumping stations were changed to the maximum ± 20% of actual capacity.

Simulation of rainfall before the installation of sensors at CSOs

For rainfall periods in which no CSO discharges (inclusive detention tanks) were indicated in the sewer model simulation or by HydroNET (CSOs of detention tanks), the model was not modified.

If the sewer model simulation indicated a CSO discharge while HydroNET measurements did not, the model was modified to decrease the CSO discharges to zero.

Simulation rainfall after the installation of sensors at CSOs

In this case, the sewer model was modified to limit the discrepancy between simulated and measured values to Factor 2, based on monitored data of 5 selected CSOs.

3.3 Water quality model

Using a hydrodynamic model, it is possible to predict the temporal and spatial variability of FIB concentrations in a study area, thereby avoiding the high expenses and long times required for the laboratory analysis of water testing. In addition, the model can be applied in situations in which water sampling is not feasible (Loucks & van Beek, 2017). Furthermore, modelling can predict the water quality following different management strategies, such as the construction of new urban canals or the effect of decreased sewer overflow discharges.
To visualise the contribution of contaminant sources and biological processes properly, the hydrological components should be correct.

In this study, the researcher focused to simulate water quality of Breda canals in a model. The model performance was evaluated by comparison of sampling results and simulated values.

3.3.1 Selecting a model concept

For this project, it was decided to set up a 1D model using SOBEK version 2.16.003, which was developed by Deltares and was used by the water boards in the Netherlands as a standard hydrological model. 1DWAQ module is linked automatically to SOBEK-Urban, which allows users to determine water quality and water fraction in an integrated rural–urban context (Deltares, 2017). The investigation results of few municipalities, such as Amsterdam and Den Bosch, also indicated that SOBEK can be an appropriate model to simulate the FIB dynamics of urban water (Waterschap De Dommel , 2016; van den Tillaart, 2017).

Water quality analysis of Breda canals indicated that *E. coli* provides more certainty to evaluate the water quality of Breda canals; thereby, only *E. coli* dynamics was simulated by SOBEK in this study.

3.3.2 Hydrological model of Breda

For this thesis, the researcher used the hydrological model that the water board of Brabantse Delta set up in 2012 as the basis of the water quality model. However, this model simulated the whole water system, which is under supervision of Brabantse Delta Waterboard. The 1D flow model is illustrated in Figure 9.

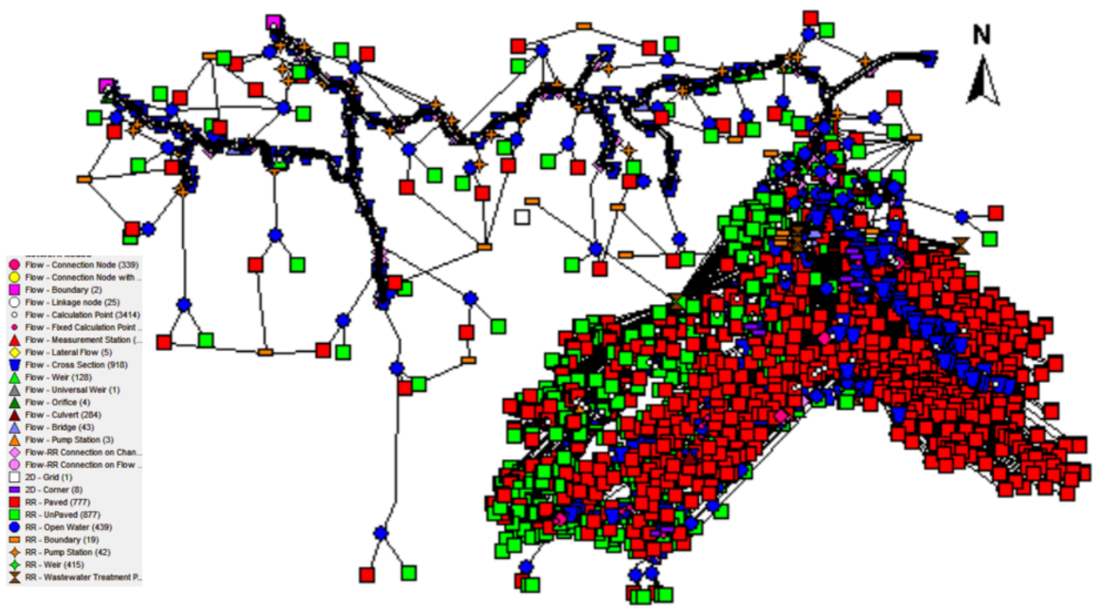

Figure 9 - 1D flow model of the whole water system, which is under supervision of Brabantse Delta Waterboard.

3.3.3 Boundaries

To simplify the model and to decrease the simulation time, the Brabantse Delta flow model was cut off, and five boundaries were defined. To develop an accurate model, downstream boundaries were placed where the stream discharge is measured hourly by Brabantse Delta Waterboard. These consist of five boundaries:

- the Weerijs boundary, at a measured point of stream discharge of Brabantse Delta after the converging of the Turfvaart and Bijloop streams (see Appendix K);
- the Mark boundary, at a measured point of stream discharge of Brabantse Delta before the converging of the Bavel and Chaam streams to the Mark River (see Appendix K);
- the Molenleij boundary;
- the Harbour boundary at Tolbrug; and
- the Belcrum boundary adjacent to Location 1 (sampling point).

At the boundary, all active substances enter into the model area from the "outside world." The final model results may be significantly affected by boundary conditions (Deltares, 2017).

Although the harbour section was not schematised in the original model, to simulate the water quality, this section was added. Since the cross-section was not the same along this section, an average value was chosen. Figure 10 shows the flow model set up for this thesis to simulate the water quality of Breda canals.

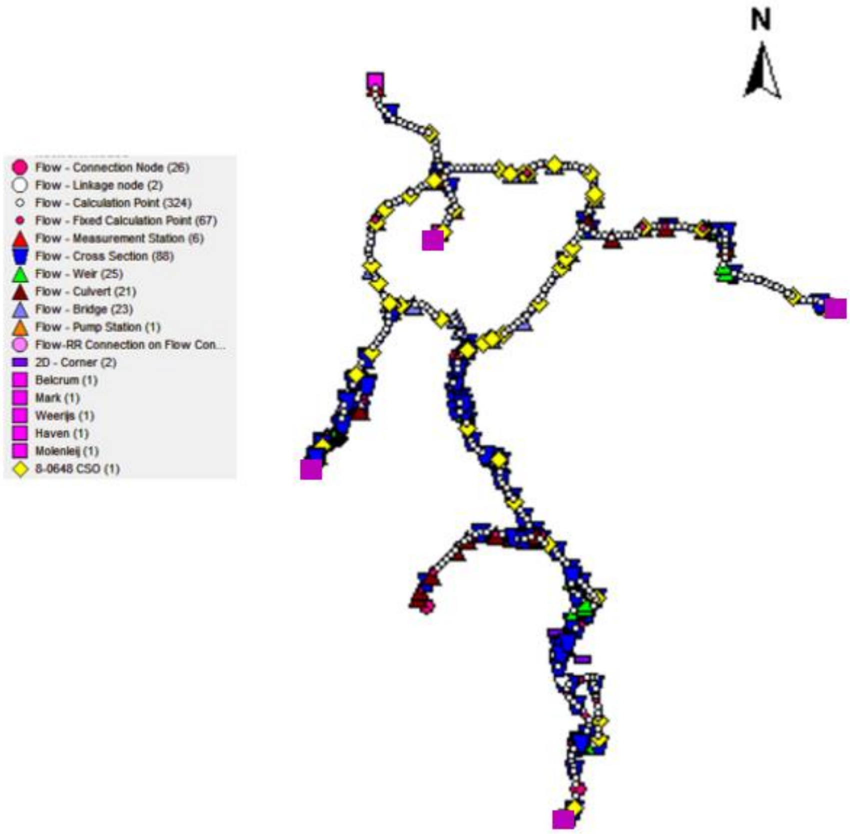

Figure 10 -1D flow model set up for this thesis to simulate water quality based on the Brabantse Delta Waterboard model. In this model, five new boundaries were defined.

3.3.4 Runoff

In the rainfall- runoff (RR) module of SOBEK, the runoff is calculated by simulating precipitation. There are two types of runoffs as the result of precipitation: runoff from rural areas (such as pasture and agriculture) and runoff from paved surface areas. The paved surfaces can be connected to sewer systems or watercourse. Since the concentrations of contamination of these sources differ, it is important to model these sources separately.

The paved surface areas of the Brabantse Delta hydraulic model and the Breda sewer model for the sewer catchment areas of 2, 7, and 8 were compared. The result indicated a 33% discrepancy, thus affecting the calculated runoff volume. In addition, the FIB concentrations of agriculture and pasture runoff vary, whereas the rural area details were not available by Brabantse Delta Waterboard.
Due to insufficient data over type of rural area and uncertainty of how paved and unpaved area were distributed in the Breda sewer model and of hydraulic model of waterboard, was decided to make a model based on the available measurement series of sewer overflows of the Breda sewer model, and only two modules of 1Dflow and 1DWAQ in SOBEK were used. It was assumed all paved surface areas are connected to sewer system.

3.3.5 Wastewater treatment plant effluent

Mark upstream received effluent of four WWTPs of Merkplas, Zonderingen, Hoogstraten, Meer and two WWTPs of Baarle-Nassau, and Chaam located in Belgium and the Netherlands, respectively. Weerijs upstream received effluent of two WWTPs of Brecht and Loenhout located in Belgium. The impact of WWTPs on water quality was considered in the model as initial *E. coli*.

3.3.6 Model input

Water discharge of boundaries

The simulation period of model was derived from flow data at all five boundaries for two periods in the summer from July 10, 2018, to September 30, 2018, and in the winter from January 1, 2019, to February 17, 2019.

For the Weerijs and Mark boundaries (at a distance of 1.5 km and 4.5 km from the sampling locations of 4 and 5), the average daily stream discharge (hourly measured by Brabantse Delta) was filled in 1DWAQ. However, the measured location of the Mark was placed before the convergence point of the Chaam and Bavel streams. These streams were estimated as 2.5% of measured flow based on the ratio of connected surface areas. The harbor section is a dead end and can only be fed by sewer overflows of the harbour. The Molenleij discharge was during bathing season of 2018 zero. Therefore, at Molenleij and the harbour, the constant value of 0 was selected. For the Belcrum boundary as a downstream boundary, the available hourly water level at Achteremer (measured by Brabantse Delta) at a distance of 2 km from the boundary location was filled in.

Sewer overflows discharge

All overflows that had a direct influence on the upstream and the study area water quality within 1,000 meters distance were modelled in SOBEK. The overflows were divided into CSO and SSO categories, with subdivided groups ''route'' and ''exc.''. The ''route'' group was related to overflows located in the study area and ''exc. ''group to overflows outside the study area. Upstream Location 5 included 8 CSOs and 3 SSOs; 2 CSOs and 3 SSOs were modelled at upstream Location 4, as well as 28 CSOs and 9 SSOs in the study area. In total, 38 CSOs and 14 SSOs were simulated in SOBEK. Based on suggested *E. coli* concentration of sewer overflows of various studies, which presented in Table 1, *E. coli* concentration of 1* E^{10} [CFU/m^3] and 1*E^8 [CFU/m^3] were selected for CSO and SSO, respectively.

Initial *E. coli* concentration

The initial E. coli concentration at the Mark and Weerijs streams' boundaries were unknown due to the absence of measuring points at these locations. By considering decay rate as a rough estimation, it was assumed that the initial *E. coli* loads at the boundaries of both the Mark and Weerijs were one log higher than the measured *E. coli* concentrations at Locations 4 and 5.

Decay rate

The decay rate in SOBEK was calculated based on Mancini Equation 3 in this paper. The decay rate can be set up based on salinity, irradiation at the surface of the water, the mortality rate by radiation, and the temperature coefficient. Various studies demonstrated that water temperature and solar radiation have the most impact on the *E. coli* decay rate (Majedul Islam, et al., 2018; Verbyla, et al., 2019; Hijnen, et al., 2007). However, solar radiation may have lower effect compared to the temperature because of the climate condition of the Netherlands.
Due to lack of input data was decided to model the decay process using constant value of mortality rate based on water temperature of samples and various studies, which presented in Table 5.

Therefore, constant values of 35 [g/m^3] (based on measured values of Brabantse Delta Waterboard at Locations a and b), 200 [W/m^2], and 1.07 [-] were selected as salinity, the irradiation at the surface of the water, and the temperature coefficient, respectively. Based on measured temperature of water samples; 21$^{°C}$ and 5$^{°C}$ in summer and winter period, the mortality rate of 0.8 and 0.14 was applied in the model for summer and winter period, respectively.

It should be taken into account that default value for water temperature in SOBEK is 15$^{°C}$.

3.3.7 Fraction calculation

A fraction calculation allows one to label the origin of the water in the water system, which helps to track the contaminant sources in a water system. To set up a water quality model, performing a fractional calculation is a basic step, which provides insights into the water balance, thereby permitting a better understanding of the model results. The fraction calculation is carried out via the water quality module in SOBEK (Royal Haskoning DHV, 2015). The representation of a fraction is relative to the total. A fraction of 0.05 for sewer overflow at a specific location means that 5% of the water source at this location has contributed to the particular sewer overflow.

The water fraction was calculated separately for two periods in the summer from July 10, 2018, to September 30, 2018, and in the winter from January 1, 2019, to February 17, 2019. Although the first samples in summer were taken on July 25, 2018, and in winter on January 29, 2019 (see Appendix D), to simulate the water balance accurately, the analysis period was selected earther than first sampling dates.

As the next step, the discharge of boundaries and sewer overflows were imported to SOBEK. For the Weerijs and Mark boundaries, the hourly stream discharges (measured by Brabantse Delta) were filled in 1DWAQ. However, the measured location of the Mark was placed before the convergence point of the Chaam and Bavel streams. These streams were estimated as 2.5% of measured discharge based on the ratio of connected surface areas. At Molenleij and the harbour, the constant value of 0 was selected. For the Belcrum boundary as a downstream boundary, the available hourly water level at Achteremer (measured by Brabantse Delta) at a distance of 2 km from the boundary location was filled in.
The calculated discharge of each overflow for simulated rainfall events by sewer model, as mentioned in section 3.2, was imported to SOBEK as a flow in function of time. As mentioned in section 4.2.3, the sensor at CSO V indicated a discharge due to clogging. For this event, at CSO 13-0724, the sensor data were imported to 1DWAQ.

3.3.8 *E. coli* calculation

By considering the WWTPs locations, decay rate, and high water age due to low stream discharge during research period, and insufficient data over upstream rural area was decided to apply impact of WWTPs contaminations and rural area runoff only by initial *E. coli* factor at Mark and Weerijs boundaries in model.

The *E. coli* source at this model was defined as sewer overflows (CSOs and SSOs) and the initial *E. coli* of upstream flows; the rural runoff and WWTP effluent were neglected.

As the initial *E. coli* value, the constant value of 250 [CFU/100ml] was filled in at the Weerijs and Mark boundaries in dry weather and one log higher than monitoring data of Locations 4 and 5 during wet weather. Based on various studies (see Table 1), the constant values of 1.E10 [CFU/m^3] and 1.E8 [CFU/m^3] were selected as *E. coli* concentration for both group of ''route'' and ''exc.'', CSOs, and SSOs, respectively.
The *E. coli* concentration of SSOs were 2 logs less than CSOs. The *E. coli* load was calculated based on Equation 1. The discharge values were same as those in the fraction calculation section of 4.3.1.

It should be taken into account that the *E. coli* concentration unit in 1DWAQ is [CFU/m^3].

3.3.9 Sensitivity of model

The sensitivity of the model was analysed for both the summer and winter periods based on three criteria:

- The initial *E. coli* concentration at the Mark and Weerijs boundaries;
- *E. coli* concentrations of CSOs and SSOs; and
- decay rate.

The variables combinations are displayed in Table 8.

Table 8 - The variable combinations tested to analyse the sensitivity of model for the summer and winter periods.

Criteria	Period	Mortality rate [1/day]	Initial *E-coli* at Weerijs boundary [CFU/m^3]	Initial *E-coli* at Mark boundary [CFU/m^3]	*E-coli* concentration CSOs [CFU/m^3]	*E-coli* concentration SSOs [CFU/m^3]
Base model	Summer	0.8	Variable (based on monitoring data)	Variable (based on monitoring data)	1.E10	1.E8
	Winter	0.14	Variable (based on monitoring data)	Variable (based on monitoring data)	1.E10	1.E8
***E. coli* Concentrations of overflows**	Summer	0.8	Base model	Base model	1.E12 1.E10 0	1.E10 0 1.E8
	Winter	0.14	Base model	Base model	1.E12 1.E10 0	1.E10 0 1.E8
Mortality rate	Summer	0.8 0.45 0	Base model	Base model	1.E10	1.E8
	Winter	0.14 0.8	Base model	Base model	1.E10	1.E8
Initial *E. coli* concentration	Summer	0.8	1.E8 0	1.E8 0	1.E10	1.E8
	Winter	0.14	1.E7 0	1.E7 0	1.E10	1.E8

3.3.10 Simplifications and assumptions

Initial *E. coli* concentration

For both the Mark and Weerijs boundaries, a constant value of 250 [CFU/100ml] was assumed during dry weather, and one log higher concentrations of monitoring data at Locations 4 and 5 during wet weather. However, it was expected that the Mark upstream location was more polluted compared to the Weerijs upstream location due to higher number of WWTPs discharge and sewer overflows.

Decay rate

The irradiation at the surface of the water and the water temperature were assumed to be constant values in the summer and winter periods, whereas in practice, they vary each day.

E. coli concentration of overflows

A constant *E. coli* concentration was assumed during the discharge event. However, the peak of FIB concentration is observed at the beginning of rainfall events, and the duration of peaks depends on prior rainfall and rainfall intensity. In addition, after a long dry period, FIB can be accumulated and built up in sediments and suspended solids of sewer systems, which will be washed off by rainfall events and enter receiving bodies of water (Pongmala, et al., 2015).

It should be noticed that no degradation or growth of FIB was assumed in the sewer system.

Cross-section of the harbour canal

The harbour section that was added to the SOBEK model had multiple cross-sections. To simplify the model, the average of the cross-section values was selected.

Connected paved surface area to the sewer system

It was assumed that all paved area runoff entered the sewer system. The rural runoff was introduced to model as initial *E. coli* concentration at boundaries due to the absence of details.

4 Results and discussions

4.1 Water quality analysis

This section reports on the temporal and spatial variations of FIB at measuring points of this research and of Brabantse Delta Waterboard, and discusses how rainfall events can affect microbial water quality. Further, the DNA source detection results at five measuring points are presented and over potential water contamination sources of Breda canals is discussed.

4.1.1 Faecal Indicator Bacteria sampling

Since the budget allowed for the analysis of *E. coli* and IE for a total of 40 samples, the results of Brabantse Delta Waterboard's sampling also support this study's findings. The total sampling results are presented in more detail in Appendices F and G.

PH and water temperature were not monitored for all samples. The measured data did not indicate significant variations in PH. Water temperature variation between the summer and winter was up to 17$^{°C}$ (from 4.7$^{°C}$ in winter to 21.2$^{°C}$ in summer). Temperature and sun radiation are main factors determining the decay rate of FIB (Liu, et al., 2006; Majedul Islam, et al., 2018). Therefore, due to the winter season, the decay rate is significantly low, and the behavior of FIB can be different than in the summer season. It is probable that *E. coli* peaks after the same rainfall events in the summer decline more rapidly.
On the other hand, the hydrological conditions of canals in the winter and summer differ. Due to the higher stream discharges, the travel time of contamination in winter can be shorter than in the summer, and the dilution factor can be higher in the spring and winter (Keller, et al., 2014). For example, discharges from the industrial area of Molenleij to Breda canals (see Figure 5) in the summer amount to 0, whereas in the winter, they can be up to 1.7 [m^3/s]. The average discharge of Mark and Weerijs (at Brabantse Delta measuring points) in the winter was up to 25 times higher than in the summer.

Researcher sampling

The samples of researcher were collected on 15 occasions from July 25, 2018, to June 13, 2019, during dry weather and rainfall events at five locations (see Figure 5). Due to a long dry period and low precipitation, the sampling phase took longer than estimated. Table 9 provides an overview of sampling dates and associated precipitation data. The rainfall graphs are presented in Appendix C.

After October 2018, the samples associated with CSOs discharge were taken based on the Koenders online database. However, the provided data were limited to five CSOs, and for the rest of CSOs, it remains uncertain whether discharges occurred.

Table 9 - Sampling dates and associated precipitation data

Date	Rainfall date	Time [hh:mm]	Rainfall [mm]	CSO discharge	Consideration	Analysis
25-7-2018	-	09:00-10:00	-	No	Dry weather	FIB
30-7-2018	-	09:00-10:00	-	No	Dry weather	FIB
10-8-2018	8-8-2018	09:00-10:00	8.8	Unknown	3rd day	FIB
17-8-2018	17-8-2018	09:00-10:00	12.2	Unknown	Day of rainfall	FIB
20-8-2018		14:00-15:00			4th day (weekend)	
27-8-2018	25-8-2018	10:00-11:00	16.95	Unknown	3rd day	FIB
29-8-2018		08:30-09:30			5th day	
31-8-2018	29-8-2018	09:00-10:00	8.7	Unknown	3rd day	FIB
20-9-2018	-	09:00-10:00	-	No	Dry weather	FIB
29-1-2019	27-1-2019	09:00-10:00	10.15	Yes	3rd day	FIB
12-2-2019	10-2-2019	09:00-10:00	33.4	Yes	3rd day	FIB
14-2-2019		09:00-10:00			5th day	
2-4-2019	-	-	-	No	Dry weather	DNA-FIB
6-6-2019	5-6-2019	14:00-15:00	10.2	Yes	2nd day	DNA-FIB
13-6-2019	12-6-2019	09:00-10:00	28	Yes	2nd day	DNA-FIB

At the end phase of research, a DNA source trace was carried out by KWR. The DNA analysis samples were collected on three occasions: one in dry weather as reference situation and two after rainfall with a CSO discharge monitored by Koender's sensors. Besides DNA source trace analysis, the *E. coli*, IE, and total coliforms concentration were measured at sampling locations (see Appendix M).

Brabantse Delta Waterboard sampling

Brabantse Delta Waterboard began to measure *E. coli* monthly starting in 2017 at Locations a and b (see Figure 5), Location a (Julianalaan) located at a distance of 500 m from Location 4 and Location b (Duivelsberglaan) at a distance of 1.7 km from Location 5. This sampling data was used as analysis of Locations 4 and 5.
The interpretation of the monthly *E. coli* measuring points of Brabantse Delta at Locations a and b, was difficult because they were not all sampled at the same date of this thesis sampling. In addition, the sampling date of both locations was different to provide an overview of *E. coli* concentration of both streams simultaneously (the Weerijs and the Mark).

Due to a City Swim event (swim to fight cancer) on September 1, 2019, Brabantse Delta also began weekly monitoring of FIB since June 11, 2019, at three locations, two of which are located at Locations 2 and 3 of this research and third one located near Valkenberg Park at a distance of 700 m from location 3. The Brabantse Delta report is presented in Appendix H.

4.1.2 Temporal and spatial variation of E. coli and IE

Figure 11 illustrates the *E. coli* distribution of total sampling per location during summer and winter in more detail; the box plots of *E. coli* concentration demonstrate, for all locations, a median *E. coli* concentration of 4.5. 10^2 [CFU/100 ml] except at Locations 1 and 2, where the median *E. coli* concentration was one order of magnitude higher at 1,5. 10^3 [CFU/100 ml].
In general, temporal and spatial variations of *E. coli* were observed specially after rainfalls. This finding means that water quality can be degraded during the bathing season by precipitation (depending on the intensity and amount of rainfall), thereby increasing health risks for the events held after rainfall. The sampling results are presented in more detail in Appendices D and E.

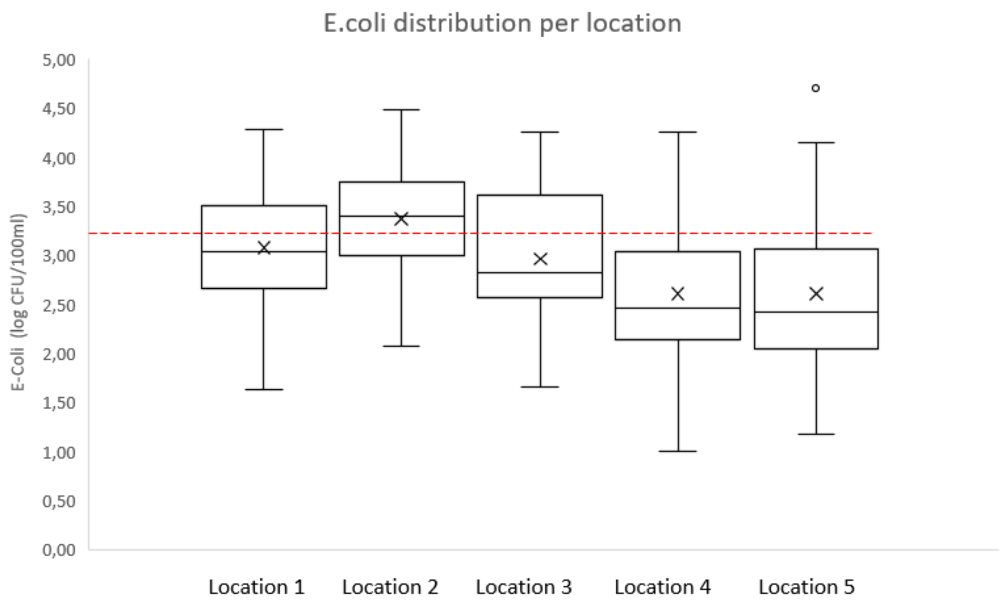

Figure 11- Boxplots of *E. coli* concentration of total sampling per location during summer and winter per unit of CFU per 100 ml (log-transformed with base 10). The horizontal line in each box is the median, and the x in the box represents the mean. The top line of the box represents the median of the top half or third quartile. The whiskers (vertical lines) extend from the ends of the box to the minimum and maximum values, and the wide dots denote extreme values. The red dashed line stands for "Beslisnotitie" guideline value of 1,800 [CFU/100 ml] for *E. coli*.

Figure 12 illustrates the IE distribution of total sampling per location during summer and winter, and the box plots of IE concentration indicate, for all locations, a median IE concentration of 7.1. 10^1 [CFU/100 ml] except at location 2, where the median IE concentration is one order of magnitude higher at 1.41. 10^2 [CFU/100 ml].
In general, the temporal and spatial variation of IE is less than that *E. coli*. Peak values were only observed after intense rainfall of 30 mm and more.

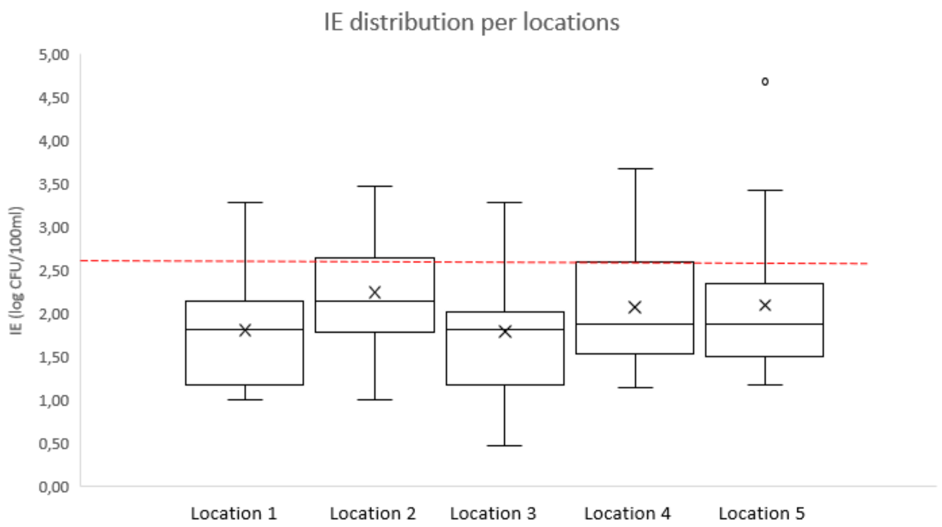

Figure 12 - Boxplots of EI concentration of total sampling per location during summer and winter per unit of CFU per 100 ml (log-transformed with base 10). The horizontal line in each box is the median, and the x in the box represents the mean. The top line of the box represents the median of the top half or third quartile. The whiskers (vertical lines) extend from the ends of the box to the minimum and maximum values, and the wide dots denote extreme values. The red dashed line stands for "Beslisnotitie" guideline value of 400 [CFU/100 ml] for IE.

4.1.3 Analysis of weather conditions

4.1.3.1 Dry weather

The sets of sampling in dry weather indicated a low value of *E. coli* in most measuring locations. An *E. coli* concentration of one log value higher was only observed once at all locations except location 3. However, the *E. coli* concentration only at location 2 and Valkenberg park exceeded ''Beslisnotitie'' guideline value of 1,800 [CFU/100 ml].

The *E. coli* concentration of measuring points in dry weather is presented in Table 10.

Table 10 - *E. coli* concentration [CFU/100 ml] per sample location during dry weather. The results indicate a low value of *E. coli* at most measuring locations. Higher *E. coli* on 25-7-2018 at Location 1 can be due to the water event of "Breda Drijft" on 22-7-2018. A probable explanation for the peak value of Location Valkenberg Park on 11-6-2019 might be the "Nassaudag" event at Valkenberg Park on 10-6-2019.

Date	Location 1 (Belcrum beach)	Location 2 (Nieuwe Mark)	Location 3 (harbor)	Location 4 (Weerijs)	Location 5 (Mark)	Valkenberg park
3-5-2017					120	
22-5-2017				78		
7-6-2017					40	
19-6-2017				120		
5-7-2017					210	
20-7-2017				250		
2-8-2017					40	
22-8-2017				140		
6-9-2017					730	
20-9-2017				110		
4-10-2017					110	
18-10-2017				200		
8-11-2017					690	
13-11-2017				220		
10-1-2018					650	
19-2-2018				210		
19-3-2018				290		
4-4-2018					660	
16-4-2018				270		
23-5-2018				110		
6-6-2018					290	
18-6-2018				130		
4-7-2018					160	
16-7-2018				30		
25-7-2018	1,400	120	710	10	930	
30-7-2018	43	380	130	260	150	
6-8-2018					15	
21-8-2018				46		
5-9-2018					140	
17-9-2018				15		
20-9-2018	370	3,700 (swing sampler) 5,700 (bucket)	46	61	700 (swing sampler) 560 (bucket)	
8-10-2018					160	
9-10-2018				94		
8-11-2018					180	
19-11-2018				15		
12-12-2018				270	1500	
2-1-2019				61	330	
6-2-2019					550	
1-4-2019					61	
2-4-2019	190	1300	300	240	100	
11-6-2019		920	660			4400

The dates on which *E. coli* peak were indicated, no sewage pump failure was reported, and no peak of the water levels at the pump stations was observed.

Higher *E. coli* levels on 25-7-2018 at Location 1 may be due to the "Breda Drijft" event on 22-7-2018 (see Figure 1). The *E. coli* travel time was longer to Location 1 because of the extreme low discharge of the stream. The average discharge of Mark and Weerijs were 0.25 [m^3/s] and 0.1 [m^3/s] in this period, respectively.

Between Locations 2 and 4, there is no SSO, and between Locations 2 and 5, there are four SSOs. Determining the impact of these four SSOs due to illicit connections is difficult because of lack of measuring point between Locations 2 and 5. However, no *E. coli* peak was observed at other sets of sampling in dry weather at Location 2 and downstream Location 1. The sampling on 24-6-2019 after rainfall event of 4 mm also indicated no *E. coli* peak at Location Valkenberg Park (see Table 10). The probability of the effect of only one SSO located within 500 m downstream (between Locations 1 and 2) is also negligible, as confirmed by low *E. coli* values at Location 1 within a distance of 700 m from this SSO. Therefore, the probability of a microbial impact due to illicit connections at Locations 2 and Valkenberg is almost nil.

Higher *E. coli* levels on 11-6-2019 at location Valkenberg Park may be due to the "Nassaudag" event at Valkenberg Park on 10-6-2019 (Whit Monday).

The probable explanations for *E. coli* peak at location 2 on 20-9-2018 and 2-4-2019 might be presence of an accidental pollution source (such as fresh animal feces) at this specific time and location. Another explanation might be the low water velocity and even stagnant water at a few places adjacent to Location 2 caused by the increasing of width of the canal. The samples could be taken by a bucket from areas of stagnant water, leading higher *E. coli* values.

The IE concentration of measuring points is displayed in Table 11. The total sampling in dry weather indicated no IE peak value except at Location 2 on 20-9-2018, at which point IE exceeded ''Beslisnotitie'' guideline value of 400 [CFU/100 ml]. The explanation of the *E. coli* peak on 20-9-2018 can be applied for IE as well.

The peak of IE concentration observed correlates with peak of *E. coli* concentration and is one to two log values lower than the *E. coli* concentration.

Table 11- IE concentration [CFU/100 ml] per sample location during dry weather. The results indicate no IE peak value except at location 2 on 20-9-2018 when IE exceeded ''Beslisnotitie'' guideline value of 400 [CFU/100 ml]. The explanation of the *E. coli* peak on 20-9-2018 can be applied for IE as well.

Date	Location 1 (Belcrum beach)	Location 2 (Nieuwe Mark)	Location 3 (harbor)	Location 4 (Weerijs)	Location 5 (Mark)	Valkenberg park
25-7-2018	10	<10	43	32	87	
30-7-2018	10	43	10	76	32	
20-9-2018	10	700 (swing sampler) 560 (bucket)	15	15	30 (swing sampler) 30 (bucket)	
2-4-2019	45	250	20	36	26	
11-6-2019						270

To check the possible distortion between the bucket and swing sampler methods, extra samples from Locations 2 and 5 were taken at the same moment on 20-9-2018. The results of two methods for both *E. coli* and IE are on same order of magnitude.

4.1.3.2 Wet weather

The interpretation of water quality as it is associated with rainfall events was complicated due to insufficient sampling data, which were taken under different temporal and spatial sampling conditions. As mentioned in section 3.1.3, it was assumed that rainfall less than 12 mm would not lead to a CSO discharge within the study area. To delete the uncertainty of CSO discharges occurring and for the calibration of sewer model, five sensors were installed in October 2018 at five CSOs.

The sampling analysis in this research is divided into three categories: rain events under 12 mm (assumed without CSO discharge), moderate rain events between 12 mm and 25 mm, and heavy rain events more than 25 mm.

Rain events less than 12 mm

In general, temporal and spatial variations of *E. coli* were observed after rainfalls less than 12 mm. The *E. coli* concentration of measuring points in rain events under 12 mm is presented in Table 12.

Table 12- *E. coli* concentration [CFU/100 ml] per sample location in relation to a rainfall event less than 12 mm. *E. coli* peaks were detected frequently at Locations 2 and 5.

Date	Rainfall [mm] [date]	Location 1	Location 2	Location 3	Location 4	Location 5	Location 6 (Galder)	Valkenberg Park	CSO discharge
11-12-2017	11.9 10-12-2017				5700				Unknown
22-1-2018	4.4 21-1-2018				1900				Unknown
7-2-2018	7.3 2-2-2018					1600			Unknown
7-3-2018	4.5 4-3-2018					2100			Unknown
10-8-2018	8.8 8-8-2018	760	1,100	450 420 750	1,100	3,900			Unknown
17-8-2018	12.2 17-8-2018	300	2,500	590	2,200	540 580 500	3,900		Unknown
20-8-2018		1,300	580	520	87	1,000			
31-8-2018	8.7 29-8-2018	580	12,000	580	230	5,400			Unknown
6-6-2019	10.2 5-6-2019	5900	>30000	<100	230	7100			Yes
24-6-2019	4 20-6-2019		480	180				560	No
5-8-2019	10.2 2-8-2019		1700	3800				4100	Yes
13-8-2019	9.7 12-8-2019		3200	4300		3400			unknown

The interpretation of *E. coli* analysis in winter period is difficult because the samples were collected by Brabantse Delta Waterboard only at Locations 4 and 5 on different dates.
The peak value was observed in all samples even at the 4th day after light rainfall of 4.5 mm, that can be due to extreme low decay rate. The *E. coli* concentration was on same order of magnitude at both Locations 4 and 5 (rainfall events of 4.5 mm). However, sampling were carried out on 2nd and 4th day of rainfall events, respectively.

E. coli peaks were detected frequently at Locations 2 and 5 in the summer period.

Two rainfall events of 8.7 mm on 8-8-2018 and 29-8-2019 had almost the same impact on water quality on the 3rd day. The *E. coli* concentrations exceeded ''Beslisnotitie'' guideline value of 1,800 [CFU/100 ml] at Locations 2 and 5.
On 8-8-2018, 8 mm of rain was collected in 3 hours. By considering the pump capacity of 0.7 [mm/h], 0.3 mm would be over the sewer storage capacity; therefore, no CSO overflows were expected, and the detention tanks sensors indicated also no CSOs discharge. Whereas *E. coli* concentration was one log higher at Location 2, 4 and 5. But, the *E. coli* concentration exceeded ''Beslisnotitie'' guideline value only at Location 5. The explanation for different *E. coli* concentration at Locations 4 and 5 can be higher discharge (5 times) and a greater number of overflows at Mark upstream.

On 10-8-2018, two extra samples from two points within 20 m and 40 m distance from Location 3 were taken to investigate variations of FIB across the length of the canal. As with Location 3, two extra samples from two points within distances of 7 m and 14 m from Location 5 were taken from the bridge to investigate variations of FIB in terms of the width of the canal. The variations of both the *E. coli* and IE concentrations were not significant. These samples were taken to investigate variations in FIB as related to the length and width of the canal, and to control bias, since a sample was taken from an exact point in each location on each occasion; thus, any bias related to selecting near these locations was eliminated.

On 31-8-2018, *E. coli* peak was detected at Locations 2 and 5, which *E. coli* concentration at Location 2 was one log higher than Location 5. The probable explanations for *E. coli* peak at Location 2 on 8-8-2018 and 31-8-2018 might be presence of an accidental pollution source (such as fresh animal feces) at this specific time and location. Another explanation might be the low water velocity and even stagnant water at a few places adjacent to Location 2 caused by the increasing of width of the canal.

In contrast to the assumption that there was no CSO discharge for rainfall events of less than 12 mm, on 6-6-2019 (at 9.6 mm cumulative rainfall until moment of CSO discharge), and 10 mm on 2-8-2019 and 12-8-2019 a discharge was detected at CSO I and CSO V, followed by the *E. coli* peak at Location 2. It indicated that the sewer system of Breda, especially near Location 2, can be vulnerable to rainfall events under 12 mm. This finding can be caused the frequent peak value at Location 2. Nevertheless, the CSO discharge due to the light rainfall of 6-6-2019 is questionable. The explanation might be failure of the pump station 3-0013 (see Figure 7), where a high water level was indicated in the HydroNET database, which could have led to a CSO discharge at CSO I. In addition, no water level was measured on 5-6-19 at pump stations 2-0044 and 4-0325 (see Figure 7), which could be due to pump failures or sensors' failures.

E. coli concentration after CSO discharge exceeded the guideline value at most locations. The *E. coli* peak at downstream Location 1 indicated the impact of CSO discharges of study area. The interpretation of *E. coli* peak at Valkenberg Park due to adjacent three CSO's discharge is difficult because the *E. coli* concentration was not measured at Location 5. However, no discharge at CSO III was detected.
The explanation for the peak of FIB on 5-8-2019 compared to results on 6-6-2019 with similar rainfall amount might be a highly intense rainfall event of 7 mm in 1.5 hour on 2-8-2019 with minimal three CSO discharges according to sensors' data. Another explanation can be inaccurate results due to the late analysis of the water samples after sampling on 6 June 2019. Within two days, the peak FIB levels had not declined to a low value.

E. coli concentrations of two rainfall events of 10 mm on 5-8-2019 and 13-8-2019 were almost the same on 2nd and 4th day after rainfall, which can be explained by the impact of hydrological conditions of the Mark and Weerijs strems in the summer; longer travel time of E. coli due to low stream discharge.

A sample set within four hours of rainfall of 12.2 mm on 17-8-2018 was taken to determine whether the light rain can impact the water quality of Breda canals rapidly. No peak was observed at Locations 1, 3, and 5. The peak value at Location 2 could be CSO discharges adjacent Location 2, especially CSO V. It should be noted that no CSO discharge was detected by the sensors at the detention tanks.
Since land use in the upstream Aa of Weerijs is largely agriculture (Waterschap Brabantse Delta, 2018), an explanation of the *E. coli* peak at Location 4 compared to that at Location 5 could be the recent spreading of manure and pasture runoff or presence of an accidental pollution source (such as fresh animal faeces) at this specific time and location.
An extra sample at Location 6 at the border of the Netherlands and Belgium within a distance of 10 km from Location 5 was taken on 17-8-2018 to determine the *E. coli* concentration entering the Netherlands. The peak value might be due to four WWTPs' effluent and sewer overflows from

Belgium introduced into the Mark upstream location. An explanation for low *E. coli* value at Location 5 might be the long travel time of *E. coli*.
The result of samples on 20-8-2018 revealed that the peak value returned to the dry weather value within 3 days at Locations 2 and 4. Although, *E. coli* concentration was one log higher at Locations 5 and location 1, the levels were still in compliance with ''Beslisnotitie'' guideline. One can conclude that the water travel time of microbial contamination from the upstream location to reach to Locations 1 and 5 was 3 days, considering the low discharge in such a dry summer.

The *E. coli* peak at harbour was detected by Brabantse Delta sampling, which located opposite from Location 3 of researcher sampling point. The probability of presence of an accidental pollution source (such as fresh animal faeces) at this specific time and location is nil because the peak was observed at both sampling occasions of Brabantse Delta. The explanation might be the low water velocity and even stagnant water at sampling point of researcher.

The IE concentration after rain events under 12 mm is displayed in Table 13. IE concentration exceeded the ''Beslisnotitie'' guideline values less than *E. coli*. After 2 days, IE concentrations were in compliance with ''Beslisnotitie'' guideline in most locations. As with dry weather, the IE concentration was from one to two log values lower than the *E. coli* concentration, which can be explained by previous studies indicating that *E. coli* concentrations of sewer overflows can be to two log values higher than IE concentrations (see Table 1).

Table 13- IE concentration [CFU/100 ml] per sample location in relation to rainfall events under 12 mm. The IE concentration at 3rd day was in compliance with ''Beslisnotitie'' guideline in most locations.

Date	Rainfall [mm] [date]	Location 1	Location 2	Location 3	Location 4	Location 5	Location 6 (Galder)	Valkenberg Park	CSO discharge
10-8-2018	8.8 8-8-2018	87	43	87	690	220			Unknown
17-8-2018	12.2 17-8-2018	65	76	87	900	53 32 76	450		Unknown
20-8-2018		76	65	10	53	110			
31-8-2018	8.7 29-8-2018	15	140	15	64	110			Unknown
6-6-2019	10.2 5-6-2019	210	2000	3	110	90			Yes
24-6-2019	4 20-6-2019		230					290	No
5-8-2019	10.2 2-8-2019		46	45				330	Yes
13-8-2019	9.7 12-8-2019		520	77				2000	Unknown

The IE concentration was less sensitive to rainfall events under 12 mm compared to the *E. coli* concentration. In contrast to peak value of *E. coli* in most locations on 6-6-2010 and 5-8-2019, when CSO discharge was occurred, IE concentration was in compliance with "Beslisnotitie" guideline except at Location 2.

Moderate rain events between 12 mm and 25 mm

Table 14 presents the *E. coli* concentration after rain events between 12 mm and 25 mm, which was assumed that would lead to a CSO discharge within the study area.

Table 14- *E. coli* concentration [CFU/100 ml] per sample location in relation to a rainfall event between 12 mm and 25 mm. An *E. coli* peak was observed on 5th day only at Location 2.

Date	Rainfall [mm] [date]	Location 1	Location 2	Location 3	Location 4	Location 5	CSO discharge
29-11-2017	12.9 27-11-2017					7100	Unknown
27-8-2018	16.95 25-8-2018	1100	1200	4300	370	13000	Unknown
29-8-2018		770	5300	860	130	500	

Based on assumption (see section 3.1.3), the samples were collected at 3rd and 5th day after a rainfall event of 16.95 mm on 25-8-2018. *E. coli* peaks at most location can be due to CSO's discharges within study area. It took 4 days for the *E. coli* peak to decline in all sample locations except Location 2—a known issue at this location. It should be mentioned that scum and oil on the surface of the water were observed during the sampling at this location.

In contrast with *E. coli* peak on 20-8-2018 at Location 5, the *E. coli* peak associated with rainfall of 16.95 mm returned to a low value faster after two days. An explanation might be extreme low average discharge of 0.07 [m^3/s] in period between 25-8-2018 to 27-8-2018 compared to average discharge of 0.48 [m^3/s] in period between 17-8-2018 to 20-8-2018.

The Table 15 displays the IE concentration after rain events within the CSO discharge.

Table 15- IE concentration [CFU/100 ml] per sample location in relation to rainfall events between 12 mm and 25mm.

Date	Rainfall [mm] [date]	Location 1	Location 2	Location 3	Location 4	Location 5	CSO discharge
27-8-2018	16.95 25-8-2018	93	140	130	77	930	Unknown
29-8-2018		15	61	15	30	15	

As in dry weather, IE concentration was one to two orders of magnitude lower than *E. coli* concentration, which can be explained by previous studies indicating that *E. coli* concentrations of sewer overflows can be up to two orders of magnitude higher than IE concentrations (see Table 1). The EI concentration exceeded the "Beslisnotitie" only at Location 5 on 3rd day.

Heavy rain events more than 25 mm

Both rainfall events on 27-1-2019 (at 11.15 mm cumulative rainfall until moment of CSO discharge), and 10-2-2019 led to CSO discharges at CSO I, CSO V, and detention tanks of 905 and 909, as expected. Table 16 presents the *E. coli* concentration after rain events more than 25 mm.

Table 16- *E. coli* concentration [CFU/100 ml] per sample location in relation to a rainfall event more than 25 mm. An *E. coli* peak was observed on 5th day only at Location 2.

Date	Rainfall [mm] [date]	Location 1	Location 2	Location 3	Location 4	Location 5	CSO discharge
2-5-2018	32.6 30-4-2018					12000	Unknown
29-1-2019	29 27-1- 2019	19000	15000	6300	14000	14000	Yes
12-2-2019	33.4 10.2-2019	4800	5800	8700	2800	5300	Yes
14-2-2019		2200	3200	3000	940	530	
14-2-2019 (BD)					1600		
13-6-2019	28 12-6-2019	18000	19000	18000	18000	51000	Yes

Monitoring the water quality of canals after rainfall events followed by CSO discharge took longer until the winter of 2019. For intense rainfall events on 27-1-2019 and 10-2-2019, even after four days, peaks of *E. coli* at three locations were observed. On 29-1-2019, the *E. coli* concentration was one log higher than on 12-2-2019. An explanation might be that it rained continuously from 27-01-2019 until 29-01-2019, whereas the rainfall of 10-2-2019 was shorter.
The 5th day sampling of rainfall on 27-01-2018 was skipped because the sample bottles were not available. The result suggests that, after such intense rains, within 4 days, the probability of health risks due to the peak of *E. coli* remains.

E-coli concentration after intense rainfall on 12-6-2019 was on the same order of magnitude as that of results on 29-1-2019 at 3rd day with rainfall amount of 29 mm, but it should be taken into account those results indicated water quality at 2nd day. An explanation for almost the same *E-coli* level at 2nd and 3rd day might be significant lower decay rate at winter period compared to summer period.

An explanation for low *E. coli* levels on 14-2-2019 at Locations 4 and 5 might be higher average streams discharge of 13.9 [m^3/s] in period between 10-2-2019 to 14-2-2019 compared to average streams discharge of 0.48 [m^3/s] in period between 17-8-2018 to 20-8-2018. Due to the higher discharge, the travel time of FIB was shorter, and the dilution factor was higher.

On 14-2-2019, the *E. coli* concentration of water sampling, which carried out by Aquon at Location 4 was one log higher than researcher water sampling. The grab sample presents the concentration at a moment in time. Therefore, the impact of temporal concentration variability of grab samples due to influence of flow rate should be taken into account.

On 2-5-2018, after rainfall of 32 mm on 30-4-2018, an *E. coli* concentration of 12,000 [CFU/100 ml] was measured by Brabantse Delta, which can be compared with results on 29-1-2019 after similar rainfall event of 29 mm. The *E. coli* concentration was a little higher in winter period but still was in same order of magnitude. This could be due to different decay rate during summer and winter period.

As in dry weather, IE concentration was in correlation with *E. coli* concentration and was one to two orders of magnitude lower than *E. coli* concentration. The major peak values are observed after rainfall events: more than 30 mm. In contrast with *E. coli*, the IE peak associated with rainfall 30 mm returned to a low value faster—at 3rd and 5th day, respectively.

The Table 17 displays the IE concentration after rain events within a CSO discharge.

Table 17-IE concentration [CFU/100 ml] per sample location in relation to rainfall events more than 25 mm.

Date	Rainfall [mm] [date]	Location 1	Location 2	Location 3	Location 4	Location 5	CSO discharge
29-1-2019	29 27-1- 2019	1600	1600	800	1300	2700	Yes
12-2-2019	33.4 10.2-2019	320	250	1500	220	290	Yes
14-2-2019		15	94	350	14	30	
13-6-2019	28 12-6-2019	1900	3000	1900	4700	48000	Yes

The IE concentration after intense rainfall on 12-6-2019 was one order of magnitude higher than IE concentration on 29-1-2019, and 12-2-2019 (with similar rainfall amount) despite the higher decay rate in summer period. The probable explanations for IE peak at Location 5 (considering *E. coli* peak at this location) might be presence of an accidental pollution source (such as fresh animal faeces) at this specific time and location. Another explanation maybe presence of ruminants' faecal bacteria entered the water system by runoff, whereas in the winter period this factor was absent.

It should be taken into account that the investigation of the time needed for the *E. coli* peak to decline after intense rainfall in the summer was based only on available data. This study is thus complicated by two main factors: the water temperature and the higher discharge and velocity of water in the winter season. More research is required to investigate how the water quality is affected by highly intense rainfall events in the summer.

4.1.4 DNA source trace sampling

4.1.4.1 Analysis of weather conditions

At the end phase of research, a DNA source trace was carried out by KWR. Besides DNA source trace analysis, the *E. coli*, IE, and total coliforms concentration were measured at sampling locations (see Appendix M).
The *E. coli* and IE analysis in dry weather and after rain events within a CSO discharge were presented at Table 11 to Table 17.

4.1.4.2 Analysis of pollution sources

The following section was taken from KWR report (KWR, 2019). This report is presented in Appendix M. For human and ruminates sources, representatives from the Bacteroides bacterial group were controlled, which a distinction can be made between humans and ruminants such as possible source. For the detection of birds, the Helicobacter bacteria was analysed. To identify dogs as a source has been focused on DNA in dog cells instead of faecally related bacteria. Faeces from dogs contain many cells from the intestinal wall.

Location 1

After both sewer overflow discharges, the human DNA marker was increased. The dog marker was detected after the second overflow, which suggests the impact of runoff after heavy rainfall of 28 mm on June 12. The absence of the dog marker after the first sewer overflow might be due to late analysis of the water samples after sampling, or lower runoff discharge after rainfall of 11.5 mm on June 5. The DNA marker for ruminants was reduced after the rainfalls compared to the reference data. An explanation might be the dilution of surface water. The birds DNA marker was not detected in any samples.

The results demonstrated sewer overflows with human faecal material as a source of faecal contamination on June 6 and June 13, whereby on June 13 dogs also played a role as a source. Human and ruminants played a role as contamination sources in all samples in the reference situation.

Location 2

After both sewer overflow discharges, the human DNA marker was increased compared to the reference data (dry weather). The DNA marker of dog and ruminant were increased after the second overflow, which suggests that contribution of surface runoff originating from rural areas was increased at this location, which can be confirmed by high *E. coli* concentration at Location 4. The DNA marker for birds was not detected in any of the sample.

The results indicated the sewer overflows with human faecal material as a source of faecal contamination on 6 and 13 June. Besides, ruminants have also a role on June 13 due to increasing rural water source.
Human, dogs, and ruminants had a role as faecal sources in the reference situation.

Location 3

Only after the second overflow, DNA markers of human and ruminant were increased, which suggests the impact of runoff after heavy rainfall of 28 mm on June 12. Increasing ruminants DNA marker contrasted with assumption of dead-end harbour and indicated that the harbour section can be fed by harbour overflows, Mark and Weerijs streams. The absence of the dog and ruminant markers after the first sewer overflow might be due to late analysis of the water samples after sampling, or lower runoff discharge after rainfall of 11.5 mm on June 5. The DNA marker for birds was not detected on any of the data.
The results indicated sewer overflows as a source of faecal contamination on 13 June, in which dogs and ruminants also played a role.

Human and ruminants played a role as faecal sources in the reference situation.

Location 4

Only after the second overflow, increase of DNA marker for humans and ruminants has been detected. On June 6, both DNA markers were lower than the reference value of April 2. This could be due to late analysis of the water samples after sampling on June 6. The dog marker was not found on any of the sample data. The DNA marker for birds was only detected on the reference date of 2 April.

The results showed the sewer overflow as a source of faecal contamination on June 13, in which ruminants also played a role (possibly due to increase of contribution of rural area runoff). Human, ruminants, and birds played a role as contamination sources in the reference situation.

Location 5

After both sewer overflow, an increase in human DNA marker has been detected. Ruminants mark was only increased on 13 June. An explanation might be late analysis of the water samples after sampling on 6 June. The dog marker was found at both overflow moments, but not on the reference date. The birds DNA marker was not found in any samples.

The results illustrated the sewer overflow of 6 and 13 June, and dog faeces, as a source of faecal contamination. The ruminants also played a role as a faecal source on 13 June (possibly due to increase of contribution of rural area runoff). However, the DNA marker for ruminants was reduced after the rainfalls in Location 1. This phenomenon was reported by (Petit & et al., 2017); they have demonstrated that ruminant-associated *E. coli* were isolated in water and sediment near to the pasture site and human-associated *E. coli* was higher at downstream of urban site. Human and ruminants played a role as contamination sources in the reference situation.

4.1.5 Sampling analysis limitations

- The samples reflect the water quality at the moment of sampling and may not represent the daily value. The *E. coli* concentration can meet ''Beslisnotitie'' guideline, whereas it exceeds them in the afternoon (Joosten, et al., 2018). However, for this thesis, the researcher tried to take the samples at the same time on each occasion.
- Compliance of water with FIB criteria does not guarantee the absence of other pathogens and harmful bacteria that can still threaten human health (Schets, et al., 2010; Hofstra, et al., 2019; Sokolova, et al., 2013).
- Concentrations of FIB cannot be accurately measured. Aquon mentioned 30–35 [%] measured uncertainties (Overzicht methoden Aquon, 2019). It should be taken into account that the interpretation of results was based on an FIB analysis of three different laboratories of Aquon, Aquallab Zuid, and KWR, which may have influenced the measured FIB values.
- Occurrence of CSOs discharges were unknown before October 2018, and interpretation of sampling results is difficult. The installation of sensors after October 2018 provided certainty about only five CSOs.
- The investigation of FIB behaviour after intense rainfall events in the summer season due to different decay rates and hydrological condition is not feasible.
- The high value of FIB at Location 2 is questionable. This location may be affected by an unknown, external contamination source.
- The measuring points are limited to five locations due to the available budget for the FIB concentration analysis. Therefore, the researchers could not investigate the water quality in all the Breda canals to identify possible hot spots. For example, Velkenberg Park as an attractive route for recreational activities can be a critical point based on the Brabantse Delta Waterboard results.

4.2 Sewer model analysis

This section evaluates the sewer model of Breda in the context of CSO discharge volumes and discusses the discrepancy between simulated and measured values.

60% of Breda sewer system is of the combined type. The area of each catchment based on the type of sewer system is presented in Appendix B. The catchment areas which directly affect the water quality of upstream and the study area are connected to a combined sewer, with the exception of one part of Catchment 4, from which storm water enters the study area via four SSOs.

To run the water quality model, overflow volumes simulated by the sewer model during the sampling period are used as input; thus, the discrepancy between the simulated and actual discharged CSO volumes should be negligible if one wishes to reach a significant correspondence between the *E. coli* concentration simulated by the water quality model and the measured *E. coli* concentration. To make the modification of the sewer model feasible, the discrepancy between the simulated and actual overflow volumes was confined to Factor 2, as explained in Section 3.2.8.

In the subsequent section that includes the sewer model analysis, the method of sewer model calibration is presented.

4.2.1 Catchment pump operation schedule

The initial simulations indicated that pump operation has scheduled automatically, and municipality had no access to this schedule. The water discharge was not measured at pumping stations; therefore, it was impossible to determine pumps' patterns, especially during rainfall by the Q-H curve. The electricity consumption of each pump at pumping stations was measured. After electricity consumption analysis of each pumping station, it was detected that the pump patterns were not identical at the same water level, which made modifications to the model rather complicated. In practice, the pump operation schedule was frequently adjusted due to maintenance work. The municipality had a notification system that registered most of the failures and maintenance work, but the maintenance projects did not follow a regular plan to apply in a sewer model.

Hence, the modification of the pumping stations was limited to the adjustment of pump capacity to the maximum ± 20% of the actual capacity in InfoWorks.

4.2.2 Rainfall before the installation of sensors at CSOs

The graphs of simulated and measured water levels at pumping stations and CSOs associated these rainfall events are displayed in Appendix J. It should be noted that, from 38 CSOs that affect the water quality of the study area, only data from 10 sewer detention tanks' overflows were measured and submitted in HydroNET.

Rainfall 8-8-2018

The model and HydroNET indicated no CSOs and detention tank discharges except at CSO I. The measured values at 0-0172 illustrated either sensor error or prevented flow to reach the pumping station due to maintenance work. Because the peak water level was indicated at pumping station 1-0187, which received sewage from 0-0172, the second explanation is not valid. At 1-0187, the measured water level was higher than the simulated value. Therefore, CSO discharges could have occurred in catchment 1, which could explain the *E. coli* peak on 10-8-2018 at Location 5.
Since the measured data were not valid at a few pumping stations, and considering the discharge volume of CSO I, the modification of the model was skipped, and the simulated discharged sewer overflows volume were exported to the water quality model directly. The simulated values of CSOs are presented in Table 18.

Table 18- Simulated CSO discharge volumes at 8-8-2018.

Location	InfoWorks [m^3/h]
CSO I	49.38
CSO II	0
CSO III	0
CSO IV	0
CSO V	0

Rainfall 17-8-2018

Whereas no CSO discharge at the detention tanks in HydroNET was indicated, the simulation data confirmed the occurrence of discharges at detention tanks' overflows 913 and 909, as well as at CSO I, CSO II, and CSO III. Therefore, the model was modified to approach 0 discharges. The pump station at 0-0172 had the same dynamic behaviour on 8-8-2018, which could be due to sensor error (see Appendix J). The data of pump 3-0013 were not available. Table 19 displays the simulated values of discharge volumes by InfoWorks before and after modification.

Table 19 - Simulated CSO discharge volumes at 17-8-2018.

Location	InfoWorks (base) [m^3/h]	InfoWorks (modified) [m^3/h]
CSO I	302.85	8.04
CSO II	269.99	0
CSO III	0	0
CSO IV	0	0
CSO V	179.05	0.2

Rainfall 25-8-2018

Whereas no CSO discharge at the detention tanks in HydroNET were indicated, the simulation data confirmed the occurrence of discharges at the detention tanks' overflows of 905, 909, and at CSO I. Hence, the model was modified to approach 0 discharges. Table 20 contains the simulated values of discharge volumes by InfoWorks before and after modification.

Table 20- Simulated CSO discharge volumes at 25-8-2018.

Location	InfoWorks (base) [m^3/h]	InfoWorks (modified) [m^3/h]
CSO I	56.47	0
CSO II	0	0
CSO III	0	0
CSO IV	0	0
CSO V	10.59	0

Rainfall 29-8-2018

Based on the water level at 1-0187 (see Appendix J), it can be concluded that, during the rainfall, the flow of catchment 0 was prevented to reach the pumping station of catchment 1. An explanation could be maintenance work or a pump failure at 1-0187. Similarly, on 8-8-2018, the higher measured water level at 1-0187 and 2-0044 can be an explanation for the *E. coli* peak on 31-8-2018 at Locations 2 and 4, where no overflow was expected after such light rainfall.
Since no CSO discharge was indicated, the simulated results of the base model were directly exported to the water quality model.

4.2.3 Simulations of rainfall after the installation of sensors at CSOs

Two intense rainfall events following CSO discharges were simulated in InfoWorks. The sensor data of five selected CSOs were applied to modify the sewer system to approach Factor 2 regarding the discrepancy between simulated and actual overflow volumes.

Rainfall 27-01-2019

The simulated water level almost corresponded with the measured value except at pumping station 2-0044, where the pump shut off due to maintenance work. Figures 13 through 17 display the graphs of measured and simulated water levels at five selected pumping stations (see Figure 7).

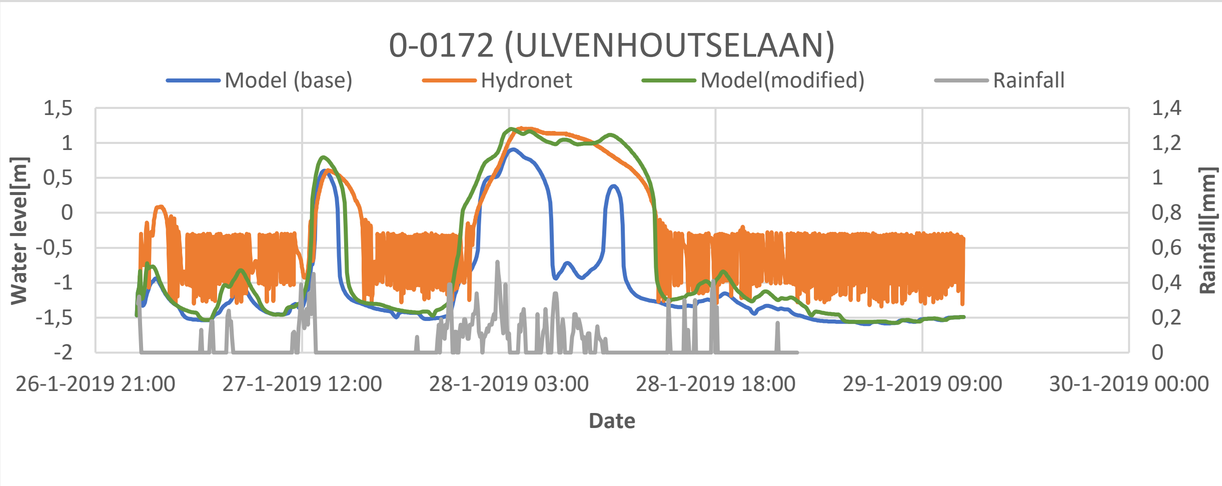

Figure 13- Simulated and measured water levels at pumping station 0-0172. An explanation for higher measured water levels might be operating pumps with lower capacity due to maintenance work. The model was modified by deleting infiltration factors at catchment 0, 1, 8 and 9.

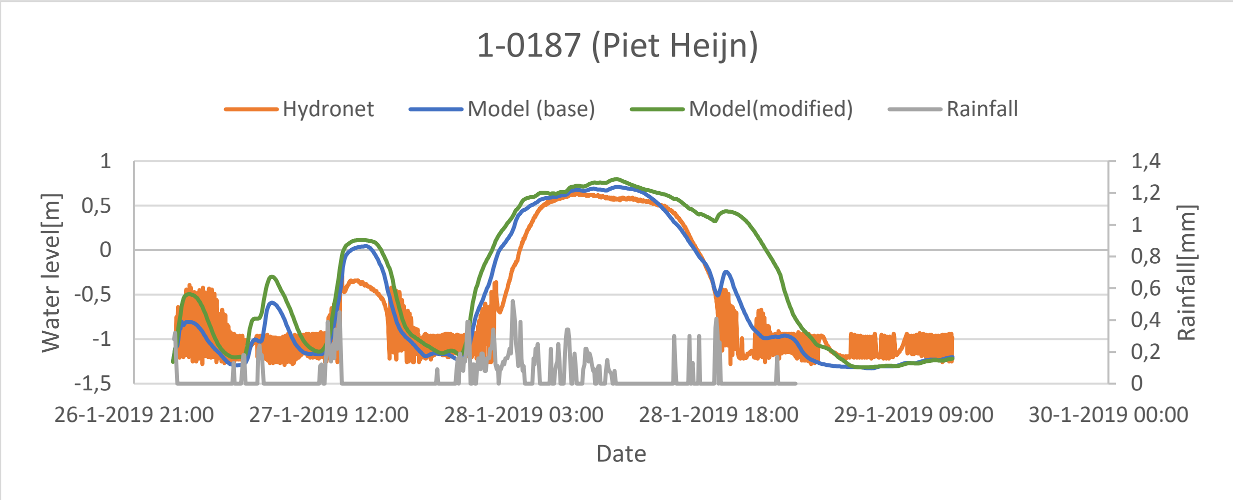

Figure 14- Simulated and measured water levels at pumping station 1-0187. The measured values almost corresponded with simulated values. However, the pump capacity was decreased to simulate CSO I discharge.

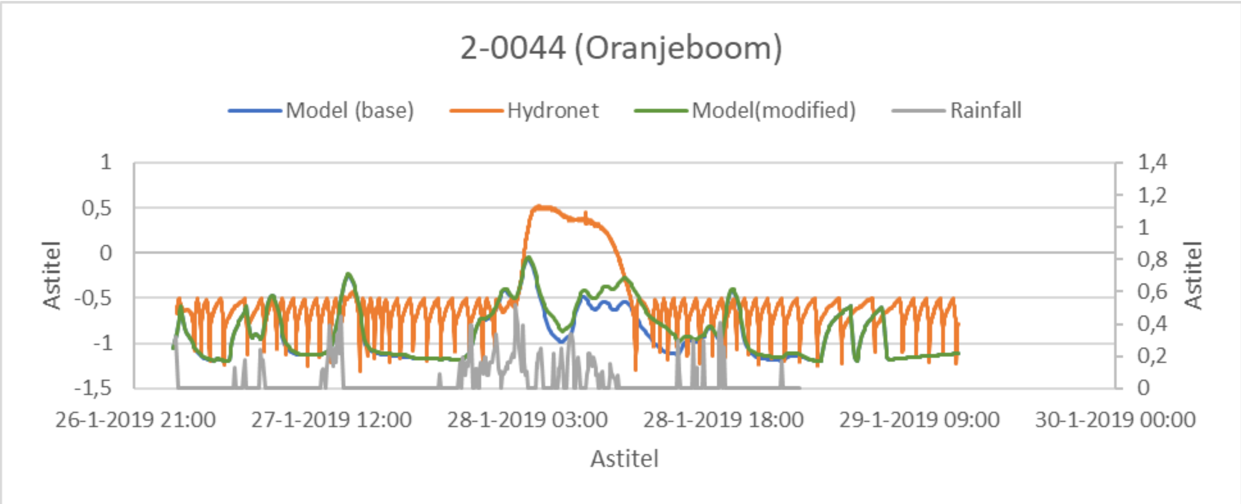

Figure 15- Simulated and measured water levels at pumping station 2-0044. The measured values almost corresponded with simulated values. However, the pump capacity was decreased to simulate CSO I discharge.

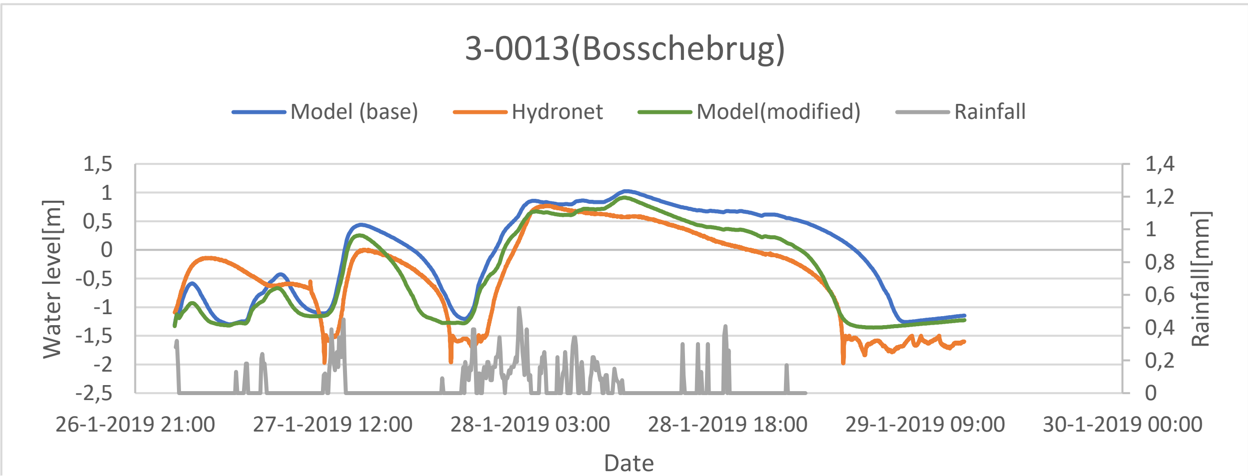

Figure 16- Simulated and measured water levels at pumping station 3-0033. The measured values almost corresponded with simulated values.

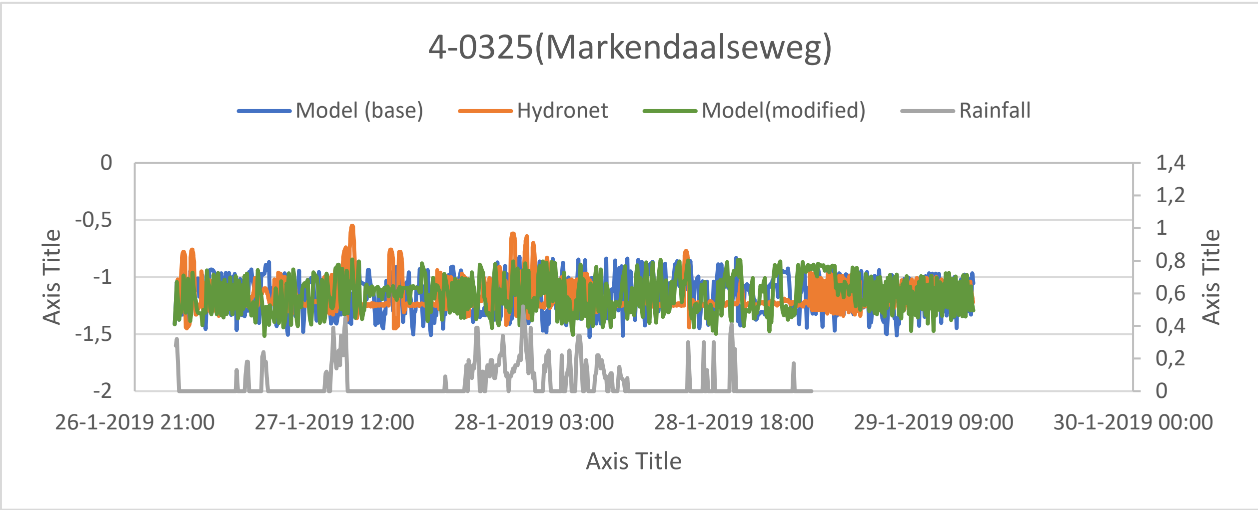

Figure 17- Simulated and measured water levels at pumping station 4-0325. The measured values corresponded with simulated values.

The simulation of the base model did not detect any combined overflow discharge, whereas the sensors identified CSO discharges at CSO I and CSO V. Figures 18 through 22 show the simulated and measured water levels at selected CSOs (see Figure 8).

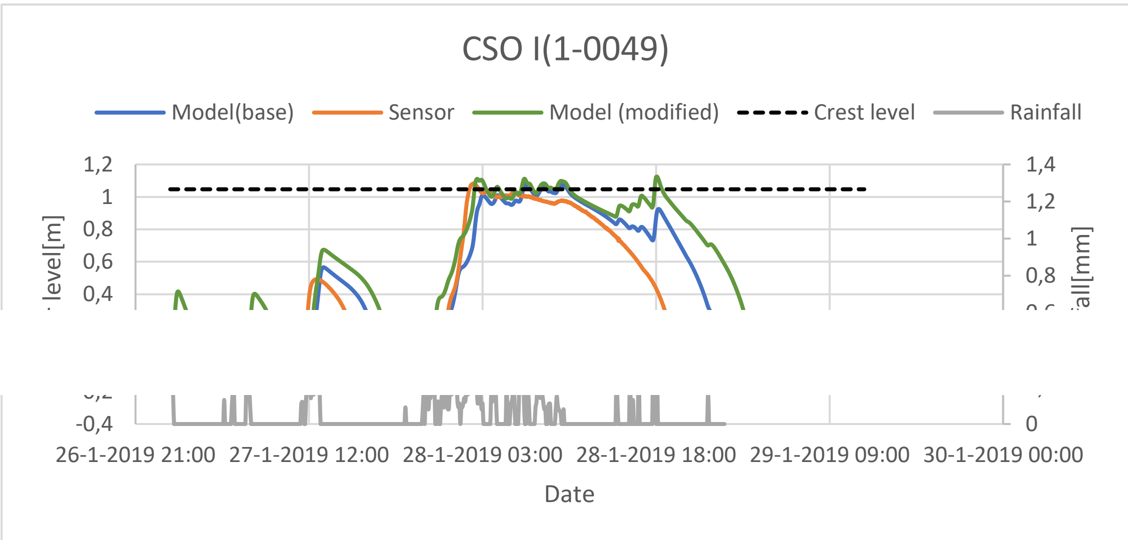

Figure 18 - Simulated and measured water level at CSO I. The model was modified by decreasing pump capacity to approach CSO discharge. The figure illustrates clearly that by increasing infiltration in modified model, the sewer system received water with delay.

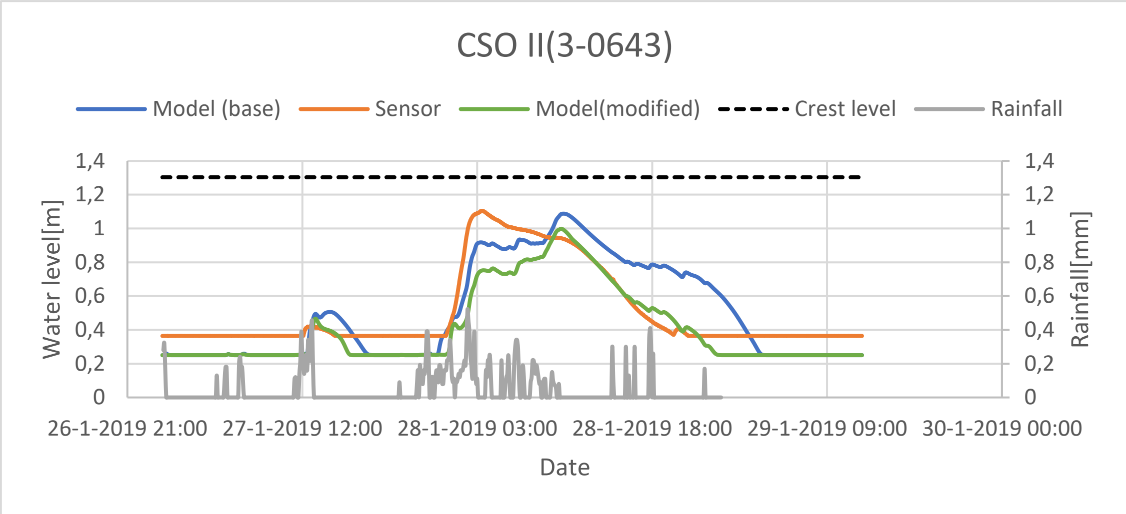

Figure 19 - Simulated and measured water level at CSO II.

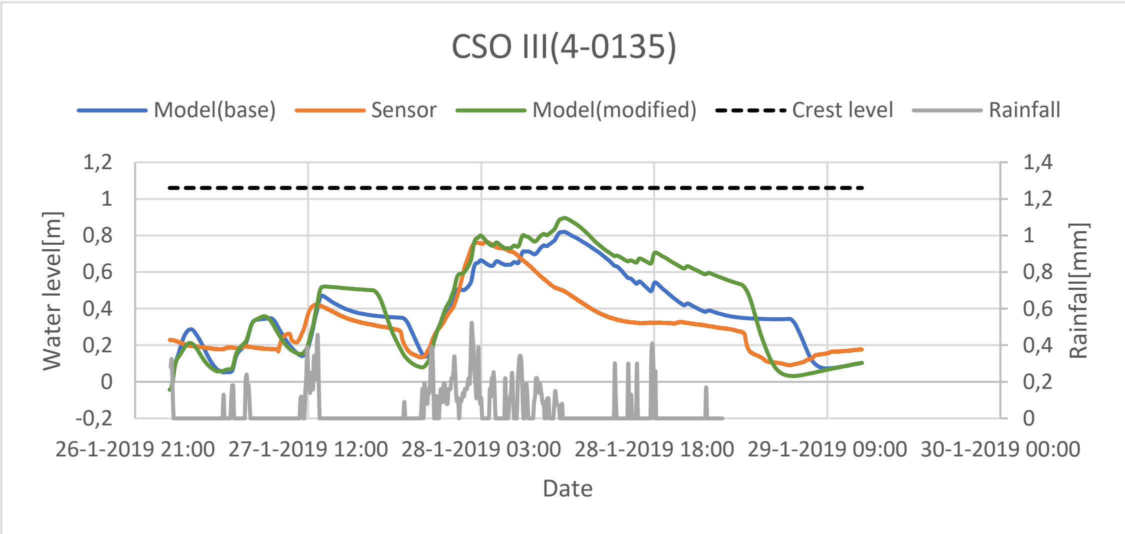

Figure 20- Simulated and measured water level at CSO III.

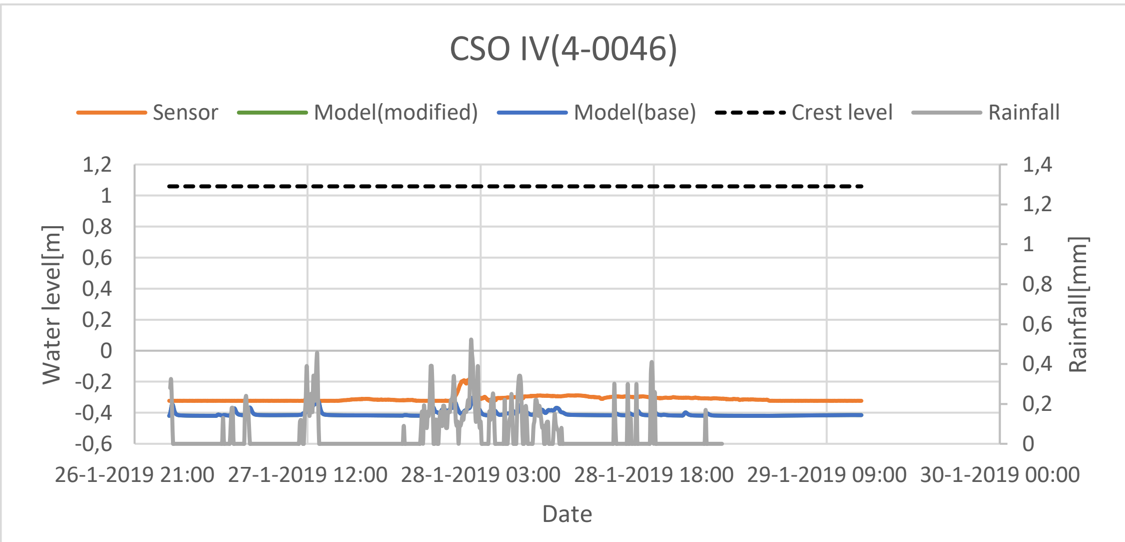

Figure 21-Simulated and measured water level at CSO IV.

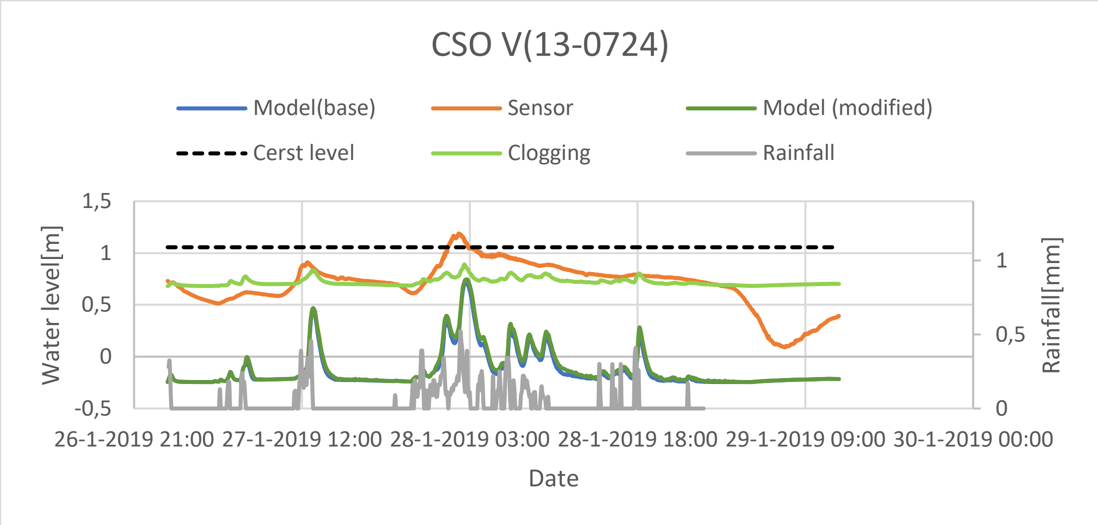

Figure 22 - Simulated and measured water levels at CSO V. The high water levels in dry weather can be due to clogging removed by rainfall. The plot of simulated clogging in InfoWorks almost corresponds with the measured values.

The figures clearly illustrate that, due to increasing infiltration in the modified model, the sewer system received the water with a delay compared to the measured values at CSOs. The sewer model was modified at pumping station 1-0187 by decreasing the pump capacity to obtain an overflow discharge at CSO I.

A total of 7.44 mm of rainfall within 13 hours on 27-01-2019 from 00:00 to 14:00 was registered in HydroNet (see Appendix C). Based on a pump capacity of 0.7 [mm/h], this amount could not cause any CSO overflow. After 5 hours of dry weather, the overflow discharge was indicated at CSO V on 28-01-2019 at 01:00 at 4.5 mm of rainfall in 4 hours, which was not a significant enough amount to cause the overflow discharge. The sensor data illustrated that the water level raised at CSO V in dry weather, and after the rainfall, the water level declined. This behaviour was replicated three times. Figure 23 clearly indicates the water dynamic at CSO V.
Since before installation of the sensor, the clogging was identified at this location, and an explanation for such a water dynamic could be clogging removed by rainfall. To control for this assumption, in the model, the clogging was simulated by removing a conduit to create a disconnection in the sewer system. The water dynamic in Figure 23 illustrates clearly that this assumption can be confirmed. It was decided to skip the modification of the model to approach the overflow discharge at CSO V. The sensors' data were exported directly to the water quality model. On 10-03-3019, a visual inspection was carried out to control for the status of CSO V, but no clogging was observed.

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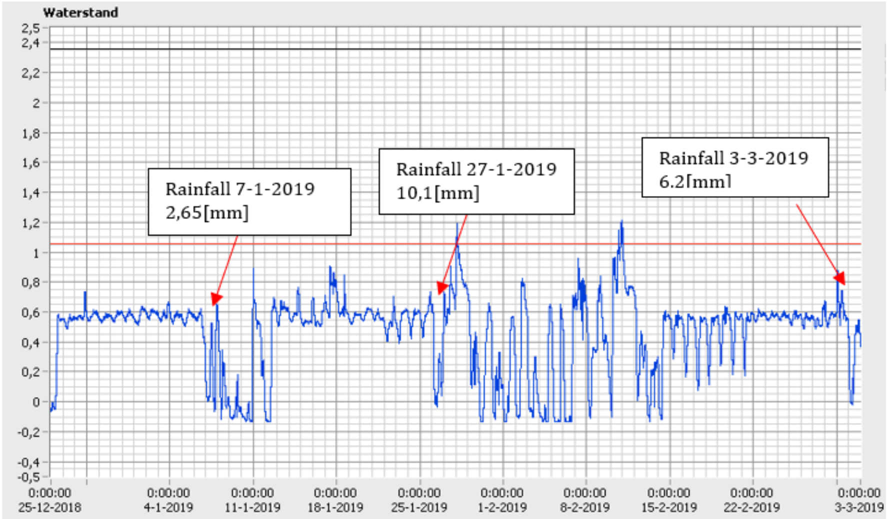

Figure 23 - Measured water level by the sensor at CSO V. The raised water level in dry weather declined after rainfall. An explanation might be light clogging at CSO removed by higher sewer discharges.

Table 21 displays the measured and simulated values of discharge volumes by InfoWorks before and after modification.

Table 21-Simulated and measured CSO discharge volumes on 27-01-2019.

Location	InfoWorks (base) [m³/h]	Sensor [m³/h]	InfoWorks (modified) [m³/h]
CSO I	0	29.19	32.98
CSO II	0	0	0
CSO III	0	0	0
CSO IV	0	0	0
CSO V	0	295.66	0

Rainfall 10-02-2019

The simulated water level corresponded with the measured value except at pumping station 2-0044, where the pump was shut off due to maintenance work. Figures 24 through 28 contain the graphs of the measured and simulated water levels at five selected pumping stations (see Figure 7).

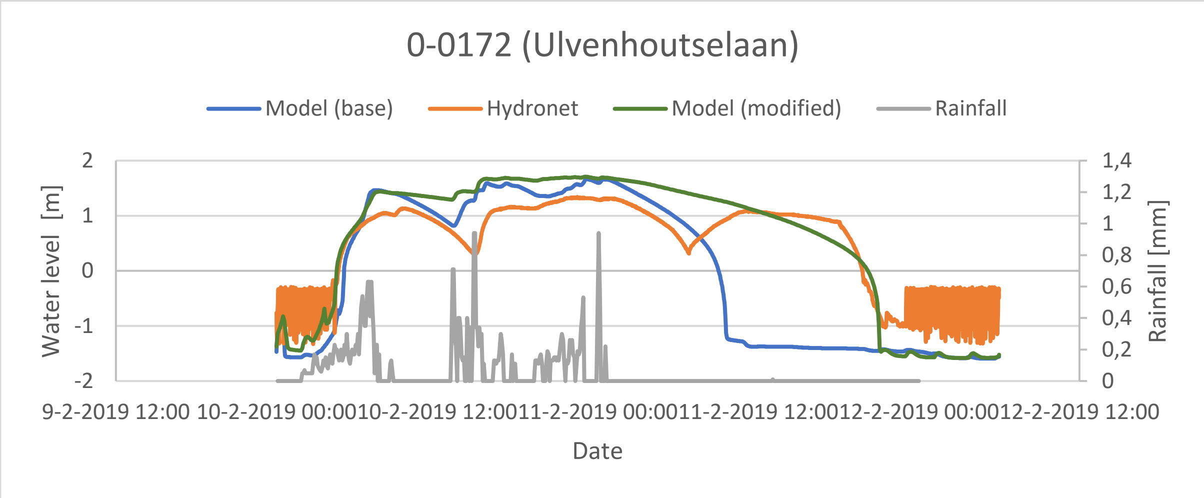

Figure 24 - Simulated and measured water levels at pumping station 0-0172. The pump was shut off due the maintenance work, thereby taking longer to system be emptied. The model is modified by decreasing pump capacity on 0-0172 and increasing Horton factors at catchments 0, 1, 2, 3, 8 and 9.

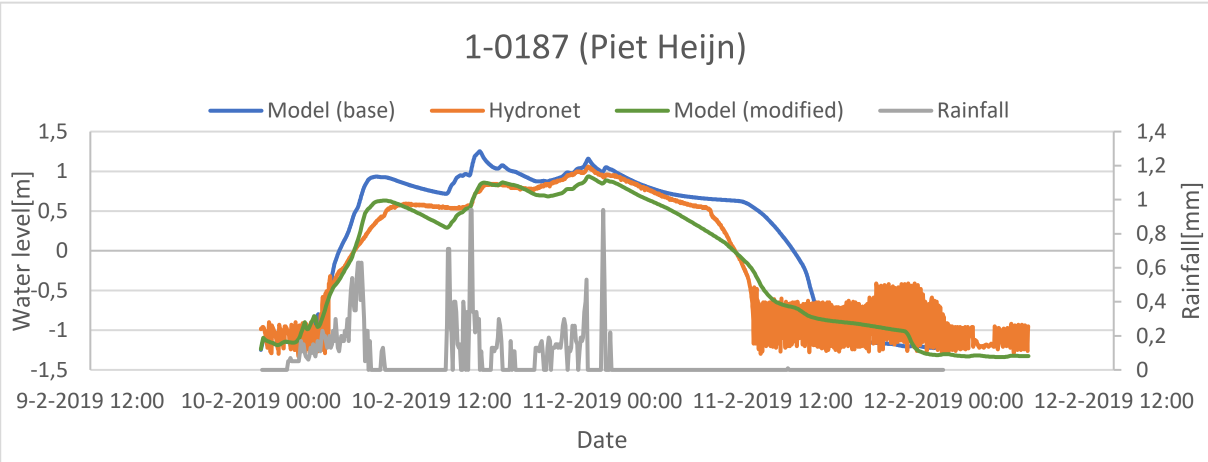

Figure 25 - Simulated and measured water levels at pumping station 1-0187.

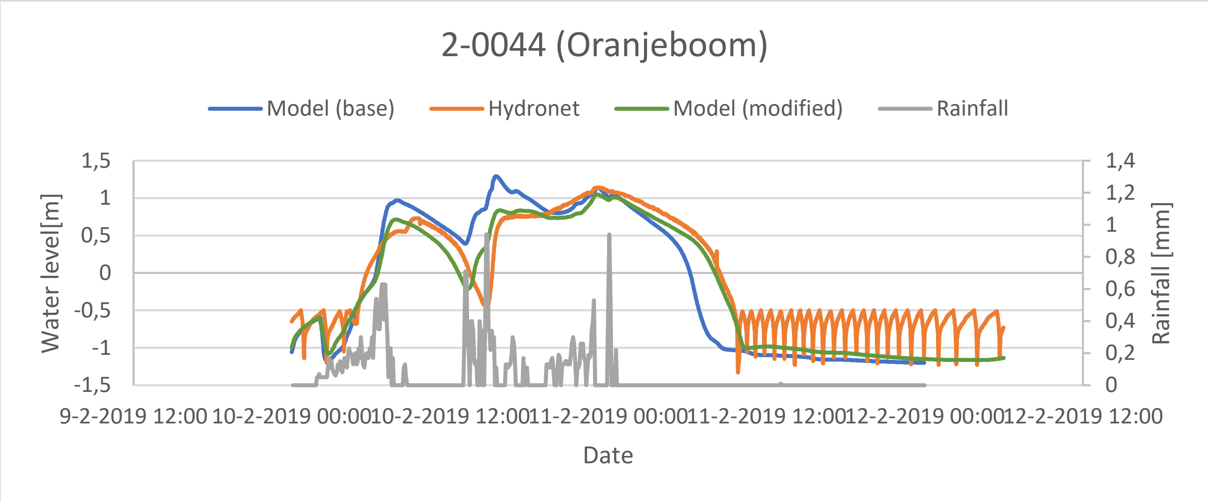

Figure 26- Simulated and measured water levels at pumping station 2-0044.

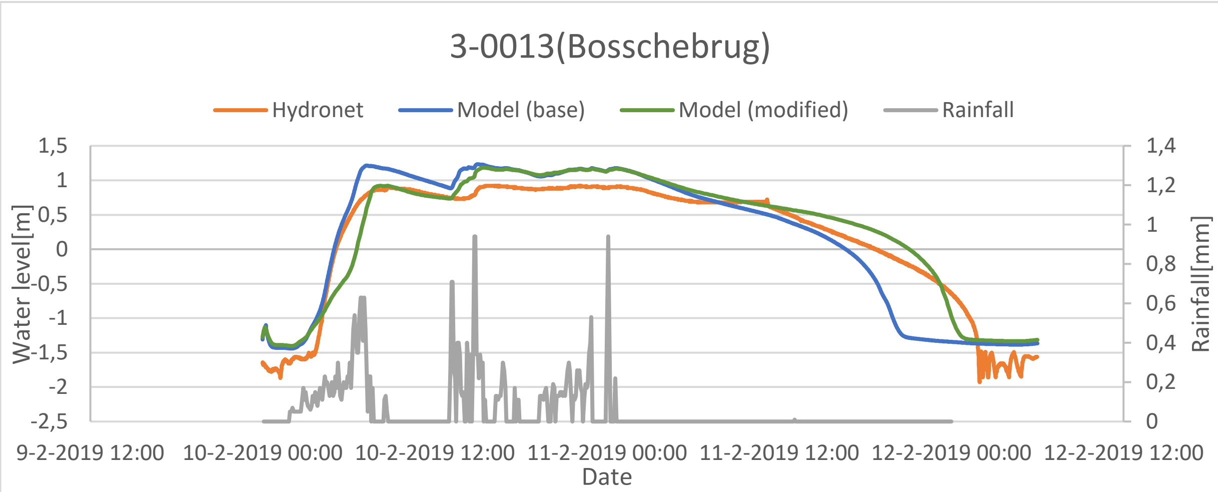

Figure 27- Simulated and measured water levels at pumping station 3-0013.

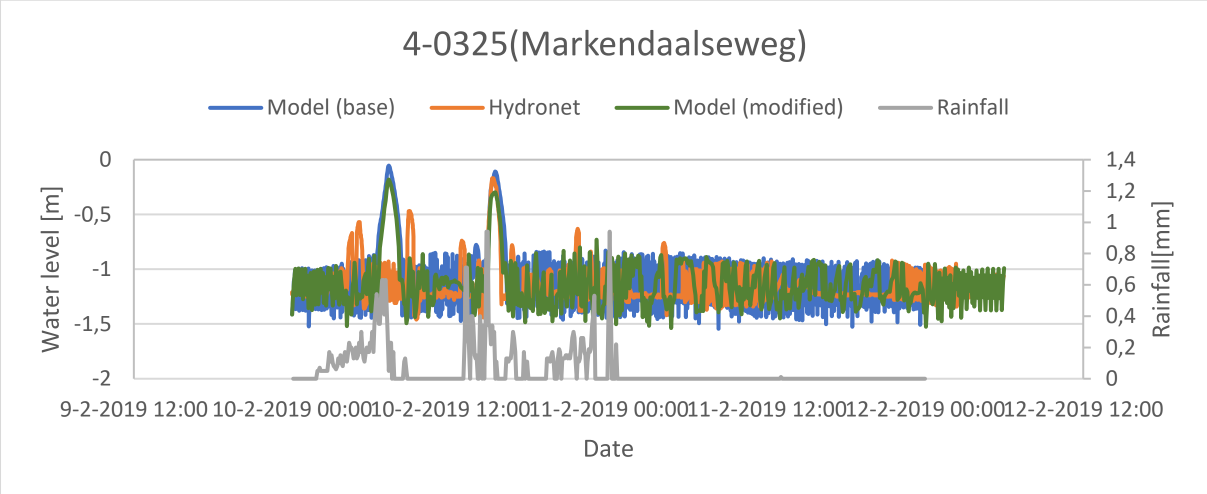

Figure 28-Simulated and measured water levels at pumping station 4-0325.

Through a simulation of the base model, the overflow discharge at detention tanks 902, 913, 909, and 901 was indicated, whereas HydroNET data only revealed discharges at detention tanks 905 and 909 (see Appendix I). Figures 29 through 33 show the simulated and measured water levels at selected CSOs (see Figure 8).
Table 22 displays the measured and simulated values of discharge volumes by InfoWorks before and after modification.

Table 22- Simulated and measured CSOs discharge volumes on 27-01-2019.

Location	InfoWorks (base) [m³/h]	Sensor [m³/h]	InfoWorks (modified) [m³/h]
CSO I	1,007.86	350.31	530.5
CSO II	915.51	0	0.45
CSO III	0	0	0
CSO IV	660.62	0	0
CSO V	802.83	954.55	389.59

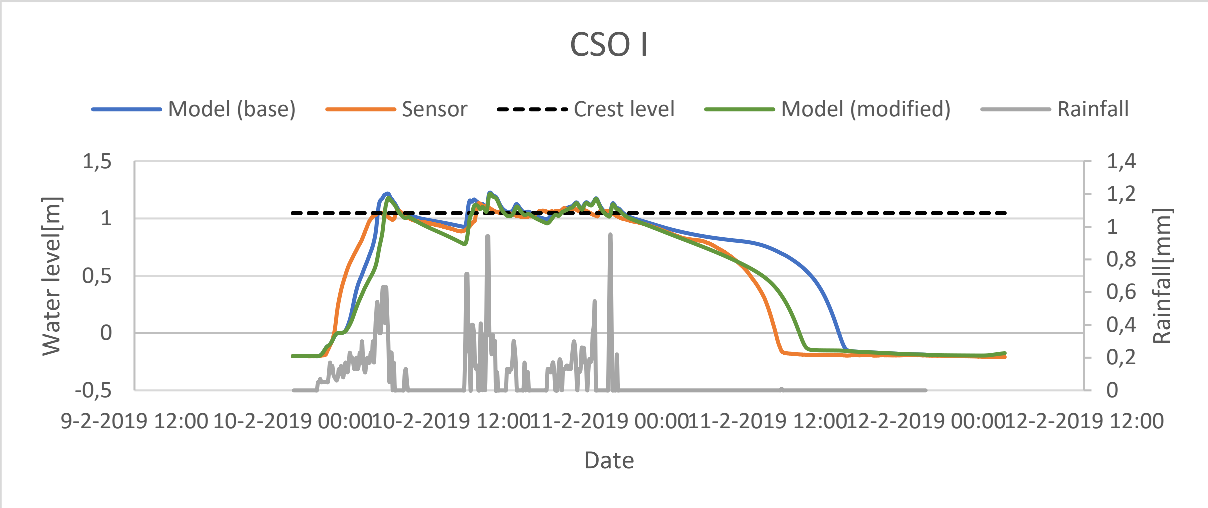

Figure 29- Simulated and measured water levels at CSO I. The model was modified by decreasing pump capacity to approach CSO discharge. The figure illustrates clearly that by increasing infiltration in modified model, the sewer system received water with delay.

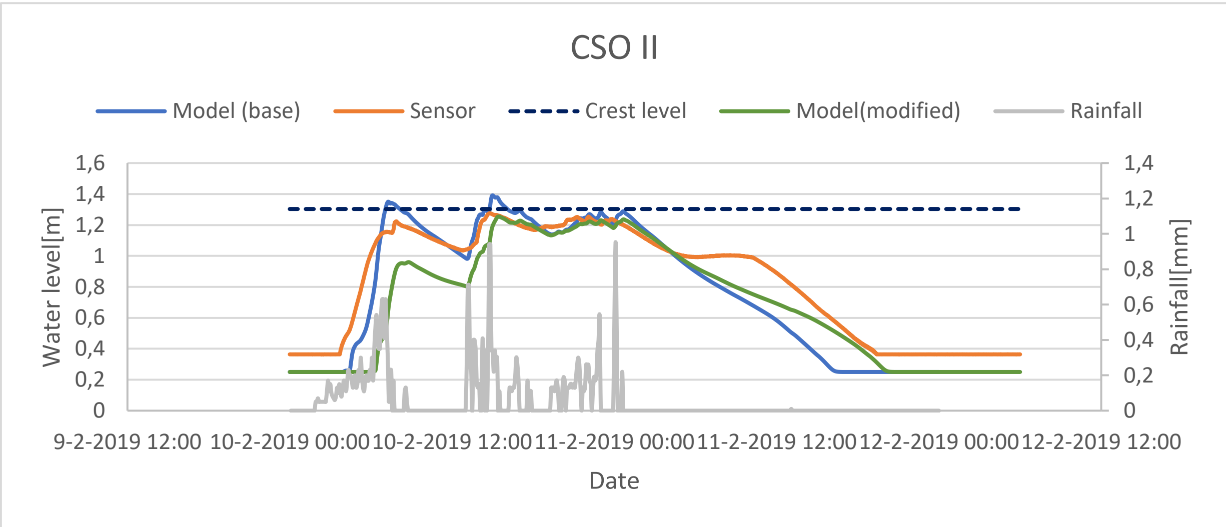

Figure 30- Simulated and measured water levels at CSO II.

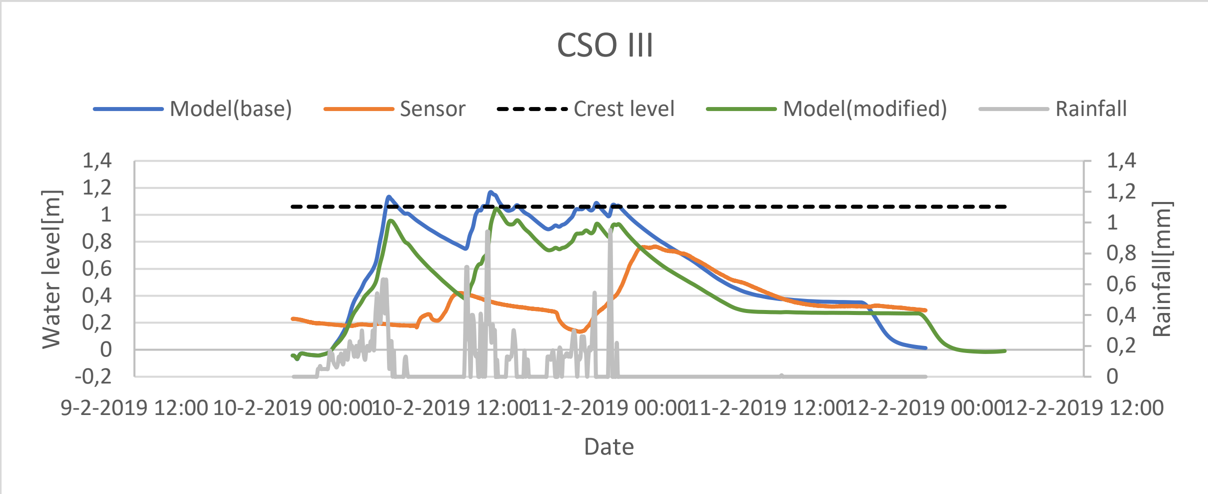

Figure 31-Simulated and measured water levels at CSO III.

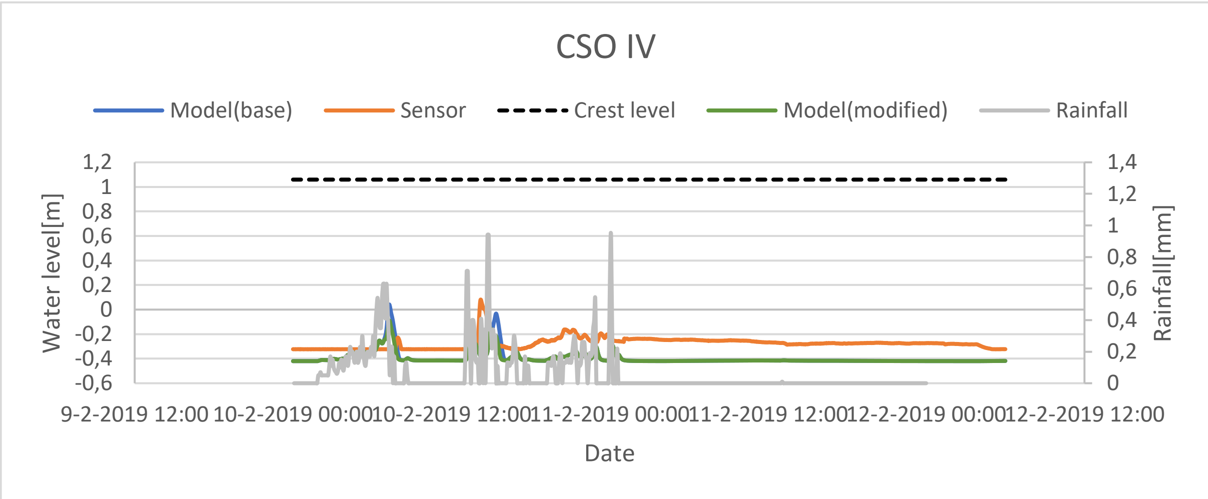

Figure 32-Simulated and measured water levels at CSO IV.

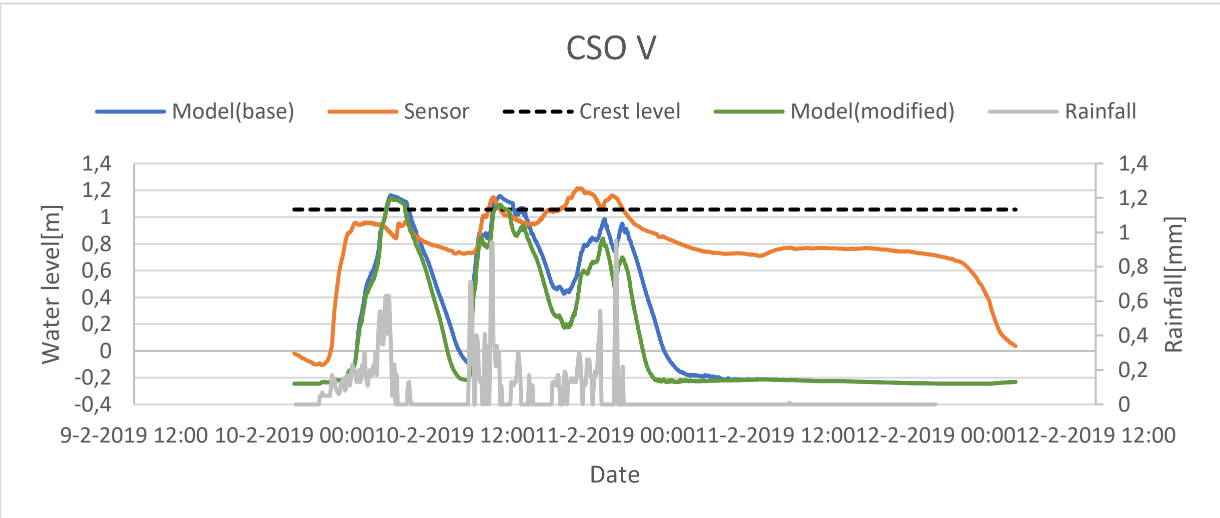

Figure 33-Simulated and measured water levels at CSO V.

4.2.4 Sewer detention tanks

The information from the municipality on sewer overflows was limited to sewer detention tanks' overflows. However, there was uncertainty over the reliability of sensors' data at CSDTs. The sensors' location for all CSDTs were not registered. In addition, after plausible maintenance, the position of sensors can be changed. Since the amount of overflow discharge was not an issue, less attention was to the CSDT sensors' reliability.

An example to support this claim is the detention tank of 905 (see Appendix I) located at catchment 1. The internal weir was deleted from this detention tank; therefore, the sewer pipe directly connected to this CSDT. Two sensors were installed, one in the sewer system within a distance of 300 m and another one at the inlet of the tank. Since the gradient difference is not significant during the peak flow and vacating the tank, both sensors should have indicated almost the same water level due to the fact that they have the same hydraulic pressure. Figure 34 shows the water level at both locations by simulating the rainfall on 10-02-2019. The discrepancy of the water level of these points was less than 2 cm, as expected. However, as Figure 35 illustrates, the sensors indicated a discrepancy of 10 cm, which is considerable, especially when a 1 cm error leads to a deviation of 1.5% on the discharged overflow volume. An analysis of other rainfall data also confirms this argument.

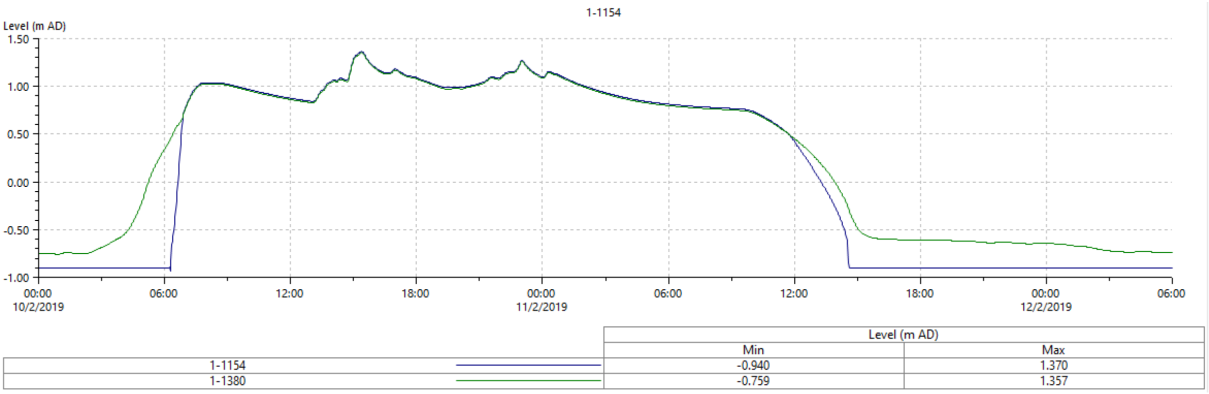

Figure 34- Water level at locations of sensors simulated rainfall on 10-02-2019 by INfoWorks. The discrepancy was under 2 cm since the hydraulic pressure at peak for both is same.

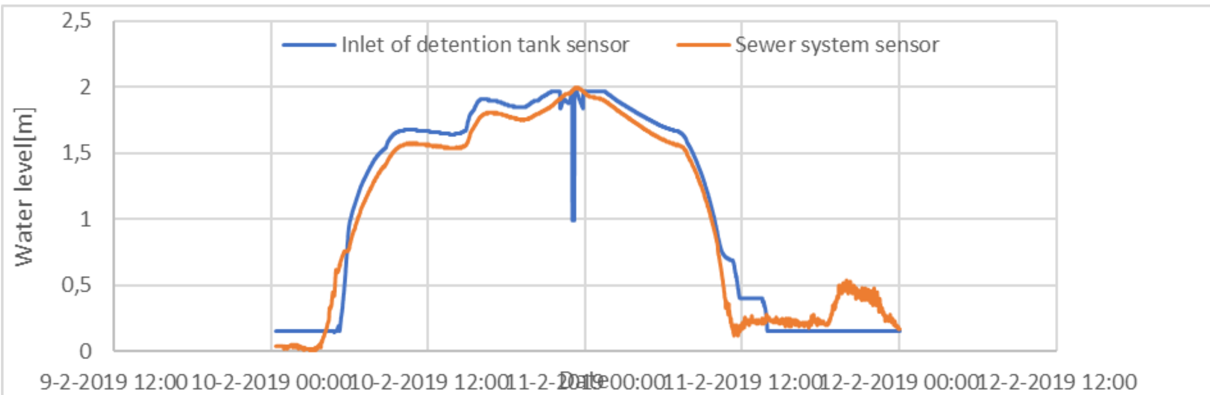

Figure 35- Water levels measured by sensors at both locations of the sewer system within a distance of 300 m and inside the detention tank. The discrepancy is 10 cm, which is considerable. This finding confirms that the level of the sensors might be changed. In addition, an error is detected at CSDT which, can be due the water level reaching a dead zone of the ultrasonic sensor.

4.3 Water quality model

This section reports on the water quality model results and discusses the discrepancy between the sampling results and the simulated values. The simulation period of model was derived from flow data at all five boundaries for two periods in the summer from July 10, 2018, to September 30, 2018, and in the winter from January 1, 2019, to February 17, 2019.
The simulated overflow discharges of modified sewer model in the summer from August 7, 2018, to August 30, 2018, and in the winter from January 27, 2019, to February 12, 2019 were imported to SOBEK.

4.3.1 Water fractions at sampling locations

The water fractions at sampling locations in the summer and winter periods were calculated by the water quality module in SOBEK. Since the contribution of overflows is too low, they are presented separately.

Location 5

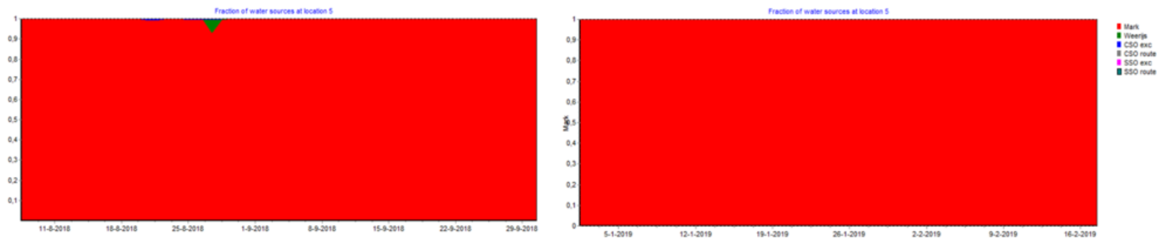

Figure 36 - Water fraction at location 5 in the summer and winter periods. The main source of water at Location 5 was the Mark stream.

As Figure 36 indicates, in the summer and winter, Location 5 was fed solely by the Mark stream. Therefore, only Mark upstream water quality affected the *E. coli* concentration at this site.

Figure 37 illustrates the fraction of overflows at Location 5 in summer period. In total 8 CSO "exc." and 3 SSO "exc." were simulated as upstream overflows of Location 5. The CSOs of the study area upstream had a maximum fractional contribution in the summer period with a value of 1%.

For the rainfalls less than 12 mm, the contribution of CSO "exc." was dominant on 10-8-2018 and 20-8-2018 and, the measured *E. coli* concentration were on the same order of magnitude. The simulated fractions on 20-8-2018 confirmed the water age of 3 days after a rainfall of 12.2 mm on 17-8-2018. The measured *E. coli* values at this location on 17-8-2018 and 20-8-2018 were 520 [CFU/100 ml] and 1,000 [CFU/100 ml], respectively.
The lower fraction of overflows on 10-8-2018 compared to 20-8-2018 can be explained by section 4.1.3.2, which indicated that the sewer system can be vulnerable to the light rainfalls more than 8 mm, thereby underestimating the discharged overflows volumes by InfoWorks.

For the rainfall events between 12 mm and 25 mm, the fractional contribution of route overflows was also indicated. However, the fractional contribution of CSO "exc." was less than the rainfall of 12 mm. The explanation might be that flowrate of Mark was 2 to 10 times lower from 25-8-2018 to 27-8-2018 compared to average discharge in this period.
The high value of *E. coli* concentration of 13,000 [CFU/100 ml] was measured on 27-8-2018, whereas the maximum fraction on 20-8-2018 was measured with an *E. coli* level of 1,000 [CFU/100 ml]. The explanations can be impact of adjacent route SSOs and CSOs on 27-8-2018, less dilution due to lower flowrate of Mark, and underestimate the overflow discharge volumes by InfoWorks on 10-8-2018.

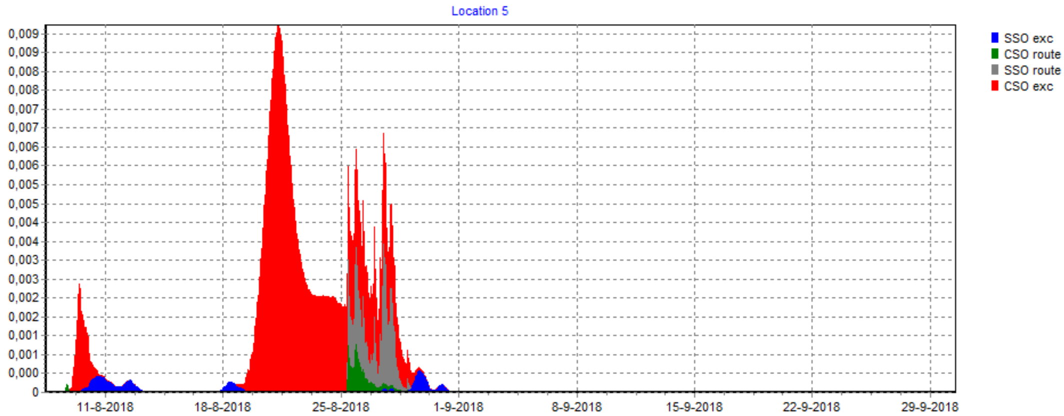

Figure 37- Overflow fraction at Location 5 in the summer period. The CSOs of upstream locations had a maximum fractional contribution of 1%.

Figure 38 illustrates the fractional contribution of overflows at Location 5 in winter period. During the winter period, as in the summer period, the maximum contribution belongs to the CSOs of Location 5 upstream. The fraction of CSO ''exc.'' decreased to 0.3%. An explanation may be the Mark discharge was on average 25 times higher in the winter than the summer period.
The highest *E. coli* concentration of 14,000 [CFU/100ml] was measured on 29-1-2019 when the CSOs ''exc.'' makes the highest contribution to the water system. Although the *E. coli* concentration of 5,300 [CFU/100 ml] was measured on 12-2-2019, but the SOBEK indicated no fraction of overflows. An explanation can be underestimated discharged overflows volume by InfoWorks and higher initial *E. coli* levels entering the water system from Belgium.

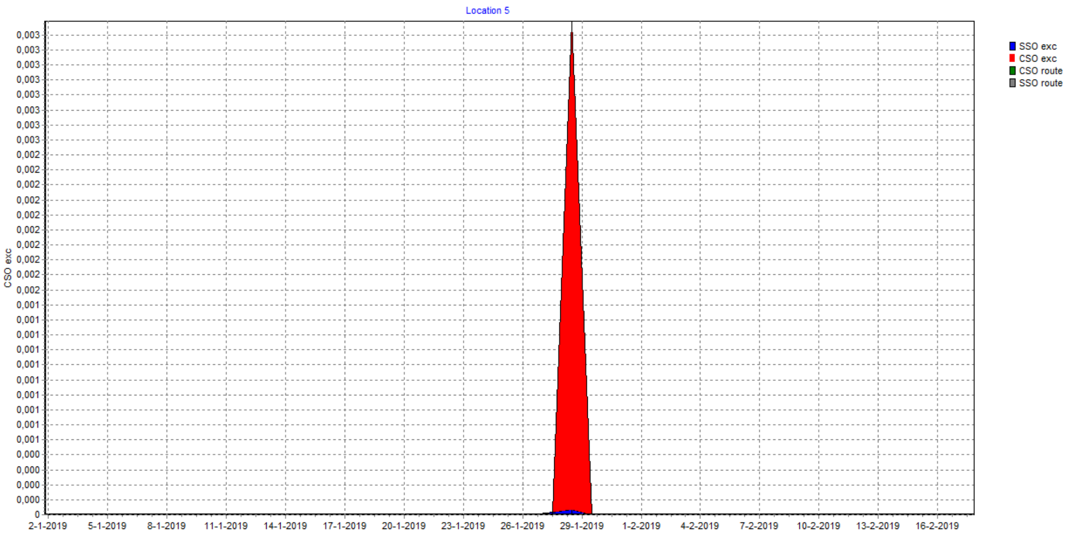

Figure 38 - Overflow fractions at Location 5 in the winter period. Same as summer period, the CSOs of upstream had a maximum fractional contribution of 0.3%.

Location 4

The water fractions of Location 4 in both the summer and winter periods are displayed in Figure 39. The water source at Location 4 was the Weerijs stream, except in the summer period and after rainfall events; at these times the Mark stream provided up to 10% of fractional contribution at this location. Thus, the water quality was only influenced by the Weerijs stream's water quality.

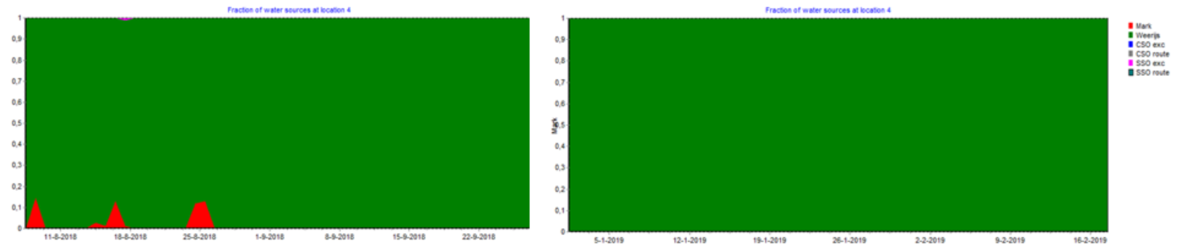

Figure 39 - Water fraction at Location 4 in the summer and winter periods. The main source of water at Location 4 was the Weerijs stream. During the summer period, after rainfall events, the Mark stream had 10 % fractional contribution.

The Figures 40 and 41 illustrate the overflow fractions in the summer and winter periods. In total 2 CSO "exc." and 2 SSO "exc."were simulated as upstream overflows of Location 4. The highest faction was related to SSOs ''exc.''on 17-8-2018 when the highest *E. coli* concentration of 2,200 [CFU/100 ml] was measured, and flowrate was 50% lower than 26-8-2018. In general, upstream SSOs (SSO ''exc.'') had more impact on water quality.
Although the *E. coli* concentrations of 14,000 [CFU/100ml] and 2,800 [CFU/100ml] were measured on 29-1-2019 and 12-2-2019, but the overflows had no fractional contribution at Location 4. A probable explanation might be the underestimation of discharged overflows volumes by InfoWorks.

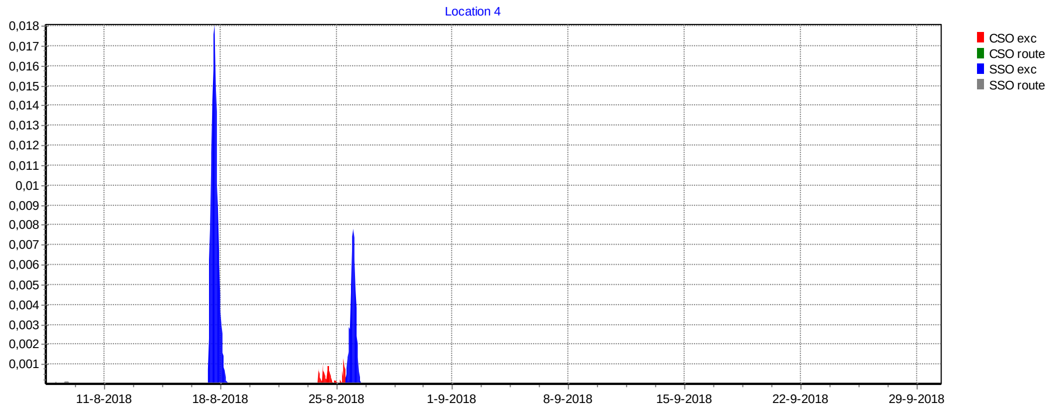

Figure 40 - Overflows fraction at Location 4 in the summer period. The SSOs of upstream had a maximum fractional contribution of 2%.

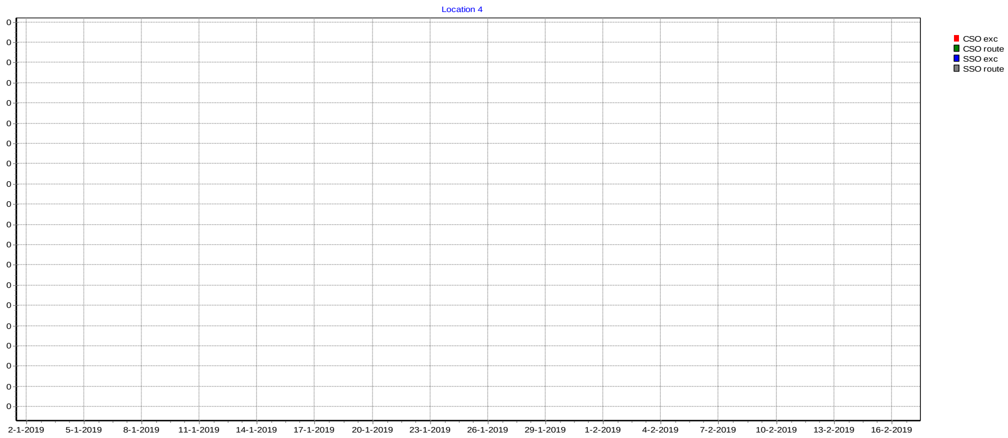

Figure 41 - Overflow fractions at Location 4 in the winter period. The overflows had no fractional contribution at this location.

Location 3

Location 3 as a dead stream had almost constant fractions of 65% and 35% for the Mark and Weerijs streams, respectively. The Mark stream had two times more impact on water quality compared to the Weerijs stream.

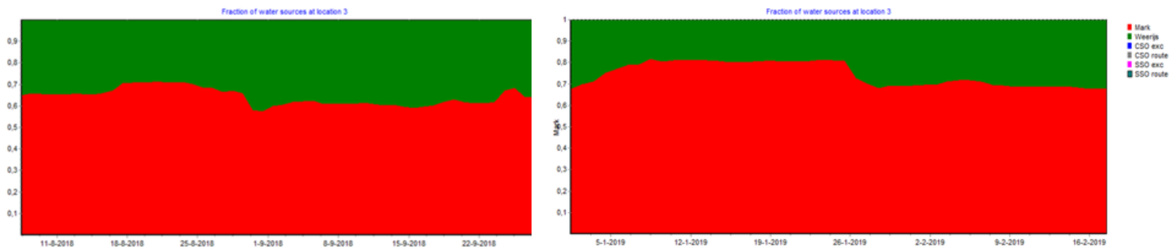

Figure 42- Water fraction at Location 3 in the summer and winter periods. Location 3 as a dead stream had almost constant fractions of 65% and 35% for the Mark and Weerijs streams, respectively.

The fractions of overflows at Location 3 in the summer and winter periods are presented in Figures 43 and Figure 44. The maximum fractional contribution of the SSO route of 0.3% and 0.2% were found in the summer and winter periods, respectively whereas the simulated discharges of 2 SSOs and 1 CSO of harbour were zero. The fractional contributions of overflows from outside of the study area were 0.2% and 0.1% during summer and winter period, respectively.
The highest *E. coli* concentration of 4,300 [CFU/100 ml] was measured on 27-8-2018, when all the overflows had a simultaneous impact on the water system. The rest of the *E. coli* concentration had an average value of 400 [CFU/100 ml]. The *E. coli* concentrations of 6,300 and 8,700 [CFU/100 ml] were measured on 29-1-2019 and 12-2-2019, respectively, whereas no fractional contribution of overflows was indicated on 29-1-2019. An explanation can be the wrong modelling of the harbour area in InfoWorks, as mentioned in section 5.3, or an underestimation of discharged overflows volume by InfoWorks. Since the harbour has a dead stream, the fractional contribution of overflows carried on longer than other locations. However, the fractional contribution of overflows contrasts with the assumption of dead-end harbour.

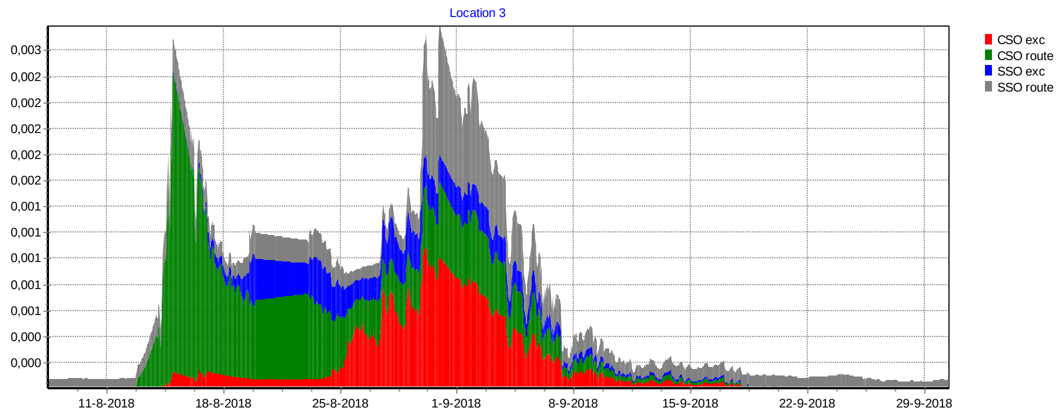

Figure 43 - Overflow fractions at Location 3 in the summer period. The SSOs of study area had a maximum fractional contribution of 0.3%.

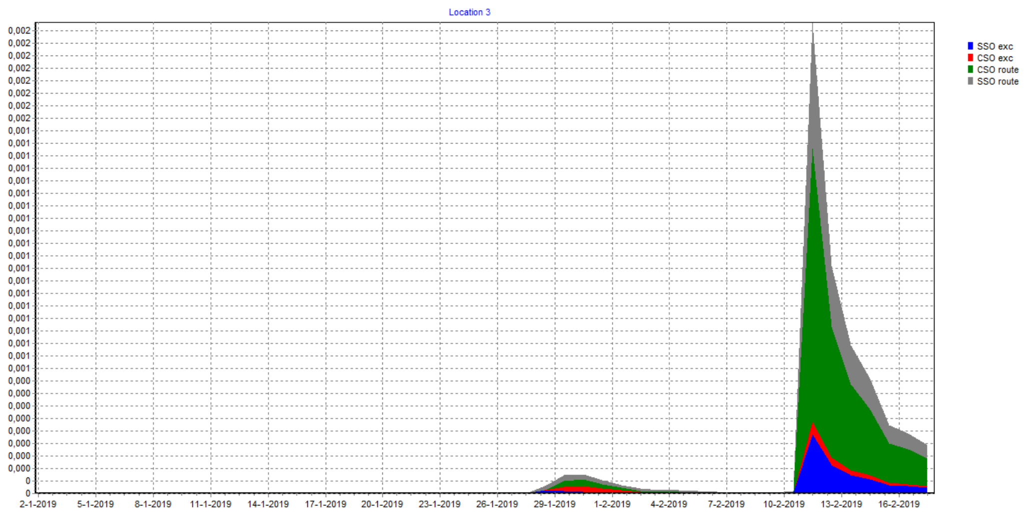

Figure 44 - Overflow fractions at Location 3 in the winter period. The SSOs of study area had a maximum fractional contribution of 0.2%.

Location 2

During the summer period, the Weerijs stream had an average fraction of 60%, and the Mark stream had a 40% contribution, whereas in the winter period, this ratio was reversed. Figure 45 displays the water fractions at Location 2 during the summer and winter periods.

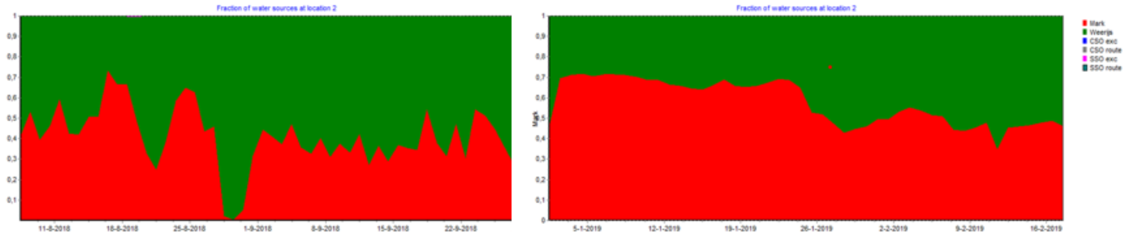

Figure 45- Water fractions at Location 2 in the summer and winter periods. The main source of water at Location 2 was the Weerijs stream in the summer period and, during winter period, the Mark stream.

Figure 46 illustrates the overflow fractions in the summer period, in which SSOs had the highest fractional contribution of 0.5% on 20-8-2018 with a measured *E. coli* concentration of 580 [CFU/100 ml]. The maximum *E. coli* concentration of 12,000 [CFU/100 ml] was measured on 31-8-2018.

In the winter period simulation, the CSOs ''exc.'' had highest fractional contribution of 0.1%. Despite the measured *E. coli* concentrations of 4,800 and 2,200 [CFU/100 ml] on 12-2-2019 and 14-2-2019, the model indicated no contribution of overflows on these dates, which can be due to an underestimation of discharged overflows volume by InfoWorks.

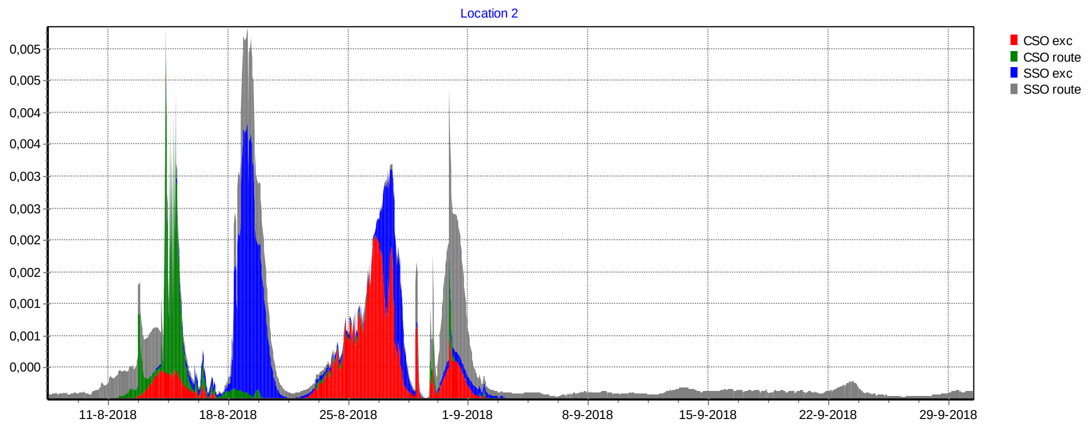

Figure 46 - Overflow fractions at location 2 in the summer period. The SSOs had a maximum contribution of 0.4%.

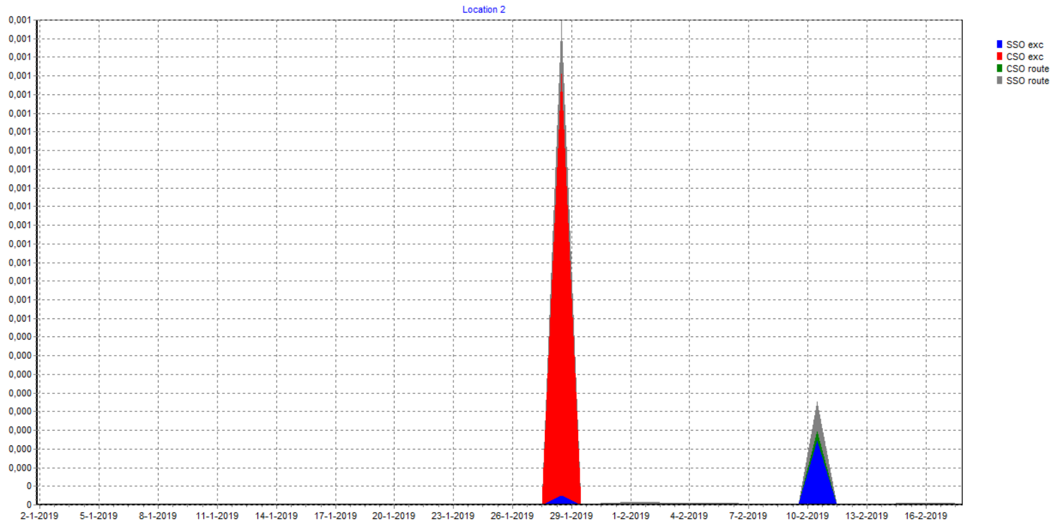

Figure 47- Overflows fractions at Location 2 in the winter period. The CSO ''exc.'' had a maximum fractional contribution of 0.1%.

Location 1

Figure 48 displays the water fractions of the summer and winter periods at Location 1. The Mark fraction of 65% in summer period was increased to 80% in the winter period.

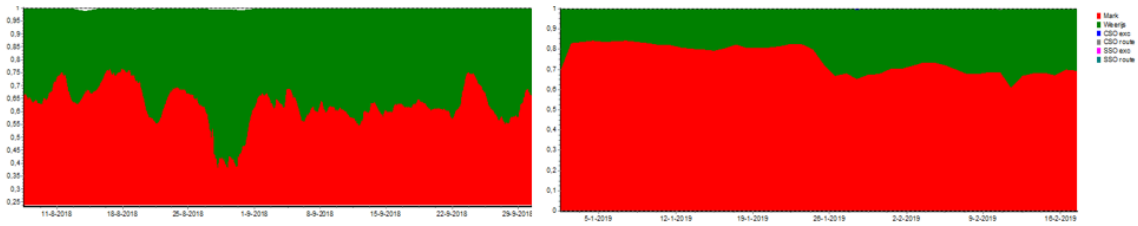

Figure 48- Water fractions at Location 1 in the summer and winter periods. The Mark fraction of 65% in the summer period increased to 80% in the winter period.

The overflow fractions of Location 1 are displayed in Figures 49 and 50. The maximum fractions of overflows were 0.7% at both CSOs ''route'' and SSOs ''route'', and 0.6% at SSOs ''route'' in the summer and winter periods, respectively. The highest *E. coli* level of 1,300 [CFU/100 ml] was measured on 20-8-2018 and 27-8-2018 in the summer period and 19,000 [CFU/100 ml] on 29-1-2019 in the winter period. Although the simulated discharge of the only overflow between Location 2 and Location 1 was zero in the summer and winter, the fractional contribution of overflows at Location 1 did not correspond to that of overflows at Location 2. An explanation might be the convergence of Mark and Weerijs streams and the widening of canal at Location 2, leading to a specific hydraulic situation at this location.

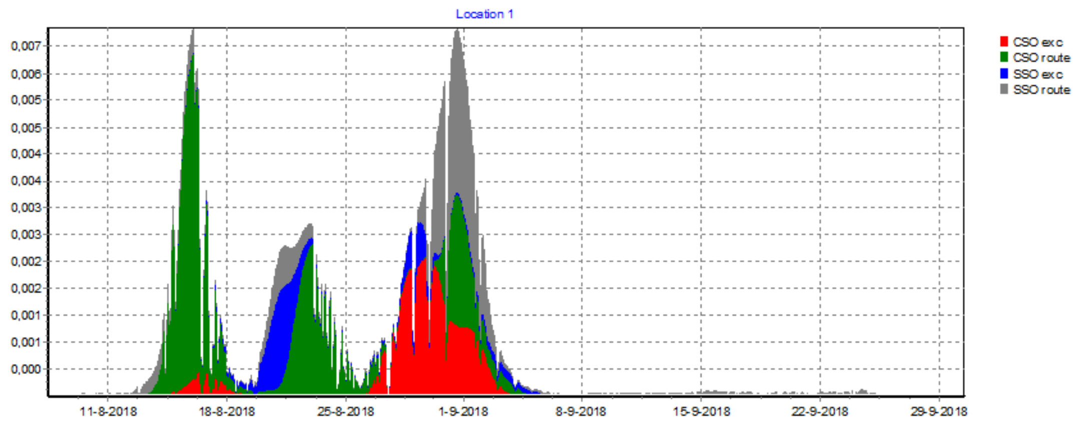

Figure 49 - Overflow fractions at Location 1 in the summer period. The "route" overflows had a maximum fractional contribution of 0.7%.

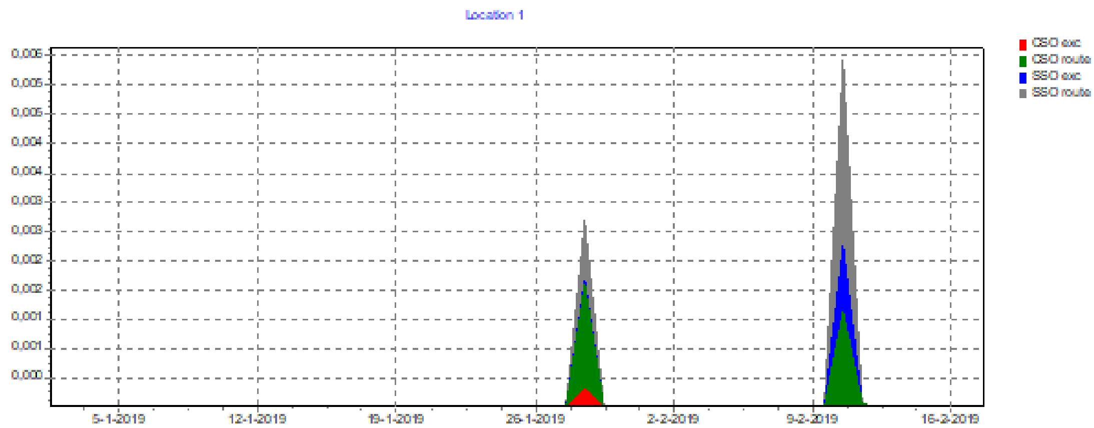

Figure 50- Overflow fractions at Location 1 in the winter period. The SSO "route" had a maximum fractional contribution of 0.6%.

4.3.2 Calculation of *E. coli* concentrations

The *E. coli* concentration of whole study area was simulated by the 1DWAQ module of SOBEK, as described in section 3.3. The results of sampling locations are presented and discussed in this section.

Location 5

Location 5 represented the water quality of the Mark stream quality entering the study area. The sampling results indicated frequently high concentrations of *E. coli* at this location. The *E. coli* dynamics were simulated separately for the summer and winter sampling periods.

In summer period, the simulated *E. coli* concentration was considerably lower than the measured *E. coli* concentration. An explanation may be an underestimation of adjacent sewer overflows by InfoWorks. In actuality, more overflow discharges occurred that were not included in the InfoWorks simulation, and earlier, the sampling results indicated that the sewer system may be more sensitive to moderate rainfall. Other explanations may be neglecting the runoff and associated pollution, and low initial *E. coli* in SOBEK. During the winter period, the calculated *E. coli* concentration was higher than the measured value of *E. coli*. An explanation may be high initial *E. coli* in SOBEK. Another explanation can be higher decay rates in the model.

The calculated *E. coli* concentration of the winter and summer periods at location 5 are displayed in Figures 51 and 52.

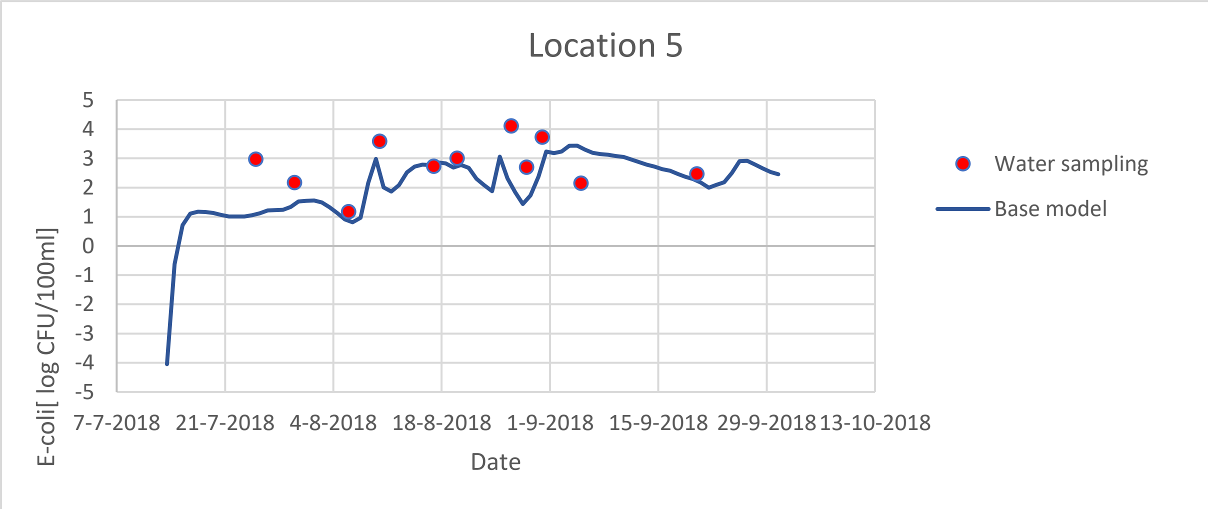

Figure 51 - Simulated *E. coli* concentrations at location 5 in the summer. The measured *E. coli* concentration were higher than the simulated values.

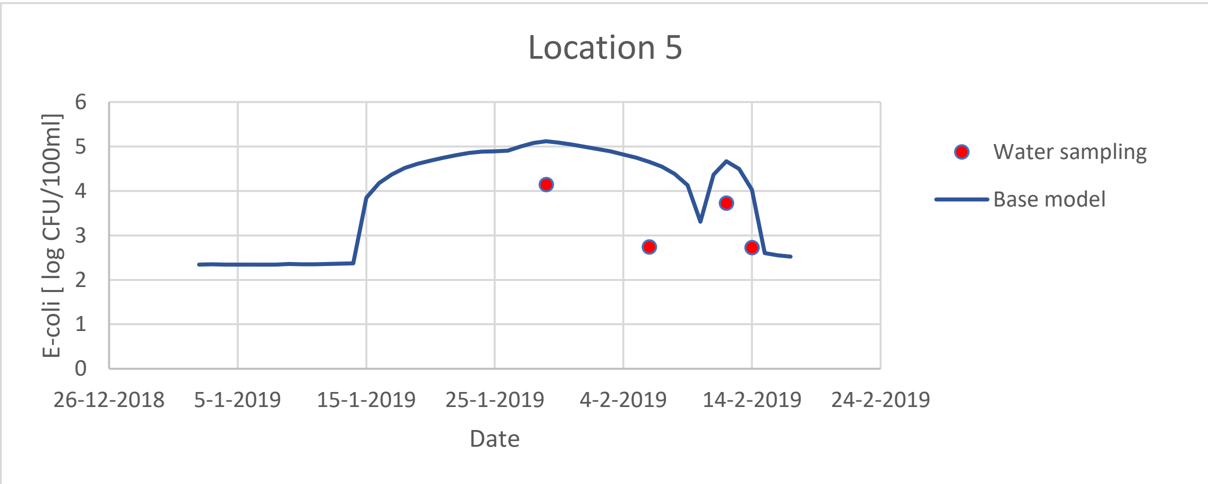

Figure 52 - Simulated *E. coli* concentrations at location 5 in the winter. The calculated *E. coli* concentrations were higher than measured *E. coli*. On 10-2-2019, the *E. coli* concentration was declined due to decrease of initial *E. coli* to 250 [CFU/100ml].

Location 4

Location 4 represented the water quality of the Weerijs stream quality entering the study area. The sampling results indicated a low concentration of *E. coli* at this location. The *E. coli* dynamics were simulated separately for the summer and winter sampling periods.

During dry weather in summer period, the simulated *E. coli* concentrations were lower than measured ones. The explanations can be low initial *E. coli* concentrations, and high decay rate in model. In both summer and winter periods, during wet weather, the simulated *E. coli* concentrations were considerably lower than the measured *E. coli* concentrations. An explanation can be an underestimation of upstream sewer overflows by InfoWorks, especially in the winter

period, and intense rainfall when the model also indicated no fractions of overflows at this location in the winter.
Another explanation for results of the winter period may be higher decay rates in the model.

The calculated *E. coli* concentrations of the winter and summer periods at Location 4 are displayed in Figures 53 and 54.

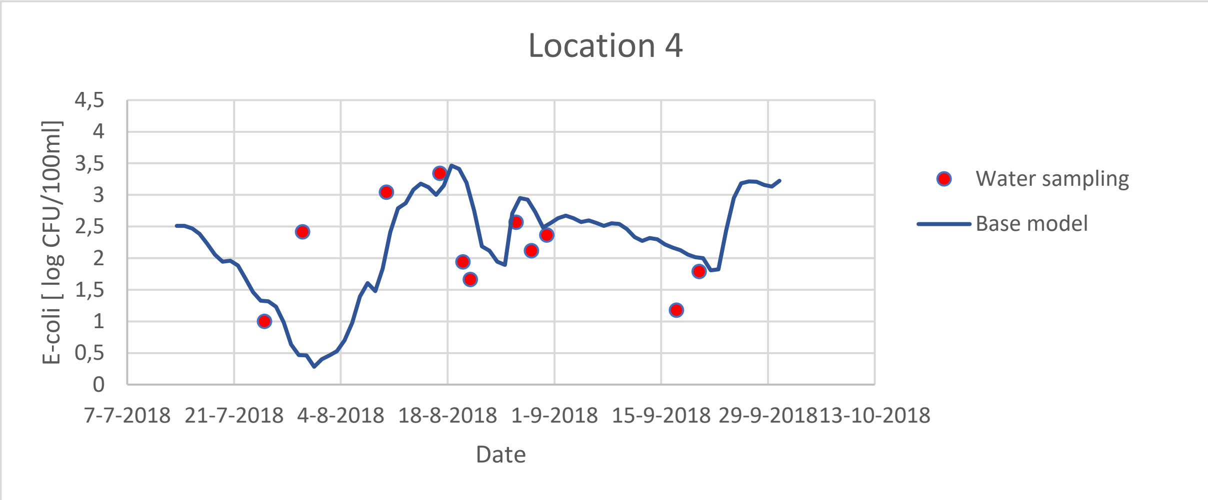

Figure 53 - Simulated *E. coli* concentrations at location 4 in the summer period. The calculated *E. coli* concentrations were lower than measured values during dry weather but in wet weather the measured values were lower than simulated values.

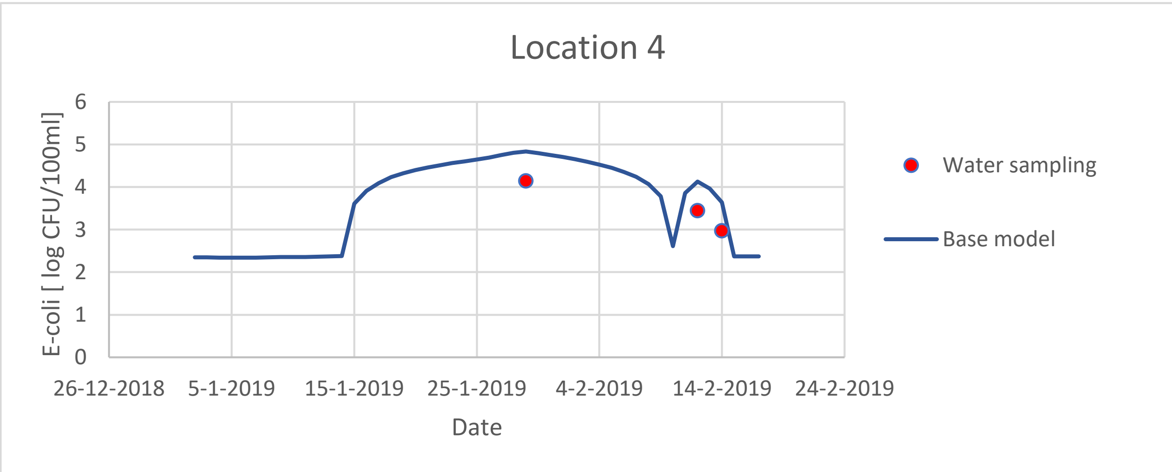

Figure 54 - Simulated *E. coli* concentrations at location 4 in the winter the calculated *E. coli* concentrations were higher than simulated values in wet weather. On 10-2-2019, the *E. coli* concentration was decreased due to decrease of initial *E. coli* to 250 [CFU/100ml].

Location 3

The harbour location as a dead-end water is fed solely by sewer overflows. The explanation for considerably lower simulated *E. coli* concentration compared to measured values in the summer period can be underestimation of overflows discharges and inappropriate modelling of this section in InfoWorks, and high decay rate in SOBEK.
The simulated *E. coli* concentration corresponded roughly with sampling results in the winter period.

The simulated *E. coli* concentration of winter and summer periods at location 3 are illustrated in Figures 55 and 56.

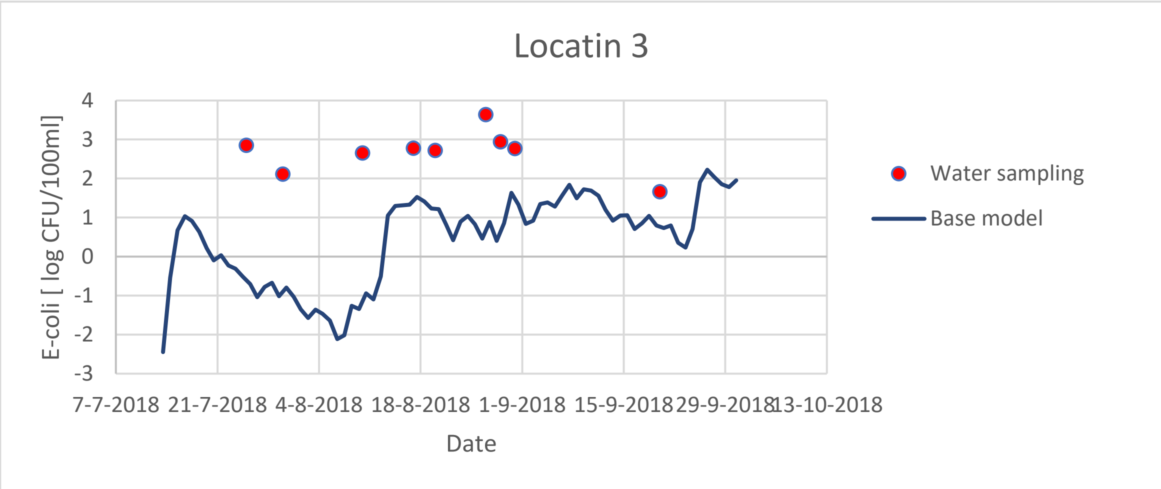

Figure 55- Simulated *E. coli* concentrations at Location 3 in the summer period. The simulated *E. coli* concentrations were higher than measured values.

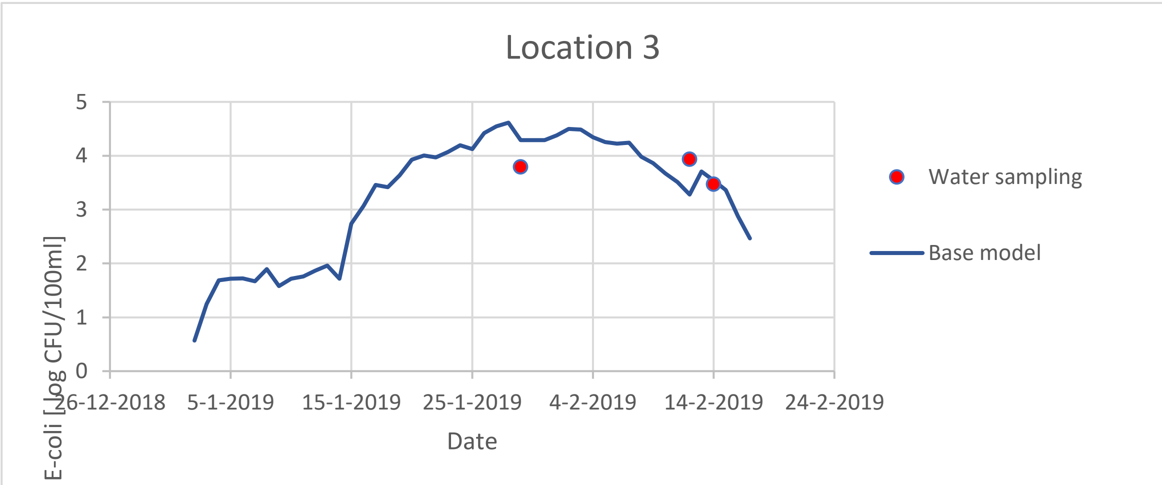

Figure 56- Simulated *E. coli* concentrations at Location 3 in the winter period. The simulated *E. coli* concentration corresponded roughly with sampling results. On 10-2-2019, the *E. coli* concentration was decreased due to decrease of initial *E. coli* to 250 [CFU/100ml].

Location 2

The Mark and Weerijs streams converge at Location 2, leading to a specific hydraulic situation at this location. The water sampling indicated frequently high *E. coli* concentrations at Location 2. The simulated *E. coli* concentrations were significantly lower than the measured *E. coli* in the summer period. Besides the same explanations mentioned above for other locations, there is also uncertainty regarding pollution sources and water sampling methods at this location, as described in section 4.3.1.

The calculated *E. coli* concentrations of the winter and summer periods at Location 2 are presented in Figures 57 and 58.

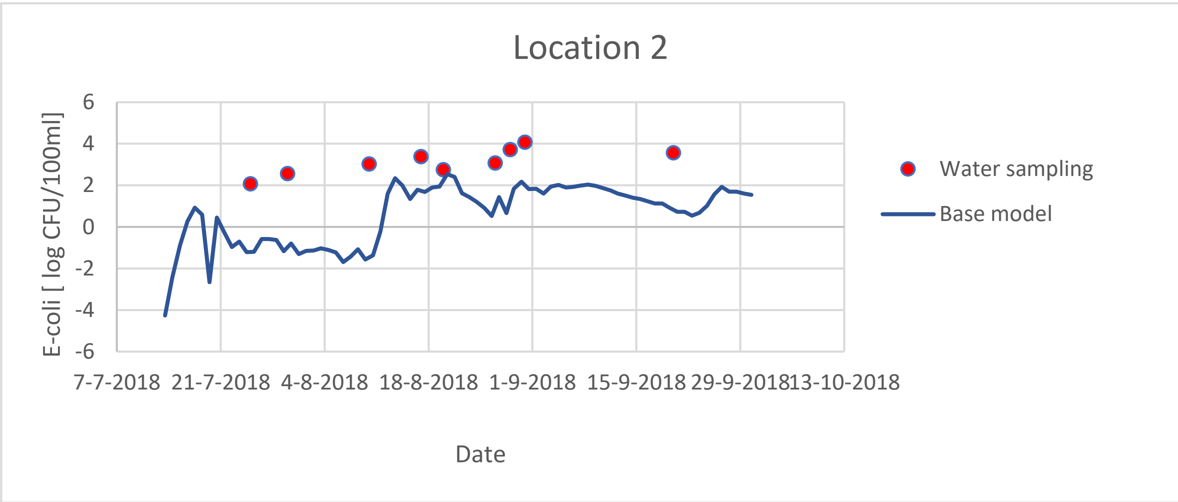

Figure 57- Simulated *E. coli* concentrations at Location 2 in the summer. The simulated *E. coli* concentrations were higher than measured values.

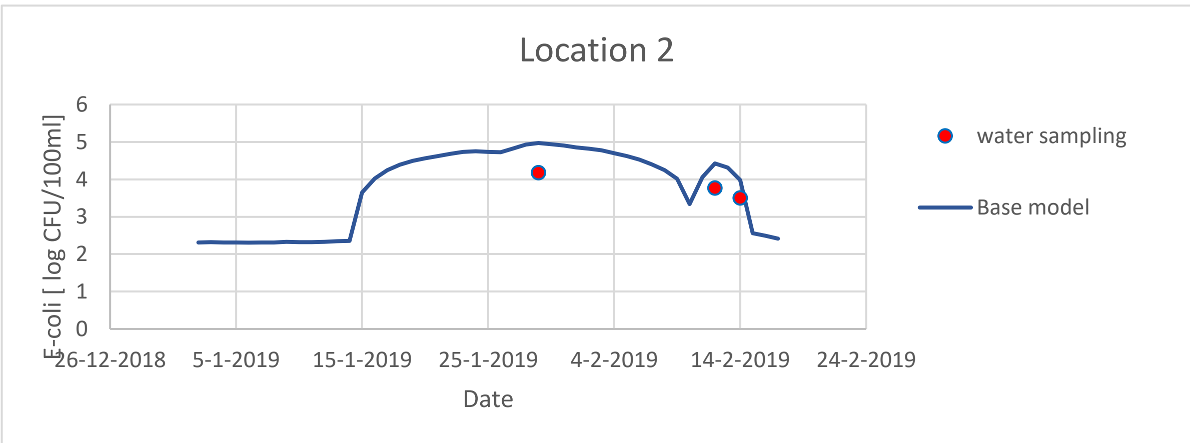

Figure 58- Simulated *E. coli* concentrations at Location 2 in the winter. The calculated *E. coli* concentrations were higher than measured values. On 10-2-2019, the *E. coli* concentration was decreased due to decrease of initial *E. coli* to 250 [CFU/100ml].

Location 1

Location 1 represented the water quality of the downstream locations of the study area. The measured *E. coli* concentrations were in compliance with “beslisnotitie” norm except the winter period.

The measured *E. coli* concentrations were considerably higher than the calculated values in the summer period. An explanation, as for the other locations, can be an underestimation of overflow discharges by InfoWorks, the neglect of the runoff source impact, and high decay rate in the water quality model.
According to water fraction modelling, the contribution of Mark is %80 in the winter period at Location 1. The explanation for kink on 26-1-2019 could be that the Mark flow rate is reduced by 50% and increased to 3 times on 1/28/2019 and 1/29/2019.

The calculated *E. coli* concentrations of the winter and summer periods at Location 1 are displayed in Figures 59 and 60.

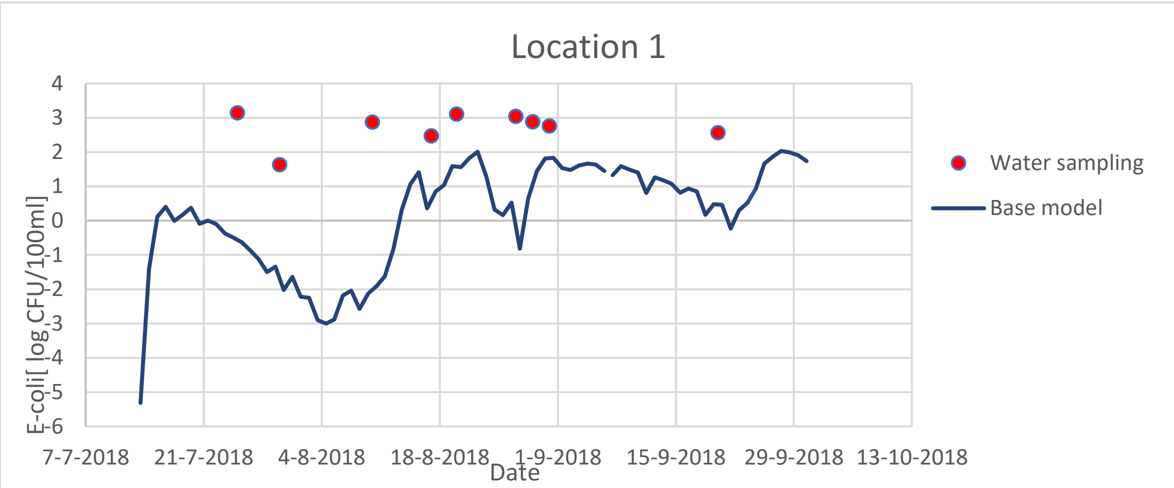

Figure 59- Simulated *E. coli* concentrations at Location 1 in the summer period. The calculated *E. coli* concentrations were lower than measured values.

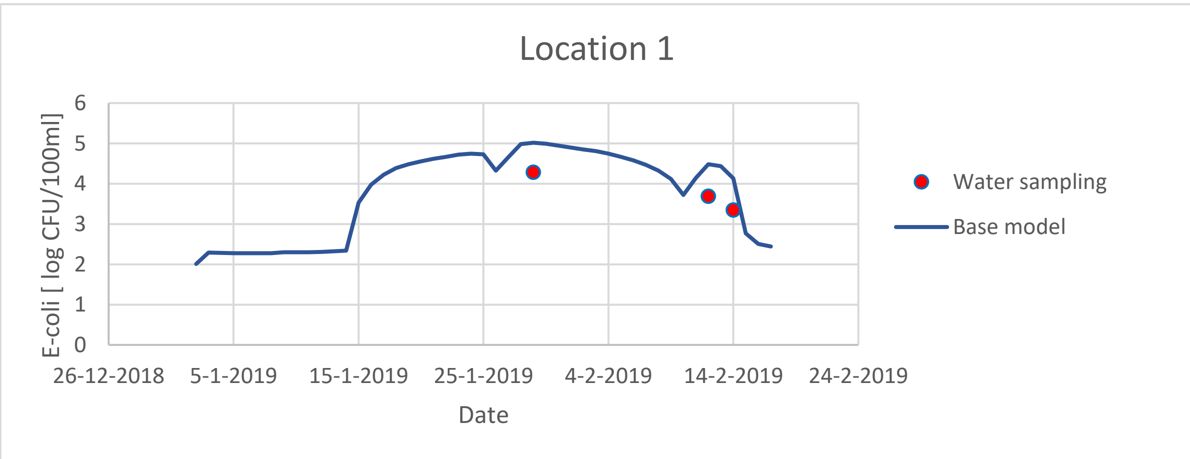

Figure 60- Simulated *E. coli* concentrations at Location 1 in the winter period. The calculated *E. coli* concentrations were higher than measured values. On 10-2-2019, the *E. coli* concentration was decreased due to decrease of initial *E. coli* to 250 [CFU/100ml].

4.3.3 Sensitivity of the water quality model

The sensitivity of the model was analysed for both the summer and winter periods based on three criteria:

- The initial *E. coli* concentration at the Mark and Weerijs boundaries;
- *E. coli* concentrations of CSOs and SSOs; and
- Decay rate.

Sensitivity of model to initial *E. coli* at the Mark and Weerijs boundaries

Initial *E. coli* at Mark and Weerijs boundaries were unknown due to absence of measuring points at these locations. By considering decay rate as a rough estimation, one log higher than measured *E. coli* concentrations at Locations 4 and 5 was assumed as initial *E. coli* at both boundaries of Mark and Weerijs. The initial *E. coli* was assumed 250 [CFU/100ml] at first date of simulation period, on 10-7-2018, 1-1-2019, 14-1-2019, and 10-2-2019.

To control the sensitivity of model, two constant value of 0 [CFU/100ml] and 10000 [CFU/100ml] in the summer period, and 0 [CFU/100ml] and 1000 [CFU/100ml] in the winter period were simulated.
The model demonstrated sensitivity to Initial *E. coli* concentration. The most impact of initial *E. coli* concentration was indicated at Location 4. The explanation could be low contribution of sewer overflows as *E. coli* source at this location (see Figure 40).
The model was only sensitive to initial *E. coli* in dry weather in the summer period at Location 5 and Location 1. It can be concluded that the impact of sewer overflows is more than the initial *E. coli* at these locations.

The sensitivity of model to initial *E. coli* concentration in the summer period are presented in Figure 61 to Figure 65.

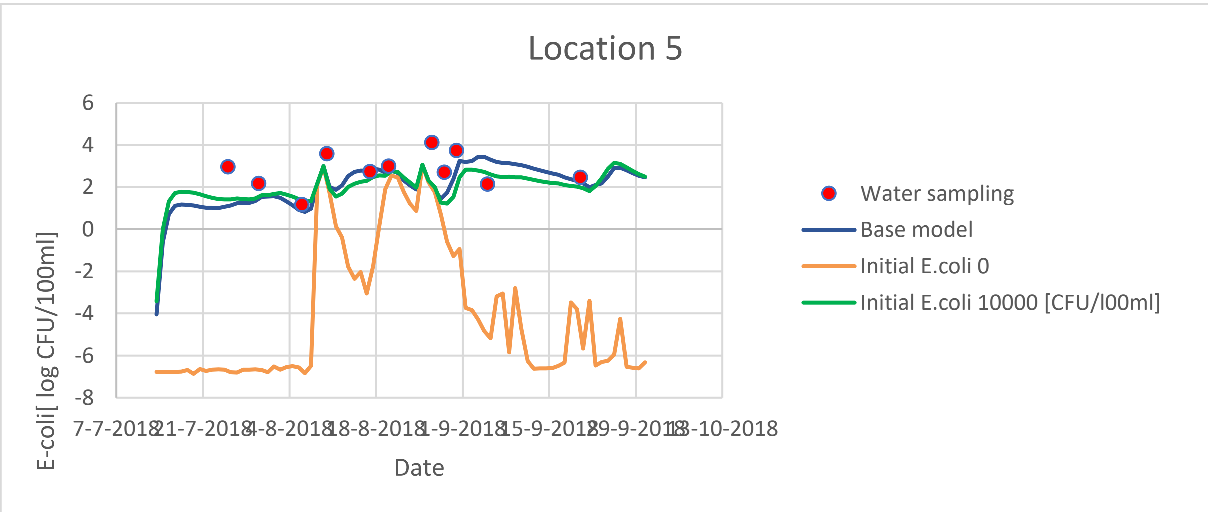

Figure 61- Sensitivity of model to initial *E. coli* concentrations at Location 5 in the summer period.

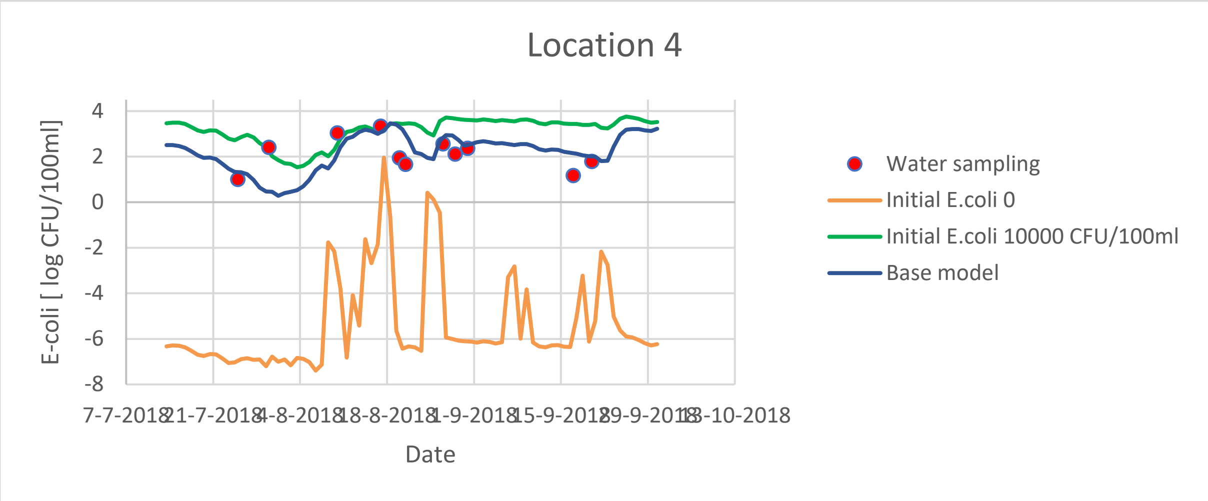

Figure 62- Sensitivity of model to initial E. *coli* concentration at Location 4 in the summer period. The most impact of initial *E. coli* concentration was indicated at Location 4. The explanation can be low contribution of sewer overflows as *E. coli* source at this location.

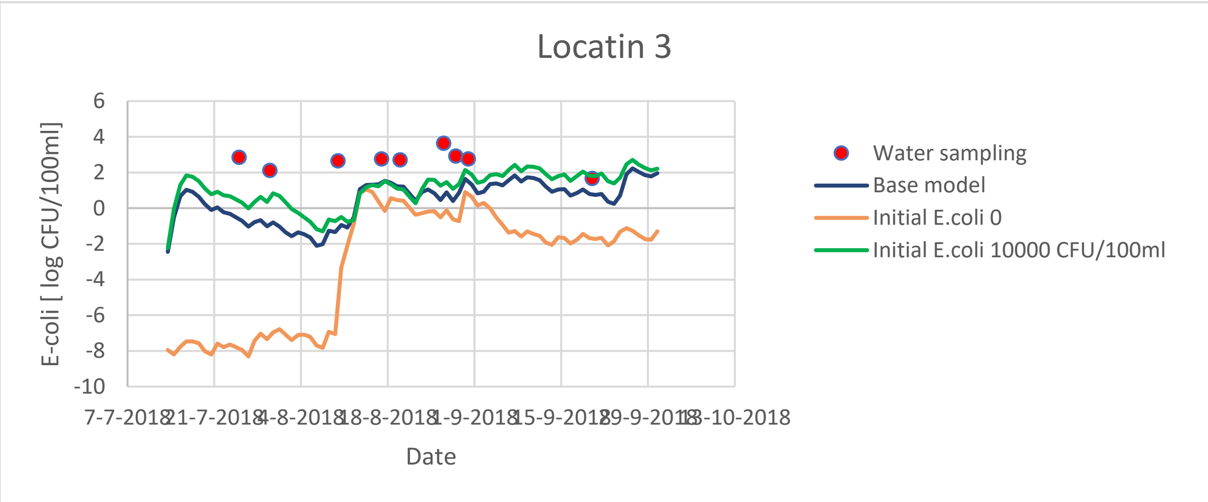

Figure 63-Sensitivity of model to initial E. coli concentration at Location 3 in the summer period.

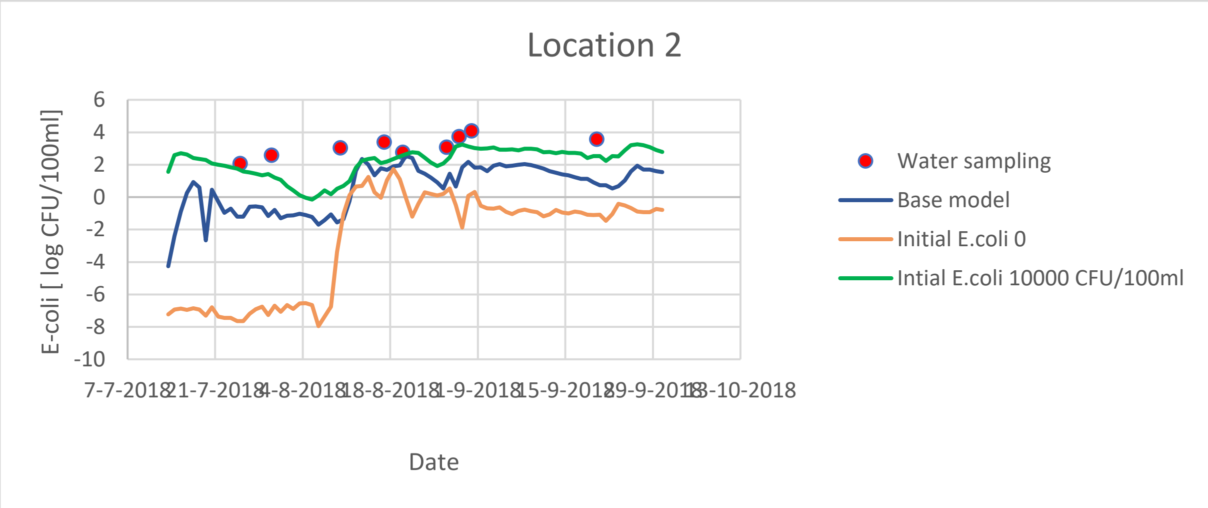

Figure 64- Sensitivity of model to initial E. *coli* concentration at Location 2 in the summer period.

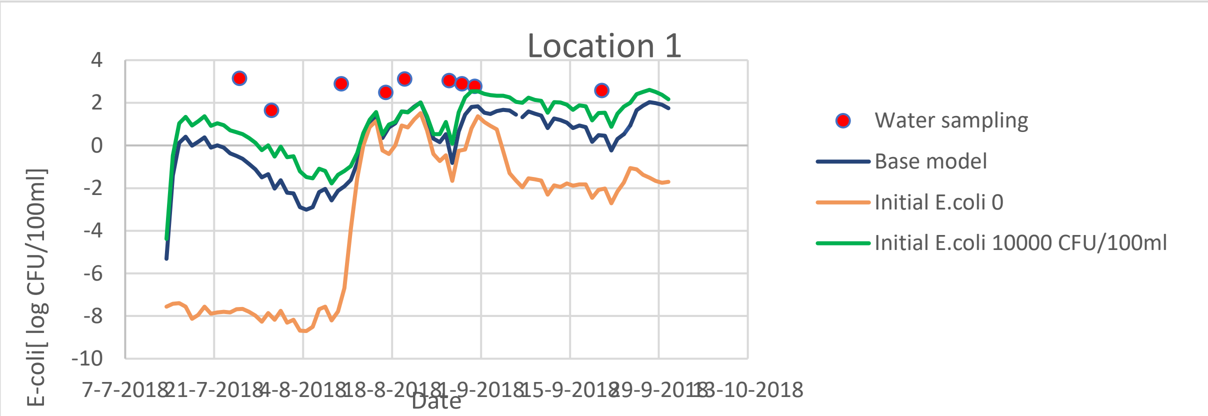

Figure 65- Sensitivity of model to initial *E. coli* concentration at Location 1 in the summer period. The model demonstrated the sensitivity to initial *E. coli* only in dry weather in the summer period. It can be concluded that the impact of sewer overflows is more than the initial *E. coli* at this location.

The impact of initial *E. coli* in the winter period was more than summer period. The explanations might be low contribution of simulated sewer overflows in the winter, lower decay rate, and higher upstream discharges, following higher initial *E. coli* load. The impact of sewer overflows after the heavy rain events were indicated as peak of *E. coli* concentration when initial *E. coli* was nil. No significant peak value was illustrated by constant initial *E. coli* 1000 [CFU/100ml].

The sensitivity of model to initial *E. coli* in the winter period are presented in Figure 66 to Figure 70.

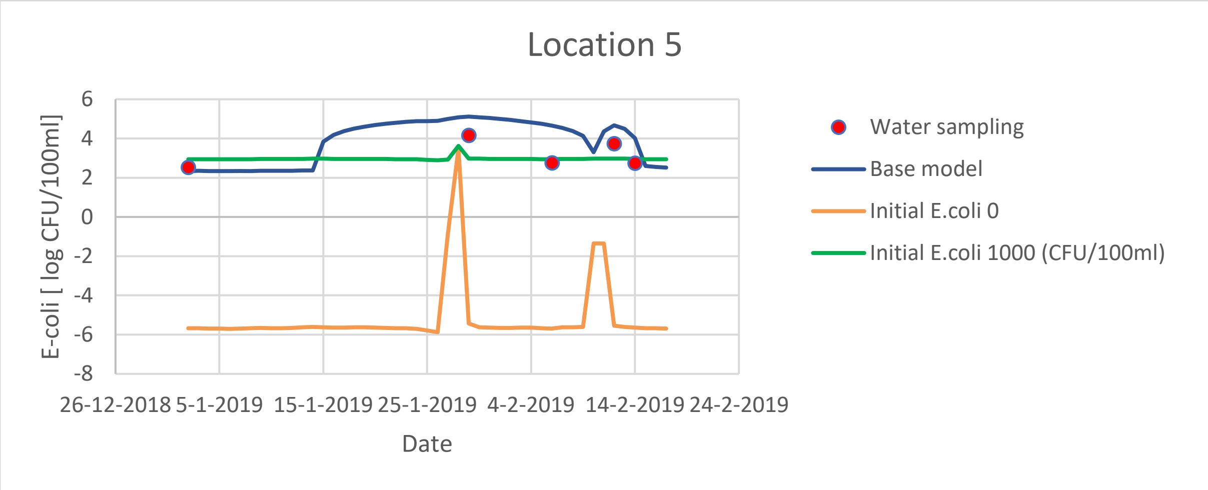

Figure 66- Sensitivity of model to initial E. *coli* concentration at Location 5 in the winter period. The impact of sewer overflows after the heavy rain events were indicated as peak of *E. coli* concentration when initial *E. coli* was nil.

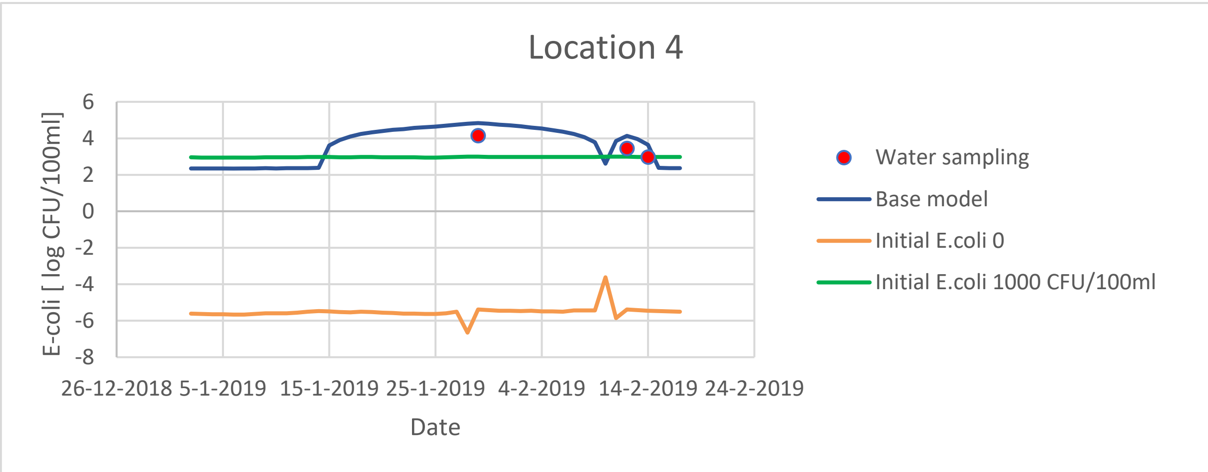

Figure 67- Sensitivity of model to initial E. *coli* concentration at location 4 in the winter period. On 28-1-2019, the *E. coli* concentration was declined when initial *E. coli* was nil. An explanation might be extremely low contribution of sewer overflows when the flow rate was 3 times higher than average flow rate.

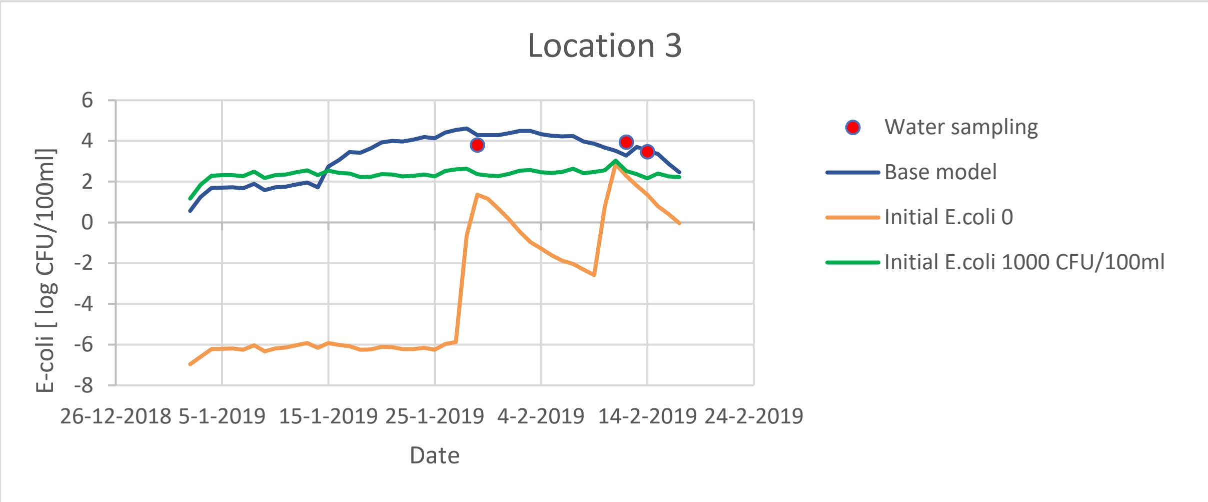

Figure 68- Sensitivity of model to initial E. *coli* concentration at Location 3 in the winter period. The impact of sewer overflows after the heavy rain events were indicated as peak of *E. coli* concentration when initial *E. coli* was nil.

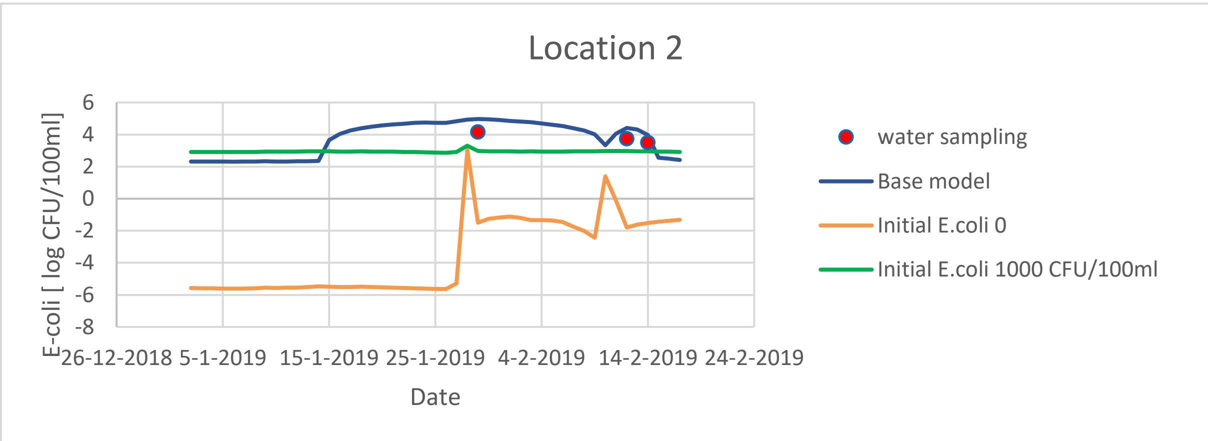

Figure 69- Sensitivity of model to initial E. *coli* concentration at Location 2 in the winter period. The impact of sewer overflows after the heavy rain events were indicated as peak of *E. coli* concentration when initial *E. coli* was nil.

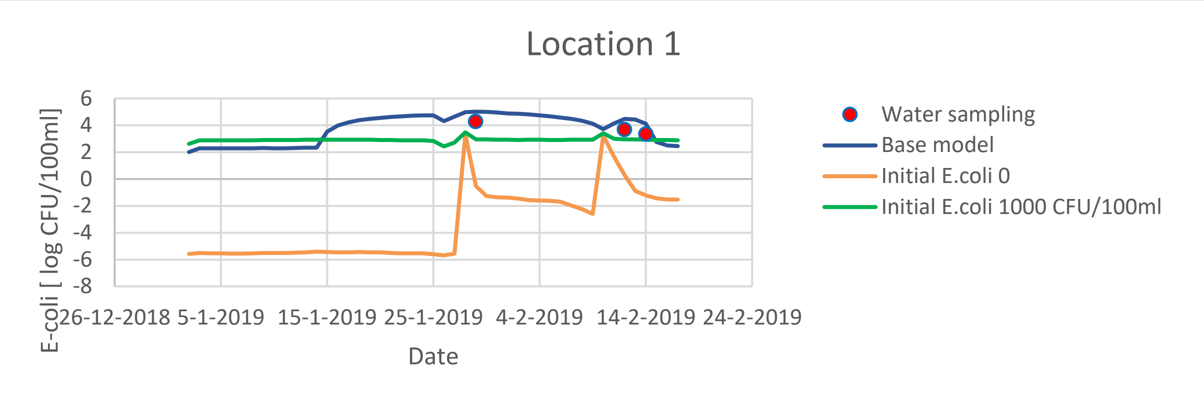

Figure 70- Sensitivity of model to initial E. *coli* concentration at Location 1 in the winter period. The impact of sewer overflows after the heavy rain events were indicated as peak of *E. coli* concentration when initial *E. coli* was nil.

Sensitivity of model to *E. coli* concentrations of CSOs and SSOs

Based on suggested *E. coli* concentration of sewer overflows of various studies (see Table 1), *E. coli* concentration of 1* E10 [CFU/m³] and 1*E8 [CFU/m³] were selected for CSO and SSO, respectively.

The model demonstrated sensitivity to *E. coli* concentration of sewer overflows in the summer period, except at Location 4. The explanation might be low contribution of sewer overflows at this location (see Figure 40). The model corresponded better to measured values when, *E. coli* concentration of 1* E12 [CFU/m³] and 1*E10 [CFU/m³] were selected for CSO and SSO, respectively. The lower *E. coli* concentration by deleting CSO discharge factor indicated that CSOs discharge affected the water quality more than the SSOs discharge in the summer period.
The sensitivity of model to *E. coli* concentrations of CSOs and SSOs in the summer period are presented in Figure 71 to Figure 75.

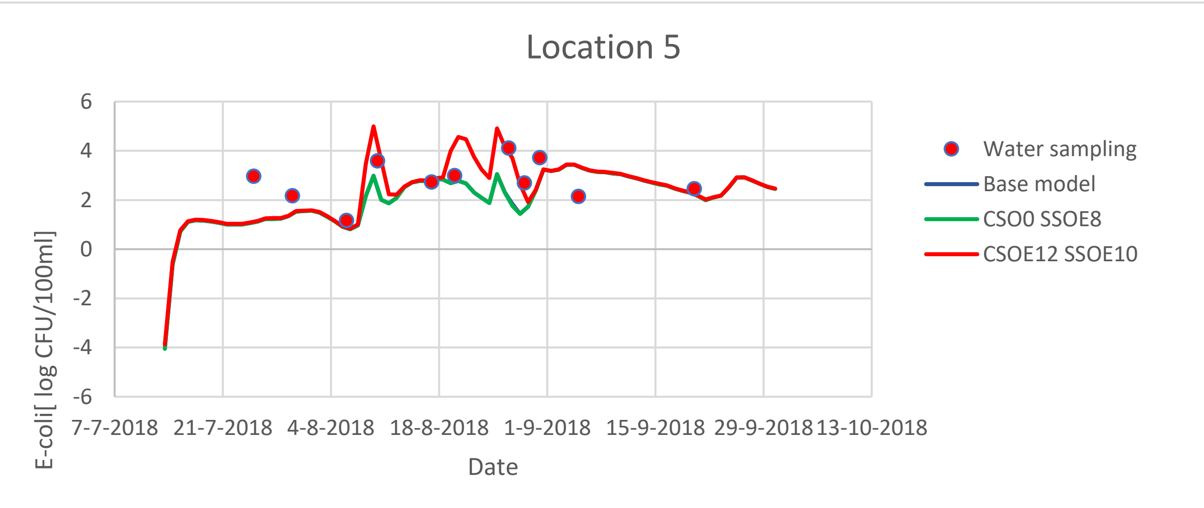

Figure 71- Sensitivity of model to E. *coli* concentration of sewer overflows at Location 5 in the summer period. The model was less sensitive to *E. coli* concentration of sewer overflows. The explanation might be the impact of decay rate on *E. coli* concentration.

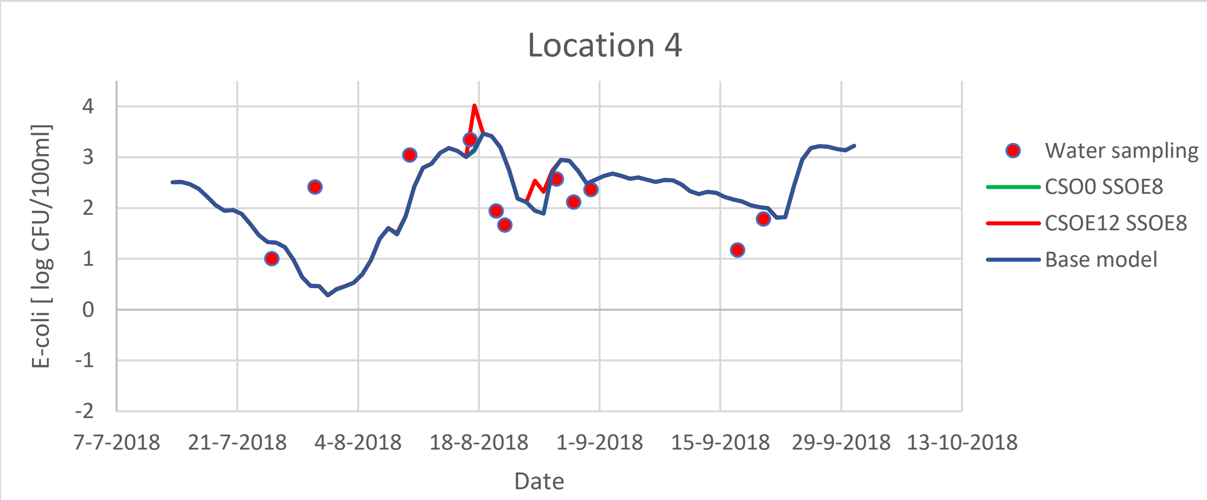

Figure 72- Sensitivity of model to E. *coli* concentration of sewer overflows at Location 4 in the summer period. The model was less sensitive to *E. coli* concentration of sewer overflows. The explanation might be low contribution of sewer overflows at this location.

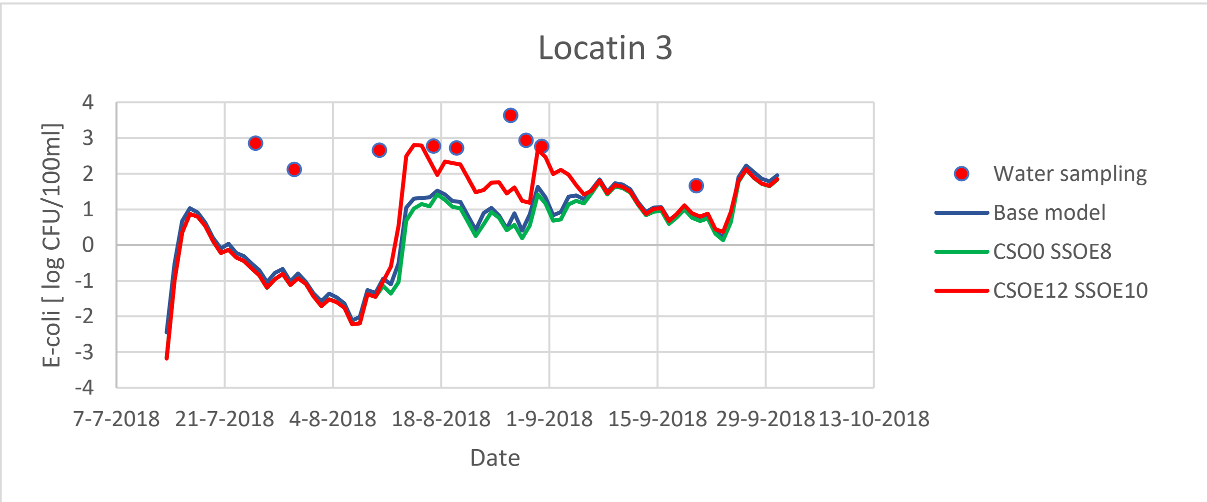

Figure 73-Sensitivity of model to *E. coli* concentration of sewer overflows at Location 3 in the summer period.

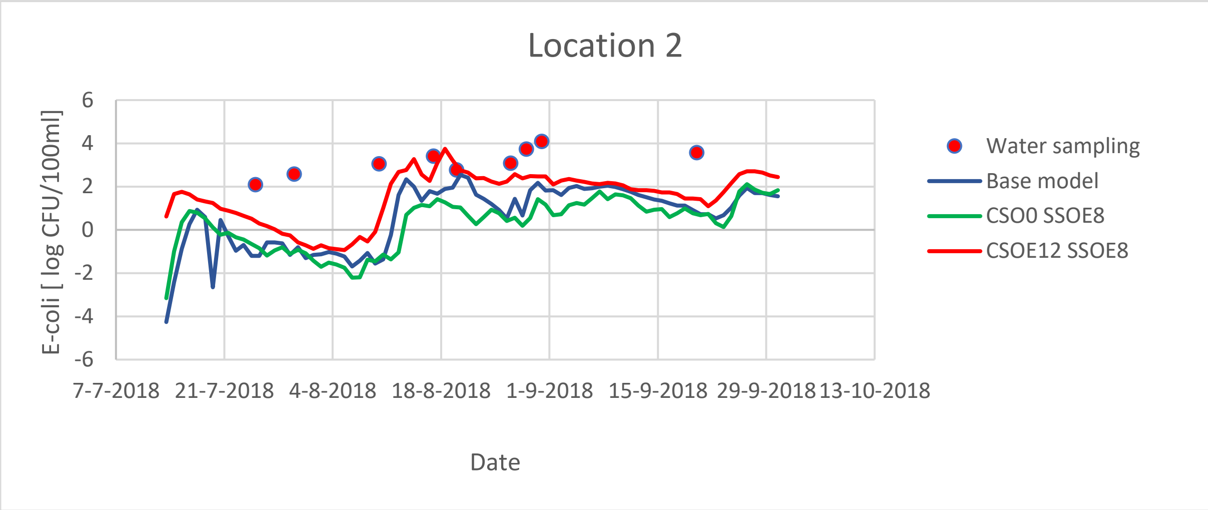

Figure 74-Sensitivity of model to *E. coli* concentration of sewer overflows at Location 2 in the summer period.

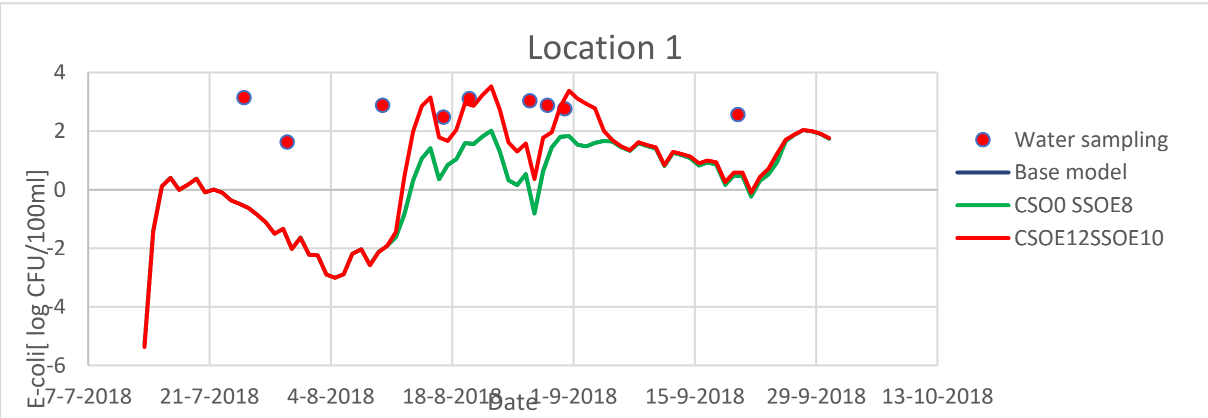

Figure 75- Sensitivity of model to E. *coli* concentration of sewer overflows at Location 1 in the summer period. The model was less sensitive to *E. coli* concentration of sewer overflows. The explanation might be the impact of decay rate on *E. coli* concentration.

The model demonstrated no sensitivity to *E. coli* concentration of sewer overflows in the winter period. However, the peak values were detected after two heavy rainfalls by *E. coli* concentration of 1* E^{12} [CFU/m^3] and 1*E^{10} [CFU/m^3] for CSO and SSO, respectively. The explanation might be extreme low simulated sewer overflows discharges and higher dilution factor.

The sensitivity of model to *E. coli* concentrations of CSOs and SSOs in the winter period are presented in Figure 76 to Figure 80.

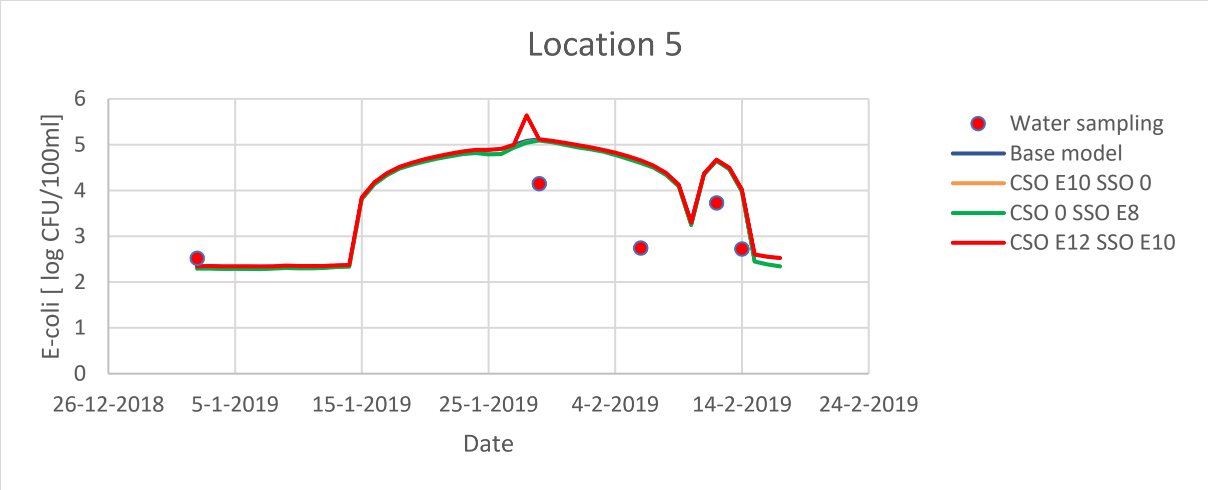

Figure 76- Sensitivity of model to E. *coli* concentration of sewer overflows at Location 5 in the winter period. The model was not sensitive to *E. coli* concentration of sewer overflows. The explanation might be extreme low simulated sewer overflows discharges and higher dilution factor.

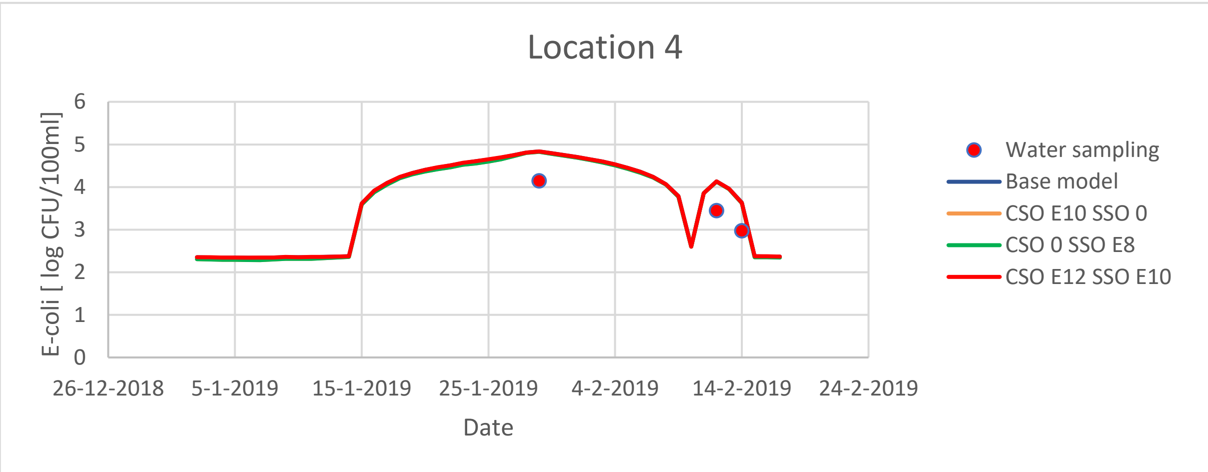

Figure 77- Sensitivity of model to *E. coli* concentration of sewer overflows at location 4 in the winter period. The model was not sensitive to E. coli concentration of sewer overflows. The explanation might be extreme low simulated sewer overflows discharges and higher dilution factor.

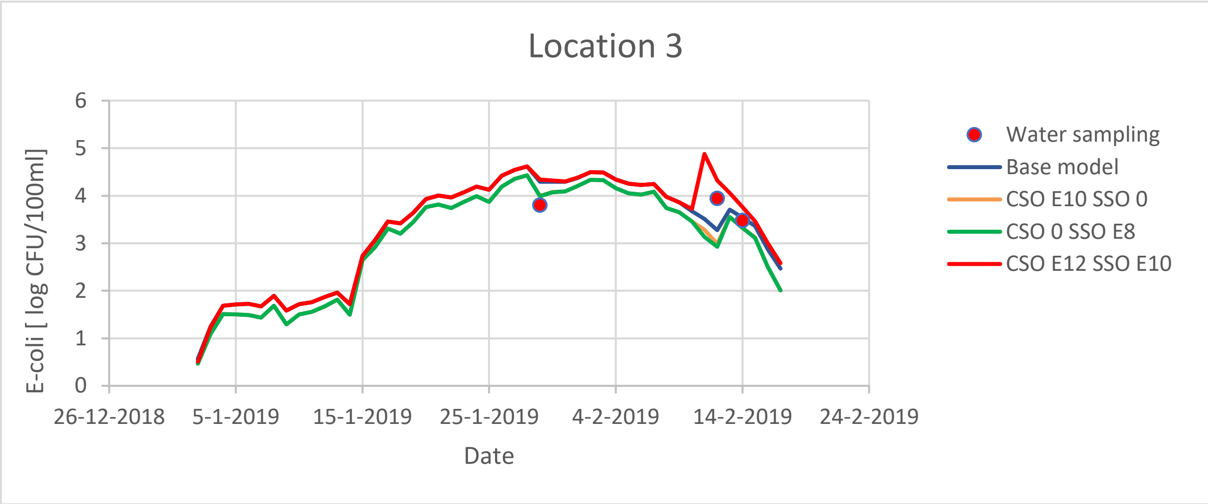

Figure 78- Sensitivity of model to E. *coli* concentration of sewer overflows at Location 3 in the winter period. The model was not sensitive to *E. coli* concentration of sewer overflows. The explanation might be extreme low simulated sewer overflows discharges and higher dilution factor.

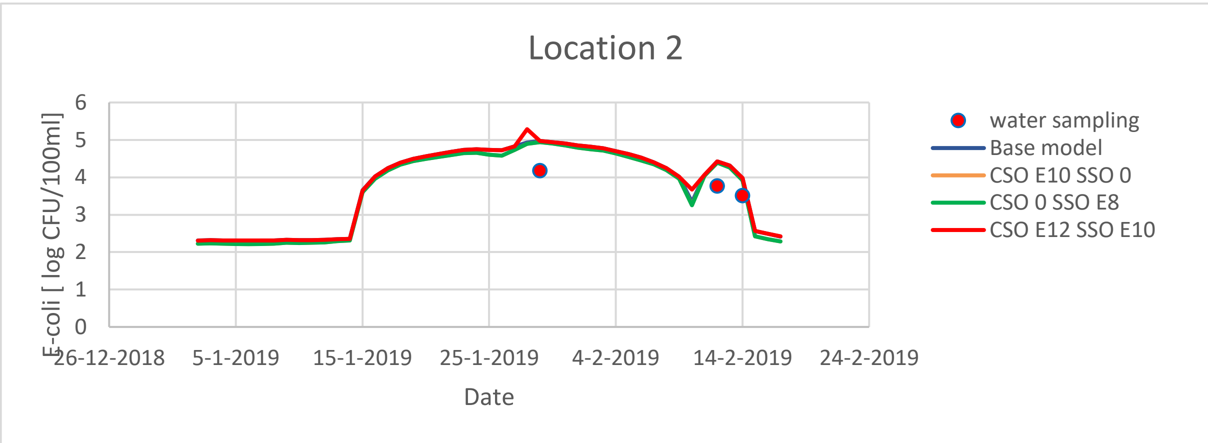

Figure 79-- Sensitivity of model to E. *coli* concentration of sewer overflows at Location 2 in the winter period. The model was not sensitive to *E. coli* concentration of sewer overflows. The explanation might be extreme low simulated sewer overflows discharges and higher dilution factor.

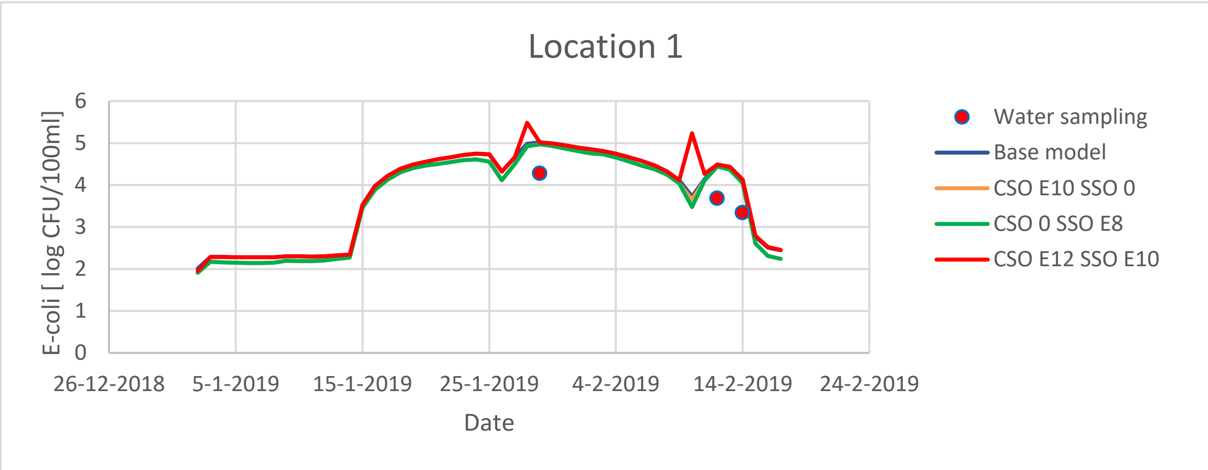

Figure 80- Sensitivity of model to E. *coli* concentration of sewer overflows at Location 1 in the winter period. The model was not sensitive to *E. coli* concentration of sewer overflows. The explanation might be extreme low simulated sewer overflows discharges and higher dilution factor. The explanation for kink on 26-1-2019 could be that the Mark flow rate is reduced by 50% and increased to 3 times on 1/28/2019 and 1/29/2019.

Sensitivity of model to *decay rate*

The sensitivity of model to decay rate was analysed with decay rates of 0, 0.45 and 0.8 in the summer period. The decay rate of 0 indicates the impact of decay rate versus the hydrology. The model was sensitive to decay rate; higher decay rate resulted in a lower *E. coli* concentration. The absence of *E. coli* dynamics with a decay rate of 0 can be explained by the extremely low discharge the of Mark and Weerijs streams, as well as simulated sewer overflow discharges in the summer period. The variation of *E. coli* concentration with a decay rate of 0 was detected only at Location 4. The decreasing discharge of the Weerijs in the period between 20-8-2018 and 25-8-2018 may provide the explanation. The order of magnitude of the *E. coli* concentration declined approximately one log with a 50% decrease in decay rate. The E. *coli* travel time of three days after rainfalls with a decay rate of 0 was confirmed by measuring results at Location 5.

The sensitivity of model to decay rate in the summer period are presented in Figure 81 to Figure 85.

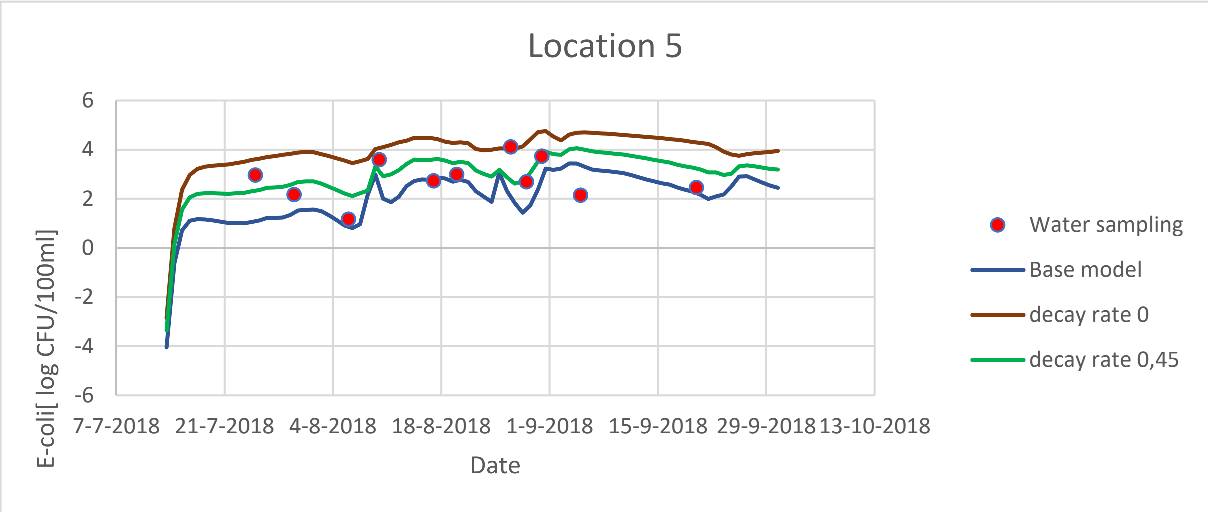

Figure 81- Sensitivity of model to decay rate at Location 5 in the summer period. The model was sensitive to decay rate; higher decay rate resulted in lower *E. coli* concentration. The *E. coli* travel time of 3 days after rainfalls with decay rate of 0 at Location 5 confirmed by measuring results.

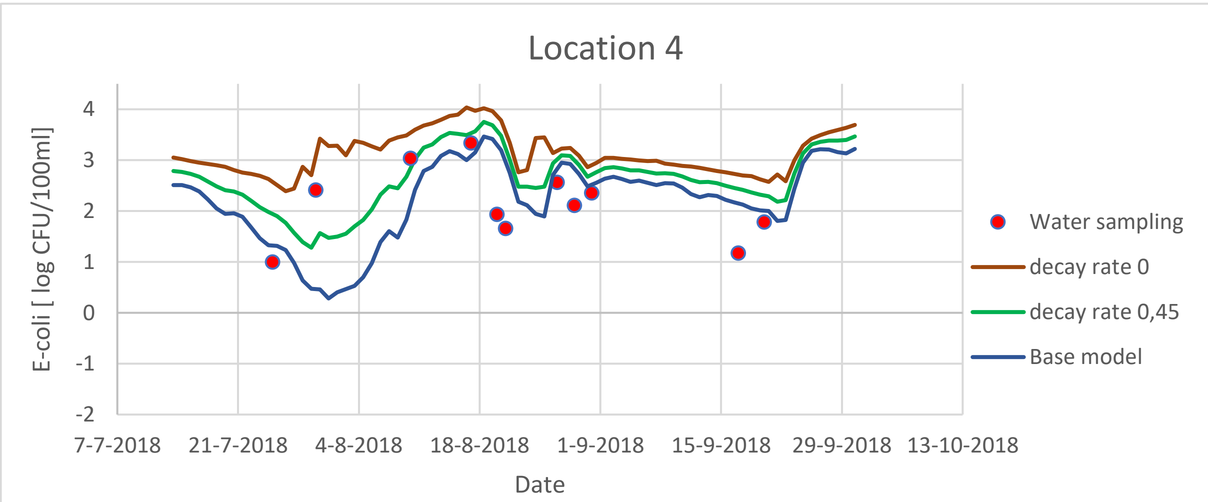

Figure 82- Sensitivity of model to decay rate at Location 4 in the summer period. The model was sensitive to decay rate; higher decay rate resulted in lower *E. coli* concentration. The variation of *E. coli* concentration with decay rate of 0 was detected only at location 4. The explanation might be decreasing the stream discharge of Weerijs in the period between 20-8-2018 and 25-8-2018.

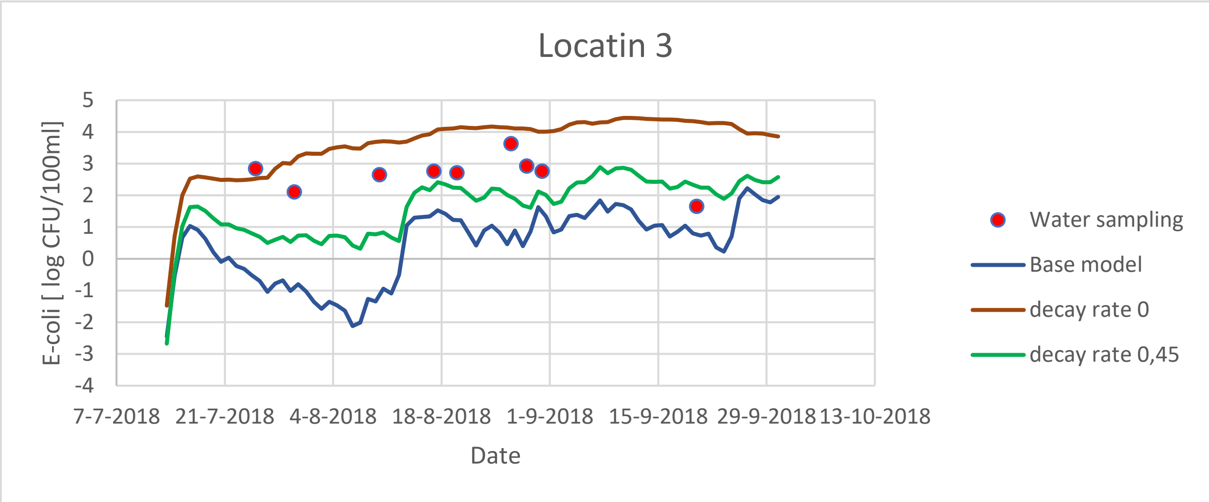

Figure 83- Sensitivity of model to decay rate at Location 3 in the summer period. The model was sensitive to decay rate; higher decay rate resulted in lower *E. coli* concentration. The magnitude of order of the *E. coli* concentration declined approximately one log with a 50% decrease in decay rate.

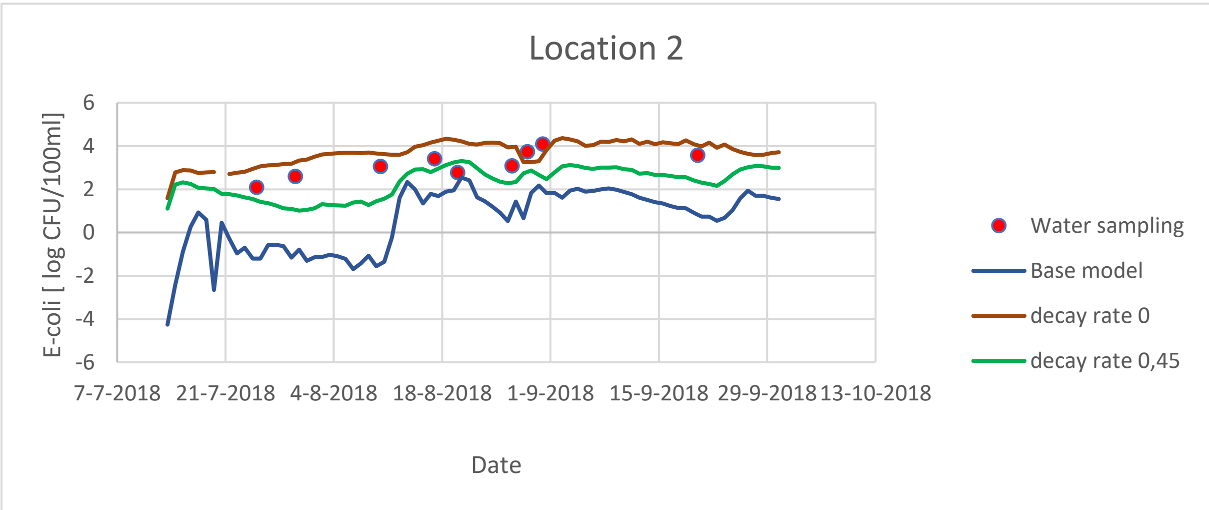

Figure 84- Sensitivity of model to decay rate at Location 2 in the summer period. The model was sensitive to decay rate; higher decay rate resulted in lower *E. coli* concentration. The magnitude of order of the *E. coli* concentration declined approximately one log with a 50% decrease in decay rate.

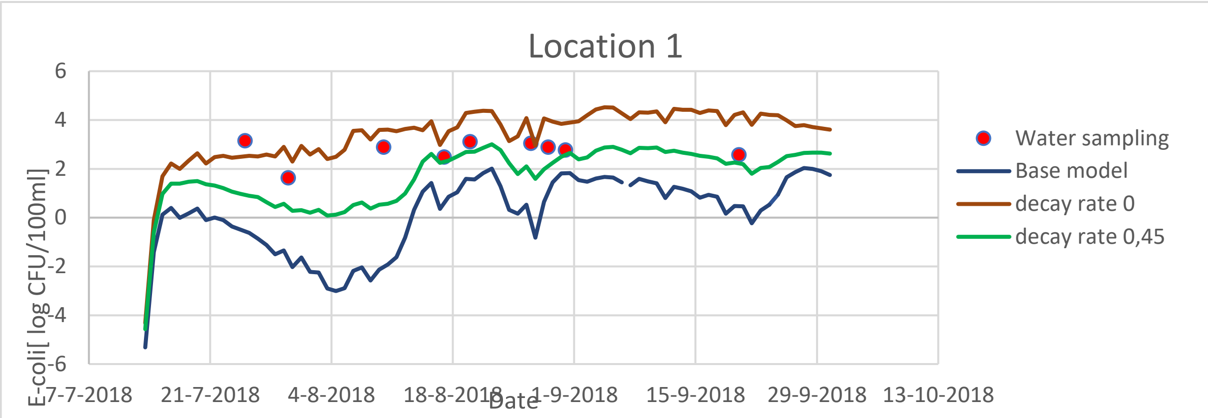

Figure 85- Sensitivity of model to decay rate at Location 1 in the summer period. The model was sensitive to decay rate; higher decay rate resulted in lower *E. coli* concentration. The magnitude of order of the *E. coli* concentration declined approximately one log with a 50% decrease in decay rate.

The discrepancy of decay rates of 0.14 (base model) and 0 was negligible. Therefore, the decay rates of 0.45 and 0.9 were analysed in the winter period. In this period, the model was not sensitive to decay rate. The explanation might be extreme low simulated sewer overflows discharges and higher dilution factor. The decay rate affected only the Location 3. An explanation can be the absence of *E. coli* dynamics at this location; therefore, the decay rate is the only factor affecting the *E. coli* concentration.

The sensitivity of model to decay rate in the winter period are presented in Figure 86 to Figure 90.

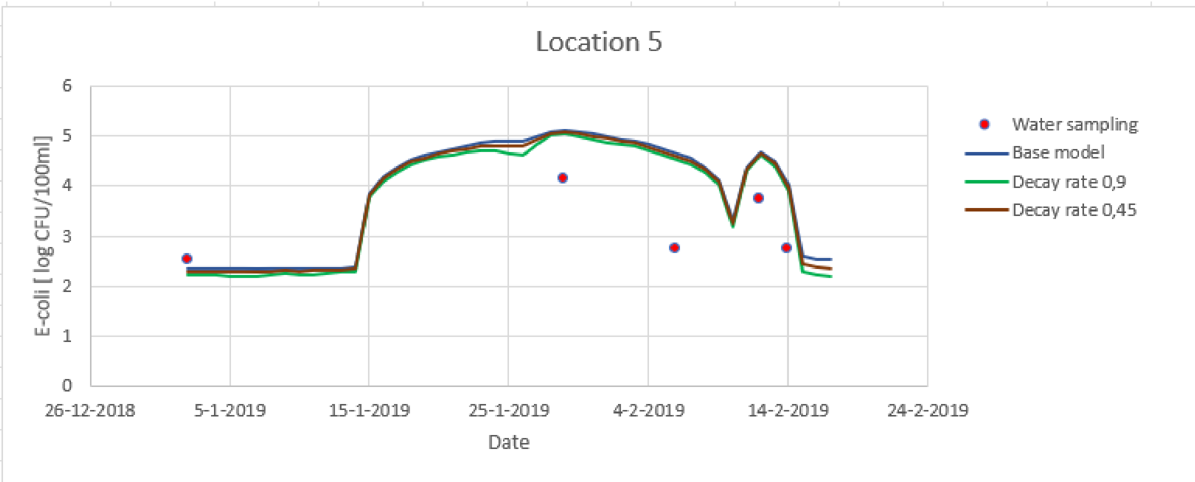

Figure 86- Sensitivity of model to decay rate at Location 5 in the winter period. The model was not sensitive to decay rate. The explanation might be extreme low simulated sewer overflows discharges and higher dilution factor.

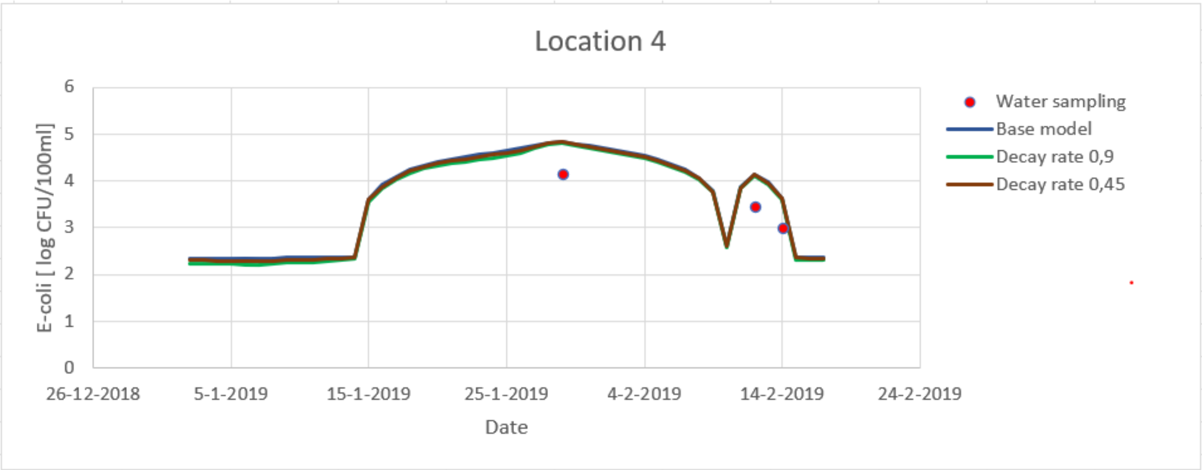

Figure 87- Sensitivity of model to decay rate at Location 4 in the winter period. The model was not sensitive to decay rate. The explanation might be extreme low simulated sewer overflows discharges and higher dilution factor.

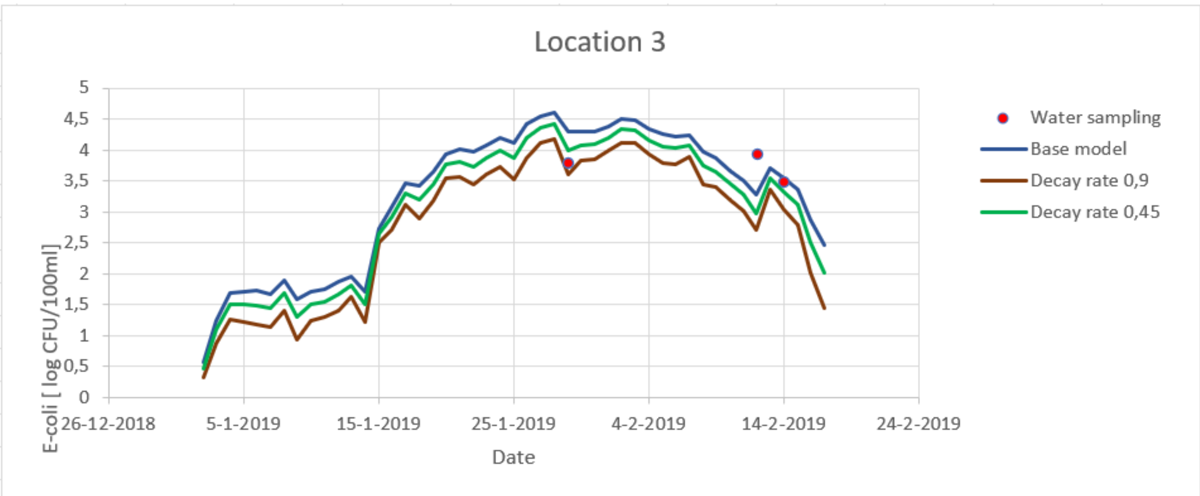

Figure 88- Sensitivity of model to decay rate at Location 3 in the winter period. The model was not sensitive to decay rate. The explanation might be extreme low simulated sewer overflows discharges and higher dilution factor. The decay rate affected only the simulated results of Location 3. An explanation can be the absence of *E. coli* dynamics at this location; therefore, the decay rate is the only factor affecting the *E. coli* concentration.

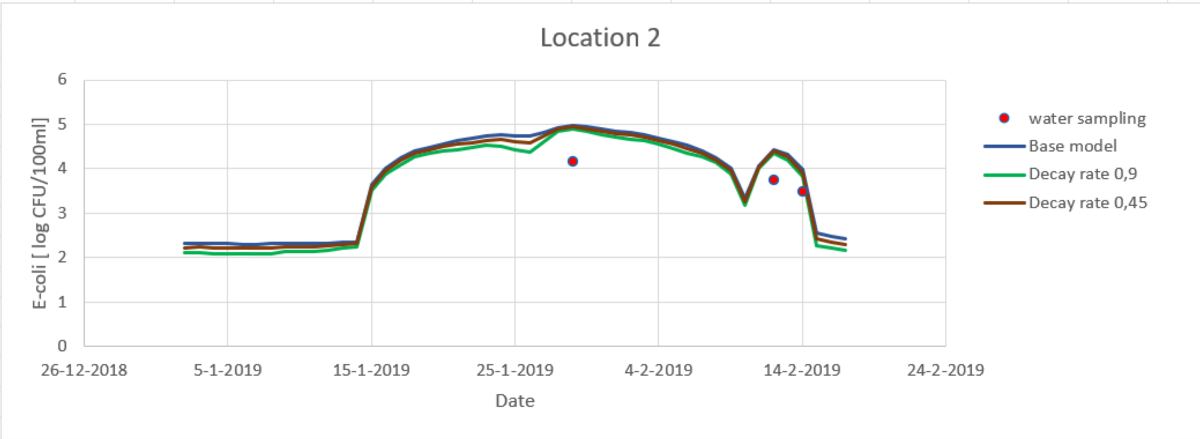

Figure 89- Sensitivity of model to decay rate at Location 2 in the winter period. The model was not sensitive to decay rate. The explanation might be extreme low simulated sewer overflows discharges and higher dilution factor.

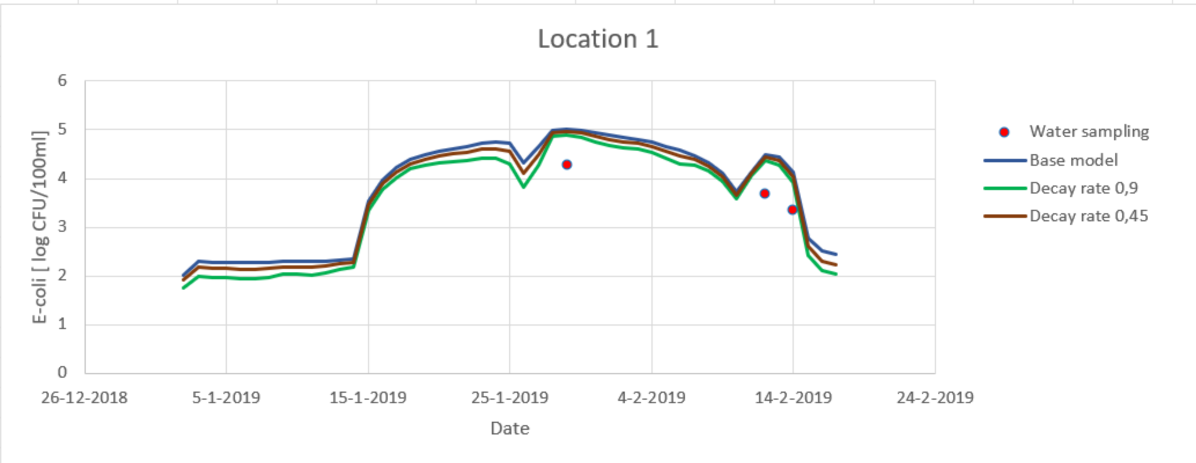

Figure 90- Sensitivity of model to decay rate at Location 1 in the winter period. The model was not sensitive to decay rate. The explanation might be extreme low simulated sewer overflows discharges and higher dilution factor. The explanation for kink on 26-1-2019 could be that the Mark flow rate is reduced by 50% and increased to 3 times on 1/28/2019 and 1/29/2019.

5 Conclusions

5.1 Water quality analysis

The first part of this study was designed to answer the sub-questions one through three, as presented in Section 1.3. It presents a water sampling analysis, analysing how water quality affects recreational water activities in Breda canals. In addition, this thesis, as an introductory study in this area, provides an overview of the microbial water quality of the Breda canals, as well as possible temporal and spatial variations of FIB during the bathing season. Taking into account the insufficiency of the exciting data on the water quality in the Breda canals, the sampling campaign of Brabantse Delta was also used in addition to this study's sampling results, to allow for a comprehensive analysis.

Microbiological water quality of the Breda canals

During dry weather, the sampling results of this study and of Brabantse Delta indicated low FIB concentrations. In the context of the "Beslisnotitie" norm, the water was safe for recreational activities.

Temporal and spatial variations of *E. coli* were observed in the Breda canals after rainfall events in the winter and summer seasons, indicating that water quality can be affected by rainfall events. The most significant peak of observed EI values was seen after rainfall events of more than 25 mm.

Interpreting a sampling analysis that is based on rainfall amount was difficult because of insufficient sampling data. Furthermore, the data were obtained under different temporal and spatial sampling conditions. In addition, due to extreme dry weather during this research period, the water sampling of intense rainfall events (above 25 mm) was only carried out in the winter period, when the hydrological conditions of streams and FIB behaviours are very different from those in the summer period.

Based on available data, it is recommended to Breda municipality to wait two days after light rainfall events of up to 12 mm with low intensity, to give permission for recreational events, for certainty's sake. After moderate rainfalls between 12mm and 25 mm, it is advised to wait a minimum of two days, depending on an event's location. After highly intense rainfall events of 25 mm or more, it is recommended that events be postponed for a minimum of four days.

Impact of weather conditions

The interpretation of water quality as it is associated with rainfall events was complicated due to insufficient sampling data, which were taken under different temporal and spatial sampling conditions, as well as uncertainty about the occurrence of CSO discharges. Additionally, sampling was only done in the winter season. Aside rainfall amount, rainfall intensity must also be taken into account.

- **Rainfall events less than 12 mm**

The peak value was observed in all winter samples from locations 4 and 5, even on the 4th day after a light rainfall of 4.5 mm. This observation may have been due to extremely low decay rate. The *E. coli* concentration was on the same order of magnitude at both locations 4 and 5 (after rainfall events of 4.5 mm).

E. coli peaks were detected frequently at locations 2 and 5 during the summer period.

In contrast to the assumption that there was no CSO discharge for rainfall events of less than 12 mm, on 6-6-2019 (9.6 mm cumulative rainfall until moment of CSO discharge), 2-8-2019 (10 mm cumulative rainfall until moment of CSO discharge; a highly intense rainfall event of 7 mm in 1.5 hour) and 12-8-2019, a discharge was detected at CSO I and CSO V. *E. coli* concentration after CSO discharge exceeded the guideline value at most locations. The *E. coli* peak at downstream location 1 indicated the impact of CSO discharges on the study area. The interpretation of *E. coli* peak, due to three CSO's discharge adjacent to Valkenberg park was difficult because the *E. coli* concentration was not measured on the sampling dates at location 5. However, no discharge at CSO III was detected. This observation indicates that the sewer system of Breda, especially near location 2, can be vulnerable to rainfall events of under 12 mm. This finding could indicate the cause of the frequent peak value of location 2.

The CSO discharge that occurred due to the rainfall event on 17-8-2018 is unknown. The result of the samples taken on 20-8-2018 revealed that the peak value returned to the dry weather value within three days at locations 2 and 4. Although, *E. coli* concentration was one log higher at locations 5 and location 1, the levels were still in compliance with the ''Beslisnotitie'' guideline. However, after the rainfall event on 2- 8-2019, which was followed by CSO discharges, the *E. coli* concentrations still exceeded the guideline value three days later, on 5-8-2019.

The IE concentration was less sensitive, compared to the *E. coli* concentration, to rainfall events of under 12 mm. In contrast to the peak value of *E. coli*, IE concentration was in compliance with "Beslisnotitie" guideline in most locations on 6-6-2010 and 5-8-2019, when CSO discharge was observed. The exception was location 2.

- **Rainfall events between 12 mm and 25 mm**

The samples were collected on the 3rd and 5th day after a rainfall of 16.95 mm on 25-8-2018. The CSO discharge that occurred is unknown. However, *E. coli* peak at most locations was detected. It took four days for the *E. coli* peak to decline in all sample locations, except location 2— the *E. coli* peak was already a known issue at this location.

The EI concentration exceeded the "Beslisnotitie" guideline only at location 5 on the 3rd day.

- **Heavy rainfall events more than 25 mm**

Rainfall events on 27-1-2019 (11.15 mm cumulative rainfall until moment of CSO discharge) and 10-2-2019 both led to CSO discharges at CSO I and CSO V, as well as detention tanks of 905 and 909, as expected.
After intense rainfall events on 27-1-2019 and 10-2-2019, *E. coli* peak was at three locations, even after four days.

The major peak values of IE were observed after rainfall events of more than 30 mm. In contrast to *E. coli*, the IE peak associated with a rainfall of 30 mm returned to a low value faster—on 3rd and 5th day.

It should be considered that the investigation of the time needed for the *E. coli* peak to decline after intense rainfall in the summer was based only on available data. This study is thus complicated by two main factors: the water temperature and the higher discharge and velocity of water in the winter season. More research is required to investigate the water quality is affected by highly intense rainfall events in the summer.

Impact of the sewer system of Breda

The *E. coli* concentration exceeded "beslisnotitie" values more often at location 5 than location 4. The connected catchment area to upstream location 5 (catchments 0 and 1) is almost 5 times larger than catchment area 2, which is connected to upstream location 4.

The connected catchment pumping stations area and the type of sewer system can affect the water quality. The FIB concentration of CSO discharge is higher than SSO discharge. In addition, a larger catchment area leads to more discharges of runoff and sewer overflows.

A highly intense rainfall event of 7 mm in 1.5 hour on 2-8-2019 led to CSO discharges, which contradicted the assumption of this study that there was no CSO discharge for rainfall events of less than 12 mm. Analysis of the results led to the conclusion that the sewer storage capacity of Breda is less than 7 mm. The high frequency of discharge of CSO V (and, probably, CSO 13-0743) led to the frequent occurrence of peak of *E. coli* concentration at location 2. Furthermore, frequent and unexpected pump failures and maintenance plans during the research period affected the sewer system performance of Breda.

After October 2018, the samples associated with CSO discharges were taken based on the Koenders online database. However, the available data were limited to five CSOs; it remains uncertain whether discharges occurred for the rest of CSOs. In addition, the data on detention tanks' water levels were not sufficient in determining whether a CSO discharge had occurred.

Plausible microbial contamination sources in the Breda canals

The results of DNA resource trace of the Breda canals, performed in 2019, suggested that, in the reference situation (dry weather), humans and ruminants have always been a source of contamination. Depending on circumstances and locations, dogs and birds may also act as contaminant source.

After rainfall events with CSO discharges, human faecal material was a source of contamination for most locations. It should be noted that effluent from the Breda WWTP does not discharge into Breda canals. Therefore, the human faecal contamination source was most likely sewer overflow discharges. Even a short duration of sewer overflow discharges, such as those caused by the rain event on 6-6-2019, can lead to increased concentrations of FIB for a few days after the rainfall, thus increasing the health risks of recreational events taking place after rainfalls. Depending on rainfall characteristics and locations, ruminants and dogs may also play a role as microbial sources.

Hot spots

E. coli peaks were detected at locations 2 and 5 more often than at other measuring points. The higher *E. coli* concentration at location 5 compared to location 4 might have been due to the higher number of CSOs and the six WWTP discharges introduced to the Mark upstream, as well as the larger connected catchment area. The CSO V near location 2 had a higher frequency of discharges compared to the other four CSOs with sensors. It can be concluded that the sewer system of this catchment is more vulnerable to even light rainfall, such as the one that took place on 6-6-2019. Further studies are needed to find a possible cause of the *E. coli* peaks at location 2.

Based on the Brabantse Delta results, the Valkenberg Park location can also be considered as a critical spot.

Water quality indicators

Both *E. coli* and IE concentration were on the same order of magnitude in dry weather. In contrast to *E. coli*, the IE peak associated with moderate and heavy rainfall events declined faster. The concentration of IE was one to two log values lower than the concentration of *E. coli*, and it had fewer temporal and spatial variations than *E. coli*.

The water quality was mostly in compliance with the "Beslisnotitie" guideline value for IE concentrations, as the results on 12-2-2019 and 5-8-2019 indicate. These results contrast the findings on *E. coli* concentrations. Therefore, *E. coli* provides more certainty when evaluating the water quality of the Breda canals; in other words, the water quality can be predicted more accurately with a modeling of *E. coli* dynamics. To avoid extra expenses, officials can solely monitor *E. coli* concentrations.

Sampling process

- Although the five measuring points covered the whole research area and provided insight into water quality, determining the impact of the sewer overflows within research area was difficult because of insufficient data on sewer overflow discharge, as well as a lack of measuring points between location 2 and upstream locations (locations 4 and 5).

- Negligible deviation was indicated by the results of the following samplings: two methods of sampling with bucket and swing sampler, at the same moment, at locations 2 and 5; two extra samples from two points within 20 m and 40 m of distance from location 3; and two extra samples from two points within distances of 7 m and 14 m from location 5. Both *E. coli* and IE were on the same order of magnitude, and the differences were not significant. These samples were taken to investigate variations in FIB as related to the length and width of the canal, and to control bias, since a sample was taken from an exact point in each location on each occasion; thus, any bias related to selecting near these locations was eliminated.

- On 14-2-2019, the *E. coli* concentration of the water sample, which was taken by Aquon at location 4, was one log higher than my water sample, which was taken one hour later. It should be taken into account that the interpretation of the results was based on a FIB analysis by three different laboratories —Aquon, Aquallab Zuid, and KWR— which may have influenced the measured FIB values. In addition, grab samples present the concentration at a specific moment in time. Therefore, the temporal variability in the concentration of grab samples, caused by the influence of flow rate, should be taken to account.

Illicit connections

The Municipality of Breda estimated a maximum 2% of connections are illicit. Between locations 2 and 4, there is no SSO, and between locations 2 and 5, there are four SSOs. Determining the impact of these four SSOs due to illicit connections was difficult because of the lack of a measuring point between locations 2 and 5. However, no *E. coli* peak was observed during other sampling in dry weather at location 2, location 3 and downstream location 1. The sampling carried out on 24-6-2019 after rainfall event of 4 mm also indicated no *E. coli* peak at the Valkenberg Parc location. The probability of an effect from only one SSO located within 500 m downstream (between locations 1 and 2) is also negligible, as confirmed by the low *E. coli* values at location 1 within a distance of 700 m from this SSO. Therefore, the probability of a microbial impact due to illicit connections in the study area is extremely low.

Impact of SSOs

It should be noted that, while SSO discharges have lower FIB concentrations than CSOs due to the dilution factor, they still contain high values of FIB; FIB contamination can then be even higher due to illicit connections. As mentioned above, however, the role of illicit connections within the study area has been eliminated.

Determining the impact of CSOs and SSOs on water quality of the study area was difficult because of uncertainty regarding whether CSO discharges occurred, as well as the absence of any measuring points between locations 2 and 5. However, the catchment areas which directly affect the water quality of upstream and the study area are connected to a combined sewer, with the exception of one part of catchment 4, from which storm water enters the study area via four SSOs.

The catchment area adjacent to the harbour is connected to a combined sewer, and only surface runoff from the streets is discharged to the harbour via two SSOs.

Since no discharge was detected at CSO IV (the only CSO that connected to the harbour) after installations of Koender ‘s sensors, the *E.coli* peak could be the result of two SSOs’ discharges. However, detection of a ruminant DNA marker at location 3, on 13-6-2019, raises doubt as to whether the harbour is solely fed by sewer overflow discharges.

Impact of decay rate and hydrological behaviour on the Breda canals

The hydrological conditions of canals differ between the winter and summer. Due to the higher flow rate, the travel time of contamination in winter is shorter than in the summer, and the dilution factor is higher in the spring and winter. However, the decay rate is significantly lower in the winter season than the summer season.

In the summer period, the FIB contamination sources in the study area can be more dominant in the first days after rainfall events than the upstream contamination sources, due to the long travel time of FIB.

Agricultural and urban runoff

Increases in the DNA markers of ruminants and dogs on 13-6-2019 suggested that agricultural and urban runoff can also be one of the major microbial sources in the Breda canals after intensive rainfall events; no variation was detected after the light rainfall event on 5-6-2019. This finding might be explained by the decreased discharge of runoff to the canals, or the late analysis of the water samples after the sampling on 6-6-2019.

The implications of the study findings

Although the results of this research mainly provide information about the microbial water quality status of the Breda canals, the study may contribute to the development of a regular water sampling plan at suitable locations in the Breda canals during the bathing season. Nevertheless, further research is needed to obtain more information on the impact of different rainfall events on FIB concentrations during the bathing season.

5.2 Sewer model analysis

The second part of this thesis involved testing the sewer model of Breda in the context of CSO discharge volumes. Since the sewer overflows are one of the major FIB contamination sources in the Breda canals, it is essential that the discrepancy between simulated and actual overflow volumes be minimised in order to create a reliable water quality model. Sensor data from five selected CSOs and catchment pumping stations were the basis of the model's modification.

Sewer model of Breda

The sewer model of Breda was designed to monitor whether the capacity of the sewer system is sufficient to avoid flooding. During wet weather, sewage directly discharges into surface water due to the overwhelm of the sewer system, main pump pressure failures, or WWTP failures. Although the sewer system is designed to limit sewage discharges into surface water, the volumes of sewer discharge entering surface water receive less priority.

The comparison of simulated and measured water levels at pumping stations indicated that, generally, the sewer model is valid, and the simulated water levels at pumping stations corresponded to the measured values. However, the model overestimated the water level due to inaccurate modeling of the pumps' operation and capacities. In practice, the pumps' capacities varied frequently, mainly due to maintenance work, so the operating conditions of available pumps in a catchment pumping station were not clear.
Hence, in the context of CSO discharge volumes, the model should be modified in order to be reliable indicator of the water quality model. However, the modification of the model according to the pumps' operational systems is complicated, as stated above.

Furthermore, the sewer model did not indicate any CSO or SSO discharges into Breda Harbour; this indication contradicts the high FIB concentrations observed on 29-1-2019, 12-2-2019, and 14-2-2019, because sewer overflow discharges are the only water sources for the harbour of Breda. The municipality was aware of the possibility of errors in harbour-area modelling. Since this location is a popular for recreational water events, further research is required to control the sewer model and SSO discharges in this area.

It should be noted that the sensors are currently the basis of the municipality's information on CSO discharges and pumping stations' water levels, but their Normal Amsterdam Peil (NAP) levels are not controlled frequently. The infrequent control affects the reliability of their data, as mentioned in Section 4.2.4.

Based on the results of this thesis, one can conclude that the best way to oversee the occurrence of CSO discharges and their quantity, as an alternative to the modification of the sewer model, is the installation of accurate sensors at 38 CSOs which affect the water quality of the study area.

Sewer system of Breda

The peak of the water level at most of the pumping stations was reached within 7.3 mm of rainfall. However, the water quality analysis of different rainfall events revealed that, after a rainfall of 7 mm in 1 hour, unexpected CSO discharges at CSO I and CSO V occurred. Based on a pumping station analysis, this observation might be explained by a failure in the pumps or a decrease in the pumps' capacity due to maintenance work.

The sensor data at CSO II confirmed the negative discharges into the sewer system. At CSO IV, no discharge was recorded, and this location responded to rainfall events gently.

It should be noted that, at CSO V, discharges into canals happened frequently, thus affecting the water quality at location 2. As the last catchment receiving sewage from the whole sewer system of Breda, this catchment should be investigated by the municipality. However, the sewer system in the area affected by CSO V will be rehabilitated, and rainfall runoff will directly discharge into canals, thereby decreasing the load on the sewer system.

Sewer overflows discharges

The sewer model of Breda generally overestimated the discharges and the water levels at the pumping stations.
The model was modified to approach factor two regarding the discrepancy between the simulated and measured discharge volumes of only five CSOs. It should be considered that, although SSO discharges have lower FIB concentrations compared to those of CSOs, they still constitute contamination sources of Breda canals. This research did not investigate the impact of model modification by increasing the infiltration and accuracy of the model to calculate SSO discharge volumes.

The implications of the study findings

The results of this study can provide valuable data on the sewer model of Breda. By comparing the measured and simulated water levels, in combination with water quality analyses, researchers can identify the vulnerable sewer catchments and uncover plausible errors in data from sensors and pumping stations.

5.3 Water quality model analysis

The third part of this study was designed to answer sub-questions four, as explained in section 1.3. This section, presents the water quality model analysis created through a simulation of InfoWorks' overflow discharges and hydraulic data in SOBEK.

Percentage contributions of water sources at sample locations

In the summer and winter, location 5 was fed solely by the Mark stream. Therefore, only Mark upstream water quality affected the *E. coli* concentration at this site. The water source at location 4 was the Weerijs stream, except in the summer period and after rainfall events; at these times the Mark stream had up to 10% contribution at this location. Thus, the water quality was only influenced by the Weerijs stream's water quality. The Mark and Weerijs streams' discharges can be up to 20 times higher in the winter period compared to the summer period. The discharges of the Mark and Weerijs streams during the sampling period is displayed in Appendix L.

Location 3, as a dead stream, had almost constant contributions of 65% and 35% for the Mark and Weerijs streams, respectively. At location 2, the Mark stream had an average contribution of 40% in the summer period, and 60% in the winter period. At location 1, the Mark stream's impact was 65% in summer period, increasing to 80% in the winter period. Therefore, the Mark stream's impact was more dominant than the Weerijs stream's at the downstream of the study area.

Percentage contributions of contamination sources in the Breda canals

The fractions of sewer overflows of the Breda sewer system, calculated through the use of the water quality model at sampling locations, indicated that the maximum fraction of sewer overflows associated with SSOs outside the study area at Location 4 in the summer period. For the rest of locations, the contribution of sewer overflows was less than 1% during the summer and winter. The simulation indicated percentage contribution of overflows at location 4 (with four upstream overflows) or location 3 in the winter period. In general, percentage contribution of sewer overflows in the summer and winter were extremely low. An underestimation of overflow discharges by InfoWorks is a possible explanation for this result.

Since the percentage contribution is calculated based on the ratio of sewer overflows to total water volume per location, comparison of the sewer overflow percentages of the locations is not an appropriate basis for analysis.

Prediction of *E. coli* concentrations based on the developed water quality model

In general, the calculated *E. coli* were underestimated in the summer period and overestimated in the winter period. There are four explanations, as listed below:

- The underestimation of sewer overflows discharges by InfoWorks had an impact. In actuality, more sewer overflow discharges occurred than were indicated in InfoWorks.
- The runoff in urban and rural areas was not simulated in the water quality model, despite that the DNA analysis indicated the animal faecal contaminations in the Breda canals entered the receiving water through runoff. The impact of rural areas is important considering that the irrigation season, which occurs during the summer, coincides with the bathing season.
- Higher initial *E. coli* concentrations at the Mark and Weerijs streams' boundaries played a role.
- The assumed decay rate, especially in the winter period, also influenced the values.

It is not feasible to develop a model to simulate the exact measured *E. coli* values, because samples represent the water quality only at a specific time and location, and the complex processes of water system are simulated with few parameters. However, the development of a reasonably accurate water quality model is feasible if more accurate input data on sewer overflows discharges are provided.

Sensitivity of the water quality model

- **Initial *E. coli* concentration**: In the summer period, the model demonstrated sensitivity to Initial *E. coli* concentration. The greatest impact of initial *E. coli* concentration was indicated at location 4. The explanation could be low fractional contribution of sewer overflows as *E. coli* source at this location (see Figure 40). The model was only sensitive to initial *E. coli* concentration in the dry weather in the summer period at location 5 and location 1. It can be concluded that the impact of sewer overflows is greater than of the initial *E. coli* concentration at these locations.

  The impact of initial *E. coli* concentration in the winter period is greater than in the summer period. Explanations may include low fractional contribution of simulated sewer overflows in the winter, lower decay rate, and higher upstream discharges, all resulting in a higher initial *E. coli* load.

  As described in section 4.3.3, initial *E. coli* concentration at Mark and Weerijs streams' boundaries were unknown due to the absence of measuring points at these locations. By considering decay rate as a rough estimation, it was assumed that initial *E. coli* loads at the boundaries of both the Mark and Weerijs were one log higher than the measured *E.coli* concentrations at locations 4 and 5. This estimate was far from the actual concentration and led to an underestimation of *E.coli* in the summer period and an overestimation of *E.coli* in the winter period. Considering the low discharges of the Mark and Weerijs streams in the summer period, the constant value of initial *E. coli* concentration can be used in the model in dry weather. However, the assumption of constant value of 250 [CFU/100ml] for *E. coli* resulted in underestimation of *E. coli* concentration

- ***E. coli* concentration of sewer overflows** :The model demonstrated sensitivity to the *E.coli* concentration of sewer overflows in the summer period, except at location 4. The explanation might be the low fractional contribution of sewer overflows at this location (see Figure 40). The model aligned more closely with measured values when *E. coli* concentration of $1*E^{12}$ [CFU/m^3] and $1*E^{10}$ [CFU/m^3] were selected for CSO and SSO, respectively. The lower *E. coli* concentration resulting from the deletion of the CSO discharge factor indicated that CSOs discharge affected the water quality more than the SSOs discharge in the summer period.

  The model demonstrated no sensitivity to the *E. coli* concentration of sewer overflows in the winter period. However, the peak values were detected after two heavy rainfalls by selecting *E. coli* concentration of $1*E^{12}$ [CFU/m^3] and $1*E^{10}$ [CFU/m^3] for CSO and SSO, respectively. The explanation might be the extremely low simulated sewer overflows discharges and a higher dilution factor.

- **Decay rate**: The sensitivity of the model to decay rate was analysed using decay rates of 0, 0.45 and 0.8 in the summer period. The decay rate of 0 indicated the impact of decay rate versus hydrology. The model was sensitive to decay rate; higher decay rate resulted in a lower *E. coli* concentration.
  The absence of *E. coli* dynamics with a decay rate of 0 can be explained by the extremely low flowrate of the Mark and Weerijs streams, as well as simulated sewer overflow discharges in the summer period. The variation of *E. coli* concentration with a decay rate of 0 was detected only at location 4. The decreasing flowrate of Weerijs in the period

between 20-8-2018 and 25-8-2018 may provide the explanation. The order of magnitude of the *E. coli* concentration declined approximately one log with a 50% decrease in decay rate. The *E. coli* travel time of three days after rainfalls with a decay rate of 0 was confirmed by measuring results at location 5.

In the winter period, the model was not sensitive to decay rate. This result might be explained by extremely low simulated sewer overflows discharges, higher dilution factor, and low initial *E. coli* load. The decay rate affected only the location 3. A possible explanation is the absence of *E. coli* dynamics at this location; therefore, the decay rate is the only factor affecting the *E. coli* concentration.

The implications of the study findings

The results of this study indicate that municipality should adopt a strategy to minimise the discrepancy between simulated and actual sewer overflow discharges. In addition, a better rain–runoff model (RR module) for conducting simulations in SOBEK can be developed by the waterboard of Brabantse Delta. However, further research is needed to accurately simulate intense rainfall events in the summer and to compare these findings with sampling results.

5.4 Overall conclusions

This section provides answers to the main question of this study: "What is the water quality of the Breda canals in the context of recreational activities during the bathing season?''

In this thesis, the "Beslisnotitie'' guideline was selected as a framework for the water quality criteria. The criteria in this guideline are defined as 1,800 [CFU/100 ml] and 400 [CFU/ 100 ml], respectively, for *E. coli* and IE. It must be noted that, during this research, STOWA released a new guideline, which states that an event can continue if no or little (< 10 mm) rainfall has occurred in the four days prior to the event. However, this advice is based on the Dommel waterboard's research on the Den Bosch water canals and cannot be generalised for all water systems. The results of this thesis have indicated that the sewer overflows of the Breda sewer system react more quickly to light rainfall, and, due to low discharges of flow in the summer, the travel time of FIB contamination can be longer.

This thesis has demonstrated that *E. coli* concentration has spatial and temporal variations during the bathing season. The water quality of the Breda canals during the dry weather of the bathing season was in compliance with the "Beslisnotitie" norm, and no peaks in *E. coli* were observed, however, an *E. coli* peak can be expected after a rainfall of 7 [mm/h]. The results analysis leads to the conclusion that the sewer storage capacity of Breda is less than 7 mm.

Based on the intensity of rainfall events, one can anticipate how long it will take for an *E. coli* peak to decrease. For light rainfall events with low intensities of up to 12 mm, the expected decrease time is two days. After moderate rainfall between 12 mm and 25 mm, it is a minimum of two days, depending on the event location. Finally, for highly intense rainfall events of 25 mm or more, a minimum of four days is required before the *E. coli* level meets the "Beslisnotitie" norm again.

It should be taken to account that samples reflect the water quality at the moment of sampling and may not represent the daily value. Moreover, even if the water quality meets the "Beslisnotitie" guideline, researchers indicated that the possible presence of other pathogens may still threaten human health.

Due to extreme dry weather during this research period, the water sampling of intense rainfall events (over 25 mm) was carried out during the winter period, when the water system and FIB behaviours are totally different from those of the summer period. Therefore, further research is required to analyse the impact of intense rainfall in the bathing season.

Since the water sampling analysis is expensive and time consuming, the municipality can investigate the water quality of the Breda canals by developing a water quality model.

For this project, a 1D model was set up using SOBEK version 2.16.003, which was developed by Deltares and is used by the water boards in the Netherlands as a standard hydrological model. 1DWAQ module, which allows users to determine water quality and water fraction in an integrated rural–urban context, is linked automatically to SOBEK-Urban.

To run the water quality model, overflows discharge simulated by the sewer model during the sampling period are used as input; thus, the discrepancy between the simulated and actual CSO discharge should be negligible if one wishes to reach a significant correspondence between the *E. coli* concentration simulated by the water quality model and the measured *E. coli* concentration. To make the modification of the sewer model feasible, the discrepancy between the simulated and actual overflow discharges was confined to Factor 2, as explained in section 3.2.8.

The results of the developed model indicated that simulated *E. coli* concentrations were significantly lower than the measured values in the summer, and higher than the measured values in the winter. There are four explanations, as listed below:

- The confinement of the discrepancy between simulated and actual overflow discharges to Factor 2 based on data from only five sewer overflows, led to an underestimation of sewer overflow discharges by InfoWorks. Most sewer overflow discharges were zero after modification of model.
- Although the runoff in urban and rural areas was not simulated in the water quality model, DNA analysis indicated that animal faecal contaminations in the Breda canals entered the receiving water through runoff. The impact of rural areas is important, considering that the irrigation season, which occurs during the summer, coincides with the bathing season.
- Considering the low discharges of Mark and Weerijs streams in the summer period, the constant value of initial *E. coli* concentration can be used in the model in dry weather. However, the assumption of constant value of 250 [CFU/100ml] for initial *E. coli* concentration in dry weather—for instance, selecting one log higher than the measured *E.coli* concentrations at locations 4 and 5 as the initial *E.coli* concentration in wet weather—resulted in an underestimation of *E. coli* concentration.
- The decay rate seems be lower than 0.8 in the summer period and be higher than 0.14 in the winter period.

The model demonstrated sensitivity to initial *E. coli* concentration in the summer and winter periods, and lack of sensitivity to decay rate and *E. coli* concentration of sewer overflows in the winter period. The explanation might be the extremely low simulated sewer overflow discharges and higher dilution factor in the winter.

The model corresponded better to measured values when *E. coli* concentration of $1^* E^{12}$ [CFU/m^3] and 1^*E^{10} [CFU/m^3] were selected for CSO and SSO, respectively. The lower *E. coli* concentration resulting from the deletion of the CSO discharge factor indicated that the CSOs discharge affected the water quality more than the SSOs discharge in the summer period. However, it should be taken into account that this analysis was based on simulated sewer overflows discharge.

Based on a sensitivity analysis of this model, it can be concluded that there is uncertainty surrounding some essential inputs of the model, including sewer overflows discharge and initial *E. coli* load. To develop a more accurate water quality model, the quality of input data should be improved. One can conclude that the best way to oversee the occurrence of CSO discharges and their quantity, as an alternative to the modification of the sewer model, is the installation of accurate sensors at 38 CSOs which affect the water quality of the study area.

However, collecting more sample data in different rainfall events sounds more feasible than sensors installation for collecting reliable data on sewer overflows discharge.

6 Recommendations

6.1 Water sampling

- As mentioned in section 5.4, the investigation of the water quality of the Breda canals after intense rainfall events in the summer period needs further research and water sampling. Furthermore, developing an accurate water quality model requires a data set of two to three consecutive years if the model is to be calibrated and validated. This research has defined the impact of weather conditions in three categories: light rainfall events of less than 12 mm, moderate rainfall between 12 mm and 25 mm, and intense rainfall of more than 25 mm. Further research would provide more information to complete the investigation on the impact of weather conditions on the water quality of the Breda canals.

- Since the Valkenberg Park is a favorite location for recreational activities, it is advised that extra samples be taken at this location

- The contribution of overflows at Location 1 did not correspond to that of overflows at location 2. An explanation might be the convergence of the Mark and Weerijs streams and the widening of the canal at Location 2, both of which led to a specific hydraulic situation at this location. . It is recommended to move Location 2 50m towards Location 1.

- Two extra sample locations at the model boundaries of the Mark and Weerijs can provide useful data on initial *E. coli* concentration.

- Considering that the Breda canals were more sensitive to *E. coli* concentrations compared to IE concentrations, as well as the fact that the model was developed based on *E. coli* concentrations, future water sampling research can be limited just to *E. coli* analyses to avoid extra expenses.

- Extra investigation is required for Location 2 due to the unexpected consequences of *E. coli* concentration. The possibility of local pollutions, illicit connections, or the frequency of adjacent CSOs should be accounted for.

6.2 Simulation of discharged sewer overflows volume

The water quality model results of this research suggest that the simulation of sewer overflows discharge varies greatly from the actual situation. The InfoWorks model underestimates sewer overflows discharge. To approach a more accurate simulation, there are two solutions, as presented below.

- The sewer model, especially at the harbour section, should be adjusted according to the modification of the catchment pump schedule; considering unexpected pump failures and maintenance plans, however, this approach is not feasible.

- Since the water quality model is the most sensitive to CSO concentration, certainty about the occurrence of CSOs is essential. The best solution to this problem is the installation of sensors at 38 CSOs of the Breda sewer system that affect the water quality of the study area. The sensors' data should be sent at shorter time intervals (in minutes, for example) instead of in 12-hour intervals from the present research.

The harbour was assumed as a dead stream by InfoWorks. However, the sample results and fractional contribution of overflows at this location contradict the assumption of a dead-end harbour. The possibility of errors in harbour-area modelling was known to the municipality. Since this location is a popular for recreational water events, further research is required to control the sewer model and SSOs discharge in this area.

6.3 Water quality model

- The decay rate in this research was simplified, and constant values such as salinity, irradiation at the surface of the water, mortality rate, and the temperature coefficient were used in the model. Although the sensitivity analysis of this research model indicated that decay rate has no significant impact on calculated *E. coli* concentrations in the winter period, further research is required to control the other factors of Equation (1), such as the impact of water temperature or solar radiation at the surface on calculated *E. coli* concentrations in the bathing period.

- In the model developed for this thesis, the urban and rural runoff were not simulated. By acquiring more details about rural areas from the water board, the rainfall–runoff (RR) module of can be simulated in SOBEK.

- The water age was not calculated in the developed model of this research because the model results were significantly lower than the measured values, and the quality of the input data should be improved. However, the water age data are essential in deciding whether a recreational water event can be held after rainfall events. This research presents advice based on results from sampling taken three and five days after rainfall events. The water age can be calculated in a new model using the tracer analysis in SOBEK.

- The impact of initial *E. coli* concentrations at the Mark and Weerijs boundaries should be analysed. In reality, it is not expected that both boundaries would have same initial values, because the number of sewer overflows and WWTP discharges is higher at the Mark stream than at the Weerijs stream. Further research will be needed to investigate appropriate values for both boundaries.

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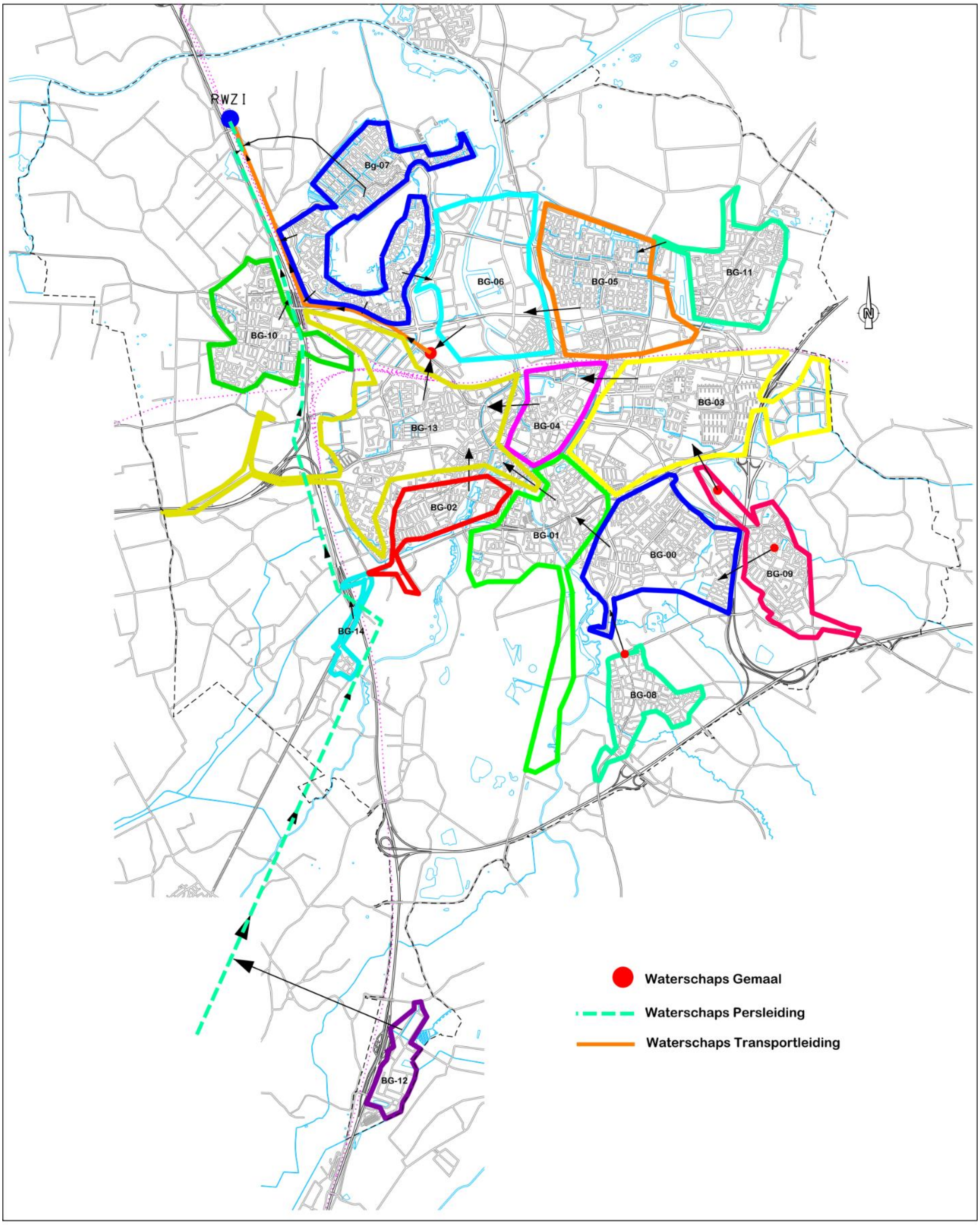
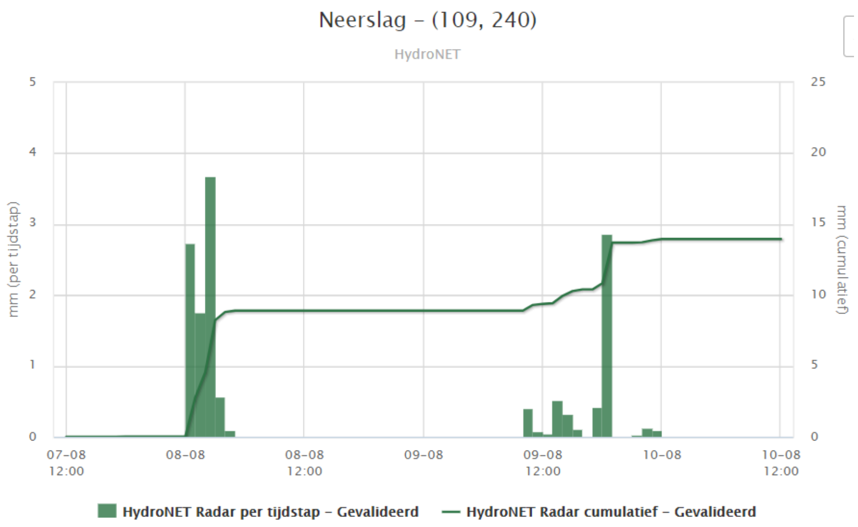
Appendix A : Pump catchments of sewer system of Breda

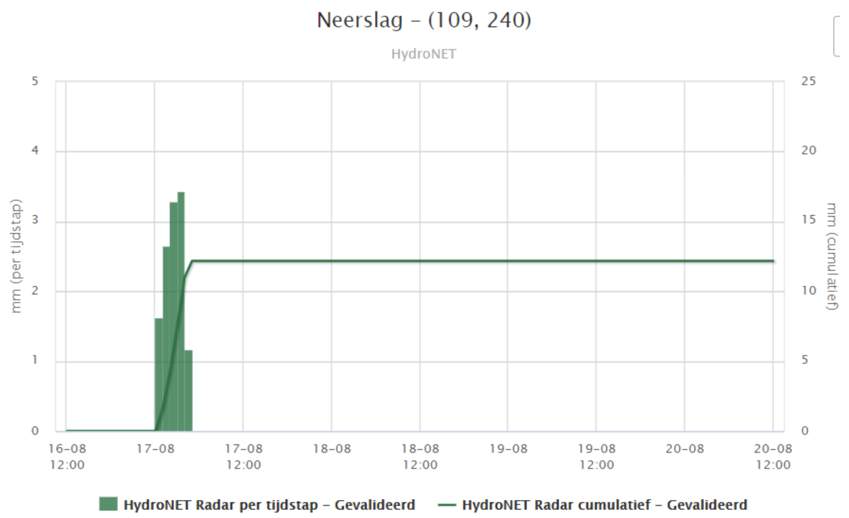


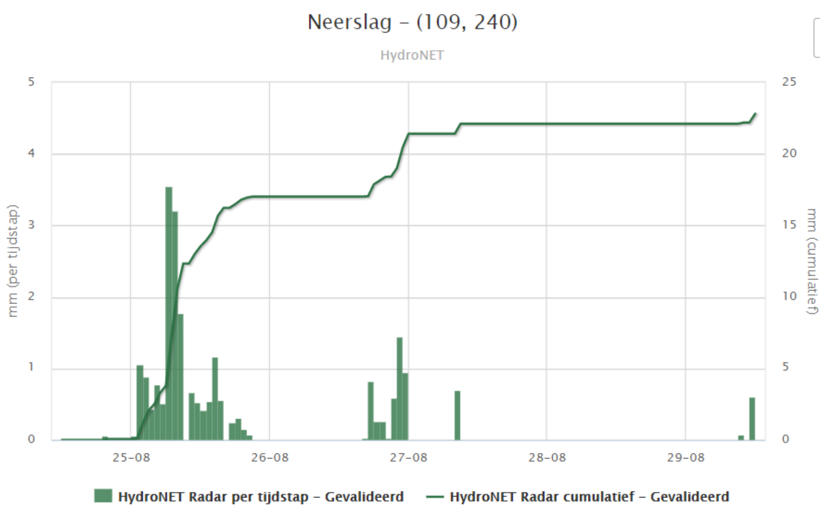
Appendix B : Catchment areas of separated and combined sewer of Breda

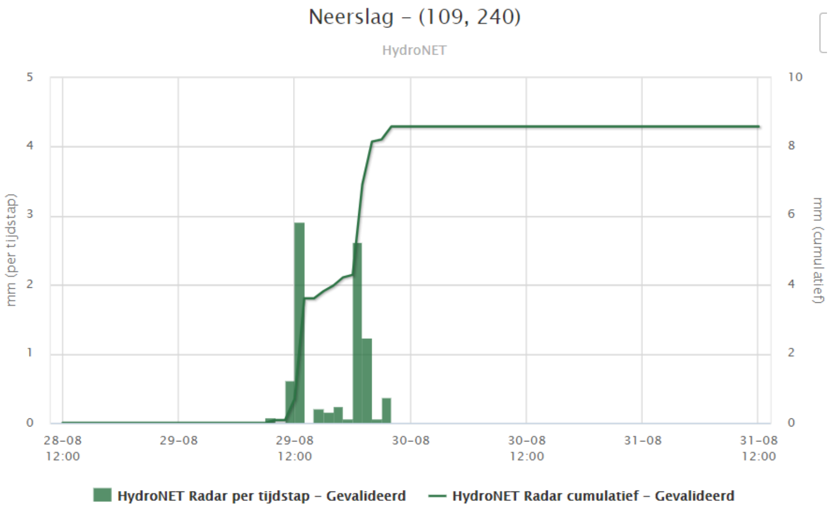
Catchment pumping station	Catchement foul sewer [ha]	Catchement storm sewer [ha]	Catchement combined sewer [ha]	Catchement other [ha]
0	56,6	49,0	239,1	
1	30,4	34,7	417,4	
2	22,9	33,6	129,6	
3	219,0	245,8	471,4	
4	35,3	45,9	127,9	
5	35,7	46,7	294,6	
6	293,9	303,8	231,1	
7	372,0	381,8	0,0	
8	14,7	18,5	113,0	
9	28,1	36,0	121,8	
11	108,5	102,4	125,5	
13	245,9	246,8	279,2	
14	4,0	6,3	17,7	
12	11,1	75,4		28,3
10	41,5	48,6	197,4	
	1519,7	1675,3	2765,8	

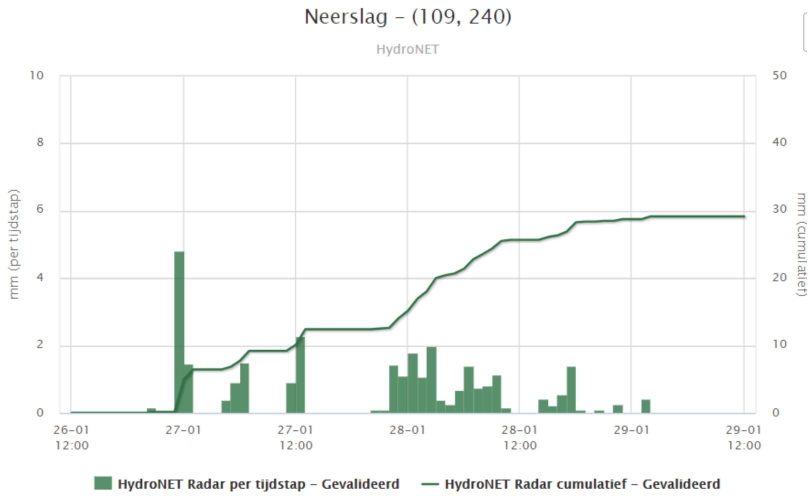
Appendix C : Rainfalls intensity and amount (in Dutch)

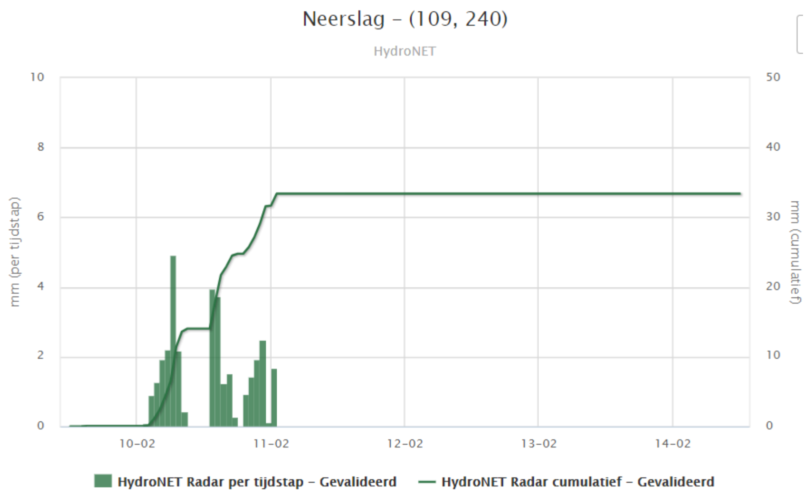


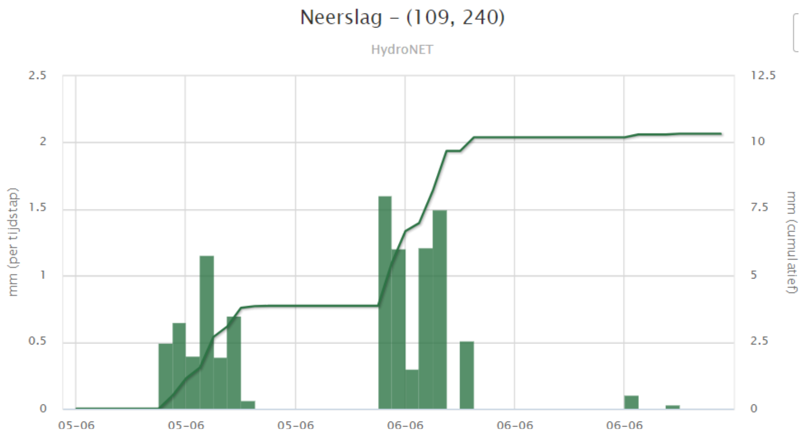


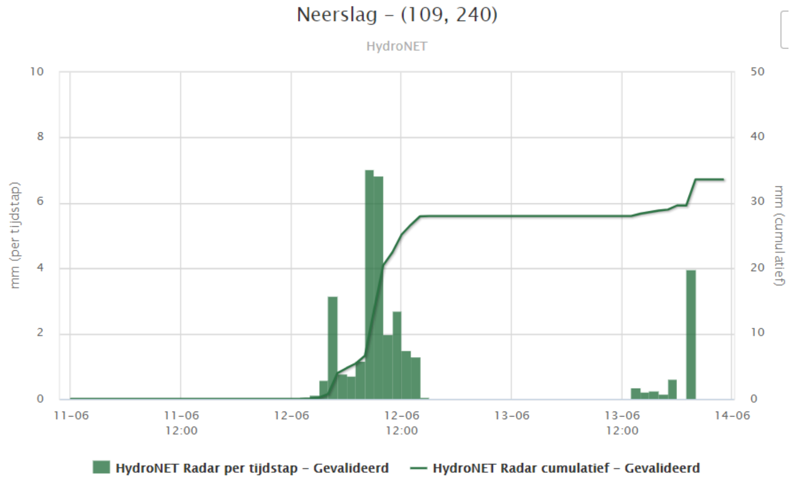


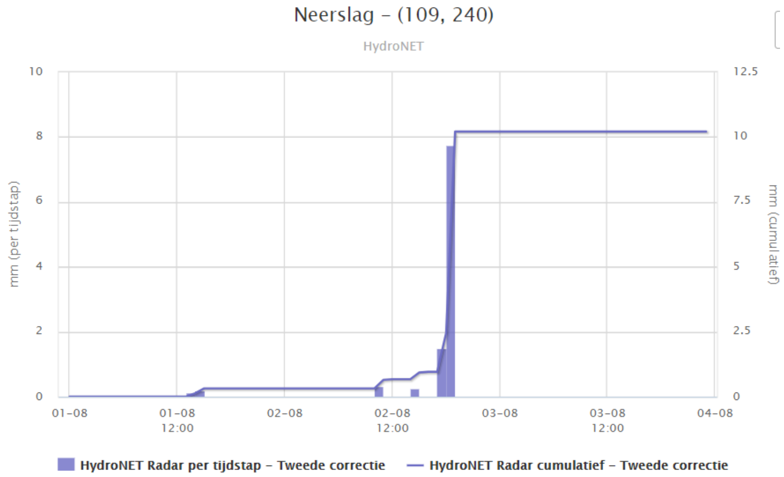

Neerslag - (109, 240)
HydroNET
mm (per tijdstap)
mm (cumulatief)
28-08 12:00
29-08
29-08 12:00
30-08
30-08 12:00
31-08
31-08 12:00
HydroNET Radar per tijdstap - Gevalideerd
HydroNET Radar cumulatief - Gevalideerd


Neerslag - (109, 240)
HydroNET
mm (per tijdstap)
mm (cumulatief)
26-01 12:00
27-01
27-01 12:00
28-01
28-01 12:00
29-01
29-01 12:00
HydroNET Radar per tijdstap - Gevalideerd
HydroNET Radar cumulatief - Gevalideerd


Neerslag - (109, 240)
HydroNET
mm (per tijdstap)
mm (cumulatief)
10-02
11-02
12-02
13-02
14-02
HydroNET Radar per tijdstap - Gevalideerd
HydroNET Radar cumulatief - Gevalideerd


Neerslag - (109, 240)
HydroNET
mm (per tijdstap)
mm (cumulatief)
2.5
2
1.5
1
0.5
0
12.5
10
7.5
5
2.5
0
05-06
05-06
05-06
06-06
06-06
06-06


Neerslag - (109, 240)
HydroNET
mm (per tijdstap)
mm (cumulatief)
10
8
6
4
2
0
50
40
30
20
10
0
11-06
11-06 12:00
12-06
12-06 12:00
13-06
13-06 12:00
14-06
HydroNET Radar per tijdstap - Gevalideerd
HydroNET Radar cumulatief - Gevalideerd


Neerslag - (109, 240)
HydroNET
mm (per tijdstap)
mm (cumulatief)
10
8
6
4
2
0
12.5
10
7.5
5
2.5
0
01-08
01-08 12:00
02-08
02-08 12:00
03-08
03-08 12:00
04-08
HydroNET Radar per tijdstap - Tweede correctie
HydroNET Radar cumulatief - Tweede correctie

Appendix D : *E- coli* concentrations of total sampling

				Escherichia coli (MPN) n/100ml per date per locatieon								
Date of sampling	Date of rainfall	Rainfall [mm]	Sample collector	Location 1 Mark singel (Belcrum beach)	Location 2 Nieuwe Mark singel	Location 3 Nieuwe Mark singel (haven)	Location 4 Weerijssingel (Bernhard brug)	Location 5 Mark singel (Juliana brug)	Location 6 Mark singel (Galderweg 99)	Location a (500m from loc.4)	Location b (1,7 km from loc.5)	Valkenberg Park
3-5-2017	-	-	Brabantse Delta-monthly							-	120	
22-5-2017	-	-	Brabantse Delta-monthly							78	-	
7-6-2017	-	-	Brabantse Delta-monthly								40	
19-6-2017	-	-	Brabantse Delta-monthly							120	-	
5-7-2017	-	-	Brabantse Delta-monthly							-	210	
20-7-2017	-	-	Brabantse Delta-monthly							250	-	
2-8-2017	-	-	Brabantse Delta-monthly							-	40	
22-8-2017	-	-	Brabantse Delta-monthly							140	-	
6-9-2017	-	-	Brabantse Delta-monthly							-	730	
20-9-2017	-	-	Brabantse Delta-monthly							110	-	
4-10-2017	-	-	Brabantse Delta-monthly							-	110	
18-10-2017	-	-	Brabantse Delta-monthly							200	-	
8-11-2017	-	-	Brabantse Delta-monthly							-	690	
13-11-2017	-	-	Brabantse Delta-monthly							220	-	
29-11-2017	27-11-2017	12,9	Brabantse Delta-monthly							-	7100	
11-12-2017	10-12-2017	11,9	Brabantse Delta-monthly							5700	-	
10-1-2018	-	-	Brabantse Delta-monthly							-	650	
22-1-2018	21-1-2018	4,4	Brabantse Delta-monthly							1900		
7-2-2018	2-2-2018	7,3	Brabantse Delta-monthly							-	1600	
19-2-2018	-	-	Brabantse Delta-monthly							210	-	
7-3-2018	4-3-2018	4,5	Brabantse Delta-monthly							-	2100	
19-3-2018	-	-	Brabantse Delta-monthly							290	-	
4-4-2018	-	-	Brabantse Delta-monthly							-	660	
16-4-2018	-	-	Brabantse Delta-monthly							270	-	
2-5-2018	30-4-2018	32,6	Brabantse Delta-monthly							-	12000	
23-5-2018	-	-	Brabantse Delta-monthly							110	-	
6-6-2018	-	-	Brabantse Delta-monthly							-	290	
18-6-2018	-	-	Brabantse Delta-monthly							130	-	
4-7-2018	-	-	Brabantse Delta-monthly							-	160	
16-7-2018	-	-	Brabantse Delta-monthly							30	-	
25-7-2018	-	-	researcher	1400	120	710	10	930		-		
30-7-2018	-	-	researcher	43	380	130	260	150				
6-8-2018	-	-	Brabantse Delta-monthly								15	
10-8-2018	8-8-2018	8,8	researcher	760	1100	450	1100	3900				
17-8-2018	17-8-2018	12,2	researcher	300	2500	590	2200	540	3900			
20-8-2018	17-8-2018	12,2	researcher	1300	580	520	87	1000				
21-8-2018	-	-	Brabantse Delta-monthly							46	-	
27-8-2018	25-8-2018	16,95	researcher	1100	1200	4300	370	13000				
29-8-2018	25-8-2018	16,95	researcher	770	5300	860	130	500				
31-8-2018	29-8-2018	8,7	researcher	580	12000	580	230	5400				
5-9-2018	-	-	Brabantse Delta-monthly							-	140	
17-9-2018	-	-	Brabantse Delta-monthly							15	-	
20-9-2018	-	-	researcher	370	3700	46	61	290			-	
8-10-2018	-	-	Brabantse Delta-monthly							-	160	
9-10-2018	-	-	Brabantse Delta-monthly							94	-	
8-11-2018	-	-	Brabantse Delta-monthly								180	
19-11-2018	-	-	Brabantse Delta-monthly							15	-	
12-12-2018	-	-	Brabantse Delta-monthly							270	1500	
2-1-2019	-	-	Brabantse Delta-monthly							61	330	
29-1-2019	27-1-2019,28-1-2019	29	researcher	19000	15000	6300	14000	14000				
6-2-2019	-	-	Brabantse Delta-monthly							-	550	
12-2-2019	10-2-2019	33,4	researcher	4800	5800	8700	2800	5300				
14-2-2019	10-2-2019	33,4	researcher	2200	3200	3000	940	530				
14-2-2019	11-2-2019	34,4	Brabantse Delta-monthly							1600		
1-4-2019	-	-	Brabantse Delta-monthly								61	
2-4-2019	-	-	researcher	190	1300	300	240	100				
6-6-2019	5-6-2019	10,2	researcher	5900	>30000	<100	230	7100				
11-6-2019	-	-	Brabantse Delta-event		920	660						4400
13-6-2019	12-6-2019	28	researcher	18000	19000	18000	18000	51000				
24-6-2019	20-6-2019	4	Brabantse Delta-event		480	180						560
5-8-2019	2-8-2019	10,2	Brabantse Delta-event		1700	3800						4100
13-8-2019	12-8-2019	20	Brabantse Delta-event		3200	4300						3400

Appendix E : IE concentrations of total sampling

Intestinale enterokokken (MPN) n/100ml per date per locatieon

Date of sampling	Date of rainfall	Rainfall [mm]	Sample collector	Location 1 Mark singel (Belcrum beach)	Location 2 Nieuwe Mark singel	Location 3 Nieuwe Mark singel (haven)	Location 4 Weerijssingel (Bernhard brug)	Location 5 Mark singel (Juliana brug)	Location 6 Mark singel (Galderweg 99)	Location a (500m from loc.4)	Location b (1,7 km from loc.5)	Valkenberg Park
10-8-2018	8-8-2018	8,8	researcher	87	43	87	690	220				
17-8-2018	17-8-2018	12,2	researcher	65	76	87	900	53	450			
20-8-2018	17-8-2018	12,2	researcher	76	65	10	53	53				
27-8-2018	25-8-2018	16,95	researcher	93	140	130	77	930				
29-8-2018	25-8-2018	16,95	researcher	15	61	15	30	15				
31-8-2018	29-8-2018	8,7	researcher	15	140	15	64	110				
29-1-2019	27-1-2019,28-1-2019	29	researcher	1600	1600	800	1300	2700				
12-2-2019	10-2-2019	33,4	researcher	320	250	1500	220	290				
14-2-2019	10-2-2019	33,4	researcher	15	94	350	14	30				
6-6-2019	5-6-2019	10,2	researcher	210	2000	3	110	90				
13-6-2019	12-6-2019	28	researcher	1900	3000	1900	4700	48000				
5-8-2019	2-8-2019	10,2	Brabantse Delta-event		46	45						330
13-8-2019	12-8-2019	10	Brabantse Delta-event		520	77						2000

Appendix F Brabantse Delta sampling results at location a

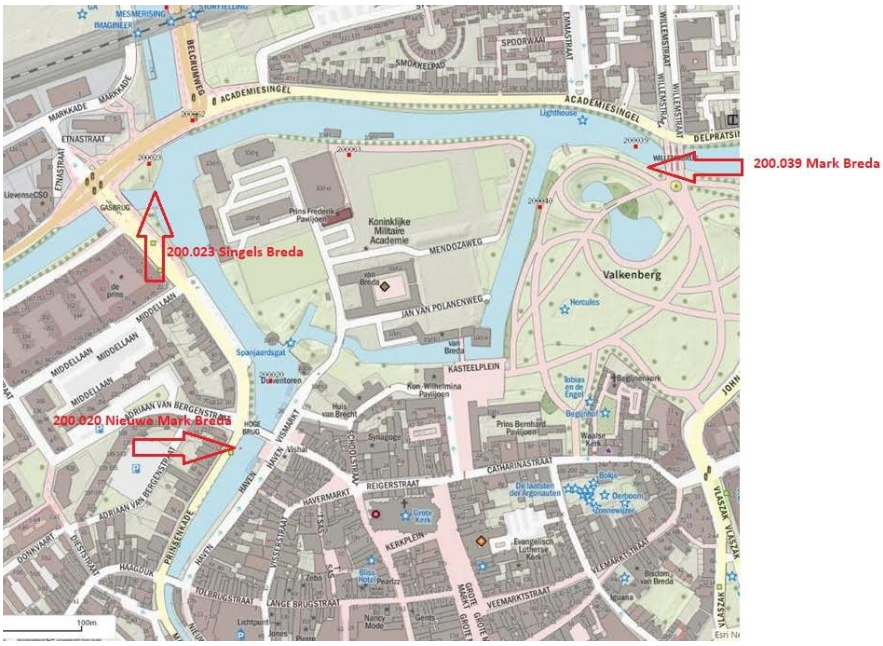
					chloride	Escherichia coli	Zuurgraad
Meetpunt	**Datum**	**Tijd**	**Omschrijving**	**Waterloop**	**Cl [mg/l] [NVT] [OW]**	**E_COLI [n/dl] [KVE] [OW]**	**pH [DIMSLS] [NVT] [OW]**
210002	03-05-2017	07:49:00	BOVENSTR. BRUG IN DUIVELSBRUGLAAN	De Bovenmark	32,30	120,00	7,50
210002	07-06-2017	07:43:00	BOVENSTR. BRUG IN DUIVELSBRUGLAAN	De Bovenmark	34,90	40,00	7,60
210002	05-07-2017	07:07:00	BOVENSTR. BRUG IN DUIVELSBRUGLAAN	De Bovenmark	41,00	210,00	7,60
210002	02-08-2017	07:56:00	BOVENSTR. BRUG IN DUIVELSBRUGLAAN	De Bovenmark	25,70	40,00	8,00
210002	06-09-2017	13:04:00	BOVENSTR. BRUG IN DUIVELSBRUGLAAN	De Bovenmark	39,20	730,00	7,40
210002	04-10-2017	07:15:00	BOVENSTR. BRUG IN DUIVELSBRUGLAAN	De Bovenmark	35,70	110,00	7,50
210002	08-11-2017	15:38:00	BOVENSTR. BRUG IN DUIVELSBRUGLAAN	De Bovenmark	37,40	690,00	7,40
210002	29-11-2017	10:35:00	BOVENSTR. BRUG IN DUIVELSBRUGLAAN	De Bovenmark	22,60	7100,00	7,20
210002	10-01-2018	14:46:00	BOVENSTR. BRUG IN DUIVELSBRUGLAAN	De Bovenmark	33,80	650,00	7,10
210002	07-02-2018	13:57:00	BOVENSTR. BRUG IN DUIVELSBRUGLAAN	De Bovenmark	33,70	1600,00	7,30
210002	07-03-2018	12:55:00	BOVENSTR. BRUG IN DUIVELSBRUGLAAN	De Bovenmark	43,00	2100,00	7,40
210002	04-04-2018	14:41:00	BOVENSTR. BRUG IN DUIVELSBRUGLAAN	De Bovenmark	26,40	660,00	7,40
210002	02-05-2018	07:04:00	BOVENSTR. BRUG IN DUIVELSBRUGLAAN	De Bovenmark	13,00	12000,00	6,80
210002	06-06-2018	12:43:00	BOVENSTR. BRUG IN DUIVELSBRUGLAAN	De Bovenmark	30,90	290,00	7,30
210002	04-07-2018	14:27:00	BOVENSTR. BRUG IN DUIVELSBRUGLAAN	De Bovenmark	44,80	160,00	8,00
210002	06-08-2018	13:43:00	BOVENSTR. BRUG IN DUIVELSBRUGLAAN	De Bovenmark	44,90	15,00	7,70
210002	05-09-2018	14:18:00	BOVENSTR. BRUG IN DUIVELSBRUGLAAN	De Bovenmark	47,40	140,00	8,20
210002	08-10-2018	14:37:00	BOVENSTR. BRUG IN DUIVELSBRUGLAAN	De Bovenmark	54,40	160,00	7,70
210002	08-11-2018	11:02:00	BOVENSTR. BRUG IN DUIVELSBRUGLAAN	De Bovenmark	33,80	180,00	7,20
210002	12-12-2018	10:18:00	BOVENSTR. BRUG IN DUIVELSBRUGLAAN	De Bovenmark	41,00	1500,00	7,00
210002	02-01-2019	11:48:00	BOVENSTR. BRUG IN DUIVELSBRUGLAAN	De Bovenmark	45,70	330,00	7,30
210002	06-02-2019	14:35:00	BOVENSTR. BRUG IN DUIVELSBRUGLAAN	De Bovenmark	49,90	550,00	7,20
210002	04-03-2019	13:50:00	BOVENSTR. BRUG IN DUIVELSBRUGLAAN	De Bovenmark	38,20	2400,00	7,20
210002	01-04-2019	07:42:00	BOVENSTR. BRUG IN DUIVELSBRUGLAAN	De Bovenmark	43,30	61,00	7,30

Appendix G Brabantse Delta sampling results at location b

					chloride	Escherichia coli	Zuurgraad
Meetpunt	Datum	Tijd	Omschrijving	Waterloop	Cl [mg/l] [NVT] [OW]	E COLI [n/dl] [KVE] [OW]	pH [DIMSLS] [NVT] [OW]
220013	22-05-2017	09:43:00	BRUG IN JULIANALAAN	Aa Of Weerijs	33,40	78,00	7,30
220013	19-06-2017	13:59:00	BRUG IN JULIANALAAN	Aa Of Weerijs	32,70	120,00	7,30
220013	20-07-2017	06:35:00	BRUG IN JULIANALAAN	Aa Of Weerijs	20,70	250,00	7,00
220013	22-08-2017	07:09:00	BRUG IN JULIANALAAN	Aa Of Weerijs	26,00	140,00	7,20
220013	20-09-2017	13:43:00	BRUG IN JULIANALAAN	Aa Of Weerijs	22,50	110,00	7,00
220013	18-10-2017	11:28:00	BRUG IN JULIANALAAN	Aa Of Weerijs	24,80	200,00	7,10
220013	13-11-2017	07:16:00	BRUG IN JULIANALAAN	Aa Of Weerijs	31,10	220,00	7,30
220013	11-12-2017	14:42:00	BRUG IN JULIANALAAN	Aa Of Weerijs	32,80	5700,00	7,00
220013	22-01-2018	15:14:00	BRUG IN JULIANALAAN	Aa Of Weerijs	18,70	1900,00	6,80
220013	19-02-2018	15:17:00	BRUG IN JULIANALAAN	Aa Of Weerijs	33,10	210,00	6,80
220013	19-03-2018	15:45:00	BRUG IN JULIANALAAN	Aa Of Weerijs	29,70	290,00	7,00
220013	16-04-2018	06:57:00	BRUG IN JULIANALAAN	Aa Of Weerijs	25,50	270,00	7,00
220013	23-05-2018	09:26:00	BRUG IN JULIANALAAN	Aa Of Weerijs	28,80	110,00	7,10
220013	18-06-2018	13:26:00	BRUG IN JULIANALAAN	Aa Of Weerijs	29,30	130,00	7,40
220013	16-07-2018	06:59:00	BRUG IN JULIANALAAN	Aa Of Weerijs	38,50	30,00	7,20
220013	21-08-2018	07:46:00	BRUG IN JULIANALAAN	Aa Of Weerijs	37,80	46,00	7,20
220013	17-09-2018	08:59:00	BRUG IN JULIANALAAN	Aa Of Weerijs	32,60	15,00	7,20
220013	09-10-2018	07:30:00	BRUG IN JULIANALAAN	Aa Of Weerijs	43,00	94,00	7,50
220013	19-11-2018	14:36:00	BRUG IN JULIANALAAN	Aa Of Weerijs	29,40	15,00	7,20
220013	12-12-2018	09:54:00	BRUG IN JULIANALAAN	Aa Of Weerijs	31,70	270,00	7,00
220013	02-01-2019	12:37:00	BRUG IN JULIANALAAN	Aa Of Weerijs	36,20	61,00	7,10
220013	14-02-2019	07:52:00	BRUG IN JULIANALAAN	Aa Of Weerijs	33,30	1600,00	6,80
220013	26-03-2019	07:52:00	BRUG IN JULIANALAAN	Aa Of Weerijs	30,20	30,00	7,10

Appendix H : Brabantse Delta City Swim measurements in summer 2019

(in Dutch)



11 juni

200.020: **E. coli 660 n/100 ml** matig verhoogde hoeveelheid, maar geen overschrijding; verder geen bijzonderheden.
200.023: **E. coli 920 n/100 ml** sterk verhoogde hoeveelheid, maar geen overschrijding; laag zuurstofgehalte.
200.039: **E. coli 4400 n/100 ml forse overschrijding** signaleringswaarde; **Intestinale Enterococcen: 270 n/100 ml** matig verhoogde hoeveelheid; laag zuurstofgehalte.

24 juni

200.020: **E. coli 180 n/100 ml** licht verhoogde hoeveelheid, maar geen overschrijding; verder geen bijzonderheden.
200.023: **E. coli 480 n/100 ml** matig verhoogde hoeveelheid, maar geen overschrijding; **Intestinale Enterococcen: 230 n/100 ml** matig verhoogde hoeveelheid; laag zuurstofgehalte.
200.039: **E. coli 560 n/100 ml** matig verhoogde hoeveelheid; **Intestinale Enterococcen: 190 n/100 ml** matig verhoogde hoeveelheid; laag zuurstofgehalte.

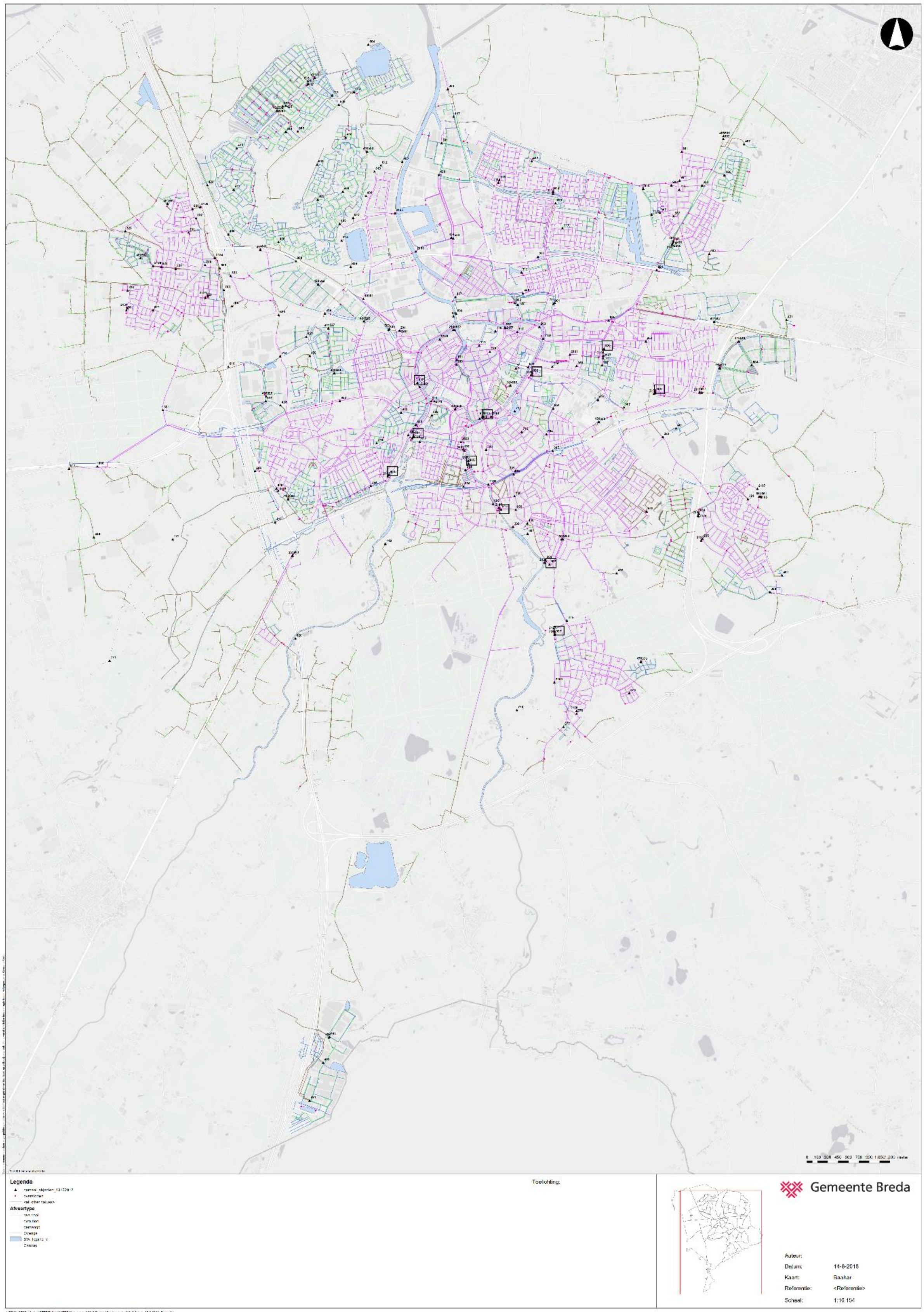
5 augustus

200.020: **E. coli 3800 n/100 ml** ***forse overschrijding***; **Intestinale Enterococcen: 45 n/100 ml** licht verhoogde hoeveelheid; verder geen bijzonderheden.
200.023: **E. coli 1700 n/100 ml** sterke verhoging, maar net geen overschrijding; **Intestinale Enterococcen: 46 n/100 ml** licht verhoogde hoeveelheid; verder geen bijzonderheden.
200.039: **E. coli 4100 n/100 ml** ***forse overschrijding***; **Intestinale Enterococcen: 330 n/100 ml** sterke verhoging; verder geen bijzonderheden.

13 augustus

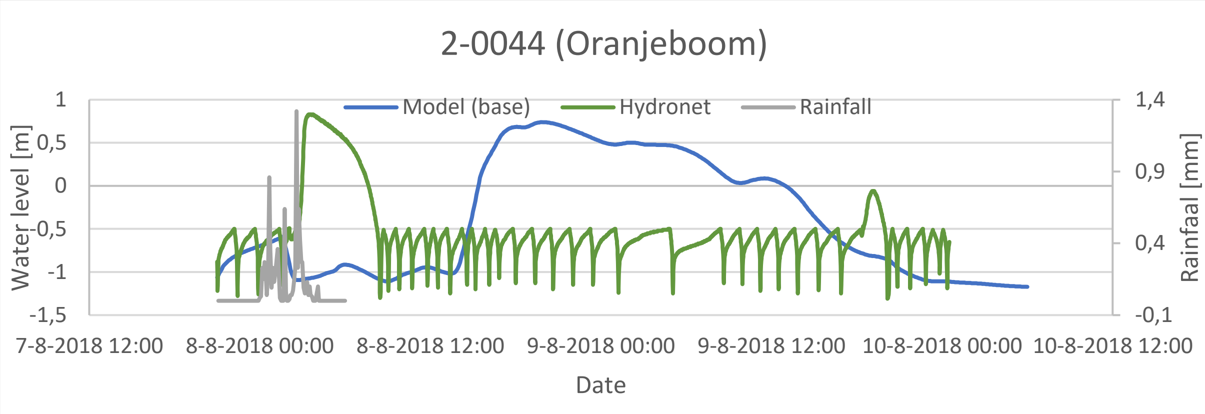
200.020: **E. coli 4300 n/100 ml** ***forse overschrijding***, met kanttekening; **Intestinale Enterococcen: 77 n/100 ml** licht verhoogde hoeveelheid; verlaagd zuurstofgehalte.
200.023: **E. coli 3200 n/100 ml** ***forse overschrijding***; **Intestinale Enterococcen: 520 n/100 ml** ***overschrijding***; verlaagd zuurstofgehalte.
200.039: **E. coli 3400 n/100 ml** ***forse overschrijding***; **Intestinale Enterococcen: 2000 n/100 ml** ***zeer forse overschrijding***, verlaagd zuurstofgehalte

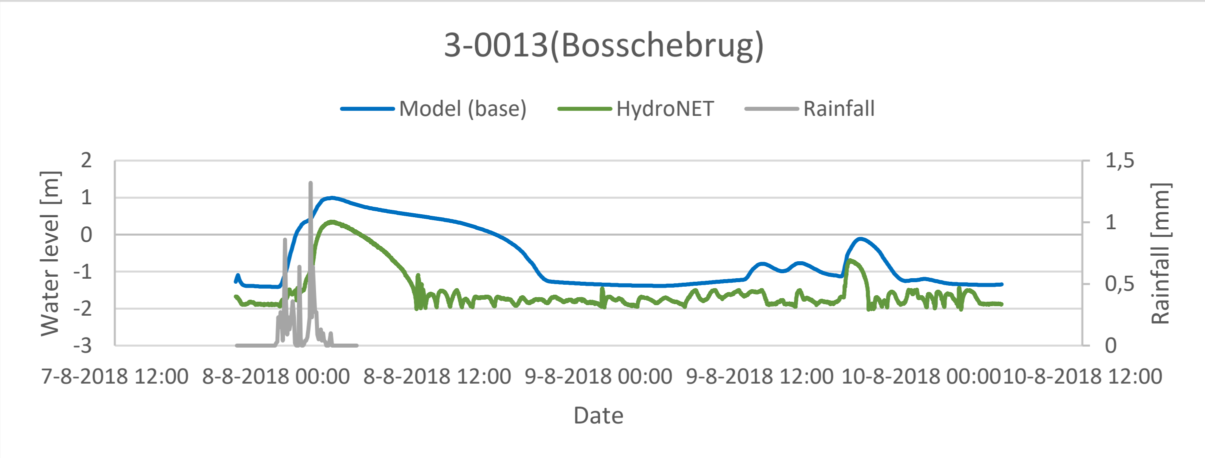
Appendix I: Location of CSDTs in catchments 0, 1, 2, 3, 4, 13

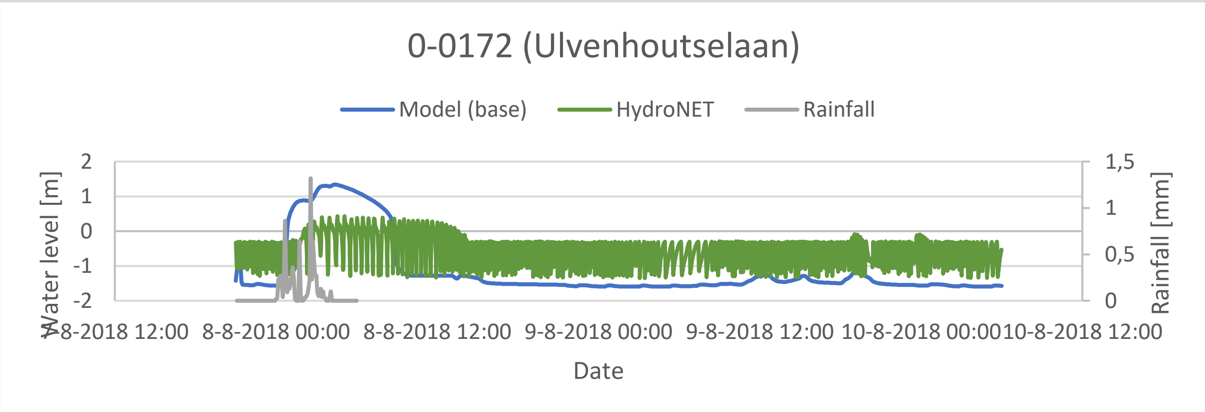


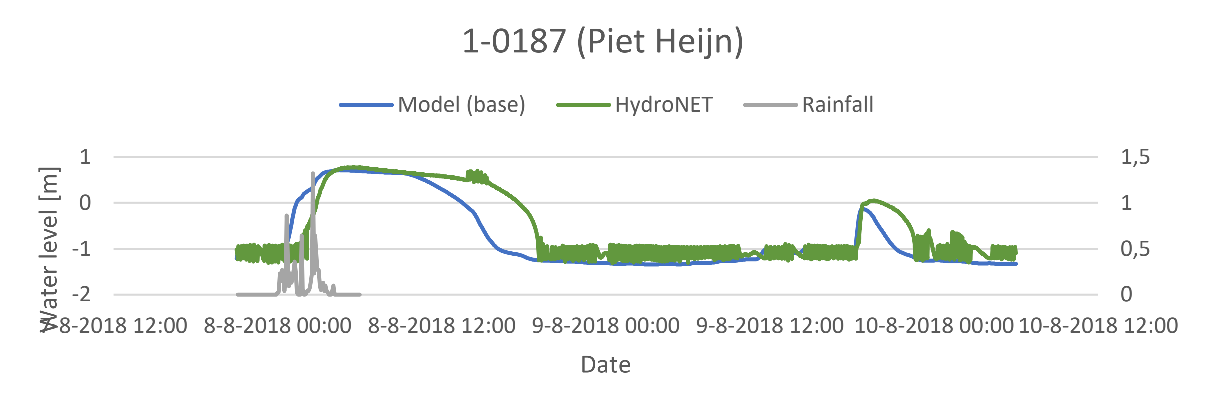
Appendix J : Results of Infoworks simulation per rainfall

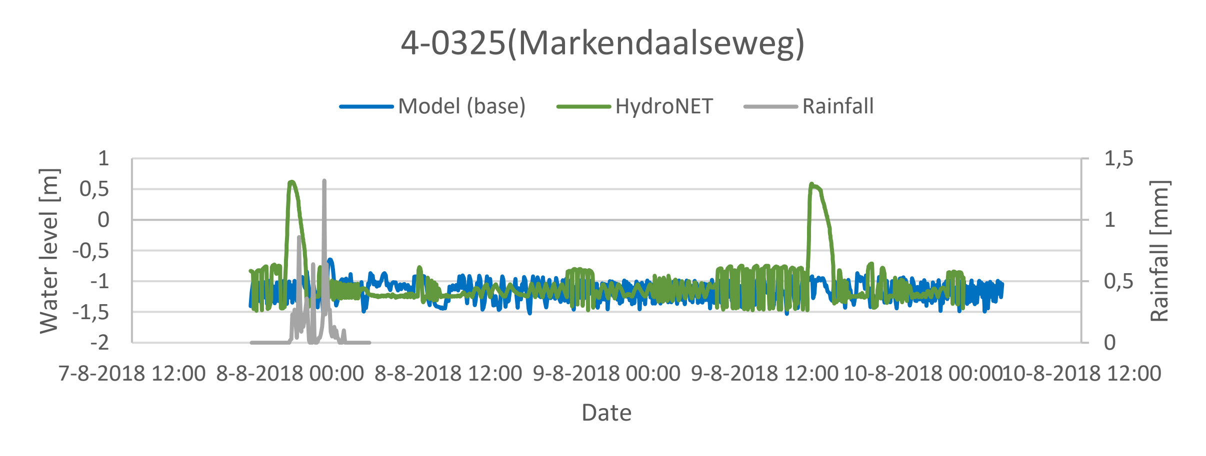
Rainfall 8-8-2018

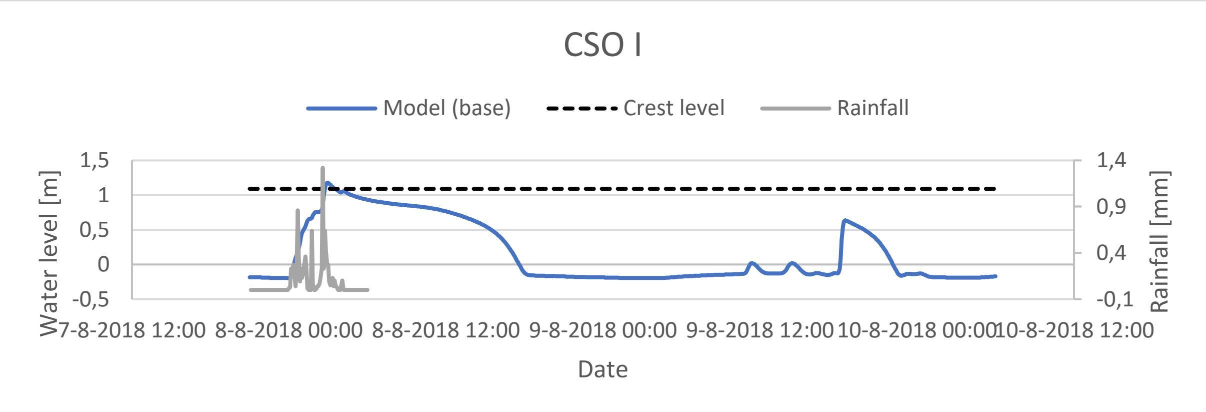


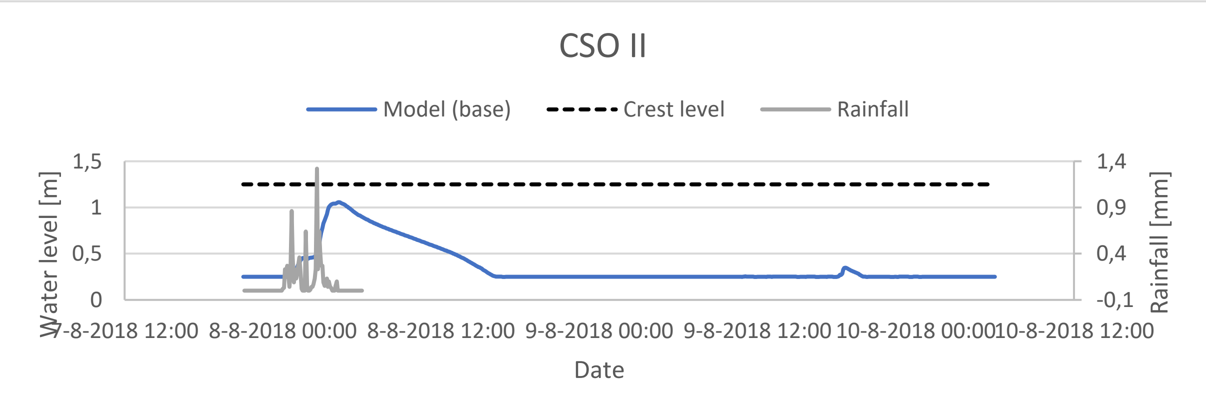


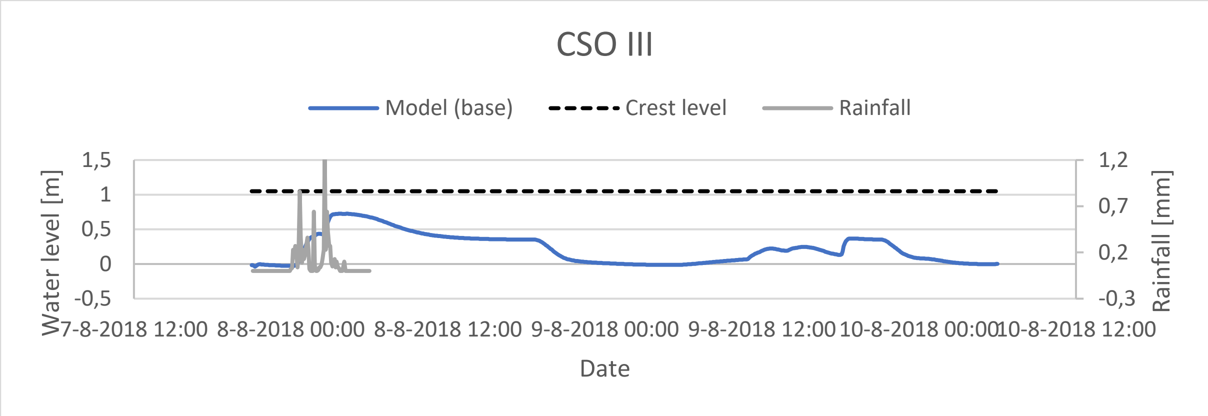


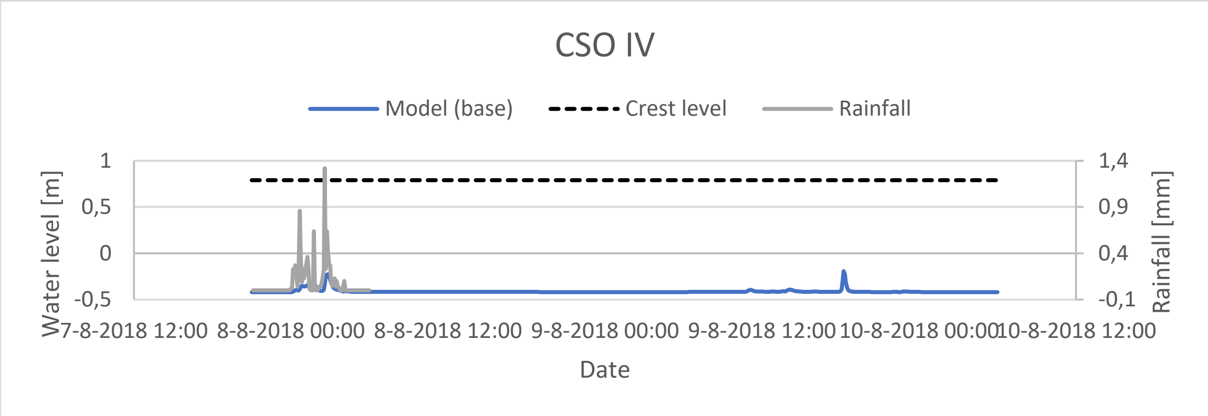

1-0187 (Piet Heijn)
Model (base)
HydroNET
Rainfall
Water level [m]
1
0
-1
-2
1,5
1
0,5
0
7-8-2018 12:00
8-8-2018 00:00
8-8-2018 12:00
9-8-2018 00:00
9-8-2018 12:00
10-8-2018 00:00
10-8-2018 12:00
Date

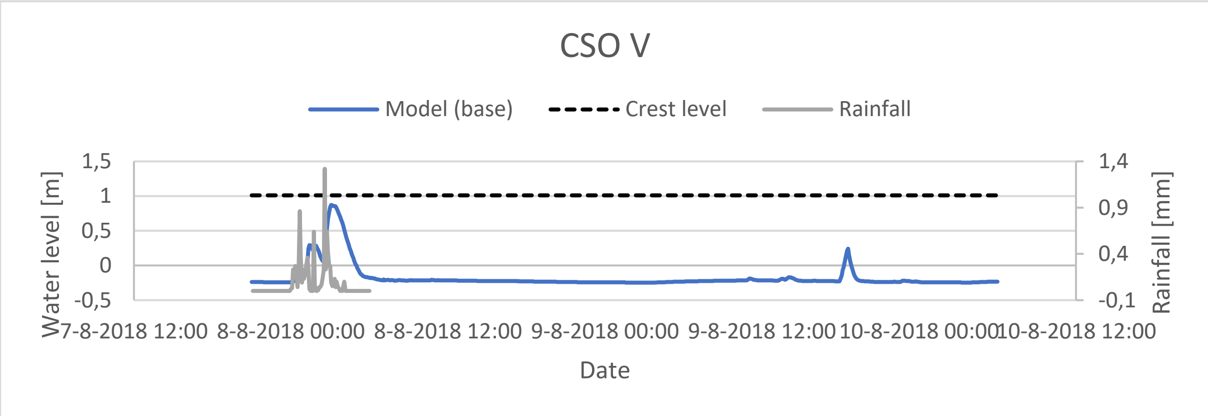

4-0325(Markendaalseweg)
Model (base)
HydroNET
Rainfall
Water level [m]
1
0,5
0
-0,5
-1
-1,5
-2
1,5
1
0,5
0
Rainfall [mm]
7-8-2018 12:00
8-8-2018 00:00
8-8-2018 12:00
9-8-2018 00:00
9-8-2018 12:00
10-8-2018 00:00
10-8-2018 12:00
Date


CSO I
Model (base)
Crest level
Rainfall
Water level [m]
1,5
1
0,5
0
-0,5
1,4
0,9
0,4
-0,1
Rainfall [mm]
7-8-2018 12:00
8-8-2018 00:00
8-8-2018 12:00
9-8-2018 00:00
9-8-2018 12:00
10-8-2018 00:00
10-8-2018 12:00
Date

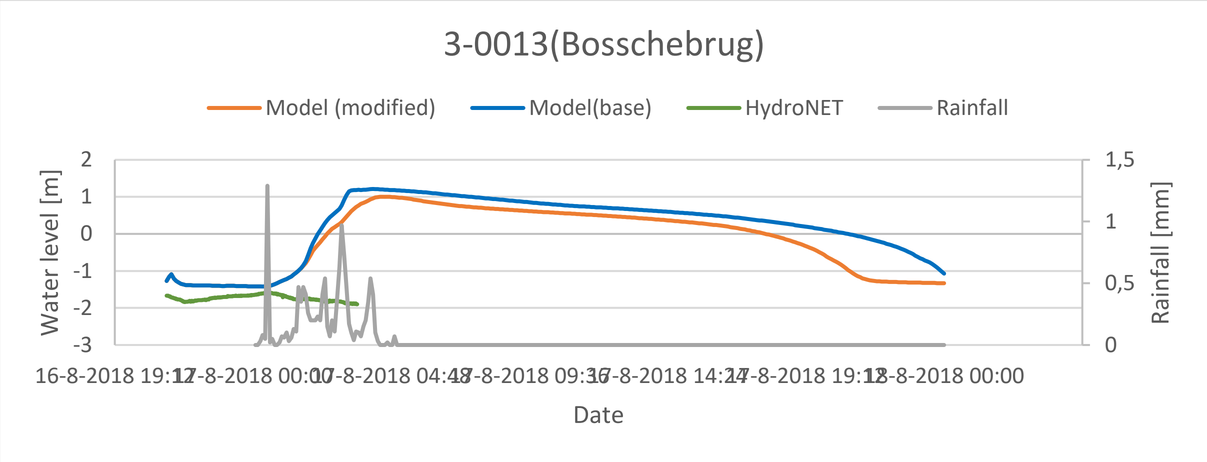

CSO II
Model (base)
Crest level
Rainfall
Water level [m]
1,5
1
0,5
0
1,4
0,9
0,4
-0,1
Rainfall [mm]
7-8-2018 12:00
8-8-2018 00:00
8-8-2018 12:00
9-8-2018 00:00
9-8-2018 12:00
10-8-2018 00:00
10-8-2018 12:00
Date

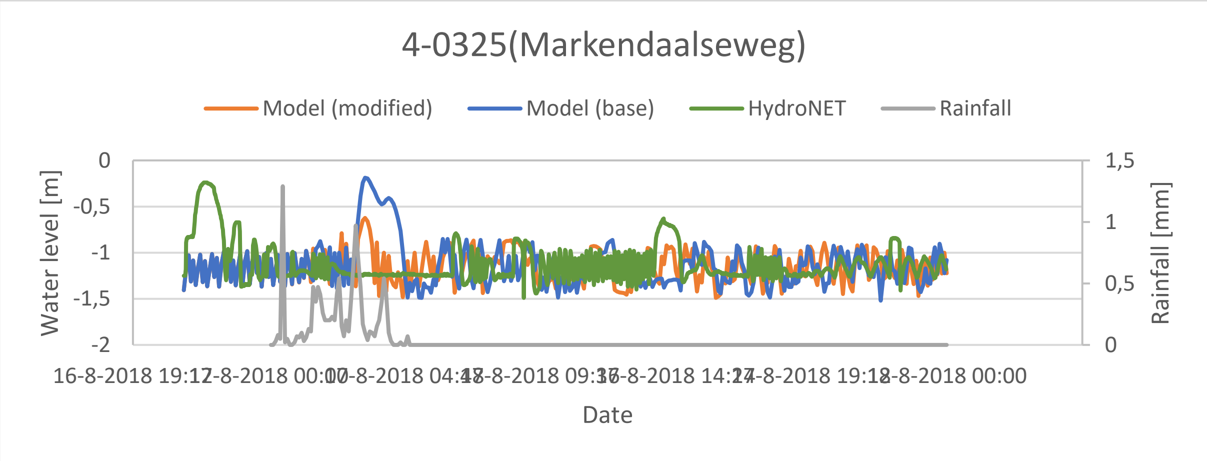

CSO III
Model (base)
Crest level
Rainfall
Water level [m]
Rainfall [mm]
Date

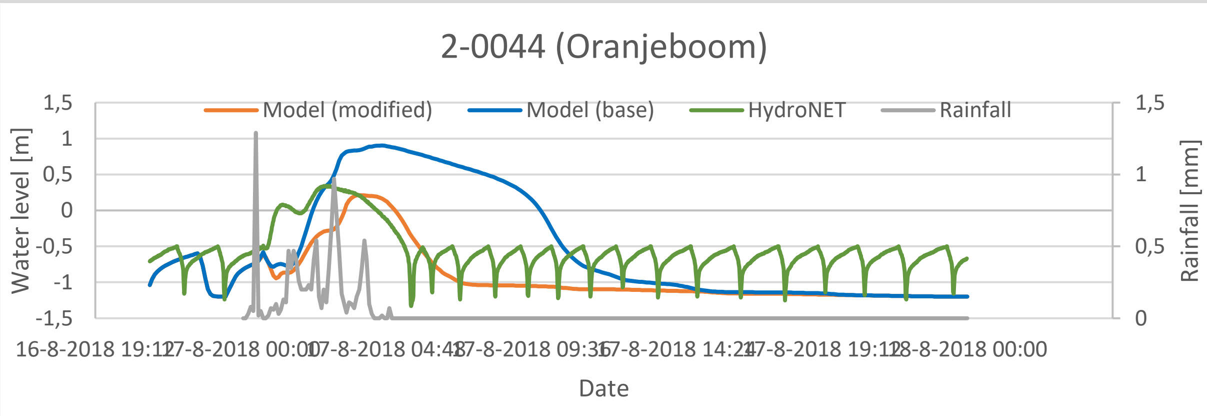

CSO IV
Model (base)
Crest level
Rainfall
Water level [m]
Rainfall [mm]
Date

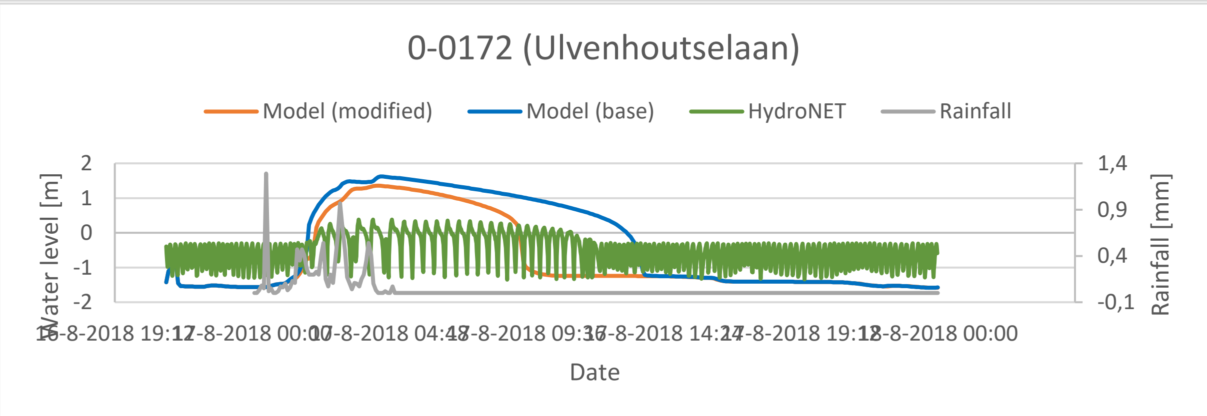

CSO V
Model (base)
Crest level
Rainfall
Water level [m]
Rainfall [mm]
Date

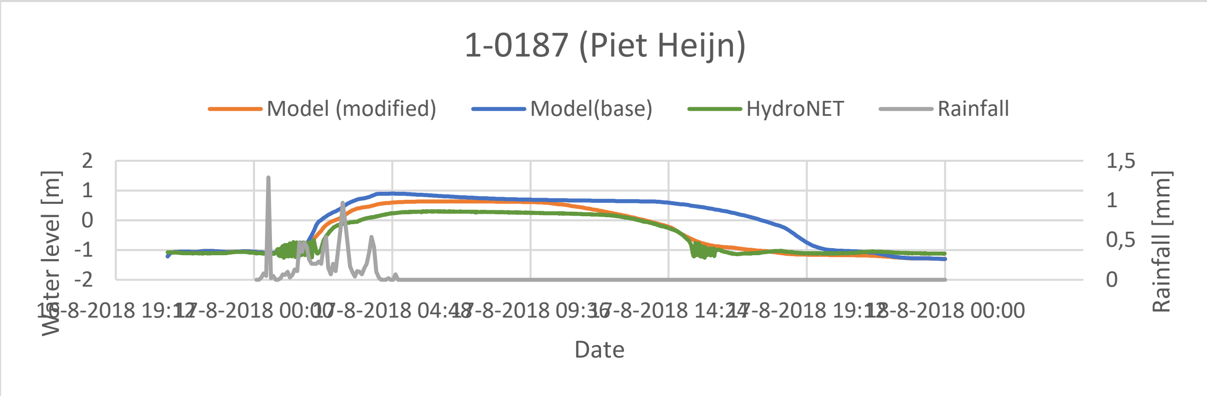
Rainfall 17-8-2018

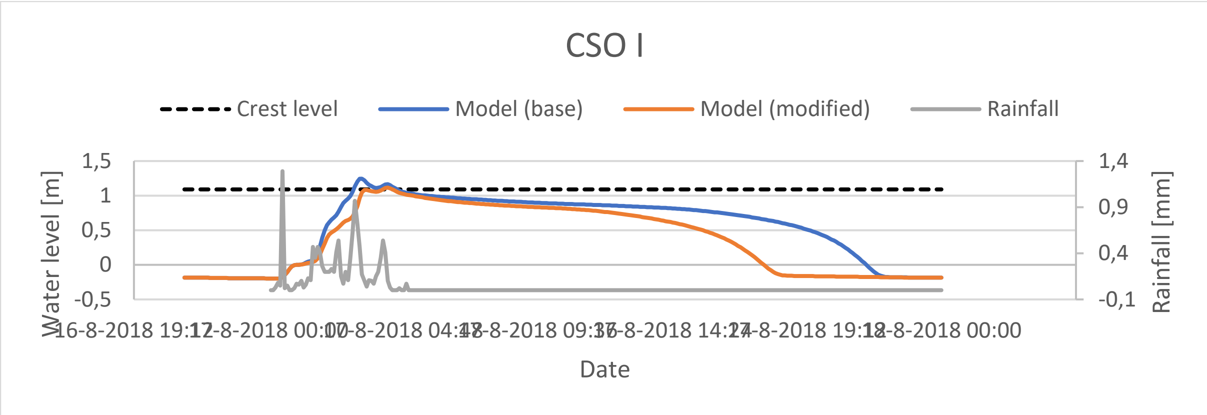


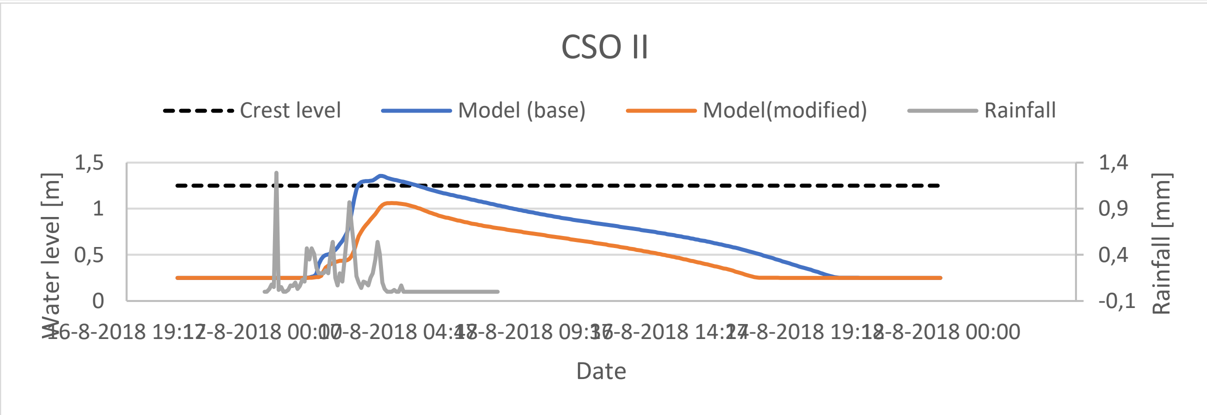


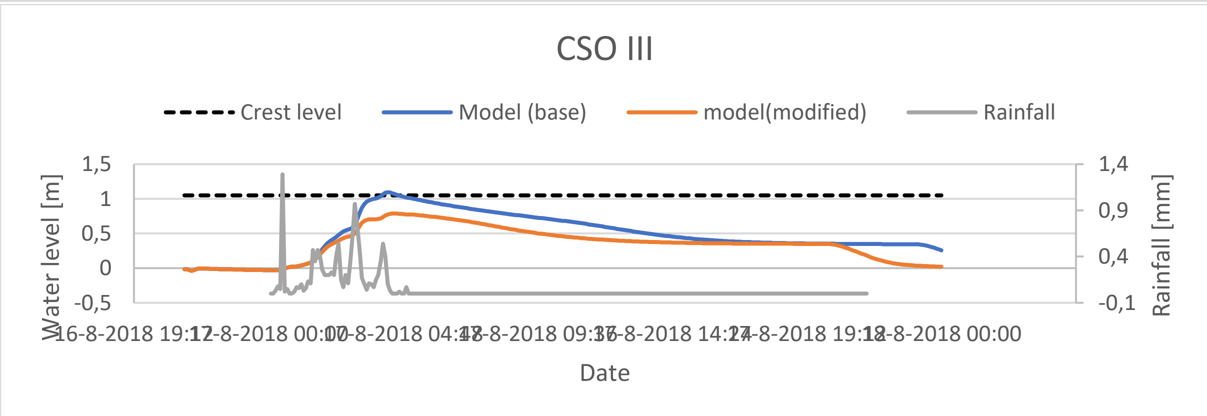


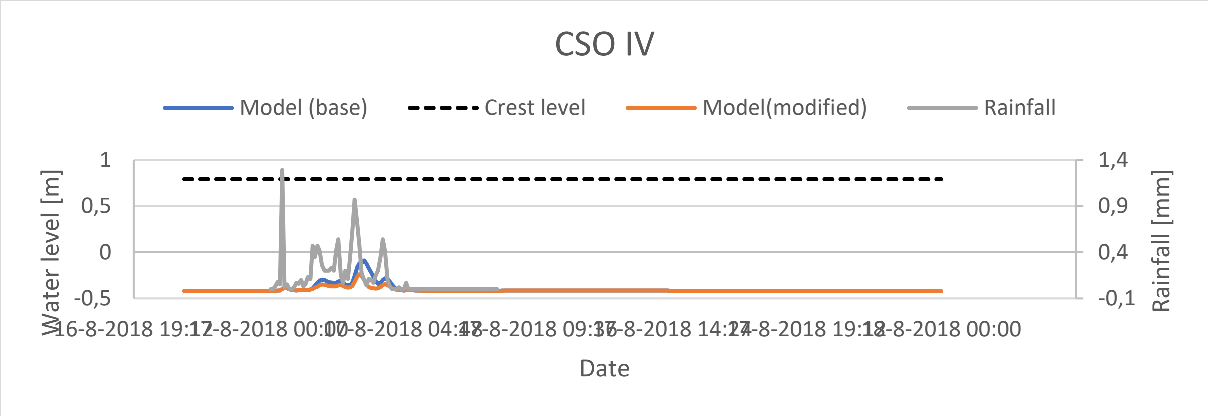



1-0187 (Piet Heijn)
Model (modified)
Model(base)
HydroNET
Rainfall
Water level [m]
Rainfall [mm]
2
1
0
-1
-2
1,5
1
0,5
0
16-8-2018 19:12
17-8-2018 00:00
17-8-2018 04:48
17-8-2018 09:36
17-8-2018 14:24
17-8-2018 19:12
18-8-2018 00:00
Date


CSO I
Crest level
Model (base)
Model (modified)
Rainfall
Water level [m]
Rainfall [mm]
1,5
1
0,5
0
-0,5
1,4
0,9
0,4
-0,1
16-8-2018 19:12
17-8-2018 00:00
17-8-2018 04:48
17-8-2018 09:36
17-8-2018 14:24
17-8-2018 19:12
18-8-2018 00:00
Date


CSO II
Crest level
Model (base)
Model(modified)
Rainfall
Water level [m]
Rainfall [mm]
1,5
1
0,5
0
1,4
0,9
0,4
-0,1
16-8-2018 19:12
17-8-2018 00:00
17-8-2018 04:48
17-8-2018 09:36
17-8-2018 14:24
17-8-2018 19:12
18-8-2018 00:00
Date


CSO III
Crest level
Model (base)
model(modified)
Rainfall
Water level [m]
Rainfall [mm]
1,5
1
0,5
0
-0,5
1,4
0,9
0,4
-0,1
16-8-2018 19:12
17-8-2018 00:00
17-8-2018 04:48
17-8-2018 09:36
17-8-2018 14:24
17-8-2018 19:12
18-8-2018 00:00
Date



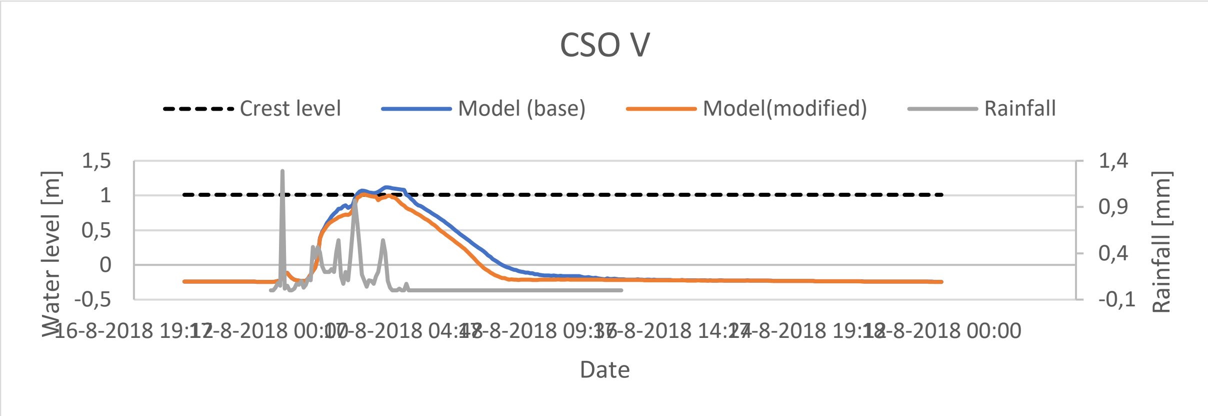
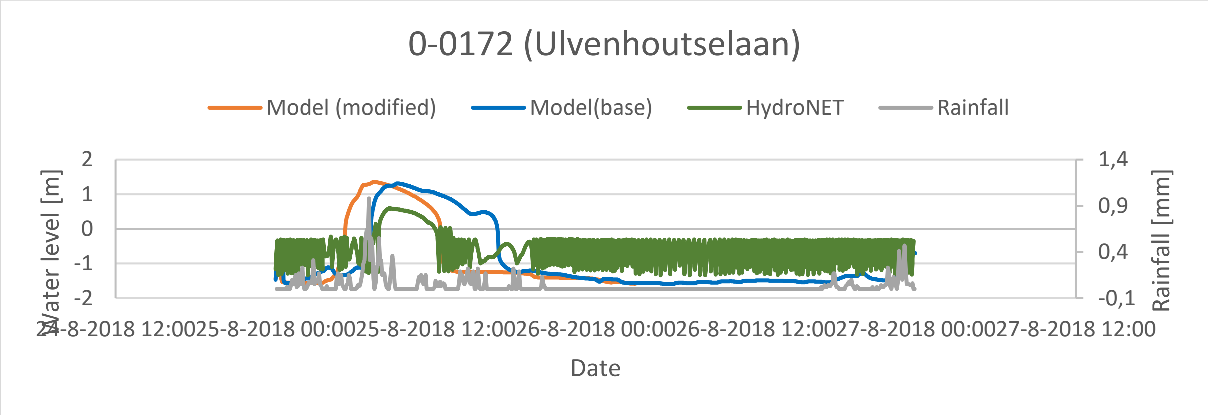
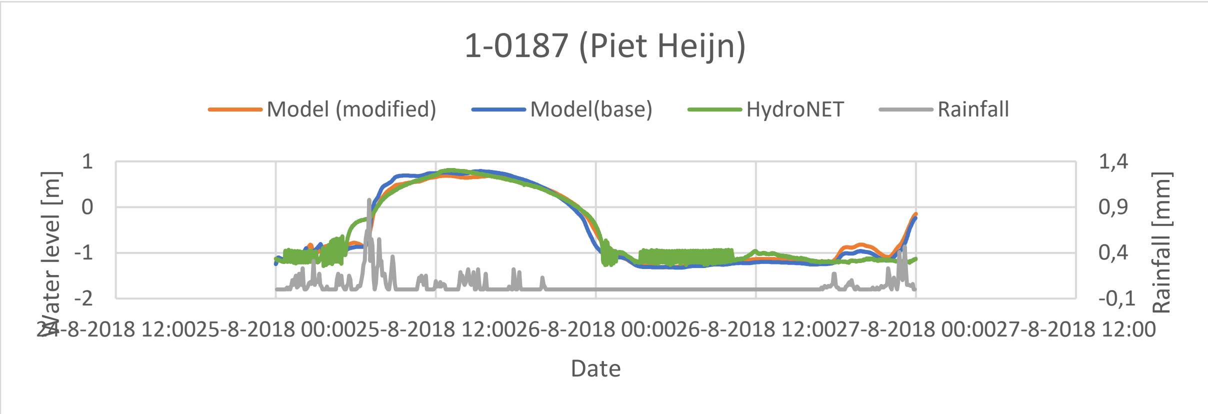


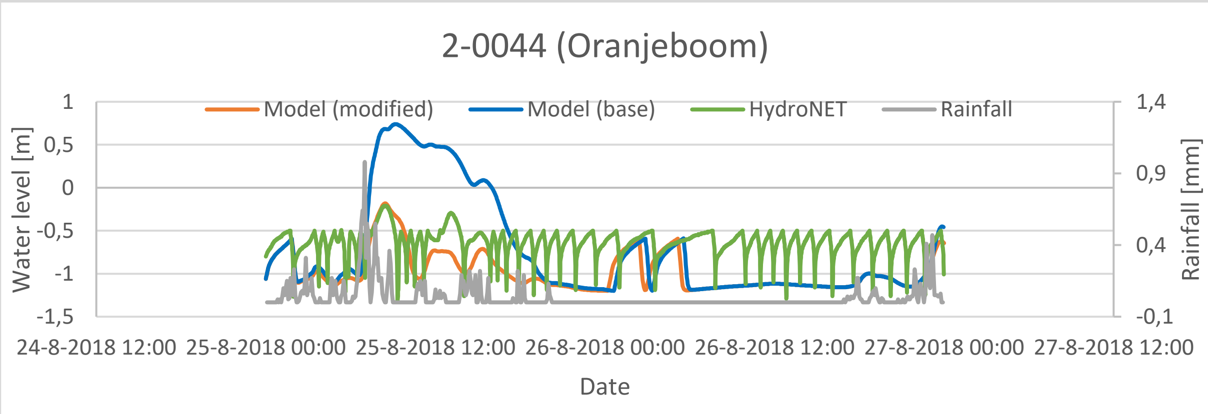
Table 23 - Modified values in InfoWorks for rainfall of 17-8-2018

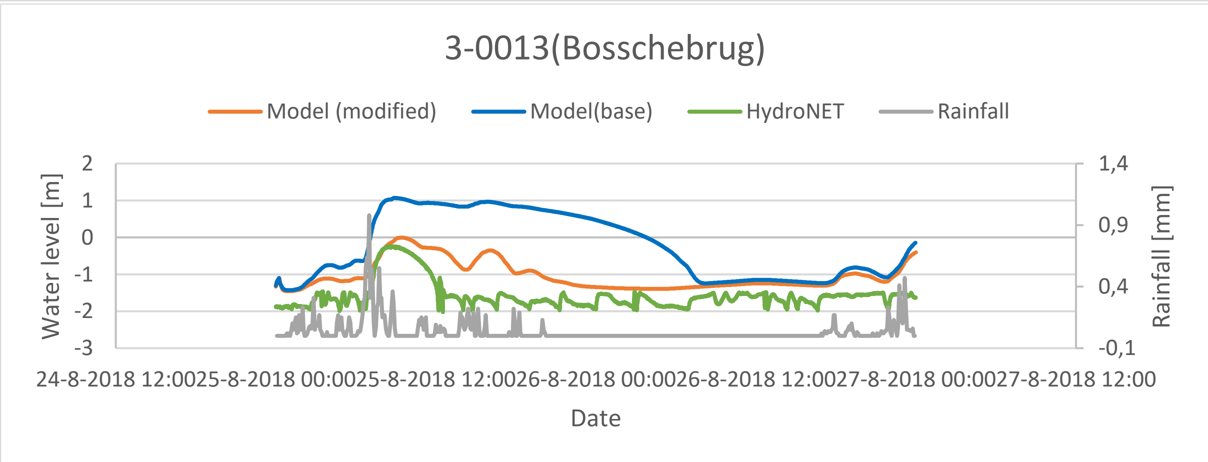
Catchment nr.	HI [mm/h]	HD [1/h]
C1	35	0.5
C2	35	0.5
C3	35	0.5
C4	35	0.5

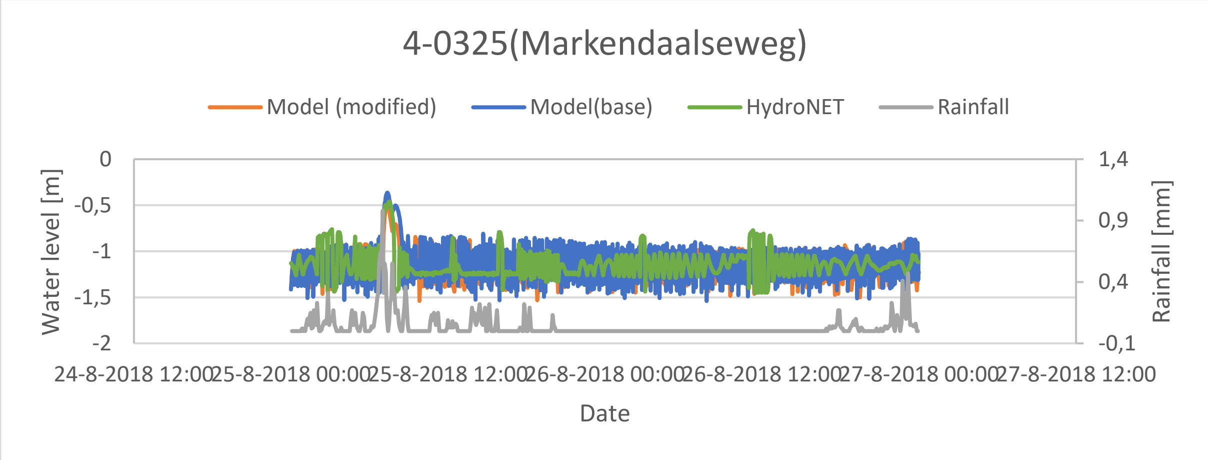
Rainfall 25-8-2018

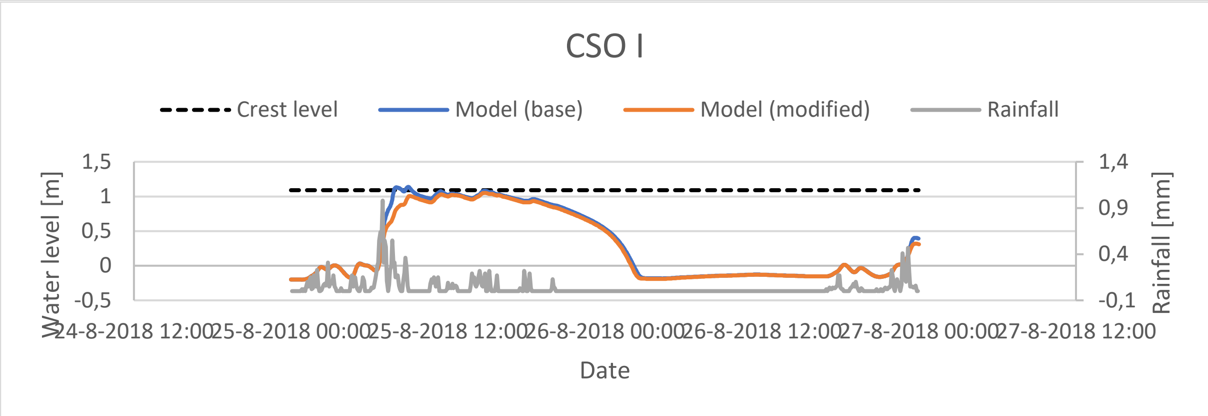


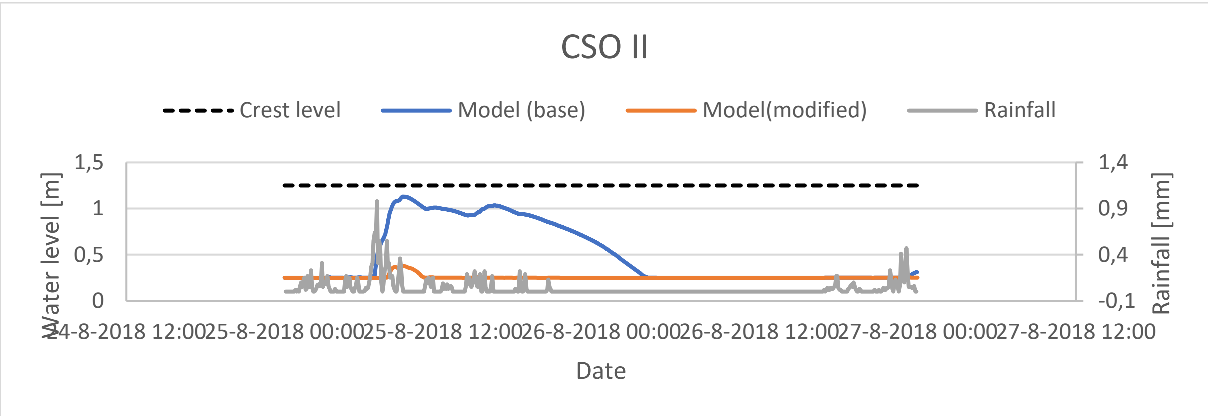


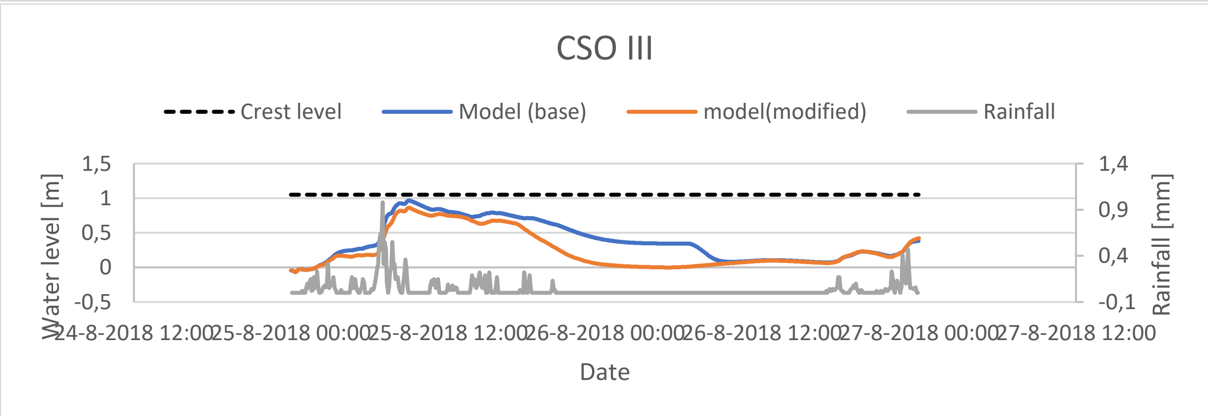


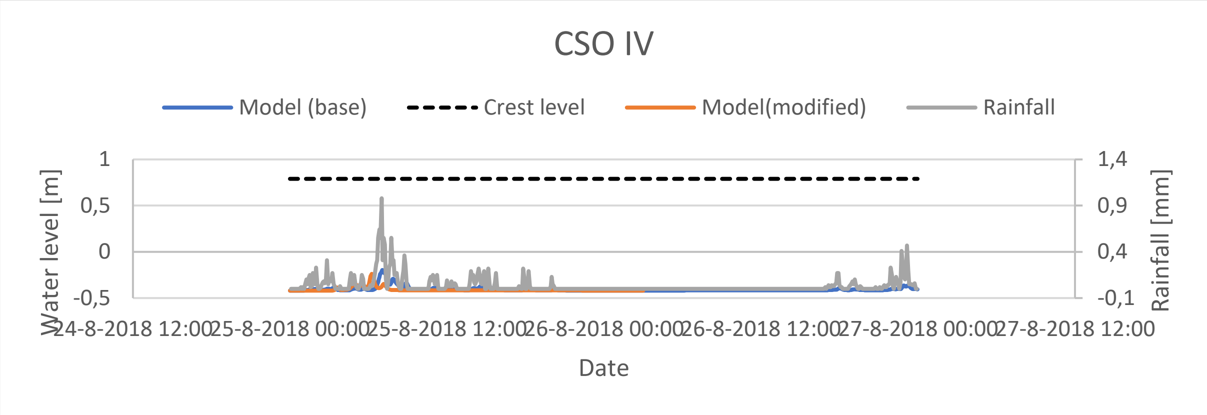



4-0325(Markendaalseweg)
Model (modified)
Model(base)
HydroNET
Rainfall
Water level [m]
Rainfall [mm]
0
-0,5
-1
-1,5
-2
1,4
0,9
0,4
-0,1
24-8-2018 12:00
25-8-2018 00:00
25-8-2018 12:00
26-8-2018 00:00
26-8-2018 12:00
27-8-2018 00:00
27-8-2018 12:00
Date


CSO I
Crest level
Model (base)
Model (modified)
Rainfall
Water level [m]
Rainfall [mm]
1,5
1
0,5
0
-0,5
1,4
0,9
0,4
-0,1
24-8-2018 12:00
25-8-2018 00:00
25-8-2018 12:00
26-8-2018 00:00
26-8-2018 12:00
27-8-2018 00:00
27-8-2018 12:00
Date


CSO II
Crest level
Model (base)
Model(modified)
Rainfall
Water level [m]
Rainfall [mm]
1,5
1
0,5
0
1,4
0,9
0,4
-0,1
24-8-2018 12:00
25-8-2018 00:00
25-8-2018 12:00
26-8-2018 00:00
26-8-2018 12:00
27-8-2018 00:00
27-8-2018 12:00
Date


CSO III
Crest level
Model (base)
model(modified)
Rainfall
Water level [m]
Rainfall [mm]
1,5
1
0,5
0
-0,5
1,4
0,9
0,4
-0,1
24-8-2018 12:00
25-8-2018 00:00
25-8-2018 12:00
26-8-2018 00:00
26-8-2018 12:00
27-8-2018 00:00
27-8-2018 12:00
Date



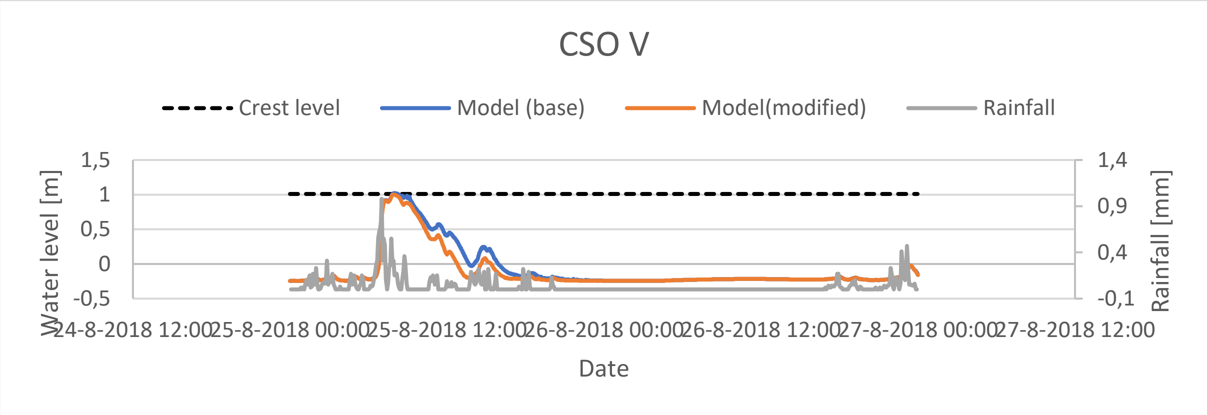
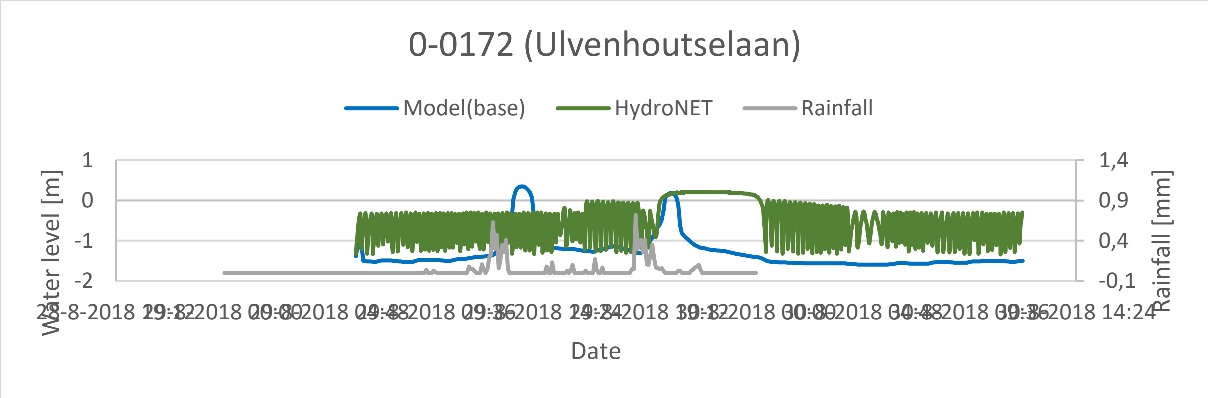
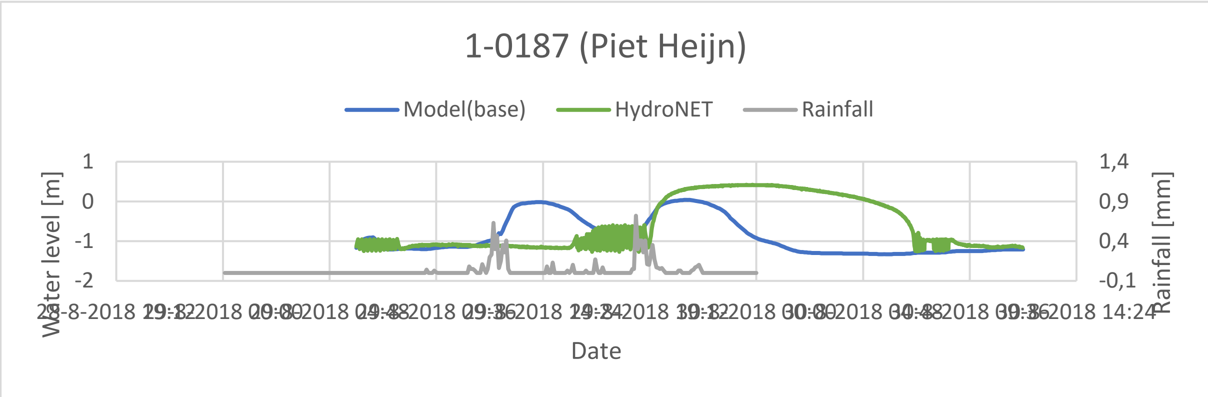


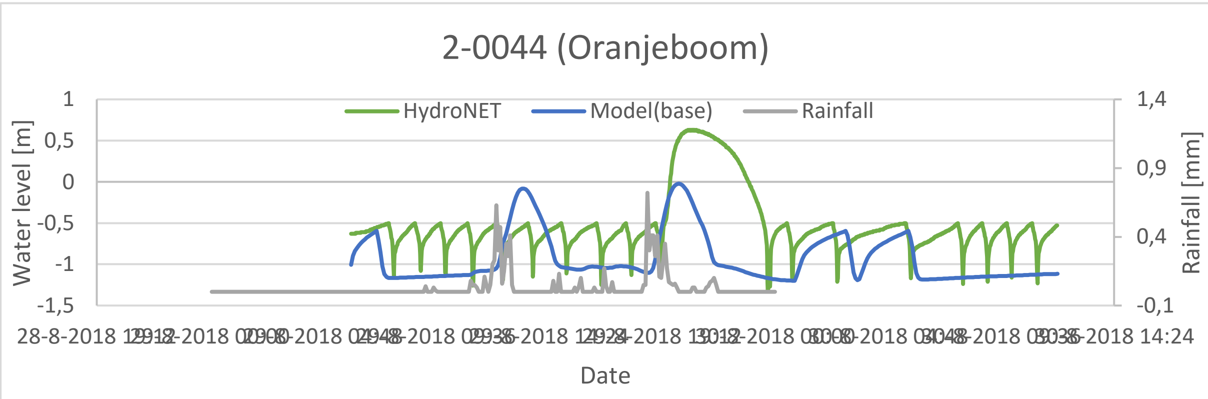
Table 24 -Modified values in InfoWorks for rainfall of 25-8-2018

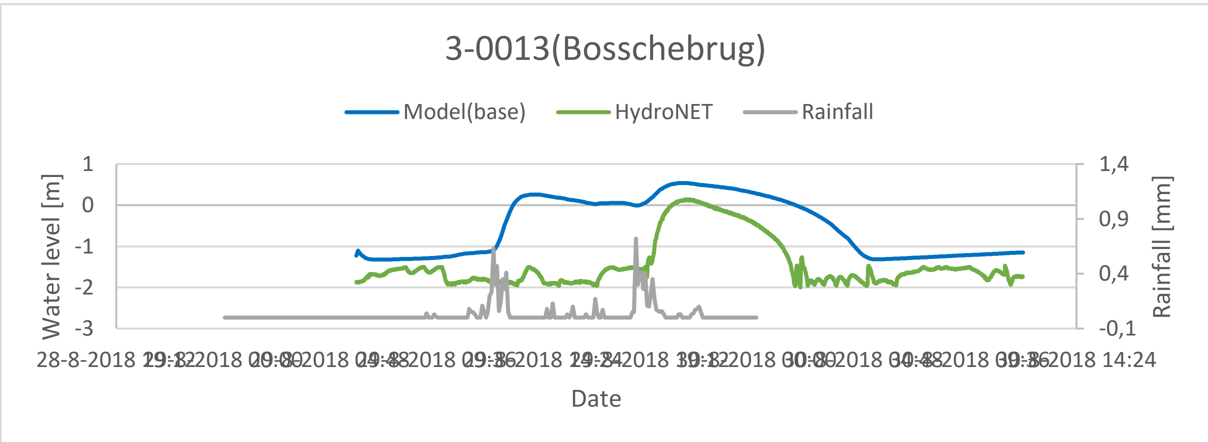
Catchment nr.	HI [mm/h]	HD [1/h]	IL [mm]	Pump capacity [m³/h]
C8	35	0.5	7	-
C9	35	0.5	7	-
C0	35	0.5	7	-
C1	15	1	1	-
C2	35	0.5	6	-
C3	35	0.5	8	-
Pump st.				
0-0172				1,100
1-0187				1,630
3-0013				1,200

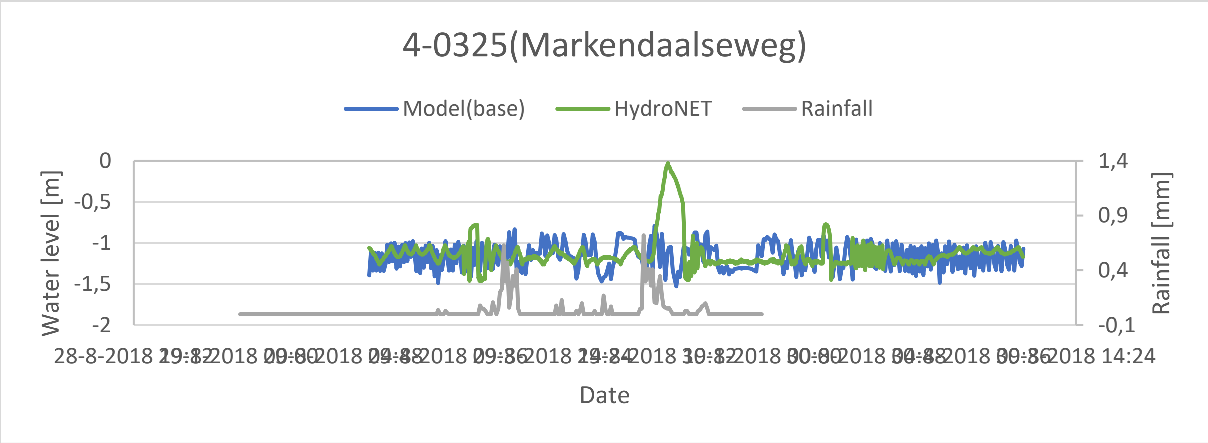
Rainfall 29-8-2018

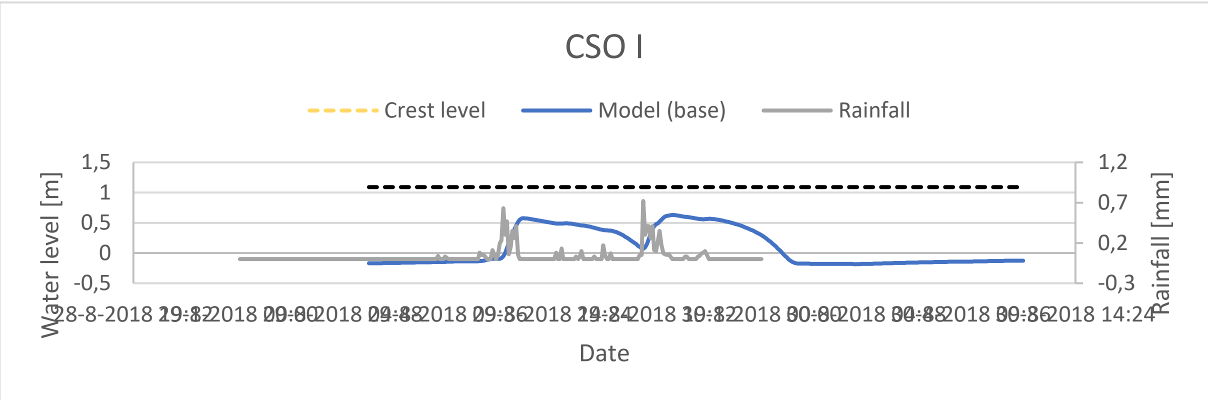


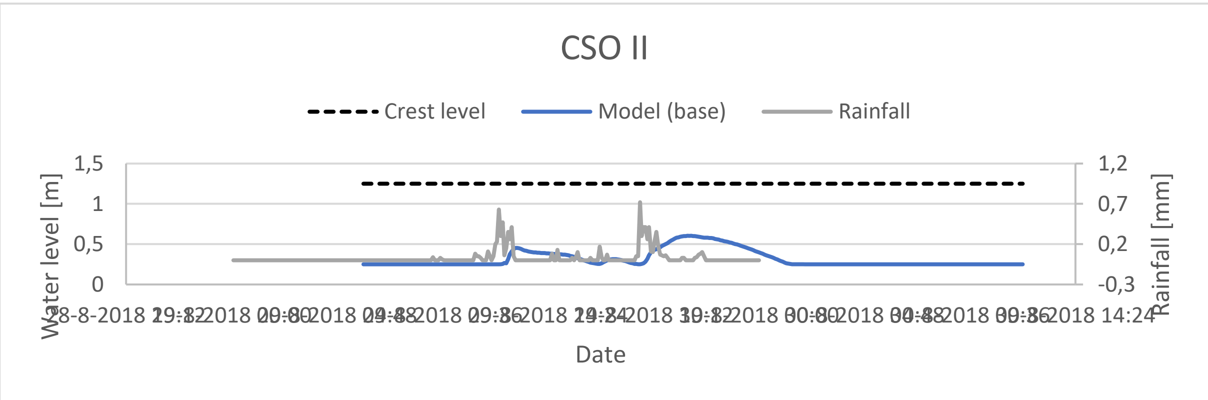


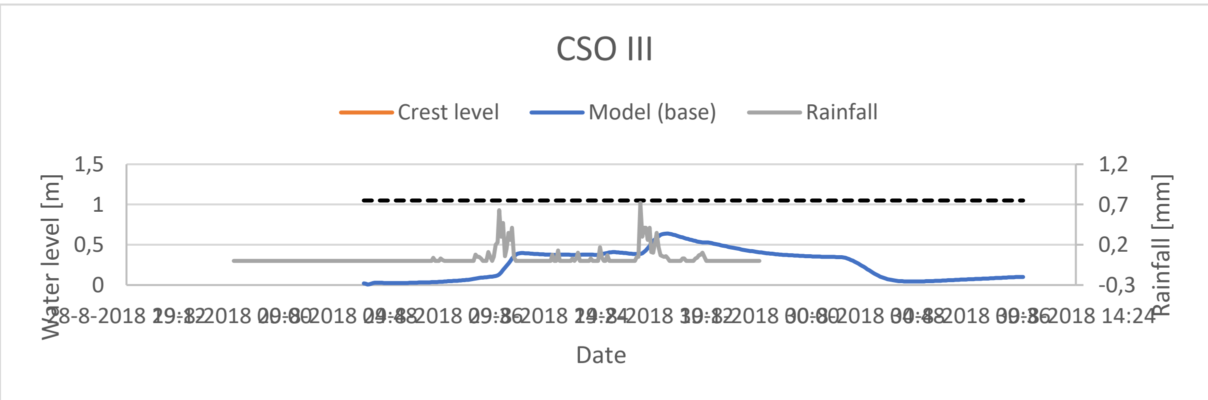


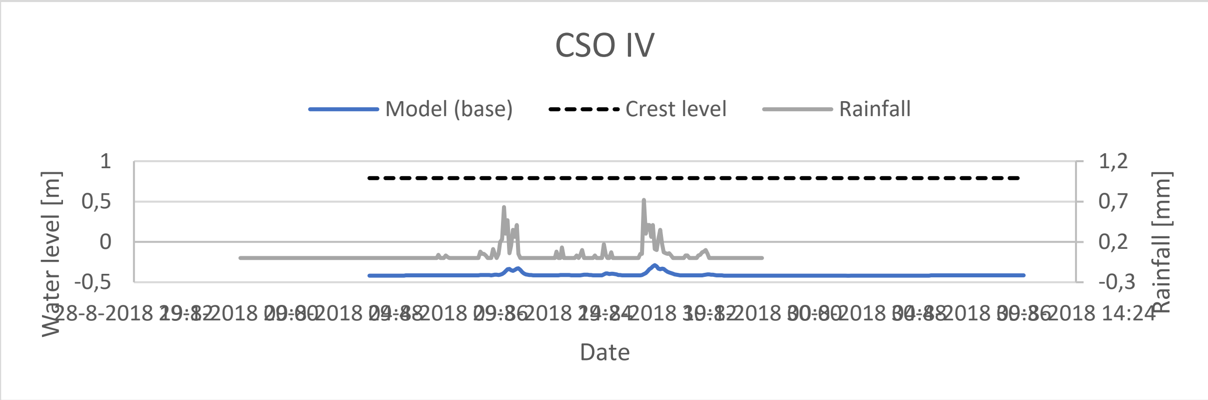


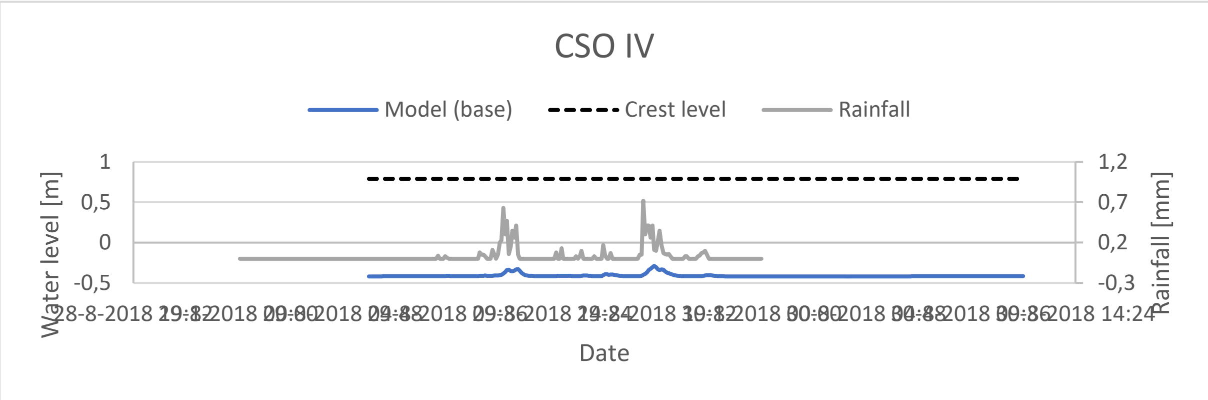

4-0325(Markendaalseweg)
Model(base)
HydroNET
Rainfall
Water level [m]
Rainfall [mm]
Date


CSO I
Crest level
Model (base)
Rainfall
Water level [m]
Rainfall [mm]
Date

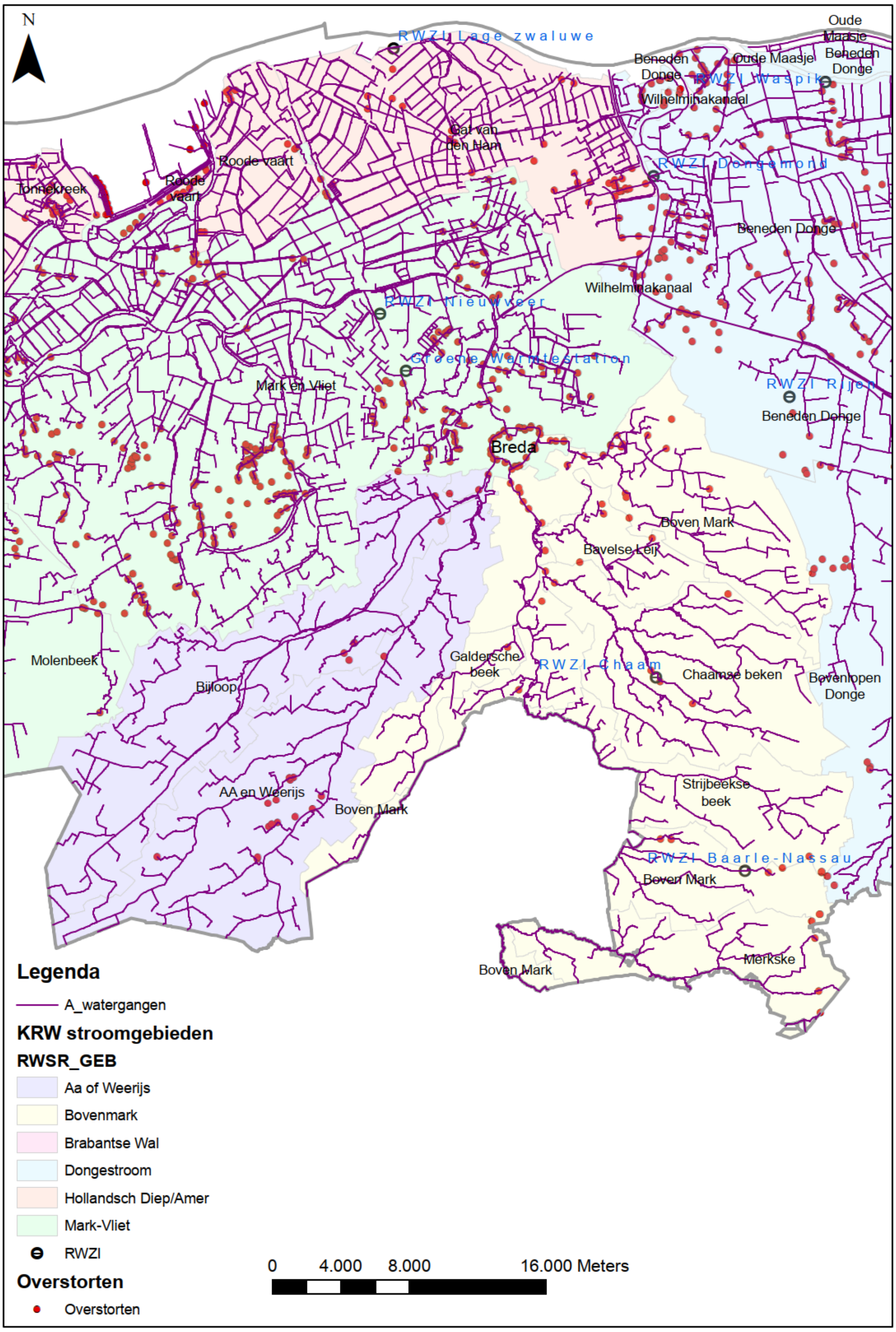

CSO II
Crest level
Model (base)
Rainfall
Water level [m]
Rainfall [mm]
Date


CSO III
Crest level
Model (base)
Rainfall
Water level [m]
Rainfall [mm]
Date


CSO IV
Model (base)
Crest level
Rainfall
1
0,5
0
-0,5
Water level [m]
1,2
0,7
0,2
-0,3
Rainfall [mm]
Date


CSO IV
Model (base)
Crest level
Rainfall
1
0,5
0
-0,5
Water level [m]
1,2
0,7
0,2
-0,3
Rainfall [mm]
Date

Appendix K : Water frame work catchment areas of Brabantse Delta



Appendix L: water discharge of Mark and Weerijs streams at Locations 4 and 5

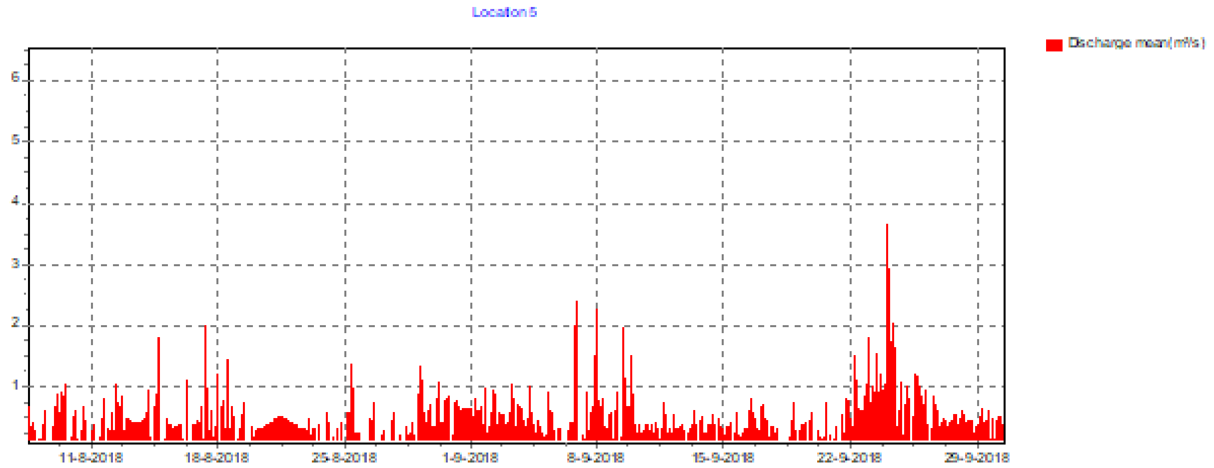


Figure 91 – Mark discharge of water sampling in summer period at location 5

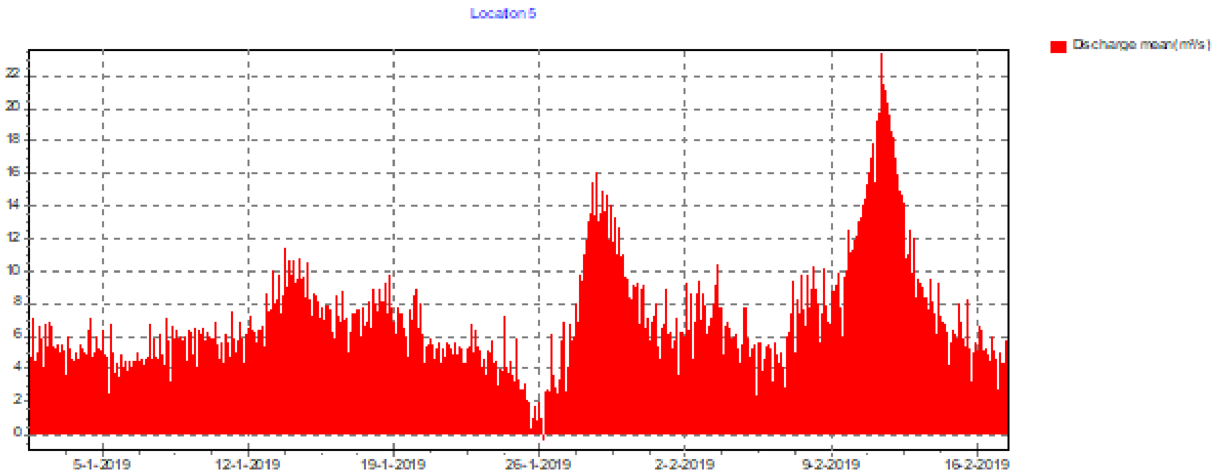


Figure 92 – Mark discharge of water sampling in winter period at location 5

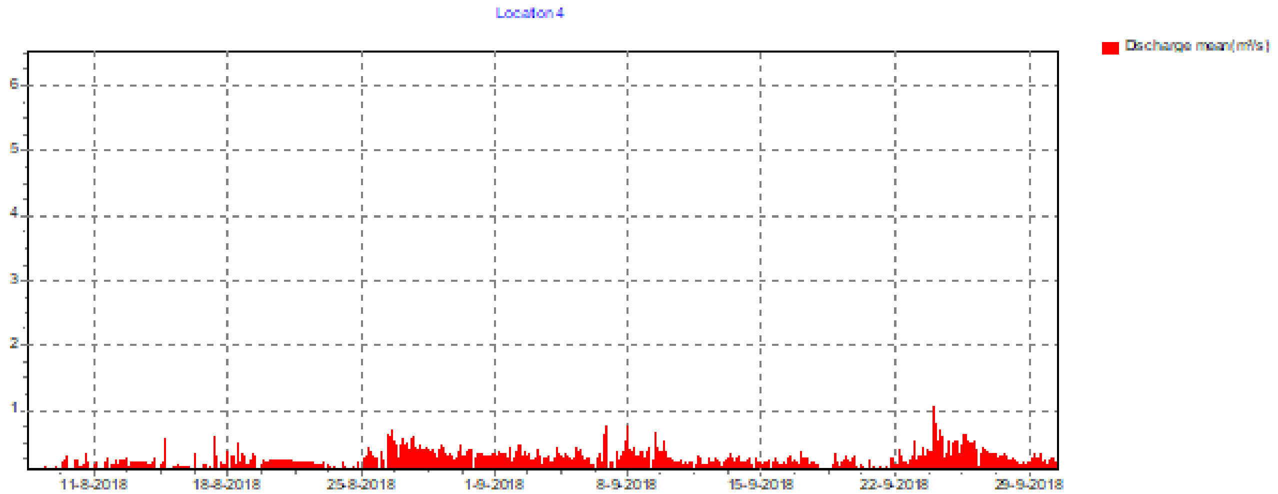


Figure 93 – Weeerijs discharge of water sampling in summer period at location 4

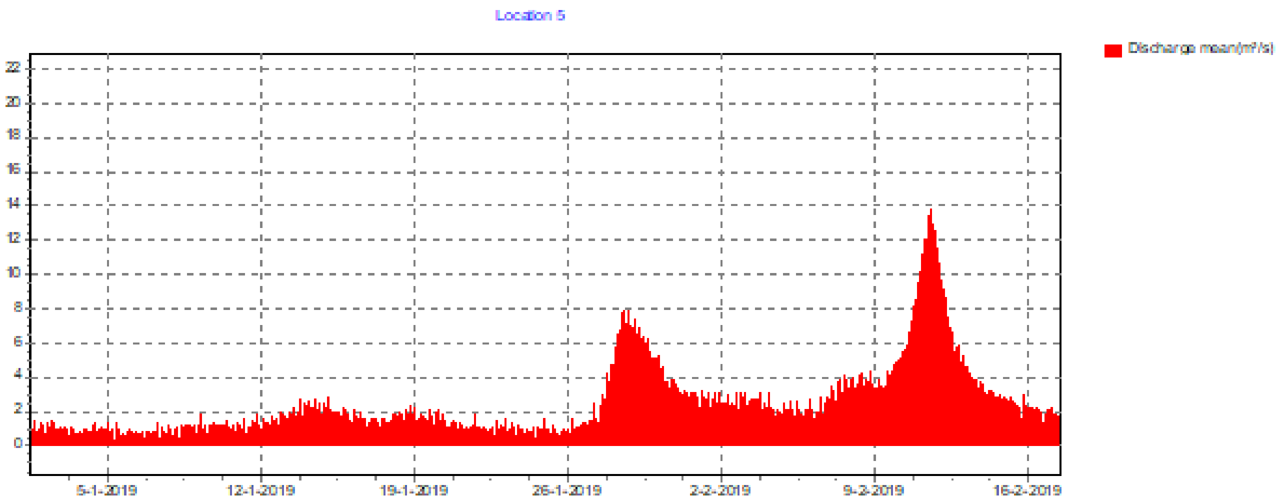


Figure 94 - Weeerijs discharge of water sampling in winter period at location 4

DNA bronopsporing in stadsingels van Breda

KWR 2019.058 | 24 juni 2019

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Inhoud

1 Inleiding

Voor waterbeheerders is het van belang om de belangrijkste bronnen in beeld te krijgen die bijdragen aan overschrijdingen in concentraties van fecale indicatorbacteriën zoals *E. coli* en intestinale enterococcen. Deze indicatorbacteriën komen algemeen voor in darmen van warmbloedige dieren en de concentratie van deze bacteriën in oppervlaktewater geeft daarom een indruk van de concentratie fecaal materiaal in het water en daarmee van de potentiële aanwezigheid van ziekteverwekkende micro-organismen, zoals virussen en bacteriën.

Fecale verontreiniging in oppervlaktewater kan afkomstig zijn van een heel scala aan bronnen. Te denken valt aan de aanwezigheid van (water)vogels, vervuiling door recreanten, afspoeling van agrarisch gebied, effluentlozing door RWZI's, overstorten uit rioolwater- of hemelwaterriolering, aanwezigheid van wilde fauna en afspoeling van honden- en/of paardenfeces. Welk van dergelijke bronnen nu bijdraagt aan de verminderde (zwem)waterkwaliteit is op basis van alleen de indicator bacteriën (*E. coli* en intestinale enterococcen) niet te achterhalen. Sinds enkele jaren maakt men daarvoor gebruik van specifieke DNA-methoden. Met dergelijke technieken is onderscheid te maken tussen diergroepspecifieke bacteriën met fecale herkomst (Heijnen & Learbuch 2013, Heijnen et al. 2014). Met behulp van DNA technieken (qPCR) kunnen dergelijke bacteriën gedetecteerd en gekwantificeerd worden. De meest relevante diergroepen die momenteel in het laboratorium van KWR met DNA merkers onderscheiden kunnen worden zijn: mensen, vogels, varkens, herkauwers (als groep), runderen (uit de groep van herkauwers), paarden en honden.

Met de resultaten van de DNA analyse in de hand kunnen (water)beheerders gericht maatregelen nemen om deze verontreinigingen te voorkomen. Dergelijke maatregelen zijn erop gericht om bronnen te minimaliseren en zo de (zwem)waterkwaliteit te verbeteren dan wel te garanderen.

In het hier gerapporteerde onderzoek is op verzoek van de gemeente Breda op drie momenten in 2019 een DNA gebaseerde bronopsporing uitgevoerd op vijf locaties in stadssingels van Breda. Het betrof de DNA concentratie van merkers voor vier groepen organismen: mensen, herkauwers, honden en vogels. Daarnaast is in dezelfde monsters de concentratie van fecale bacteriegroepen bepaald. De eerste meting vond plaats in een periode zonder veel regenval, zodat er geen riool overstorten waren en dus een referentie beeld kon worden verkregen van de gemiddelde waarden voor deze parameters. Daarna zijn monsters genomen op twee momenten nadat een riool overstort had plaatsgevonden.

2 Methoden en aanpak

2.1 Verzamelen van watermonsters

Watermonsters zijn verzameld op 2 april (referentie; door KWR bemonsterd), 6 juni (na overstort moment; bemonsterd door de opdrachtgever) en 13 juni (na overstort moment; bemonsterd door de opdrachtgever). De door de opdrachtgever gekozen locaties bevinden zich in Breda in de singels bij Markkade 52, Nieuwe Prinsenkade, Haven 20, Weerijssingel 29 en de Julianabrug. De monsters genomen door de opdrachtgever op 6 juni konden niet binnen de gewenste periode van 24 uur worden geanalyseerd. Indien monsters echter later dan 24 uur na monstername aangeleverd worden bestaat de kans op dusdanige degradatie van de monsters dat de uiteindelijke resultaten minder betrouwbaar worden. Op uitdrukkelijk verzoek van de opdrachtgever zijn deze monsters alsnog in behandeling genomen, maar de betreffende resultaten moeten dus onder dit voorbehoud worden geïnterpreteerd.

2.2 Filtratie en kweek

Voor DNA analyse is van elk watermonster een volume van 100-150 ml onder vacuüm gefiltreerd over een polycarbonaat membraan filter (Track-edge filters, Sartorius), met een poriegrootte van 0,2 µm en een doorsnede van 4,5 cm. Vervolgens is het DNA geïsoleerd en gezuiverd. Bij elke monsternameronde is ook een blanco filter (DNA-vrij water) geprepareerd.

De detectie van fecale indicator bacteriën is in het laboratorium van KWR uitgevoerd voor coliformen (inclusief *E. coli*), *E. coli* en intestinale enterococcen. De bepalingen zijn uitgevoerd met standaard door KWR gehanteerde en gecertificeerde procedures. De methode voor het aantonen, kwantificeren en bevestigen van bacteriën van de coligroep en van *E. coli* in water is gelijkwaardig aan NEN-EN-ISO 9308-1. Voor het aantonen, kwantificeren en bevestigen van intestinale enterococcen in water is conform NEN-EN-ISO 7899-2 gewerkt. De resultaten worden in de hierna volgende tabellen en figuren weergegeven op een lineaire schaal als kolonievormende eenheden (kve) / l.

2.3 DNA-analyse

De DNA-analyse is op te splitsen in een aantal stappen: DNA-isolatie, DNA-analyse (met qPCR) en kwaliteitscontrole. Zowel voor de DNA-isolatie als voor de qPCR-analyses is gebruik gemaakt van gestandaardiseerde KWR-werkvoorschriften.

Voor dit onderzoek hebben de analyses zich in overleg met de opdrachtgever gericht op de detectie van DNA dat indicatief is voor fecale verontreiniging afkomstig van mens, herkauwers, hond en vogels. Voor de eerste twee mogelijke bronnen is daarbij gebruik gemaakt van vertegenwoordigers uit de bacteriegroep *Bacteroides* waarmee onderscheid kan worden gemaakt tussen mensen en herkauwers als mogelijke bron. Voor het opsporen van vogels is gebruik gemaakt van de in vogeluitwerpselen voorkomende *Helicobacter* bacterie. Voor het aantonen van honden als bron is een methode gebruikt die zich richt op DNA in hondencellen in plaats van fecaal gerelateerde bacteriën. Uitwerpselen van honden bevatten veel cellen afkomstig van de darmwand. De resultaten worden in de hierna volgende tabellen en figuren weergegeven op een loglineaire schaal als DNA-kopieën/l.

De kwaliteitscontrole bestaat uit twee onderdelen. In de DNA analyse wordt gebruik gemaakt van een interne controle zodat zicht ontstaat op de efficiëntie van de DNA-extractie en de qPCR-analyse. De tweede controle wordt uitgevoerd doordat een collega-laborant alle gerapporteerde uitkomsten controleert op juistheid.

3 Resultaten en discussie

3.1 Overzicht resultaten DNA analyse en kweek

De rendementen van de DNA-extracties bleken voor vrijwel alle geanalyseerde monsters en de bijbehorende blanco's zeer goed (> 45 % rendement). De uitkomst geeft aan dat de watermonsters afkomstig van deze locaties zich goed lieten behandelen. Alleen het rendement van het monster van 13 juni uit de Weerijssingel was wat laag (12,8%); dit is echter nog voldoende voor het doen van betrouwbare uitspraken.

In onderstaande Tabel 1 zijn de DNA concentraties van de aangetroffen bronnen van fecale herkomst aangegeven. In Tabel 2 staan de met kweek vastgestelde concentraties van de drie onderzochte bacteriegroepen. In de geanalyseerde monsters is in alle gevallen met de DNA detectie tenminste één bron vastgesteld. In alle monsters is het DNA afkomstig van mensen aangetoond. Herkauwers werden alleen niet vastgesteld in de locatie Haven op 6 juni. De hoogste concentraties *Bacteroides* DNA indicerend voor mensen en herkauwers werden aangetroffen op 13 juni bij de Julianabrug. DNA van vogel indicerende *Helicobacter* bacteriën werd op slechts één locatie gevonden (op 2 april, in de Weerijssingel). DNA van honden werd vrijwel alleen na overstort momenten gevonden.

Tabel 1. DNA concentraties (kopieën/l) zoals gedetecteerd voor de vijf onderzochte locaties in stadssingels in Breda.

Datum monster		Locatie	Mens	Herkauwer	Hond	Vogel
				DNA kopieën/l		
2-4-2019	Breda - nulmeting	Markkade 52	1,4E+06	5,4E+04	<1,9E+03	<9,6E+03
6-6-2019	Breda overstort	Markkade 52	2,2E+06	2,4E+03	<1,4E+03	<6,8E+03
13-6-2019	Breda overstort	Markkade 52	1,9E+07	3,4E+03	7,4E+03	<7,1E+03
2-4-2019	Breda - nulmeting	Nieuwe Prinsenkade	4,1E+06	2,4E+04	7,0E+03	<1,1E+04
6-6-2019	Breda overstort	Nieuwe Prinsenkade	2,0E+07	1,5E+04	4,6E+04	<7,3E+03
13-6-2019	Breda overstort	Nieuwe Prinsenkade	9,6E+06	9,1E+05	1,5E+03	<6,9E+03
2-4-2019	Breda - nulmeting	Haven 20	8,3E+05	1,2E+04	<1,9E+03	<9,3E+03
6-6-2019	Breda overstort	Haven 20	5,5E+04	<1,2E+03	<1,2E+03	<6,00E+03
13-6-2019	Breda overstort	Haven 20	1,9E+07	3,4E+03	7,4E+03	<7,1E+03
2-4-2019	Breda - nulmeting	Weerijssingel 29	3,7E+05	7,3E+03	<2,0E+03	1,7E+04
6-6-2019	Breda overstort	Weerijssingel 29	1,6E+04	3,9E+03	<1,4E+03	<7,2E+03
13-6-2019	Breda overstort	Weerijssingel 29	7,4E+05	4,4E+04	<5,2E+03	<2,6E+04
2-4-2019	Breda - nulmeting	Julianabrug	2,4E+05	1,3E+05	<2,1E+03	<1,0E+04
6-6-2019	Breda overstort	Julianabrug	1,1E+06	3,8E+04	1,5E+03	<6,6E+03
13-6-2019	Breda overstort	Julianabrug	2,2E+07	1,8E+07	1,8E+04	<9,2E+03

Gegevens met een '<' betreffen waarden beneden de detectiegrens

Tabel 2. Kweekresultaten (kolonievormende eenheden, kve/l) voor de drie onderzochte bacteriegroepen zoals gedetecteerd voor de vijf onderzochte locaties in stadssingels in Breda.

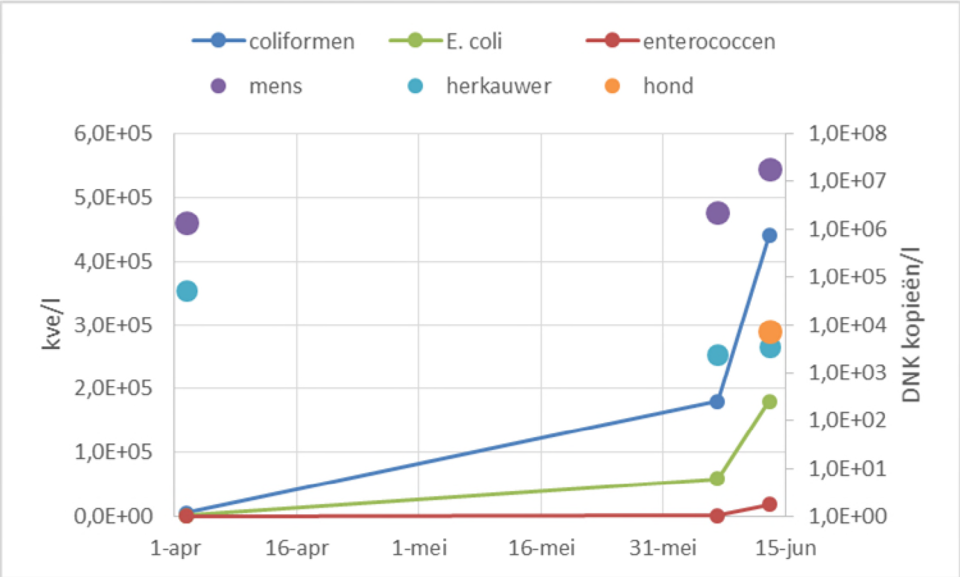
Datum monster		**Locatie**	**coliformen (incl *E. coli*)**	***E. coli***	**intestinale enterococcen**
			Kolonievormende eenheden (kve/l		
2-4-2019	Breda - nulmeting	Markkade 52	5,70E+03	1,90E+03	450
6-6-2019	Breda overstort	Markkade 52	1,80E+05	5,90E+04	2,10E+03
13-6-2019	Breda overstort	Markkade 52	4,40E+05	1,80E+05	1,90E+04
2-4-2019	Breda - nulmeting	Nieuwe Prinsenkade	4,30E+04	1,30E+04	2,50E+03
6-6-2019	Breda overstort	Nieuwe Prinsenkade	>2,7E+06	>3,0E+05	2,00E+04
13-6-2019	Breda overstort	Nieuwe Prinsenkade	2,10E+05	1,90E+05	3,00E+04
2-4-2019	Breda - nulmeting	Haven 20	9,00E+03	3,00E+03	200
6-6-2019	Breda overstort	Haven 20	1,30E+04	<1,0 E+03	30
13-6-2019	Breda overstort	Haven 20	4,40E+05	1,80E+05	1,90E+04
2-4-2019	Breda - nulmeting	Weerijssingel 29	2,40E+04	2,40E+03	360
6-6-2019	Breda overstort	Weerijssingel 29	4,60E+03	2,30E+03	1,10E+03
13-6-2019	Breda overstort	Weerijssingel 29	2,00E+05	6,10E+04	4,70E+04
2-4-2019	Breda - nulmeting	Julianabrug	1,90E+04	<1,0 E+03	260
6-6-2019	Breda overstort	Julianabrug	1,40E+05	7,10E+04	900
13-6-2019	Breda overstort	Julianabrug	5,60E+05	5,10E+05	4,80E+05

Gegevens met een ‘<’ betreffen waarden beneden de detectiegrens. Gegevens voor locatie Nieuwe Prinsenkade op 6 juni zijn aangegeven met ‘>': de betreffende waarden bleken dermate hoog dat zij boven de gehanteerde grens voor kwantificeren uitstegen. Deze waarden zijn in de betreffende figuur aangegeven met respectievelijk de waarden ‘6,00E+05’ (coliformen) en ‘3,00E+05’ (*E. coli*) om de verticale as voor de koloniewaarden vergelijkbaar te houden met die van de andere locaties.

3.2 Markkade 52

In Figuur 1 zijn de resultaten weergegeven voor de drie met kweek bepaalde bacteriegroepen en de DNA waarden voor vastgestelde merkers van fecale bronnen in water van de singellocatie bij Markkade 52. De bacteriewaarden voor coliformen, *E. coli* en intestinale enterococcen zijn verhoogd na de eerste overstort, en ze zijn na de tweede overstort nog hoger (zie ook Tabel 2).

Na de eerste en vooral na de tweede overstort is er een verhoging van de DNA merker voor mens gedetecteerd, en na de tweede overstort wordt ook de merker voor hond gevonden. Gezien de afwezigheid van de merker voor hond op de beide eerdere data suggereert dit het afspoelen van uitwerpselen van honden als gevolg van de regenval op 13 juni. De afwezigheid van de hondmerker na de eerste overstort kan worden veroorzaakt door de late analyse van de watermonsters na de monstername van 6 juni. De DNA merker voor herkauwers is na de eerste en tweede overstort juist verlaagd ten opzichte van de referentie waarde van 2 april. Dit is mogelijk het gevolg van verdunning van oppervlaktewater afkomstig uit landelijk gebied met water afkomstig van riooloverstorten en afspoelend water uit de directe omgeving. De DNA merker voor vogels werd op geen van de monsterdata gedetecteerd. De resultaten wijzen naar riooloverstorten met menselijk fecaal materiaal als bron van fecale verontreiniging op 6 en 13 juni, waarbij in ieder geval op 13 juni ook honden als bron een rol hebben gespeeld. Daarnaast is er op alle data een rol weggelegd voor mensen en herkauwers als bron.

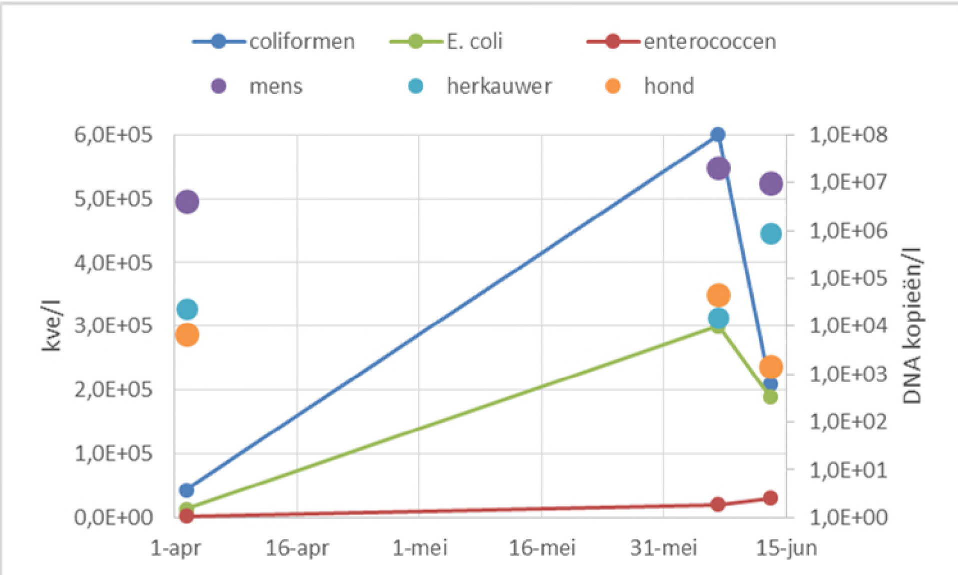


Figuur 1. Meetwaarden voor coliformen, E. coli en enterococcen op de locatie Markkade 52 en de bijbehorende positieve DNA-resultaten van potentiële fecale bronnen. Coliformen, E. coli en intestinale enterococcen zijn uitgedrukt in een lineaire schaal in kve (kolonievormende eenheden) per l; de DNA-merkers zijn uitgedrukt op een loglineaire schaal in DNA-kopie aantallen / l.

3.3 Nieuwe Prinsenkade

In Figuur 2 zijn de resultaten weergegeven voor de drie met kweek bepaalde bacteriegroepen en de DNA waarden voor vastgestelde merkers van fecale bronnen in water van de singellocatie bij de Nieuwe De kve-waarden voor coliformen en *E. coli* zijn sterk verhoogd na de eerste overstort op 6 juni. Op deze datum zijn de waarden voor coliformen en *E. coli* zelfs hoger dan de maximale telwaarden. Om in Figuur 2 de as voor de kweekresultaten vergelijkbaar te houden met die van de figuren voor de andere locaties, en om in Figuur 2 een waarde aan de beide variabelen te kunnen toekennen, is op deze datum ter indicatie voor coliformen de kve-waarde van >2,7E+06 afgebeeld als 6 E+05; voor *E. coli* is de waarde van >3,0E+05 afgebeeld als 3 E+05. Na de tweede overstort op 13 juni zijn de waarden voor deze beide bacteriegroepen lager dan op 6 juni maar nog steeds hoger dan op de referentiedatum van 2 april. De intestinale enterococcen zijn op 6 en 13 juni eveneens verhoogd ten opzichte van 2 april.

Na de eerste en de tweede overstort is er een verhoging van de DNA merker voor mens gedetecteerd ten opzichte van de referentie datum. Na de eerste overstort is er ook een verhoogde waarde voor de hondmerker gevonden, die na de tweede overstort weer beduidend lager is dan op de referentie datum. Die verlaging zou kunnen zijn veroorzaakt doordat aanwezige uitwerpselen van honden als gevolg van de regenval op 6 juni zijn afgespoeld, en kennelijk niet meer zijn aangevuld in de periode tot 13 juni. De DNA merker voor herkauwers is alleen na de tweede overstort verhoogd ten opzichte van de referentie waarde van 2 april en de waarde van 6 juni. De verhoging van de herkauwer merker op 13 juni suggereert een dan op deze locatie verhoogde toevoer van oppervlaktewater afkomstig uit landelijk gebied. Op geen van de monster data werd de DNA merker voor vogels gedetecteerd. De resultaten wijzen naar riooloverstorten met menselijk fecaal materiaal als bron van fecale verontreiniging op 6 en 13 juni, waarbij in ieder geval op 6 juni ook honden als bron een rol hebben gespeeld. Daarnaast is er op 13 juni ook een rol weggelegd voor herkauwers als verhoogde bron. Naast mensen spelen honden en herkauwers ook een rol als bron in de referentie situatie.

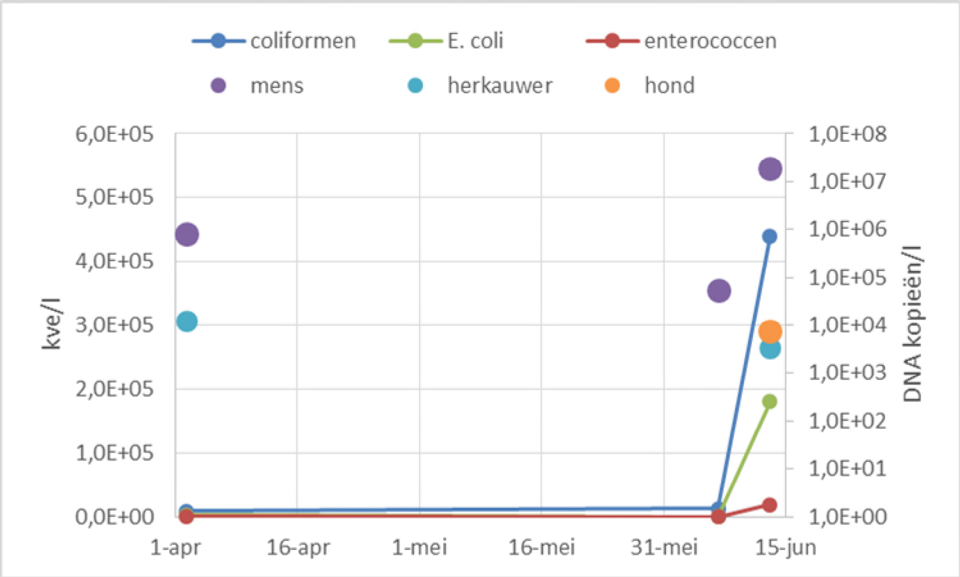


Figuur 2. Meetwaarden voor coliformen, E. coli en enterococcen op de locatie Nieuwe Prinsenkade en de bijbehorende positieve DNA-resultaten van potentiële fecale bronnen. Coliformen, E. coli en intestinale enterococcen zijn uitgedrukt in een lineaire schaal in kve (kolonievormende eenheden) per l; de DNA-merkers zijn uitgedrukt op een loglineaire schaal in DNA-kopie aantallen / l.

3.4 Haven 20

In Figuur 3 zijn de resultaten weergegeven voor de drie met kweek bepaalde bacteriegroepen en de DNA waarden voor vastgestelde merkers van fecale bronnen in water van de singellocatie bij de Haven 20. De kve-waarden voor coliformen, *E. coli* en intestinale enterococcen zijn alleen verhoogd na de tweede overstort op 13 juni.

Alleen na de tweede overstort is er een verhoging van de DNA merker voor mens gedetecteerd ten opzichte van de referentie datum. De DNA merker voor herkauwers is alleen op de referentiedatum en na de tweede overstort gevonden; op 13 juni is deze vergelijkbaar met de referentie waarde van 2 april. De afwezigheid van herkauwermerker op 6 juni kan worden veroorzaakt door de late analyse van de watermonsters na de monstername van die datum. De hondmerker werd alleen aangetroffen op 13 juni. Dit suggereert een dan op deze locatie verhoogde toevoer van uitwerpselen van de hond als gevolg van afspoeling. De DNA merker voor vogels werd op geen van de data gedetecteerd. De resultaten wijzen naar riooloverstorten met menselijk fecaal materiaal als bron van fecale verontreiniging op 13 juni, waarbij ook honden als bron een rol hebben gespeeld. Naast mensen spelen ook herkauwers een rol als bron in de referentie situatie.

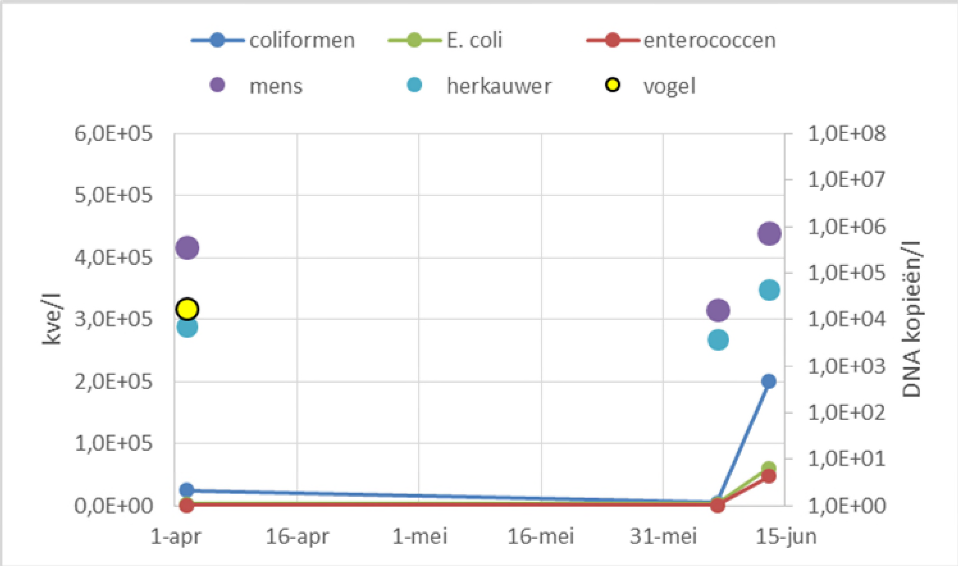


Figuur 3. Meetwaarden voor coliformen, E. coli en enterococcen op de locatie Haven 20 en de bijbehorende positieve DNA-resultaten van potentiële fecale bronnen. Coliformen, E. coli en intestinale enterococcen zijn uitgedrukt in een lineaire schaal in kve (kolonievormende eenheden) per l; de DNA-merkers zijn uitgedrukt op een loglineaire schaal in DNA-kopie aantallen / l.

3.5 Weerijssingel 29

In Figuur 4 zijn de resultaten weergegeven voor de drie met kweek bepaalde bacteriegroepen en de DNA waarden voor vastgestelde merkers van fecale bronnen in water van de singellocatie bij de Weerijssingel 20. De kve-waarden voor coliformen, *E. coli* en intestinale enterococcen zijn eigenlijk alleen verhoogd na de tweede overstort op 13 juni.

Alleen na de tweede overstort is er een verhoging van de DNA merker voor mens en herkauwers gedetecteerd ten opzichte van de referentie datum. Op 6 juni zijn deze beide wat lager dan de referentie waarde van 2 april. Dit kan mogelijk zijn veroorzaakt door de late analyse van de watermonsters na de monstername van 6 juni. De hondmerker werd op geen van de monsterdata aangetroffen. De DNA merker voor vogels werd alleen op de referentie datum van 2 april gedetecteerd. De resultaten wijzen naar de riooloverstort van 13 juni met menselijk fecaal materiaal als bron van fecale verontreiniging op 13 juni, waarbij ook herkauwers als bron een rol hebben gespeeld (mogelijk door een dan wellicht verhoogde wateraanvoer vanuit het achterland). Naast mensen spelen ook herkauwers en vogels een rol als bron in de referentie situatie.

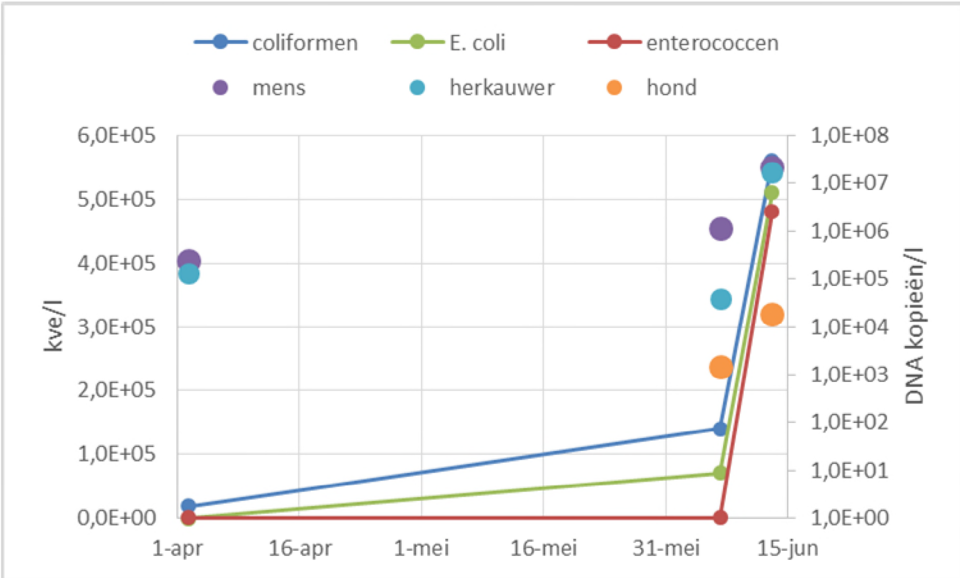


Figuur 4. Meetwaarden voor coliformen, E. coli en enterococcen op de locatie Weerijssingel 29 en de bijbehorende positieve DNA-resultaten van potentiële fecale bronnen. Coliformen, E. coli en intestinale enterococcen zijn uitgedrukt in een lineaire schaal in kve (kolonievormende eenheden) per l; de DNA-merkers zijn uitgedrukt op een loglineaire schaal in DNA-kopie aantallen / l.

3.6 Julianabrug

In Figuur 5 zijn de resultaten weergegeven voor de drie met kweek bepaalde bacteriegroepen en de DNA waarden voor vastgestelde merkers van fecale bronnen in water van de singellocatie bij de Julianabrug. De kve-waarden voor coliformen en *E. coli* zijn verhoogd na beide overstort momenten, de intestinale enterococcen zijn met name verhoogd na de tweede overstort op 13 juni.

Na beide overstort momenten is er een verhoging van de DNA merker voor mens gedetecteerd ten opzichte van de referentie datum. Voor herkauwers geldt dat hun merker alleen op 13 juni is verhoogd ten opzichte van de referentie waarde van 2 april. Dit kan mogelijk zijn veroorzaakt door de late analyse van de watermonsters na de monstername van 6 juni. De hondmerker werd op beide overstortmomenten aangetroffen, maar niet op de referentiedatum. De DNA merker voor vogels werd op geen van de data gevonden. De resultaten wijzen naar de riooloverstort van 6 en 13 juni met menselijk fecaal materiaal, en afspoeling van uitwerpselen van honden, als bron van fecale verontreiniging op deze data, waarbij ook herkauwers als bron op 13 juni een rol hebben gespeeld (mogelijk door een dan wellicht verhoogde wateraanvoer vanuit het achterland). Naast mensen spelen ook herkauwers een rol als bron in de referentie situatie.



Figuur 5. Meetwaarden voor coliformen, E. coli en enterococcen op de locatie Julianabrug en de bijbehorende positieve DNA-resultaten van potentiële fecale bronnen. Coliformen, E. coli en intestinale enterococcen zijn uitgedrukt in een lineaire schaal in kve (kolonievormende eenheden) per l; de DNA-merkers zijn uitgedrukt op een loglineaire schaal in DNA-kopie aantallen / l.

4 Conclusies

De resultaten wijzen voor de meeste locaties bij het optreden van riooloverstorten naar menselijk fecaal materiaal als bron van fecale verontreiniging op zowel 6 als 13 juni, waarbij locatie en datum afhankelijk onder die omstandigheden ook herkauwers en honden als bron een rol hebben gespeeld. Daarnaast is er in de referentie situatie steeds een rol weggelegd voor mensen en herkauwers als bron, waarbij onder die omstandigheden en locatie afhankelijk, soms ook honden en vogels als bron optreden. De op 6 juni soms (erg) lage DNA concentraties zijn mogelijk veroorzaakt doordat de monsters van 6 juni niet tijdig konden worden geanalyseerd.

5 Referenties

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