

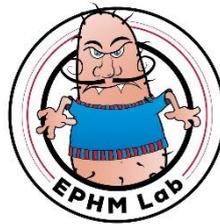


MONASH University

Modelling *Escherichia coli* Dynamics in an Urban Estuary

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A thesis submitted for the degree of *Doctor of Philosophy*
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Abstract

Receiving waters such as rivers, urban estuaries and coastal waters are under increasing pressure from anthropogenic impacts due to population growth, rapid urbanisation rates and increased pollution levels. Microbial water quality is of particular concern for uses such as fishing and recreation, and it is therefore essential to understand the levels and dynamics of faecal microorganisms in these water bodies. This can help us estimate the human health risks associated with recreational uses, and will also provide valuable information to develop targeted and cost effective mitigation strategies.

This research project focuses on the city of Melbourne, Australia, as a case study for the analysis of faecal microbial contamination of receiving waters. Melbourne is a large metropolitan area located on the banks of the Yarra River, which forms an urban salt-wedge estuary. Estuaries are particularly complex environments because faecal microbial levels are influenced by a wide range of parameters: inputs of faecal contamination, survival of microorganisms in the water column and sediments, sediment-microbe interactions, and complex estuarine hydrodynamics. Currently, the only practical and possible method to assess faecal contamination is to use complex numerical models; however, existing models either do not appropriately characterise inputs of faecal microorganisms, do not account for all important dynamic processes within the estuary, and/or are not robust enough due to small data sets and inadequate testing.

The main aim of this research project was therefore to develop and test a more comprehensive and robust estuarine microorganism model, using the Yarra River estuary as a case study. The final model is based on a substantial data set and compiles a range of different components, which ensure that all inputs and estuary processes related to movement and survival of faecal microorganisms are accurately characterised.

The thesis conducted an extensive data collection campaign which included high resolution measurements of water levels, flow velocity, temperature, salinity, pH, dissolved oxygen, turbidity and *Escherichia coli* (*E. coli* - a common faecal indicator organism) over a period of nearly two years. To characterise the faecal microbial levels within the estuary with a high temporal and spatial resolution, this thesis collected *E. coli* samples as time series and depth profiles in the water column: 3500 *E. coli* samples were collected from two locations for the time series, and to assess the stratification in the estuary, over 80 depth profiles were undertaken in four locations. The data set was supplemented with 1600 *E. coli* samples taken from various freshwater sources entering the estuary to appropriately characterise faecal microorganism inputs into the system.

Analysis of the collected data revealed that the main driver of *E. coli* concentrations within the estuary was the freshwater input from the Yarra River itself, highlighting the critical need for accurate characterisation of faecal contamination inputs. Using the extensive data set collected for this thesis, this thesis assessed the existing MicroOrganism Prediction in Urban Stormwater (MOPUS) model for its robustness on a variety of water systems and spatial scales. The model was able to predict wet weather microbial dynamics not only in stormwater drains, but also in an urban creek and in the Yarra River itself. This thesis therefore included MOPUS (coupled with a simple dry weather estimation using the collected data points) to model the inputs / boundary conditions at a fine temporal scale for the estuarine model.

The collected data also revealed that spatial variability of *E. coli* was closely related to salt-wedge dynamics and that concentration fluctuations over a tidal cycle were correlated to estuarine flow velocity. As such, it was particularly important to accurately model estuarine hydrodynamics. This thesis found that the hydrodynamic model TUFLOW FV performed well because it predicted the high stratification (salinity and temperature levels) of the Yarra River estuary accurately in addition to standard hydrodynamic variables such as water levels and flow velocity. This model was integrated into the final model to assess estuarine dynamics.

The large data set allowed robust development and testing of the final estuarine model and the results show that it accurately predicts *E. coli* dynamics within the Yarra River estuary. The model accounts for all complex parameters influencing *E. coli* in the estuary; a simple sensitivity analysis revealed that these are primarily influenced by the inputs and hydrodynamic transport and mixing and that conversely, *E. coli* die-off and sediment-microorganism interactions had limited impact on model performance.

In addition to meeting its initial aim of creating a comprehensive and robust estuarine faecal microorganism model for the Yarra River estuary, this research project paves the way for practical applications for the management of this iconic water body. Scenario testing will allow the development of specific risk management strategies for recreational use of the Yarra, and will facilitate the selection of appropriate mitigation strategies to improve microbial water quality within the estuary.

Finally, this research project contributes greatly to the growing body of research around faecal microbial characterisation of urban waters. It provides a robust methodology for the prediction of *E. coli* inputs into a receiving water body at a fine temporal scale. For stratified estuarine environments in particular, it offers a unique study of the spatial distribution of *E. coli*, shows that it is tidal velocity, rather than tidal water levels, which is the determining factor for *E. coli* concentrations, and that *E. coli*

dynamics is primarily impacted by hydrodynamic processes in these environments. This particular estuarine model can be used as a reliable basis to develop and test models for other (similar) estuarine environments.

Publications during enrolment

Journal publications (First author; in chronological order):

JOVANOVIC, D., HENRY, R., COLEMAN, R., DELETIC, A. & MCCARTHY, D. 2015. Integrated conceptual modelling of faecal contamination in an urban estuary catchment. *Water Science and Technology*, 1472-1480.

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Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes five original publications published in peer reviewed journals and one manuscript under internal review for submission to a peer reviewed journal. Additionally, two co-authored journal publications and two conference publications were produced based on the work conducted within this research project. The core theme of the thesis is water quality modelling. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the Department of Civil Engineering under the supervision of Associate Professor David McCarthy and Professor Ana Deletic.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of Chapters 4, 5, 6 and 7 my contribution to the work involved the following:

Thesis Chapter	Publication Title	Status	Nature and % of student contribution	Co-author name(s) Nature and % of Co-author's contribution	Co-author(s), Monash student Y/N
4	<i>Spatial variability of E. coli in an urban salt-wedge estuary</i>	Published 2017 <i>Marine Pollution Bulletin</i>	<i>Initiation, ideas, experimental work, data analysis, interpretation and write up; 70%</i>	1) <i>Rhys Coleman, data interpretation, reviewing of manuscript; 5%</i> 2) <i>Ana Deletic, data interpretation, reviewing of manuscript; 5%</i> 3) <i>David McCarthy, ideas, data interpretation, reviewing of manuscript; 20%</i>	1) N 2) N 3) N
4	<i>Tidal fluctuations influence E. coli concentrations in urban estuaries</i>	Published 2017 <i>Marine Pollution Bulletin</i>	<i>Initiation, ideas, experimental work, data analysis, interpretation and write up; 70%.</i>	1) <i>Rhys Coleman, data interpretation, reviewing of manuscript; 5 %</i> 2) <i>Ana Deletic, data interpretation, reviewing of manuscript; 5%</i> 3) <i>David McCarthy, ideas, data interpretation, reviewing of manuscript; 20%</i>	1) N 2) N 3) N
5	<i>Conceptual modelling of E. coli in urban stormwater drains, creeks and rivers</i>	Published 2017 <i>Journal of Hydrology</i>	<i>Initiation, ideas, experimental work, modelling work, data analysis, interpretation and write up; 65%.</i>	1) <i>Jon Hathaway, data interpretation, reviewing of manuscript; 5%</i> 2) <i>Rhys Coleman, data interpretation, reviewing of manuscript; 5%</i> 3) <i>Ana Deletic, data interpretation, reviewing of manuscript; 5%</i> 4) <i>David McCarthy, ideas, data interpretation, reviewing of manuscript; 20%</i>	1) N 2) N 3) N 4) N
6	<i>Modelling shallow and narrow urban salt-wedge estuaries: evaluation of model performance and sensitivity to optimise input data collection</i>	Published 2019 <i>Estuarine, Coastal and Shelf Science</i>	<i>Initiation, ideas, experimental work, modelling work, data analysis, interpretation and write up; 55%.</i>	1) <i>Simone Gelsinari, data interpretation, write up; 5%</i> 2) <i>Louise Bruce, data interpretation, reviewing of manuscript; 5%</i> 3) <i>Mathew Hipsey, data interpretation, reviewing of manuscript; 5%</i> 4) <i>Ian Teakle, data interpretation, reviewing of manuscript; 5%</i> 5) <i>Mathew Barnes, data interpretation, reviewing of manuscript; 5%</i> 6) <i>Rhys Coleman, data interpretation, reviewing of manuscript; 5%</i> 7) <i>Ana Deletic, data interpretation, reviewing of manuscript; 5%</i> 8) <i>David McCarthy, ideas, data interpretation, reviewing of manuscript; 10%</i>	1) Y 2) N 3) N 4) N 5) N 6) N 7) N 8) N
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I have not renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student signature:

Date:

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor signature:

Date:

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Chapter 1

Introduction

1.1 Introduction

Estuaries across the world are increasingly being developed and managed for recreational purposes. However, at the same time, they are placed under environmental stressors, leading to excessive pollution and thereby limiting their benefits (Wolanski and Elliott, 2016). The majority of the adverse influences affecting estuarine health are anthropogenic (e.g. population growth, urbanization, climate change). This is not surprising considering that estuaries and continental shelf areas comprise 5.2% of the earth surface and around 60% of the global population lives alongside these systems (Lindeboom, 2002). Current population growth predictions suggest that population in coastal areas is doubling every 20 years (Wolanski and Elliott, 2016), hence, the environmental pressures on estuaries are likely to increase in the future.

Faecal microorganisms are the leading cause of pollution in urban estuaries (Burton and Pitt, 2002), and they can have significant impact on the public health. Medical treatment of illnesses associated with recreational waters can represent significant economic burden. For example, estimated cost for treatment of these illnesses was \$3.3 million per year for only two beaches in California, USA (Dwight et al., 2005).

For the above reasons, increased effort has been placed around mitigation strategies for improvement of health of urban estuaries. However, faecal microorganisms are influenced by myriad sources/inputs (e.g. rivers and creeks, urban stormwater, seawater, bed and bank sediments and other non-host habitats, direct deposition by wildlife and humans), various processes (survival in water column and sediments, sedimentation, re-suspension) and an array of hydrological factors (e.g. flow, velocity, tide, hydrodynamic/density driven mixing). This complexity often makes it difficult to develop accurate mitigation strategies that focus on the underlying causal source/mechanism.

It is therefore hypothesised that adequate mitigation cannot occur without a full understanding and appreciation of all the inputs and processes which occur within the system. This lends itself to the use of modelling tools which can incorporate these complex dynamics and then be used to explore (by means of hypothesis testing) various methods of mitigation and the influence of future externalities on the system's behaviour, including climate change and population growth. Such a tool could be part of a wider decision making process. Furthermore, this tool would not only be valuable for assessing different mitigation options but also to provide real-time data and warnings to users of estuaries. **However, the development of microorganism models for estuaries is hindered by the large knowledge and data gaps present in the literature, the uncertainty involved in microorganism quantification, and the complex hydrodynamics in urban estuaries.**

There have been a few attempts to model microbial dynamics in estuaries. However, due to their complexity, these models include only some of the many possible faecal pollution sources, sinks and processes. Modelling was mostly restricted to the water column (Salomon and Pommepuy, 1990; Kashefipour et al., 2002; Garcia-Armisen et al., 2006; de Brauwere et al., 2011; Gao et al., 2015), rarely including interactions between sediments and overlaying water layer (sedimentation/re-suspension)(de Brauwere et al., 2014a), and influence of the stratification due to salt wedge and temperature (i.e. modelling estuary as layered system). **As such, there is a need for the development of complex coupled hydrodynamic-microbial models that could accurately predict concentrations of faecal microorganisms in estuaries in different hydrological/environmental conditions.**

The main aim of this research is to develop a hydrodynamic-microorganism model that can simulate complex microbial dynamics in urban estuarine environments. The objective is to develop a model that would help water managers to better understand faecal pollution dynamics, identify most important inputs and develop effective mitigation strategies. The new modelling tool will focus on predicting microorganism concentrations in the water column of urban estuaries. Since there are many different faecal microorganisms present in urban estuaries, the objective is also to develop a model that could be easily modified to model a range of different faecal microbes.

The Yarra River estuary was used as a case study, because it is the iconic waterway of the city of Melbourne, commonly used for recreation and has a complex catchment. A data set including measurements of water level, flow rates, flow velocities, electrical conductivity (salinity), temperature, pH, DO, Turbidity and *E. coli* concentrations was collected from the Yarra River estuary, urban stormwater drains as well as the upstream Yarra River to enable the development of a model that can holistically account for the sources, sinks and processes influencing faecal microbial dynamics in urban estuarine environments.

1.2 Thesis scope

There are many aspects of developing a model of faecal microorganism dynamics in urban estuaries. However, within the limited time and resources available, it is not possible to explore each of these aspects to a great extent in this thesis. Rather, time and resource allocation to certain aspects was based on the hypothesised level of importance and available knowledge/data in the literature. This section outlines the scope of this thesis and discusses not only the aspects that were considered but also what was not considered.

The main focus of the thesis will be development and testing of an estuarine microorganism model. The model will predict concentrations of the microorganisms in estuarine environment. As such, the model will not explicitly assess the health risk to the users of the estuary. Therefore, health risks assessments will not be implemented as a part of the new model and as such remain in domain of future development and additions to the model. Nevertheless, addition of health risk assessment feature to the model can be achieved by adding a risk assessment tool based on Quantitative Microbial Risk Assessment (QMRA) framework.

A major part of the thesis was collecting sufficient data for model testing and development. In addition to hydrology/hydrodynamic data (e.g. water levels, flow velocity etc.) needed for testing the hydrodynamic model of the estuary, significant amount of data was collected for development and testing of faecal microorganism model. These included *E. coli* concentrations within the estuary as well as within the major inputs to the estuary, and various water quality parameters such as, water temperature, electric conductivity/salinity, turbidity, pH and dissolved oxygen. Due to resource constraints only a standard indicator organism (i.e. *E. coli*) and not specific pathogens, could be monitored. However, the model will be flexible for adapting to the range of other indicator and pathogenic microorganisms. As such, testing of the model using a range of pathogens is part of the future research plan for this model.

The collected data was used to analyse the dynamics of *E. coli* within the Yarra River estuary and identify major processes governing its levels, so that the model could be more accurately constructed. Inputs were hypothesised to be extremely important for accurate prediction of the *E. coli* dynamics within the estuary, hence, a major task involved characterising all significant inputs of *E. coli* as best as possible. This was achieved by employing existing models for microorganism prediction.

Hydrodynamics is known to significantly impact the water quality and as such, validating and sensitivity testing of the hydrodynamic model of the Yarra River estuary was another major task. However, whilst accurate hydrodynamics is necessary for accurate pollutant prediction, hydrodynamic modelling is not the focus of this research. As such, a commercial software for hydrodynamic modelling was used to develop a hydrodynamic model of the Yarra River estuary.

1.3 Outline of the thesis

The thesis consists of eight chapters in total (discussed briefly below). The four main chapters of this thesis (Chapters 4 to 7) contain publications that have been published or are currently under internal review. Additional discussions have also been included to supplement these publications.

Chapter 2: Literature review. This chapter provides a review of the published literature from perspective of modelling pathogens/faecal microbes in urban estuaries. The existing microorganism models are assessed based on selection of key criteria formulated from the review of literature. Current research gaps are identified and the objectives and main hypotheses underlined in the present thesis are presented.

Chapter 3: Monitoring program and collected data. This chapter describes the comprehensive data collection campaigns conducted as one of the essential parts of this research project in order to collect data needed for model testing and development. Monitoring site locations, equipment used and data collected are presented.

Chapter 4: *E. coli* dynamics within the Yarra River estuary. This chapter presents analysis of the *E. coli* dynamic within the Yarra River estuary using the newly collected datasets. The chapter consists of three main sections: 1) exploration of estuarine hydrodynamics and *E. coli* levels; 2) a journal paper titled "*Influence of tides on E. coli levels in an urban estuary*" published in *Marine Pollution Bulletin* in 2017 and; 3) a journal paper titled "*Spatial variability of E. coli in an urban estuary*" also published in *Marine Pollution Bulletin* in 2017.

Chapter 5: Modelling flow and *E. coli* inputs to the Yarra River estuary. This chapter describes modelling of the flow and *E. coli* concentrations from main inputs to the Yarra River estuary. The chapter consists of three main sections: 1) a journal paper titled "*Conceptual modelling of E. coli in urban stormwater drains, creeks and rivers*" published in *Journal of Hydrology* in 2017; 2) a section describing how the models tested in a journal paper above were used for providing continuous inputs of flow and *E. coli* concentrations for all stormwater, creek and riverine inputs to the estuary, and; 3) sections of the paper "*Integrated conceptual modelling of faecal contamination in an urban estuary catchment*" published in *Water Science and Technology* in 2015 relevant for the input assessment.

Chapter 6: Modelling hydrodynamics of the Yarra River estuary. Main focus of this chapter is hydrodynamic model of the Yarra River estuary. The chapter presents assessment of the model performance as well as the sensitivity of the model outputs to a range of input data and model parameters. This work is described in a journal paper titled "*Modelling shallow and narrow urban salt-*

wedge estuaries: Evaluation of model performance and sensitivity to optimise input data collection" published in *Estuarine, Coastal and Shelf Science* in 2019.

Chapter 7: Modelling *E. coli* dynamics in the Yarra River estuary. This chapter presents modelling of *E. coli* dynamics within the Yarra River estuary. The chapter consists of two main sections: 1) a journal paper "Integrated conceptual modelling of faecal contamination in an urban estuary catchment" published in *Water Science and Technology* in 2015, presenting a simple conceptual model of the *E. coli* dynamics in the Yarra River estuary, and; 2) a journal paper titled "*Integrated modelling of fate and transport of E. coli within an urban salt-wedge estuary*", currently under internal review, to be submitted to *Water Research*.

Chapter 8: Conclusions, strengths and weaknesses of the research. The final chapter provides a summary of the key findings, a discussion of the strengths and weaknesses of the thesis and a summary of the areas requiring further investigation.

Appendix A: Supplementary materials. This appendix contains supplementary materials of all journal articles presented in the above chapters.

Appendix B: Conference papers. This appendix contains two conference papers produced during the course of this research project.

Appendix C: Co-authored journal papers. This appendix encloses two journal papers co-authored by the candidate during the course of this research project.

Chapter 2

Literature Review

2.1 Introduction

The following literature review has five sections. The first introduces waterborne pathogens and faecal indicators and presents the justification of the faecal indicator selected for this study. The second section gives an overview of the inputs of faecal contamination from an estuarine perspective, while the third section gives background information on estuarine environment (i.e. estuarine hydrodynamics and water quality). The fourth section identifies the key processes influencing levels of faecal microorganisms in estuarine environments. Finally, the last section summarises what is needed from an estuarine hydrodynamic-microorganism model, reviews the currently available models and highlights their benefits and deficiencies.

The scope of this literature review will cover three main groups of microorganisms: bacteria, protozoa and viruses. Although other groups exist, such as worm and fungi, they are not considered in this literature review, as the most of the waterborne pathogens come from the first three groups mentioned. Furthermore, the focus of this literature review will be on faecally derived human pathogens, due to the dominant exposure pathway being faecal-oral route. The overall focus of the research project will be on estimating waterborne pathogen concentrations. Whilst this is only one of the factors in estimating the risk of contracting a waterborne disease, the outputs of this project could possibly later feed into a risk assessment framework to estimate human health risks due to waterborne pathogens.

2.2 Microorganisms in aquatic environment and public health risk

The term *microorganism* refers to wide range of organisms that are too small to be seen clearly by the unaided eye (CWP, 2000; Willey et al., 2008). Microorganisms are ubiquitous in the environment and exist on nearly every surface of the earth. They are very diverse and can be divided by different criteria. From a water pollution perspective, there are three main groups of microorganisms of interest: bacteria, protozoa and viruses (there are others, such as worm and fungi, but these are outside the scope of this research). Bacteria are single-celled organisms with cells 0.4 - 14 μm in length and 0.2 - 1.2 μm in width. They are the major inhabitants of human skin, mouth, and intestines (Willey et al., 2008), and inhabit almost all living creatures and environmental systems. Protozoa are a large group of usually motile unicellular eukaryotic organisms ranging in size from 2 - 100 μm (CWP, 2000; Horan, 2003b; Willey et al., 2008). Protozoa are widely distributed through almost every aquatic environment (Horan, 2003b). They are normally inhabitants of animal intestinal tracts where they help with digestion of complex materials (Willey et al., 2008). Viruses are acellular entities that have to invade

host cell in order to replicate (Willey et al., 2008). They are the smallest of all microorganisms, ranging in size from 0.02 - 0.09 μm .

2.2.1 Waterborne pathogens

Microorganisms can be beneficial to humans, have no impact at all or they can cause illness/disease (CWP, 2000). Microorganisms that are known to cause disease are called *pathogens* (CWP, 2000; Willey et al., 2008) and their ability to cause disease is called *pathogenicity* (Willey et al., 2008). The actual risk of contracting a waterborne disease depends on a number of factors: pathogen concentration, way and time of exposure or transmission (inhalation, ingestion or penetration), infectious dose, and age and immune system status of the exposed individual (CWP, 2000; Pond, 2005). The focus of this project will be on just one of these factors in risk estimation: that is, estimating waterborne microbial concentrations.

Table 2 - 1, Table 2 - 2 and Table 2 - 3 provide lists of pathogenic bacteria, protozoa and viruses (respectively) commonly found in water bodies. Adverse effects of these microorganisms on human health vary, but mostly involve gastrointestinal symptoms with diarrhoea and some infections can result in hospitalisation, surgery or death (Pond, 2005). These tables show that some pathogens are autochthonous to aquatic environments (i.e. those which are naturally present in the environment, e.g. *Legionella spp.*, *Vibrio spp.*, *Naegleria Fowleri*), but most are allochthonous (i.e. introduced in aquatic environments, e.g. faecal microbes from human and/or animal faeces). Although autochthonous free-living pathogens can cause severe health effects, infections by these agents are much rarely reported (Pond, 2005). Conversely, allochthonous faecally derived pathogens are identified as a major concern to public health (WHO, 2011). Indeed, of the pathogens listed in Tables 2.1 to 2.3, the faecal-oral route is the dominant exposure pathway for causing disease, meaning these allochthonous faecal microbes are of most concern for mitigating waterborne disease. Therefore, the focus of this research project will be on faecally derived pathogens.

Table 2 - 1 Bacterial pathogens found in aquatic environments with their sources/origins, exposure route and mechanism of infection and effects on human health

Pathogenic Bacteria	Sources/Origin	Exposure route / Mechanism of infection	Adverse Health Effects
<i>Campylobacter spp.</i>	Faecal – animals ^{1,4} (esp. birds ^{1,4})	Faecal-oral/ ingestion of contaminated water ^{1,4}	Gastrointestinal infections (GI) ^{1,4}
<i>E. coli O157:H7</i>	Faecal – humans ¹ and animals ¹	Faecal-oral / ingestion of contaminated water ¹	GI ¹ ; bloody diarrhoea ¹ ; vomiting ¹
<i>Helicobacter pylori</i>	Faecal – humans ¹	Faecal-oral / ingestion of contaminated water ¹	GI ^{1,4}
<i>Legionella</i>	Free-living aquatic environment ^{1,4} , heating/cooling systems, soils ⁴	Inhalation of aerosols ^{1,4} ; ingestion of contaminated water ¹	Pneumonic legionellosis ^{1,4} ; Pontiac fever ^{1,4}
<i>Leptospira spp.</i>	Kidneys of animal hosts ^{1,2}	Inhalation of aerosols ^{1,2} ; Penetration through skin ^{1,2}	Leptospirosis ^{1,2} ; kidney and liver failure ^{1,2} ; severe muscle pain ¹
<i>Mycobacterium</i>	Almost every environment in contact with humans and animals ^{1,4}	Ingestion of contaminated water ¹	Fever ¹ ; Lung damage ^{1,4} ; haemoptysis ¹ ; chest pain ¹
<i>Salmonella spp.</i>	Faecal – humans ^{1,3,4} and animals ^{1,3,4}	Faecal-oral / ingestion of contaminated water ¹	GI ^{1,4} ; Typhoid and paratyphoid fever ^{1,4}
<i>Shigella spp.</i>	Faecal – humans ^{1,4} and gorillas ¹	Faecal-oral / ingestion of contaminated water ^{1,4}	Shigellosis ¹ – bacillary dysentery ^{1,4} ; GI ^{1,4}
<i>Vibrio spp.</i>	Free-living in estuarine and marine environments ^{1,2}	Ingestion of contaminated water ^{1,2} ; inhalation of aerosols ² ; consumption of contaminated shellfish ²	Necrotising wound infections ^{1,2} ; GI ^{1,2,4} ; primary septicaemia ¹ ; cholera ^{2,4} ; pneumonia ²
<i>Yersinia spp.</i>	Faecal – animals ⁴	Faecal-oral / ingestion of contaminated water ⁴	GI ⁴
<i>Aeromonas spp.</i>	Free-living in aquatic environments ²	Water contact through open wounds ² ; ingestion of contaminated water ² ; consumption of contaminated shellfish ²	GI ^{2,4} ; diarrhoea ^{2,4} ; septicaemia ⁴
<i>Pseudomonas aeruginosa</i>	Faecal – human and animal ⁴ ; free-living in water and soil ⁴	Water contact through open wounds ⁴ or injured body parts ⁴	Destructive lesions ⁴ ; septicaemia ⁴ ; rashes ⁴

¹(Pond, 2005); ²(WHO, 2003); ³(CWP, 2000); ⁴(NHMCR, 2011)

Table 2 - 2 Protozoan pathogens found in aquatic environments with their sources/origins, exposure route and mechanism of infection and effects on human health

Pathogenic Protozoa	Sources/Origin	Exposure route / Mechanism of infection	Adverse Health Effects
<i>Cryptosporidium</i> spp.	Faecal – livestock ^{1,3,4} and infected humans ^{1,3,4}	Faecal-oral/ ingestion of contaminated water ^{1,4}	Cryptosporidiosis ¹ ; diarrhoea ^{1,4} ; abdominal pain ¹ ; fever ¹
<i>Giardia</i> spp.	Faecal – humans ^{1,3} and animals ^{1,3,4}	Faecal-oral/ ingestion of contaminated water ^{1,4}	Giardiasis ^{1,4} ; diarrhoea ^{1,4}
<i>Amoebae</i> (<i>Naegleria Fowleri</i>)	Free-living in environmental waters ^{1,2,4} and soil ¹	Intranasal adsorption of water ^{1,4} , contact with contaminated water ²	Primary amoebic meningoencephalitis (PAM) ^{1,2,4}
<i>Amoebae</i> (<i>Acanthamoeba</i>)	Free-living in environmental waters ^{2,4} and soil ^{2,4}	Contact with contaminated water ¹	Granulomatous amoebic encephalitis (GAE) ^{2,4} ; Keratitis ^{2,4}
<i>Microsporidia</i>	Faecal – animals ¹	Faecal-oral/ ingestion of contaminated water ¹	Microsporidiosis ¹ – GI ¹ ; infections of reproductive, respiratory, muscle, excretory, and nervous tissues ¹

¹(Pond, 2005); ²(WHO, 2003); ³(CWP, 2000); ⁴(NHMCR, 2011)

Table 2 - 3 Viral pathogens found in aquatic environments with their sources/origins, exposure route and mechanism of infection and effects on human health

Pathogenic Viruses	Sources/Origin	Exposure route / Mechanism of infection	Adverse Health Effects
Adenovirus	Faecal – humans ^{1,2}	Faecal-oral/ ingestion of contaminated water ¹ ; Inhalation of aerosols ²	Fevers ^{1,2} ; upper respiratory tract symptoms ¹ ; conjunctivitis ^{1,2} ; GI ²
Enterovirus	Faecal – humans ^{2,3}	Faecal-oral/ ingestion of contaminated water ²	GI ² ; sore throat ^{2,3} ; rashes ² ; aseptic meningitis ^{2,3} ; conjunctivitis ^{2,3}
Coxsackievirus (A and B)	Faecal – humans ^{1,3}	Faecal-oral/ ingestion of contaminated water ¹ ; inhalation of aerosols ¹	Rashes ¹ ; headaches ¹ ; fever ¹ ; haemorrhagic conjunctivitis ^{1,3} ; heart disease ^{1,3} ; meningitis ^{1,3} ; encephalitis ^{1,3}
Echovirus	Faecal – humans ¹	Faecal-oral/ ingestion of contaminated water ¹ ; inhalation of aerosols ¹	GI ¹
Hepatitis A	Faecal – humans ^{1,2,3}	Faecal-oral/ ingestion of contaminated water ^{1,2} ; consumption of contaminated shellfish ^{1,2}	Fever ^{1,2} ; GI ^{1,2,3} ; rashes ¹
Hepatitis E	Faecal – animals ¹ and humans ^{2,3}	Faecal-oral/ ingestion of contaminated water ^{1,2} ; consumption of contaminated shellfish ^{1,2}	Fever ^{1,2,3} ; GI ^{1,2} ;
Norovirus	Faecal – humans ^{2,3}	Faecal-oral/ ingestion of contaminated water ² ; consumption of contaminated shellfish ²	GI ^{2,3} ; vomiting ^{2,3} ; fever ² ;
Rotavirus	Faecal – humans ^{2,3}	Faecal-oral/ ingestion of contaminated water ²	Diarrhoea ² ; GI ^{2,3}

¹(Pond, 2005); ²(NHMCR, 2011); ³(Moe, 2002)

2.2.2 Faecal indicator microorganisms

As highlighted above, to quantify the risk that waterborne pathogens pose to humans, it is important to not only determine the presence of infectious agents but also their concentration (Hurst, 2002; NHMCR, 2008). However, detection of waterborne pathogens is impractical for number of reasons such as (Moe, 2002; Toranzos et al., 2002; Savichtcheva and Okabe, 2006): large number of different pathogens, low concentration and intermittent presence of pathogens in environmental waters, problems with microbiological procedures and labour-intensive and expensive detection methods. Furthermore, new pathogens are still emerging and for some of the known pathogen detection/quantification methods are yet to be developed. Therefore, it is almost impossible to assay all pathogens for water quality monitoring purposes on a routine basis. As result, faecal indicator organisms (FIOs) are used to indicate potential microbial risk (CWP, 2000). They are more easily detected than pathogens, thus allowing greater monitoring frequency in a timely manner (Yan and Sadowsky, 2007). Properties of an ideal faecal indicator organism are summarized as follows (Edberg et al., 2000; NHMCR, 2011; WHO, 2011):

- non-pathogenic themselves
- universally present in faeces of humans and animals in large numbers
- do not multiply in natural waters
- at least as resistant as pathogens to environmental conditions and treatment processes
- readily detected by simple, inexpensive culture methods

From a review of the literature, it becomes evident that one single indicator that possesses all of these properties does not exist (Horan, 2003a; Savichtcheva and Okabe, 2006). However, indicators are still commonly used, not just for the reasons outlined above, but also because some epidemiology studies have found a direct dose-response relationship between illness rates in recreational water bodies and FIO concentrations (Harrington et al., 1993; Kay et al., 1994; Prüss, 1998; Dorevitch et al., 2012). The following provides a description of some of the conventional and alternative indicators.

Conventional indicators

Most commonly, detection of faecal contamination of water has relied on bacterial indicators. Coliform bacteria have been used as indicators of faecal pollution for decades. They are typically found within intestines of warm-blooded animals and humans and include total coliforms, faecal coliforms and the group *Escherichia coli* (CWP, 2000).

Total coliforms include a wide range of aerobic and facultative anaerobic, Gram-negative, non-spore forming bacilli (WHO, 2011). The coliform group comprises of bacteria that utilize lactose to produce gas and acid, or possess the enzyme β -D-galactosidase (Edberg et al., 2000; Horan, 2003a). In the past

they have been used routinely as indicators of the general bacteriological quality of water but are no longer recommended for this use. They have been marked as a poor parameter for measuring potential faecal contamination because they were found to be able to grow in water and soil environments in the absence of faecal contamination (NHMCR, 2011).

Faecal coliforms are coliforms of exclusively faecal origin and able to grow and ferment lactose at 44°C (Horan, 2003a). They are often known interchangeably as **thermotolerant coliforms** because high temperature is supposed to suppress bacteria of non-faecal origin. However, some non-faecal coliforms may also grow at these higher temperatures (Horan, 2003a). Hence, the more appropriate name is thermotolerant coliforms. Given that they encompass bacteria that are present in the environment as well as in faeces, they are not a guarantee for true assessment of faecal contamination (Paruch and Maehlum, 2012). Public health authorities have traditionally used faecal coliforms extensively for indicating potential risk, and to set water quality standards for drinking water, recreational activities and shellfish consumption (CWP, 2000).

Escherichia coli as a FIO has been seen as far superior to all other coliform bacteria. It satisfies most of the criteria for an ideal indicator organism: most of the strains are non-pathogenic, they are found in faeces in large numbers (10⁹ org per 1g of faeces (Edberg et al., 2000) [97% of the coliforms normally present in intestines of humans (Makepeace et al., 1995; NHMCR, 2004) and 94% in animals (NHMCR, 2004) are *E. coli*], do not multiply *appreciably* in the environment (Edberg et al., 2000), and are readily detectable by simple and inexpensive methods (Edberg et al., 2000). Furthermore, it was found to be the indicator microorganism best correlated with health outcomes in freshwater systems (Prüss, 1998). Therefore, many water quality guidelines and monitoring programs today have implemented *E. coli* for indication of faecal pollution (EPA, 1986; EEC, 2006; Yarra Watch). Edberg et al. (2000) states that at the end of 20th century the *E. coli* was the best single biological indicator for drinking water safety. While *E. coli* is a good indicator of bacterial pathogens, its quality as a viral or protozoan indicator has been questioned (Horan, 2003a). Additionally, *E. coli* has been found in pristine environments (Rivera et al., 1988) and has been shown to grow in soils of tropical regions (Byappanahalli and Fujioka, 1998; Solo-Gabriele et al., 2000).

Additional conventional bacterial indicator microorganisms are **intestinal enterococci**. Enterococci are subgroup of larger group of organisms known as **faecal streptococci** which are facultatively anaerobic, Gram-positive and non-spore-forming cocci and consist of the species *E. faecalis*, *E. faecium*, *E. durans* and *E. hirae* (WHO, 2011). Enterococci are found in high concentrations in excreta of mammals, although they are 10 to 1000-fold less numerous than *E. coli* (Edberg et al., 2000). Most of the enterococcus spp. do not multiply in the environment (WHO, 2011). A particular characteristic of

enterococcus is its resistance to salinity and alkaline pH levels, which makes it a good indicator of faecal pollution of estuarine and ocean waters (Edberg et al., 2000; WHO, 2011). There are a number of simple and cost-efficient cultural methods for detection of enterococci that can be performed routinely (Edberg et al., 2000). All of the above-mentioned properties make enterococci a good faecal indicator organism, together with the many epidemiological links to recreational illnesses (Harrington et al., 1993; Kay et al., 1994; Prüss, 1998; Dorevitch et al., 2012). Accordingly, enterococci are used as an indicator organism in guidelines and standards related to recreational activities in marine environments (EPA, 1986; WHO, 2003; EEC, 2006; NHMCR, 2008).

Alternative indicators

Clostridium perfringens are anaerobic sulphite-reducing spore-forming bacilli (Horan, 2003a; WHO, 2011). Spores of *C. perfringens* are extremely resistant to environmental stressors and persist for a longer time than other indicator bacteria (e.g. *E. coli* or enterococci) and most pathogens (Medema et al., 1997; WHO, 2011). Accordingly, it has been proposed as an indicator of protozoa in treated drinking-water supplies, and Harrington et al. (1993) suggested it as an indicator of illness for recreational marine waters. *C. perfringens* do not multiply in environment (WHO, 2011) but they are widely spread in nature (NHMCR, 2004), possibly due to their enhanced survival and variety of transport pathways. Therefore, it is one of the most conservative indicators of faecal pollution and its presence can indicate a remote pollution event that occurred a long time ago (Fujioka, 2002). Costs related to performing clostridium assays are two to three times higher than for other indicator bacteria because of the enhanced technical skill required, anaerobic incubation conditions, and more difficult controls (Edberg et al., 2000). Therefore, they are generally used when faecal coliforms or faecal enterococci cannot be detected (Horan, 2003a). They have not been adopted as faecal indicator organisms by any regulatory body and their use remains largely in research (WHO, 2003; EEC, 2006).

***Bacteroides* spp.** are anaerobic non-spore-forming bacilli. They are primarily found in the gastrointestinal tract of humans and animals in much higher numbers than *E. coli* (NHMCR, 2011). Because of their anaerobic nature, they do not survive long outside the intestinal environment, hence if detected, represent recent faecal contamination. They can be enumerated using anaerobic culturing methods or specific molecular methods (NHMCR, 2011). *Bacteroides* spp. can be used not only to detect faecal contamination but also to discriminate between the human or animal sources. This makes them suitable for tracking sources of faecal contamination.

Bacteriophages are viruses that use bacteria as hosts for replication. They have been proposed and used as microbial indicators because they behave similarly to human enteric viruses which pose a health risk to water users (Horan, 2003a; NHMCR, 2004). **Somatic coliphages** are bacteriophages that

infect members of the total coliform group (Edberg et al., 2000). They replicate in the intestines of warm-blooded animals, but are also found to replicate in water environments (WHO, 2011). **F- (male) specific RNA (FRNA) coliphages** are unlikely to multiply in environments other than the gastrointestinal tract of warmblooded animals (NHMCR, 2011). Both somatic coliphages and FRNA coliphages are found in sewage, although somatic coliphages are found in higher numbers. However, since FRNA coliphage are unlikely to grow in the environment, they can be used as specific faecal indicators. It is noted that there is no direct correlation between numbers of coliphages and numbers of enteric viruses (NHMCR, 2011). There are standard culturing tests for the detection of coliphages, although testing is more expensive than for bacterial indicators and includes certain limitations (Fujioka, 2002). Furthermore, they have been found less useful for assessing surface waters because their concentrations tend to be low (NHMCR, 2011).

Bacteroides fragilis bacteriophage is a virus that infects Bacteroides bacteria. It is extremely resistant to environmental stressors unlike its bacterial host (WHO, 2011). Furthermore, these bacteriophages are found exclusively in human faeces which make them a host-specific faecal indicator. However, these indicators are found in relatively low numbers in sewage and polluted water environments which makes them hard to detect (WHO, 2011). Hence, they are best used in laboratory investigations and possibly water treatment validation testing.

Which indicator?

The literature review shows a variety of indicator microorganisms that could be used as an indication of waterborne faecal pathogens. Indeed, none of these microorganisms fulfil all of the conditions of an ideal indicator organism. This project aims to model microbial concentrations in a complex estuarine environment and, as with many other projects where a microbial model was developed (Haydon and Deletic, 2006; McCarthy et al., 2011b), a large number of data points (i.e. water samples) are required to adequately test the model. Hence, the methods used in this project for enumerating microbial numbers has to be simple, efficient and cost-effective. Although some alternative indicators have been proposed because of their ability to better represent recent faecal pollution or a specific source of pollution, methods for enumerating these indicators require complex procedures, higher technical skills, large-volume water samples and are more costly (Fujioka, 2002). Therefore, conventional indicators for which there are readily available simple inexpensive methods of enumerating (e.g. Colilert®, (IDEXX Laboratories, 2013)) should be used. However, it should be noted that the culture-based methods do not detect viable but not culturable cells (VBNC cells) and as such may an underestimate of the true microorganism concentration.

Currently most commonly used conventional indicators of microbial water quality are *E. coli* and enterococci. Because of its higher resistance to salinity, enterococci have been proposed as better indicators of faecal contamination than *E. coli* in marine environments. However, it is noted that recent research by Sinigalliano et al. (2010) showed that even when using four different detection methods for enterococci, no specific links could be made between illness levels in recreators of marine waters and enterococci concentrations. Even so, many recreational water quality guidelines for marine environments provide a guideline values for this indicator organism (EPA, 1986; WHO, 2003; NHMCR, 2008). However, some guidelines (e.g. EEC (2006)) give values for both *E. coli* and enterococci even for marine environments. In addition, estuarine environments have highly variable salinity levels, which can range from completely fresh to completely saline waters (Dyer, 1997), hence sometimes being more marine-like and sometimes more freshwater-like. Furthermore, health risks from recreational activities are commonly linked to the top water layer (i.e. where the most of recreational activities are conducted) and in highly stratified estuaries, this layer is often low in salinity and is mainly freshwater.

E. coli is the chosen indicator organism for this study. Further to the above discussions, this indicator was also chosen because of local context; indeed, all monitoring done to date on the Yarra River and its estuary has been done using this organism (e.g. Yarra Watch ()). Quantification of the *E. coli* concentration will be achieved by using the IDEXX Colilert method. This method has previously been applied for enumerating *E. coli* in water and sediments in estuarine setting (Schang et al., 2016b) demonstrating that the method is suitable for the use in this project. Furthermore, IDEXX method is the most widely used method for *E. coli* enumeration and it strongly correlates with other commercially available culture-based method for *E. coli* enumeration TECTA (Schang et al., 2016a), which will enable comparison of the data collected in this study with data from literature.

It is very important to note that this research project is aimed at modelling microbial dynamics in urban estuaries; not to directly estimate or predict risks. As such, it is of secondary importance what particular microorganism is used to help develop and test the model, as the model will account for any inadequacies in any given indicator (i.e. the fact that *E. coli* is more susceptible to salinity than enterococci is irrelevant as the model will account for this die-off). Finally, while the model will be tested and validated on this particular organism, the model's structure will be such that it can be re-calibrated and tested on another organism. Indeed, several modelling studies applied a similar model structure for range of microorganisms, both pathogens and indicator organisms (Dorner et al., 2006; Hipsey et al., 2008; de Brauwere et al., 2014b).

2.3 Conceptual diagram of faecal microorganisms in urban estuaries

Figure 2 - 1 shows a conceptual diagram of faecal microorganisms in an estuarine context. Faecal microorganisms which enter the estuary are derived from a range of sources with differing magnitudes (these inputs are discussed in Section 2.4). Once in the estuary, faecal microorganisms are influenced by the complex estuarine environment (general estuarine characteristics are described in Section 2.5). Indeed, the faecal microorganisms will undergo a number of processes while in this environment, which could lead to their transport, sedimentation, resuspension, death or even growth (these processes are described in Section 2.6). Finally, Section 2.7 presents the requirements of a coupled hydrodynamic-microorganism model which can simulate the fate and transport of faecal microorganisms in urban estuaries. Sections 2.7.3 and 2.7.4 then review existing hydrodynamic and microorganism models in surface waters respectively.

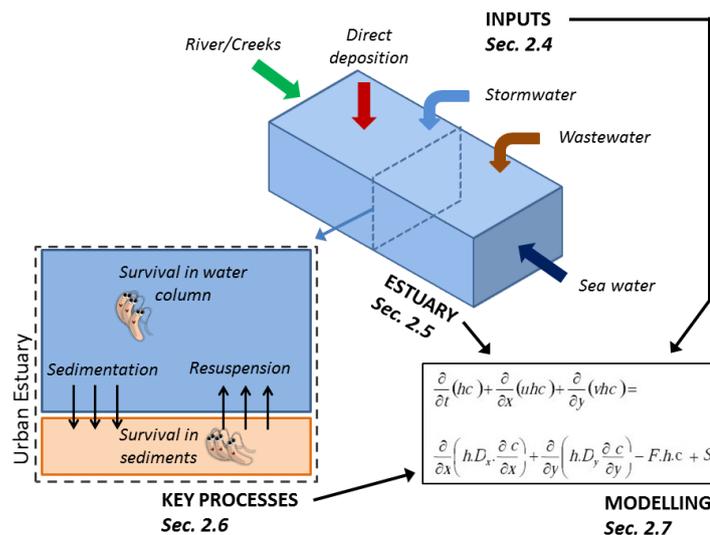


Figure 2 - 1 Microorganisms in urban estuaries

2.4 Inputs of faecal contamination from an estuarine perspective

As concluded in Section 2.2.1, most pathogens are transmitted through the faecal-oral route, meaning that faecal pollution is generally regarded as a major contributor of pathogens to waterways (Yan and Sadowsky, 2007). Therefore, the first step in properly modelling levels of faecal microorganisms in estuarine environments is to determine the potential inputs of faecal contamination and, if possible, to assess the relative importance of each input. Inputs of faecal contamination from an estuarine

perspective are (Figure 2 - 2): rivers and creeks, stormwater, sewage and wastewater, direct deposition by wildlife and discharge from boats (Milliken and Lee, 1990; CWP, 2000).

Rivers and creeks that feed into an estuary can be significant contributors of faecal contamination (Martinez-Manzanares et al., 1992; Daly et al., 2013). The river flow can often carry with it most of the other inputs identified above (e.g. stormwater, sewage, wildlife faecal deposits etc.) in addition to agricultural and stormwater run-off from upper catchment areas, which can contain high number of faecal microorganisms (CWP, 2000). Additionally, if the energy of the flow is high enough it can cause re-suspension of river sediments, which are known reservoirs of faecal microorganisms (Pachepsky and Shelton, 2011), and increase microbial levels even further (Wilkinson et al., 1995; Yakirevich et al., 2013). Daly et al. (2013) demonstrated the significance of upstream river flow as a source of faecal microorganisms in the case of Yarra River estuary. It was shown that loads from river flow feeding in estuary are 1.5 orders of magnitude higher than those from the two largest stormwater drains (>3m in diameter). Consequently, it was estimated that 30 drains of similar size are needed to discharge in estuary so that their total load is comparable to that of river inflow. Rivers and creeks are often considered the most significant continuous inputs of pollution to the estuary environment (especially during dry weather periods, and high *rural* flow periods) and therefore their influence should not be neglected.

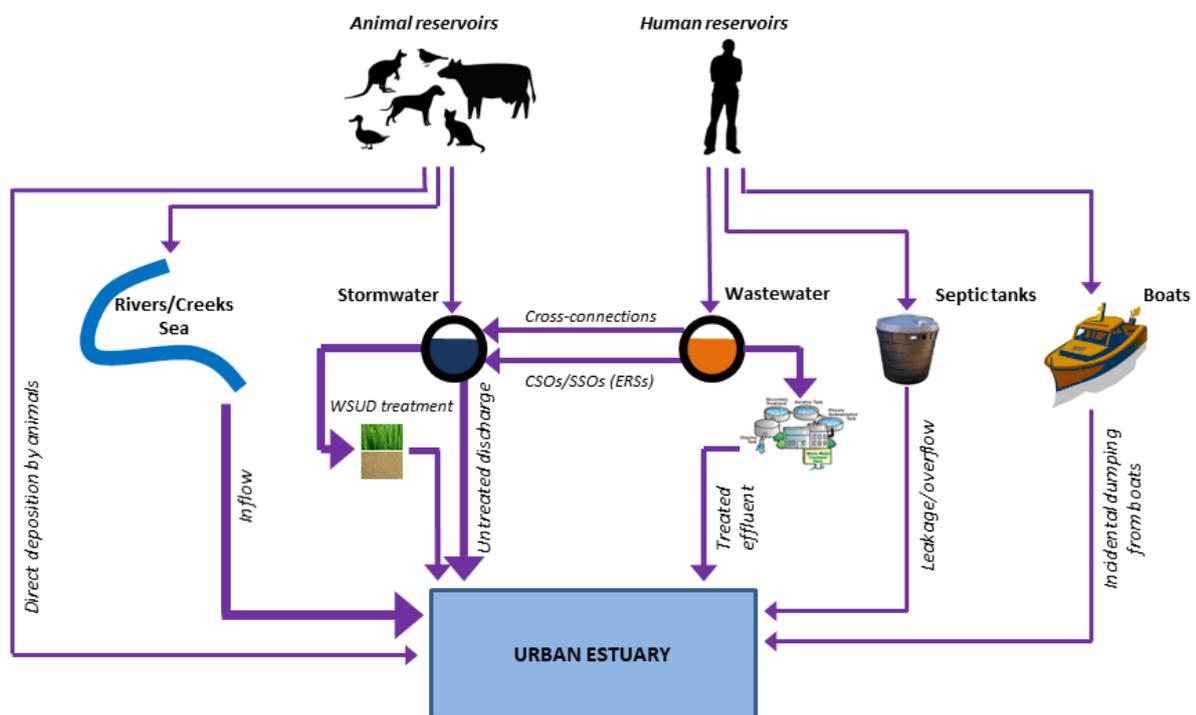


Figure 2 - 2 Inputs of faecal microorganisms from an estuarine perspective. The thickness of the arrows indicates the hypothesised importance of each input from the faecal contamination perspective.

Stormwater has been identified as an important source of faecal contamination for receiving waters (CWP, 2000; Burton and Pitt, 2002; Jeng et al., 2005; McCarthy et al., 2011b; Mallin and McIver, 2012). It can contribute significant loads of faecal microorganisms during wet-weather episodes (Weiskel et al., 1996; Jeng et al., 2005; Daly et al., 2013). Variability of FIOs in stormwater is very large and can even range in orders of magnitude between events at the same sampling location (CWP, 2000; Burton and Pitt, 2002; McCarthy et al., 2008). Significant seasonal variation has also been reported (CWP, 2000). Additionally, dry weather flows are common for stormwater drainage systems. Dry-weather flow includes natural drainage of groundwater but sewage can also be an important component of the dry weather flow (Burton and Pitt, 2002). This flow often has low discharge rates but can be highly contaminated with faecal matter. Its influence on water quality will depend on frequency and quantity of sewage discharge and the flow rate of receiving water body, hence it is highly site-specific (Burton and Pitt, 2002).

Stormwater inputs will particularly be important in urban areas, especially during wet weather periods when large quantities of surface run-off washes deposited faecal material from the catchment into receiving waters (or when wastewater leakage or overflows enter the stormwater pipe). Furthermore, this input is highly intermittent as storm events can finish within couple of hours. On the other hand, dry weather flow is not likely to be driving faecal microorganism concentrations considering the high degree of dilution which can occur in some receiving waterways (this is hypothesised to be true even if wastewater cross connections or leakages occur during dry weather). However, the occurrence of SSOs (i.e. ERSs) during dry weather might significantly impact microbial levels in receiving waterways. The actual effect of the overflow event will depend on the microbial load delivered during the event as well as on the buffering capacity of the waterway. Overall, stormwater influence during dry weather is hypothesised to be important only locally in the area of discharge in systems which carry enough buffering capacity, such as the Yarra River estuary.

Wastewater is a highly concentrated input of pathogens and FIOs and its effect will depend upon dilution effects within the estuary (de Brauwere et al., 2011). Ideally, sanitary drainage network and wastewater treatment provides efficient collection, conveyance and treatment of wastewater. In reality, many wastewater drains are still an episodic or chronic source of faecal microorganisms. Potential inputs of sewage include combined sewer overflows (CSOs), sanitary sewer overflows (SSOs), wastewater treatment plant (WWTP) effluent which still can contain significant numbers of faecal microorganisms, cross connections with stormwater drains, leakages from wastewater drains and failing septic systems (CWP, 2000).

Combined Sewer Overflows (CSOs) and Sanitary Sewer Overflows (SSOs). Commonly, many older cities have drainage systems that transport both wastewater and stormwater together, i.e. combined sewers. During heavy rainfall when the drainage system capacity is exceeded, diluted wastewater is directly discharged into the surface waters without treatment (CWP, 2000). This is known as *combined sewer overflow (CSO)*. CSOs have extremely high bacterial levels and are comparable to raw sewage (Table 2 - 4). More recently, common practice is to separate the stormwater and wastewater drainage networks (as in most cases in Australia). However, even in the case of separate drainage networks, sewage can be introduced into surface waters through *sanitary sewer overflows (SSOs)*, also known as *Emergency Relief Structures (ERSs)*. SSOs are caused by the exceedance of the drainage capacity due to high rates of infiltration and inflow during wet weather periods, blockages (during dry weather periods), or power supply failure at pumping stations (CWP, 2000). Overall, CSOs and SSOs are most likely to occur during heavy rainfall events. Therefore, CSOs and SSOs are considered as intermittent direct inputs of faecal contamination. The impact of CSOs and SSOs on microbial levels might be substantial depending on the quantity of water delivered during overflow episode as compared to the flow in the estuary, and the degree of dilution occurring. However, little comprehensive data are available to quantify SSO frequency and bacteria loads in most catchments (CWP, 2000). Therefore, the overall significance of these types of inputs remains unclear, but it is hypothesised that these inputs might be important both during wet and dry weather because of the high microbial load they can carry. As an example, in the Yarra River estuary, there are nine documented sewer overflows which enter the estuary directly, and hence the impact these inputs have on faecal contamination should be considered in an estuarine microbial model.

Wastewater treatment plants effluent. WWTPs provide treatment of raw sewage in order to remove a range of pollutants before releasing effluent into environment. However, pathogen removal is poor in some wastewater treatment plants (Curtis, 2003). Removal of both pathogens and indicators will greatly depend on the level of treatment. Values reported in the literature indicate that levels of indicator organisms are extremely high even after the secondary treatment level (e.g. 3.3×10^5 cfu/100ml faecal coliforms) (Kay et al., 2008). Therefore, effluent of secondary wastewater treatment plants is considered a significant input of faecal contamination if discharging directly into estuary. Indeed, a number of studies (Salomon and Pommepuy, 1990; Kashefipour et al., 2002; Garcia-Armisen et al., 2006; Bedri et al., 2011; de Brauwere et al., 2014a; Gao et al., 2015) assessed the impact of wastewater treatment plant effluent on water quality of receiving water bodies.

Sewer Cross Connections with Stormwater Drains. Sewage can be introduced to receiving waterway through stormwater drains. Due to the hundreds of kilometres of underground wastewater and stormwater drains found in urban areas, it is not surprising that improper connections are made between drains. However, not all of the misconnected drains contain sewage (i.e. floor drains, sinks etc.). Because of the random nature of cross connections, the influence of improper connections on faecal levels of the receiving water body is very hard to assess. Temporal variability of these inputs is more likely to be related to water consumption over the day, season, rather than weather conditions (i.e. dry/wet weather). Furthermore, this input is not a direct input to the estuary, as it enters the estuary with stormwater. Therefore, it is hypothesised that with proper monitoring and modelling of stormwater inputs, influence of sewage drain cross connections would be implicitly taken into account.

Leakage from wastewater drains can occur due to cracks in pipes or due to pipes simply falling apart because of poor joints or pipe materials (CWP, 2000). Leachate can then infiltrate into the stormwater drain and ultimately discharge in surface water. Sercu et al. (2011) showed that most of the sewage exfiltration contamination happened in areas with aged clay sanitary sewer pipes which were above stormwater drains and cross or run parallel with stormwater drains within 5 m. Unless all conditions were met, sewage infiltration was negligible. Leakage inputs are continuous with time but are hypothesised to contain a medium level of faecal contamination, because they are relatively low in flow rates and a certain degree of filtration will occur between the two drainage systems (Sercu et al., 2011). Furthermore, these inputs are not direct inputs to the estuary, but instead will primarily contribute to other inputs before entering the estuary (e.g. these sources will enter stormwater drains or creeks before they enter the estuary, and hence should be inherently included in these input estimations). As such, for an urban estuary, leakages are not considered a significant input.

Failing septic tanks are suggested as source of faecal contamination by number of studies (Lipp et al., 2001; Pang et al., 2004; Mallin and McIver, 2012). In one coastal area study, high levels of enterococci were correlated with 24h and 48 antecedent rainfall, while there was no correlation with rainfall on the day of sampling (Mallin and McIver, 2012). It was hypothesised that this is due to formation of a hydraulic gradient at the water table, which induced significant lateral movement of water that contained leachate from septic tanks. It must be noted that sampling was conducted in drainage ditches that were very close to the last rows of houses that had this type of wastewater treatment and the soil was highly permeable (sands, cracked limestone) with elevated water table from 0.3-1.2m below the surface. In another study in New Zealand, recommendation for minimum septic tank setback distance from surface water bodies was evaluated based on microbial water quality standards (Pang et al., 2004). A model was developed to simulate fate and transport from leaking septic tanks to the

surface water body. Although there were many assumptions and simplifications, for the worst case scenario (i.e. highest hydraulic conductivity and gradient measured in field, removal rates of faecal indicators determined from experiment, assuming absence of unsaturated zone and continuous discharge of raw sewerage) setback distances of 20 meters for the fulfilment of recreational water quality standards and 50 meters for the fulfilment of drinking water requirements were confirmed. Additionally, Weiskel et al. (1996) compared different sources of faecal coliforms, including septic tank leachate and their relative contributions within Buttermilk Bay, Massachusetts and estimated that septic tanks would contribute only 0.01% of total yearly faecal coliform load to the bay. Even in malfunctioning septic systems, 5 log attenuation was shown in 2 m radius of the septic system (Weiskel et al., 1996). It is clear that contribution of septic systems is dependent on their relative density in area, soil type, level of ground water table and influence of tide in coastal areas. Indeed, septic tanks present inputs but their influence seems rather limited. In addition, they are not direct inputs, i.e. they are being injected into upstream river or an upstream creek system which then enters the estuary. Therefore, for an urbanised estuary septic systems are not considered significant inputs (as there are few septic systems in highly urbanised which would directly enter an urban estuary).

Table 2 - 4 Comparison of faecal bacterial concentrations in different types of water

Faecal Microorganism	Raw sewage [org/100ml]*	Treated sewage** [org/100ml]	CSO [org/100ml]	Failed septic system [org/100ml]	Stormwater [org/100ml]
Total Coliform	3.9×10^7	$5.5 \times 10^3 - 3 \times 10^7$	$10^4 - 10^7$	$10^4 - 10^7$	$7 - 18 \times 10^6$
Faecal Coliform	$10^6 - 10^7$	$1.3 \times 10^3 - 1 \times 10^7$	$10^4 - 10^6$	$10^4 - 10^6$	$0.2 - 1.9 \times 10^6$
Faecal Streptococci	1.2×10^6	N/A	10^5	10^5	$0.3 - 1.4 \times 10^6$
Enterococci	$10^5 - 10^6$	$3 \times 10^2 - 1.3 \times 10^6$	N/A	9.3×10^5	N/A
<i>E. coli</i>	$10^6 - 10^7$	N/A	N/A	1.2×10^6	$12 - 4.7 \times 10^3$
<i>Salmonella</i>	$10^1 - 10^3$	N/A	N/A	N/A	Up to 4.5×10^3
<i>Campylobacter</i>	$10^1 - 10^3$	N/A	N/A	N/A	N/A

(Makepeace et al., 1995; CWP, 2000; Curtis, 2003; Pang et al., 2004; Kay et al., 2008)

* units of microbial concentrations are different for different authors ([MPN/100ml]; [CFU/100ml]; [org/100ml]) but here for consistency are all expressed as [org/100ml].

** depends on treatment level (i.e. primary, secondary or tertiary treatment).

Direct deposition by wildlife. Direct deposition herein means direct excretion of faeces into a water system. Table 2 - 5 gives insight in bacterial densities in warm-blooded animals and daily production of faeces. For example, Weiskel et al. (1996) estimated that 67% of the total load of faecal coliforms to Buttermilk Bay, Massachusetts, was coming from waterfowl compared to 16% through stormwater,

8% through stream flow and less than 3% through resuspension. Additionally, it was shown that the direct deposition is seasonal, depending on the variation of waterfowl populations over the year. However, even though wildlife was contributing a major load of FC to the bay, data suggested dominance of surface water inputs in controlling the FC concentrations in the bay.

It is hypothesised that these inputs are potentially a significant source of faecal contamination in the estuary, especially during dry weather periods, and periods of low riverine flow rates. This hypothesis is demonstrated through the following example, using the Yarra River as a case-study. If we assume that average flow rate entering the estuary from the upper Yarra River is $11\text{m}^3/\text{s}$ (Daly et al., 2013), 410 ducks would need to defecate into the river every day to increase the level of *E. coli* entering the estuary by 100 MPN/100mL (see Table 2-5 for defecation rates; we assume that the defecation rate of faecal coliforms and *E. coli* are similar). During summer months, when inflow rates from the upper Yarra River are much lower (e.g. $4\text{m}^3/\text{s}$ (Beckett et al., 1982), less than 150 ducks are required for the same increase. Daly et al (2013) showed that during dry weather conditions, the Yarra River at Kew contains around 200MPN/100mL of *E. coli*; this could theoretically be caused by 300 ducks per day defecating directly into the system. However, inclusion of this source is difficult from the modelling standpoint due to its random nature. Sufficient data is required to characterise the dynamics of direct deposition (i.e. when and where the deposition occurs and what amount of faeces is discharged into water column). For instance, it is likely that temporal dynamics of defecation rate will change over time and over seasons. Moreover, in most urbanised areas, hot spots (i.e. places with higher population of waterfowl) are likely to form at certain locations along the estuary where waterfowl would search for shelter (e.g. around and under the bridges). As such, this source should be taken into account, provided that a good quality dataset exists to characterise it properly.

Table 2 - 5 Bacterial densities in faeces of warm-blooded animals with daily defecation rates

Origin	Faecal coliform org/1g	Faecal streptococci org/1g	Defecation rate g/day
Human	1.3×10^7	3×10^6	160
Cat	7.9×10^6	2.7×10^7	70
Dog	2.3×10^7	9.8×10^8	145
Rat	1.6×10^5	4.6×10^7	35
Cow	2.3×10^5	1.3×10^7	7000
Duck	3.3×10^7	5.4×10^7	70
Waterfowl	3.3×10^7	N/A	80-160

* Adapted from (CWP, 2000)

Discharge from boats is a potential input of faecal contamination (Milliken and Lee, 1990). Sanitary wastes from boat occupants may be discharged into the surrounding water legally, through inbuilt sanitation devices with some treatment, or illegally, through the discharge of raw sewerage (Milliken and Lee, 1990). Illegal dumping of raw sewage from boats can be important considering the high microbial concentrations in raw sewage (Table 2 - 4) and its impact will depend on the quantity of raw sewage discharged. There have been studies (e.g. (Faust, 1982; Sobsey et al., 2003)) positively correlating increased levels of faecal coliforms with numbers of boats present. However, the influence of boat discharge on faecal microorganism levels is hard to assess as these are coming from different sources simultaneously. Faust (1982) concluded that the relative contribution of faecal matter from boats is seasonal and usually low compared to contribution by run-off. Taking into account that boat traffic is normally restricted in areas where recreational activities are conducted, it is not considered as important input of faecal contamination.

2.4.1 Conclusions

Urban estuaries can receive a variety of inputs of faecal contamination. Some inputs are hypothesized to be more important during wet weather (e.g. stormwater), some during dry weather (e.g. direct deposition) and some to be equally important regardless of weather conditions (e.g. rivers, creeks and sewer overflows). From a temporal perspective, some of the inputs are continuous in time (e.g. river and creeks, WWTP effluent) and some are highly intermittent (e.g. stormwater, CSO, SSO).

In most water systems (e.g. rivers) the focus is placed on inputs entering upstream and along the waterway. However, in tidal estuaries certain microbial loads can enter the estuary downstream and can move upstream with the tide (de Brauwere et al., 2011). Therefore, some representation of loads entering from downstream should be made.

In summary, major inputs of faecal contamination from an estuarine perspective are: river and creeks, stormwater, WWTP effluent, CSOs and SSOs and direct deposition by wildlife. The overall significance of each input has been depicted in Figure 2 - 2 by the size of the lines which were adjusted to their hypothesised importance.

2.5 Estuary as an environment

Once the microorganisms from the aforementioned sources enter an estuary, they will be impacted by a variety of processes. In order to understand the fate and transport of faecal microorganisms in urban estuaries, it is first necessary to understand estuaries as an environment. Estuarine hydrodynamics is major driver of key processes controlling the levels of faecal microorganisms in an estuary. Estuarine hydrodynamics directly controls the physical processes of transport, sedimentation and re-suspension (discussed in Sections 2.6.2, 2.6.4) and indirectly controls the survival of microorganisms through temporal and spatial distribution of environmental factors, such as salinity, temperature, nutrients, etc. (discussed in Sections 2.6.1 and 2.6.3).

2.5.1 What is an estuary?

“An estuary is an inlet of the sea, reaching into the river valley as far as the upper limit of tidal rise, usually being divisible into three sectors: a) a marine or lower estuary, in free connection with the open sea; b) a middle estuary, subject to strong salt and freshwater mixing; c) an upper or fluvial estuary, characterized by fresh water but subject to daily tidal action” (Dyer, 1997).

The degree of mixing which occurs in an estuary is very dependent on the type of estuary system. Indeed, there are three main groups: highly stratified, partially mixed and well-mixed/homogenous estuaries (Dyer, 1997). Highly stratified estuaries include salt-wedge and fjord type estuaries and are commonly microtidal (with tidal range <2m). River flow, having lower density than saline water, will separate itself and flow over the top of the sea water forming a sharp halocline at the fresh-saline water interface (Wolanski, 2007)(Figure 2 - 3). Partially-mixed estuaries are usually mesotidal (tidal range 2-4m) as more energy is required for mixing saline and fresh water. The salinity gradient over depth is much more uniform than in salt wedge estuaries (Wolanski, 2007). Additionally, within partially mixed estuaries there can be considerable variation of salinity structure along the estuary, with highly stratified conditions near the head, where the tidal range diminishes, and well-mixed conditions near the mouth where current velocities are higher. Finally, in well-mixed estuaries, the tidal range is often large relative to water depth and the turbulence produced by velocity shear stress on the bottom is large enough to mix the water column completely and make the estuary vertically homogeneous (Wolanski, 2007). Therefore, these estuaries are usually macrotidal (tidal range >4m).

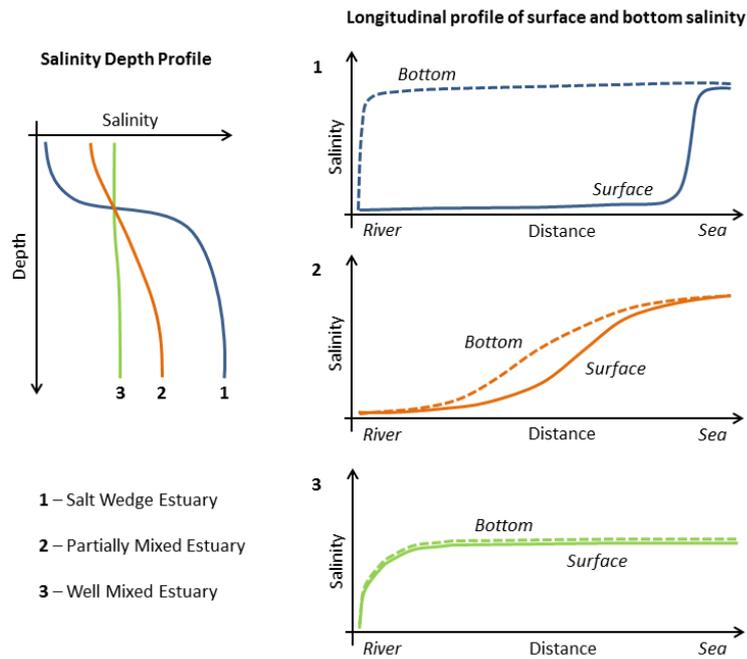


Figure 2 - 3 Depth and longitudinal salinity structure in an estuary: (1) In salt wedge estuaries significant depth stratification exists. The top layer is mostly fresh water while the bottom layer is comprised of sea water, hence the halocline forms at the interface between two layers. Longitudinally, an increase in bottom salinity marks the start of the salt wedge and increase in surface salinity marks the extent until which freshwater influence is noticeable; (2) In partially mixed estuaries the density gradient both vertically and longitudinally is much more uniform and salinity is increasing constantly with the depth and from head towards the mouth of the estuary; (3) In well mixed estuaries, there is no density gradient halocline along the depth, hence the whole water column has uniform salinity. Longitudinally, salinity is increasing from the head towards the mouth of the estuary.

2.5.2 Estuarine hydrodynamics

Hydrodynamics of the estuary represent complex nonlinear interaction of tides, currents, bathymetry, sea and fresh water inputs and sediment transport (Dyer, 1997; Hardisty, 2007). This section briefly introduces these topics.

Tides

Tides are the regular rising and falling of the sea level due to the gravitational attractions of the moon and to a lesser extent of the sun. The crest of the tidal wave is called *high water*, or high tide, and the

minimum water depth with respect to the datum is called *low water*, or low tide (Wolanski, 2007). *Tidal range* is the vertical distance between high water and low water and is not constant over time but will go through cyclic increases and decreases depending on the relative positions of the moon and the sun (Dyer, 1997) (Figure 2 - 4). *Spring tide* is the period of the maximum tidal range and *neap tide* is the period of the minimum tidal range. Generally, spring/neap tides occur fortnightly. The *tidal period* is the time interval between the occurrence of high waters or low waters or any other two corresponding points on a tidal wave. Usually tides are semi-diurnal, i.e. there are two high tides and two low tides each day (Dyer, 1997). The volume of water within the tidal range is defined as the *tidal prism* (Wolanski, 2007) (Figure 2 - 4). For example, in high tidal range estuaries, tidal prisms are large compared with low tide volumes.

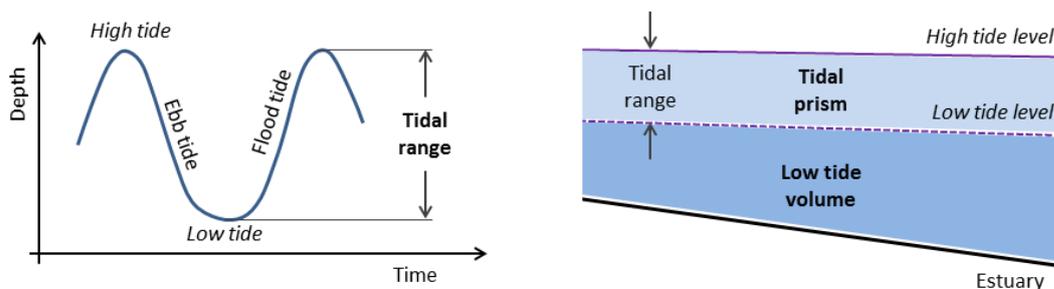


Figure 2 - 4 Tidal Range and Tidal Prism

Currents

Both rising and falling of the water level due to tides and input of fresh water from catchment sources will generate currents within the estuary (Hardisty, 2007). To some extent, locally and for short periods, wind may also become a significant driver for currents within an estuary (Dyer, 1997). With respect to tidal action, water can move towards the estuary, i.e. *flood tide*, or out of the estuary, i.e. *ebb tide* (Hardisty, 2007). Generally maximum flood and ebb tides (i.e. maximum longitudinal velocity) occurs around mid-tide in estuarine environments (Vieira et al., 2000; Hardisty, 2007)(Figure 2 - 5).

The movement of the water, i.e. estuarine currents, will cause mixing of the water column in the estuary. Vertical mixing is carried out by boundary layer turbulence generated by shear at the estuarine bed and banks, internally by turbulence generated by the shear at the halocline, and by turbulence induced by wind on top (Dyer, 1997; Wolanski, 2007)(Figure 2 - 6). Internally, at the halocline, mixing is carried by means of entrainment, turbulent diffusion and internal waves (Dyer, 1997). In most

estuaries, mixing will be a combination of the three and their magnitudes will vary in both space and time. For instance, in the Derwent estuary, Tasmania, mixing in the upper estuary is controlled by entrainment by fresh water and is proportional to discharge, while in the lower estuary, mixing is dominated by a combination of tidally-driven and wind-driven mixing (Davies and Kalish, 1994). The resultant mixing will be reflected in the density structure. On the other side, the presence of the stratification may cause modification of the circulation of water (Dyer, 1997). This is known as density driven circulation (Dyer, 1997). Although, there is only 2% density difference between fresh and sea water, it is sufficient to influence the flow.

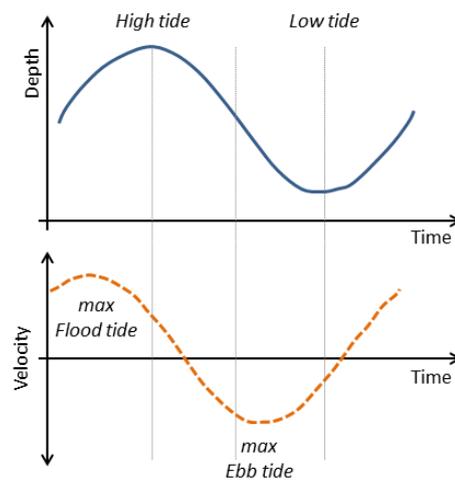


Figure 2 - 5 Typical change of currents over tidal wave

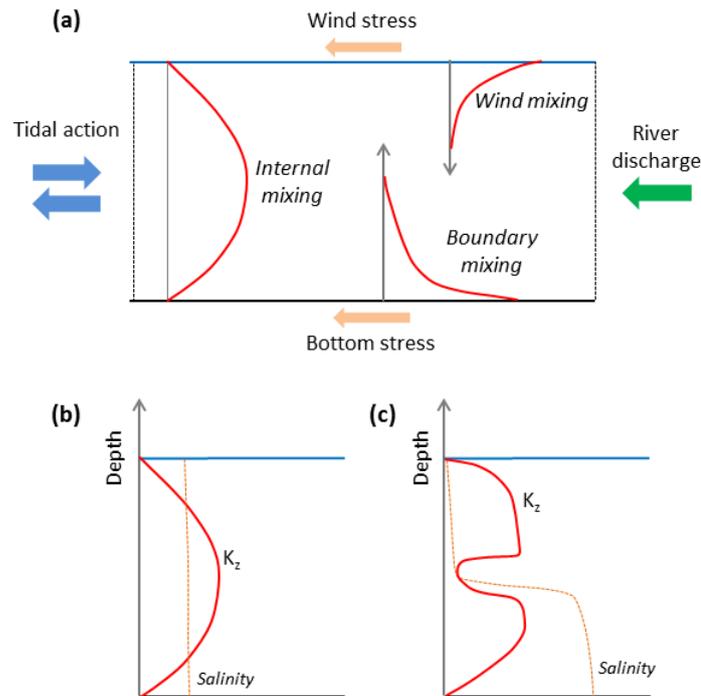


Figure 2 - 6 Vertical mixing in estuary; (a) mixing processes in an estuary; (b) vertical profile of K_z in well mixed estuary; (c) vertical profile of K_z in highly stratified estuary (adopted from Wolanski (2007))

Vertical mixing is parameterised by the vertical eddy diffusion coefficient K_z . In vertically well mixed systems, K_z is at its maximum in mid-waters (Wolanski, 2007)(Figure 2 - 6). In contrast, where significant stratification exists (i.e. salt-wedge estuaries), K_z will be smallest at the density interface, due to the buoyancy effects inhibiting mixing (Wolanski, 2007)(Figure 2 - 6).

In addition, water circulation in estuaries varies markedly across the estuary's width which provides additional mixing (Wolanski, 2007). The flow along the estuary is affected by bends which causes secondary flows within the cross-section in a clockwise sense. These are lateral components of velocity in the plane normal to that of main flow and tend to be few orders of magnitude lower than the longitudinal velocity. Circulation within the cross section will depend on tidal current, flood or ebb tide, magnitude of the river discharge as well as the degree of stratification (Dyer, 1997).

2.5.3 Water quality of estuary

It is commonly known that the microbiological water quality of water systems is influenced by physical/chemical parameters such as temperature, salinity, DO, pH and suspended sediments (Crane and Moore, 1985). This section summarises available literature on how these water quality parameters can vary in estuarine environments, while the subsequent section focuses on how these parameters influence microbes in urban estuaries.

The temperature of estuarine waters varies on daily and seasonal time scales and also spatially depending upon the relative temperatures of the tidal and freshwater inputs (Hardisty, 2007; Vaz and Dias, 2008; Navarro et al., 2011). Generally, fresh water is colder than sea water in winter and conversely sea water is colder than fresh water in summer (Hardisty, 2007; Vaz and Dias, 2008). However, water temperature variations are also closely related with meteorological forcing like air temperature, solar radiation, etc. This will particularly influence the top layer of the estuarine water column, or even whole column if estuary is shallow (Vaz and Dias, 2008).

Salinity variations in estuaries depend on the magnitude of fresh and sea water inputs and the degree of mixing of the two. For example, in Guadalquivir estuary, Spain, Navarro et al. (2011) found that salinity had much higher variability than temperature, which was exhibiting clear seasonal patterns. Furthermore, salinity showed high correlations with heavy discharges of fresh water. The maximums of discharges coincided with decreases in salinity and increases in turbidity. On the other hand, Stephens and Imberger (1996) showed seasonal patterns in salinity and dissolved oxygen (DO) in the Swan River estuary, Australia, which is classified as a salt wedge estuary. Similar seasonal patterns were observed by Davies and Kalish (1994) in a salt wedge/partially mixed estuary of the Derwent River in southern Tasmania. Additionally they found an overall negative correlation between DO and salinity. A study of the Douro estuary, Portugal, also confirmed a decreasing trend in DO with increased salinity (Azevedo et al., 2008).

The reported pH range within estuarine systems was between 6.0 – 10.0, with the vast majority of reported values falling in the near neutral range, i.e. 7.0 – 8.0 (De Mora, 1983; Howland et al., 2000; Ortega et al., 2009; Feely et al., 2010; Pereira et al., 2012). More alkaline conditions were observed in the Tweed estuary, UK, during periods of low river discharge and predominantly in upper parts of the estuary, while in the lower estuary the pH was around 8 (Howland et al., 2000). Alkaline conditions resulted from ground water inputs during low flow conditions. Additionally, seasonal variations of pH have been reported and the main driver of variability was river discharge. Biological factors were also identified, but were of secondary importance.

The majority of the sediments interchanged between fluvial and marine systems is in the form of suspended sediments (Dyer, 1997). The concentration of suspended sediments varies not only due to tidal range and mixing, but also throughout tidal cycles and in response to fresh water inputs. In general, the concentration of suspended solids will increase with increases in shear stresses at the sediment-water interface. On the other hand, particles continually settle under gravity, reducing concentration of suspended solids in the water column and hence overall concentration of the suspended sediments in water will be the net result of the two processes.

2.6 Key factors and processes governing the levels of faecal microorganisms in urban estuaries

As highlighted above, estuaries are complex hydrodynamic systems. Understanding the key processes affecting the levels of faecal microorganisms in estuarine environments is essential for proper modelling of their fate and transport. The following processes are discussed in this section: survival of microorganisms in the water column (Section 2.6.1) and in the sediments (Section 2.6.3), association with sediments and settling (Section 2.6.2), re-suspension and entrainment in the water column (Section 2.6.4.).

2.6.1 Survival of faecal microorganisms in the water column

Survival of microorganisms in aquatic environments has been broadly attributed to a variety of interacting physical, chemical and biological factors. Die-off/survival is most commonly parameterized through a first-order decay function (Chick, 1908):

$$\frac{C}{C_0} = e^{-kt} \quad (2 - 1)$$

where C [org/100mL] – is concentration at time t ; C_0 [org/100mL] – concentration at time t_0 ; k [1/day] – first order die-off rate ; and t [day] – time.

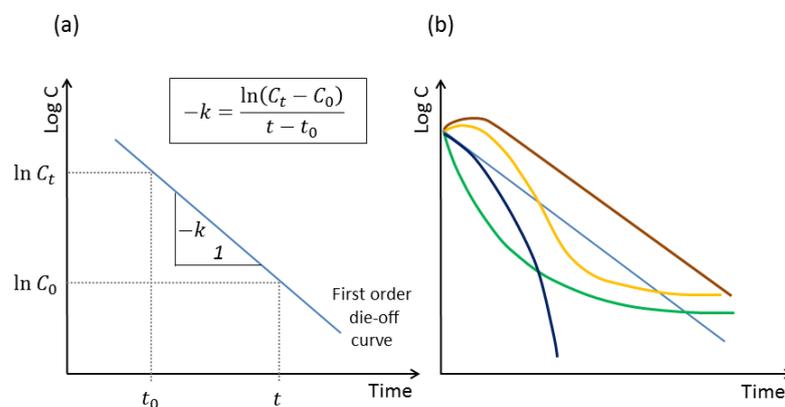


Figure 2 - 7 (a) - determination of die-off rate from observed data; (b) - different observed survival curves (after Crane and Moore (1985))

For a constant die-off rate (k – from Equation (2 - 1) the shape of the survival curve (i.e. concentrations-over-time curve) is linear on a log-transformed concentration graph. Other shapes of the survival curve have been observed in die-off experiments. Hence, modifications to the first-order decay function by changing the form of Equation (2 - 1) and keeping the constant die-off rate, were proposed by various authors as summarised by Crane and Moore (1985). However, later the problem was overcome by modelling die-off rate dynamically as function of a number of environmental factors and the overall die-off rate would simply be the sum of die-off rates due to individual environmental influences at each time step:

$$k(t) = k_T(t) + k_{sal}(t) + k_{pH}(t) + \dots \quad (2 - 2)$$

where $k(t)$ – is the overall die-off rate; k_T – die-off rate due to temperature; k_{pH} – die-off rate due to acidity/alkalinity.

Hence, the form proposed by Chick (1908) remained the most widely used in modelling survival of faecal microorganisms (de Brauwere et al., 2014b) and is therefore primarily used in the following discussions.

Temperature

It has been shown that for many enteric bacteria temperature is inversely related to their survival in aquatic environments (Orlob, 1956; McFeters and Stuart, 1972; Faust et al., 1975; Mancini, 1978; McCambridge and McMeekin, 1980; Flint, 1987; Šolić and Krstulović, 1992; Blaustein et al., 2013) (Figure 2 - 8). Different relationships have been found between water column temperatures and die-off rates (k) but all demonstrated an inverse relationship, i.e. lower survival at higher temperatures. For example, Faust et al. (1975), Šolić and Krstulović (1992) and Barcina et al. (1986) demonstrated that there was an exponential relationship between the survival rate and temperature in sea and fresh water, while Faust et al. (1975) found a linear relationship between temperature and *E. coli* survival rates in an estuarine water system. Finally, Mancini (1978) proposed a power relationship between the two variables (Eq. (2 - 3)); this is the most common form used in literature.

$$k_T = k_* \times \theta^{(T-T_*)} \quad (2 - 3)$$

where k_T [1/day] – is the die-off rate at temperature T [°C]; k_* [1/day] - is the die-off rate at reference temperature T_* (usually 20°C); θ [-] – is the temperature sensitivity parameter with a typical value of 1.07 (Hipsey et al., 2008).

Independent of the type of relationship, temperature is regarded as one of the most important factors for controlling die-off rates of faecal microorganisms in water systems (Crane and Moore, 1985; Blaustein et al., 2013; de Brauwere et al., 2014b). In fact, Faust et al. (1975) showed that temperature was the most influential parameter controlling the survival of *E. coli* in the Rhode River estuary. As such, it is hypothesised that temperature impacts on the survival of faecal bacteria must be incorporated in an estuarine microbial model.

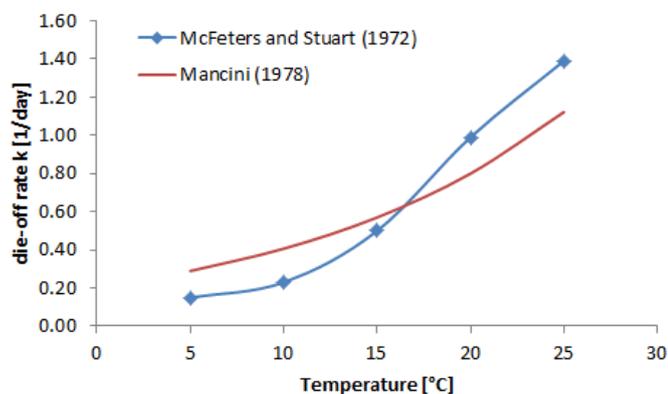


Figure 2 - 8 Relation between FC die-off rate and temperature in a creek as observed by McFeters and Stuart (1972) and regression model proposed by Mancini (1978) (Equation (2 - 3))

Salinity

A number of studies have shown that inactivation of faecal microorganisms is pronounced in salt water (Carlucci and Pramer, 1960; Mancini, 1978; Fujioka et al., 1981; Šolić and Krstulović, 1992). Similarly to temperature, Šolić and Krstulović (1992) reported an inverse relation between salinity and the survival

of faecal coliforms (Figure 2 - 9). There have been only a few authors who have proposed a relationship between the survival of faecal microbes and salinity. Mancini (1978) developed the following relationship between the die-off rate and salinity, based on data reported in literature:

$$k_{\text{sal}} = 0.8 + 0.006 (\% \text{ sea water}) \quad (2 - 4)$$

In salt-wedge estuaries, salinity will impact faecal microorganisms only in the salt-wedge. Hence, the upper fresh water parts of the estuary are hypothesised to be largely free of this impact. Furthermore, in the salt-wedge region, not all microorganisms will be impacted to same extent. The ones found in bottom layer (i.e. within the actual salty layer) will be impacted more than ones in the top layer (i.e. the area comprised of mixed salt and fresh water). It is hypothesised that salinity is an important factor in controlling the survival of faecal microorganisms, especially in environments with marked oscillation in salinity.

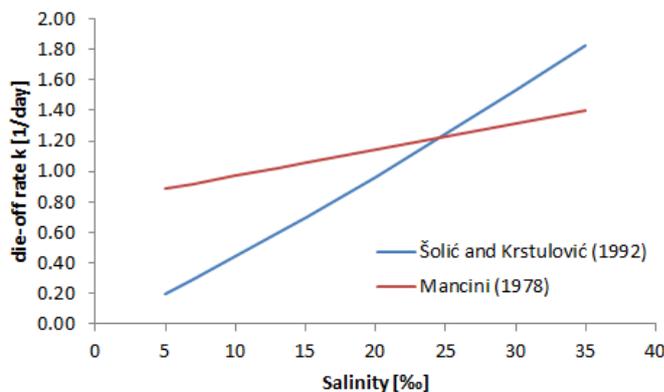


Figure 2 - 9 Regression models of FC die-off due to salinity proposed by Mancini (1978) and Šolić and Krstulović (1992)

Solar radiation

Solar radiation has been shown to have a detrimental effect on faecal microorganisms, particularly enteric bacteria (Fujioka et al., 1981; McCambridge and McMeekin, 1981; Davies and Evison, 1991; Šolić and Krstulović, 1992; Noble et al., 2004). Sunlight can be generally divided into the visible fraction and UV fraction. The latter was found to have a much more significant influence on die-off (Davies and

Evison, 1991), although the visible band should not be neglected (Fujioka et al., 1981). For instance, McCambridge and McMeekin (1981) have shown that survival of *E. coli* and *Salmonella typhimurium* was directly related to the total radiation received by the estuarine water sample.

The light transmission through water is highly dependent on the concentration of suspended matter (or turbidity, as its surrogate). Suspended material in the water column scatters and adsorbs the light causing its attenuation over the depth (Davies-Colley and Smith, 2001). Kay et al. (2005) found that solar radiation impact on survival of enterococci in estuarine and coastal waters was significantly reduced by the level of turbidity. Moreover, it was shown that enterococci decay in experiments with turbidity >200 NTU was similar to decay observed under dark conditions. As such, turbidity should be considered when assessing the impact of solar radiation on the survival of enteric bacteria in estuarine waters.

Interestingly, many authors showed that die-off rates were much higher in sea water than in fresh water when exposed to sunlight, indicating that solar radiation and salinity superimpose on each other; that is, their combined impact is higher than the sum of the individual impacts of solar radiation and salinity (Fujioka et al., 1981; Davies and Evison, 1991; Šolić and Krstulović, 1992). This might be of importance in coastal and estuarine areas.

The above indicates that solar radiation is an important factor in controlling survival of faecal microorganism, and that this could be particularly exacerbated in areas of an estuary which has increased salinity (both because of the interactive effects described above, but also because of the typically lower turbidity of highly saline water/sea water). In areas of high turbidity (e.g. in the freshwater sections of an estuary as identified by Kay et al. (2005)), the impact of sunlight will be minimal. In salt-wedge estuaries with highly turbid freshwater, microbes in the underlying water column might be protected from sunlight impacts.

pH

Enteric bacteria have been shown to rapidly die-off at both high and low pH values (Carlucci and Pramer, 1960; McFeters and Stuart, 1972; Reddy et al., 1981; Crane and Moore, 1985; Šolić and Krstulović, 1992)(Figure 2 - 10). Different authors have found different optimum pH ranges for the survival of faecal coliforms. (Šolić and Krstulović, 1992) found it in pH range 6-7, (McFeters and Stuart, 1972) between pH 5.5 and 7.5 and (Carlucci and Pramer, 1960) at pH 5.

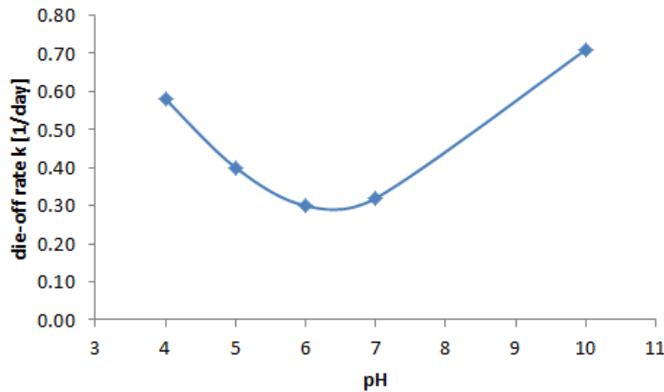


Figure 2 - 10 *E. coli* die-off rates at different pH values in aquatic environments (Reddy et al., 1981)

Based on the synthesis of the data from above studies, Hipsey et al. (2008) proposed a model of pH impact on bacterial survival where pH ranging from 6 – 8 does not have any influence at all. Considering that the reported pH values in estuarine environments fall in range pH 6-10 (most of the values fall in range pH 7-8 (See Section 2.5.3), it most likely that pH will not be the governing factor for *E. coli* survival. As such, inclusion of the effect of pH should be only considered in estuaries where pH is not within the neutral range (i.e. 6-8).

Dissolved oxygen (DO)

Relatively few studies have investigated the impact of DO levels on the survival of enteric bacteria or FIO in surface waters. For instance, Hanes et al. (1964) reported prolonged survival of both coliform and enterococci bacteria at low DO concentrations (0.4 mg/l) and more rapid die-off at higher DO concentrations (7.8 to 38 mg/l) with little differences in survival between the latter two DO concentrations. Similarly, Daly et al. (2013) found a negative correlation between *E. coli* concentrations and DO at some sites along the Yarra and its tributaries. Faust et al. (1975) reported contradictory results; they found strong positive correlations between levels of *E. coli* and DO in the Ronde River estuary. Nonetheless, they finally concluded, based on multiple linear regression analysis, that the effect of DO on survival of *E. coli* was limited. From the current perspective, the role of DO levels on survival of enteric bacteria remains unclear, and could be partially confirmed through modelling work.

Nutrient levels

The presence of excess nutrients in aquatic environments is known to extended survival and even promote growth of faecal microorganisms, primarily bacteria, and practically offset the bactericidal effect of other environmental factors (Orlob, 1956; Crane and Moore, 1985). This was suggested as an

explanation for often observed lag period in die-off curves, as seen in Figure 2 - 7 where there is a period of time before the exponential die-off begins (Crane and Moore, 1985).

Carlucci and Pramer (1960) investigated the effect of nutrients on the survival of *E. coli* in sea water. They did so by adding two different salts containing phosphorus (P) and nitrogen (N) as inorganic nutrients, and organic nutrients in the form of glucose, peptone and domestic sewage. It was shown that both P and N prolonged the survival of the *E. coli*, where the latter appeared to be of greater importance. Glucose (as carbon source) had no influence on the extended survival of *E. coli*, while peptone and sewage were found to favour survival of *E. coli*.

Lim and Flint (1989) investigated the effect of nutrients on the survival of *E. coli* in lake water by adding synthetic sewage. Similarly to Carlucci and Pramer (1960), they found that the addition of sewage prolonged the survival of *E. coli* and increases in survival times were proportional to the amount of sewage added. They further focused on the main groups of nutrients found in sewage, namely phosphorus, carbon and nitrogen. Even though phosphate is an important element in metabolism and cell structure and most fresh water is considered phosphate-limited. It was found that the addition of phosphorus to lake water did not lead to increased survival times. It was not clear whether this was because the inoculated *E. coli* had sufficient endogenous reserves of phosphate or phosphate plays little role in survival of *E. coli* in fresh water. Addition of readily-utilisable carbon did not increase survival times, while the addition of nitrogen greatly increased survival of *E. coli* (Figure 2 - 11). However, Thomas et al. (1999) argued that levels of nitrogen Lim and Flint (1989) used in their experiment greatly exceed levels normally present in surface waters, and that in naturally present concentrations nitrogen would be expected to have little effect on survival.

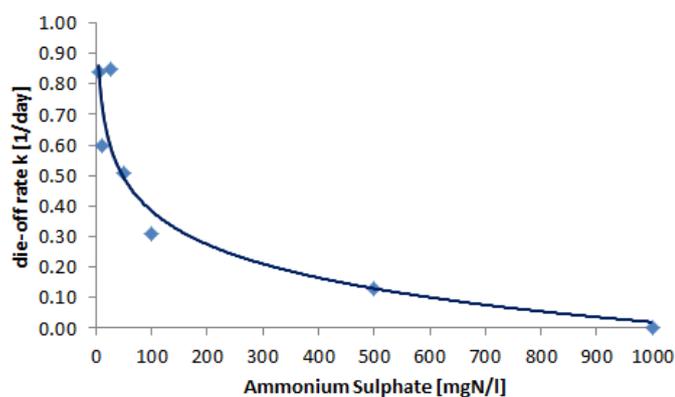


Figure 2 - 11 Influence of nitrogen level on die-off rates of *E. coli* in lake water (Lim and Flint, 1989)

Predation and competition – interactions with indigenous microorganisms

While nutrient levels can directly influence the survival of faecal microbes, they can have an indirect role by promoting other competitors or predators which can then in-turn hinder their survival. It has been suggested that *E. coli* has difficulties competing for nutrients with indigenous microorganisms (Carlucci and Pramer, 1960; Lim and Flint, 1989). For example, Carlucci and Pramer (1960) found that addition of organic matter prolonged the survival of indigenous populations to a greater extent than it did for *E. coli*. Further, Lim and Flint (1989) explained that the reason for limited increase in the survival times of *E. coli* even with the addition of a high carbon source was the presence of, and competition by, indigenous microorganisms. Flint (1987) examined the influence of naturally present microorganisms by filtering river water, hence removing parts of the population; he found that competition for nutrients with indigenous bacteria was a primary factor governing *E. coli* disappearance. Therefore, it is most likely that competition will be important factor in governing survival of *E. coli* in estuarine water.

Predation of enteric microorganisms by the naturally present microbial population is well studied (Orlob, 1956; Rhodes and Kator, 1988; Barcina et al., 1997). McCambridge and McMeekin (1979) investigated protozoan predation of *E. coli* in estuarine water and concluded that the decline in *E. coli* population was primarily associated with the presence and the concentration of protozoa. Additionally, they found that predacious bacteria (as opposed to protozoa) were of secondary importance and their effect was only exerted when protozoan populations were artificially removed. Barcina et al. (1997) summarised many similar studies and confirmed that predation by protozoa is much more significant than predation by bacteria, or infection by viruses. They concluded that predation may be the main factor controlling bacterial populations in aquatic systems.

McCambridge and McMeekin (1980) demonstrated there was an interactive effect between temperature and the importance of predators on the survival of *E. coli* and *Salmonella typhimurium*. Indeed, they found that temperature had a significant influence on these predators, with minimum influences at around 15°C (Figure 2 - 12). Rhodes and Kator (1988) also showed that peak in autochthonous microorganism population was dependent on temperature. Therefore, as with sunlight and salinity, the interactive effects of temperature and predation might need to be accounted for when modelling the survival of faecal microorganisms. Furthermore, the effect of predation will depend on the initial population of both prey and predators (McCambridge and McMeekin, 1979)

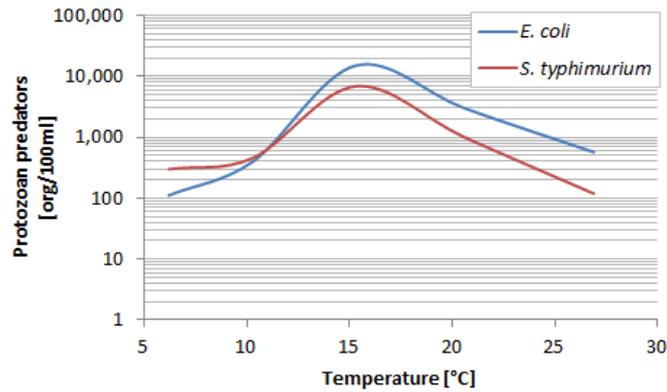


Figure 2 - 12 Effect of temperature on numbers of protozoan predators (two experiments with different prey *E. coli* and *S. typhimurium*)(McCambridge and McMeekin, 1980).

The above indicates that both competition and predation will have an important influence on survival of *E. coli* in urban estuaries. However, it is hard to numerically account for these effects, because indigenous microorganisms are influenced by a number of environmental factors in similar ways as enteric populations. Furthermore, it is likely that studies that measured survival of *E. coli* as function of some abiotic factor (e.g. temperature, salinity, sunlight) in natural conditions, also have implicitly taken into account the effect of biotic factors (i.e. competition and predation) on die-off rates. As such, only one modelling study in literature proposed a functional relationship for die-off rate due to protozoan predation, which was accounting for the effects of the temperature and the concentration of the enteric microorganisms (i.e. concentration of the prey) on activity of protozoan predators (Hipsey et al., 2008). Therefore, the effects of predation and competition on survival of enteric bacteria could be included in a model through a functional relationship with environmental factors (such as temperature). However caution is needed to ensure that predation and competition effects are not accounted for twice.

Conclusion

Survival of faecal microorganisms is affected by various environmental factors, but a review of the literature suggests that in estuarine environments, the following are most likely to be of importance for survival:

- Temperature
- Salinity & Solar radiation
- Competition and Predation

Additionally, the observed combined impact of salinity and solar radiation might be especially important in estuarine areas, due to the marked oscillation in salinity. It is acknowledged that

competition and predation play an important role in the survival of enteric microorganisms in aquatic environments, yet it is hard to determine their impact as they are influenced by the physical/chemical factors. Therefore, this should be further investigated and findings should assist in proper modelling of the survival. It is hypothesised that the impact of environmental factors can be taken into account by developing functional relationships between die-off rate and a particular environmental variable.

2.6.2 Association with particles and sedimentation

Enteric bacteria have been shown to associate with sediment particles, typically fine grained sediments (< 60 μm), i.e. clay and silt (Orlob, 1956; Gannon et al., 1983; Auer and Niehaus, 1993; Pachepsky and Shelton, 2011). The particle association influences transport characteristics of microorganisms, as those associated with denser inorganic particles tend to settle out of water column more quickly. Many studies have investigated the degree of faecal microorganism partitioning to sediment particles and reported partitioning rates in a wide range, from < 20% to 100% (i.e. all organisms attached to sediment particles) (Schillinger and Gannon, 1985; Auer and Niehaus, 1993; Characklis et al., 2005; Jamieson et al., 2005; Fries et al., 2006).

Settling was shown as important in lakes, and impoundments (Gannon et al., 1983). In a slow moving stream Russo et al. (2011) concluded that sedimentation was not significant as modelling results showed that <10% of sediment-associated faecal coliforms settled into bed sediments. However, in salt-wedge estuaries, sedimentation might be an important factor in removing attached bacteria from water column. Kostaschuk and Luternauer (1989) showed that during rising tide, the salt-wedge migrates into the estuary and leads to rapid deposition of suspended material. This was related to the salt-wedge interfering with the flow-bed sediment exchange pattern and reduced turbulence in the upper layer. However, it should be noted that the sediments in their study were predominantly sands and that sedimentation might be less pronounced in estuaries with less coarse sediments (i.e. clay and silt), such as the Yarra River estuary. In fact, sedimentation of attached *E. coli* was measured using the water collected from the Yarra River estuary and it was determined that there was no settling of *E. coli* in the first 24h indicating that the *E. coli* was attached to particles of less than 1.5 μm in diameter (McCarthy et al., 2011a), which agrees well with high percentage of clay particles (less than 2 μm) found in the Yarra River estuary (Ellaway et al., 1982). Furthermore, this was reinforced by the minimal settling within a six to seven day period (McCarthy et al., 2011a). Nevertheless, sedimentation should be included in estuarine hydrodynamic-microorganism model and its significance tested through model sensitivity analyses.

Because of highly variable microbial partitioning to sediments reported, there have been generally two approaches in modelling sediment-bacteria interaction. One assumes that bacteria are “free” phase

(i.e. unattached to sediments, but still settle overtime; (de Brauwere et al., 2011; Yakirevich et al., 2013), while others differentiate between free floating bacteria and those associated with suspended sediments (Jamieson et al., 2005; Hipsey et al., 2008; Gao et al., 2011b; Liu and Huang, 2012; de Brauwere et al., 2014a). Furthermore, the majority of studies that made distinction between free and attached bacteria, accounted only for settling of sediment attached bacteria (Garcia-Armisen et al., 2006; Gao et al., 2011b; Liu and Huang, 2012; de Brauwere et al., 2014a; Liu et al., 2015), as the settling velocities of free bacteria are negligible, due to their small size and density. Nevertheless, whichever approach is used, sedimentation is commonly parameterised as a function of settling velocity, which was calculated assuming Stokes law or derived from settling experiments conducted on a particular water body.

Additionally, attachment to sediment particles provides a certain degree of protection against adverse environmental impacts (Pachepsky and Shelton, 2011; de Brauwere et al., 2014b). While the survival of the microbes attached to sediments is still impacted by all previously mentioned environmental factors (Section 2.6.1), the die-off rate of attached microorganisms is typically taken as a fraction of that of free floating microorganisms (Garcia-Armisen et al., 2006; de Brauwere et al., 2014a).

2.6.3 Survival of faecal microorganism in estuarine bed and bank sediments

Faecal microorganisms, especially those associated with denser inorganic solids, can settle out of the water column into the sediment layer where they can be protected from environmental factors such as UV radiation, high salinity and attack by bacteriophages and be provided with sufficient nutrients found in sediments (Davies et al., 1995). However, differentiation should be made between faecal microorganisms in bed sediments (i.e. sediments that are completely submerged in water) and bank sediments (i.e. those which encounter periodical wetting and drying due to the tidal action), as their survival will be different.

Survival in bed sediments

While the microbes within the bed sediments of water systems are sheltered from external stressors, some factors can still result in net removal of microbes from these environments. As such, researchers have begun to study and understand the influence of these stressors. For example, temperature has been identified as a main factor (Pachepsky and Shelton, 2011) for controlling the survival of *E. coli* and faecal coliforms in bed sediments and this was modelled using the same equation as for the water column (see Equation (2 - 1)). In addition, Anderson et al. (2005) showed that salinity has influence on faecal coliform and enterococci survival in sediments. Calculated decay rate for faecal coliform in salt water sediments was 65 times higher than in fresh water sediment although it was 2.5 times lower

than in the overlying water column showing a degree of protection that sediments provide to faecal microorganisms.

Many studies also observed growth of microbial populations in autoclaved/sterile sediments which was attributed to excessive nutrients found in the sediments and the removal of the other competing and predating microbes (Gerba and McLeod, 1976; Davies et al., 1995; Desmarais et al., 2002). However, in natural conditions, net die-off was confirmed (Davies et al., 1995; Schang et al., 2016b) suggesting that presence of indigenous microflora, particularly protozoan predators, but also competition with other microorganisms, have an influence on the survival in natural conditions.

Survival of faecal microorganism in sediments was also related to sediment texture (Burton et al., 1987; Davies and Bavor, 2000; Desmarais et al., 2002; Pachepsky and Shelton, 2011). Burton et al. (1987) showed that a number of human-associated bacteria exhibited better survival in sediments with higher clay content (>25%) compared to more coarse sediments. This was interpreted by Davies and Bavor (2000) as a result of better protection from predators, which were excluded from small pores containing bacteria due to their large size.

It is clear that bed sediments will provide a certain degree of sheltering of faecal microorganisms from detrimental environmental factors. However, similar factors to that shown in Section 2.6.1 for water column survival were seen to still influence their die-off or growth rates in sediments (albeit at rates slower than that found in the water column). Therefore, this process should be included in microbial water quality models. Most commonly, the survival rate in sediments is taken as a fraction of survival rate of free (or attached) microorganism in the water column (Pachepsky and Shelton, 2011; de Brauwere et al., 2014a; de Brauwere et al., 2014b).

Survival in bank sediments

Far more attention has been given to survival of faecal microorganisms in bed sediments than for survival in tidally influenced bank sediments. Just a few authors investigated levels of faecal microorganisms in banks of tidally influenced waterways. For example, Solo-Gabriele et al. (2000) first indicated that water content plays major role in controlling levels of *E. coli* in bank sediments of a tidal influenced river in subtropical region. Subsequently, Desmarais et al. (2002) further investigated this finding on the same waterway. They measured levels of *E. coli*, enterococci and *C. perfringens* across the river bank and found that numbers of *E. coli* and *C. perfringens* were considerably higher in the first 50 cm where water content was the highest and decreased with distance from the bank confirming that soil moisture is an important factor for the survival of these microorganisms in bank soils. Enterococci were not found to vary along the bank, but were found in low numbers. Additionally,

it was identified that a higher fraction of fine sediment particles and a higher content of organic matter will even promote the growth of *E. coli*. Schang et al. (2016b) studied the presence and survival of *E. coli* and *Campylobacter* in the bank sediments of the Yarra River estuary and showed results consistent with the studies discussed above. *E. coli* and *Campylobacter* were able to survive for extended periods of time in bank sediments and the measured microbial concentrations were positively related with sediment moisture content.

While more research is needed in order to better understand the survival of faecal microorganisms in the banks of tidal estuaries, this process is potentially important in estuaries where large sections of bank and bed (i.e. tidal flats) are exposed to wetting/drying cycles during tidal water level oscillations.

2.6.4 Re-suspension and entrainment in water column

The importance of sediments as an input of faecal contamination is based on the fact that microorganisms associated with sediments can be resuspended by both natural (e.g. currents, tide) and man-made activities (e.g. recreational boating, dredging). This resuspension will ultimately influence the microbial quality of water column. However, in the absence of turbulence and resuspension (i.e. during base flow), sediments contribute very little of the bacterial load to water column (Pachepsky and Shelton, 2011).

A number of studies investigated the significance of microbes in the sediment as an input into the water column. This was done by creating artificial flood events (i.e. releasing significant volumes of water into the stream in the absence of rainfall) to induce the resuspension of bottom sediments without other faecal inputs from the catchment (Wilkinson et al., 1995; Muirhead et al., 2004; Yakirevich et al., 2013). For example, Wilkinson et al. (1995) showed that peak concentrations of faecal coliforms produced by artificial hydrographs during dry-weather are in the order of those observed during natural wet-weather events. Additionally it was also shown that sediment stores can be depleted of microorganisms if hydrodynamic conditions causing resuspension last for sufficiently long periods of time.

With respect to microbial densities in bed sediments, the literature shows that the highest concentrations of faecal coliforms and *E. coli* are found in top few centimetres of the sediment profile (Desmarais et al., 2002), with a significant decrease in concentration with increasing depth (Pachepsky and Shelton, 2011; Schang et al., 2016b). This suggests that potential effects of sediment resuspension on bacterial concentrations in the water column should be estimated only from this top layer. This further explains the observed depletion of the microorganism sediment store if hydrodynamic conditions which promote resuspension last sufficiently long (Wilkinson et al., 1995).

In addition to river forcing, resuspension in estuarine environments can be induced by a variety of mechanisms, such as: tides, salt-wedge movement and boat traffic. Solo-Gabriele et al. (2000) suggested that soils along the bank of a tidally influenced waterway were the primary source of *E. coli* to the water column during dry weather periods (i.e. between storm events). It was suggested that *E. coli* were entrained during the water-soil interface at high tide. However, this was only hypothesised and no further measurements or testing of this hypothesis was conducted. Influence of the salt-wedge movement on resuspension was examined by Kostaschuk and Luternauer (1989). They showed that resuspension begins at ebb tide, as the tip of the salt-wedge moves seaward, which was attributed to increased turbulence in this region which was enhanced with high river discharge. Additionally resuspension may be induced by motor-powered boat traffic. Boat-induced resuspension is heavily dependent on the characteristics of the boat itself, the speed at which the boat is moving, and the intensity of boat traffic (Garrad and Hey, 1987). Garrad and Hey (1987) showed that patterns of suspended sediment concentrations correlated with the frequency of boat movement. However, in areas of recreational activities, motor boat traffic is usually restricted or at least there is a speed limit, and hence influence on sediment resuspension is likely to be limited.

The literature review showed that in-stream sediments can be a significant internal input of faecal microorganisms. They can be a source of microbes if they are capable of growth in these sheltered environments. Considering the impact they can have on the concentration of enteric bacteria in the water column if resuspended, and the variety of ways for sediment resuspension to occur in estuarine environments, this process should be incorporated in an estuarine-microorganism model.

Modelling of resuspension of sediments and attached bacteria due to hydrodynamic forcing is most commonly done as function of shear stress related to the critical shear stress above which resuspension starts to occur (Hipsey et al., 2008; Gao et al., 2011b; de Brauwere et al., 2014a). However, the effects of salt-wedge movement on the resuspension of sediments and associated microorganisms has not been modelled or analysed in any of the microorganism modelling studies in literature.

2.7 Modelling microorganisms in urban estuaries

2.7.1 Modelling objectives for estuarine models of enteric bacteria

The estuarine microorganism model needs to fulfil a number of objectives in order to be useful for both research and water quality management applications. These objectives are related to basic model structure and will help support the choice of the most appropriate model structure.

The model must predict estuarine microorganism concentrations. The main output of the microorganism model will be concentration of the microorganism at current timestep, i.e. number of microorganism per unit volume. Microorganism concentration is important for both recreational and seafood harvesting use as well as other water extraction uses and is inbuilt in microbial water quality legislation. Since human infection from pathogens is directly related to exposure concentrations it is essential that the model is able to predict microorganism concentration accurately.

The model must take into account all important estuarine processes related to microbial dynamics. The model must account for all important processes influencing the microbial dynamics in urban estuaries. These included microbial survival and sediment-microorganism interaction. As discussed above, the complexity of microorganism dynamics in urban estuaries is directly related to processes influencing microorganism concentration. As such, it is necessary that the model accounts for these processes. This will enable model to be useful tool for exploration of microbial dynamics in urban estuaries and design of effective mitigation strategies for water quality management.

The model must use a timestep appropriate for dynamics of urban estuaries and their catchments. The microorganism model is intended to be able to model microbial dynamics at fine temporal scale and not only predict average daily concentrations. Therefore, the maximum timestep used in the model will be limited by the most dynamic input/processes affecting microorganism concentration. For example, urban stormwater inputs are most commonly directly discharged into the receiving water environment and a storm event can start and finish within an hour. As such, the estuarine microorganism model would need to operate on sub-hourly timesteps.

The model's spatial dimensionality must be such that can cover a range of different estuarine systems. In the cases of well-mixed estuaries a one-dimensional model may be sufficient for addressing spatial characteristic of the system. However, in highly-stratified estuaries two-dimensional or three-dimensional models are required to address the spatial extent of the modelled system. Therefore, the developed microorganism model should have ability to be able account for different spatial dimensionality.

The model must be able to be applied to a range of urban estuaries. The model structure needs to be flexible in allowing the model to be applied to a range of different estuaries with different dominant faecal pollution inputs.

2.7.2 Modelling requirements for estuarine models of enteric bacteria

Previous sections of the literature review have identified many requirements for the urban estuary hydrodynamic-microorganism model. This section focuses on summarizing these requirements in concise manner, and reviewing the available hydrodynamic and estuarine microorganism models based on the identified requirements.

Appropriate representation and modelling of inputs into the estuary. As highlighted in Section 2.4, there are a number of significant inputs of faecal contamination which enter urban estuaries. As such, it is essential that the following inputs are either modelled accurately or represented well by data sources (i.e. it is required to have a continuous time series of microbial concentrations entering the estuary that will form boundary conditions for the microorganism model). The following inputs were hypothesised to be the governing sources (see Section 2.4): *Rivers and creeks, stormwater, WWTP effluent, CSO/SSO and direct deposition by wildlife.*

Appropriate modelling of key processes governing the level of microorganisms in urban estuaries. The key processes identified in Section 2.6 needs to be well represented in an urban estuarine microorganism model. Specifically, the following requirements have been identified:

- Survival of faecal microbes in the water column and sediments is affected by a number of environmental factors (see Sections 2.6.1 and 2.6.3). Furthermore, it was shown that certain environmental factors act synergistically in controlling survival of faecal microorganism (e.g. salinity & sunlight) or that the impact of certain factors depends on another (predation-temperature, irradiance-turbidity). As such, an urban estuarine model should include some representation of the impact these factors (including their interrelations) have on faecal microbes. It is hypothesised that models which employ a simple, constant decay rate will not yield adequate results, and instead, this decay rate should be modelled dynamically as a function of the key environmental factors (see Section 2.6.1), which may vary during each month, season or year.

- Microorganism-sediment interactions (i.e. settling/resuspension) have been shown to significantly influence the concentrations of faecal microorganism in the water column and hence should be a crucial element of the model.
- The overall movement of the estuarine water will significantly affect the spatial distribution and transport of the microorganism throughout the estuary. This is particularly important in estuaries compared to other systems (rivers, creeks, stormwater), as the dominant flow direction can change a few times during the day, depending on the tide. Therefore, an estuarine microorganism model must include appropriate transport processes. Furthermore, at the downstream boundary, a part of the water that exits with ebb tide can come back with the next flood tide and bring back a certain load of microorganisms; this can therefore influence the quality of the water column. As such, this should also be addressed in the microorganism model.

Temporal resolution (i.e. time step). Considering the significant variability of microorganisms with time (as highlighted in Section 2.4), it is essential that a microorganism model of any water system uses a time step which is small enough to account for this variability. The temporal resolution requirements of an estuarine microorganism model will depend on the timescale of various hydrological factors, such as: river discharges and tides, various inputs of faecal microorganisms and the temporal variability of environmental factors (de Brauwere et al., 2011). For instance, tides are mostly semi-diurnal (see Section 2.5.2) which makes highly unsteady conditions in the estuary and changes the flow direction four times a day. Some inputs are highly intermittent; for example, urban stormwater rainfall/runoff events can last for as little as a couple of hours (Burton and Pitt, 2002) and still have highly variable microorganism levels during this period (McCarthy et al., 2011b). Similarly, sewage overflows and their duration of discharge into the estuarine environment may be less than an hour. The variability of environmental factors (such as temperature, solar radiation, salinity) is also significant over the day. To satisfy all of the above requirements, the minimum time step required for modelling microbial dynamics in estuarine systems will be in the order of minutes. Additionally, the model should be able to perform simulations continuously in time, meaning that it needs to be able to reproduce both dry-weather concentrations (base conditions) and wet weather concentrations (during rain events). It is essential that wet weather periods are simulated as many authors have shown positive relationships between wet weather events and bacterial loads and peaks in concentrations (hence wet weather periods may pose the most significant risks). Dry weather periods are also important; firstly because most recreational activities occur during dry weather and secondly that in an estuarine environment, it is hypothesised that the wet weather loads can remain in the estuary for a significant duration (i.e. sloshing can occur).

Spatial dimensionality. In well-mixed conditions, which are found in most of the rivers, streams and stormwater drains, microorganism concentrations will vary most significantly along the waterway, and hence the modelling problem can often be reduced to only one dimension, i.e. a 1D model (as the cross-sectional variation is minimal). However, in estuarine environments there is a significant degree of vertical stratification, and this is particularly exacerbated in salt-wedge estuaries. Stratification will influence the vertical distribution of environmental factors which are known to impact the survival of microorganisms. It also has effect on sedimentation, resuspension and mixing within the estuary (see Sections 2.5 and 2.6). Finally, it also represents very different sources of water, meaning that the pollution levels will vary with this stratification (e.g. freshwater from upstream catchments will have a different level and type of microbial population as compared to the seawater which may enter at the bottom of a salt-wedge estuary). These results clearly demonstrate that a depth averaged model is not appropriate for modelling microorganisms in urban estuaries. Indeed, Bedri et al. (2011) showed the inadequacy of a depth averaged model in the presence of high stratification. Furthermore, lateral distribution of microbial concentrations (along the cross section) can be influenced by inputs discharging along the estuary (e.g. stormwater drains discharge) or, if the estuary is wide enough, by the formation of preferential flow paths due to the Coriolis effect (Dyer, 1997). Indeed, for accurate spatial representation of faecal microorganism concentrations in urban estuaries, a microorganism model should be three dimensional (3D).

Data availability and model complexity. Process-based models are the most complex type of models and are built on a deep understanding of the modelled process. Figure 2 - 13 shows that increasing complexity of the model has to be followed by an increase in data availability in order for the model performance to be improved or even maintained. Therefore, the development of a process-based estuarine microorganism model has to be done simultaneously with the collection of sufficient amounts of data which will help not only in the process understanding and the development of the model, but also in proper calibration of the model parameters and validation. Datasets should cover as many different conditions of the studied system as possible, i.e. wet/dry weather, seasonal variation in meteorological conditions, high and low magnitudes of inputs into the system, etc. For example, if the dataset is consisted of mostly dry weather data, calibration of the model which is based on rainfall/flow processes will be very poor. Furthermore, the temporal resolution of datasets have to be able to capture the variable dynamics of the system, i.e. daily/weekly measurements of the microbial levels in the system are not sufficient to allow understanding of processes and proper calibration/validation of the model. Similarly, spatial cover of the dataset should allow an understanding of the variability of microorganisms across the system and has to be adequate for the adopted dimensionality of the model (i.e. if model is three-dimensional, the dataset needs to contain

information on all necessary environmental variables and microbial concentrations in all three dimensions – along the estuary, along the cross section and along the depth). However, the creation of good quality microbial datasets is costly and time consuming, thus model complexity should always be kept to a minimum (i.e. the model should only be as complex as the data and knowledge of processes allows). In order to understand which processes are important, appropriate sensitivity testing of the model parameters could be conducted. This lends itself back to data availability and the need for a rich dataset that would encompass microbial dynamics in estuarine systems in various possible conditions. For example, it could be wrongly concluded that solar radiation is not important in governing survival of microorganisms, if sensitivity testing of the model is done on a dataset collected during winter, when the sky is cloudy.

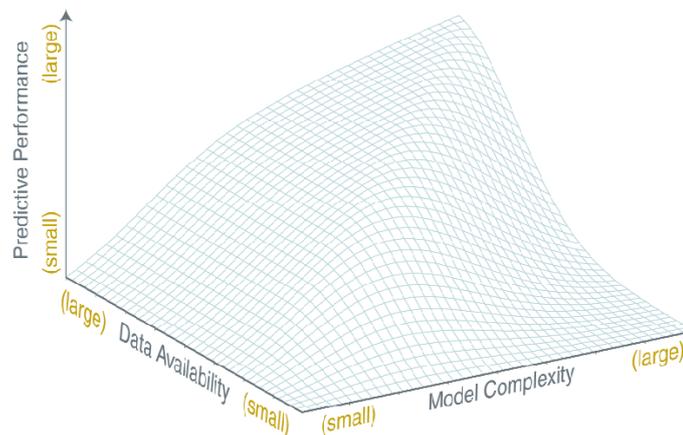


Figure 2 - 13 Conceptual relationship between model complexity, data availability and predictive performance of the model (after Grayson and Blöschl (2001))

2.7.3 Hydrodynamic models to support the microorganism model

Hydrodynamics within the estuary is the main driver of microbial transport, mixing, sedimentation and resuspension. Additionally, the hydrodynamics will have a significant impact on the spatial and temporal distribution of environmental factors, which influence the survival of microorganisms within the estuary. Therefore, the hydrodynamic model needs to accurately predict velocity fields and appropriately represent mixing within the estuary. This is very important for highly stratified estuaries where significant density gradients exist. The hydrodynamic model also needs to account for the many different inputs into the estuary and account for forcing factors such as tides and wind. Furthermore, if the hydrodynamic model is not able to simulate sediment transport processes, it would need to provide appropriate outputs (i.e. velocity/shear stress values especially at the sediment-water interface) which could be easily coupled with a sediment transport model. Additionally, the

hydrodynamic model will need to be coupled to a bio-geochemical model which can predict the distribution of the key environmental factors (such as temperature, salinity, pH, DO or even nutrients). Finally, the hydrodynamic model will need to have the same dimensionality as the microorganism model (i.e. 3D hydrodynamic model is required), with a similar or smaller time step (i.e. in order of minutes). Furthermore, high temporal and spatial resolution will increase computational requirements and consequently increase time of simulation. Therefore, the hydrodynamic model needs to be time-efficient (e.g. developed for parallelized computing).

There are a number of three-dimensional hydrodynamic models that are developed/evolving which can simulate estuarine and coastal hydrodynamics, such as: ELCOM (Hodges and Dallimore, 2006), TELEMAC-3D (EDF R&D, 2013), MIKE 3 (DHI, 2013), TUFLOW FV (BMT WBM, 2014).

ELCOM (Estuary and Lake Computer Model)(Hodges and Dallimore, 2006) solves the unsteady, viscous Navier-Stokes equations for incompressible flow using the hydrostatic assumption for pressure. This model can simulate transport of salt, heat and passive scalars and processes such as rotational effects, tidal forcing, wind stresses and surface thermal forcing. It uses a rectangular grid, but allows variable spacing along the x and y axes. However, rectangular grids do not provide much flexibility in adjusting to the modelled area. Additionally computational time might increase because of an unnecessarily high number of cells. Furthermore, the model is not developed for parallelization on multiple cores, hence with high spatial and temporal resolution, time efficiency might be very poor. Vertical discretisation is possible only with a z-level coordinate system which allows variable thickness of the layers. This model does not simulate sediment transport or water quality, hence coupling with external models is required. However, ELCOM has been previously coupled with a microorganism model (Hipsey et al., 2008). The microbial model was incorporated into the water quality model CAEDYM (Hipsey et al., 2005), which also provided necessary environmental information, such as sediment transport. This coupling was only tested in a freshwater lake.

TELEMAC-3D (EDF R&D, 2013) is a modelling software developed by the LNHE (Laboratoire National d'Hydraulique et Environnement). It solves the Navier-Stokes equations for 3D free surface flow and transport-diffusion for salinity and temperature. TELEMAC-3D can take into account influence of temperature and salinity on density, Coriolis effect, influence of air pressure and wind and consideration of thermal energy exchange with the atmosphere. Spatial discretisation is done through a flexible mesh that is comprised of triangles in horizontal plane. This gives more flexibility compared to structured grids and can reduce computational time. Vertical discretisation is done with sigma-coordinate transformation. Furthermore, this model can run in parallelised mode on multi-thread machines, which can further reduce time of simulation. TELEMAC-3D offers several approaches of

different complexity for modelling vertical and horizontal turbulence. There is a readily available sediment transport module but no water quality module. Hence, the use of TELEMAC-3D might not be straight forward in producing the necessary outputs to feed into an urban estuarine microorganisms model. Telemac was used in the past for providing a hydrodynamic basis for a microorganism model in Dublin Bay and it was coupled with an external water quality model (Bedri et al., 2011).

MIKE 3 (DHI, 2013) is a hydrodynamic modelling software developed by the Danish Hydraulic Institute. It solves the Navier-Stokes equations both with and without the hydrostatic pressure assumption using a finite volume approach. It can model salinity and temperature, and take into account their effect on water density. It uses an unstructured mesh, which provides an optimal degree of flexibility in the representation of complex geometries. Free-surface is taken into account using a sigma-coordinate transformation approach or using a combination of z-level and sigma coordinate systems. MIKE 3 takes into account a range of boundary conditions such as water levels, discharges, wind speed and direction, and tides. Within the MIKE modelling framework there are readily available modules for simulation of sand and mud transport as well as modelling water quality.

TUFLOW-FV (BMT WBM, 2014) is a 3D hydrodynamic model which solves the conservative integral form of the non-linear shallow water equations (NLSWE) using the finite volume solution method. The model also simulates advection-dispersion, including heat balance and density coupling of temperature, salinity and sediment concentrations. It uses a flexible mesh for discretisation of the spatial domain which consists of triangular and quadrilateral elements of different sizes, thus spatial discretisation is adjustable to the areas of interest (i.e. finer around the area of interest and coarser elsewhere), which can improve simulation time. For the vertical discretisation of the spatial domain, three options are available: z-level, sigma-transformation coordinate system or a combination of the two; this provides much more flexibility than that of the other models above. Additionally, TUFLOW-FV provides by far the most options in terms of modelling turbulent mixing, including an external turbulence mixing model. Sediment transport and WQ modules are readily available for coupling with the main hydrodynamic module, hence the use of this model in providing necessary information is straightforward. The TUFLOW-FV hydrodynamic module is the only model (out of the ones above) that the author has seen successfully applied to salt-wedge estuaries (Bruce et al., 2014).

Conclusions. The models listed above use some form of the Navier-Stokes equations for simulating 3D free surface flow hydrodynamics, although the solution method varies between the models. Furthermore, they all can model salinity and heat transport and take into account the effects of temperature and salinity on water density. They all offer a range of boundary conditions such as discharge, water levels, tides, wind and take into account the effect of the Coriolis force and barometric

pressure gradients. However, in terms of spatial discretisation, TELEMAC-3D, MIKE 3 and TUFLOW-FV offer more flexible discretisation of the modelling domain which can help in producing more usable results and improve efficiency of the model. Moreover, TUFLOW-FV offers the most options in terms of vertical discretisation, which can be of importance in salt-wedge estuaries. Turbulent mixing is of great importance in highly stratified conditions where TUFLOW-FV offers a range of turbulent mixing models (even the option of using an external mixing model).

Simulation of sediment transport and environmental conditions can only be done through coupling of a hydrodynamic model with appropriate sediment transport and biogeochemical modules. In this regard, only MIKE 3 and TUFLOW-FV have readily available sediment and water quality modules as part of their modelling framework. Hence use of these models can be straightforward in providing necessary information for a microorganism model.

All of the above indicates that TUFLOW-FV seems to be the most appropriate for this research project. Furthermore, there are local experts from the TUFLOW-FV development team who can assist with its use. Moreover, there is already a preliminary hydrodynamic model developed for the Yarra River estuary using TUFLOW-FV (Bruce et al., 2014) and hence it can be easily adapted to this research project.

2.7.4 Review of available estuarine microorganism models

Empirical microorganism models (also known as regression-based models or black box models; Figure 2 - 14) were not considered in this literature review, although many such models exist. These models are not necessarily based on causal (or mechanistic) relationships between variables, but are instead based on correlations between response variables (i.e. microbial concentration) and explanatory variables (e.g. temperature, salinity, flow velocity, wind etc.). As such, they cannot be used for an in-depth understanding of faecal microorganism dynamics, to scientifically inform mitigation strategies nor to assess long-term management practices. Therefore, these models were often developed with the aim to be used for real-time prediction (nowcasts) of recreational water quality. For more information on empirical microorganism models and a review of existing ones see the comprehensive literature review by de Brauwere et al. (2014b).

This literature review focuses on process-based models of microbial dynamics in urban estuaries. Nine such models were identified in the literature. Additionally, one generic process-based microorganism model, intended to be applicable to all water bodies, has been reviewed as well. Interestingly, there has not been an attempt to model microorganisms in urban estuaries using a simpler approach, such as a conceptual model. This could be linked to the assumption that, since the hydrodynamics of

estuarine systems are highly complex, physically based models are required for accurate microbial predictions.

Table 2 - 6 shows a brief overview of the reviewed models and whether each of them met the modelling requirements outlined in Section 2.7.2. The following paragraphs provide details of each model, and further highlight their limitations and benefits.

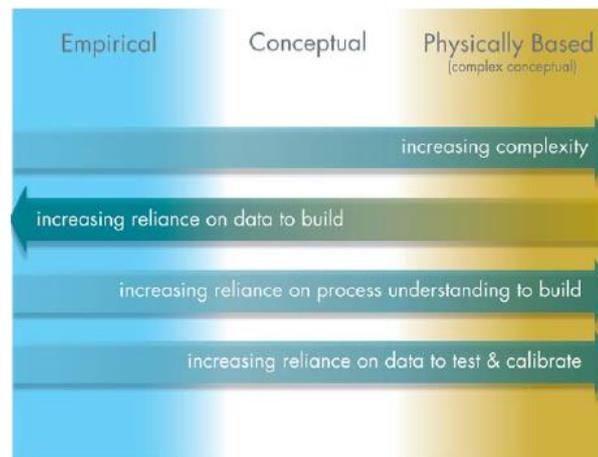


Figure 2 - 14 Some features of different types of models (adapted from CRC for Catchment Hydrology (2013))

Table 2 - 6. The six models which have been developed and tested for estuaries, and whether they meet the requirements outlined in Section 2.7.2

ESTUARINE MICROORGANISM MODELS					
REQUIREMENTS	<i>Salomon and Pommepuy (1990)</i>	<i>Kashefipour et al. (2002)</i>	<i>Garcia-Armisen et al. (2006)</i>	<i>de Brauwere et al. (2011)</i>	<i>Gao et al. (2011b)</i>
Type of estuary	Well-mixed	Partially-mixed	Not known	Well-mixed	Well-mixed
Hydrodynamics	2D/1D coupled model	2D/1D coupled model	3D	2D/1D coupled model	2D/1D coupled model
Inputs	WWTP effluent	Rivers/creeks, WWTP effluent, CSO	Rivers, WWTP effluents	River, WWTP effluent	River, WWTP
<i>Were inputs measured or modelled?</i>	Measured	Measured	Modelled	Measured	Unknown
Survival in water column	Constant die-off rate based on in-situ survival studies	Pseudo-dynamic; different die-off rates for day/night, dry/wet weather, coastal river water	Constant survival rates – different for free and attached bacteria	Temperature dependent survival rate	Turbidity and solar radiation dependent survival rate in water
Survival in sediments	NM	NM	NM	NM	Modelled, but unclear how
Settling	NM	NM	Only attached fraction of bacteria – const. sett. vel.	All bacteria – constant settling velocity	Only attached fraction of bacteria – const. sett. vel.
Resuspension	NM	NM	NM	NM	Only attached fraction of bacteria
Data availability	8 sampling longitudinal profiles with 10 data points	6 sets of one day observations	Not clear. Data collected by authors + external data	Monthly samples and two one day cruises along the estuary.	Poor, only 15 points presented
Sensitivity testing	NC	NC	NC	Conducted	Conducted
Efficiency (how well the model worked?)	NME	Event 1 APE=25.5% Event 2 APE<40%	NME	NME	NME

* NM – not modelled; NC – not conducted; NME – no measure of efficiency only visual assessment (i.e. graphs); APE – average percentage error

Table 2 - 6 (Continued)

REQUIREMENTS	ESTUARINE MICROORGANISM MODELS			GENERIC MODEL	
	<i>Liu and Huang (2012)</i>	<i>de Brauwere et al. (2014a)</i>	<i>Gao et al. (2015)</i>	<i>Liu et al. (2015)</i>	<i>Hipsey et al. (2008)</i>
Type of estuary	Well-mixed	Well-mixed	Not know	Well-mixed	N/A
Hydrodynamics	2D (laterally averaged)	2D/1D coupled model	2D/1D coupled model	3D	3D
Inputs	River, downstream boundary input	River,- WWTP effluent	River, WWTP, CSOs	River, downstream boundary input	N/A
<i>Were inputs measured or modelled?</i>	Modelled/Measured	Modelled/Measured	Measured (Rivers)	Measured	
Survival in water column	Temperature dependent survival rate	Temperature dependent survival rate - different for free and attached bacteria	Temperature, salinity and light dependent survival rate	Temperature dependent survival rate	Dynamic survival rates – temperature, salinity, pH, sunlight and predation
Survival in sediments	NM	Temperature dependent survival rate, albeit set to zero	Unknown	NM	Dynamic survival – temperature, salinity, pH and predation
Settling	Only attached fraction of bacteria – const. sett. vel.	Only attached fraction of bacteria – Stokes sett. vel.	Unknown	Only attached fraction of bacteria – const. sett. vel.	Both free and attached fraction
Resuspension	NM	Only attached fraction of bacteria	Unknown	NM	Both free and attached fraction
Data availability	Monthly samples	Monthly samples at several points along the estuary.	Not clear – around 30 data points presented	Poor, two days of monitoring	Poor – few days of observations with low temporal resolution
Sensitivity testing	Conducted	Conducted	Conducted	Conducted	N/A
Efficiency (how well the model worked?)	NME	NME	Root Mean Square Error (RMSE), Relative RMSE and Mean Absolute Error	Root Mean Square Error (RMSE), Relative RMSE and Mean Absolute Error	N/A

* NM – not modelled; NC – not conducted; NME – no measure of efficiency only visual assessment (i.e. graphs); APE – average percentage error

Salomon and Pommepuy (1990) proposed one of the first estuarine models of faecal pollution. They developed a coupled 2D/1D model of bacterial contamination of the Morlaix estuary, France. The upper part of the estuary was modelled using a 1D model and the lower part using 2D depth averaged model. Three dimensional hydrodynamic model was discarded due to complexity and financial costs. Yet it was shown that the hydrodynamic model had problems with reproducing salinity particularly in the upper estuary which was clearly stratified and where the 1D model was applied. Therefore, the application of this model in salt-wedge estuaries is inappropriate. WWTP effluent was the only input taken into account, and modelling of other inputs was not attempted. The microbial model was comprised of an advection-dispersion equation and a simple first order decay function, where survival rate was a constant which, as explained in Section 2.7.2, is not suitable for microorganism prediction in urban estuaries. Furthermore, modelling of any of the processes related to bacteria-sediment interaction was not conducted (i.e. association with particles, sedimentation and resuspension were not represented by the model). These processes should not be omitted from the model, considering their importance (see Sections 2.6.2 and 2.6.4). Data availability was poor and consisted of just 8 daily measurements at 10 surface points along the estuary which was not enough for the development of an appropriate process-based microorganism model or for appropriate calibration and validation. Furthermore, no sensitivity testing was conducted at all, meaning that it is not known which parameters (i.e. processes they represent) are important. Furthermore, size of the dataset used is not appropriate for proper sensitivity testing of the model. Overall, this model does not satisfy any of the requirements identified above.

Kashefipour et al. (2002) conducted a modelling study of faecal coliforms in the Ribble estuary, Great Britain, using a coupled 2D(depth averaged)/1D modelling approach similar to Salomon and Pommepuy (1990). Similar to the previous model, the application of this model is not adequate for use in vertically stratified estuaries. It is noted that 34 inputs of faecal pollution to the estuary were identified including WWTP effluent, upstream inputs from three rivers and several creeks and CSOs. However, it is not clear how these inputs were taken into account, i.e. it is unknown whether these inputs were modelled or considered in some other way. The survival of faecal coliforms in the water column was not modelled dynamically, although different constant die-off rates were used for coastal and riverine waters, for day or night and for wet and dry weather conditions. This is an advancement compared to the model developed by Salomon and Pommepuy (1990) but still does not match the requirements described in Section 2.7.2 for dynamic survival rate modelling. Sediment-bacteria interaction was not modelled, and it was shown previously that this is hypothesised to be an important process. Data for calibrating and validating the model consisted of six daily surveys at four points along

the estuary, and the temporal resolution of the dataset was unclear. The size of the dataset was too small for proper calibration and validation of this model. Sensitivity testing was not conducted.

Garcia-Armisen et al. (2006) conducted a faecal coliform modelling study on the Seine River estuary, France, and proposed a new model for faecal coliforms (FC-SIAM-3D). The microbial model was coupled with a three-dimensional hydrodynamic model (SIAM-3D) which was able to simulate tides, currents, suspended matter concentrations and salinity within the estuary. However, data available for calibration consisted of samples taken just below the surface, thus modelling results were only presented for the surface layer. In this modelling study, inputs of faecal contamination (river upstream, tributaries along the estuary and WWTP effluent) were modelled using simple linear regressions. As such, this is the first model that actually describes how inputs are taken into account. The microbial model simulated processes of bacterial mortality and sedimentation, where a differentiation between free living and attached coliforms was made by using a constant coefficient for describing the attached fraction. This constant coefficient was derived from experiments. Sedimentation was simulated for the attached fraction only. Subsequently, the difference in mortality rates was calculated based on experiments which showed that the mortality rate of free living bacteria was twice the mortality rate of the attached fraction. However, survival was modelled using constant mortality rates (i.e. they did not vary with water column physical or chemical parameters). Interestingly, even though a significant amount of attention was devoted to modelling the sedimentation process, resuspension was not modelled. This is a drawback of the model considering the importance of this process (see Section 2.6.4). Similarly to the microorganism models described above, sensitivity analysis was not conducted in this study.

de Brauwere et al. (2011) developed a new model for simulating *E. coli* in estuaries, SLIM-EC and tested it on the Scheldt estuary, Belgium. This is a coupled 2D (depth-averaged)/1D model and bacteria was modelled as a single type of reactive tracer. As stated before, 2D depth averaged models cannot account for vertical stratification found in salt wedge estuaries, and therefore its application to the Yarra estuary is inappropriate. WWTP effluent and the upstream river were two inputs considered in this model. Both inputs delivered constant loads of microorganisms calculated based on field measurements (i.e. they did not vary with wet or dry weather conditions). Two processes were included in the model, namely temperature impact on survival of *E. coli* and sedimentation through a constant settling velocity. The impact of solar radiation on survival was omitted because of high turbidity. It must be noted the model did not consider impact of other environmental factors on microbial survival (primarily salinity) which is unacceptable for microorganism models in estuarine environments. No modelling of the resuspension of sediments was attempted, similar to the previously

discussed models. Datasets for validating the model were rather scarce, consisting of two longitudinal profiles with 9 points, collected during a two day cruise along the estuary and 16 monthly samples. The necessary observations and boundary conditions were not available to evaluate model's performance at high temporal resolution. de Brauwere et al. (2011) state that their model is not fit for "point predictions" at a precise time and location, which on the other hand is exactly what is required from a microbial model used for assessing public health risks in urban estuaries. However, this is the first modelling study that conducted sensitivity analysis of the developed model by removing one process/forcing at a time in order to determine what processes are important in controlling long term median concentrations and variability.

Subsequently, de Brauwere et al. (2014a) published an improved version of the SLIM-EC model called SLIM-EC2. As shown in the Table 2 - 6, there are considerable improvements in comparison with the previous version of the model which include:

- 1) Coupling of the estuarine model with the upstream catchment microorganism model SENEQUE-EC (Ouattara et al., 2013) which provided upstream boundary conditions SLIM-EC2.
- 2) Division of the *E. coli* pool into three fractions, free floating, attached to the suspended sediments and those present in the bed sediments, with their own transport, survival and settling/resuspension dynamics.

While coupling with the catchment model provided an improvement in boundary condition characterisation, the catchment model only produced outputs with a 10 day time step. Consequently, catchment inputs were linearly interpolated to 15 min values to provide boundary conditions for SLIM-EC2. As such, the model still does not meet the temporal resolution criterion. Indeed, de Brauwere et al. (2014a) asserts, these boundary conditions are not highly resolved in time and hence will not represent extreme conditions, which is needed for recreational risk assessment. The die-off of the microbes was modelled in the same way as in the previous model (as a function of temperature only), although the three *E. coli* fractions did have different mortality constants. For example, the die-off rate of attached *E. coli* was half of free *E. coli* and the die-off constant of the sediment *E. coli* was set to zero (i.e. effectively no die-off in sediments). Settling and resuspension was enabled only for attached microbes, where the sediment microbial store could be depleted in case of prolonged resuspension. This improvement reflects well the knowledge about microbial sediment dynamics outlined in Section 2.6. The resolution of the validation dataset was not appropriate for high temporal assessment of the model's performance, nor could the model reproduce such dynamics due to the coarse resolution of boundary conditions, as indicated previously. Therefore, the model's ability to represent monthly and seasonal variation was assessed. Similar to the previous study, the sensitivity analysis was conducted

to explore the effects of model inputs/processes on model results. It was found that resuspension/settling impacted the predicted concentrations and concluded that these processes should be explicitly represented in the model. Furthermore, it was also found that the model was sensitive to estimated values of die-off rates, particularly for the free fraction of *E. coli*.

Gao et al. (2011b) particularly focused efforts on appropriate modelling of sediment-bacteria interaction processes in the Severn estuary, Great Britain. They used a coupled 2D/1D hydrodynamic model with sediment transport model. Similar to the above 2D/1D models, this approach is not suitable for salt wedge estuaries. Model inputs were effluents from 34 WWTPs and river discharges from 29 rivers, although it is not described how these inputs were taken into account. Furthermore, other identified inputs in the requirements section (see Section 2.7.2) were not considered. Different decay rates for the water column and sediments were incorporated, where decay in the water column was modelled as a function of solar radiation and turbidity only. The impact of salinity on the survival of microorganisms was neglected, yet it was shown previously that it can have a significant influence on the survival of enteric microorganisms. Differentiation between free-living and attached bacteria was made by using partitioning coefficients where the local equilibrium is assumed to be reached instantly, i.e. that the adsorption/desorption process is fast. It is not clear from the paper what was the spatial and temporal resolution of the dataset used (only comparison against 15 data points is presented) and sensitivity analysis was not conducted, possibly due to the low resolution dataset.

Gao et al. (2015) more recently published another modelling study on the same estuary Kashefipour et al. (2002) published previously, the Ribble estuary, Great Britain. As indicated by the author, the model applied was the same one described above (i.e. 2D/1D model), although there was no information on values of parameters applied. One notable difference was the parametrisation of microbial decay in water as a function of temperature, salinity and solar radiation. 31 inputs delivered faecal microbes in the model domain including 3 rivers, WWTP discharges and CSOs. No detailed information was given on how these inputs were taken into account except that measured data was used. Although limited data were presented for model testing (i.e. around 30 data points), unlike previous studies, model fit parameters were calculated and presented (including: Root Mean Square Error - RMSE, Relative RMSE and Absolute Mean Error – AME). Furthermore, sensitivity analysis was conducted to assess the effect of different input data (i.e. boundary conditions).

Liu and Huang (2012) made an effort to model faecal coliform dynamics in an estuary in Taiwan. The approach was similar to de Brauwere et al. (2011), although they used a laterally averaged (along cross section) two-dimensional model. This approach might be appropriate for very narrow highly stratified estuaries and may allow proper simulation of bed resuspension, because space is discretised vertically;

however, this type of modelling implicitly assumes that lateral inputs are instantly mixed across the width of cross-section and indeed cannot be applied to estuaries where any lateral variability exists. Inputs of faecal microorganisms were three river boundaries upstream; *E. coli* levels at these inputs were modelled as power functions of the flow rate in the river. For the first time, an input on the downstream boundary (at the estuary mouth) was included in the model, and was estimated using measured data and kept constant during the simulation. No other inputs identified in Section 2.7.2 were modelled. Survival of faecal coliforms was a function of temperature only, and the process of sedimentation was included in the die-off rate coefficient, but only for the fraction of faecal coliforms attached to sediment particles. Resuspension was not included in the model. Datasets used for faecal coliform model testing were scarce, consisting of monthly microorganism concentration values. As such, proper testing and validation was not possible. Although sensitivity analysis was conducted, it is not very robust considering the size of the available dataset. However, analysis showed that die-off rates play an important role in determining bacterial concentrations in a tidal estuary.

Liu et al. (2015) published another study on faecal microorganism modelling in the same estuary, the Danshuei estuary, Taiwan. The microorganism model presented is identical to the one published previously, but it was coupled to a 3-dimensional hydrodynamic model. This is only the second modelling study that applied full 3D modelling. Unlike the modelled riverine inputs previously, constant *E. coli* concentrations were applied to characterise the inputs in this study. A different dataset was used to assess the model performance, which consisted of single *E. coli* measurements at approximately 15 stations along the estuarine system. Furthermore, Liu et al. (2015) assessed model performance by calculating the same model fit parameters that Gao et al. (2015) used to assess their model (i.e. Root Mean Square Error - RMSE, Relative RMSE and Absolute Mean Error – AME). It remains unclear why the authors applied 3-dimensional model for simulating the microbial dynamics when the estuary is well mixed and no data was presented to support proper testing and application of full spatial dimensionality.

Hipsey et al. (2008) developed a generic model of microbial dynamics in aquatic systems which can simulate protozoan, bacterial and viral microorganisms, and both pathogens and indicators. However, little evidence has been reported in the literature to demonstrate all of these applications. This model is developed using a 3D approach and hence is suitable for modelling highly stratified estuaries. In comparison to previously described estuarine microorganism models, this model includes dynamic survival in both water column and sediments. This is the only model in the literature that actually represents the influences of all environmental factors identified in Section 2.7.2. However, at times the model seems over parameterized. For instance, sun light inactivation has been divided into

inactivation by separate band widths. Although, it was shown that separate bandwidths of solar radiation affect microorganisms to different extent, it might not be necessary to parameterize this effect. Furthermore, even the author states that for many of the parameters, insufficient data exist for accurate estimation of the parameter values. Sediment-microorganism interaction has been taken into account through settling and resuspension of both free and attached microorganisms. The model was tested in three fresh water environments (lakes) with two event based simulations and one long term simulation of 1 year. However all were done with scarce datasets, and none on estuarine environments. Therefore, it is unknown how this model would perform in salt-wedge estuaries. Furthermore, not all of the model's structure was tested; indeed, the salinity and the pH components of the survival in water column were neglected in all applications of this model. Sensitivity analysis of the model has not been conducted. There is no doubt that much of this model's structure can be implemented for microorganism modelling in urban estuaries. But, as highlighted above, there are still some notable deficiencies including that the highly complex nature of this model requires a large dataset for proper calibration and validation, and sensitivity testing.

Conclusions. As showed above, there have been a number of attempts to model faecal microorganism dynamics in estuarine environment. However, all developed models fail on some of the modelling requirements described in Section 2.7.2:

- 1) All models except two use 2D/1D coupled hydrodynamic models. 2D depth averaged and 1D model are not suitable for vertically stratified environments such as salt wedge estuaries. Additionally, 2D laterally averaged models may account for vertical stratification but it is not appropriate for simulating inputs entering along the estuary. 3D hydrodynamics was only used in two studies, but there was no clear reason why this was done, as the calibration dataset only contained values from the top of the water column.
- 2) In terms of inputs of faecal contamination, none of the existing models appropriately characterise the input dynamics (see Section 2.7.2). In fact, inputs are mostly simulated as constant fluxes of microbial loads into the model and only a couple of studies applied simple single variable regression models. Nevertheless, in one study, proper coupling with the upstream catchment model was presented, even though the temporal resolution of catchment model outputs was low (i.e. 10-day time step) and inappropriate for comprehensive analysis of microorganism dynamics in urban estuaries. Additionally, inputs from the downstream boundary (i.e. water that comes upstream with the tide) was only modelled in one study. Therefore, currently there is no holistic model that includes accurate characterisation of the inputs that are likely to be driving forces of microbial levels in urban estuaries (Daly et al.,

- 2013). It is hypothesised that without proper modelling of inputs, it is not possible to predict microorganism levels in an estuary.
- 3) The survival of faecal microorganisms in the water column was mostly modelled using a constant die-off rate. Some studies made an effort to include dependence of the survival rate on temperature, turbidity and solar radiation, but none of the models attempted a truly dynamic model of survival including all important environmental factors and their interactions. Furthermore, only two studies included survival of microorganism in bed sediments, one through a constant survival rate and the other through temperature dependant survival. The generic microorganism model developed by Hipsey et al. (2008) included all of the environmental factors identified in Section 2.7.2 into a truly dynamic representation of the survival of microorganisms. Some of the model's structure seems unnecessary complex, with many parameters which would be difficult to estimate because of the paucity of data. Furthermore, the model structure has not been tested on an estuarine system and therefore it is not clear how it would perform. Nevertheless, many of the proposed parameterisations could be used for microorganism modelling in urban estuaries.
 - 4) Some of the models include sedimentation of faecal microorganisms but only two included resuspension of bed sediments and attached microorganisms. Again, both settling and resuspension were taken into account in the generic microorganism model by Hipsey et al. (2008).
 - 5) In some modelling studies (de Brauwere et al., 2011; Gao et al., 2011b; Liu and Huang, 2012; de Brauwere et al., 2014a; Gao et al., 2015; Liu et al., 2015), datasets of microbial concentrations were rather scarce, i.e. sampling was not conducted in appropriate temporal and spatial resolution. In other studies (Salomon and Pommepuy, 1990; Kashefipour et al., 2002), authors have made effort to observe the modelled system with the higher resolution but on the other side, this was done for an insufficient period of time with too few data points (i.e. there was only couple of days of hourly monitoring for a modelling period of a year or longer). Hence, it is very likely that a small range of possible states of the system were observed. Therefore, developed models were not tested properly. Furthermore, most of the authors assessed their model's performance visually, and no numerical measure of efficiency is reported (i.e. RMSE, AME, R^2 , E – Nash-Sutcliffe efficiency etc.), hence it hard to assess how well the models performed. Additionally, sensitivity analysis of the models was conducted in six modelling studies (de Brauwere et al., 2011; Gao et al., 2011b; Liu and Huang, 2012; de Brauwere et al., 2014a; Gao et al., 2015; Liu et al., 2015). However, since the scarcity of data (i.e. monthly samples) sensitivity analysis was not robust and hence conclusions are not firm.

The above discussions indicate that there is the need for a new coupled hydrodynamic-microorganism model for urban estuaries; one which includes all model requirements outlined in Section 2.7.2, and one which balances the complexity of the model with the amount of data available for accurate estimation of the model parameters. Sensitivity testing of such a model could then be applied, to help in the identification of key processes in urban estuaries.

2.8 Conclusions from the literature review

Microorganism dynamics in urban estuaries is very complex. It is influenced by a myriad of microbial inputs such as rivers and creeks, stormwater, wastewater and emergency release structures, wildlife deposition etc. Furthermore, once microorganisms are in the estuarine environment, their dynamics is led by a number of processes related to the survival of microorganisms in the water column and sediments, and microorganisms' interaction with sediments (i.e. attachment and settling/resuspension) which are all influenced by complex estuarine hydrodynamics. Therefore, modelling of microbial dynamics within the estuary requires a holistic approach that will take into account all of the various important factors.

Predictive microorganism models for estuaries have been developed previously, however it was concluded that none of these models satisfied all the requirements of an appropriate holistic estuarine microorganism model. Therefore, there is need for the development of a new coupled hydrodynamic-microorganism model. Furthermore, existing models have been tested with scarce datasets, hence the true performance of these models are not known, and conclusions drawn from such models are not robust. As such, there is need for extensive field studies in order to collect sufficient amounts of data for better understanding estuarine microbial dynamics as well as proper calibration and testing of the coupled hydrodynamic-microorganism model.

2.9 Research aims and objectives

The overall aim of this research project is **to develop a coupled estuarine hydrodynamic-microbial model, using the Yarra River estuary as a case study**. The Yarra River is a microtidal, salt-wedge estuary located in the City of Melbourne, Australia. As shown in the literature review, currently there is no appropriate estuarine microorganism model that includes all of the important processes. Furthermore, modelling of microbial inputs, which are shown to be the driving forces of microbial dynamics, has not been conducted in most of the studies. Without this, it is not possible to really

understand the microbial dynamics within the estuary, and not possible for industry to understand the important inputs of faecal contamination which require mitigation. Additionally, none of the models have been tested properly due to data deficiencies. As such, development of a new estuarine hydrodynamic-microorganism model is necessary.

The following outlines the main research questions and hypotheses in this research:

1) What are the most important inputs of faecal microorganisms in an urban estuary?

It is hypothesised that main inputs of faecal microorganisms are (in order of highest to lowest):

- a. *Yarra River upstream of Dights Falls* – Yarra River upstream of Dights Falls is a significant input of *E. coli* both during dry and wet weather with respect to other sources (e.g. stormwater) and will be the most important in determining overall levels of microorganism in the estuary.
- b. *Urban stormwater* – is likely an important contributor to the *E. coli* levels in the estuary during wet weather, while during dry weather its impact may not be influential on overall levels of *E. coli*; hence its effect on *E. coli* levels is hypothesised to be noticeable locally in the area of drain outlet.
- c. *Bed and bank sediment stores of faecal microorganisms* – it has been reported in literature previously and it is hypothesised that sediment bacterial storage may have substantial influence on microbial levels in the estuary, particularly during wet weather when it is likely that significant resuspension will occur due to higher flow velocities.

2) What are the most important processes (including transport pathways) of faecal microorganisms in an urban estuary?

It is hypothesised that the main processes affecting the levels of faecal microorganisms in the Yarra River estuary are:

- a. *Die-off/Survival in the water column* – it is hypothesized that die-off will be variable in different areas of the estuary. In the top most layer of the estuary, die-off is influenced mostly by solar radiation and temperature, while at bottom, salt wedge dynamics may

be the leading cause of microbial die-off. Die-off is hypothesized to be a significant sink during dry weather conditions, but is expected to have very limited impact on microorganism levels during wet weather conditions, when the microorganism levels are expected to be dominated by inputs and processes such as resuspension of sediments.

- b. *Settling and subsequent resuspension* is hypothesised to be dependent on microorganism characteristics, particle association, water velocity and turbulence, tidal fluctuation and salt wedge movement. It is hypothesised that deposition will occur only within estuarine areas when velocities are low (i.e. during dry weather), leading to a possible sink term. Conversely, it is hypothesised that resuspension will cause significant increase in microorganism concentrations especially during wet weather. During dry weather, resuspension of banks due to tidal action and wind may occur.
- c. *Transport of microbes throughout the estuary* – it is hypothesised that estuarine hydrodynamics will be main factor in explaining spatial and temporal variability of *E. coli*.

3) What are the most appropriate methods to model microbial dynamics in salt-wedge estuaries? What complexity is required?

It is hypothesised that due to the complexity of microbial processes in salt wedge estuaries and to fully cover the spatial extent of the environment, a discretised 3D process-based model is required. However, for modelling the surface layer, which poses the greatest risk to public health, it is hypothesised that application of a simpler conceptual model might be possible. The literature review showed that there have not been attempts to conduct modelling of the microbial dynamics in narrow estuaries using simplified conceptual approaches.

4) What are the essential input data that need to be measured accurately in order to predict the parameters required for modelling faecal microorganisms in urban estuaries?

It is hypothesised that transport, mixing and sediment resuspension/settling within the estuary is important for accurate prediction of *E. coli* concentrations. These processes are intrinsically associated with flow velocity. Furthermore, accurate prediction of salinity and

temperature distribution within the estuary is also linked with velocity trough mixing and these are hypothesised to be main environmental factors governing the die-off of *E. coli*. Therefore, it is essential that velocity fields are accurately predicted. It is hypothesised that the following inputs are important for the accurate velocity prediction (from higher to lower importance):

- a. Yarra River, Gardiners Creek and stormwater flow rates for both surface and bottom velocity prediction.
- b. Accurate bathymetry data, particularly for bottom velocity prediction.
- c. Wind data, particularly for the surface velocity prediction.

Chapter 3

Monitoring program and collected data

3.1 Introduction

As indicated in the literature review (Chapter 2), one of the main issues with the existing hydrodynamic-microorganism models is the lack of proper testing of the models due to limited data availability. This constrains the appropriate performance testing of these models and may limit their application. As such, there is pressing need for extensive field studies in order to collect sufficient data for better understanding of the estuarine microbial dynamics, as well as proper calibration and testing of the coupled hydrodynamic-microorganism model. Therefore, data collection represented a significant part of this research project.

This chapter focuses on the monitoring program developed to collect the necessary data. The data included water level and flow measurements, *E. coli* concentration and other water quality measurements. The chapter begins with a description of monitoring sites (Section 3.2), then the sampling regime and laboratory assays are described (Section 3.3) and finally, brief mention of externally-sourced data is made (Section 3.4).

3.2 Establishment of monitoring sites

A thorough field monitoring campaign was established to collect hydrologic, hydraulic and water quality data from the Yarra River estuary; such data is necessary for the development and testing of a coupled hydrodynamic-microorganism model. For this research project, five monitoring sites have been carefully selected and established by Monash University (Figure 3 - 1).

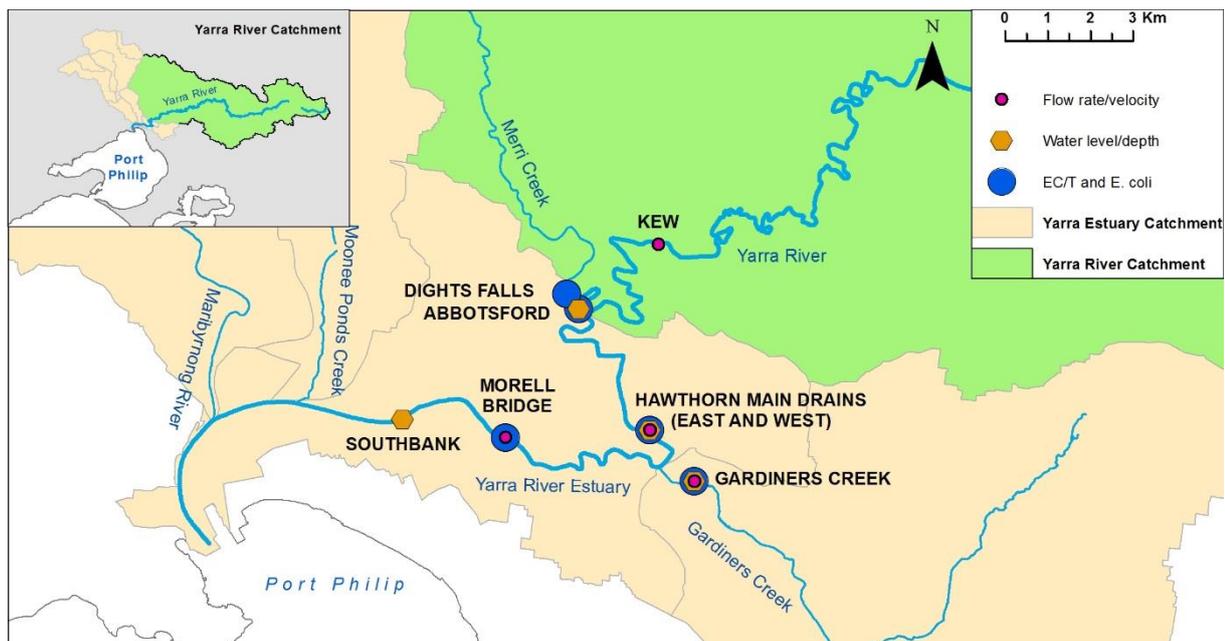


Figure 3 - 1 Monitoring sites/stations within the Yarra River and estuary catchments

Estuarine monitoring stations. Two sites were located within the Yarra River estuary: Abbotsford (ABB) at the very upstream of the estuarine section of the Yarra River (which was selected to represent the region with little influence from the salt-wedge, but still impacted by tidal changes) and Morell Bridge (MOR), located in the downstream part of the estuary (selected to represent an area highly impacted by the salt-wedge). The sites became fully operational in October 2012. Both sites were equipped with refrigerated automated samplers (Hach SD900) for the collection of water samples. The water intake to the auto-sampler at Abbotsford was fixed at approximately 40 cm above the estuary bed, while at Morell Bridge, the intake was attached to a flotation device and samples were taken from 10 cm depth (from the water surface) regardless of the tidal stage. This was predominately fresh water and considered to pose the higher risk to recreational users. Both sites had continuous measurements of

electrical conductivity (EC) and temperature (T) at the position of water intake, while Morell Bridge had continuous measurements of EC and T near the estuarine bed.

The Abbotsford site was equipped with a depth probe for measuring water depth, while the Morell Bridge site was equipped with two Acoustic Doppler Current Profilers (ADCPs) for measuring velocity components in all three directions (i.e. x, y and z directions) at 1 min interval. One ADCP was positioned in the shallower part of the cross section (shallow ADCP), closer to the auto sampler intake pipe, while the other was positioned in the deepest part of the cross section (deep ADCP). Both ADCPs functioned similarly. The water column was vertically divided into layers (cells) of user-specified thicknesses where in each layer all three velocity components were measured, hence a depth velocity profile can be derived. For the shallow ADCP the cell thickness was 0.5 m and for the deep ADCP the cell thickness was 0.7 m). Additionally, both ADCPs had a surface dynamic measurement cell. The thickness of this cell was also user-specified but the position of the cell dynamically adjusted to the water level (by using the in-built pressure sensor) so that it measured all three components of the velocity at the top of the water column. The thickness of surface dynamic cell was 0.5 m for shallow ADCP and 1.0 m for deep ADCP.



Figure 3 - 2 Left – Abbotsford monitoring site – sample intake point below the bank; Top right - Morell Bridge monitoring site (sample intake and top EC/T measurements point is at the end of the fishing pier); Bottom right – sampling stations setup.

Input monitoring stations. The Dights Falls (DF) site (Figure 3 - 1 and Figure 3 - 3) has been established for monitoring the upstream river inputs (i.e. the Yarra River just before it enters the estuary). This site

is located at the weir which physically divides the estuarine and riverine sections of the Yarra River. The site is equipped with a non-refrigerated automated sampler and an EC/T probe. However, this site was established much later than the two estuarine monitoring sites, in September 2013, and was used continually until the end of sampling campaigns in August 2014.



Figure 3 - 3 Dights Falls monitoring site.

Two other sites were established for monitoring the urban stormwater inputs into the estuary, namely Gardiners Creek (GAR) and the two main drains at Hawthorn (HMDs; Figure 3 - 1 and Figure 3 - 4). Hawthorn main drains are some of the biggest stormwater drains discharging directly into the estuary, while Gardiners creek is the largest source of water other than the Yarra River upstream of Dights Falls. Each of the stormwater monitoring sites (GAR, HMD east and HMD west) were equipped with non-refrigerated automated samplers, EC/T sensors and depth/velocity probes.



Figure 3-4 Top - Gardiners creek - sample intake point (left) and sampling station setup (right); Bottom – Hawthorn main drain west – sample intake point (left) and sampling station setup (right).

3.3 Sampling regime and laboratory assays

Sampling was conducted from November 2012 to August 2014 during both dry and wet weather periods.

Dry weather conditions. Automated samplers at all sites were started manually and time-based sampling was conducted (i.e. samples were taken using regular time intervals). The aim of the dry weather monitoring was to capture the background microorganism concentrations in the stormwater and riverine inputs and in the estuary when there are no wet weather events. As such, there was no need for flow-based sampling, typically applied when event characteristics need to be estimated (i.e. event mean concentrations, event loads etc.). The initial sampling time-interval was 1 hour at all sites (i.e. 1 L sample taken once every hour). At the Abbotsford site however, this was changed due to technical issues with the auto-sampler (i.e. pump failure occurring frequently due to a large pump head). As such, at the Abbotsford site the sampling time-interval was changed to 15 minutes (i.e. 1 L sample consisted of four samples from four different time points - 250 mL taken every 15 minutes). This change in sampling time-interval also helped achieve a better representation of the background levels over one hour. For monitoring consistency, the sampling interval was also changed at Morell

Bridge. Occasionally, due to logistical constraints, it was not possible to take samples from all sites during all monitoring campaigns, and as such sometimes daily (or more regular) grab samples were only taken.

Wet weather conditions. Automated samplers in the estuarine stations (Morell Bridge and Abbotsford) were triggered remotely (using telemetry). The samplers were triggered when the average rainfall in the urban part of the Yarra River catchment was over 1 mm per hour. The rainfall data was obtained from weather radar observations for Melbourne, retrieved in real-time from the Bureau of Meteorology website (<http://www.bom.gov.au/products/IDR024.loop.shtml#skip>). The sampling continued over the wet weather event and for two days after the wet weather event (i.e. two dry weather days) in order to capture the return of the estuarine microorganism concentrations to its base levels. While flow-based sampling is preferable during wet-weather, time-based sampling was applied at Morell Bridge and Abbotsford for the following reasons: 1) calculating volumes in estuarine environments is very difficult and involves a high level of uncertainty because of bi-directional water movement and, 2) the model testing will involve prediction of instantaneous microbial concentration and as such, event characteristics such as event mean concentrations are not needed. For the input monitoring stations (i.e. stormwater sites and the Dights Falls site), the automated samplers were triggered by a change in flow rate. Up to 24 x 1 L samples were then taken according to flow-weighted intervals. Similarly to dry weather, if automated samplers were not used, grab samples were taken at least once each day of monitoring.

Sample assay. All collected samples were transported to the Environmental and Public Health Microbiology (EPHM) laboratory at Monash University in coolers on ice and analysed using Colilert method (IDEXX Laboratories, 2013) within 24h of collection. As shown previously, auto-samplers at Dights Falls, Gardiners Creek and Hawthorn main drains were not refrigerated. Considering the importance of the temperature effect on survival of microorganism (McFeters and Stuart, 1972; McCambridge and McMeekin, 1980; Barcina et al., 1986) there was some concern whether the storage time in unrefrigerated samplers will have impact on the measured *E. coli* levels at these sites. However, McCarthy et al. (2008) conducted a thorough analysis of uncertainty of *E. coli* levels in stormwater including storage uncertainty, and showed that storage time up to 24h in unrefrigerated conditions was not a significant factor in explaining variability in *E. coli*.

The Colilert method allowed for detection of *E. coli* from only 1 Most Probable Number (MPN)/100mL up to 2,400 MPN/100mL. As such, suitable dilution rates are normally required in order to detect levels higher than 2,400 MPN/100mL. The optimum dilution rates were determined using trial and error. On most occasions a 1 in 10 dilution was suitable for Dights Falls, Abbotsford and Morell Bridge sites, while

stormwater typically required a 1 in 100 dilution to ensure an optimal detection range. However, sometimes *E. coli* levels were outside the detection range and these samples were reported to have qualitative values only (i.e. 'greater than' or 'less than' based on the dilution used and the concentration of the sample). Since it is impractical to use these values for calibrating and testing a water quality model, it was decided that these points are to be neglected during the testing of the microorganism model.

Collected water samples. In total, slightly over six thousand water samples were collected and analysed for *E. coli* (Table 3 - 1). More than half of the samples (around 3500) was collected from the Yarra River estuary (i.e. Abbotsford and Morell Bridge). 914 samples were collected from the Yarra River just before it enters the estuary over Dights Falls. And the rest of the samples (just slightly under 1700) were collected from the urban inputs to the Yarra River estuary (i.e. Gardiners Creek and Hawthorn Main Drains). Based on rainfall measurements around the Yarra River catchment, the data were divided into wet and dry weather periods. Samples from the Yarra River (i.e. Dights Falls, Abbotsford and Morell Bridge) were considered wet weather samples if the cumulative rainfall over the proceeding 24 hours was greater than 1 mm in the lower part of the estuarine catchment (urbanised part of the catchment), or if the cumulative rainfall over the proceeding 72 hours (estimated time of concentration for the upper Yarra River catchment) was greater than 3 mm in the upstream rural parts of the catchment. Samples from the urbanised inputs (i.e. Gardiners Creek, Hawthorn Main Drain east and west) were categorised as wet weather samples if the rainfall was greater than 1 mm in their respective catchments. Around one third of samples was collected in dry weather conditions while two thirds of samples were wet weather samples (Table 3 - 1)

Table 3 - 1 Number of collected and analysed water samples in total, wet and dry weather conditions.

	Number of samples collected		
	Total	Wet weather	Dry weather
Dights Falls	914 (100%)	701 (77%)	213 (23%)
Abbotsford	1679 (100%)	1195 (71%)	484 (29%)
Morell Bridge	1777 (100%)	1281 (72%)	496 (28%)
Gardiners Creek	869 (100%)	464 (53%)	405 (47%)
Hawthorn Main Drain (HMD) west	343 (100%)	299 (87%)	44 (13%)
Hawthorn Main Drain (HMD) east	477 (100%)	211 (44%)	266 (56%)
TOTAL	6059 (100%)	4151 (68%)	1908 (32%)

3.4 Other available datasets

Additional hydrologic data was obtained from the Melbourne Water Corporation. Data included: (1) water level measurements within the estuary at Abbotsford, Hawthorn, Burnley and South Bank, (2) Yarra River flow rate measurements at Kew (Figure 3 - 1) and (3) rainfall data from 18 rainfall gauges within the Yarra River catchment, all in 6 minute time-steps, which were used for dividing the data into wet and dry weather periods and (4) air temperature in 6 minute time-steps and solar radiation data in 1 minute time-steps were obtained from Australian Bureau of Meteorology.

Chapter 4

E. coli dynamics within the Yarra River estuary

4.1 Introduction

As indicated in the literature review (Chapter 2), one of the main issues with the existing hydrodynamic-microorganism models is the lack of proper testing of the models due to limited data availability, which constrains the appropriate performance testing of these models and may limit their application. As such, there is a pressing need for extensive field studies in order to collect sufficient amounts of data for better understanding of the estuarine microbial dynamics and proper calibration and testing of the coupled hydrodynamic-microorganism model. Therefore, data collection is a significant part of this research project.

This chapter focuses on addressing (in whole or in part) the following key research questions and hypotheses through exploring the estuarine hydrodynamics and *E. coli* dynamics of by analysing the large dataset collected during this project. We hypothesised that the most important inputs of faecal microorganisms to the Yarra River estuary are the Yarra River, urban stormwater and microbes stored in the bed and bank sediments (Section 2.9, Chapter 2 – Literature Review). In this chapter, we specifically focus on testing the hypothesis about the bed and bank sediments as an input of faecal microorganisms. Furthermore, we hypothesised that the main processes influencing the levels of faecal microorganisms are: die-off in the water column, settling and resuspension to/from bed and bank sediments, and transport of the microorganism throughout the estuary. We also tested this hypothesis in this chapter by analysing the collected data.

The chapter is comprised of three main parts. Section 4.2 is an exploration of estuarine hydrodynamics and *E. coli* levels in the Yarra River estuary using measured data. Section 4.3 discusses the links between tides (i.e. tidal water levels and currents) and *E. coli* levels in the Yarra River estuary and is presented in the form of a published journal paper ("*Tidal fluctuations influence E. coli concentrations in urban estuaries*", in *Marine Pollution Bulletin*, 2017, VOL 119(1), pp. 226-230). The final part (Section 4.4) explores the vertical, lateral and longitudinal variability of *E. coli* within the Yarra River estuary by analysing the large dataset of depth profiles collected during this research project. This section is presented as an accepted journal paper ("*Spatial variability of E. coli in an urban estuary*", *Marine Pollution Bulletin*, 2017, VOL 114(1), pp. 114-122). The chapter finishes with a discussion that integrates the findings of this chapter with the broader aims and objectives of this thesis (Section 4.1).

4.2 Estuarine hydrodynamics and *E. coli* dynamics

4.2.1 Introduction

The aim of this section is to highlight the dynamics of salt-wedge estuaries, and to highlight some of the factors which could be important for modelling *E. coli* in these complex systems. This was done by analysing the dataset collected during the monitoring program and the data obtained from other sources described in Chapter 3. An overview of the estuarine hydrodynamics and the physical water properties during both dry and wet weather flow periods is given in Section 4.2.3, followed by an overview of the levels of *E. coli* found during the monitoring campaign along with a discussion of the plausible links with hydrologic and environmental parameters in Section 4.2.4.

4.2.2 Methods

Pair-wise comparisons between the sites and comparisons between dry and wet weather samples at each site were performed using the Wilcoxon rank-sum test. This statistical test is a two-sided rank-sum test of the null hypothesis that two samples are independent samples from identical continuous distributions with equal medians, against the alternative that they do not have equal medians (Zar, 1999). The data were divided into wet and dry weather periods according to rainfall observations from 18 gauges within the Yarra River and estuary catchment. A sample was considered to be influenced by wet weather if the cumulative rainfall over the proceeding 24 hours was greater than 1 mm in the lower part of the estuarine catchment (urbanised part of the catchment), or if the cumulative rainfall over the proceeding 72 hours (estimated time of concentration for the upper Yarra River catchment) was greater than 3 mm in the upstream rural parts of the catchment.

To determine if there is a link between *E. coli* dynamics at Morell Bridge and hydrological and environmental variables, simple Spearman rank and Pearson correlation analysis (Zar, 1999) between *E. coli* concentrations and the flow rate at Kew, water temperature in the top layer of the water column and solar radiation has been conducted. For Pearson correlation analyses, both *E. coli* and explanatory variables were log-transformed in an attempt to increase the normality of the data (and hence to meet the requirement of simple linear regression to have normally distributed residuals).

4.2.3 Variability of water levels, flows and environmental factors within the Yarra River estuary

Variability of water level, flow velocity, top and bottom electrical conductivity and temperature at Morell Bridge are shown in Figure 4 - 1, while variability of water level, electrical conductivity and temperature at Abbotsford are shown in Figure 4 - 3.

Morell Bridge. The Yarra River estuary has semi-diurnal tidal regime and maximum and minimum velocities occur around mid-tides. Tides are controlling the flow velocity during dry weather, while during wet weather, river forcing is dominant over the tidal forcing, resulting in high velocities almost always in the downstream direction (i.e. positive flow velocities). During dry weather, due to the low flow velocities (e.g. - 0.15 to 0.40 m/s in the Figure 4 - 1), it is unlikely that significant amounts of resuspension from the bed sediments will occur; the estuarine sediments are predominantly fine grained (over 60 % is <20 μm ; (Ellaway et al., 1982) and the velocity needed to cause resuspension of muds of such composition needs to be higher than 0.35 m/s (van Rijn, 1993; Yang, 1996). Dry weather velocities higher than 0.35 m/s only occur for only a limited period of time, suggesting that resuspension will be limited. However, wet weather flow velocities can be around 1 m/s and significant resuspension could occur during these higher flow events. However, it is important to note that bank resuspension could occur even during dry weather where estuary velocities are low because of local effects, wind, boat movements, etc. causing turbulence at the bank-water interface.

Stratified conditions of the estuary are confirmed by electrical conductivity (EC) measurements. During dry weather, the salinity of the bottom layer is constant and EC measurements indicate that the bottom layer is predominantly sea water ($EC_{ave} = 46 \text{ mS/cm}$ – average over the monitoring period; while $EC_{sea} = 54 \text{ mS/cm}$ (Eaton et al., 2005)), while EC measurements of the top layer indicate that water is predominantly fresh ($EC_{ave} = 10 \text{ mS/cm}$ – average over the monitoring period). As indicated in the literature review (Chapter 2), many authors reported the detrimental effect of salinity on survival of faecal microorganism in sea water; i.e. die-off increases with increase in salinity (Carlucci and Pramer, 1960; Fujioka et al., 1981; Šolić and Krstulović, 1992). Therefore, the survival of faecal microorganisms will largely be impacted by salinity in the bottom layer of the water column. Salinity will also impact the survival of microorganisms in the surface layer, but to a lesser extent. As shown in Figure 4 - 1, during wet weather events, fresh water pushes the salt wedge further downstream and reaches the bottom EC/T sensor at depth of 2.5 m, demonstrating that the salt wedge is no longer present at this site during this event. However, it can be seen that quickly after the event finished, the salt wedge began to return to its previous position. Additionally, significant oscillations in EC can be seen at Morell

Bridge, indicating that river flow rate and tidal stage are governing factors of the position of the salt wedge within the estuary.

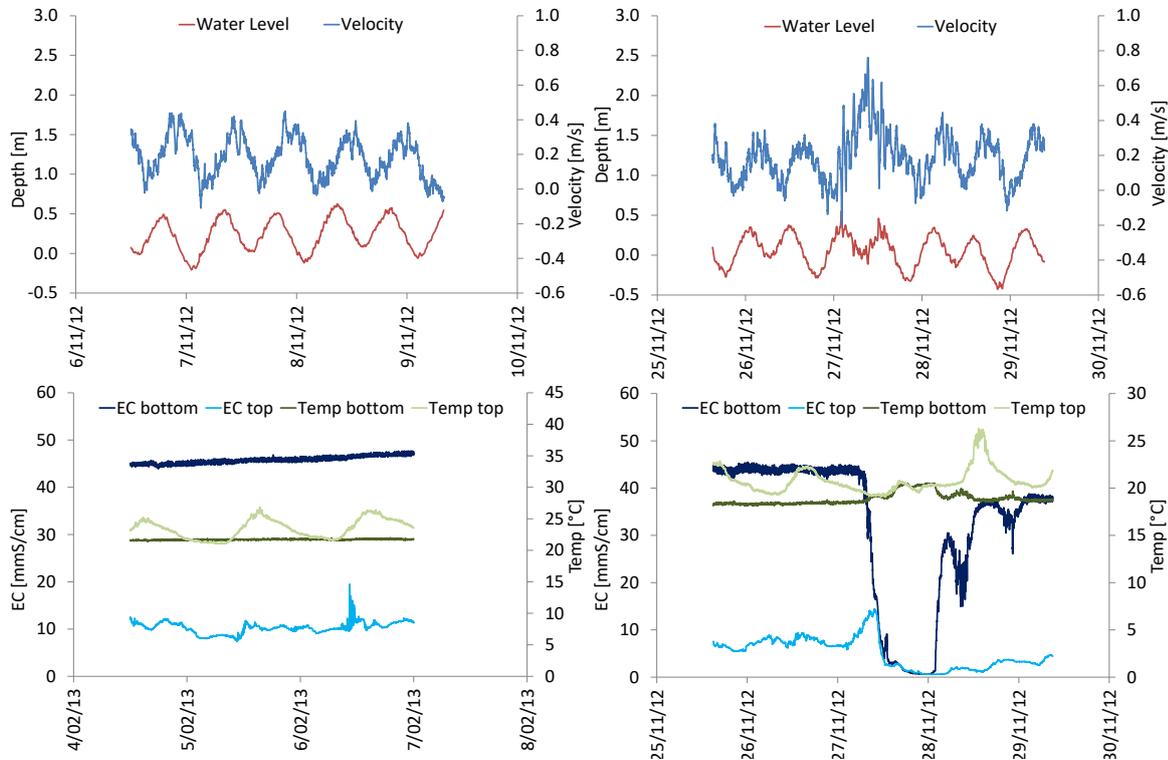


Figure 4 - 1 Morell Bridge monitoring site. Top - depth and velocity during dry weather (left) and wet weather event (right); Bottom - EC/T measurements from top and bottom of water column during dry weather (left) and wet weather event (right).

Temperature is considered to be the most significant environmental factor influencing the survival of faecal microorganism (Blaustein et al., 2013). Thus, it is important to examine its variability within the estuary. Temperature of the top layer has diurnal oscillations following atmospheric and meteorological conditions (i.e. air temperature and cloud cover/sunlight). Indeed, a significant positive correlation between air temperature and the top water layer temperature was found (Spearman's rank correlation coefficient $\rho=0.77$ $p<0.001$). The temperature of the bottom layer is constant over the day, possibly (i) as a result of this water being sourced from the bay which has a stable temperature and being (ii) protected from the diurnal effects of air temperature and irradiance. In any case, the daily oscillation in the temperature of the top of the water column is shown to be significant (in the example in Figure 4 - 1 the change in temperature is 6.1°C , while the maximum change in temperature in any one day over the monitored period was 11.8°C), and hence is likely to have an impact on the survival of microbes in the estuary. The observed diurnal temperature differences are likely the consequence of the longer residence time of the water in the estuarine environment. Unlike rivers

which have unidirectional flows, water movement within estuaries is bidirectional. In fact, in the Yarra River estuary, flow direction changes four times a day, with a semi-diurnal tidal pattern. Together with the limited mixing of the surface water and the salt-wedge, these flow directions enable the large temperature fluctuations within the surface water layer.

On a seasonal scale, water temperature in the estuary increases during spring and summer, and decreases during autumn and winter (Figure 4 - 2). Furthermore, during autumn/winter, the bottom layer is warmer than the top layer, while in autumn/winter the opposite is true. Therefore, temperature is likely to have significant impact on seasonal survival of microbes in the estuary as the die-off rate of *E. coli* at 22°C (average summer temperature) is more than three times higher than at 10°C (average winter temperature) (Hipsey et al., 2008).

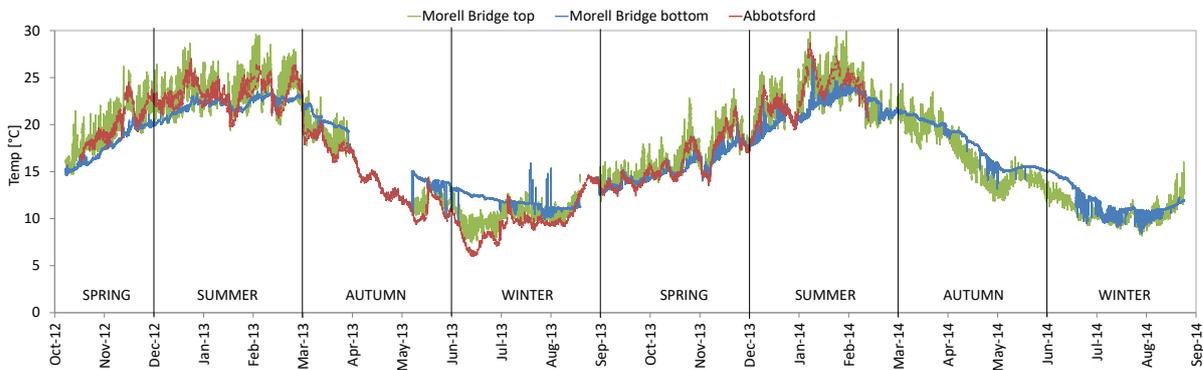


Figure 4 - 2 Seasonal temperature change in the Yarra River estuary.

Abbotsford. Dry weather water level at Abbotsford is controlled by tidal fluctuations (Figure 4 - 3), and the tidal range is attenuated along the estuary (i.e. it is around 40 cm at Abbotsford compared to around 60 cm at Morell Bridge). However, during wet weather, river flow overcomes any tidal influences and water level is therefore mainly controlled by the river.

Mean EC levels measured at the Abbotsford monitoring site are around 0.2 mS/cm (average over monitoring period, maximum 1.5 mS/cm), which is fifty times less than the time-averaged EC of the top layer at Morell Bridge, meaning that salt wedge did not reach this site during the monitoring period and hence survival of the microorganism at this site is not likely to be impacted by salinity.

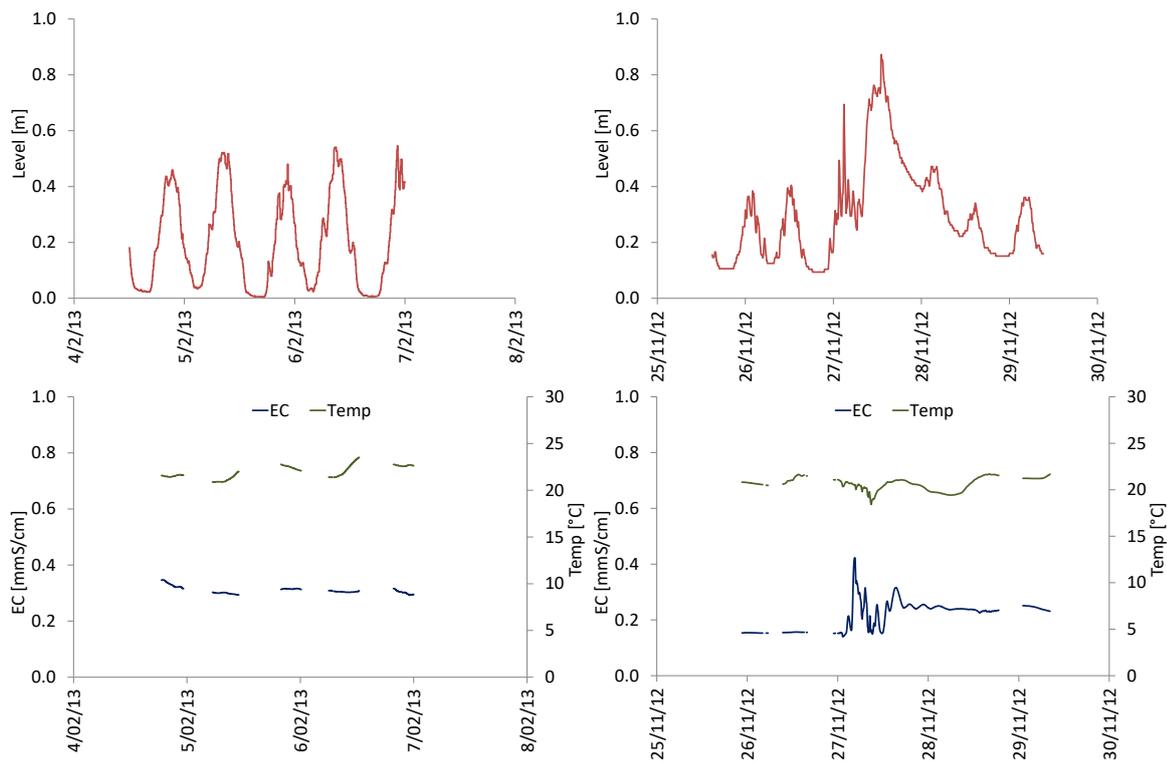


Figure 4 - 3 Abbotsford monitoring site. Top – Water level during dry weather (left) and wet weather event (right); Bottom - EC/T measurements during dry weather (left) and wet weather event (right).

Temperature fluctuations at Abbotsford are diurnal, correlating to atmospheric and meteorological conditions (similarly to top layer temperature at Morell Bridge). However, the extent of the diurnal variation at Abbotsford is smaller than what was observed at Morrell Bridge. The maximum change in temperature in any one day during the monitoring period at Abbotsford was 3.9°C. A couple of reasons could be contributing to the lower diurnal variation at Abbotsford. Firstly, due to the weakened tidal influence at Abbotsford, the flow is mostly unidirectional (downstream) resulting in a shorter residence time which in turn prevents the water from warming up. Secondly, the dense vegetation on the banks of the Yarra River upstream protects the river from direct solar radiation, which also limits the diurnal temperature variation. Therefore, daily temperature oscillations are likely to have less impact on microbe survival in upper parts of the estuary. However, seasonal changes in temperature at Abbotsford has a similar pattern to that at Morell Bridge (Figure 4 - 2). Thus, temperature changes (seasonally) at Abbotsford are likely to have significant impact on microorganism survival in the estuary on seasonal scale as described above.

4.2.4 *E. coli* levels in the Yarra River estuary

Figure 4 - 4 shows descriptive statistics for measured *E. coli* concentrations in samples from Dights Falls, Abbotsford and Morell Bridge. A large variability in *E. coli* concentrations can be seen between individual samples taken from each of the monitoring sites. Between-sample variability of *E. coli* in the estuary is an order of magnitude lower than the variability in *E. coli* reported for urban stormwater (McCarthy, 2008) (10 MPN/100mL – 10000 MPN/100mL compared to 10 MPN/100mL – 100000 MPN/100mL). This is due to the large buffering capacity of the estuary. Additionally, there are significant differences in the distributions of *E. coli* concentrations during wet weather and dry weather at all sites ($p < 0.001$, Wilcoxon Rank Sum test) indicating differences in sources/processes controlling the *E. coli* levels during different weather conditions.

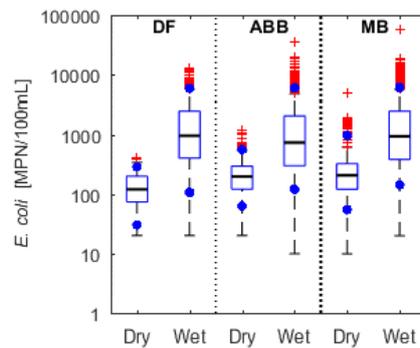


Figure 4 - 4 Variability of the *E. coli* in the Yarra River estuary and its major drains during dry and wet weather (On each box, the central mark is the median and blue markers are 5th and 95th percentiles).

Both wet and dry weather *E. coli* distributions at Dights Falls, Abbotsford and Morell Bridge are quite similar. Indeed, measured *E. coli* concentrations at these sites are significantly positively correlated: Dights Falls and Abbotsford - $\rho = 0.88$ and $p < 0.001$; Dights Falls and Morell Bridge $\rho = 0.75$ and $p < 0.001$; and Abbotsford and Morell Bridge - $\rho = 0.78$ and $p < 0.001$ (Figure 4 - 5). This suggests that the Yarra River is the main driver of the *E. coli* levels within the estuary as previously was indicated by Daly et al. (2013).

Figure 4 - 6 shows dynamics of the *E. coli* during dry weather. The levels of *E. coli* at both sites are within the same order of magnitude, and they roughly follow similar patterns, demonstrating that the sources and/or processes which govern both sites are similar. This indicates that during dry weather, the major driving force is the riverine inputs upstream. Furthermore, it can be seen that *E. coli* levels are linked with the flow rate at Kew (i.e. when flow decreases *E. coli* decreases).

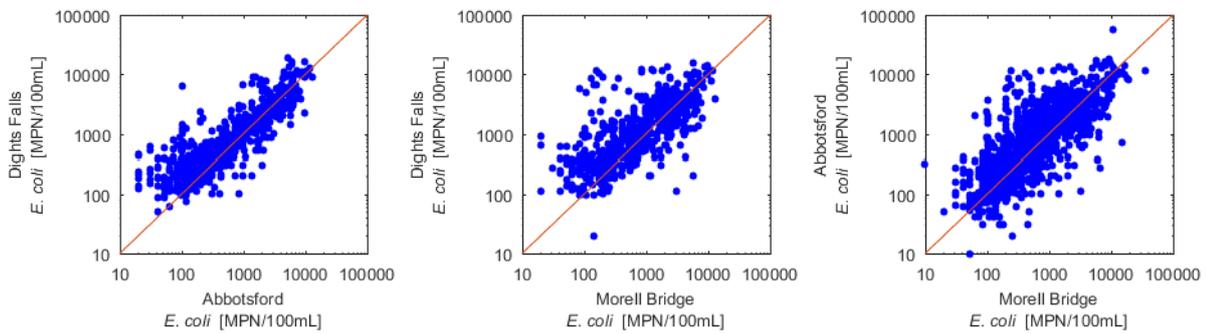


Figure 4 - 5 Correlations between sites: left – Dights Falls and Abbotsford ($\rho = 0.88$ and $p < 0.001$), middle – Dights Falls and Morell Bridge ($\rho = 0.75$ and $p < 0.001$) and left – Abbotsford and Morell Bridge ($\rho = 0.78$ and $p < 0.001$)

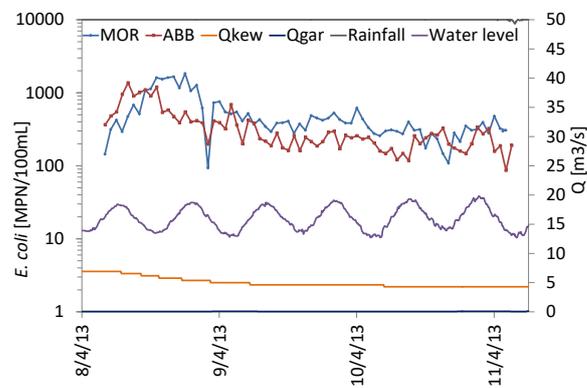


Figure 4 - 6 *E. coli* levels at the Abbotsford and Morell Bridge monitoring sites during dry weather

E. coli dynamics at Abbotsford and Morell Bridge during wet weather is presented in Figure 4 - 7. The overall pattern is driven by the wet weather inputs. Indeed, as hypothesised above, the riverine inputs upstream of Dights Falls seem to be the major driving force for the estuary, and hence it was expected that these sites behave in a similar manner. However, there are certain differences between the two sites. For example, Figure 4 - 7 shows that *E. coli* levels at Morell Bridge are consistently higher (although the difference are small) than that at Abbotsford, possibly indicating that the additional sources of *E. coli* which exist downstream of Abbotsford are increasing the *E. coli* levels (stormwater inputs, Gardiners Creek, etc.). Particularly, the peak in *E. coli* concentration during the event on the 26th February at Morell Bridge (where concentrations are almost an order of magnitude higher than Abbotsford) may be linked to the Gardiners Creek input (which sits between the two sites). In addition, resuspension of the sediments during wet weather events might also be contributing to the observed higher levels (as mentioned above, resuspension from the bed sediments is hypothesised to be a small

during dry weather but wet weather events may cause noticeable resuspension because the measured velocities are above 0.35 m/s; the critical velocity required to induce resuspension of bed sediments of the estuary (van Rijn, 1993; Yang, 1996)).

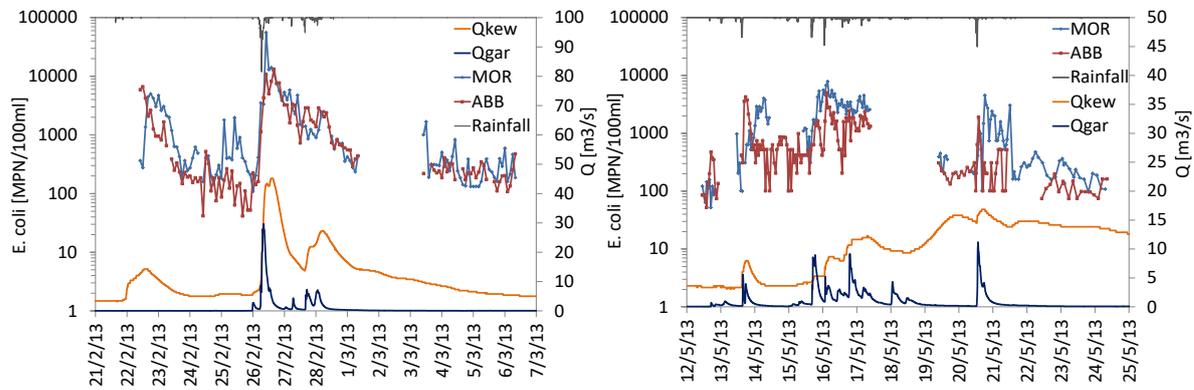


Figure 4 - 7 *E. coli* levels at Abbotsford and Morell Bridge monitoring site during wet weather

Simple Spearman rank and Pearson correlation analysis between *E. coli* concentrations and hydrological and environmental parameters at Abbotsford and Morell Bridge showed that *E. coli* is significantly correlated with flow rates at Kew, which could explain 20 per cent of the observed *E. coli* variability at both sites (Table 4 - 1). This indicates that dynamics of *E. coli* is related to the hydrological conditions of the estuary, as previously suggested.

E. coli concentrations at Morrell Bridge and Abbotsford were also negatively correlated to environmental parameters. These correlations were slightly weaker than the correlations with flow (see Table 4 - 1). Higher *E. coli* concentrations commonly occur during and after wet weather events (as shown above), when incoming water can be colder. Additionally, during wet weather, solar radiation is typically lower due to the cloud cover which reflects some of the incoming radiation. High temperatures and solar radiation are all known to have detrimental effect on survival of enteric bacteria (Crane and Moore, 1985). Interestingly, slightly weaker negative correlations with temperature at Abbotsford may reflect shorter residence times at this location and the existence of dense bank vegetation found here that may be keeping the water cool and also providing shade, thereby enhancing *E. coli* survival.

Table 4 - 1 Spearman rank and Pearson correlation coefficients, p-values and coefficients of determination between *E. coli* concentrations and hydrologic and environmental variables at Abbotsford and Morell Bridge monitoring sites

Location			Q_{kew} [m ³ /s]	Variables Temperature [°C]	Solar radiation [W/m ²]
Abbotsford	<i>E. coli</i> [MPN/100mL]	Spearman ρ (p-value)	0.50 (<0.001)	-0.22 (<0.001)	-0.10 (<0.001)
		Pearson ρ (p-value)	0.44 (<0.001)	-0.22 (<0.001)	-0.16 (<0.001)
		R ²	0.20	0.05	0.03
Morell Bridge	<i>E. coli</i> [MPN/100mL]	Spearman ρ (p-value)	0.46 (<0.001)	-0.31 (<0.001)	-0.12 (<0.001)
		Pearson ρ (p-value)	0.44 (<0.001)	-0.30 (<0.001)	-0.16 (<0.001)
		R ²	0.20	0.09	0.03

4.2.5 Conclusions

This section presented the initial analysis of the collected hydrologic and water quality data set. The main conclusions can be summarised as follows:

- The stratified nature of the Yarra River estuary is confirmed through measurements. These conditions significantly impact the overall hydrodynamics and the distribution of the environmental parameters (salinity and temperature). As such, it is essential that the hydrodynamic model applied in this research project is able to reproduce the salt-wedge conditions in the estuary.
- Observed velocities indicate that sediment (and hence *E. coli*) resuspension within the estuary during dry weather will be very limited, while during wet weather more significant resuspension of the bed and bank sediments is likely to occur.
- Overall die-off of the *E. coli* is likely to be more pronounced in summer than in winter due to the seasonal variation in temperature.
- Die-off of the *E. coli* in the lower estuary (i.e. at Morell Bridge) is likely to be more significant than in the upper estuary (i.e. at Abbotsford), due to larger variation in temperature, higher electrical conductivity/salinity, direct exposure to solar radiation and longer residence times.
- The Yarra River is controlling the overall levels of *E. coli* within the estuary. Nevertheless, *E. coli* levels at Morell Bridge are consistently higher than the ones at Abbotsford indicating the existence of additional inputs and/or processes occurring along the estuary that contribute to increase in concentrations.

4.3 Tidal fluctuations influence *E. coli* concentrations in urban estuaries

The supplementary material for this publication is provided in Appendix A.1.

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Tidal fluctuations influence *E. coli* concentrations in urban estuaries



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ABSTRACT

This study investigated the influence of water level and velocity on *Escherichia coli* levels over multiple tidal cycles in an urban microtidal estuary in Melbourne, Australia. Over 3,500 *E. coli* samples and high resolution water level and velocity measurements from two locations within the estuary were used for the analysis. *E. coli* negatively correlated with water level in the upper estuary which was proposed to be linked to increased resuspension of estuarine sediments during low tide. No relationship was found in the lower estuary, likely due to wet weather inputs dwarfing subtler tidal-related processes. Removal of wet weather data enabled significant relationships to emerge in the lower estuary: 1) positive with water level (when a 9-h shift applied corresponding to the phase shift between water levels and velocities) and; 2) positive with velocity (no shift applied). This supports a link between increased *E. coli* levels and tidal-related resuspension.

1. Introduction

Relatively few studies have examined the role of estuarine or coastal hydrodynamics on faecal bacteria levels (Bedri et al., 2013; Rippy et al., 2013). This is especially true in regards to the effect of tidal forcing (i.e. tidal stage and tidal currents) and where studies have occurred, contrasting conclusions have been reported. Solo-Gabriele et al. (2000) found that *Escherichia coli* levels were positively correlated with water level in a tropical estuary in Florida, USA. Conversely, a significant negative correlation between tidal stage and *E. coli* was reported by Mallin et al. (1999) and Perini et al. (2015) for three different coastal creeks in North Carolina, USA and a coastal lagoon in Venice, Italy. An Australian study showed similar results, with higher *E. coli* levels corresponding to low water levels (Mill et al., 2006).

The contradictory results reported in the literature could be a reflection of either (1) limited sampling effort, whereby authors attempted to draw link using small datasets (e.g. some studies simply monitored one to six tidal cycles only), or (2) water velocities were not taken into consideration, yet they are known to be an important driver of faecal microorganism transport, mixing, settling and resuspension processes (Martinez-Manzanas et al., 1992; Mallin et al., 2007; Pachepsky and Shelton, 2011), or (3) at different locations the relationship between water level and *E. coli* levels may change because of the change in the relationship between water level and velocity, which is hypothesised to be main factor in determining *E. coli* variability across

the tidal cycle. Therefore, further studies, which are based on large datasets that include velocity measurements, are required to help define the role that tides play in governing the levels of faecal microorganisms (such as *E. coli*) in estuaries.

The aim of this study was to assess the impact of water level and flow velocity on *E. coli* in an urban salt-wedge estuary in Melbourne, Australia. This was done using a large *E. coli* dataset that includes over 3500 samples collected over two years covering both wet and dry weather periods. Data analyses are conducted to link these *E. coli* levels to high resolution stage and flow velocity measurements.

2. Methods

2.1. Study site

The Yarra River in Melbourne, Australia, has a total length of 242 km and drains a catchment of 4,000 km² - comprising forested headwater reaches, predominantly rural mid-reaches and urbanized lower reaches before flowing into Port Phillip Bay. The lower 22 km reach of the river represents its estuarine section (Fig. 1), with a well-defined upstream boundary - an artificial weir known as Dights Falls, which prevents tidal propagation upstream. The estuary is used for secondary contact aquatic recreation (especially rowing, kayaking and fishing) while primary contact recreation is either restricted due to boat navigation or is not recommended due to frequently high levels of

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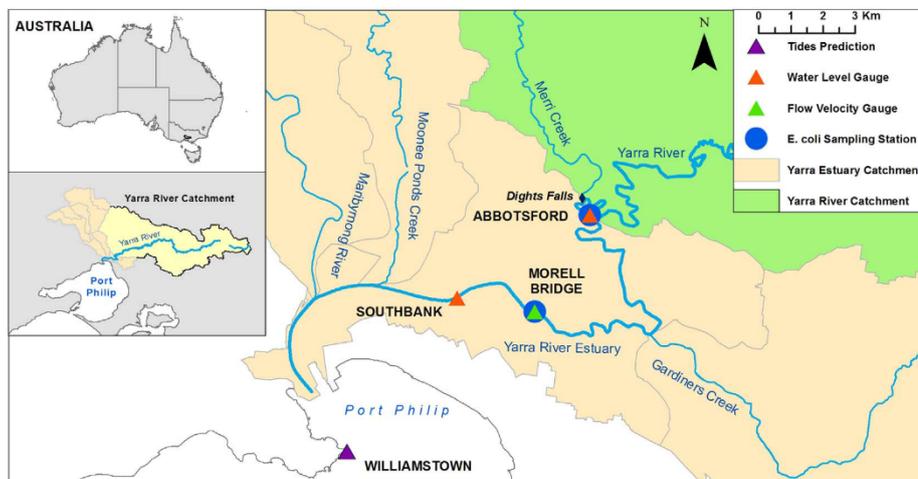


Fig. 1. Yarra River catchment and the estuary catchment with environmental monitoring sites.

faecal indicator microbes (Department of Sustainability and Environment, 2012).

Water level fluctuations within Port Philip Bay average around 0.5 m, but vary between 0.3 and 0.9 m. The tidal pattern is semi-diurnal with a significant diurnal variation (Beckett et al., 1982). The major freshwater inputs to the estuary include the Yarra River upstream of Dights Falls (about 70% of the total flow at the estuary mouth; (Sokolov and Black, 1996)), Gardiners Creek, Maribyrnong River, Moonee Ponds Creek and 208 stormwater drains discharging directly to the estuary (Daly et al., 2013; Jovanovic et al., 2015).

2.2. Data collection

Water samples were collected between November 2012 and August 2014 during both wet and dry weather conditions using two refrigerated automated samplers (Hach SD900); one installed in the upper estuary at Abbotsford (1736 samples collected) and one in the lower estuary at Morell Bridge (1838 samples collected) located approximately 12 km downstream of Abbotsford (Fig. 1).

The water intake to the auto sampler at Abbotsford was fixed at approximately 40 cm above the estuary bed, while at Morell Bridge where tidal water level fluctuations are much greater, the intake was attached to a flotation device and samples were withdrawn at 10 cm depth regardless of the tidal stage. The mean depth at the water intake was approximately 0.85 m at Abbotsford and 1.7 m at Morell Bridge. While there was significant difference in the positions at which the samples were collected at these two sites, due to the well-mixed conditions along the water column at Abbotsford the depth at which the samples were collected at this site was unlikely to cause high levels of uncertainties in measured *E. coli* concentrations (see Jovanovic et al., 2017). At Morell Bridge, however, due to extensive stratification of the water column the highest levels of *E. coli* are found in the surface layer while the bottom salt-wedge contains minimal numbers of *E. coli* (Jovanovic et al., 2017). Therefore, in order to observe the *E. coli* level fluctuation over the tidal cycle, it was important that the samples were always collected from the top of the water column.

Time-based water samples at intervals ranging from 15 min to 1 h, with each 1 L sample either consisting of one sample from single time point, or up to four samples from four different time points (i.e. 250 mL taken every 15 min). All samples were analysed by the Environmental and Public Health Microbiology Laboratory (EPHM Lab) at Monash University, Clayton, within 24 h of collection (McCarthy et al., 2008), using the Colilert method (IDEXX Laboratories, 2013).

Additionally, an Acoustic Doppler Current Profiler (ADCP) was installed at Morell Bridge for continuous monitoring of flow velocity at 1-min intervals. Measurements of water level at Abbotsford (Yarra River at Johnston Street, Collingwood; 229622A) and Southbank (Yarra River at Crown Casino, Spencer Street, Southbank; 229663A) at 6-min intervals were obtained from Melbourne Water (unpublished data, <https://www.melbournewater.com.au/waterdata/Pages/waterdata.aspx>).

2.3. Data analysis

The relationship between *E. coli* levels and tides was investigated by aggregating water level, flow velocity and *E. coli* concentrations into twelve intervals ('bins'), equally spread over each tidal cycle defined using astronomical predictions of high/low tides for Williamstown station (Fig. 1). This means that all our measured data were placed into twelve bins which represented various tidal conditions, allowing us to determine whether *E. coli* levels were consistently higher/lower in particular tidal conditions.

Because the Yarra River estuary is microtidal (i.e. having a tidal range < 2 m), tidal forcing is dominant during dry weather, when inputs from the Yarra River and tributaries are small. Thus, it is likely that the tidal effects will be more clearly seen during these periods. In fact, Solo-Gabriele et al. (2000) observed that *E. coli* concentrations were correlated with tidal stage two days after the end of a storm event, suggesting that *E. coli* levels are governed by other processes during wet weather. Other studies analysed the impact of tides on *E. coli* levels during dry weather only (Mallin et al., 1999; Mill et al., 2006; Perini et al., 2015). As such, we further separated our data into wet and dry weather periods according to rainfall observations from 18 gauges within the Yarra River catchment. A sample was considered to be influenced by wet weather if the cumulative rainfall over the preceding 24 h was > 1 mm in the estuarine urbanized catchment, or if the cumulative rainfall over the preceding 72 h (estimated time of concentration) was > 3 mm in the upstream rural parts of the catchment.

Spearman rank correlation coefficients (ρ) and corresponding p -values were calculated between *E. coli* and water level, and *E. coli* and flow velocity using the median values of each of the 12 tidal cycle bins. Because a phase shift (i.e. time difference between occurrence of low/high tide and minimum/maximum tidal current) commonly exists between water levels and flow velocities in estuaries (Beckett et al., 1982; Dyer, 1997), additional Spearman rank correlation analyses were performed, where *E. coli* median values of from each bin were fixed and

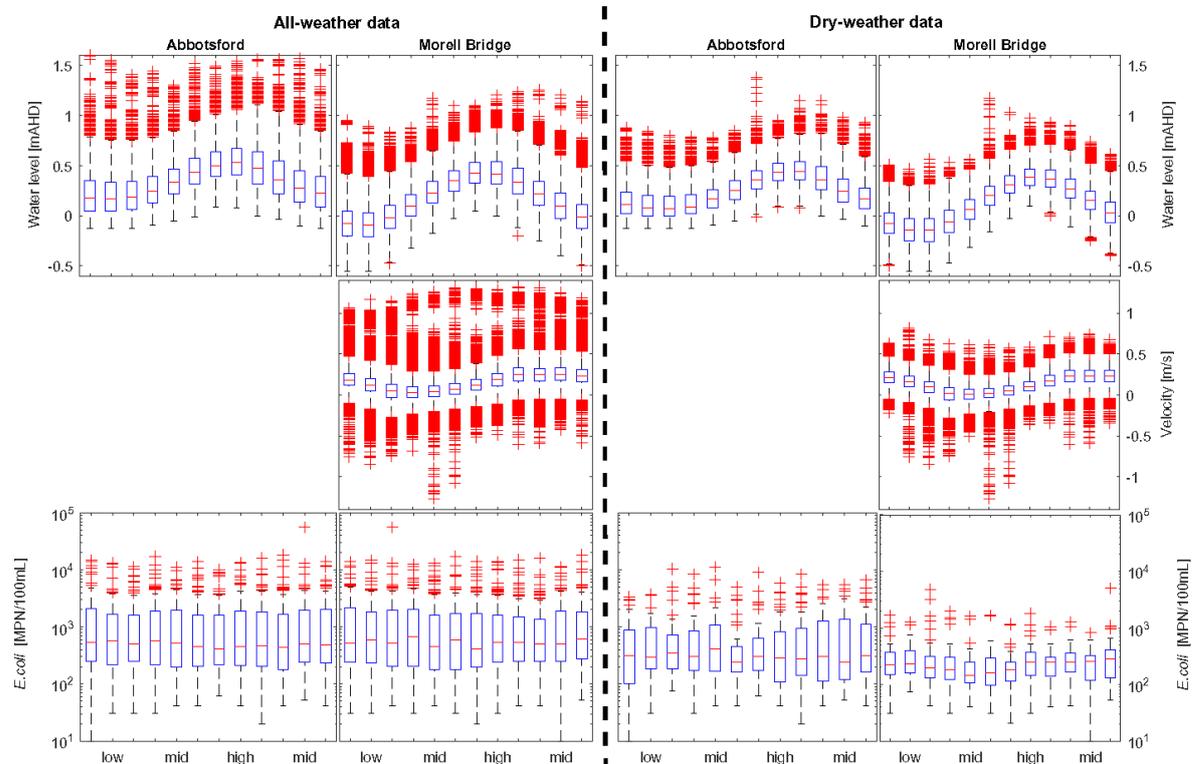


Fig. 2. Boxplots of measured water level, flow velocity, *E. coli* concentrations at Abbotsford and Morell Bridge binned into twelve equally-spaced intervals across each tidal cycle. Positive velocities are flowing in a downstream direction while negative velocities are flowing in an upstream direction. *E. coli* units are in Most Probable Number (MPN) per 100 mL. Left hand plots are for all-weather data; right hand plots are for dry-weather only data.

water level and flow velocity median values of each bin were shifted by increments of 1 h up to 11 h so that the whole tidal cycle was covered. For each shift increment, a Spearman rank correlation coefficient (ρ) and an associated p-value were calculated.

3. Results and discussion

Measured water level, flow velocity and *E. coli* concentrations at Abbotsford and Morell Bridge binned into twelve equally-spaced intervals over each tidal cycle are presented in Fig. 2 for both all-weather data (left) and dry weather data only (right). On average, *E. coli* concentrations oscillate around 500 MPN/100 mL, with 5th and 95th percentiles around 100 MPN/100 mL and 5000 MPN/100 mL at both sites, respectively. These statistics all reduce when considering dry weather data only (Fig. 2; right) with median *E. coli* concentrations oscillating around 200 MPN/100 mL.

At Abbotsford for all-weather data, the most significant negative correlations between *E. coli* and water levels were found with no shift (i.e. 0 h – $\rho = -0.79$, $p < 0.01$; Fig. 3, top left). This translates to higher *E. coli* levels corresponding to lower water levels (and vice versa). While there were no measurements of flow velocity at Abbotsford, it is likely that existing correlations with high/low tidal stage are actually caused by variations in flow velocity (hence shear forces) which could, for example, cause resuspension of bed sediments. The phase shift between maximum water level and flow velocity changes along the estuary and is influenced by relative magnitudes of tidal and riverine forcing, topography of the estuary and bottom friction (Dyer, 1997). In fact, at upstream boundaries of estuaries, like Abbotsford, which are shallow and act like a river during low water levels, the phase

shift is likely close to six hours (i.e. at high tide, velocities are close to their minimum because of high water depths, while at low tide velocities are close to their maximum because the system is acting like a river and directly conveying upstream inputs). For dry weather only data (Fig. 3, top right), similar correlation patterns persist, albeit not being statistically significant. This is likely because the dry weather conditions result in lower velocities and hence reduced resuspension contributions at this site. The sediments found at Abbotsford are much coarser, ranging from fine to coarse sands, than silt/clay sediments found in lower estuarine sections (Ellaway et al., 1982), thus require higher shear stress/flow velocity to be resuspended, which supports the hypothesis above.

While similar patterns were observed between *E. coli* and water levels at Morell Bridge, two notable differences were observed between this site and Abbotsford. First, Morell Bridge's all-weather *E. coli* concentrations were never significantly associated with tidal stage (Abbotsford's were). The inclusion of wet weather data at Morell Bridge may have skewed the all-weather data analysis, whereby multiple sources of *E. coli* exist, hence dwarfing any, more subtle, within-estuary processes, such as resuspension. Indeed, Yarra River and Gardiners Creek, together with 208 stormwater drains between Abbotsford and Morell Bridge, discharge directly into the estuary during storm events delivering up to 50% of the total daily load of *E. coli* (Jovanovic et al., 2015). This is much more complex than what occurs at Abbotsford, where one river feeds this site.

Second, Morell Bridge's dry weather *E. coli* concentrations were significantly correlated to water level and the best positive correlation was observed at 9 h phase shift (the best positive correlation for Abbotsford occurred when a shift was applied at 6 h). It is hypothesised

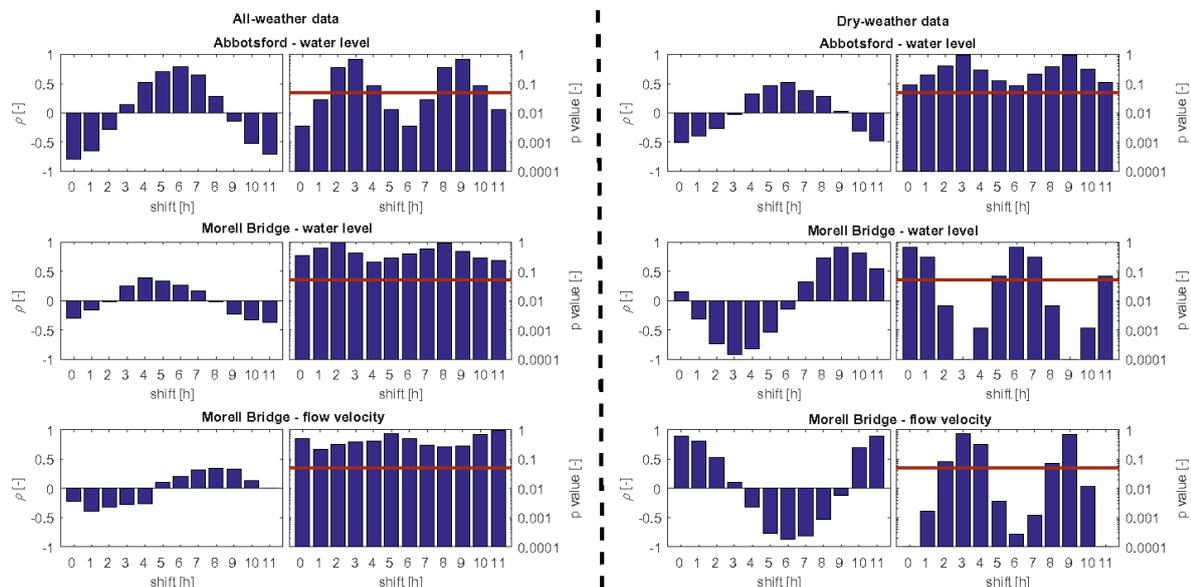


Fig. 3. Spearman rank correlation coefficients (ρ) and corresponding p-values between *E. coli* levels and water levels at Abbotsford (top), *E. coli* levels and water levels at Morell Bridge (middle) and *E. coli* levels and flow velocity at Morell Bridge (bottom) and applying various shift intervals to: all-weather data (left) and dry weather data only (right). The red line indicates a p-value of 0.05. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

that excluding the wet weather data means that the subtler processes (such as resuspension from bed and bank sediments) may be observed. The optimum phase shift of 9 h between *E. coli* and water levels is thought to be linked to the fact that a similar phase shift is seen between velocity and water level at Morell Bridge (Fig. 2 right; correlations between velocity and water level are shown in more detail in Supplementary material - Fig. S1). This hypothesis is further reinforced by the significant positive correlations between *E. coli* and flow velocity (no shift) found at Morell Bridge during dry weather ($\rho = 0.89$, $p < 0.001$; Figs. 2 and 3), suggesting that higher velocities, and hence shear related processes, result in higher *E. coli* levels.

It is speculated that a number of processes could be contributing to the observed relationship between *E. coli* and flow velocity at Morell Bridge during dry weather. Firstly, *E. coli* stored in bed sediments of the estuary (i.e. all sediments located below the level of low tide) might be introduced into the water column via resuspension caused by high flow velocities during mid tides (Pachepsky and Shelton, 2011). Secondly, estuarine bank sediments (i.e. sediments exposed to periodic wetting and drying during tidal cycle) are a known source of *E. coli* and provide favourable environment for its survival and growth (Solo-Gabriele et al., 2000; Desmarais et al., 2002). This was also confirmed for the Yarra River estuary (Schang et al., 2016). As such, *E. coli* stored in the bank sediments may be introduced into the water column by resuspension during the high velocities of mid-tides. It is possible that the relationship between *E. coli* and tides was not statistically significant at the uni-directional Abbotsford site during dry weather because it is located at the upstream estuarine boundary, with only small bank/bed sediment areas upstream (meaning that contribution from these could be limited as compared to Morell Bridge which is located at the downstream end of the estuary and has 16 km of bed and banks upstream to contribute to these effects).

There are other potential processes that are not resuspension-related that could explain some of these relationships. For example, salinity changes due to tidal fluctuations could contribute to the link between water level and *E. coli* level (i.e. high tides result to higher salt intrusion from the bay, and hence lower *E. coli* because of die-off related processes; (Fujioka et al., 1981; Šolić and Krstulović, 1992; Mallin

et al., 1999; Mill et al., 2006)). However, due to the stratified nature of the Yarra River estuary, salinity concentrations in top of the water column where the *E. coli* levels were analysed never exceeded 8 psu (Jovanovic et al., 2017). This level is not known to cause excessive die-off as compared to fresh water (Mancini, 1978; Hipsey et al., 2008) and the salinity variation over the tidal cycle is likely to be low due to limited mixing between the salt-wedge and overlaying freshwater layer. As a result, the influence of salinity to the observed correlations are expected to be minimal if any.

4. Conclusions

A large dataset of *E. coli* concentrations (over 3500 samples) along with high resolution water level and flow velocity measurements, was used to examine relationships between *E. coli* levels, water levels and flow velocities at two locations within an urban salt-wedge estuary.

E. coli levels at Abbotsford were significantly negatively correlated with water levels. While there were no flow velocity measurements at this site to test the relationship with *E. coli*, it is likely that observed relationship with water level is actually caused by variation in flow velocity and related resuspension of bed sediments and associated *E. coli*. Due to weakened tidal influences at Abbotsford, the maximum flow velocities/resuspension are likely to occur near low tide and vice versa. When wet weather data was removed a similar pattern of correlation coefficients was observed but no significant correlations were found, probably due to the limited ability of flow to cause resuspension during dry weather.

Contrary to Abbotsford, no significant correlation with water levels or flow velocity was observed at Morell Bridge, likely due to many *E. coli* inputs entering between Abbotsford and Morell Bridge and dwarfing the subtler within-estuary processes, like resuspension. Indeed, removal of wet weather data enabled significant correlations with water level and flow velocity to emerge. The strongest positive correlation with water level was observed when a 9 h shift was applied, which was similar to the phase shift between water levels and flow velocity at this site. The strongest positive correlation with flow velocity (no shift) supported the hypothesis that the observed relation-

ships could be related to bed and bank sediment resuspension.

These findings may also explain contrasting reports about *E. coli* levels and tidal stage relationships found by previous studies where flow velocities and time shifts in different sections of estuaries have not been taken into consideration.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.marpolbul.2017.04.004>.

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4.4 Spatial variability of *E. coli* in an urban salt-wedge estuary

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journal homepage: www.elsevier.com/locate/marpolbulSpatial variability of *E. coli* in an urban salt-wedge estuaryDusan Jovanovic^a, Rhys Coleman^b, Ana Deletic^a, David McCarthy^{a,*}^a Environmental and Public Health Microbiology (EPHM) Laboratory, Monash Infrastructure Institute, Department of Civil Engineering, Monash University, Clayton, Victoria 3800, Australia^b Melbourne Water Corporation, Docklands, Victoria 3008, Australia

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ABSTRACT

This study investigated the spatial variability of a common faecal indicator organism, *Escherichia coli*, in an urban salt-wedge estuary in Melbourne, Australia. Data were collected through comprehensive depth profiling in the water column at four sites and included measurements of temperature, salinity, pH, dissolved oxygen, turbidity, and *E. coli* concentrations. Vertical variability of *E. coli* was closely related to the salt-wedge dynamics; in the presence of a salt-wedge, there was a significant decrease in *E. coli* concentrations with depth. Transverse variability was low and was most likely dwarfed by the analytical uncertainties of *E. coli* measurements. Longitudinal variability was also low, potentially reflecting minimal die-off, settling, and additional inputs entering along the estuary. These results were supported by a simple mixing model that predicted *E. coli* concentrations based on salinity measurements. Additionally, an assessment of a sentinel monitoring station suggested routine monitoring locations may produce conservative estimates of *E. coli* concentrations in stratified estuaries.

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1. Introduction

Increased faecal contamination of estuarine and coastal waters around the world represents major issue in water quality management today due to its implications on public health through associated aquatic recreation and aquaculture. Additionally, elevated faecal pollution levels can have substantial economic implications through costs associated with medical treatment of waterborne illnesses caused by faecal pathogens directly, or indirectly by impacting tourist activities and associated local businesses (e.g. closure of beaches). For example, estimated cost for treatment of waterborne illnesses for only two beaches in California, USA was \$3.3 million per annum (Dwight et al., 2005). As such, increased efforts are needed to understand faecal microorganism dynamics in estuarine environment, which in turn would lead to effective mitigation strategies and, ultimately, to improved recreational water quality.

Understanding faecal microorganism dynamics in an estuarine system starts with collecting information about the levels of faecal pollution. This is achieved through monitoring programs, which commonly involve collection of water samples from discrete points within the analysed estuary. Often, due to logistical and financial constraints, sampling sites are sparsely distributed along the system, and water samples collected from these sites are assumed being representative of faecal microbe levels within an entire estuarine reach. However, the choice

of the precise sampling location can have significant impact on the measured faecal microorganism levels especially in the case of such complex environments. For example, Quilliam et al. (2011) highlighted the importance of spatial variability by showing that samples taken within the same cross-section, but on opposite sides of an estuary, led to contrasting classifications of microbial water quality. Nevertheless, in spite of the importance of the issue, literature lacks comprehensive studies on spatial variability of faecal microorganisms within estuarine environment, thus leaving a significant knowledge gap in understanding this aspect of faecal microorganism dynamics.

Furthermore, numerical models are increasingly being used to help understand faecal microorganism dynamics in estuaries and serve as a management tool for defining effective mitigation strategies (Salomon and Pommepuy, 1990; Kashefipour et al., 2002; Garcia-Armisen et al., 2006; de Brauwere et al., 2011; Gao et al., 2011; Liu and Huang, 2012; Bedri et al., 2013; de Brauwere et al., 2014; Gao et al., 2015; Liu et al., 2015). These models are typically complex 1D/2D or 3D hydrodynamic-microorganism models – consistent with the complexity of the environment and the processes modelled. It is well established that as the complexity of a model increases, so does the need for data quantity and quality for model testing (Grayson and Blöschl, 2001), in order to be able to fully exploit model capabilities. Despite this, data supporting the spatial dimensionality of models is often missing. For example, some studies have collected longitudinal profiles of estuarine faecal microorganism concentrations (Salomon and Pommepuy, 1990; de Brauwere et al., 2011), but none have measured vertical concentration profiles of faecal microorganisms, which is essential for testing 3D models. It is clear that lack of understanding the spatial variability of faecal microbes within a water body can hinder the robustness of such models and limit

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their application. Finally, application of improperly validated models and associated decision-making could have serious implications both in terms of management costs and water quality benefits.

The aim of this study was to characterize the spatial variability of a commonly used faecal indicator organism (*Escherichia coli*) in a highly stratified, salt-wedge estuary in Melbourne, Victoria, Australia. Data were collected and analysed to understand the factors influencing vertical variability (i.e. along the depth of water column), transverse variability (i.e. across the estuary), and longitudinal variability (i.e. along the estuary). An additional objective was to test the assumption of representativeness of a single *E. coli* sample collected from a fixed, routine monitoring station of the overall *E. coli* levels within that cross-section.

2. Methods

2.1. Study site

The Yarra River is located in south-eastern Australia and is the major river which flows through the urban metropolis of Melbourne. It has a total length of 242 km and drains a catchment of 4000 km² comprising forested headwater reaches, predominantly rural mid-reaches and urbanised lower reaches, before discharging into Port Philip Bay. The last 22 km represent its estuarine section, which has a well-defined upstream boundary (i.e. an artificial weir, Dights Falls, which prevents tidal propagation upstream). The estuary is used for secondary contact water recreation (especially rowing, kayaking, and fishing) while primary contact recreation is either restricted due to boat navigation or is not recommended due to frequently high levels of faecal indicator microbes (Department of Sustainability and Environment, 2012).

The Yarra River estuary is categorised as a highly-stratified, salt-wedge estuary (Beckett et al., 1982) and is micro-tidal (i.e. having a tidal range <2 m, (Dyer, 1997)). Water level fluctuations within Port Philip Bay average 0.5 m but vary between 0.3 and 0.9 m. The tidal pattern is semi-diurnal with a significant diurnal variation (Beckett et al., 1982).

The major input of fresh water to the estuary is the Yarra River, which contributes about 70% of the total flow at the estuary mouth (Sokolov and Black, 1996). Other freshwater inputs include Gardiners

Creek in the upper estuary (~7.5 km downstream of the Dights Falls) and Maribyrnong River and Moonee Ponds Creek in the lower part of the estuary. Additionally, there are >200 stormwater drains discharging directly to the estuary, some of which have pipe diameters >3 m (Daly et al., 2013; Jovanovic et al., 2015).

2.2. Depth profiling

The depth profiling was done at four key cross-sections, spanning the entire estuary: at Abbotsford, Hawthorn, Morell Bridge and South Bank, located 0.5, 6.2, 10.1 and 12.4 km from the beginning of the estuary, respectively (Fig. 1). To understand the spatial variability of *E. coli* within the estuary, depth profiling was conducted by collecting water samples and taking in-situ water quality measurements at increments of 25 to 50 cm below the water surface (increments depended on the position of salt-wedge, with coarser resolution applied below the halocline, where variability in water quality was significantly lower). In-situ water quality measurements were made using a multi-parameter probe (Hydrolab DS5X): Temperature (Temp; °C), Salinity (Sal; psu), Turbidity (Turb; NTU), pH, and dissolved oxygen (DO; mg/L). Water samples were retrieved using a peristaltic pump which had the inlet suction pipe attached to the multi-parameter probe. Samples were collected into 200 ml sterile PET bottles and stored in coolers on ice until transported to the Environmental and Public Health Microbiology Laboratory (EPHM Lab) at Monash University where they were analysed for *E. coli*, well within 12 h of collection (McCarthy et al., 2008), using the Colilert method (IDEXX Laboratories, 2013).

Single- and double-pass depth profiling methods were used to provide the necessary data for assessment of the spatial variability of *E. coli*. The specific aim of the single pass profiling was to provide data for assessing cross-sectional and transverse variability of *E. coli*. This procedure entailed moving in one direction along the estuary (i.e. from South Bank to Abbotsford) and sampling three verticals across the width of each cross-section at each monitoring site. One vertical was positioned at the thalweg, while the other two were distributed evenly across the width of the estuarine cross-section depending on the thalweg position. All three depth profiles within the cross-section were obtained within 10–30 min of each other. This enabled a cross-sectional analysis of

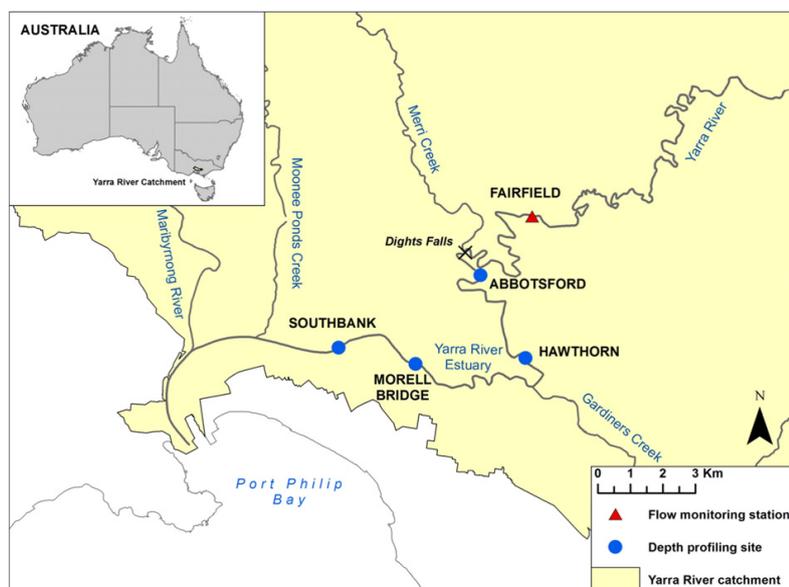


Fig. 1. Yarra River Estuary and four depth profiling sites: Abbotsford, Hawthorn, Morell Bridge and South Bank.

variability at each site, at approximately the same tidal stage and hydrodynamic conditions.

Double pass depth profiling aimed at assessing the impact of salt-wedge intrusion on water quality along the depth of the water column. The extent of the salt-wedge intrusion into the estuary is dependent on tidal stage and the riverine flow rate (Dyer, 1997), so this double pass profiling aimed at collecting data both during different tidal stages and different riverine flow rates. Each double pass depth profiling day consisted of monitoring the same vertical (i.e. the deepest vertical - at the position of thalweg) at each site, twice; by repeating this on the same day, we could capture two different tidal conditions while maintaining very similar riverine flow rates. The first pass was conducted moving upstream from South Bank to Abbotsford. Following a 3 h intermission at Abbotsford, to allow for change in tidal conditions and hence salt wedge position, a second pass was conducted in the downstream direction, from Abbotsford to South Bank. This double pass profiling experiment was repeated on different dates to capture the effect of different riverine flow rates.

Ten monitoring campaigns (5 single pass depth profiling and 5 double pass depth profiling) were conducted across a 17 month period with 84 depth profiles monitored in total (Table 1). Due to technical issues with the probe, some water quality parameters were not available for some campaigns (Table 1). Also three campaigns had to be terminated due to issues with the boat, hence the monitoring was done only partially. These instances were marked as incomplete but still used in the analyses where possible.

2.3. Cross sectional sampling uncertainty

2.3.1. Transverse uncertainty

The transverse uncertainty of *E. coli* (i.e. the uncertainty attributed to only sampling on the edge or thalweg) was estimated following Eq. 1 which uses *E. coli* concentrations made at discrete depths in the three verticals sampled at each monitoring site during the single pass depth profiling. This uncertainty was then compared to the analytical uncertainty of the laboratory method used for enumeration of *E. coli* (McCarthy et al., 2008).

$$\%unc. = \frac{\Delta x_i}{x_i} \approx \frac{2u(x_i)}{x_i} = \frac{2(s/\sqrt{n})}{x_i} \quad (1)$$

where: x_i is the sample mean of the *E. coli* concentrations obtained a similar depths across the cross-section (in most cases, samples were within 10 cm of each other, but sometimes up to 20 cm), $\Delta x_i/x_i$ is the relative uncertainty of the *E. coli* concentration, $u(x_i)$ is the standard error

of the mean, s is standard deviation of *E. coli* concentration and n is the size of sample (i.e. $n = 3$; samples at similar depths from three verticals within the cross-section). Equation follows that of McCarthy et al. (2008).

2.3.2. Single sampling point uncertainty

The Morell Bridge site has been used for the past four years as a fixed, routine monitoring site for the estuary. The site has an automatic sampler installed on the right bank for the collection of samples during both dry and wet weather periods. A sampling tube inlet is attached to a floatation device which enables water sampling approximately 100 mm below the water surface at all times (i.e. it samples the part of the water column most likely to come into contact with those undertaking recreational activities). An attempt was made in this study to compare the water quality observations made at the routine monitoring site (i.e. via the automatic sampler) to the water quality observations made using more robust measurement methods (i.e. the depth profiling data described above). Specifically, the aim of this activity was to assess how representative a single, discrete water sample collected by the autosampler is of microbial levels within the entire cross-section. As such, the automatic sampler was used to retrieve a water sample immediately after depth profiling measurements; the *E. coli* concentration of this single sample was compared to the area-weighted average cross-sectional *E. coli* level calculated using the depth profiling measurements. Additionally, to determine if the monitoring station's discrete sample was representative of the top freshwater layer only, the top freshwater layer *E. coli* level was also compared to the average freshwater cross-sectional *E. coli* level calculated using only depth profiling samples located above the halocline.

2.4. Mixing modelling

E. coli concentration, and thus its spatial variability, within an estuary is largely governed by transport processes (i.e. hydrodynamics) and interactions within the estuarine environment (e.g. sediment-bacteria interaction and impact of environmental factors on *E. coli* survival). Therefore, to help understand how these processes impact on the spatial variability of *E. coli*, simple mixing calculations were performed (see Eqs. (2) and (3)). These calculations used salinity measurements as a tracer compound to estimate the expected turbidity and *E. coli* concentration profiles; these expected profiles were then compared to actually measured profiles to better understand processes. The assumptions of these calculations were that sea water is free of turbidity and *E. coli* (i.e. has turbidity and *E. coli* concentration equal to zero). This is a reasonable assumption, especially since faecal indicator microorganisms are not able to withstand extreme salinities (Carlucci and Pramer, 1960; Mancini, 1978; Fujioka et al., 1981; Šolić and Krstulović, 1992), and our data indicate minimal *E. coli* levels in deep marine waters. We also assumed a constant salinity of 35 psu for sea water and a constant salinity of 0.1 psu for freshwater; again, both of these assumptions match literature (Eaton et al., 2005) and were also supported by our datasets for the Yarra River estuary. The use of a conservative tracer implicit in these calculations allows us to understand the importance of various neglected sources, processes and die-off kinetics in governing *E. coli* concentrations in the estuary. Furthermore, comparisons between *E. coli* and turbidity (which has no known die-off kinetics but does exhibit strong settling properties) also allows inference about major processes.

$$Turb_{estimated} = Turb_{river} * \left(1 - \frac{Sal_{measured} - 0.1}{35}\right) \quad (2)$$

$$E.coli_{estimated} = E.coli_{river} * \left(1 - \frac{Sal_{measured} - 0.1}{35}\right) \quad (3)$$

where: $Turb_{estimated}$ – estimated turbidity [NTU] at particular time and position within the water column based on using salinity as a

Table 1
Description of depth profiling monitoring events.

Date	Profiling method	Water quality variables measured						Measurements complete/incomplete
		Temp	Sal	Turb	DO	pH	<i>E. coli</i>	
23/04/2013	Single pass	✓	✓	✓	✓	✓	✓	Incomplete ¹
30/04/2013	Double pass	✓	✓	✓	✓	✓	✓	Complete
04/06/2013	Single pass	✓	✓	✓		✓	✓	Complete
26/06/2013	Single pass	✓	✓		✓	✓	✓	Complete
30/07/2013	Double pass	✓	✓	✓	✓	✓	✓	Complete
12/12/2013	Single pass	✓	✓	✓	✓	✓	✓	Complete
09/05/2014	Single pass	✓	✓	✓	✓	✓	✓	Complete
06/06/2014	Double pass	✓	✓	✓	✓	✓	✓	Incomplete ²
06/08/2014	Double pass	✓	✓	✓	✓		✓	Incomplete ³
19/08/2014	Double pass	✓	✓	✓	✓	✓	✓	Complete

¹ – no measurements at Abbotsford; ² – no measurements at Morell Bridge and Southbank and only one pass conducted at Abbotsford and Hawthorn; ³ – only one pass conducted at all sites;

conservative tracer; $Turb_{river}$ – measured turbidity [NTU] of the upstream riverine input, which is estimated as the average measured turbidity of the samples located in top third of the water column at Abbotsford on a particular date of monitoring; $Sal_{measured}$ – the measured salinity [psu] at a particular time and position within the water column; $E. coli_{estimated}$ – the estimated *E. coli* concentration [MPN/100 ml] at particular time and position within the water column based on using salinity as a conservative tracer; and $E. coli_{river}$ – *E. coli* concentration [MPN/100 ml] of the upstream riverine input, which is estimated as the average measured concentration of the samples located in the top third of the water column at Abbotsford on a particular date of monitoring.

Once the estimated concentrations were obtained, they were paired with measured turbidity and *E. coli* levels taken at the same site/depth and ratios of measured/estimated levels could then be calculated. A ratio > 1 (i.e. measured level was greater than estimated) suggests an unidentified input/process is contributing to an increase in turbidity or *E. coli*, whereas a ratio < 1 suggested the opposite (i.e. an unidentified input/process is contributing to a decrease in turbidity or *E. coli*).

2.5. Data analyses

Standard descriptive statistics were calculated for each water quality variable. Boxplots were used to explore the longitudinal and vertical variability of salinity, turbidity, and *E. coli* concentrations. Data from each monitoring site were binned according to the sampling position within the water column i.e. top, middle, and bottom sections of the water column. When a salt-wedge was present at a monitoring site, the top of the water column was the section above the halocline, the middle was the section of the salinity gradient (i.e. halocline layer), and the bottom was the section below the halocline. When there was no salt-wedge, the top, middle, and bottom sections were simply split evenly across the water depth. In order to determine possible links, non-parametric correlation analyses (using Spearman Rank correlation coefficients) were performed with *E. coli* concentrations as the response variable and each of the other five measured water quality variables as predictor variables.

3. Results and discussion

3.1. Vertical variability of *E. coli*

Salinity and *E. coli* depth profiles in the Yarra River estuary are summarised in Fig. 2 while Fig. 3 provides detailed information from a single pass depth profiling conducted on 12th December 2013. Both the depth of the salt-wedge and *E. coli* concentrations varied significantly over the monitoring period (Fig. 2). At the two upstream sites (i.e. Abbotsford and Hawthorn) *E. coli* concentrations generally ranged over an order of magnitude, while at the two downstream sites (i.e. Morell Bridge and South Bank) *E. coli* varied by over two orders of magnitude.

When the salt-wedge was absent at the monitoring site (e.g. Fig. 2 – Abbotsford), vertical variability of *E. coli* showed no particular pattern other than random variation around median values, indicating well-mixed conditions along the depth. This was also apparent in the other water quality parameters which also demonstrated well-mixed conditions of the estuary in the absence of the salt-wedge (e.g. Fig. 3 – Abbotsford). Conversely, when the salt-wedge was present, a negative gradient of *E. coli* concentrations with depth was observed (i.e. with the increase in depth there was a decrease in *E. coli* concentration). This was reflected by a significant negative correlation that was found between salinity and *E. coli* concentrations at sites where a salt wedge was observed (i.e. Morell Bridge $\rho = -0.45, p < 0.001$; and South Bank $\rho = -0.58, p < 0.001$; Fig. 2). It is well established that increased salinity reduces the survival of faecal bacteria (Carlucci and Pramer, 1960; Mancini, 1978; Fujioka et al., 1981; Šolić and Krstulović, 1992); therefore, seawater typically has low *E. coli* levels and fresh riverine

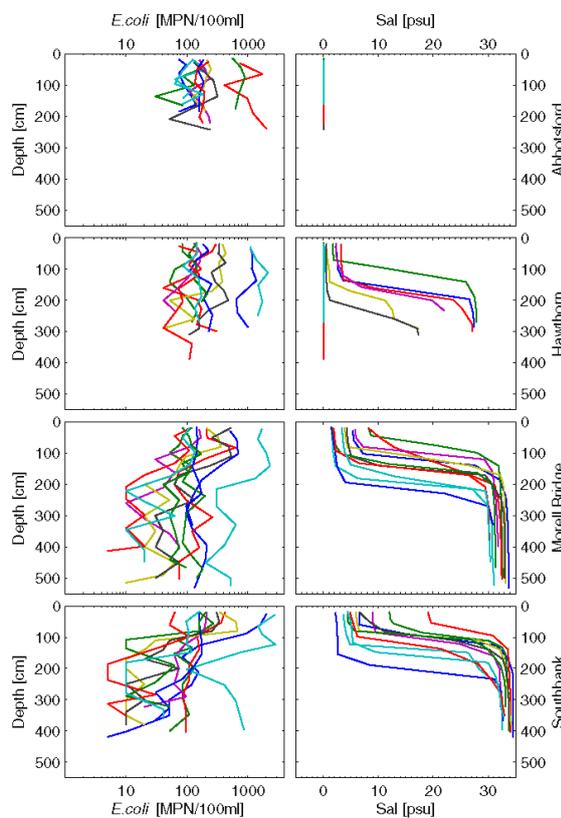


Fig. 2. Vertical profiles of *E. coli* and salinity at four sites in the Yarra River estuary. Results are from 10 monitoring events during 2013 and 2014 using all collected profiles. Each colour represents different profile.

water may contain much higher levels. The existing correlations are more likely to be the consequence of the minimal mixing between fresh and seawater layers in highly stratified estuaries (Dyer, 1997), rather than acute exposure of *E. coli* to saline waters. This is because the majority of the mixing between the salt-wedge and the overlying freshwater tends to occur near the toe of the salt-wedge, enabling *E. coli* (and other pollutants) to penetrate into salt-wedge. At downstream sites, mixing is significantly reduced and hence limits the supply of *E. coli* into the salt-wedge, thus creating stronger negative correlations at these sites as opposed to upstream sites. To further examine this observation, relationships between *E. coli* and other water quality parameters were explored.

A number of significant correlations were identified between *E. coli* concentrations and other water quality parameters. For instance, there was a weak (but significant) positive correlation between *E. coli* concentration and temperature within the whole dataset ($\rho = 0.09, p < 0.05$). Yet it is well documented that die-off of faecal microbes increases with increasing temperature in both riverine and sea water (Orlob, 1956; McFeters and Stuart, 1972; Faust et al., 1975; Mancini, 1978; McCambridge and McMeekin, 1980; Flint, 1987; Šolić and Krstulović, 1992; Jamieson et al., 2004). Additionally, a strong negative correlation with pH ($\rho = -0.57, p < 0.001$) was found, in spite of the measured pH range within the Yarra River estuary (i.e. 7–8) being well within the range of negligible impact on survival of faecal microbes (i.e. 6–8) (Reddy et al., 1981; Hipsey et al., 2008). Finally, significant positive correlations were observed between *E. coli* concentrations and both DO

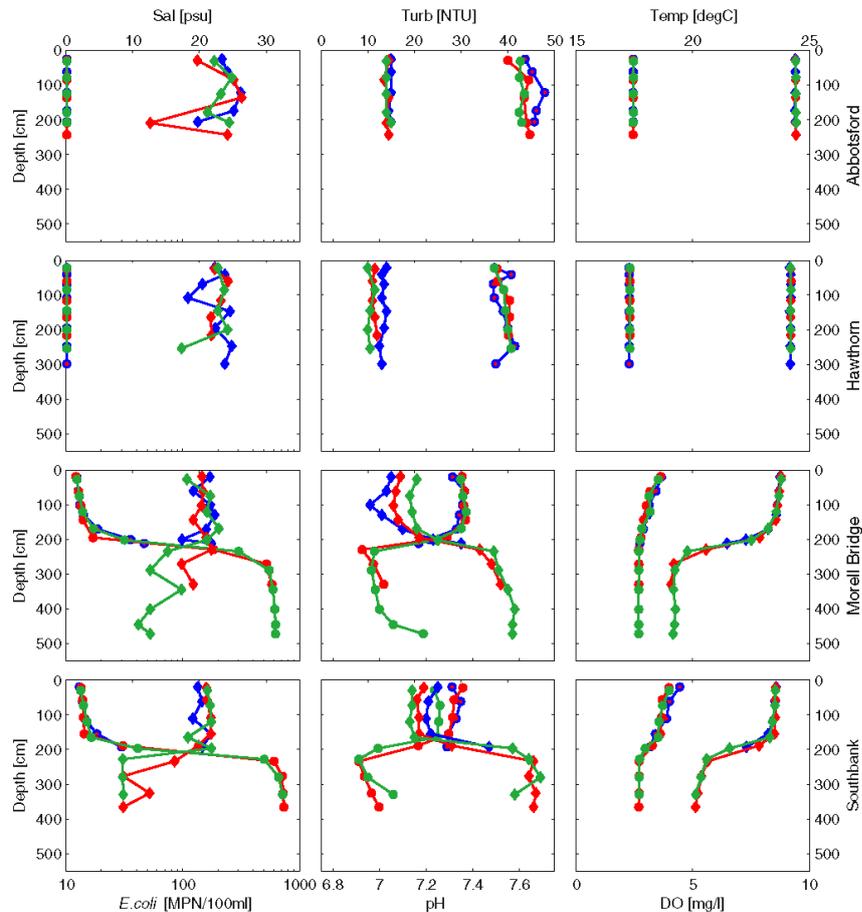


Fig. 3. Single pass depth profiling results on 12th December 2013. Blue - left vertical; red - middle vertical and green - right vertical. Circles correspond to variable labels on the top of the graph (i.e. Salinity ('Sal'), Turbidity ('Turb') and Temperature ('Temp')), while diamond markers correspond to variable labels on the bottom of the graphs (i.e. *E. coli*, pH and dissolved oxygen ('DO')).

($\rho = 0.31, p < 0.001$) and turbidity ($\rho = 0.60, p < 0.001$). These correlations are attributed to differing water quality characteristics of the freshwater layer and salt-wedge, and not necessarily causal relationships linked to acute microbial kinetics; i.e. freshwater is oxygen-rich, highly turbid and carries high loads of *E. coli* while the salt-wedge is often limited in dissolved oxygen, suspended sediments, and largely free of *E. coli* (Fig. 2).

3.2. Influence of flow and tides on salt-wedge dynamics and vertical distribution of *E. coli*

As identified above, the presence of the salt-wedge within the water column has a substantial influence on the vertical variability of *E. coli* in the Yarra River estuary. As shown in Fig. 2, at Morell Bridge on some occasions the halocline was present at <1 m below the water surface, while on another occasion it was below 2.5 m deep at the same site. Two main factors determine the extent of salt wedge intrusion into estuary: flow rate and tides (Dyer, 1997). As shown in the Fig. 4, there is a strong power relationship between the Yarra River flow rate at Fairfield and the depth to salt wedge at all three sites where the salt wedge was observed (i.e. Hawthorn, Morell Bridge and South Bank). As expected, during higher freshwater flow rates, the salt-wedge is forced downstream, hence the depth to salt-wedge increases. For example, when

the freshwater flow of the Yarra is $5 \text{ m}^3/\text{s}$, the depth to the halocline at Morell Bridge is around 1 m, while during flows of $30 \text{ m}^3/\text{s}$ the depth to the salt-wedge is over 2 m at the same site. This fluctuating depth to the salt-wedge implies that the toe of the salt-wedge also moves up and down the estuary. Also, as the freshwater flow rate decreases below $10 \text{ m}^3/\text{s}$ the salt-wedge was detectable at Hawthorn (i.e. depth to salt-wedge is <3 m which is the maximum depth measured at this site; Fig. 4).

Tides are the other major factor determining the extent of salt-wedge intrusion into the estuary. Beckett et al. (1982) reported that the salt-wedge position can change a few kilometres between high and low tides in the Yarra River estuary. Fig. 5 shows the results of double pass depth profiling conducted on 30th July 2013. There was no rainfall that day, and the freshwater flow rate of the Yarra River at Fairfield ranged between 6 and $7 \text{ m}^3/\text{s}$, indicating that the only hydraulic changes were due to tidal variations. The salt-wedge was not observed at Abbotsford, hence there was no change in vertical structure of both salinity and *E. coli* depth profiles. Conversely, the salt-wedge was present at the other three monitoring sites (Hawthorn, Morell Bridge, and Southbank), indicated by a distinct change in salinity and *E. coli* depth profiles. There is a clear shift in the position of the salt-wedge between the first and second pass sampling times. However, the vertical shift of the salt-wedge was greatest at Hawthorn (~80 cm), while it was barely

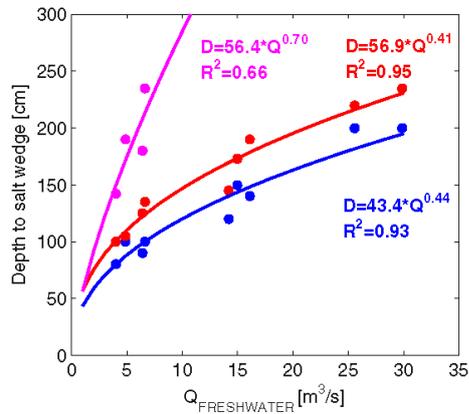


Fig. 4. Influence of Yarra River flow rate at Fairfield on depth to salt-wedge at Hawthorn (magenta), Morell Bridge (red) and Southbank (blue).

noticeable at Southbank. Indeed, at any given time point, the depth to the salt wedge increased in the upstream direction indicating the existence of a longitudinal gradient in the salt-wedge (Fig. 4). Furthermore, the vertical shifts in *E. coli* depth profiles follow the shift in salinity profiles. This further demonstrates that vertical variability of *E. coli* within the Yarra River estuary is closely related to salt-wedge dynamics.

3.3. Transverse variability and uncertainty

There was no noticeable transverse variability (i.e. across the cross-section) in *E. coli* concentrations for any site (e.g. see Fig. 3 for sampling done on 12th December 2013). Conversely, Quilliam et al. (2011) found significant transverse variability, but this was likely linked to their extreme estuary width (on average, 10 times the Yarra River estuary), which influences transport and mixing processes. Furthermore, we hypothesise that any detectable transverse variability that may exist in the Yarra estuary is dwarfed by the analytical and storage uncertainties involved in measuring *E. coli* concentrations. Fig. 6 presents distributions of relative uncertainty between the samples taken across each cross section at similar depths. These results show that the relative transverse uncertainties largely fall within the range of the analytical uncertainties reported for *E. coli* (McCarthy et al., 2008; Harmel et al., 2016b). In addition to analytical uncertainty, another source of uncertainty is introduced by the storage conditions between sample collection and analysis (Harmel et al., 2016a). For example, McCarthy et al. (2008) found that the combined analytical and storage uncertainty in a discrete *E. coli* sample can be as high as 67%. Therefore, we conclude that the transverse variability of *E. coli* in the Yarra River estuary is, on average, less than the associated storage and analytical uncertainties.

3.4. Longitudinal variability of *E. coli*

Longitudinal distributions of measured salinity, turbidity, and *E. coli* along the Yarra River estuary in top, middle and bottom sections of the water column are given in Fig. 7. Salinity increased from the head to the mouth of the estuary in all three sections of the water column (i.e. 'top', 'middle' and 'bottom'). The salt-wedge was never observed at Abbotsford during our study; while at Hawthorn, it was intermittently present as shown by the high variability of the salt-wedge at this site (Fig. 7). In contrast, the salt-wedge was always present at Morell Bridge and Southbank as indicated by elevated salinity in the bottom sections at these monitoring sites (Fig. 7).

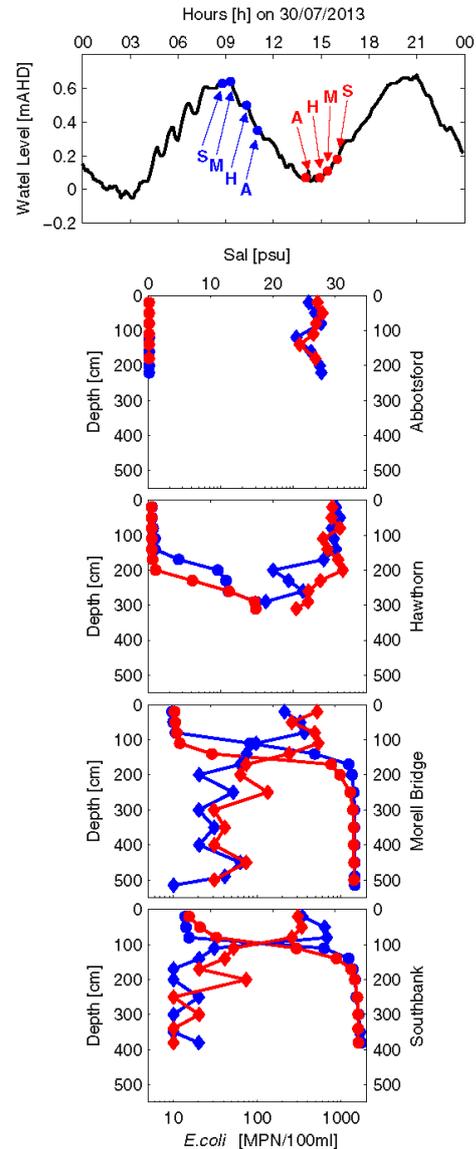


Fig. 5. Salinity and *E. coli* concentration double pass depth profiling results on 30th July 2013. The top graph shows measured water level at Southbank and indicates the times and water levels when depth profiles shown below were taken at each site ('A' = Abbotsford, 'H' = Hawthorn, 'M' = Morell Bridge, 'S' = Southbank). Circles show salinity profiles, while diamonds show *E. coli* concentration profiles.

Unlike salinity, turbidity generally decreased from the head to the mouth of the estuary. A number of processes could be contributing to this observation: 1) sediment settling 2) the entrainment and mixing with the low turbid sea water in sections downstream of Hawthorn when the salt-wedge was present, and 3) existence of cleaner water sources entering the estuary e.g. dry weather stormwater flow or groundwater. Simple modelling that accounted solely for mixing with sediment-free sea water (i.e. assumed turbidity equals zero), predicted higher turbidity levels in the top section of the water column than measured – suggesting that additional settling occurs or that additional cleaner water sources are entering the system. Sedimentation experiments using water from the Yarra River estuary (McCarthy et al.,

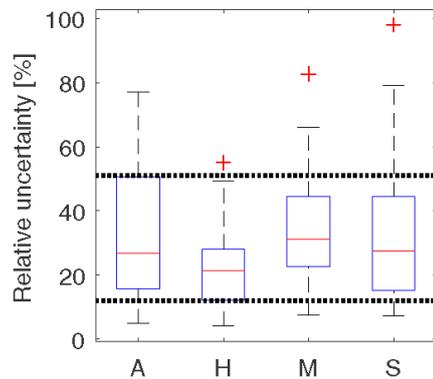


Fig. 6. Relative uncertainty of discrete water samples *E. coli* concentrations at approximately same depth from three depth profiles obtained during single pass sampling at Abbotsford (A), Hawthorn (H), Morell Bridge (M) and South Bank (S). Dashed line represents maximum and minimum relative analytical uncertainty in stormwater sample as reported by McCarthy et al. (2008)

2011), report that TSS levels decreased by about five times over a five hour experiment period, with the most significant decline occurring in the first 2 h. Using the mean measured upstream and downstream flow velocity (0.06 m/s and 0.18 m/s, respectively) and their relative duration (water moves upstream 18% of time; and downstream 82% of time (Jovanovic et al., 2015)), the average settling rate of 7.5 m/day (McCarthy et al., 2011) and the average depth in the upper section of the estuary (~2 m) most settling of sediments is expected to occur within 3 km downstream of Abbotsford. However, reductions in turbidity between Abbotsford and Hawthorn do not correspond to those predicted based on the rates derived from McCarthy et al. (2011). This difference could relate to sedimentation rates measured in still water compared to the turbulence of Yarra River estuarine flows that may retain fine sediment particles in suspension for longer periods (~70% of particles are <20 μm (Ellaway et al., 1982)) limiting the settling within the estuary. Additionally, Jovanovic et al. (2015) reported 208 stormwater drains along the estuary, together with a major urban creek, that discharge directly into the Yarra River estuary. These additional inputs could have impacted turbidity levels in top of the water column.

While saline water is often considered to be very low in turbidity, measurable (and sometimes high) levels of turbidity were detected in the salt-wedge (Fig. 7). Furthermore, the results of the simple mixing model also indicate turbidity levels at the bottom of the estuary are higher than expected based solely on mixing of the two water sources. There are several reasons for this. (1) Settling of sediment particles from the top of the water column could be increasing the turbidity levels in the salt-wedge. For example, Kostaschuk and Luternauer (1989) showed that migration of the salt-wedge into the estuary during a rising tide can cause rapid deposition of suspended material. (2) Re-suspension of the bottom sediments due to the salt-wedge movement within the estuary.

Measured *E. coli* concentrations in the top of the water column remain fairly constant along the estuary (Fig. 7), which could be explained by a combination of factors:

- Limited impact from environmental stressors. Despite an increase in salinity along the estuary, levels of salt found in the top of the water column may not be sufficient to increase the die-off rate of *E. coli* significantly compared to die-off rates in fresh water. In fact, based on a synthesis of experimental data from seven studies, Hipsey et al. (2008) showed that the die-off rate of 0.5/day below 20 psu is constant and equal to that of fresh water. Sunlight is also known to be detrimental to enteric microorganisms due to a photo-oxidative effect,

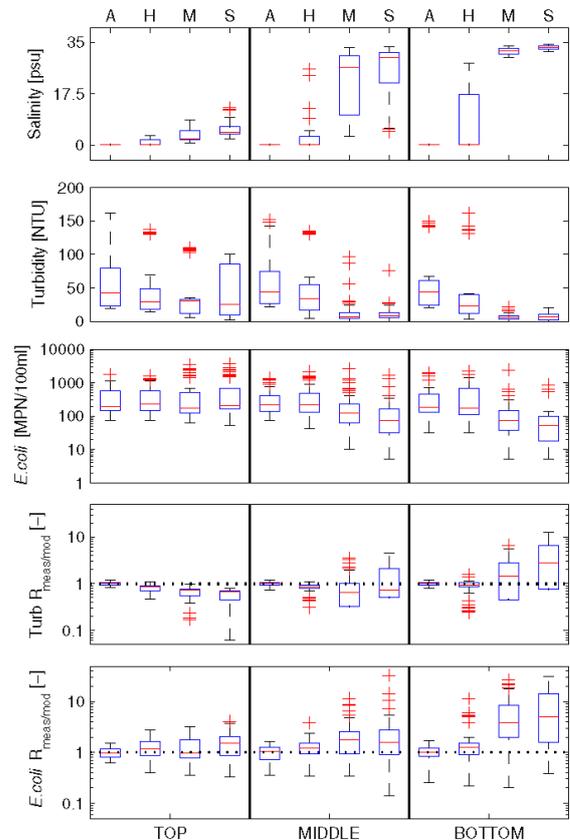


Fig. 7. Longitudinal variability in measured salinity, turbidity, *E. coli* and measured to modelled ratios of turbidity ($\text{Turb } R_{\text{meas/mod}}$) and *E. coli* ($E. coli R_{\text{meas/mod}}$) along the Yarra River estuary in top, middle and bottom sections of the water column. 'A' = Abbotsford, 'H' = Hawthorn, 'M' = Morell Bridge and 'S' = Southbank.

particularly in clear waters (Crane and Moore, 1985), and this effect is enhanced at high salinities (Šolić and Krstulović, 1992). However, the high turbidity of the freshwater layer in the Yarra estuary might substantially reduce the impact of sunlight on *E. coli* survival.

- Limited sedimentation. Faecal microbes are known to be able to attach to sediment particles, particularly fine grained sediments (<60 μm) i.e. silt and clay (Orlob, 1956; Gannon et al., 1983; Auer and Niehaus, 1993; Pachepsky and Shelton, 2011). This particle association influences the transport characteristics of microorganisms, as those associated with denser inorganic particles will tend to settle out of water column more quickly. Many studies have investigated the partitioning of faecal microbes to sediments and reported a wide range of rates from <20% to 100% (Schillinger and Gannon, 1985; Auer and Niehaus, 1993; Characklis et al., 2005; Jamieson et al., 2005; Fries et al., 2006). However, sedimentation experiments conducted by McCarthy et al. (2011) suggested that *E. coli* within the Yarra River estuary are associated with particles <1.5 μm , indicating sedimentation might have a very limited effect on *E. coli* disappearance from the water column.
- Additional inputs along length of estuary. The Yarra River estuary receives discharges from 208 stormwater drains and a major urban creek, Gardiners Creek (Jovanovic et al., 2015). Although the contribution from stormwater was found to be low (i.e. between <1% and 10% of total input into the system; (Jovanovic et al., 2015)), this may still have an impact on top layer *E. coli* concentrations along the estuary

Table 2

Comparison of *E. coli* concentrations obtained from the Morell Bridge autosampler (single intake point sample) with the cross-sectional area-weighted average *E. coli* concentration (entire cross-section and freshwater surface layer only).

Date	Profiling method	<i>E. coli</i> concentration [MPN/100 ml]			Ratio [–]	
		Autosampler	Entire cross-sectional average	Freshwater surface layer cross-sectional average	Entire cross-section	Freshwater surface layer
		(1)	(2)	(3)	(2)/(1)	(3)/(1)
23/04/2013	Single pass	546	302	504	0.55	0.92
30/04/2013	Double pass	254	191	322	0.73	1.22
04/06/2013	Single pass	2000	1448	2177	0.72	1.09
26/06/2013	Single pass	150	91	148	0.61	0.98
30/07/2013	Double pass	337	194	409	0.57	1.21
12/12/2013	Single pass	149	128	153	0.86	1.03

and potentially compensate for the small amount of die-off or settling that may still be occurring.

E. coli levels in the mid and bottom sections of the water column showed a decreasing trend along the estuary, largely attributable to the low levels of *E. coli* found in the salt-wedge water which is mainly present at the downstream sites (Fig. 7). Considering the high salinities of the salt-wedge water (and the detrimental effect it has on *E. coli*) it was expected that the salt-wedge would contain little to no *E. coli*; however, low *E. coli* levels (on average < 100 MPN/100 ml) were still present. In fact, measured *E. coli* concentrations were, on average, five times higher than expected based on the mixing model employed at the two downstream sites. This may suggest bed-sediment resuspension or other inputs of *E. coli* were contributing to the middle and bottom layers.

3.5. Can cross-sectional *E. coli* levels be represented by a single sample?

E. coli levels obtained from the autosampler (i.e. single intake point sample just below the water surface) are not representative of the overall cross-sectional average *E. coli* levels. Cross-section ratios indicate that the single intake point sample over estimates the cross-sectional average (Table 2). This was expected considering that sample was taken just below the water surface in the fresh water layer which contains majority of *E. coli* within the cross-section, while the salt-wedge is largely *E. coli*-free.

At the same time, the single intake point sample was a good representation of the average *E. coli* concentration within the freshwater layer of the channel cross-section (Table 2). The *E. coli* concentration obtained using the autosampler falls within $\pm 10\%$ of the freshwater cross-sectional average, except in the case of double pass sampling when a slightly higher discrepancy was seen (i.e. around 20%). The difference between the single and double pass results is likely the consequence of the central limit theorem (Zar, 1999), and the number of samples used for calculating the cross-sectional averages (indeed, on average 12 samples are used to calculate the cross-sectional average concentration in the single pass sampling, while 4 were used in the double pass sampling). Nevertheless, these results are quite encouraging given the analytical uncertainty associated with the Colilert method used for enumeration of *E. coli* is around 30% (McCarthy et al., 2008; IDEXX Laboratories, 2013). Furthermore, routine monitoring locations may be a conservative estimate of *E. coli* concentrations in stratified estuaries, since *E. coli* concentrations are the highest at the surface and any mixing introduced by recreational activities would cause decrease of *E. coli* levels. As such, the use of surface water grab samples for routine monitoring of the microbial water quality in the Yarra River estuary for recreational activities associated with surface water contact is supported.

4. Conclusions

The first comprehensive monitoring of *E. coli* (a common faecal indicator organism) by means of depth profiling was conducted on the Yarra River estuary to assess its spatial variability i.e. vertical, transverse, and longitudinal. Vertical variability was closely related to salt-wedge dynamics. When the salt-wedge was absent, vertical variability was low and reflected a well-mixed system. In contrast, when the salt-wedge was present, vertical variability increased significantly. *E. coli* cross-sectional variability (i.e. transverse variability) was lower than other sampling uncertainties (such as storage and analytical uncertainties). There was no decreasing trend in *E. coli* concentrations in the top section of the water column along the estuary length, likely due to the limited die-off and settling processes which are potentially offset by additional inputs of faecal contamination. Mid and bottom sections, however, showed a decreasing gradient of *E. coli* levels moving downstream, which was linked to the presence of the salt-wedge at the two most downstream sites and the low levels of *E. coli* typically found in saline waters. Nevertheless, a simple mixing model revealed that levels found within the salt-wedge are much higher than expected, and imply the existence of other sources or water-sediment interactions within the salt-wedge. Overall, the salt-wedge seems to be a major driver of *E. coli* spatial variability in the Yarra River estuary.

A single intake point sample was not able to characterize the overall level of *E. coli* within the Morell Bridge cross-section due to the pronounced vertical variability of *E. coli* concentrations (driven by the salt-wedge). However, there was good agreement between the *E. coli* levels found in the discrete water sample and the cross-sectional average of the fresh surface waters – supporting its use for routine monitoring of microbial water quality in the Yarra River estuary for predicting potential public health risks associated with recreational activities that involve contact with surface waters.

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4.5 Discussion and conclusions

Variability of *E. coli* concentrations within the estuary is significantly linked to the salt-wedge dynamics (Section 4.4). The flow velocity is the main hydrodynamic variable responsible for fluctuation in *E. coli* levels over the tidal cycle (Section 4.3). As such, to achieve the overarching aim of this thesis and develop an accurate predictive microorganism model for estuaries, the model needs to be able to reproduce the estuarine hydrodynamics well, including salt-wedge dynamics, correct distributions of velocity fields/mixing, temperature and salinity profiles, as these are essential for simulating *E. coli* dynamics properly.

This chapter demonstrated that the survival of *E. coli* within the estuary is likely to be governed by temperature, salinity and to some extent solar radiation (Section 4.2 and Section 4.4). Seasonal temperature dynamics are likely to have a significant effect on the die-off of *E. coli* as the average temperature in winter is around 10 °C and in summer is around 22 °C. The temperature variability between summer and winter can lead to die-off rates up to three times higher in summer compared to winter. Diurnal temperature variation might also impact the die-off of *E. coli*, although to lesser extent than seasonal variation due to the lower temperature variation range and shorter duration.

Salinity might also impact, albeit to lesser extent, the survival of *E. coli*, primarily in the regions where the salt-wedge is present. The impact of salinity will vary both spatially and temporally. For example, it is expected that salinity will have no impact on die-off at the most upstream section of the estuary as the presence of salt-wedge was never recorded at Abbotsford. Moreover, in Section 4.4, it was shown that the vertical depth to the salt-wedge (and thus, the longitudinal extent of the salt-wedge intrusion) is function of the Yarra River flow rate, where depth to salt-wedge decreases with lower flow rates. As such, die-off due to salinity will be most pronounced during dry weather, when the Yarra River flow rates are low, the depth to the salt-wedge is small and the entrainment of the salt into the overlaying freshwater column is the highest.

The Yarra River is highly turbid and therefore the impact of solar radiation on *E. coli* survival might be limited. However, the extent of solar radiation effect on *E. coli* survival needs to be further investigate, especially knowing that due to the high stratification of the water column majority of *E. coli* are located in top layer, which is directly exposed to irradiation. pH is likely to have minimal effect, if any, on *E. coli* die-off within the Yarra River estuary due to it being in the near neutral range from 6 to 8.

In conclusion, the effects of temperature, salinity and solar radiation on the survival of *E. coli* need to be accounted for in the microorganism model. Nevertheless, it should be noted that the effects of die-

off on the *E. coli* levels in the Yarra River estuary might be dwarfed by additional inputs along the estuary and resuspension of sediment-associated *E. coli*. This was demonstrated by relatively constant longitudinal levels of *E. coli* in the top part of the water column (Section 4.4).

It was shown that the main driver of the *E. coli* levels within the estuary is the Yarra River itself. Additionally, as mentioned previously, inputs along the estuary could be contributing to increased levels of *E. coli* in the lower estuary and therefore it is important that these inputs are accounted for in the microorganism model. As such, appropriate modelling of these inputs is required to correctly capture the *E. coli* dynamics within the estuary.

In summary, to achieve the major aim of this thesis and to develop a coupled estuarine hydrodynamic-microorganism model, the following items should be considered:

- 1) The model needs to be able to reproduce the estuarine hydrodynamics, including accurate velocity fields, mixing and salt-wedge dynamics.
- 2) The model needs to account for sediment-microorganism interactions, as this can potentially significantly impact the levels of *E. coli*.
- 3) Survival of *E. coli* needs to be modelled as a function of temperature, salinity and solar radiation.

Chapter 5

Modelling flow and *E. coli* inputs to the Yarra River estuary

5.1 Introduction

The literature review (Chapter 2) showed a number of significant inputs of faecal contamination that contribute to the levels of faecal microorganism within urban estuaries. Furthermore, it was hypothesised that dynamics of faecal microbes in inputs will have immense impact on the dynamics of faecal microbes within the estuary and as such it is essential that faecal microorganism inputs are represented accurately. Therefore, it was important that faecal contamination inputs to the Yarra River estuary are well represented and the easiest way of achieving this objective was through modelling.

The work presented in this chapter is crucial for providing the boundary conditions for both hydrodynamic and microorganism models. Furthermore, it will allow testing of hypotheses and answering many research questions related to dynamics of *E. coli* in the Yarra River estuary. In particular, the outcome of the work conducted herein will help to address parts of the Research Question 1 (Section 2.9, Chapter 2 – Literature Review) related to the most important inputs of faecal microorganisms in the Yarra River estuary. We hypothesised that the Yarra River will be main input of *E. coli* during both wet and dry weather conditions, while the stormwater may only be important during wet weather and its dry weather flow may only have an influence locally around the drain outlets.

This chapter consists of two main parts. Section 5.2 presents testing of the model for microorganism prediction in urban stormwater (MOPUS - McCarthy et al. (2011b)) to the flow and *E. coli* measurements from Chapter 3 (i.e. stormwater flow and *E. coli* data collected from the drains of urban catchments monitored during this project). Additionally, the microorganism component of the MOPUS model was tested for simulating the Yarra River *E. coli* input. This section is presented in the form of a published journal paper (*“Conceptual modelling of E. coli in urban stormwater, creeks and rivers”*, in *Journal of Hydrology*, 2017, VOL 555, pp. 129 - 140). Section 5.3 explains how the calibrated models presented in the previous section were extrapolated to generate continuous time series of flow rates and *E. coli* concentrations for all stormwater, creek and riverine inputs of faecal contamination to the Yarra River estuary. Section 5.4 presents parts of the journal paper *“Integrated conceptual modelling of faecal contamination in an urban estuary catchment”* published in *Water Science and Technology*, 2015, VOL 72 (9), pp. 1472-1480, relevant to assessment of the inputs. Finally, the chapter finishes with the discussion of the main findings (Section 5.5).

5.2 Conceptual modelling of *E. coli* in urban stormwater drains, creeks and rivers

The supplementary material for this publication is provided in Appendix A.2.

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Research papers

Conceptual modelling of *E. coli* in urban stormwater drains, creeks and rivers



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ABSTRACT

Accurate estimation of faecal microorganism levels in water systems, such as stormwater drains, creeks and rivers, is needed for appropriate assessment of impacts on receiving water bodies and the risks to human health. The underlying hypothesis for this work is that a single conceptual model (the MicroOrganism Prediction in Urban Stormwater model – i.e. MOPUS) can adequately simulate microbial dynamics over a variety of water systems and wide range of scales; something which has not been previously tested. Additionally, the application of radar precipitation data for improvement of the model performance at these scales via more accurate areal averaged rainfall intensities was tested. Six comprehensive *Escherichia coli* (*E. coli*) datasets collected from five catchments in south-eastern Australia and one catchment in Raleigh, USA, were used to calibrate the model. The MOPUS rainfall-runoff model performed well at all scales (Nash-Sutcliffe *E* for instantaneous flow rates between 0.70 and 0.93). Sensitivity analysis showed that wet weather urban stormwater flows can be modelled with only three of the five rainfall runoff model parameters: routing coefficient (*K*), effective imperviousness (*IMP*) and time of concentration (*TOC*). The model's performance for representing instantaneous *E. coli* fluctuations ranged from 0.17 to 0.45 in catchments drained via pipe or open creek, and was the highest for a large riverine catchment (0.64); performing similarly, if not better, than other microbial models in literature. The model could also capture the variability in event mean concentrations ($E = 0.17–0.57$) and event loads ($E = 0.32–0.97$) at all scales. Application of weather radar-derived rainfall inputs caused lower overall performance compared to using gauged rainfall inputs in representing both flow and *E. coli* levels in urban drain catchments, with the performance improving with increasing catchment size and being comparable to the models that use gauged rainfall inputs at the large riverine catchment. These results demonstrate the potential of the MOPUS model and its ability to be applied to a wide range of catchment scales, including large riverine systems.

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1. Introduction

Faecal microorganism are a leading cause of pollution in surface waters worldwide (Burton and Pitt, 2002; De Brauwere et al., 2014b). These elevated pollution levels represent a major concern for public health and have led to increased efforts by water managers across the world to mitigate these risks. Since frequent monitoring of faecal microorganisms can be costly, time-consuming

and essentially infeasible when a large number of surface waters are being investigated, modelling of microorganisms provides a practical solution for understanding and managing faecal pollution (De Brauwere et al., 2014b). As such, there has been an increase in faecal microorganism models published in the literature (Wilkinson et al., 1995; Kashefpour et al., 2002; Garcia-Armisen et al., 2006; de Brauwere et al., 2011; Gao et al., 2011; Liu and Huang, 2012; Ouattara et al., 2013; Yakirevich et al., 2013; De Brauwere et al., 2014a; Gao et al., 2015; Liu et al., 2015; Niazi et al., 2015). These models are typically coupled hydrodynamic-microorganism models having a microorganism module attached to a hydraulic/hydrologic module.

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An important input for microorganism models at the receiving waterbody-scale is a time series of the targeted microbe's concentrations for all source inputs. In some modelling studies, these input data from contributing sources (and the variability therein) have been neglected and either: simply represented as a constant value (de Brauwere et al., 2011), generated based on interpolation between the available measured points (Gao et al., 2015), predicted using simple correlations with flow (Garcia-Armisen et al., 2006; Liu and Huang, 2012) and/or predicted based on suspended sediment concentrations (Ghimire and Deng, 2013). Without accurate quantification of the concentrations and loads of faecal microorganisms in each source input feeding a receiving water (such as a lake, river, estuary, bay or ocean), these models can lead to ill-informed, non-effective, and costly management solutions. The neglect of input data from contributing sources arise from insufficient data for calibration and validation and an inability to account for spatially- and temporally-variable inputs of microorganisms based on source characteristics. For this reason, there has been a recent shift in modelling approaches to provide more accurate characterisation of faecal contamination inputs to the receiving water environment. This is achieved by applying either existing or newly developed microorganism models to a given source area, and using these model outputs as the source derived input for integrated models of receiving water bodies (De Brauwere et al., 2014a).

A substantial, and challenging to represent, source of microbial pollution is stormwater runoff. However, models specifically developed for simulating microbial dynamics in stormwater are rare, particularly ones able to simulate both between-event and within-event variability of faecal microorganisms. Currently, the most comprehensive model of faecal microorganisms in urban stormwater is the Micro Organism Prediction in Urban Stormwater (MOPUS) model, developed by McCarthy et al. (2011). MOPUS was able to successfully simulate wet weather discharges and faecal microorganism concentrations at the outlets of four urban catchments located in Melbourne, Australia. Indeed, the Nash-Sutcliffe efficiencies observed by McCarthy et al. (2011) for instantaneous flow rates ranged from 0.62 to 0.89, while the efficiencies for instantaneous concentrations of *E. coli* (a commonly used faecal indicator organism) were in the range of 0.25–0.45. These efficiencies were quite reasonable considering the high uncertainties associated with the microorganism data (McCarthy et al., 2008; Harmel et al., 2016a), and the water quality performance of models for other urban pollutants (Dotto et al., 2011). However, MOPUS was developed and tested on catchments ranging in size from 10 to 100 ha (McCarthy et al., 2011), leaving a knowledge gap as to how it performs for catchments above or below this range.

Whilst MOPUS was developed to simulate microorganism concentrations in urban stormwater runoff, there are some similarities between MOPUS and other spatially-lumped conceptual models for simulating the microorganisms in rural stormwater runoff (e.g. Haydon and Deletic, 2006). Firstly, constant deposition rates describing the faecal microorganism loading on the surface of the catchment have been used previously for both urban and rural microorganism models (e.g. Haydon and Deletic, 2006; McCarthy et al., 2011). Secondly, the environmental factors controlling the survival of the faecal microorganism on the surface of the catchments are similar regardless of the catchment type (i.e. urban/rural). For example, McCarthy et al. (2011) used relative humidity and vapour pressure to characterise the survival of microorganisms in urban catchments while Haydon and Deletic (2006) used potential evapotranspiration. These climatic variables are interrelated, both inferring processes related to temperature and moisture. Finally, in both models, wash-off of the microbes is described using rainfall intensity. Therefore, it is reasonable to further explore the

MOPUS microorganism concept for a large rural riverine catchment. Such an effort would support the expanded use of MOPUS in catchments with varied land use/land cover. The two MOPUS sub-models, rainfall-runoff and microorganism, can be run independently of each other; thus the rainfall-runoff model can be substituted with a more appropriate hydrologic model for rural catchments.

Rainfall intensity is the key factor controlling the amount of water discharged and microorganism wash-off from the catchment surfaces (McCarthy et al., 2013). As such, it is an essential input for hydrologic and water quality models. Traditionally, rainfall intensity inputs are obtained from gauges measuring incident rainfall within the catchment. However, due to the spatial variability of rainfall, the rainfall gauge measurements (depending on their density and distribution) might not represent the average precipitation over the catchment needed for spatially-lumped models. Furthermore, several studies indicated that large variations in flow can be caused by the spatial-temporal variation in rainfall (Obled et al., 1994; Arnaud et al., 2002; Syed et al., 2003; Smith et al., 2004). Investigations into how rainfall variability effects spatially lumped models are needed to better understand the value of advances in rainfall estimation in models such as MOPUS.

There is a critical need to both improve and to test the applicability of models developed to characterize the fate and transport of microbes in catchments. The objective of this study was to assess the applicability, adaptability and flexibility of the MOPUS model by calibrating the model to a wide range of scales and catchment types. We therefore, for the first time, applied the model on urban catchments smaller than 10 ha and larger than 100 ha, to cover a broader range of urban catchment sizes. Understanding variability in microbial export based on catchment size and composition is critical to adapting this model to a more diverse set of conditions and making it more widely applicable. We then apply the microorganism component of the MOPUS model to the Yarra River catchment (a large and predominantly rural catchment) to test its ability to capture *E. coli* dynamics in mixed land-use catchments. Furthermore, this would also provide an opportunity to test the ability of microorganism model to run independently from the chosen rainfall runoff model, providing the ability for users to choose their own rainfall-runoff model. Last, this study compared the performance of the MOPUS model using two different rainfall intensity inputs: those from conventional rain gauges, and those derived from readily available weather radar observations. The underlying hypothesis was that the estimated rainfall intensity from weather radar would be a better approximation of the overall rainfall across the catchment than a single rainfall gauge measurement, thus improving model performance.

2. Methods

2.1. Description of the MOPUS model

MOPUS is a spatially-lumped conceptual model composed of two sub-models: 1) a rainfall-runoff model which predicts instantaneous flow rates at the catchment outlet and; 2) a microorganism model which predicts instantaneous microorganism concentrations. Basic processes (such as evaporation, surface and subsurface runoff, microbial build-up, survival and wash-off) are separated to some extent, but the equations used to describe these processes are essentially calibrated input-output relationships. This algorithm complexity indicates conceptual nature of the MOPUS model, as opposed to more simple empirical or more complex process-based modelling algorithms (for more information on model classification see Grayson and Blöschl (2001)).

2.1.1. MOPUS – rainfall-runoff model

The rainfall-runoff model simulates processes on both impervious and pervious surfaces and thus consists of the two stores. Each store has its own capacity (S_{impmax} and $S_{pervmax}$), with excess rainfall being routed to the catchment outlet.

The routing in MOPUS is composed of two steps; attenuation/redistribution and translation. The outflows from the impervious and pervious stores are added to a routing store (i.e. reservoir) and the water is released from the store as a function of two parameters (K – routing coefficient and m – exponent). Additional shifting of the centroid of the inflow hydrograph is provided by translation, the amount of which is called time of translation (TOT). Together TOR and TOT equates to the time of concentration (TOC) which is the time taken for the whole catchment to contribute to flow. For a summary of MOPUS's rainfall runoff model equations please see [Supplementary material](#) (for full details and justification of model structure, please see [McCarthy et al., 2011](#)).

In summary, the MOPUS rainfall-runoff model has six calibration parameters: S_{impmax} , $S_{pervmax}$, IMP (effective imperviousness of the catchment), K , m and TOC . The capacity of the impervious store (S_{impmax}) was fixed to 1 mm as per [McCarthy et al. \(2011\)](#), because the model was not sensitive to this parameter in the range of 0.5–2.0 mm. Other input data required for the rainfall-runoff model consists of the size of catchment area, rainfall intensities, and potential evapotranspiration rates.

2.1.2. MOPUS – microorganism model

The microorganism model has two stores: a surface store that accounts for microorganism sources on the catchment surface, and a sub-surface store for in-pipe sources (e.g. sanitary sewers cross-connections and resuspension). Deposition in the surface store is modelled as a constant rate, while the die-off and survival of the microorganisms is described using antecedent vapour pressure (hPa) and relative humidity (%) ([McCarthy et al., 2011](#)). The wash-off and transport of microorganisms during rain events is a function of routed and translated rainfall intensity. As such, the microorganism model does not require the simulated flow rates from the rainfall-runoff model and is therefore, independent from the rainfall-runoff model. Furthermore, separate rainfall routing is conducted to ensure that the rainfall-runoff and microorganism models can run independently of each other, thus the only piece of hydrologic information required for the microorganism model is calibrated TOC . A linear routing technique is applied in the microorganism model with a fixed routing coefficient of $K = 0.2$ per [McCarthy et al. \(2011\)](#). For a summary of MOPUS's microorganism model equations, please see [Supplementary material](#) (for full details and justification of model structure, please see [McCarthy et al., 2011](#)).

The MOPUS microorganism model has five calibration parameters: $PsCoeff$ – describing the microorganism loading rate on the catchment surface, $VPCoeff$ and $RHCoeff$ – describing microorganism survival once deposited on the catchment; $PssCoeff$ – describing the microorganism loading rate of sub-surface sources, and V – the threshold velocity capable of flushing the deposited microorganisms from the pipe. Instead, of the last calibration parameter (V), the routed rainfall intensity (RI) capable of causing the threshold in-pipe velocity was used in this study. These two calibration parameters are intrinsically linked and can be used interchangeably (see [McCarthy et al., 2011](#)).

2.2. Site descriptions, monitoring and data collection

Data were collected from four urban catchments in Melbourne, Victoria, Australia, one urban catchment in Raleigh, North Carolina, USA, and larger, predominantly rural, Yarra River catchment in Melbourne, Australia. The urban catchments varied in size, with

the four in Melbourne being larger than the catchments previously used for MOPUS testing and development, and the Raleigh catchment being two times smaller than the smallest catchments used in the original study ([McCarthy et al., 2011](#)). Catchment characteristics are presented in [Table 1](#).

Land use was relatively uniform throughout each urban catchment, being predominantly medium to high density residential. All catchments were highly urbanized with total impervious areas being 35% at Raleigh and around 50% at the Melbourne sites. Stormwater systems in all catchments were separate (i.e. not combined sewers), which drained to a single outlet.

The Yarra River catchment is about 4020 km² ([Sinclair et al., 1989](#)). The upper part is largely forested, with a substantial portion of the area (app. 40%) being protected to ensure a quality water supply for metropolitan Melbourne. The lower portion is predominantly urban, while the rest of the catchment is extensively used for agricultural purposes ([Sokolov and Black, 1996](#)). The monitoring site was located just upstream of the estuarine section of the river.

All urban monitoring sites were equipped with either Doppler based flow meters (Melbourne sites), or a bubbler flow meter paired with a weir (Raleigh site). For water sampling, non-refrigerated autosamplers were deployed in Melbourne, and a refrigerated sampler was utilized in Raleigh. Examination by [McCarthy et al. \(2008\)](#) and [Harmel et al. \(2016b\)](#) showed minimal error is introduced by storing microbes for limited periods in autosamplers, even in non-refrigerated conditions. As per [Hathaway et al. \(2014\)](#), autosampler tubes were rinsed prior to sample collection, and were also sterilised and replaced between each event at the Raleigh site. Autosamplers were triggered to collect based on site-specific flow-weighted intervals. In the case of the Yarra River, the autosampler was triggered manually and time-based samples were taken hourly or every two hours depending on the stage of the hydrograph. The samples were transported to the Environmental and Public Health Microbiology (EPHM) laboratory at Monash University in case of the Australian catchments and to North Carolina State University for the Raleigh catchment. Samples at all locations were analysed within 24 h of collection. All samples were analysed for *E. coli* using IDEXX Colilert-18*/Quaty-Tray2000* method ([IDEXX Laboratories, 2013](#)).

2.3. Model testing

2.3.1. Data used for testing

All MOPUS components (rainfall-runoff and microbe modules) were tested using wet weather data collected per the described methods for the urban catchments. The data consisted of instantaneous stormwater flow rates and *E. coli* concentrations. The number of events used for calibration ranged from 10 to 21, with 74 to 373 individual stormwater samples used in total across the events. However, at Prahran Main Drain, only 10 events with one composite sample per event were available for testing.

MOPUS's microorganism model was tested on the Yarra River catchment without its associated model for flow prediction, as MOPUS's hydrological model did not perform satisfactorily for large rivers ([Jovanovic et al., 2015](#)). Therefore, this was a test of the ability of the MOPUS microorganism model to work independently from the rainfall-runoff model, which was originally intended by [McCarthy et al. \(2011\)](#). Independence from rainfall-runoff model enables the microorganism model to be coupled with a more suitable hydrologic model or measured flow data. Due to the lack of a rainfall-runoff model, the time of concentration (TOC) used in microorganism model became a calibration parameter. Furthermore, the routing coefficient K was also estimated through calibration since it was assumed that a fixed value of 0.2 applied in the microorganism model would not be appropriate

Table 1
Characteristics of the six study catchments and *E. coli* data used to test the models.

	Raleigh	Hawthorn Main Drain east	Hawthorn Main Drain west	Prahran Main Drain	Gardiners Creek	Yarra River
Land Use	Medium density residential	Medium density residential	Medium density residential	High density residential	Low density residential	25% Very low density residential; 35% agricultural; 40% undeveloped/reserves
Area (ha)	5	534	597	696	19,010	402,000
Catchment slope (%)	6.0	5.2	4.6	4.7	6.7	12.0
Total imperviousness (%)	35	51	51	58	47	6
Pipe diameter (m)/Outlet cross-sectional area (m ²)	0.61/0.29	3.00/7.07	3.35/8.84	–/12.38	–/37.85	–/≈70.00
Number of rainfall gauges used	1	1	1	1	5	16
Rainfall gauge distance from outlet (m)	190	3415	3415	540	8725 (3580, 11,830) ^c	34,241 (2146, 72,326) ^c
Rainfall gauge distance from centroid (m)	390	1820	1600	1560	3870 (1620, 5380) ^c	22,327 (4391, 36,767) ^c
Weather radar distance from centroid (m)	–	25,100	24,900	21,503	30,048	60,058
Number of monitored wet weather events	20	11	21	10	19	13
Range of event rainfall totals (mm)	4.1–56.0	4.2–19.0	1.2–24.8	3.6–54.4	1.7–46.4	4.2–26.5
Number of samples used for calibration	202	74	177	10 ^a	373	914
Median storm event sample <i>E. coli</i> level (MPN/100 mL) ^b	14,977 ^b (412–197,739)	8554 (2098–90,060)	8301 (856–170,979)	12,000 ^a (2300–20,000)	6802 (306–111,874)	975 (20–12712)
Median dry weather <i>E. coli</i> level (MPN/100 mL)		2080	2178		405	229

^a Prahran Main Drain monitoring site had only 10 events with flow weighted composite water samples, hence only event median concentrations were available at this site.

^b Minimum and maximum values are shown in the parenthesis. MPN = Most Probable Number.

^c Average (minimum, maximum) distance of 5 rainfall gauges within the Gardiners Creek catchment.

for such a large catchment. Only the processes occurring on the catchment surface were simulated by employing the surface store of the microorganism model. In total, there were five calibration parameters: *K*, *TOC*, *PsCoeff*, *VPCoeff* and *RHCoeff*.

The Yarra River microorganism model was tested using the whole dataset available, including 914 samples representing mixed wet- and dry-weather flow. This usage of multi-condition flow was necessary due to difficulties in separation of wet weather and dry weather samples from such a large and anthropogenically modified catchment (presence of dams, extraction of irrigation water, etc). However, during the 13 sampling occasions utilized herein, the sum of the rainfall in the previous 24 h before collection was greater than 3 mm. These events were categorized as wet weather 'events' (Table 1). Additional input data, including potential evapotranspiration (mm), vapour pressures (hPa) and maximum relative humidity (%), were obtained from the Bureau of Meteorology for sites within Melbourne and the State Climate Office of North Carolina for the Raleigh site.

Arguably the most important input data for both the rainfall-runoff model and microorganism model is a rainfall time series. Unlike the original study by McCarthy et al. (2011), site scale monitoring of rainfall was not conducted as part of monitoring program, except for the Raleigh catchment. Therefore, rainfall for catchments within Melbourne was obtained from the Bureau of Meteorology and Melbourne Water Corporation. Typically, observations from one rain gauge closest to the centroid of the catchment were used (Table 1). The exceptions were Gardiners Creek and the Yarra River catchment where measurements from 5 and 16 rain gauges within the catchment, respectively, were available and area-averaged rainfall intensity was used as input for the models.

Weather radar rainfall intensity inputs. An attempt was made to better account for the spatial variability of rainfall over the Melbourne catchments to improve this input for the MOPUS models. For this purpose, the Melbourne weather radar reflectivity observations with 1 × 1 km and 6 min resolution in period 2011–2014 were used (Bureau of Meteorology). The weather radar was located approximately 19 km west-south-west from Melbourne's central

business district at 44 m above the sea level with radar cover range of 256 km in diameter (see http://www.bom.gov.au/australia/radar/info/vic_info.shtml#melbourne02). Distances between the weather radar and the centroids of the Melbourne catchments ranged from 21 to 60 km (Table 1), with no significant obstructions. The radar reflectivity is converted into the rainfall on the ground by using the following relationship (Steiner et al., 1999)

$$Z = A \times I^b \quad (1)$$

where: *Z* – weather radar reflectivity; *I* – rainfall intensity; *A* and *b* – calibration coefficients.

Parameter *b* was fixed at 1.6 according to a previous study on Australian weather radar which included Melbourne area radar observations (Seed et al., 2002). The value of parameter *A* was estimated through Monte Carlo storm event-based calibration as described below.

To condition the radar measurements, two sets of the rain gauge measurements were used; one containing 14 rain gauges for the area covering the four urban catchments and the other containing 16 rain gauges covering the Yarra River catchment. The following procedure for the estimation of parameter *A* was applied for each set of rain gauges to allow different values of *A* for each region. Firstly, rain gauge data were used to parse the storm events over the period 2011–2014. Only events with a minimum of 1 mm average rainfall per rain gauge in a given set were utilized. Secondly, measured weather radar reflectivity from the pixels corresponding to the locations of the rain gauges were extracted, and the event-based calibration of parameter *A* was performed by comparing the measured event total rainfall at each rain gauge with the radar-predicted rainfall at each corresponding pixel. The least-squares objective function was used for calibration, while Nash-Sutcliffe efficiency was calculated to assess the prediction performance for each event.

Once optimised, the parameter *A* value was established for each event and each set of rain gauges, allowing use of the above relationship (Eq. (1)) to produce the rainfall depths for each storm event over the entire radar coverage area. During dry weather, a default parameter *A* value of 200 was used (Seed et al., 2002).

Finally, a single rainfall time series for each of the four urban catchments and the Yarra River catchment were calculated as an area-average rainfall intensity of the pixels falling within the corresponding catchment areas.

2.3.2. Model calibration and performance assessment

Both model calibration and a performance assessment were conducted following the same methodology described by McCarthy et al. (2011) to facilitate comparable results. The rainfall-runoff and microorganism models were calibrated independently by comparing the predicted and measured instantaneous flow rates and *E. coli* concentrations, respectively. The model parameters were optimised using a Monte-Carlo approach which also allowed exploration of parameter sensitivity. The least squares objective function was used for both the rainfall-runoff and microorganism models. Although this objective function favours peaks (Criss and Winston, 2008), its application is appropriate given that high flow rates and peak microorganism concentrations are important when assessing or mitigating human health risks associated with polluted stormwater (McCarthy et al., 2011). The calibration procedure entailed forming model parameter sets by random sampling within the specified parameter ranges using uniform distributions, performing the model simulation for given parameter set and calculating the value of the objective function. The procedure was repeated at least 50 000 times for each test catchment (for complete parameter sets for each catchment please see Supplementary material). The parameter ranges for both rainfall runoff and microorganism models are shown in Table 2. If a parameter was sensitive within the initial range, but no peak was obtained, the parameter range was adjusted until the optimal parameter value was obtained.

Once calibrated, performance of the rainfall-runoff model at each site was assessed by calculating the Nash-Sutcliffe efficiency (E_N) using predicted and measured values (Nash and Sutcliffe, 1970) for three flow characteristics:

- E_{Q_i} : instantaneous flow rates (L/s)
- E_{EQ_i} : instantaneous flow rates (L/s) for each event (where i goes from 1 to N – the number of events listed in Table 1); and
- E_{V_i} : total event volumes (L)

Similarly, performance of the microorganism model at each site was assessed in five ways:

- E_C : instantaneous *E. coli* concentrations (MPN/100 mL);
- E_{EC_i} : instantaneous *E. coli* concentrations (MPN/100 mL) for each event (where i goes from 1 to N – the number of events listed in Table 1);

- E_{EMC} : Event Mean Concentrations – EMC (MPN/100 mL) calculated for each of the N events at each site.
- E_{Peak} : maximum *E. coli* concentrations (MPN/100 mL) from each of the N events; and,
- E_{Load} : *E. coli* loads for each of the N events.

The Nash-Sutcliffe efficiency also favours peaks in model predictions, however it was deemed appropriate for the same reasons described above for the objective function.

Validation of the model (i.e. assessing the model performance for a portion of the dataset not used in the calibration process) is an important part of model testing, however, the aim of this study was to determine whether the MOPUS model could be calibrated to a range of different catchments and not validation of the model. Nevertheless, thorough validation of the model (including different validation techniques, such as 50:50 split sampling and cross-validation) using the data from four other catchments from Melbourne has been conducted previously. For results of this validation testing please see McCarthy (2008).

3. Results and discussion

3.1. Weather radar calibration

Fig. 1 shows the Melbourne weather radar calibration results. Overall, radar-estimated rainfall (Eq. (1)) totals matched gauged rainfall totals. However, total rainfall was underestimated for some smaller events by the weather radar data. Difficulties predicting smaller events were expected because of the higher uncertainty in measuring small amounts of reflectivity; however, these results may also be related to the fixed minimum reflectivity threshold value in the radar measurements. This threshold marks the minimum reflectivity that will cause rainfall on the ground, although it is likely that this value will vary for different types of storms.

The distribution of the calibration parameter A appeared consistent with previous studies, with median values ($A = 149$ for urban catchments and $A = 219$ for Yarra River catchment) comparable to the literature value (i.e. 200 (Seed et al., 2002)) for both calibrations.

3.2. MOPUS – rainfall-runoff model

3.2.1. Model performance

Optimised parameter values and the rainfall-runoff model performance are presented in Table 3. Considering its simplicity and the small number of parameters, the model performed well. As an illustration, Dotto et al. (2011) reported similar performance

Table 2
Initial parameter ranges for MOPUS rainfall runoff and MOPUS microorganism models applied during Monte Carlo calibration procedure.

Rainfall runoff model parameters					
	$S_{per\ max}^a$ [mm]	K^b [-]	m [-]	IMP [-]	TOC [min]
Minimum	1	0.10	1	0.10	0
Maximum	50	0.60	3	0.50	120
Microorganism model parameters					
	$PsCoeff$ [-]	$VPCoeff^c$ [-]	$RHCoeff^d$ [-]	$PssCoeff^e$ [-]	RI [mm]
Minimum	4	-3	-3	4	0.001
Maximum	6	3	3	6	0.300

Exponents indicated that initial parameter range needed to be adjusted to obtain optimal model performance for following catchments.

^a Raleigh, Prahran main drain and Gardiners Creek.

^b Gardiners Creek.

^c Hawthorn main drain east.

^d Hawthorn main drain east.

^e Raleigh and Hawthorn main drain west.

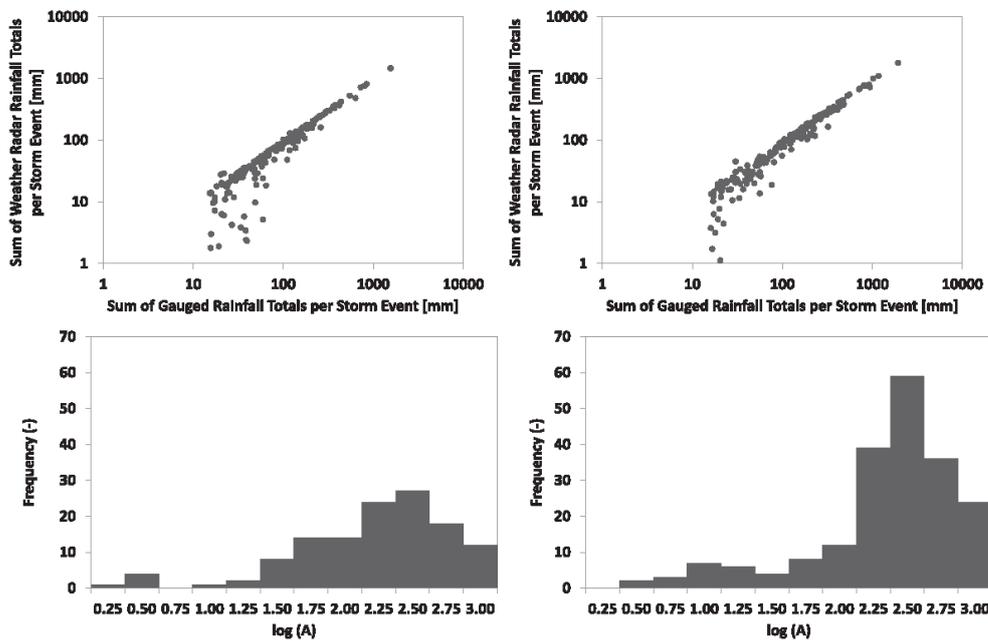


Fig. 1. Top – Sum of weather radar derived rainfall totals per storm event versus sum of rain gauge totals per storm event; Bottom – Distribution of calibrated parameter A values; Left – urban catchment area; Right – Yarra River catchment.

using a conceptual spatially lumped model with 12 calibration parameters. The performance for simulating instantaneous flow rates varied between the catchments, being the lowest at Hawthorn Main Drain west ($E_Q = 0.70$) and the highest at Gardiners Creek ($E_Q = 0.93$). Nevertheless, these are well within or above the range reported previously for MOPUS (i.e. $E_Q = 0.62$ – 0.89 (McCarthy et al., 2011)) and for more complex models (i.e. Dotto et al. (2011), $E_Q = 0.49$ – 0.81). The performance of the model when simulating individual event instantaneous flow rates (E_{EQ_i}) varied, with some events even being negative. In many cases, timing issues were identified (i.e. the predicted and measured hydrographs were misaligned) as shown in Fig. 2, resulting in substantial reductions in performance. The same issue has been reported previously and was related to the fact that, in reality, time of concentration (TOC) is variable between events as opposed to being a

constant for all events as applied in the model (McCarthy et al., 2011). Prediction performance for the events volumes (E_V) ranged from 0.74 to 0.99, which also corresponded well to the range reported previously by McCarthy et al. (2011), i.e. 0.74–0.96. These results demonstrate that the rainfall-runoff model was able to reliably simulate wet weather stormwater flows well over the range of urban catchment sizes, from small catchments of just few hectares, to large catchment of thousands of hectares. Thus, the model is relatively adaptable in runoff simulation with regard to scale.

When the gauged rainfall inputs were substituted with the radar derived rainfall, the model's performance in predicting instantaneous flow rates decreased. This was unexpected, considering the underlining hypothesis that the model would perform better with radar rainfall estimates which accounted for spatial variation in rainfall over the catchment. Nevertheless, the

Table 3

The optimised parameter values and the performance statistics for the rainfall-runoff model at the five urbanized catchments.

	Raleigh ^a		Hawthorn Main Drain east		Hawthorn Main Drain west		Pahran Main Drain		Gardiners Creek	
	RG	RADAR	RG	RADAR	RG	RADAR	RG	RADAR	RG	RADAR
<i>Optimised parameters</i>										
$S_{pervmax}$ (mm)	57	–	13	23	45	21	82	121	68	50
IMP (–)	0.11	–	0.41	0.35	0.45	0.41	0.21	0.22	0.21	0.23
K (–)	0.516	–	0.119	0.242	0.142	0.224	0.229	0.243	0.022	0.016
m (–)	1.43	–	1.95	2.98	1.46	1.78	1.31	2.68	1.06	1.31
TOC (min)	12	–	36	42	36	42	18	30	102	108
<i>Model performance</i>										
E_Q	0.79	–	0.77	0.39	0.70	0.23	0.92	0.76	0.93	0.88
E_{EQ_i} min	0.46	–	0.11	–3.76	–1.12	–3.29	–0.35	–6.62	–0.34	–3.63
E_{EQ_i} median	0.72	–	0.73	0.53	0.53	0.20	0.51	–0.16	0.64	0.45
E_{EQ_i} max	0.94	–	0.94	0.85	0.93	0.86	0.90	0.62	0.95	0.96
E_V	0.76	–	0.89	–0.70 ^b	0.74	0.71	0.99	0.97	0.81	0.66

^a Only Melbourne weather radar data was used to derive rainfall intensities, hence no results are available for Raleigh catchment.

^b The volume prediction performance was caused by one poorly predicted event. When this event was removed, the recalculated performance was 0.52.

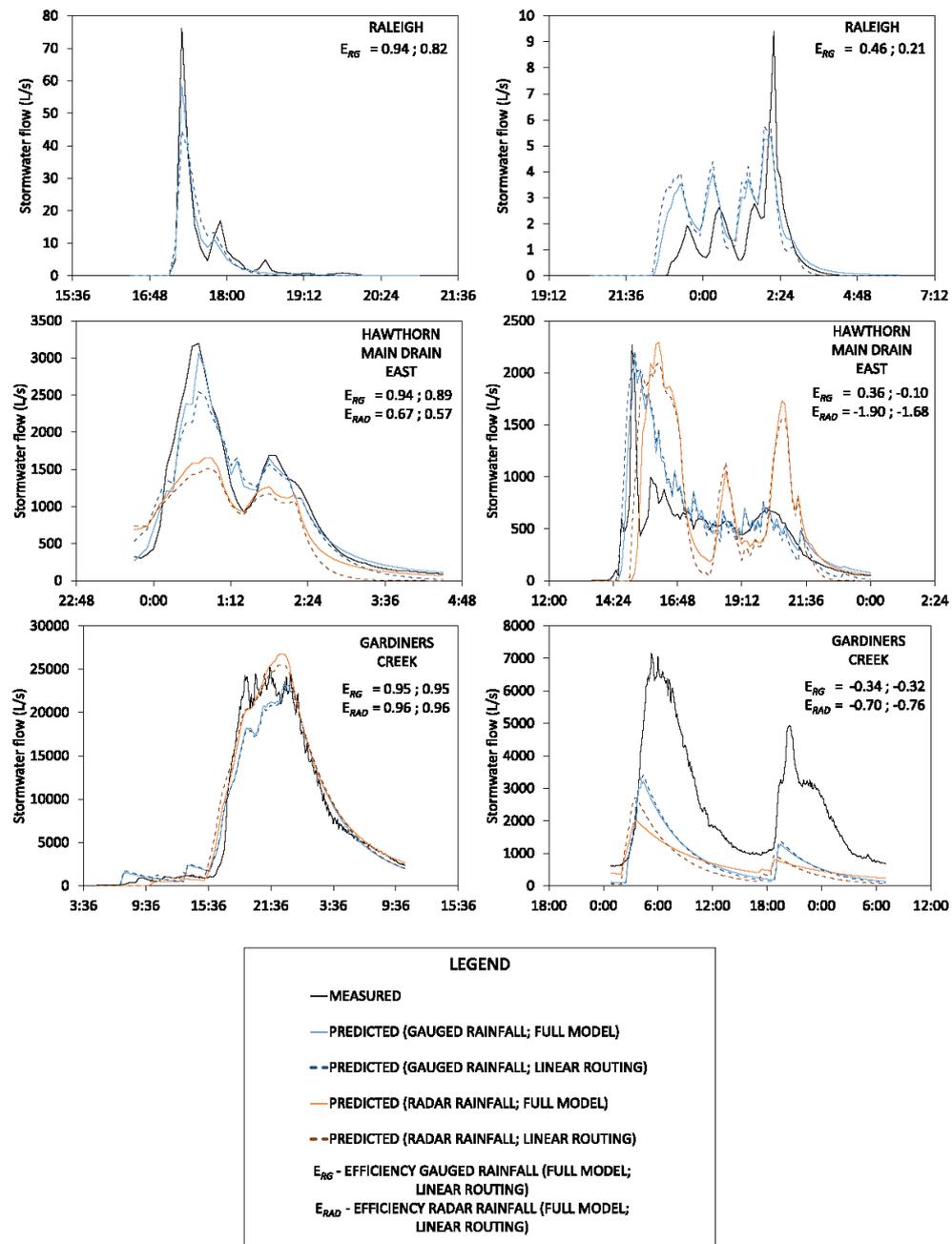


Fig. 2. Rainfall-runoff model prediction at Raleigh (top), Hawthorn Main Drain east (middle) and Gardiners Creek catchments (bottom) with a representative well predicted storm event on left and poorly predicted storm event on right. N.B. Only gauged rainfall intensities were used as input at Raleigh.

performance varied between the catchments. The model's performance was lowest at the two Hawthorn Main Drains, while at Gardiners Creek it was similar to that when using gauged rainfall inputs. Most likely, this could be explained by the larger area associated with catchments such as Gardiners Creek that incorporates more radar cells for producing average rainfall inputs for the model. In a recent study, [Ochoa-Rodriguez et al. \(2015\)](#) showed

that the impact of rainfall resolution decreases as the drainage size increases. Therefore, model performance at Hawthorn Main Drain east, Hawthorn Main Drain west and Prahran Main Drain would probably have been better if the radar resolution was higher. Indeed, the preferred spatial resolution of weather radar rainfall for urban drainage modelling applications is suggested to be in 100–500 m range ([Fabry et al., 1994](#)), since the variability of rain-

fall that occurs below the typical 1 km resolution can have a significant impact on simulated flows (Gires et al., 2012). In addition, model performance could also be reduced due to the need to convert weather radar observations to rainfall intensity, which involves estimation of the conversion parameters, while the rain gauges measure incident rainfall on the ground directly.

3.2.2. Optimised parameter sets

The optimised maximum water storage capacity of the soil ($S_{pervmax}$) varied considerably between sites, regardless of the type of rainfall input. While McCarthy et al. (2011) found a negative correlation between the maximum amount of water the soil can hold and the extent of urban development (as total imperviousness), no such relationship was found in this study. The distribution of the $S_{pervmax}$ parameter for all catchments showed that the model was sensitive to this parameter below a threshold after which it became insensitive (as shown in Fig. 3 for the smallest – Raleigh – and the largest – Gardiners Creek-test catchments). At the same time, the maximum outflow from the pervious store ($Q_{perv_out\ max}$) was plotted against the pervious store size, and it was found that the highest model performance was obtained when there was

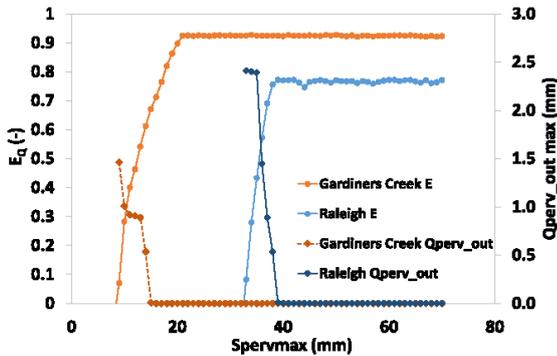


Fig. 3. Model efficiency in predicting instantaneous flow rates as a function of pervious store capacity ($S_{pervmax}$) and maximum pervious store outflow ($Q_{perv_out\ max}$) as function of pervious store capacity ($S_{pervmax}$) at the largest (Gardiners Creek) and the smallest (Raleigh) catchment.

no outflow from the pervious store (Fig. 3). Essentially, model efficiency improves as the pervious store size increases, and as the pervious store size increases, outflow from this store decreases. This means that the model was effectively removing the pervious model store contribution to the total stormwater flow – indicating that only the impervious component is important for modelling stormwater flows in these urban catchments and that the model structure could potentially be simplified further by reducing the number of parameters. Similar results have been reported previously in the literature for other conceptual rainfall-runoff models (Dotto et al., 2011).

Directly connected impervious area (*IMP*) varied across the study sites (Table 3). In both Hawthorn Main Drain east and west, the *IMP* value was similar to the total imperviousness of the catchment (Table 1), while in other cases it was significantly different from the total impervious area. Similar observations have been made previously by McCarthy et al. (2011), with a number of hypotheses presented for these trends such as a portion of the impervious areas being disconnected from stormwater systems for stormwater harvesting purposes, exfiltration occurring due to damaged drainage network, uncertainties in measured flow rate, and/or incorrect estimation of total imperviousness in the catchment.

At the scale of small urban catchments, time of concentration (*TOC*) has been shown to be strongly related to the slope of the catchment (McCarthy et al., 2011). However, no significant relationship between these two parameters was found in this study – most probably because at larger scales *TOC* is influenced by a combination of factors such as slope, drainage infrastructure, size of the catchment and its imperviousness, as well as the rainfall movement within the catchment.

An attempt was made to reduce the number of rainfall-runoff model parameters and to avoid the inherent cross-correlation between *K* and *m* parameters (for more details see Supplementary material) by applying a linear reservoir routing technique (i.e. $m = 1$) instead of the non-linear as originally proposed. Application of the linear reservoir routing technique resulted in a minimal decrease in model performance for all test catchments for both gauged and weather radar-derived rainfall inputs (see Table S3 in Supplementary materials). As shown in Fig. 3, the shape of the predicted flow hydrograph by the model with a linear routing routine

Table 4

The optimised parameter values and the performance statistics for the microorganism model at the five urbanized catchments and the Yarra river catchment when using gauged and weather radar derived rainfall intensities. *K* indicates the routing coefficient used in microorganism model.

	Raleigh		Hawthorn Main Drain east		Hawthorn Main Drain west		Prahraan Main Drain ^a		Gardiners Creek		Yarra River ^b	
	RG K = 0.2	RADAR K = 0.2	RG K = 0.2	RADAR K = 0.2	RG K = 0.2	RG K = 0.2	RG K = 0.2	RADAR K = 0.2	RG K = 0.02	RADAR K = 0.02	RG K = 0.017	RADAR K = 0.011
<i>Optimised parameters</i>												
Ps_{Coeff}	5.47	-	5.64	5.50	5.20	5.17	5.17	5.11	5.03	5.17	4.51	4.50
$RHCoeff$	-0.30	-	-9.99	-9.71	-1.94	-1.82	2.61	2.93	2.18	0.30	2.63	3.06
$VPCoeff$	-0.22	-	2.72	3.02	2.39	1.96	-0.71	-0.41	0.32	0.57	1.01	1.18
$PssCoeff$	6.04	-	4.54	5.53	5.87	6.66	1.66	4.43	5.10	5.03	-	-
Ri	0.045	-	0.115	0.134	0.109	0.083	0.025	0.155	0.111	0.110	-	-
<i>Model performance</i>												
E_C	0.17	-	0.30	0.14	0.24	0.47	-	-	0.45	0.41	0.64	0.65
$E_{ECI\ min}$	-5828	-	-223.39	-94.11	-75.33	-92.82	-	-	-5.93	-5.68	-	-
$E_{ECI\ median}$	-1.09	-	-1.61	-1.03	-3.96	-4.18	-	-	-0.46	-0.08	-	-
$E_{ECI\ max}$	0.76	-	0.34	0.84	0.32	0.97	-	-	0.60	0.76	-	-
E_{EMC}	0.17	-	0.37	0.45	0.57	-2.91	0.54	0.35	0.39	0.33	-	-
E_{Peak}	-13.66	-	0.30	-1.38	-0.25	-21.90	-	-	0.38	0.86	-	-
E_{Load}	0.32	-	0.65	-0.28	0.58	-0.52	0.97	0.97	0.56	-0.07	-	-

^a Only event mean concentrations were available at Prahraan Main Drain catchment. Therefore only model efficiencies for prediction of event mean *E. coli* concentrations and event loads are presented.

^b Calibrated *TOC* for the Yarra River catchment was 114 min when using measured flow and gauged rainfall intensity and 102 min when using radar estimated rainfall intensity.

follows closely that of the full model. However, the prediction of the flow peaks is slightly worse, which impacted overall performance efficiencies. These results suggest the number of model parameters can be reduced and parameter cross correlation can be effectively avoided without compromising the model performance significantly.

Altogether, the stormwater wet weather flows from urban catchments can be simulated quite well using the MOPUS rainfall-runoff model with only three calibration parameters: k , IMP and TOC .

3.3. MOPUS – microorganism model

3.3.1. Model performance

Table 4 summarises the performance of the MOPUS microorganism model for the five urban catchments and the Yarra River catchment and Fig. 4 presents measured and predicted pollutographs and hydrographs for two events, as well as predicted versus measured plots of instantaneous concentrations, event mean concentrations, event peaks and event loads for median performing catchment – Hawthorn Main Drain east (for result plots from other

test catchments please see Figs. S2–S5 in Supplementary materials). Model performance in representing instantaneous *E. coli* concentrations at the outlet of the urban catchments was in the range of $E_c = 0.17–0.45$ and comparable to the previously reported range $E_c = 0.25–0.41$ (McCarthy et al., 2011). Moreover, similar model performances have been reported for other faecal microorganisms models (e.g. Niazi et al. (2015), $E_c = 0.03–0.39$), or even less dynamic, more traditional stormwater pollutants. For instance Dotto et al. (2011) found $E = 0.07–0.46$ for total suspended sediments and $E = 0.04–0.36$ for total nitrogen. Remarkably (because the model was developed for small urban catchments), the model performed better for the Yarra River catchment, with an E_c value of 0.64. In particular, the model was able to represent high *E. coli* concentrations, which commonly occur during wet weather (Fig. 5). This could be related to the fact that the model was essentially developed for wet weather *E. coli* prediction but potentially also an artefact of the objective function which puts an emphasis on larger values.

The ability of the model to represent *E. coli* concentrations for individual events was highly variable (as indicated by differences in maximum, median and minimum individual event efficiencies

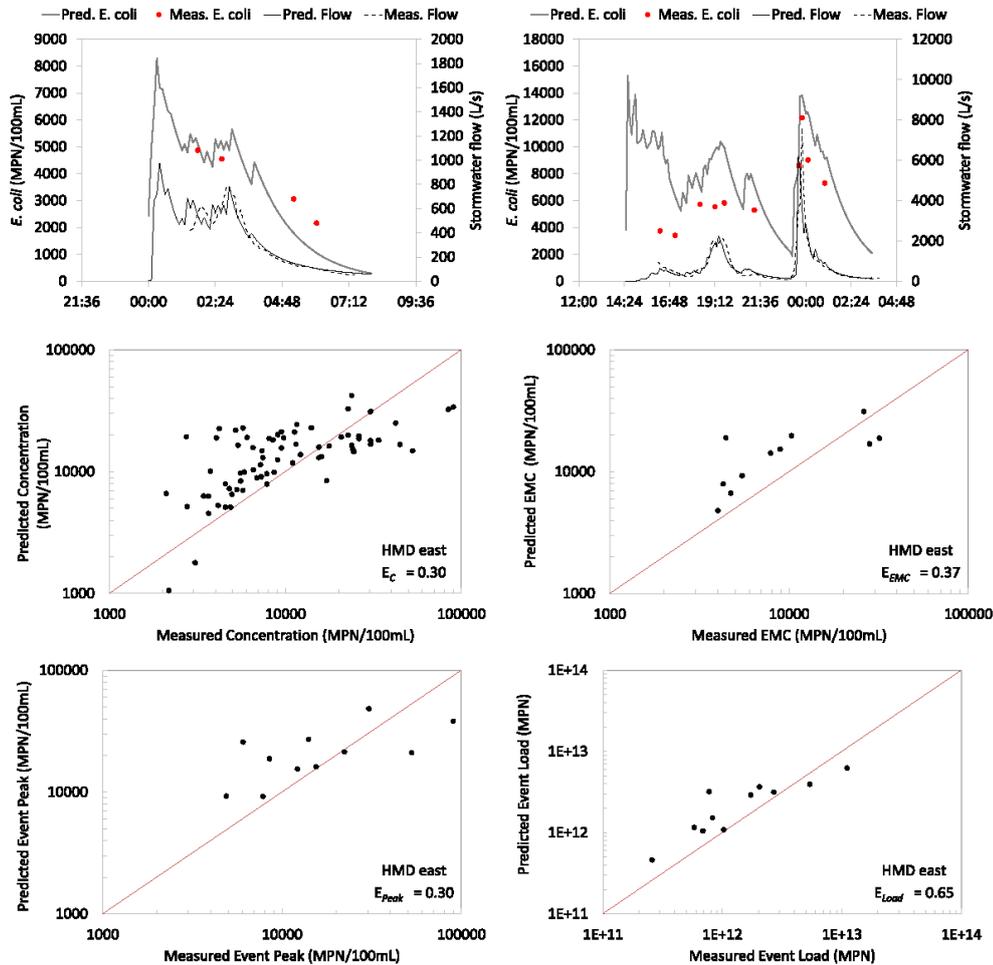


Fig. 4. Detailed results for median performing catchment when using gauged rainfall inputs – Hawthorn Main Drain east (HMD east). Top – measured and predicted *E. coli* pollutographs and hydrographs for two events, Middle Left – Predicted versus measured instantaneous *E. coli* concentrations, Middle Right – predicted versus measured *E. coli* event mean concentrations (EMCs), Bottom Left – predicted versus measured event peaks, Bottom Right – predicted versus measured event loads.

in Table 4). Nevertheless, median individual event performances are comparable to those published by McCarthy et al. (2011) suggesting, as was observed for the rainfall-runoff model, that the microorganism sub-model is robust across various catchment scales. Lastly, the performance of the model for event mean concentrations was generally similar to the performance for instantaneous concentration. Of note is the variability observed in replicating event peak *E. coli* levels. It was found that poor performance for the peak concentrations was related to the high concentrations predicted at the beginning of some events caused by the sub-surface store contributions. However, ability of the model to represent event loads (E_{load}) was good and ranged from 0.32 to 0.97, slightly higher than the 0.05–0.86 range reported by McCarthy et al. (2011).

When radar derived rainfall data were used as an input, the overall model's performance decreased similar to the trend noted for the rainfall-runoff model. It is hypothesised that this occurred for the same reasons outlined above. The model performance was observed to improve with an increase in catchment size and ultimately become similar to the performance of the model when using gauged rainfall inputs at the largest catchment (Gardiners). This is even more evident in the example of the Yarra River catchment, where the model using radar estimated rainfall inputs seem to more accurately represent *E. coli* levels, particularly in the region of low concentrations (Fig. 5).

3.3.2. Optimised parameters

A fixed routing coefficient of 0.2 (K) was originally proposed for the rainfall routing conducted as part of the microorganism sub-model, while the TOC was adopted from the calibrated rainfall-runoff model. In most cases this routing coefficient produced reasonable results (Table 4). However, for the largest urban catchment tested (i.e. Gardiners Creek), MOPUS was not able to reproduce the observed *E. coli* levels with the default K value. This

was expected since the flow response to the storm events at such a large catchment lasts significantly longer than at smaller urban catchments (Fig. 2). As such, the routing coefficient value utilized for the microorganism model was adopted from the calibrated rainfall-runoff model, which reproduced reasonable results. As can be seen in Table 4, the routing coefficient adopted was 10 times smaller than the one originally proposed. This was also true for the large riverine catchment, except there was no rainfall-runoff model, thus both K and TOC were used as calibration parameters. Surprisingly, the optimised values of the K and TOC did not differ greatly from those of Gardiners Creek, even though the Yarra River catchment is more than 20 times larger. This may be related to substantial water extraction for domestic and agricultural use in the middle and upper reaches of the Yarra River (MWC and PPWCMA, 2004). As such, the flow and *E. coli* dynamics at the outlet of the catchment are expected to be significantly influenced by urbanized areas within the lower reaches of the Yarra River system.

In MOPUS's conceptual microorganism model, the $PsCoeff$ aims to represent the deposition rate of the microorganisms on the surface of the catchment. McCarthy et al. (2011) showed that the $PsCoeff$ value is positively correlated with the median level of *E. coli*, indicating that sites with higher *E. coli* levels are expected to have higher loading rates and, thus, higher $PsCoeff$ value. Similar results were found in this study with the exception of Prahran Main Drain (Spearman rank correlation coefficient $R_s = 0.71$ and $p = 0.14$ for all sites, $R_s = 0.90$ and $p = 0.08$ for all but Prahran Main Drain), yet the result for this site should be treated with care as only 10 median event concentrations were available for model calibration for this catchment.

MOPUS's $PssCoeff$ attempts to account for a number of subsurface mechanisms of *E. coli* leading to inputs into the stormwater drains. However, McCarthy et al. (2011) hypothesised it would be primarily related to the severity of illegal sanitary sewer cross

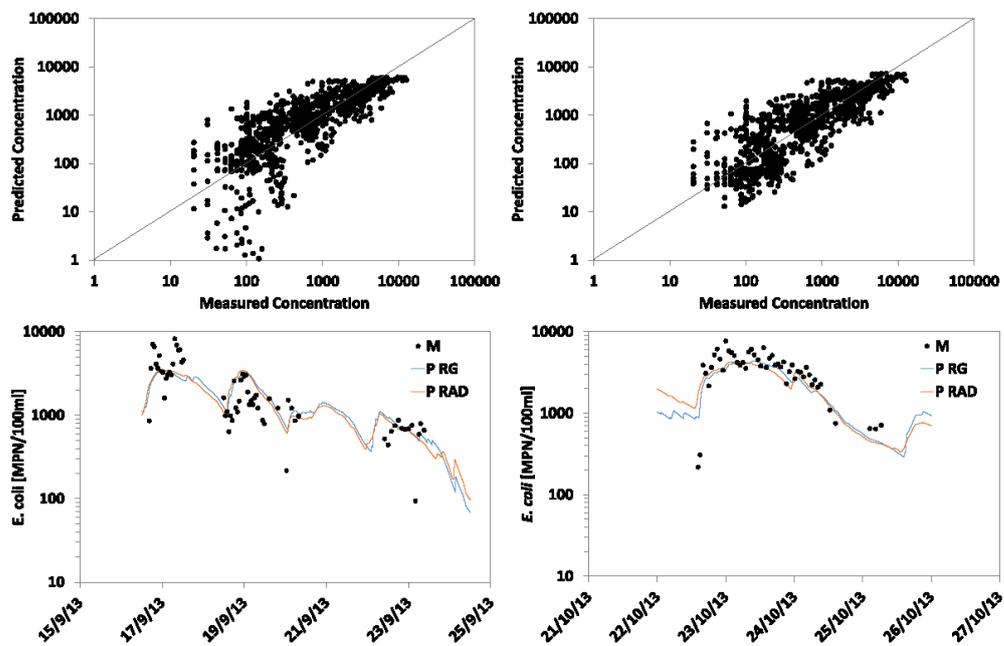


Fig. 5. Top – Predicted versus measured *E. coli* concentrations from the Yarra River catchment when using gauged rainfall (left) and radar rainfall (right); Bottom – Measured and predicted *E. coli* concentrations for two events. M – measured *E. coli* concentrations; P – predicted *E. coli* concentrations; RG – prediction using gauged rainfall intensities as input; RAD – predictions using weather radar derived rainfall intensities as input.

connection which deposit microbes into the stormwater drain (particularly during dry weather). A positive relationship between *PssCoeff* values and median *E. coli* dry weather concentrations in the McCarthy et al. (2011) study provided some support for the hypothesis, however, no such relationship was found in this study ($R_s = 0.5$ and $p = 1$). This might be related to the fact that results from only three catchments (i.e. Hawthorn main drains east and west and Gardiners Creek) were used to examine the relationship and the fact that Hawthorn main drain east model was not sensitive to value of *PssCoeff*. Its optimised value (4.54) is very different to Hawthorn main drain west (5.87) even though they had similar dry weather *E. coli* levels (2080 MPN/100 mL and 2178 MPN/100 mL respectively). Nevertheless, Gardiners Creek which had much lower dry weather *E. coli* levels (405 MPN/100 mL) than Hawthorn main drain west, also had a lower optimised *PssCoeff* value (5.10), giving some support to the hypothesis above.

(McCarthy et al., 2013) found that a positive relationship between *E. coli* levels during wet weather events and previous day's vapour pressure could be related to the enhanced survival/growth in moist conditions, when vapour pressure is higher. When McCarthy et al. (2011) tested MOPUS they did report positive values of *VPCoeff* in all test catchments, reflecting their statistical findings (McCarthy et al., 2013). In this current paper, *VPCoeff* was found to be positive in all cases except in the case of the Raleigh and Prahran Main Drain catchments, albeit in these cases the value of *VPCoeff* was very small which indicates that the previous day's vapour pressure impact on *E. coli* concentrations was limited.

Similarly to McCarthy et al. (2011), *RHCoeff* varied in this paper from being positive to negative for different catchments. For the poorest performing sites (Hawthorn main drain east and Hawthorn main drain west and Raleigh; Table 4), the values of *RHCoeff* were negative, while for the best performing and largest sites (Prahran main drain, Gardiners and Yarra River), the value of *RHCoeff* was positive. McCarthy et al. (2011) explains the variability in *RHCoeff* on the variation of the prominent sources of *E. coli* in each catchment, each of which may differ in response to atmospheric moisture content changes. The same could be confirmed in these catchment through the use of highly targeted microbial source tracking techniques (e.g. Sidhu et al., 2013; Henry et al., 2016).

While the aim of this paper was not the sensitivity analysis of the model parameters, the obtained model parameters sets and associated Nash-Sutcliffe efficiencies for each of the five catchments tested are provided in Supplementary material. Furthermore, a comprehensive sensitivity analysis of the microorganism model was conducted previously and presented in McCarthy et al. (2010).

4. Conclusions

An existing model for predicting microorganisms in urban stormwater (MOPUS) was calibrated on a greater range of urban catchment sizes and types than previously attempted. One small urban catchment, four large urban catchments (including an urban creek) and a large riverine catchment were analysed. This allowed a better understanding of how adaptable the MOPUS model is over a wide range of scales, with implications for its application in new locations.

The rainfall-runoff model performed well in all cases. The Nash-Sutcliffe efficiency for predicting instantaneous flow rates ranged from $E_Q = 0.70$ – 0.93 and was well in the range of previously reported values. These results show that the model is able to simulate wet weather flow rates over the range of catchment sizes. Additionally, it was shown that some simplification can be made to model structure without compromising the model's performance, such as removing the pervious store component (and the related calibration parameter) and the application of a linear reservoir

routing technique instead of non-linear reservoir routing which further reduced the number of model parameters.

The ability of the microorganism model to represent instantaneous *E. coli* fluctuations at the outlet of the four urban catchments was in the range of $E_C = 0.17$ – 0.45 and comparable to the previously reported values $E_C = 0.25$ – 0.41 . Furthermore, model efficiencies reported herein are similar to those reported for other pathogen models (as well as the models of some less dynamic urban pollutants). Additionally, the microorganism model achieved a Nash-Sutcliffe efficiency for instantaneous *E. coli* concentrations of $E_C = 0.64$.

The application of radar-derived areal average rainfall intensities did not improve the model performance for flow or *E. coli* at small catchment scales when compared to model's that use gauged rainfall inputs, while the performances were comparable in larger catchments. This was likely the consequence of the radar observations resolution and the need to convert weather radar observations to rainfall intensity.

The results of this study indicate that MOPUS is able to represent stormwater flow rate and microbial dynamics well over a range of catchment sizes from just a few hectares to tens of thousands of hectares. Furthermore, the ability of the MOPUS microorganism model to simulate large catchment dynamics and processes in catchments with non-urban characteristics gives some basis for further exploration of the MOPUS modelling concept in these systems. This is particularly important for estuarine and coastal microorganism models, which require appropriate representation of upper catchment inputs in order to accurately simulate the estuarine microorganism dynamics.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.jhydrol.2017.10.022>.

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5.3 Generation of flow and *E. coli* inputs to the Yarra River estuary

While the previous section describes models used to represent the flow and *E. coli* dynamics of rivers, creeks and urban stormwater drains, the models could only be calibrated and validated on a small number of catchments, for a defined period of time. Indeed, it was impossible to monitor all 208 stormwater drains entering the estuary for the full simulation period, and hence these models were only calibrated on a subset of these stormwater drains. As such, this section describes in detail how continuous time series of flow rates and *E. coli* concentrations were generated for all inputs to the estuary by application (and extrapolation) of the MOPUS model (McCarthy et al., 2011b). Section 5.3.1 describes the generation of continuous time series of flows and Section 5.3.2 describes the generation of continuous time series of *E. coli* concentrations.

5.3.1 Generation of input flow rates

For the Yarra River and Gardiners Creek, continuous measured flow data were available (obtained from Melbourne Water monitoring stations) and were used to characterize these inputs. Modelled outputs from the study presented in the Section 5.2 were used to provide inputs from the Hawthorn main drains (east and west) and the Prahran main drain. However, in addition to the above-mentioned inputs, another 205 stormwater drains discharge directly into the Yarra River estuary for which stormwater flow needed to be estimated. To achieve this, the rainfall-runoff component of MOPUS model was applied.

As indicated in Section 5.2, urban stormwater flow can be effectively simulated (i.e. without compromising the model performance) by only considering effective impervious area contributions to the stormwater flow. As such, to run the MOPUS flow model, estimates of three model parameters were required for each of 205 stormwater drains: routing coefficient (K), time of translation (TOT) and effective imperviousness of the catchment (IMP). Additionally, rainfall intensities and catchment areas needed to be provided for estimation of stormwater flows for each stormwater drain.

The routing coefficient for each of the stormwater drains was randomly sampled using a uniform distribution from the range formed by the calibrated values of routing coefficients obtained in Section 5.2 and in McCarthy et al. (2011b) (i.e. $K = 0.12 - 0.41$).

Time of translation (TOT) was calculated from estimates of time of concentration for each catchment based on a derived relationship with catchment slope (s) found in McCarthy et al. (2011):

$$TOC = -91.9 \log(s/100) + 73.9 \quad (5 - 1)$$

where *TOC* is in minutes and *s* is in percent (estimated from GIS elevation data).

Instead of estimating effective imperviousness (*IMP*) and catchment area separately, a relationship between effective impervious area ($EIA = IMP \times Catchment\ Area$) and pipe cross sectional area (*A*) was found in McCarthy et al. (2011) and was used to directly estimate *EIA* for each of the stormwater drains discharging into the estuary:

$$EIA = 14.357 \times A^{1.0673} \quad (5 - 2)$$

where *EIA* is in hectares and *A* is in square meters.

Pipe diameters of the drains discharging into the estuary (obtained from Melbourne Water and Melbourne Councils GIS datasets) range from 150 mm to over 3 m, with more than 90% of the drains having pipe diameter less than 1.65 m (maximum pipe diameter in McCarthy et al. (2011)) and as such, data obtained from our study (Section 5.2) were not applied for developing the above relationship.

To provide required rainfall intensity inputs to the model, the closest rain gauge to the centroid of the each of the stormwater drain catchments was applied. In total, four rain gauges in the estuarine catchment were used as inputs for sixty three catchments with an average distance to catchment centroid of 1234 m (min distance 205 m; max distance 2540 m).

5.3.2 Generation of input *E. coli* concentrations

As shown in Section 5.2, MOPUS was able to predict the *E. coli* dynamics in the Yarra River, Gardiners Creek, Hawthorn Main Drains (HMD east and west) and Prahran Main Drain (PMD). Therefore, MOPUS was used to provide *E. coli* concentrations for the other 205 stormwater drains discharging into the Yarra River estuary.

Firstly, a parameter set pool of all 5 model parameters (*PsCoeff*, *RHCoeff*, *VPCoeff*, *PssCoeff* and *RI*) was created using the thousand best performing parameter sets from each of the seven urban catchments located in Melbourne: three from this current study (i.e. Hawthorn main drain east and west, and Prahran main drain from Section 5.2) and the four catchments used in McCarthy et al. (2011b). Then, 205 parameter sets were randomly withdrawn from the parameter set pool. Finally, the selected parameter sets were used to produce the *E. coli* input from each of the stormwater drains.

MOPUS was developed for the predicting the wet weather stormwater *E. coli* concentrations and the predictions are a function of routed rainfall intensity (McCarthy et al., 2011). As such, during dry weather when there is no rainfall, the model was systematically under predicting the *E. coli* concentrations. To avoid this issue, *E. coli* concentrations during dry weather were estimated by sampling from a distribution of measured dry weather *E. coli* concentrations. The Yarra River and Gardiners Creek dry weather inputs were estimated using datasets collected at Dights Falls and Gardiners Creek respectively, while the dry weather inputs from stormwater drains were estimated using data set collected at Hawthorn Main Drain east and west where a substantial amount of dry weather flow monitoring was conducted. Since data were not normally distributed (Shapiro-Wilk test, $p < 0.001$), before estimating the normal distribution parameters, the data were log-transformed. The distribution parameters are shown in Table 5 - 1.

To avoid having large discrepancies between the values of predicted *E. coli* concentrations during dry weather particularly at the 6 minutely time steps applied in the model, we examined autocorrelation within the hourly measured data and applied the obtained correlation coefficients in producing the dry weather *E. coli* concentrations of the Yarra River, Gardiners Creek and stormwater drains (Equation (5 - 3)).

$$C^t = r_s C^{t-1} + (1 - r_s) 10^{[C_D^t \sim N(\mu, \sigma^2)]} \quad (5 - 3)$$

where C^t is dry weather *E. coli* concentration at time t [MPN/100mL], r_s – Pearson’s autocorrelation coefficient [-] and C_D^t is dry weather *E. coli* concentration [log(MPN/100mL)] at time t obtained by sampling dry weather normal distribution with median μ and standard deviation σ (Table 5 - 1).

Table 5 - 1 Medians, standard deviations and Pearson’s auto-correlation coefficients obtained for Dights falls, Gardiners Creek and Hawthorn main drain east dry weather *E. coli* data sets using log-transformed values.

	Median μ [log(MPN/100mL)]	St. dev. σ [log(MPN/100mL)]	r_s [-]
Dights Falls	2.24	0.27	0.61
Gardiners Creek	2.72	0.40	0.72
Hawthorn main drain east (applied to all other stormwater drains)	3.41	0.48	0.67

Finally, MOPUS predicted *E. coli* concentrations were substituted with the dry weather estimated concentrations during periods when model predictions were lower than the median measured dry weather *E. coli* concentrations. An example for the Yarra River input *E. coli* concentrations is presented in Figure 5 - 1. As shown, sampling from the distribution of measured dry weather *E. coli* concentrations helps eliminate the underestimation of the model due to the lack of rainfall. However, even though the time series are not completely random (i.e. an autocorrelation coefficient was applied during sampling) the variability during dry weather is still around 0.5 log. This may influence the estuarine model dry weather prediction and cause the model to be poorly calibrated to the measured data, particularly at the upstream end of the estuary (i.e. Abbotsford) where the *E. coli* levels are heavily influenced by the Yarra River inputs.

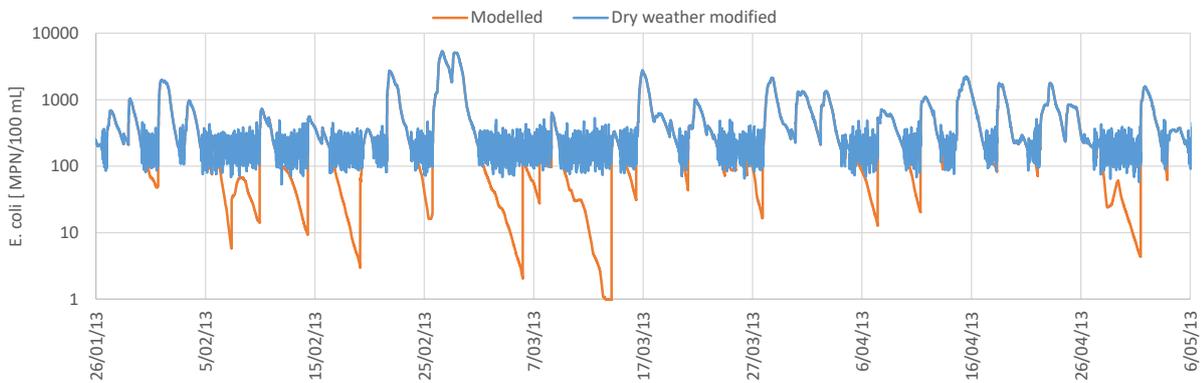


Figure 5 - 1 Example graph of the modelled and dry weather modified *E. coli* concentrations for the Yarra River input.

5.4 Assessment of the importance of urban stormwater inputs for the *E. coli* dynamics in the Yarra River estuary

This section presents parts of the journal paper “*Integrated conceptual modelling of faecal contamination in an urban estuary catchment*” published in *Water Science and Technology*, 2015, VOL 72 (9), pp. 1472-1480, relevant to assessment of the inputs of *E. coli* into the Yarra River estuary. The full paper is presented later in Chapter 7.

Integrated conceptual modelling of faecal contamination in an urban estuary catchment

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INTRODUCTION

Urban estuaries around the world are highly valued assets to the local community, as they provide aesthetics, improved microclimate and recreational opportunities (Mallin et al., 2000). Like many other urban estuaries, the Yarra River estuary has elevated levels of faecal contamination (Daly et al., 2013), which is of public health concern for recreational users. Faecal microorganisms have been identified as the leading cause of pollution of environmental waters (Ortega et al., 2009, Lipp et al., 2001, Burton and Pitt, 2002).

Urban stormwater has been recognized as an important input of faecal contamination to these waterways (Burton and Pitt, 2002; McCarthy et al., 2011). As such, increased efforts have been made towards mitigating the impacts of direct stormwater inputs (i.e. the stormwater drains that discharge directly into the estuary), including the Yarra River estuary (e.g. Melbourne Water, 2013). However,

despite these efforts, minimal improvement in compliance figures was observed for this particular system, implying that there may be other, more significant, inputs which require mitigation.

The major hypothesis of this work was that the importance of direct urban stormwater was minimal during dry weather periods, but increased during urban wet weather periods, especially when lower riverine flow rates were combined with higher amounts of urban rainfall. The impact of direct wet weather stormwater inputs could be important even in the case of uniformly distributed rainfall across a whole catchment, as stormwater could be entering the estuary much sooner than the riverine input due to the higher imperviousness and shorter time of concentration that characterize urbanised areas.

METHODS

The estuary and monitoring sites. The Yarra River estuary (Melbourne, Victoria, Australia) is a highly stratified, salt-wedge estuary (Beckett et al., 1982) and extends for about 22 km from Port Philip Bay to Dights Falls - a weir which represents the upper boundary of the estuary. Monitoring sites were selected and established for data collection (Figure 1). Two of the sites were within the estuary, Abbotsford at the very beginning of the estuarine section of the Yarra River (represents the region with little influence from the salt-wedge, but still impacted by tidal changes) and Morell Bridge, located in the lower part of the estuary (highly impacted by the salt-wedge). Both sites were equipped with refrigerated automated samplers, depth sensors and had continuous measurements of electrical conductivity (EC) and temperature (T) at 100mm below the surface. The Morell Bridge site was also equipped with an Acoustic Doppler Current Profiler (ADCP) for 3D measurements of velocities at 1 minute intervals.

Monitoring of upstream river inputs was conducted at Kew (Figure 1) where only grab samples were taken and water levels and flow rates were measured at 6 minute intervals by Melbourne Water (the local water management authority).

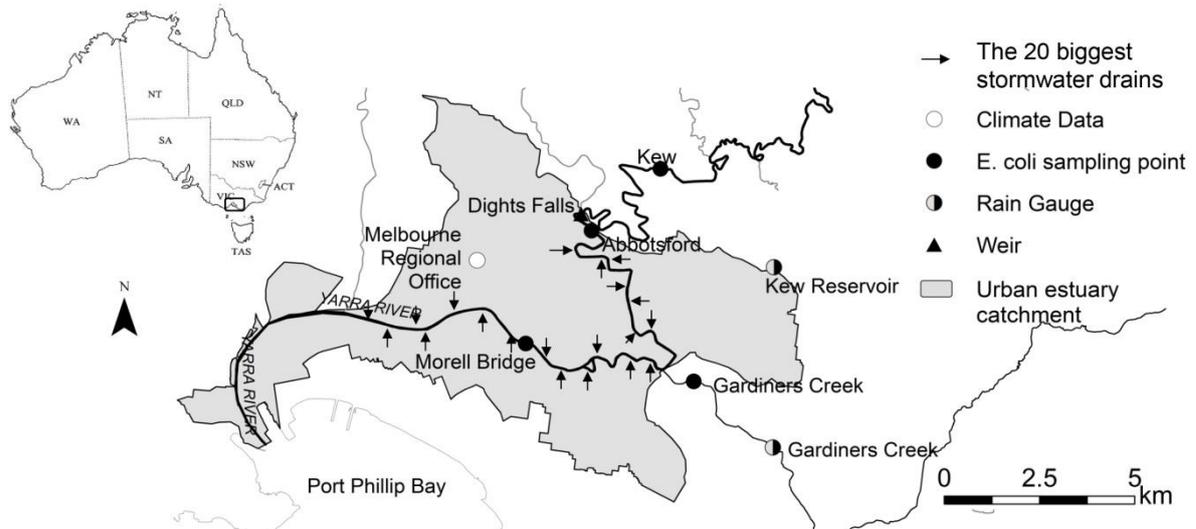


Figure 1. Monitoring stations in the Yarra River catchment (stations: Heidelberg and Coldstream (rain data) and Viewbank and Melbourne airport (climate data) are positioned outside the figure boundary. Shaded area represents the urban estuary catchment with the biggest 20 of the 216 modelled drains shown.

Monitoring of stormwater inputs was done at Gardiners Creek, a heavily channelized creek which is the largest input of water other than the Yarra River upstream of Dights Falls. The site has been equipped with an automated sampler, EC/T sensors and a depth/velocity probe. Climate data was obtained from Australian Bureau of Meteorology and Melbourne Water for different locations in the Yarra River catchment (Figure 1). Gardiners Creek is considered to be an open channel stormwater drain because its catchment is completely developed with total impervious fraction of 47%. Furthermore, observed range of the *E. coli* concentrations (944; 6203; 17673 MPN/100ml; 5th, 50th, 95th percentile) is well within the range reported for urban stormwater (Makepeace et al., 1995, Burton and Pitt, 2002).

Sample collection and analysis. Estuarine and riverine samples were taken approximately 100mm below the surface where the health exposure to recreational users is expected to be the highest. In the period of November 2012 to July 2013, 2106 samples were collected; 1500 during dry weather and 606 during wet weather conditions. All collected samples were transported to the Environmental and Public Health Microbiology (EPHM) laboratory at Monash University in coolers on ice and analyzed for

E. coli content using the Colilert method (IDEXX Laboratories, 2013) within 24h of collection. A large range of other indicators and reference pathogens were tested, but not reported here.

Riverine model. Hydrology of the upper Yarra River catchment (river inflow at Dights Falls into the estuary, Fig. 1) was modelled using MUSIC – SimHyd which is a spatially lumped catchment rain-runoff model (eWater, 2012). The model was applied with some slight variations: (1) a linear-reservoir routing routine was employed (instead of MUSIC’s standard Muskingum Cunge method) as it has been demonstrated previously that this simpler and more stable form of routing produces equivalent results (McCarthy, 2008); (2) the model was employed using a constant 6-minute timestep (as opposed to MUSIC’s standard method of daily simulation and subsequent disaggregation). This method improved the computational efficiency of the model, without compromising the results. Model inputs were areal averaged rainfall (Heidelberg, Kew, Kew Reservoir, Coldstream and Viewbank stations) and daily potential evapotranspiration, calculated using FAO Penman-Monteith method (data from Coldstream, Viewbank and Heidelberg stations). The MUSIC-SimHyd model was calibrated with a Monte-Carlo approach using a least squares objective function comparing the predicted flow rates with untransformed measured flow rates at Kew. The performance of the hydrologic model was assessed using the Nash-Sutcliffe coefficient of efficiency E_Q (Nash and Sutcliffe, 1970). Parameter sensitivity was also explored using the Monte-Carlo results, as per others in the literature (e.g. Dotto et al., 2010).

For the prediction of riverine microbial concentrations, a modified version of the EG pathogen-hydrologic catchment model (Haydon and Deletic, 2006) was applied. The main variation was that the loss of microorganisms from the subsurface store was estimated to be inversely proportional to the soil moisture instead of directly proportional which was originally proposed by Haydon and Deletic (2006), as many studies report extended survival of faecal microorganisms at higher soil moisture contents (Desmarais et al., 2002, Schäfer et al., 1998). The model had 6 parameters: one parameter described build-up, two were loss coefficients and three were related to wash-off processes. Inputs to the model were time series potential evapotranspiration and flow components as calculated by MUSIC – SimHyd. The model was calibrated against Abbotsford’s *E. coli* concentration dataset. Although there are obvious issues with this methodology (i.e. calibrating the upstream model to a site within the estuary), it was considered adequate for the following reasons: (1) Daly et al. (2013) showed that Kew and Abbotsford have similar distributions, (2) the correlation between the *E. coli* from the two sites was 0.83 (Pearson correlation coefficient, $p < 0.001$), and (3) the Abbotsford dataset had many more calibration points (776 compared to 43 at Kew) which could allow for a better calibrated model. The optimized parameter set for the EG model was obtained using a least squares objective function and by observing the Pareto front formed when calibrating using untransformed and log-transformed *E.*

coli concentrations. Additional calibration of the model parameters was conducted using the Generalized Reduced Gradient method, without limiting the parameters and using a criterion which added the two components of the Pareto front. The model's performance was assessed by the Nash-Sutcliffe efficiency calculated using untransformed and log-transformed *E. coli* concentrations - E_C and E_{Clog} respectively.

Stormwater model. Modelling of the urban stormwater input of Gardiners Creek was performed using Micro-Organism Prediction in Urban Stormwater, MOPUS (McCarthy et al., 2011), where the pervious component of the rain-runoff model was excluded. As shown previously by Dotto et al. (2011), the parameters which are used to model the pervious component are less sensitive than those used to model impervious areas, therefore demonstrating the importance of impervious areas in urbanized catchments. The rainfall runoff module of MOPUS was calibrated against the untransformed flow rates measured at the Gardiners Creek monitoring station using the same procedure outlined above for the riverine model.

MOPUS's microorganism model has five model parameters; three which represent the build-up and die-off of microorganisms on the surface of the catchment, and two others which represent the same for the subsurface (i.e. in the stormwater drain). The inputs to the model include: time series of rainfall, relative humidity and vapour pressure. MOPUS was calibrated using the 383 *E. coli* samples taken from Gardiners Creek during dry and wet weather periods and assessed using the same procedure as the EG model.

In addition to Gardiners Creek, there are 219 stormwater drains of various sizes that drain directly into the Yarra River estuary (Figure 1 – the 20 biggest shown). MOPUS was further used to generate a time series of stormwater flow rates and microorganism concentrations for each of these stormwater inputs. This was achieved by generating 219 different parameter sets. Firstly, the impervious area (IA) for each of the drains was estimated using an empirical relationship between impervious area and drain cross-sectional area (McCarthy, 2008). Then, due to the lack of measured data, the five microorganism model parameters were obtained by random sampling within parameter ranges defined by the optimized values from Gardiners Creek Catchment (this study) together with optimized values from literature which has used the MOPUS model on four other stormwater drains in Melbourne, Australia (McCarthy et al., 2011); Finally, the MOPUS model was executed for all 219 drains, using the relevant input data: rainfall, relative humidity and vapor pressure from Melbourne Regional Office station (Figure 1).

Input Analysis. Predicted stormwater flow rates and microorganism concentrations were used to calculate daily delivered volumes and loads to the estuary. A similar approach was taken with the riverine input, but instead of using predicted flow rates (which were substantially underestimated during base flow periods by the MUSIC model) measured data from Kew were used to achieve more realistic results. To assess the contribution of stormwater in dry and wet weather, both in terms of daily delivered volumes and loads, a ratio of stormwater over total inputs (sum of stormwater and river inputs) was calculated. Similarly, a ratio of daily delivered stormwater volume to the average estuary volume (estimated using GIS and bathymetry data to be $4 \times 10^6 \text{ m}^3$) was also used to assess the impact of direct stormwater inputs.

RESULTS AND DISCUSSION

Input modelling. The MUSIC-SimHyd model reproduced the observed flow pattern reasonably well ($E_Q = 0.51$); however, during base flow periods there was substantial underestimation of flow rates (probably a result of the model being modified for urbanized catchments). There were also timing issues with the prediction of the peak flows. The stormwater rainfall-runoff model had quite high performance in prediction of flow rates for Gardiners Creek, with an efficiency of $E_Q = 0.81$. It performed particularly well in the region of very high flow rates ($>10 \text{ m}^3/\text{s}$), which was expected as the model was essentially developed and calibrated for the prediction of wet weather flows.

The efficiencies of the two microorganism input models were similar; $E_C \approx 0.20$ and $E_{Clog} \approx 0.40$. Although these are not high efficiencies, they agree well with the performance reported in the literature for similar microorganism models (McCarthy et al., 2011). The pathogen-catchment model reproduced *E. coli* patterns well, although there are certain peak prediction time issues similar to that described by Haydon and Deletic (2006). The MOPUS concentration predictions are better in the region of high concentrations which are commonly observed during wet weather periods. Indeed, the current model structure was developed for modelling wet weather microbial dynamics in stormwater, hence it is expected to give better predictions during wet weather.

Inputs analysis. The relative contribution of stormwater discharging directly to the estuary during dry weather ranged from $<0.5\%$ to 10% (5th and 95th percentile), suggesting limited influence of stormwater on overall *E. coli* levels in the estuary during these periods (Figure 2.a). As expected, wet weather stormwater proportions were higher (2% to 50% ; 5th and 95th percentile), yet the average

daily contribution under these conditions remained marginal (median 10%). These findings agree well with those of Daly et al. (2013) suggesting the median daily *E. coli* loads coming into the estuary from the three biggest drains (two of them 3m in diameter and one 6x2 m) are about 1.5 orders of magnitude lower than the riverine inputs. However, it is important to note that our results also demonstrate that some conditions can produce high stormwater contributions, especially during periods of low riverine flows and high urban rainfall amounts (see Figure 2.b and Figure 2.c). It is also possible for urban stormwater to enter the estuary much faster than riverine inputs due to the higher imperviousness and the smaller time of concentration of urban catchments. Hence, at finer temporal scales (i.e. time step <1 day), stormwater could have a significant impact on overall faecal contamination levels within the estuary. Furthermore, stormwater might be significantly influencing faecal microbe distribution locally around the drain outlets. All issues stated above would certainly require further investigation, which is not within the scope of this paper.

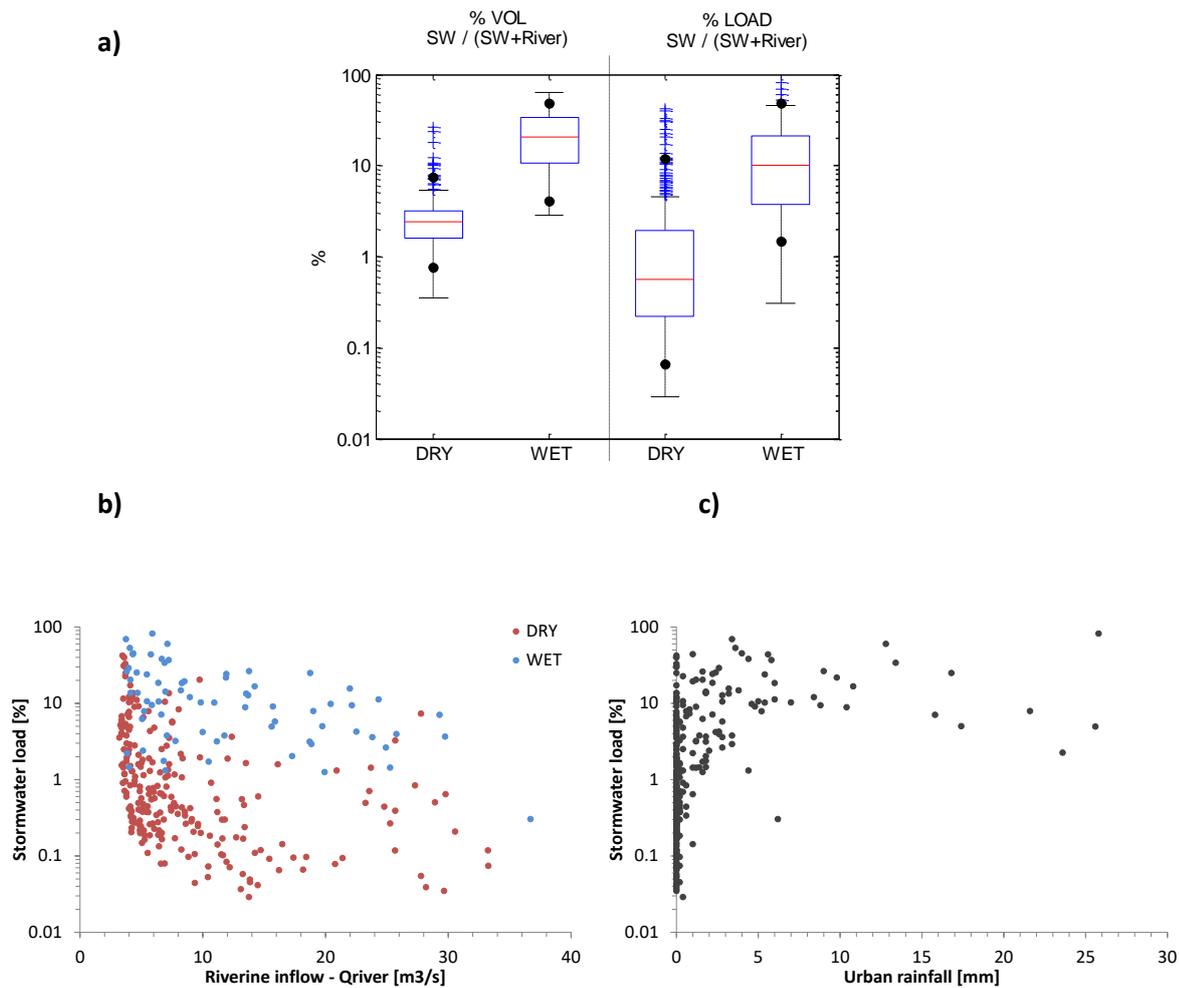


Figure 2. **a)** Modelled daily stormwater contributions during dry/wet weather conditions as a percentage of total delivered water volume (%VOL) and *E. coli* load (%LOAD) to the estuary (black dots represent 5th and 95th percentiles) for the simulated period of November 2012 – August 2013; **b)** the relationship between percentage daily stormwater load and riverine input flowrate during dry and wet weather; **c)** the relationship between percentage daily stormwater load and urban rainfall (Melbourne Regional Office station).

CONCLUSIONS

The mass balance analysis using model predictions of daily faecal microorganism loads delivered to the Yarra River estuary via riverine input, Gardiners Creek and 219 stormwater drains discharging directly to the estuary revealed limited influence of urban stormwater on the estuary during dry weather. Wet weather contributions from stormwater drains were significant in some cases (95th percentile of 50%);

however, the average contribution remained marginal (median 10%). Input analysis confirmed previous studies showing *E. coli* loads derived stormwater drains are dwarfed by other inputs. Nevertheless, it is essential to note that these results also demonstrate that some conditions reveal the opposite; high proportions from stormwater are possible when combined with low riverine inputs and high urban rainfall amounts. This study focuses on the overall impacts of direct stormwater inputs on faecal contamination levels within the estuary, and localised impacts require further investigation.

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5.5 Discussion and Conclusions

We hypothesised that accurate characterisation of inputs of *E. coli* to the Yarra River estuary is very important for accurate prediction of *E. coli* dynamics within the estuary. Indeed, in Chapter 4 it was shown that *E. coli* levels within the estuary were significantly correlated to the levels in the Yarra River upstream of Dights Falls. Furthermore, these levels are highly temporally variable (McCarthy et al., 2012), thus to accurately characterise input *E. coli* dynamics, concentrations entering the estuary are required at high temporal resolution, i.e. at sub-hourly time steps. This is especially true for the urban estuaries where urban stormwater may present an important input (Jovanovic et al., 2015). While continuous monitoring of all the inputs is impossible for practical reasons, the only way to account for these complex dynamics is by using models for the characterisation of microbial dynamics in inputs and providing boundary conditions for the estuarine microorganism model.

In this chapter, an existing model for microorganism prediction in urban stormwater (MOPUS model (McCarthy et al., 2011b)) was tested on a range of monitored catchments. The model was able to reproduce the dynamics of *E. coli* at the outlets of these catchments. After successful prediction of the flow rates and *E. coli* concentrations from a few urban catchments, the MOPUS rainfall-runoff and MOPUS microorganism models were applied to predict flow rates and *E. coli* concentrations from an additional 205 ungauged stormwater drains that discharge into the estuary. Moreover, the model was also successful in simulating the *E. coli* dynamics from the large Yarra River catchment. Indeed, the model achieved the highest performance efficiency for this catchment. This is very important since Yarra River inputs were previously shown to govern the overall *E. coli* levels within the estuary and without proper characterisations of inputs from the Yarra River, performance of the estuarine microorganism model would be compromised. Finally, since MOPUS was essentially developed for wet weather stormwater prediction, a method was presented for overcoming the model's poor performance (under-predictions) during dry weather using measured *E. coli* data, which enabled generation of a continuous time series of flow rates and *E. coli* concentrations needed for characterising all inputs of faecal contamination to the estuary (i.e. providing the boundary conditions for the estuarine hydrodynamic-microorganism model).

Whilst the MOPUS model was successfully applied for prediction of the Yarra River and urban stormwater input into the Yarra River estuary, it should be noted that the uncertainty of the predictions were not examined and the application of the produced inputs may affect the accuracy of the predictions of estuarine microorganism model.

Comparison of daily urban stormwater contributions to the overall *E. coli* load entering the Yarra river estuary revealed that stormwater inputs were dwarfed by the Yarra River inputs. Even during wet weather, stormwater inputs accounted on average for only 10% of the total load. However, it was also shown that in some cases (rainfall over the urban area of the catchment) these inputs can account for over 50% of the total load. Furthermore, the conducted analysis was based on daily loads and at sub-daily timescale, stormwater inputs may have an important impact on overall level of *E. coli* in the estuary.

In summary, accurate modelling of microorganism dynamics in urban estuaries requires provision of boundary conditions that characterise the input dynamics well. Inaccurate characterisation of input dynamics may lead to poor estuarine microorganism model performance and consequently, misleading conclusions about the most significant inputs and processes of faecal microorganism dynamics. This in turn can lead to ineffective and costly management strategies.

Chapter 6
Modelling hydrodynamics
of the Yarra River estuary

6.1 Introduction

Estuarine hydrodynamics is the main driver of the microbial transport, mixing, sedimentation and resuspension within the estuary. Additionally, it governs the spatial and temporal distribution of environmental parameters, such as temperature and salinity, which influence the survival of microorganism. Therefore, the hydrodynamic model needs to accurately predict the velocity fields and mixing within the estuary. Moreover, this is an essential requirement in case of highly stratified estuaries such as the Yarra River estuary. Hence, the aim of this chapter is to present set up and testing of the hydrodynamic model of the Yarra River estuary, to which a microorganism model will be coupled. For this purpose, TUFLOW FV modelling platform has been selected according to the criteria outlined in the literature review (Chapter 2).

This chapter aims at addressing the research question related to identifying the most important hydrodynamic model input data needed for accurate prediction of flow velocity, which is intrinsically linked to number of processes influencing the faecal microorganism dynamics such as, transport and mixing within the estuary, sediment resuspension/settling and temperature and salinity distribution (Section 2.9, Chapter 2 – Literature Review). It was hypothesised that inflow rates, bathymetry and wind data will be the most important for accurate prediction of velocity fields.

The main component of this chapter is a journal paper titled *“Modelling shallow and narrow urban salt-wedge estuaries: Evaluation of model performance and sensitivity to optimise input data collection”* published in *Estuarine, Coastal and Shelf Science*, 2019, VOL 217, pp. 9 – 27, (Section 6.2). Part of this work was initially presented in form of conference paper at the 21st International Congress on Modelling and Simulation (MODSIM) in Gold Coast, Australia in 2015 (the conference paper can be found in Appendix B.2). The chapter finishes with a discussion that integrates the findings of this chapter with the broader aims and objectives of this thesis (Section 6.3).

6.2 Modelling shallow and narrow urban salt-wedge estuaries: Evaluation of model performance and sensitivity to optimise input data collection

The supplementary material for this manuscript is provided in Appendix A3.



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Modelling shallow and narrow urban salt-wedge estuaries: Evaluation of model performance and sensitivity to optimise input data collection



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ABSTRACT

Complex three-dimensional estuarine hydrodynamic models require large quantities of high-resolution data for model forcing and initialisation. The data are often expensive and difficult to collect with high accuracy (e.g. bathymetry data, riverine flows, water depths, etc.). It may be possible to reduce input data requirements, whilst maintaining predictive capabilities. This is the first study that assesses the sensitivity of a three-dimensional hydrodynamic model of a shallow and narrow urban salt-wedge estuary to input data used for model forcing and initialisation. The model was built using the TUFLOW FV modelling platform and its performance was tested against high-resolution water level, flow velocity, vertical salinity and temperature distribution data. A number of scenarios were used in which data used for model forcing and initialisation, including flow rates, salinity and temperature, wind, bed roughness, bathymetry and vertical mesh discretisation were systematically varied. To assess the sensitivity of model outputs, model predictions were compared to the optimised model predictions for ten periods covering different hydrologic and hydrodynamic conditions. The analysis showed that all model outputs (i.e. water level, velocity, temperature and salinity) were influenced by large and localised water inputs. Due to limited wind fetch of narrow water bodies, wind inputs are expected to have limited impact on hydrodynamic model outputs. However, in this study, flow velocity, salinity and temperature outputs were all influenced by wind inputs. Whilst, accurate bathymetry data are considered essential for developing three-dimensional hydrodynamic models of shallow regions, in this study, uncertainty in the bathymetry data had limited influence on model outputs. Removal of stormwater inputs (i.e. 208 stormwater drains), setting constant salinity for fresh water inputs, weekly averaging of temperature and errors in bathymetry all had minimal impact on model outputs. The results of this case-study can help inform future modelling exercises of narrow and shallow salt-wedge estuaries by focussing efforts on the most important input data. This would potentially lead to substantial reductions in cost and time needed to set up the model.

1. Introduction

Complex three-dimensional hydrodynamic models are increasingly being used as drivers of water quality models for the assessment purposes. These include assessment of aquatic ecosystem processes and interactions, development of pollution mitigation strategies, evaluation of management actions and simulation of possible future scenarios

(Janssen et al., 2015). The need for coupled hydrodynamic and water quality models arises from dynamic feedbacks between hydrodynamic and environmental variables, which form critical interactions within ecosystems (Ganju et al., 2016).

Interactions between hydrodynamics and water quality are especially complex in urban estuarine environments, where a myriad of factors need to be considered such as: riverine and tidal forcing, mixing

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between fresh and saline water with various degrees of stratification, and mixing of different water sources with various temperatures and salinities (e.g. urban stormwater, or wastewater overflows from sewers). Accounting for these interactions requires models with input data for multiple water sources, often at a fine temporal resolution (e.g. sub-daily flow rates and physical water quality parameters such as temperature and salinity) or spatial resolution (e.g. bathymetry). Collection of these data sources is often time-consuming and costly. The collected data always contains uncertainties which may be propagated from the hydrodynamic model into subsequent water quality model predictions. Because water quality modelling can be very sensitive to hydrodynamic conditions (Allen et al., 2007), robust evaluation of the hydrodynamic component is essential (Ganju et al., 2016). This evaluation can be achieved by performance and sensitivity assessment of model outputs which inform users about which datasets are more sensitive (and hence require more attention when being collected to limit its uncertainty and subsequent propagation) compared to those which do not influence the results significantly (i.e. those that do not require as much attention as they do not impact the model's results as much) (Bennett et al., 2013).

Tests of sensitivity are fundamental for assessing and validating hydrodynamic models and can be applied to almost all boundary or model input data/parameters of the models (e.g. input flow rates, salinity, temperature, atmospheric conditions, bathymetry etc.), generating knowledge on the sensitivities associated with them and the relationship between input data/parameters and model predictions (Simionato et al., 2004; Harcourt-Baldwin and Diedericks, 2006). Constructing a three-dimensional hydrodynamic model requires a large amount of resources, both in terms of data and time needed to set up the model and the related costs. The generated knowledge regarding the most influential inputs shown in this study, can be used for decreasing both the related costs and time to set up a functioning hydrodynamic model. While uncertainty and sensitivity analysis of the model outputs has received increased attention of the last couple of decades as part of water resource modelling, very limited work has been done around estuarine hydrodynamics (Camacho et al., 2014). Only a few studies have applied sensitivity testing to models quantifying the impact of datasets on estuarine model predictions (Simionato et al., 2004; Harcourt-Baldwin and Diedericks, 2006; Cea and French, 2012; Camacho et al., 2014; Weaver et al., 2016; Chao et al., 2017; Kang et al., 2017).

These studies have increased our understanding of the relationships between uncertainty in input data and the quality of the model outputs for the modelled estuaries. However, the modelled systems were predominantly large bays (Simionato et al., 2004; Harcourt-Baldwin and Diedericks, 2006; Camacho et al., 2014; Chao et al., 2017; Kang et al., 2017) with the exception of the two studies were a lagoon-type estuary (Weaver et al., 2016) and a drowned river-valley estuary (Cea and French, 2012) were modelled. Additionally, the modelled estuaries were mainly well-mixed systems (Simionato et al., 2004; Harcourt-Baldwin and Diedericks, 2006; Cea and French, 2012; Camacho et al., 2014; Kang et al., 2017). Therefore, there is need for further studies that cover other types of estuarine systems, both in terms of scale (i.e. a range of different sizes), morphology (i.e. drowned river-valley, fjord-type, lagoon-type or tectonic estuary) and salinity structure (i.e. well-mixed, partly-mixed or highly-stratified estuaries). Furthermore, the studies in literature analyse the model sensitivity in relation to only a few selected boundary conditions/model parameters such as: fresh-water inflows (Harcourt-Baldwin and Diedericks, 2006; Camacho et al., 2014), tides (Harcourt-Baldwin and Diedericks, 2006; Camacho et al., 2014; Kang et al., 2017), bathymetry (Simionato et al., 2004; Harcourt-Baldwin and Diedericks, 2006; Cea and French, 2012; Camacho et al., 2014), roughness (Cea and French, 2012), wind (Simionato et al., 2004; Harcourt-Baldwin and Diedericks, 2006; Weaver et al., 2016; Kang et al., 2017) and water temperature (Harcourt-Baldwin and Diedericks, 2006). As such, a more comprehensive sensitivity analysis that will

include a suite of different boundary conditions/model parameters is required. Many research questions remain unanswered by existing studies, including: (a) how sensitive are the model outputs to distributed ungauged stormwater inputs? (b) is it important to collect high resolution measurements of salinity and temperature for each input in order to accurately simulate salinity and temperature distribution in stratified estuarine systems? (c) what is the effect of uncertainty in wind measurements on model outputs? (d) is highly accurate bathymetry data required for accurate predictions in salt-wedge estuaries?

This study focusses on the sensitivity of outputs to various input data using a three-dimensional hydrodynamic numerical model of a highly stratified (salt-wedge) estuary – the Yarra River estuary, Melbourne, Australia. The initial aim of this study was to evaluate the predictive ability of the hydrodynamic model using a comprehensive dataset of high-resolution measurements. Additionally, the performance of the model was compared to the previous generation of the Yarra River estuary model developed by Bruce et al. (2014). More importantly, the main aim of this study was to test the sensitivity of the model to various input data used to initialise and force the model. The sensitivity analysis included the testing of the model's response to variation in flow rates, salinity and temperature boundary conditions, wind, bathymetry, vertical mesh discretisation and bottom roughness. Noting that end-users will have very different modelling objectives, the effects of this sensitivity analysis was assessed for various model outputs (e.g. water levels, flow velocity, salinity and temperature). For the first time on narrow and shallow estuaries, we tested the following hypotheses in this paper: (1) uncertainty in the bathymetry input datasets for shallow and narrow estuaries will have a significant impact on water levels and flow velocity; (2) wind inputs will not have a significant impact on model outputs due to limited wind fetch; (3) vertical mesh discretisation will have significant impact on bottom salinity prediction (i.e. prediction of the salt-wedge dynamics). This work provides the first evidence of which input datasets are important for future modellers of narrow, shallow, river driven salt-wedge estuaries, for a wide range of modelling objectives. Results of this study may be used as a reference when building other three-dimensional hydrodynamic models of similar estuaries as well as a guide to plan and prioritize data collection campaigns to support model development and verification.

2. Methods

2.1. Study site

The Yarra River estuary is an urban estuary located within the city of Melbourne, Australia. The estuary spans 22 km between Port Phillip at the downstream end and an artificial weir, Dights Falls, at the upstream end (Fig. 1). There are two distinctive sections within the Yarra River estuary. The upper section of the estuary with depths from < 1 m to 5 m and widths from 30 m to 100 m which extends 15.5 km downstream of Dights Falls and the lower section of the estuary that has been significantly modified by dredging of bed material to depths > 10 m and widths from 100 m to 250 m to accommodate port activities (Beckett et al., 1982; Ellaway et al., 1982).

According to salinity structure classifications, the estuary has been previously categorised as a highly-stratified, salt-wedge type estuary (Beckett et al., 1982). Salt-wedge estuaries have a large fluvial to tidal flow ratio and typically occur along microtidal coasts where the tidal range is less than 2 m (Dyer, 1997). The Yarra River estuary hydrodynamic regime is consistent with this classification, with tidal water level fluctuations varying between 0.3 and 0.9 m (mean ~ 0.5 m). The tidal pattern is semi-diurnal, having significant diurnal variation (Beckett et al., 1982). The average fluvial flow rate in the lower Yarra River is estimated to be 10 m³/s (Sokolov and Black, 1996).

The major input of fresh water to the estuary is the Yarra River above Dights Falls, which contributes about 70% of the total flow

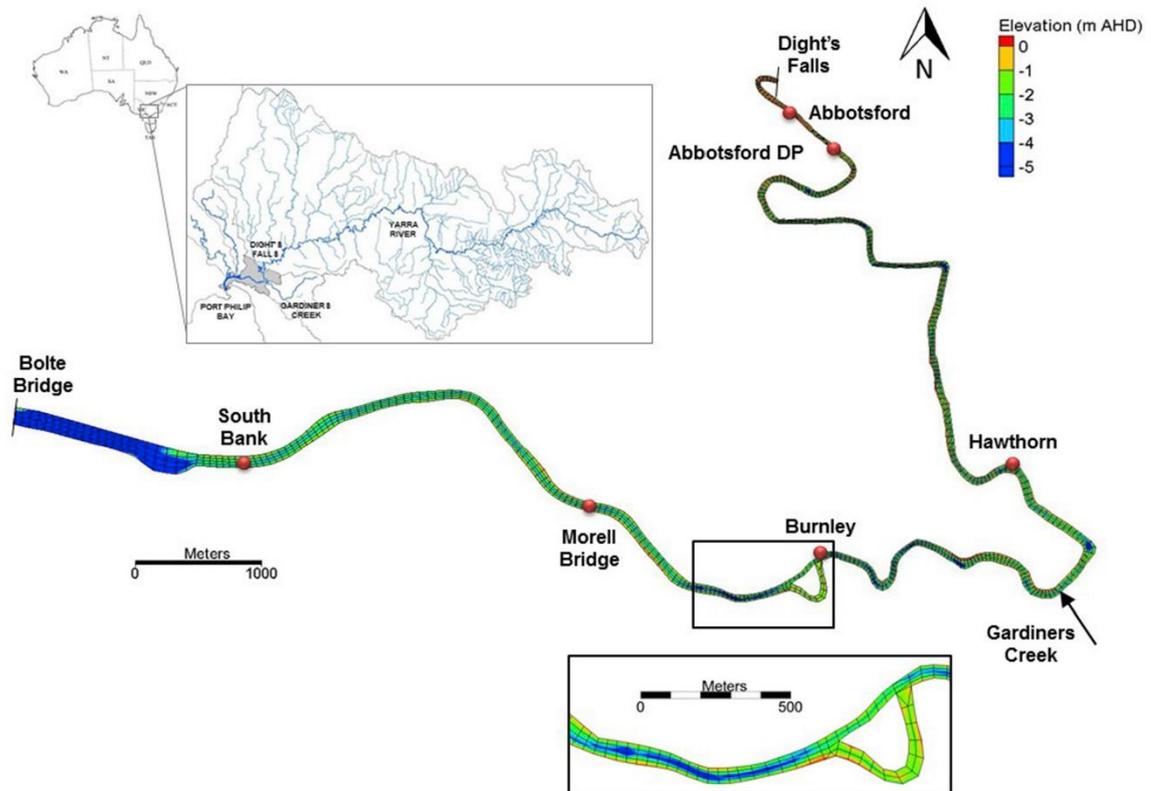


Fig. 1. Yarra River catchment and estuary section model mesh with monitoring stations: water level – Abbotsford, Hawthorn, Burnley, South Bank; flow velocity – Morell Bridge; electrical conductivity and temperature (EC/T) – Abbotsford, Morell Bridge; salinity and temperature depth profiles – Abbotsford DP, Hawthorn, Morell Bridge and South Bank. Adopted from Jovanovic et al. (2015a).

(Sokolov and Black, 1996). The other 30% of fresh water inputs include Gardiners Creek in the upper estuary (~7.5 km downstream of the Dights Falls), Maribyrnong River and Moonee Ponds Creek in the lower estuary, and several stormwater drains discharging directly to the estuary along its entire length. For example, more than 200 stormwater drain outlets were identified in the upper section of the estuary alone, some of which have pipe diameters > 3 m (Daly et al., 2013; Jovanovic et al., 2015b).

2.2. Model description

TUFLOW FV is a three-dimensional numerical hydrodynamic model able to solve a wide range of hydrodynamic systems such as open channels and floodplains through to estuaries, coasts and oceans (BMT WBM, 2013; BMT WBM, 2014). TUFLOW FV solves the conservative integral form of non-linear shallow water equations (NLSWE) by employing a finite volume numerical scheme. The NLSWE are a system of equations describing the conservation of fluid mass/volume and momentum in an incompressible fluid, under the hydrostatic pressure and Boussinesq assumptions (Leveque, 2002). The model also simulates the transport of scalar constituents, such as salinity and temperature, and includes their effect on the hydrodynamic solution through baroclinic coupling using the UNESCO equation of state (Fofonoff and Millard, 1983). Horizontal mixing is taken into account through constant eddy viscosity or the Smagorinsky model for momentum transfer and constant scalar diffusivity, Smagorinsky or Elder models for scalar transfer.

Vertical momentum and scalar mixing is parameterised through constant viscosity/diffusivity values, a zero-equation parametric turbulence model, or any external turbulence model coupled with TUFLOW FV through an in-built linking interface. The model also accounts for surface momentum exchange and heat transfer if appropriate boundary conditions are supplied. Two short wave and five long wave radiation models are available for heat transfer calculations (BMT WBM, 2013). Both first and second order spatial integration schemes are available in the model. The temporal integration scheme is explicit and employs mode splitting and dynamically varying time step, subject to Courant-Freidrich-Levy (CFL) and Peclet constraints, to maximise computational efficiency.

TUFLOW FV solves the NLSWE on regular structured grids or unstructured (flexible) meshes. The flexible mesh allows for seamless boundary fitting along complex coastlines or open channels as well as accurately and efficiently representing complex bathymetries with a minimum number of computational elements. Furthermore, a range of scales can be resolved in a single model without requiring multiple domain nesting. In the vertical dimension, the mesh discretisation can be defined using sigma coordinates, z coordinates or a hybrid sigma-z coordinates.

This study adopts the unstructured (flexible) mesh approach described above with estuarine hydrodynamics resolved using a combination of triangular and quadrilateral elements (in the horizontal dimension). The main difference in comparison with the previous generation of the Yarra River model (Bruce et al., 2014) was in relation

to the mesh. Firstly, the new mesh covered a longer reach of the estuary, approximately 17 km section from Dight's Falls to Bolte Bridge at the downstream boundary (Fig. 1), as compared to 14.5 km in the previous mesh. Secondly, the spatial resolution of the new mesh was significantly increased, with the total number of mesh elements being 1644 compared to the 397 in the previous mesh. In the new mesh, four elements were typically used to define the estuary cross-section, whereas previously the cross-section was defined by only one element. Thirdly, bed bathymetry was determined from bed elevation data from three hydrographic surveys of the Yarra River estuary supplied by Parks Victoria, Melbourne Water and Red Mapping without applying bed elevation smoothing function as done previously. Finally, vertical discretisation was done using a hybrid sigma-z coordinate system similarly to the previous model. However, the number of sigma layers above elevation of -1.0 m AHD (Australian Height Datum) was doubled (i.e. eight compared to four previously). The thickness of the sigma layers was subject to the water level change. An additional z coordinate vertical layer each 0.2 m below -1.0 m AHD, rendered a total of 26702 computational bins.

In the current hydrodynamic model of the Yarra River estuary, the Smagorinsky model was applied for horizontal mixing of momentum and scalars with coefficients $C_s = 0.2$ and $C_\sigma = 0.2$, respectively, while vertical mixing was calculated using the General Ocean Turbulence Model (GOTM) k-omega scheme with default parameters (Umlauf and Burchard, 2003). GOTM (Umlauf et al., 2003) was coupled with TUFLOW FV through the external turbulence Application Programming Interface (API). Previous testing of different vertical mixing schemes showed that application of the k-omega scheme was critical for simulating stratification in the Yarra River estuary (Jovanovic et al., 2015a).

While TUFLOW FV Surface Heat Exchange Module has a range of options for addressing the atmospheric surface forcing, in the current model, surface heat transfer was calculated by taking into account the effects of penetrative radiation (i.e. solar radiation) and non-penetrative radiation (i.e. long-wave radiation). The first was calculated based on the input of latitude, time, air temperature and cloud cover. The second was calculated based on the incoming long-wave radiation due to cloud cover and water-emitted long wave radiation due to the temperature difference between the air and the water surface.

2.3. Initial and boundary conditions

Discharge data for the Yarra River, Merri Creek and Gardiners Creek, as well as corresponding salinity and temperature measurements, were either supplied by Melbourne Water or collected by Monash University. These data were used to characterise the flow boundary conditions. Additionally, discharges from 208 stormwater drains were estimated through a rainfall-runoff model developed by McCarthy et al. (2011) that was further described by Jovanovic et al. (2015b) and Jovanovic et al. (2017). Temperature and salinity data collected by Monash University from two major stormwater drains discharging into the estuary were also used to characterise stormwater inputs. Tidal surface water elevations at Southbank supplied by Melbourne Water were used as a downstream boundary condition. All flow and water level boundary conditions were defined at a 6-min interval.

Salinity at the downstream boundary was assumed to be constant and set to the salinity of seawater (i.e. 35) across the whole boundary cell face. Indeed, salinity measurements from the commercial vessel navigating through the Port Phillip from October 2013 to September 2014 indicated median salinity of 34.8 with 5th and 95th percentile being 32.0 and 35.7, supporting the chosen value. However, due to the stratification of the Yarra River estuary at downstream boundary this assumption may have introduced some error in the predicted position of the halocline, but there was no vertical salinity distribution data available to define a variable salinity profile at the downstream boundary. Nevertheless, this set boundary condition is only active during the times of upstream flow (i.e. when flux enters into the model

domain from the downstream boundary). Measurements of the flow velocity at Morell Bridge obtained between November 2012 and September 2014 indicate that upstream flow occurs only 13% of the time and the average velocity of the upstream flow is nearly three times lower than in the downstream direction (-0.07 m/s and 0.19 m/s, respectively). Therefore, the effect of the set boundary condition is expected to have only a localised impact on the model prediction, mainly in the most downstream part of the model domain.

Measured water temperature was also obtained from the commercial vessel navigating the Port Phillip and used to further characterise downstream boundary conditions. Since no diurnal variation in the temperature of the bay was observed, a weekly time step was applied. As with the salinity boundary condition, the uniform distribution of temperature at the downstream boundary condition is expected to have mainly localised impacts on model predictions.

Meteorological data supplied by the Bureau of Meteorology were also used as input to the model. This included precipitation, air temperature and relative humidity measured at Melbourne Regional Office (www.bom.gov.au). Measurements at Essendon Airport (approximately 10 km to the north of the study area) were adopted for wind speed, wind direction, and total cloud cover. All meteorological boundary conditions were applied at 6-min intervals, with the exception of total cloud cover which was applied at a 3-h interval.

Salinity and temperature values were set to 20 and 20°C , respectively, throughout the model domain as initial conditions. Consequently, an additional month of 'warm up' was added to the beginning of the simulation period to allow the model to adjust to a dynamic equilibrium prior to undertaking any assessments. This ensured that the results were not biased by the initial conditions assumption. In total, the simulation period covered nearly 2 years, spanning from 1st October 2012 to 1st September 2014.

2.4. Observational data

The predicted variation in water level was assessed against 6-min interval measurements obtained from Melbourne Water at four gauging stations: Abbotsford, Hawthorn, Burnley and Southbank (Fig. 1). The water level data covered the whole simulation period of nearly two years.

The ability of the model to reproduce flow velocity was assessed against measurements conducted at Morell Bridge (Fig. 1). All three components of velocity (i.e. east V_x , north V_y , and vertical W) were measured by two Acoustic Current Doppler Profiler (ADCP) devices at 1-min intervals. One ADCP device was positioned in the deepest point of the cross-section (i.e. deep ADCP with bin size of 1 m) while the other was positioned closer to the right bank (i.e. shallow ADCP with bin size 0.5 m). Both deep and shallow ADCP devices had an additional measuring bin – surface dynamic bin which corresponded to the top 1 m and 0.5 m of the water column at any time, respectively. Measured velocity was averaged over a 6-min interval (to be consistent with the model outputs) before it was used for the assessment of model performance. Measurements of the flow velocity were available for the period from 1st December 2012 to 1st August 2014, covering nearly whole simulation period.

The direction of flow at Morell Bridge is well aligned with an East-West direction (Fig. 1), thus the main flow velocity component is V_x . As shown in Table 1, the measured V_x velocity component magnitude is, on average, five times larger than the velocity component along the north direction (V_y). Additionally, measured vertical velocity (W) is negligible (on average no vertical movement of water) throughout the water column (with 5th and 95th percentiles around $+25$ mm/s and -25 mm/s, respectively). Limited vertical flow, and thus limited mixing, is expected in highly stratified systems such as the Yarra River estuary in order for the salt-wedge to form and remain stable. Moreover, the measured vertical velocities are in range of the standard error of the measurements for this component (i.e. st. err. = 20 mm/s). Thus, the

Table 1

Median (5th percentile; 95th percentile) values of measured flow velocity components (V_x – east velocity component; V_y – north velocity component; W – vertical velocity component) at Morell Bridge using deep the ADCP device in different bins along the water column. Bin 1 - the deepest measurement bin; Bin 4 – the shallowest measurement bin and; Surface dynamic bin – top 1 m of the water column at any time.

Location	V_x	V_y	W
	[m/s]	[m/s]	[m/s]
Bin 1	0.043 (–0.116; 0.187)	–0.006 (–0.087; 0.076)	0.000 (–0.025; 0.027)
Bin 2	–0.039 (–0.144; 0.204)	–0.007 (–0.081; 0.070)	0.000 (–0.022; 0.022)
Bin 3	–0.020 (–0.308; 0.200)	0.040 (–0.076; 0.091)	0.000 (–0.022; 0.022)
Bin 4	–0.148 (–0.410; 0.123)	0.030 (–0.059; 0.120)	0.001 (–0.022; 0.025)
Surface dynamic bin	–0.140 (–0.414; 0.149)	0.029 (–0.080; 0.138)	0.001 (–0.030; 0.032)

performance of the model was not assessed against the measured vertical velocities.

The ability of the model to predict salinity and temperature was assessed using two datasets:

- 1) depth profiles dataset (including a total of eighty four temperature and salinity profiles at four depth-profiling sites along the estuary: Abbotsford DP, Hawthorn, Morell Bridge and Southbank). This dataset was collected on ten different occasions in the period from April 2013 to August 2014.
- 2) continuous temperature and salinity measurements at Abbotsford and Morell Bridge (Fig. 1) collected in the period September 2012–September 2014. The continuous measurements of temperature and salinity were performed at a fixed location within the water column at Abbotsford (approximately 40 cm from the river bed), while at Morell Bridge, measurements were conducted approximately 10 cm below the water surface (by attaching the Electrical Conductivity (EC)/Temperature (T) sensor to a flotation device, so that EC/T measurements corresponded to velocity measurements in the surface dynamic bin) and at the bottom of the water column (by attaching the EC/T sensor to the housing of the shallow ADCP device). Technical faults with the EC/T sensors meant that only continuous temperature measurements were suitable for model validation purposes.

2.5. Model performance evaluation

The predictive skill of numerical models is typically assessed using measures of performance (i.e. model fit parameters). In this study, five model fit parameters were selected to assess model performance and enable a comparison with previous studies in literature. More particularly, the same model fit parameters were used by Bruce et al. (2014) for the assessment of the previous version of the Yarra estuary TUFLOW FV model. The model fit parameters were:

- 1) Normalised Mean Absolute Error (NMAE):

$$NMAE = \frac{\sum_{i=1}^N |O_i - P_i|}{NO} \quad (1)$$

- 2) Root Mean Square Error (RMSE):

$$RMSE = \sqrt{\frac{\sum_{i=1}^N (O_i - P_i)^2}{N}} \quad (2)$$

- 3) Nash-Sutcliffe Efficiency (E) (Nash and Sutcliffe, 1970):

$$E = 1 - \frac{\sum_{i=1}^N (O_i - P_i)^2}{\sum_{i=1}^N (O_i - \bar{O})^2} \quad (3)$$

- 4) Index of Agreement (IOA; also known as Model Skill Score) (Willmott, 1981; Willmott et al., 1985):

$$IOA = 1 - \frac{\sum_{i=1}^N (O_i - P_i)^2}{\sum_{i=1}^N (|P_i - \bar{O}| + |O_i - \bar{O}|)^2} \quad (4)$$

- 5) Correlation coefficient (r):

$$r = \frac{\sum_{i=1}^N (P_i - \bar{P})(O_i - \bar{O})}{\sqrt{\sum_{i=1}^N (P_i - \bar{P})^2} \sqrt{\sum_{i=1}^N (O_i - \bar{O})^2}} \quad (5)$$

where: N is the number of observations, O_i and P_i are the “ i th” observed (measured) and model predicted data and \bar{O} and \bar{P} are the mean observed and mean predicted data, respectively.

2.6. Model sensitivity

Due to the computationally heavy hydrodynamic model used in this study, this paper applies an ad hoc “One at a Time” (OAT) sensitivity analysis method. While this limits our ability to detect interactive effects, the approach has been chosen to strategically gain insights into our research questions without requiring excessive computational power that is likely unreproducible due to time and cost constraints.

2.6.1. Sensitivity scenarios

Fifteen scenarios were developed to test the sensitivity of the model's outputs to the input data (Table 2). These scenarios were then compared to the base case scenario that utilised the most accurate datasets (highest resolution and no simulated errors). Each sensitivity scenario explores the effect of different input uncertainties on the modelled results i.e. uncertainties in input flow data (e.g. uncertainties in the measurements, missing inputs, etc.), uncertainties in salinity and temperature for water inputs, uncertainties in wind inputs, uncertainties of the channel bed roughness coefficient, uncertainties in the bathymetry data and the resolution of the vertical mesh. These scenarios are each explained in detail below.

2.6.1.1. Flow rates. The Yarra River is the largest water input to the estuary and represents 93% of the total fresh water volume delivered during the simulation period. Additionally, the modelled estuarine reach receives discharges from Gardiners Creek (4% of the total volume delivered) and 208 stormwater drains (3% of the total volume delivered). While the contributions of the urban inputs may seem small, Jovanovic et al. (2015b) showed that during the wet weather events these inputs can contribute up to 50% of the total volume delivered to the estuary. Therefore, two scenarios were performed to test the sensitivity of the model to urban inputs (i.e. Gardiners Creek and 208 stormwater drains). In the first scenario (Table 2 - SW), stormwater drain inputs were removed, while fresh water was delivered via the Yarra River and Gardiners Creek. In the second scenario (Table 2 - SW_GC), both the stormwater drains and Gardiners Creek contributions were removed, hence, only the Yarra River was delivering fresh water to the estuary. While these two test scenarios enable assessment of the model's sensitivity to urban water inputs as

Table 2
Summary of the sensitivity test scenarios performed.

Simulation name	Input data varied	Simulation description
Base case	None	This represents the full model as explained in Section 2.4. All input data were at the highest resolution possible, with no simulated errors.
SW	Flow	The stormwater inputs were removed. Thus, Yarra River and Gardiners Creek remained the only water inputs.
SW_GC	Flow	The stormwater and Gardiners Creek inputs were removed. Thus, the Yarra River remained the only water input.
1.25Y	Flow	Yarra River flow rate was increased by 25%, while Gardiners Creek and stormwater inputs remained as an input and unchanged.
0.75Y	Flow	Yarra River flow rate was decreased by 25%, while Gardiners Creek and stormwater inputs remained as an input and unchanged.
SAL	Salinity	Constant salinity for all water inputs.
TEMP	Temperature	Constant weekly temperature applied for the Yarra River input and constant daily temperature for Gardiners Creek and stormwater inputs.
0.5W	Wind	Wind velocity decreased by 50%.
1.5W	Wind	Wind velocity increased by 50%.
Vb_W	Wind	Wind velocity taken from a different station – Viewbank.
K5	Bed roughness	Bottom roughness increased 5 times (i.e. 5 mm) compared to the one adopted in the model (i.e. 1 mm).
K10	Bed roughness	Bottom roughness increased 10 times (i.e. 10 mm) compared to the one adopted in the model (i.e. 1 mm).
D+15	Bathymetry	Bed depth increased by 15 cm.
D-15	Bathymetry	Bed depth decreased by 15 cm.
D-50	Bathymetry	Bed depth decreased by 50 cm.
V.RES	Vertical mesh resolution	Number of vertical layers reduced by half

whole, they also allow for the comparison of the extent of impact Gardiners Creek and stormwater drain inputs have on model outputs.

Sensitivity of model outputs was also tested to the Yarra River input, due to its significance. In general, the uncertainty in flow rate measurements can vary from 5% to 45% (Pelletier, 1988; Di Baldassarre and Montanari, 2009; Camacho et al., 2014) and arise from an array of factors, such as: cross-section geometry, homogeneity of the flow in the cross-section, measurement resolution, precision and condition of monitoring equipment and measurement method (e.g. rating curve as in the case of the Yarra River) (Pelletier, 1988; Mccarthy et al., 2008; Di Baldassarre and Montanari, 2009; Camacho et al., 2014). Two test scenarios were performed to assess the impact of uncertainty in flow measurements, where the measured rate was varied by $\pm 25\%$, which is the average of the reported uncertainty range (Tables 2–1.25Y/0.75Y).

2.6.1.2. Salinity and temperature. The salinity and temperature of inputs can have a strong influence on hydrodynamics (Chua and Fringer, 2011), therefore, a significant amount of time and resources is often spent on data collection in order to define variable salinity and temperature boundary conditions in hydrodynamic models – which in turn is expected to increase the model performance.

The influence of salinity measurements on model performance was assessed by the SAL Scenario (Table 2), whereby a constant salinity value was set for each input. The salinity of the fresh water inputs was set to the mean measured value for each water input (Yarra River 0.10, Gardiners Creek 0.15 and stormwater drains 0.50). The salinity of the downstream boundary remained the same (35).

Measured water temperatures in the Yarra River estuary are known to exhibit seasonal fluctuation patterns, hence, it was not reasonable to set a constant temperature for each input for the entire two-year simulation period. Instead, to test the influence of not having continuously measured temperatures on model performance, an appropriate averaging interval was adopted for each input in the TEMP scenario (Table 2 – TEMP). In the case of urban stormwater inputs (i.e. Gardiners Creek and stormwater drains) a daily average temperature was applied, since wet weather events in urbanised areas typically last a few hours (Burton and Pitt, 2002). In the case of the Yarra River and the downstream boundary conditions, the weekly mean was adopted to account for the seasonal temperature fluctuations.

2.6.1.3. Wind. Wind inputs can be particularly important for hydrodynamic models due to the potential effect on water circulation and mixing (Kuang et al., 2011), however, wind measurements are often not available in close proximity to study sites. Hou et al. (2013) showed that in urbanised areas, wind velocities could be reduced by

20% when compared with velocities measured at the station. Bergström and JUUSO (2006) showed that valley form could enhance the wind velocity by up to 50%.

To test the impact of the wind uncertainty on model performance, three test scenarios were considered: 1) wind velocity reduced by 50% (Tables 2–0.5W); 2) wind velocity increased by 50% (Tables 2–1.5W) and; 3) wind velocity measurements taken at a different station (i.e. Viewbank station around 15 km away from the model centroid (www.bom.gov.au) – Fig. 1; Table 2 – Vb.W). Differences in measured wind speed between the Essendon and Viewbank are on average around 50% giving some support to the selected scenarios (Table 3).

2.6.1.4. Bed roughness. Bed roughness is one of the most commonly varied parameters in hydrodynamic modelling. In this TUFLOW FV application, the bottom drag model assumes a log-law velocity profile and required specification of a surface roughness length-scale, k_s . The k_s value adopted in the model was 1 mm. While this value is relatively low for natural streams, it was representative of fine bed sediment composition (Ellaway et al., 1982). Therefore, two test scenarios were performed to test the effect of the bed roughness on model performance by increasing roughness fivefold in the first scenario (i.e. $k_s = 5$ mm; Table 2 – K5), and tenfold in the second scenario (i.e. $k_s = 10$ mm, Table 2 – K10).

2.6.1.5. Bathymetry. Uncertainty in bathymetry measurements arises from small random measurement errors related to the precision of survey method or potentially more significant systematic errors typically related to imprecise reference to the datum or geographical positioning (Byrnes et al., 2002; Cea and French, 2012). Importantly, systematic errors have been shown to be more important in error propagation than small random errors (Dotto et al., 2014). The magnitude of these errors can be estimated to be in range 0.1–0.2 m when modern surveying practice are used, such as multi-beam sonar or airborne LIDAR surveys (Byrnes et al., 2002; Cea and French, 2012). However, it is not uncommon for hydrodynamic modelling studies to rely upon bathymetry data derived from digitised hydrographic charts, where related uncertainties can be much larger.

To test the sensitivity of the model to mesh bathymetry, two test scenarios were run where the elevation of the mesh bins was varied by ± 0.15 m i.e. the average expected uncertainty in the case of Yarra River bathymetry measurements, resulting in a deeper/shallower model bathymetry (Table 2 – D+15/D-15). To test the model sensitivity further, an additional test scenario outside of the likely model bathymetry uncertainty range was performed (Table 2 – D-50). In this scenario, the mesh bathymetry was elevated by 0.50 m (i.e. the mesh was made

Table 3
Characteristics of events used for sensitivity analysis.

Period	1	2	3	4	5	6	7	8	9	10
Start date	27/11/2012	31/05/2013	07/06/2013	16/09/2013	25/09/2013	12/11/2013	08/04/2014	10/01/2014	01/03/2014	27/08/2014
End date	28/11/2012	03/06/2013	09/06/2013	19/09/2013	28/09/2013	22/11/2013	17/04/2014	14/01/2014	09/03/2014	30/08/2014
Period length [day]	1	3	2	3	3	10	9	4	8	3
Volume delivered ^a [10^5 m^3]										
Yarra River	16.5 (61.1%)	169.1 (82.9%)	27.0 (75.1%)	53.5 (74.9%)	81.1 (83.3%)	254.1 (90.1%)	101.4 (81.9%)	187.2 (98.3%)	22.4 (97.7%)	36.2 (99.5%)
Gardiners Creek	4.9 (18.3%)	19.2 (9.4%)	4.5 (12.6%)	8.7 (12.2%)	8.4 (8.6%)	19.9 (7.1%)	14.4 (11.7%)	0.2 (1.0%)	0.2 (1.0%)	0.08 (0.2%)
Stormwater drains	5.6 (20.6%)	15.6 (7.7%)	4.4 (12.2%)	9.2 (12.9%)	7.8 (8.1%)	7.8 (2.8%)	8.0 (6.5%)	0.1 (0.8%)	0.3 (1.3%)	0.1 (0.3%)
Sum of rainfall 24h prior and during event [mm]										
Estuary (urban rainfall)	22	65	17	36	35	26	43	0	0	0
Yarra river (rural rainfall)	15	68	17	43	26	46	58	0	0	0
Average Salinity [-]										
Yarra River	0.09	0.11	0.14	0.10	0.09	0.08	0.11	0.10	0.10	0.08
Gardiners Creek	0.07	0.08	0.08	0.11	0.12	0.13	0.10	0.34	0.28	0.13
Stormwater drains	0.03	0.22	0.51	0.38	0.58	0.79	0.84	1.00	1.00	1.00
Average Temperature [°C]										
Yarra River	20.8	12.8	12.1	14.0	14.8	15.8	18.1	22.2	20.9	11.9
Gardiners Creek	17.9	14.1	12.4	14.5	15.2	16.7	17.9	24.1	21.5	13.9
Stormwater drains	16.6	15.1	13.8	15.1	15.5	15.6	16.1	18.0	17.3	14.4
Wind speed [m/s] and direction [deg]										
Essendon	4.6 (176)	4.5 (159)	4.3 (253)	3.9 (185)	6.9 (216)	5.3 (216)	4.0 (156)	4.7 (168)	4.7 (179)	2.4 (189)
Viewbank	3.0 (167)	2.6 (159)	2.6 (131)	2.5 (169)	4.7 (188)	3.3 (191)	2.4 (128)	3.0 (169)	2.9 (178)	1.5 (138)

^a percentage in the brackets indicates the proportion of total water input volume delivered (i.e. Yarra River + Gardiners Creek + Stormwater drains).

shallower), as decreasing mesh depth has been shown to have larger impact than increasing depth (Camacho et al., 2014). This scenario was expected to indicate the maximum sensitivity of model outputs to uncertainty in mesh bathymetry.

2.6.1.6. Vertical mesh discretisation. Vertical mesh discretisation becomes important when modelling hydrodynamics of highly stratified environments, such as the Yarra River estuary, and it is essential for reproducing steep halocline gradients (Jovanovic et al., 2015a). However, increased vertical resolution causes significant increases in model computational time, because each new vertical layer increases the number of computational bins by the number of horizontal 2D bins. To test the sensitivity of the model to vertical mesh discretisation, the vertical resolution of the mesh was decreased by 50%. Instead of 8 sigma surface layers (i.e. above -1 m AHD) and a z layer every 0.20 m (i.e. below -1 m AHD), 4 sigma layers and a z layer every 0.4 m was applied and tested.

2.6.2. Evaluation of model sensitivity

As the data generated would be too great to practically interpret the outputs, we did not use the entire simulation results for the two-year period for performance assessment described in Section 2.4 above. Although the model was still run over the full two-year period for each sensitivity scenario, outputs from ten sub-periods (from 1 to 10 days in length) were extracted from each scenario and compared to those of the base-case simulation. The ten sub-periods were carefully selected to explore different hydrologic, temperature, salinity and wind conditions experienced during the simulation period (Table 3), including seven wet weather periods (Table 3: Periods 1–7) and three dry weather periods (Table 3: Periods 8–10). The wet weather periods included rainfall totals of different magnitudes ranging from 17 mm to 68 mm. Furthermore, the amount of water delivered by the Yarra River, Gardiners Creek and stormwater drains was also different for different periods. For example, during period 1, the Yarra River contributed around 60% of the water, whereas Gardiners Creek and stormwater drains contributed around 20% each. In contrast, during period 6, the Yarra River contributed almost all of the fresh water (90%), while only

10% was delivered via urban stormwater.

The sensitivity of a number of model outputs to the input data were assessed for each scenario, including water levels, horizontal flow velocities (east and north), salinity and temperature, by calculating the following two statistics:

1) Overall bias (B):

$$B = \overline{P_b} - \overline{P} \quad (6)$$

2) Relative overall bias (B_r):

$$B_r = \frac{\overline{P_b} - \overline{P}}{|\overline{P_b}|} \times 100 \quad (7)$$

where: $\overline{P_b}$ is the mean prediction of the base case simulation and \overline{P} is the mean predictions of the test scenario simulation. The overall bias gives an indication of sensitivity of model in terms of mean change, while relative overall bias gives an indication in terms of relative (percentage) change compared to the magnitude of the base simulation output.

3. Results and discussion

3.1. Model performance

3.1.1. Water levels

Overall, the model performed well at all sites (Table 4 and Figure S-1 in supplementary material) with similar or better model performance compared to other three-dimensional estuarine models in literature (Yang and Khangaonkar, 2009; Chua and Fringer, 2011; Camacho et al., 2014). Semi-diurnal and diurnal tidal ranges corresponded well to the measured data (Fig. 2), however, the model fit parameters at the three downstream sites (Hawthorn, Burnley and Southbank) are slightly better (e.g. Nash-Sutcliffe efficiencies $E > 0.90$) than at the most upstream site at Abbotsford (e.g. $E = 0.78$). The decrease in performance at Abbotsford is most likely caused by under prediction of water levels during low tide (Fig. 2). This is likely to be related to the limited

Table 4
Model performance parameters for water level, velocity, temperature and salinity predictions.

Variable	Location	B	Br	NMAE	RMSE	E	IOA	r	
Water level - WL		[m]	[%]	[-]	[m]	[-]	[-]	[-]	
	Abbotsford	0.04	10	0.25	0.13	0.78	0.96	0.91	
	Hawthorn	-0.06	-24	0.36	0.08	0.90	0.98	0.98	
	Burnley	-0.03	-12	0.20	0.05	0.96	0.99	0.99	
	Southbank	-0.02	-9	0.14	0.03	0.99	0.99	0.99	
Shallow ADCP	Flow velocity - V_x	[m/s]	[%]	[-]	[m/s]	[-]	[-]	[-]	
	Bin 1	-0.02	-22	0.43	0.06	0.76	0.94	0.90	
	Bin 2	-0.03	-19	0.33	0.07	0.75	0.94	0.90	
	Surface dynamic bin	-0.01	-2	0.36	0.08	0.66	0.92	0.85	
	Flow velocity - V_y	[m/s]	[%]	[-]	[m/s]	[-]	[-]	[-]	
	Bin 1	0.01	45	0.72	0.02	0.36	0.75	0.73	
	Bin 2	0.01	37	0.54	0.02	0.37	0.79	0.75	
	Surface dynamic bin	0.01	21	0.62	0.03	0.30	0.73	0.60	
	Deep ADCP	Flow velocity - V_x	[m/s]	[%]	[-]	[m/s]	[-]	[-]	[-]
		Bin 1	0.01	17	0.61	0.06	0.61	0.85	0.81
Bin 2		0.00	7	0.51	0.06	0.74	0.92	0.86	
Bin 3		0.00	1	0.35	0.06	0.87	0.96	0.93	
Bin 4		-0.02	-11	0.28	0.07	0.85	0.96	0.93	
Surface dynamic bin		-0.01	-2	0.30	0.07	0.84	0.96	0.92	
Flow velocity - V_y		[m/s]	[%]	[-]	[m/s]	[-]	[-]	[-]	
Bin 1		0.00	-7	0.97	0.02	0.01	0.31	0.16	
Bin 2		0.00	-1	0.84	0.02	0.28	0.58	0.56	
Bin 3		0.00	10	0.69	0.02	0.51	0.76	0.77	
Bin 4	0.01	34	0.56	0.03	0.30	0.59	0.80		
Surface dynamic bin	0.01	27	0.60	0.03	0.46	0.78	0.74		
Depth profiles	Temperature	[°C]	[%]	[-]	[°C]	[-]	[-]	[-]	
	Overall	-0.5	-4	0.09	1.68	0.72	0.93	0.88	
	Abbotsford	0.2	1	0.02	0.35	0.99	0.99	0.99	
	Hawthorn	-0.5	-4	0.09	1.53	0.75	0.94	0.90	
	Morell Bridge	-0.6	-4	0.12	2.15	0.52	0.89	0.80	
	Southbank	-0.7	-5	0.09	1.67	0.69	0.92	0.88	
	Salinity	[-]	[%]	[-]	[-]	[-]	[-]	[-]	
	Overall	-0.8	-7	0.22	5.04	0.86	0.97	0.94	
	Abbotsford	0.0	7	0.07	0.01	0.91	0.97	0.99	
	Hawthorn	1.4	44	0.45	3.64	0.71	0.90	0.89	
Morell Bridge	0.3	2	0.17	4.32	0.89	0.97	0.95		
Southbank	-4.0	-19	0.22	7.32	0.63	0.92	0.88		

bathymetry data used for defining the mesh in the upper most section of the estuary, where only a few transects were available. Nevertheless, water level predictions at high tides agree well with the measured data. When compared to performance of the previous Yarra estuary model, significant improvement in water level prediction was observed at Burnley ($E = 0.96$ for our study compared to $E = 0.69$ in Bruce et al. (2014)), which is likely related to better mesh structure in proximity of the gauging station, while for other gauging stations performances are similar between the two models.

3.1.2. Flow velocity

The model performed well at predicting the main velocity component at Morell Bridge (i.e. V_x – east velocity) at both shallow and deep ADCP positions (Table 4). Performance for the other velocity component (i.e. V_y – north velocity) was lower than that of the main velocity component throughout the water column. However, as shown in Fig. 4, there is a high linearity between measured and predicted velocity for both velocity components i.e. the model is consistently predicting

velocity in direction shifted for a small angle compared to the measured velocity. The reason behind this is likely related to the small errors in defining the model mesh in the area around Morell Bridge. Nevertheless, the reported model performance parameters compare favourably with previously reported values for similar three-dimensional hydrodynamic models in estuarine (e.g. Yang and Khangaonkar, 2009; Camacho et al., 2014).

Generally, the model performance in flow velocity prediction increases from bottom to the top of the water column for both velocity components (Table 4). There are two reasons for this, namely: 1) the model generally is predicting surface water velocities better due to mesh discretisation and 2) the magnitude of the mean surface water velocity is much greater than that of the lower cells, hence relative bias is decreased. Furthermore, the north velocity component is particularly under-predicted in the deepest section of the water column (Table 4; Fig. 3). Again, this is possibly due to the model mesh, in particular, the discretisation of the estuary cross section. At most locations, four mesh cells are used to define the estuary cross section and each mesh cell has

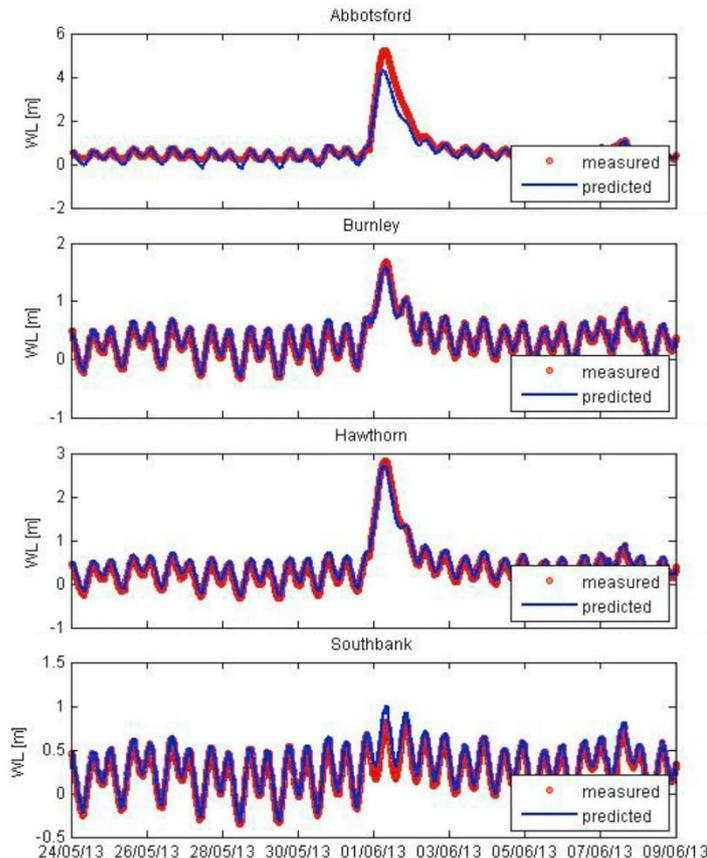


Fig. 2. Comparison of predicted and measured water levels at Abbotsford, Hawthorn, Morell Bridge and Southbank in the period 24th May 2013 to 9th June 2013, which included one of the events used for sensitivity analysis (i.e. Period 2 - Table 3). The peak flow rate of the Yarra River was $195 \text{ m}^3/\text{s}$, which is more than 15 times larger than the average flow rate over the simulation period ($12.5 \text{ m}^3/\text{s}$).

a specific bed elevation. Often the cells located next to the banks had bed elevations that are on average half of those located in the middle of the cross section (e.g. -1.6 m AHD compared to -3.5 m AHD). This relatively steep gradient in the model cross sectional profile at some locations may lead to minor inaccuracies in velocity prediction, especially at the bottom of the water column.

3.1.3. Temperature and salinity

Overall, the model performed well at predicting salinity dynamics within the Yarra River estuary with a Nash Sutcliffe model efficiency of 0.86 (Table 4). Performance at specific locations along the estuary ranged from 0.63 to 0.91 depending on the depth profiling site (see Figure S-2 in supplementary material). The abrupt change in salinity, from nearly fresh to saline water, typically occurs at over a 0.5 m change in vertical position. The model is able to reproduce this extreme stratification at Morell Bridge ($E = 0.89$; Table 4). However, in some cases there were issues with reproducing the sharp halocline e.g. on 26th June 2013 (Fig. 5), where the model did not reproduce the high salinity gradient as accurately as the other two occasions (for other depth profiles see supplementary materials for other depth profiles). This is likely to be related to the wind conditions on the particular day, which can influence mixing within the water column (Foreman et al., 2009; Kuang et al., 2011). For example, on the first occasion the average wind speed 3 h before depth profiling was much lower than on

the other two occasions i.e. 0.8 m/s compared to 5.2 and 3.4 m/s , respectively. This probably led to less mixing between the surface layer and the salt-wedge and, thus, the lower predicted salinity at the very top of the surface layer. Additionally, in some cases the salinity of the salt-wedge is over-estimated, which is likely to be the consequence of the downstream boundary condition set to a constant salinity of 35 across the boundary face. Yang and Khangaonkar (2009) developed a three-dimensional hydrodynamic model of the highly-stratified Skagit River estuary (US) and obtained a RMSE for salinity profiles in the range of 0.00–4.76 which is comparable to the RMSE range reported in Table 4.

Our model also performed well at predicting temperature dynamics within the Yarra River estuary. The overall Nash Sutcliffe model efficiency was 0.72 but ranged from 0.52 to 0.99 depending on the depth profiling site along the estuary (see Figure S-2 in supplementary material). The lowest performance is achieved in terms of the temperature prediction at the Morell Bridge depth profiling site (i.e. $E = 0.52$; Table 4), where differences between measured and predicted temperatures can be as high as a few degrees Celsius. Nevertheless, as shown in Fig. 5, good agreement between the measured and predicted temperature is apparent. On the first occasion, the temperature of the surface layer is slightly underestimated, most likely due to the same reason as for salinity, considering that the salt-wedge was warmer than the fresh water layer above. The temperature of the salt-wedge is

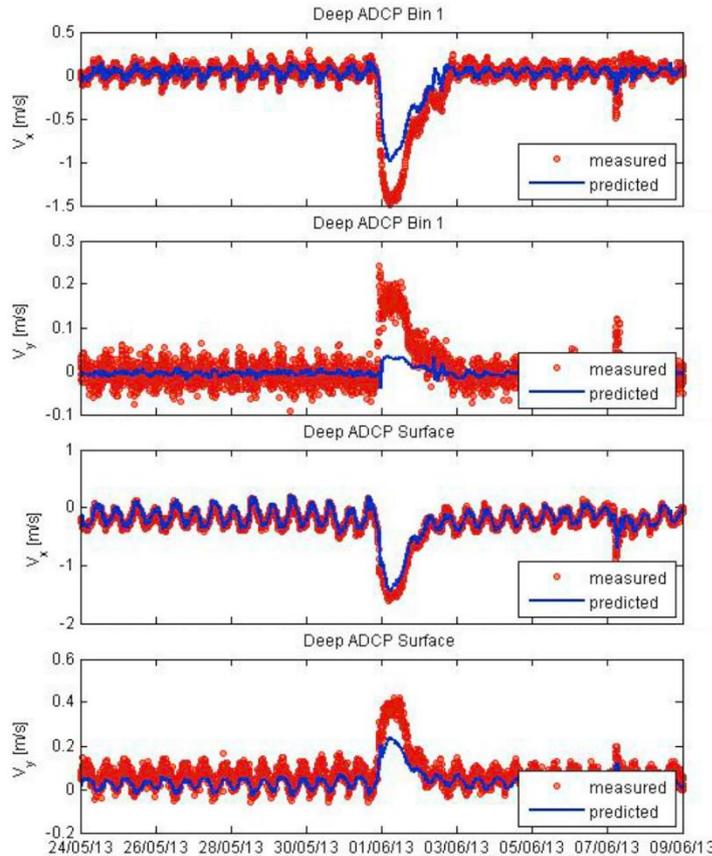


Fig. 3. Measured and predicted components of flow velocity (V_x – east and V_y – north velocity) at Morell Bridge at the surface and bottom (i.e. Bin 1) in the period 24th May 2013 to 9th June 2013.

reproduced consistently well, likely due to the continuous temperature measurements of Port Phillip that were used as a boundary condition. Temperature predictions were also assessed against the continuous temperature measurements at Abbotsford and Morell Bridge. The ability of the model to reproduce temperature dynamics within the Yarra River estuary was confirmed by obtaining similar model performance parameters as the depth profile data (see Supplementary

material: Section S.1, Tables S–1 and Figure S-6).

3.2. Model sensitivity

3.2.1. Flow rate scenarios (SW, SW_GC, 1.25Y and 0.75Y)

3.2.1.1. Scenario SW – removal of 208 urban stormwater drains. The removal of all 208 urban stormwater drains indicated that model

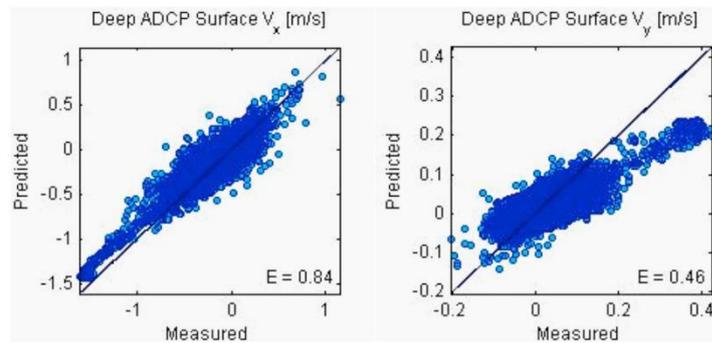


Fig. 4. Predicted vs. Measured plots of velocity components (V_x – east velocity and V_y – north velocity) in the surface layer at Morell Bridge at the position of the deep ADCP device.

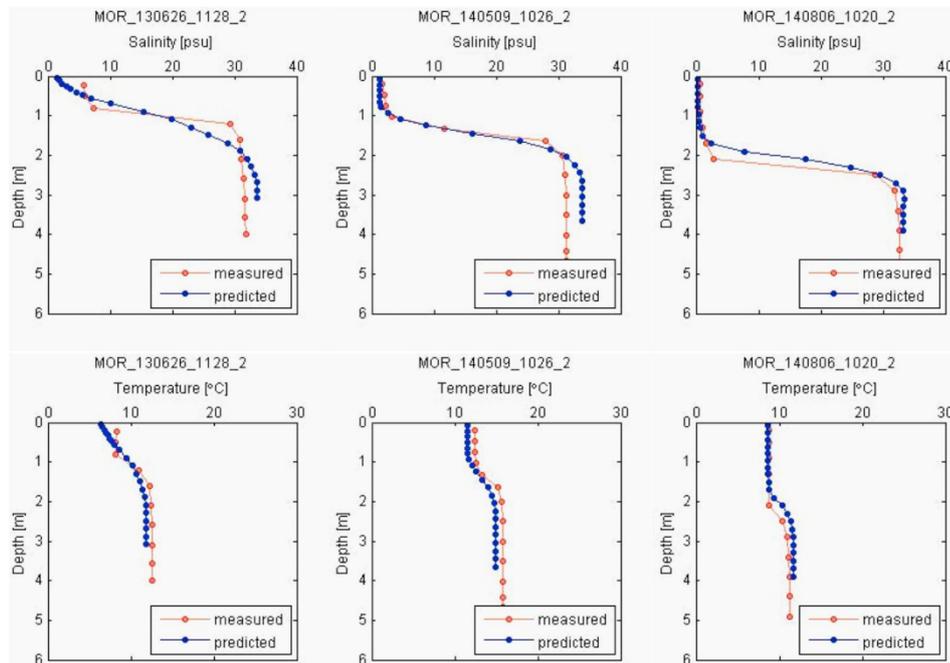


Fig. 5. Measured and predicted salinity (top) and temperature (bottom) depth profiles at Morell Bridge (MOR) on 26th June 2013 at 11:28 am (left; average Yarra River flow rate in the 24 h before the depth profiling $Q_{24h} = 4.9 \text{ m}^3/\text{s}$ – representative of low flow conditions; average wind speed over 3 h prior to depth profiling $S_{W_3h} = 0.8 \text{ m/s}$), 9th May 2014 at 10:26 am (middle; $Q_{24h} = 14.9 \text{ m}^3/\text{s}$ – representative of mean flow conditions; $S_{W_3h} = 5.2 \text{ m/s}$) and 6th August 2014 at 10:20 am (right; $Q_{24h} = 32.2 \text{ m}^3/\text{s}$ – representative of high flow conditions; $S_{W_3h} = 3.4 \text{ m/s}$). Measured and predicted salinity and temperature depth profiles on 26th June 2013 at Abbotsford, Hawthorn and Southbank are presented in Figure S-3, Figure S-4 and Figure S-5 respectively in the supplementary material.

outputs for water level and velocity, salinity and temperature are insensitive to stormwater inputs (simulation SW). For example, the bias in water level and velocity predictions was on average less than 0.01 m and 0.01 m/s, respectively. While this was expected for dry weather periods (i.e. periods 8–10) when the contributions from stormwater are small, it was surprising for wet weather events where the contribution from stormwater comprised up to 20% of the total water discharged into the estuary. This finding may be associated with the dispersed nature of stormwater inputs along the model mesh where, even though the total quantity of water delivered was significant in some periods, it was spread over 16 km of the estuary which weakened its impact on model outputs.

3.2.1.2. Scenario SW_GC – removal of 208 urban stormwater drains and Gardiners Creek inputs. In this scenario (simulation SW_GC), both urban stormwater drains and inputs from Gardiners Creek were removed and only the Yarra River above Dight's Falls was the contributing flow to the estuary. In terms of water level predictions, there was a slight underestimation compared to the base case scenario, with the change being less than 0.02 m on average but no higher than 0.04 m (or less than 5% relative bias). Similarly, the maximum change in surface velocity predictions was less than 0.05 m/s (or less than 10%). Bottom velocity predictions also showed little sensitivity to the removal of the creek and stormwater drains, with fewer events exhibiting noticeable bias compared to surface velocity. This is consistent with the stratified nature of the Yarra River estuary, where most of the fresh water flow occurs near the surface (i.e. on top of the salt-wedge) with insufficient vertical mixing to influence the bottom layer. However, relative bias for some events was much higher (> 20%) which was the consequence of small flow velocities in salt-wedge. The impact of inputs from Gardiners Creek and the stormwater water drains on salinity and temperature

predictions was minimal (always less than 10%). Note that the surface salinity relative bias is high due to the small salinities that exist in this layer and that absolute differences were always less than 1. Furthermore, sensitivity of the model to inputs from Gardiners Creek and the stormwater drains is only noticeable during wet weather periods (i.e. periods 1–7; Fig. 6 - Fig. 9), while during dry weather contributions from urban inputs is limited (i.e. < 3%; Table 3) and their removal has no impact on model outputs.

The comparison between the two urban inputs (i.e. stormwater drains and Gardiners Creek) revealed that, despite the two sources in some cases contributing equivalent total water volumes (Table 3), inputs from Gardiners Creek are more important for model performance compared to the 208 stormwater drains. For example, during period 5 (Fig. 10) both Gardiners Creek and 208 stormwater drains delivered around 8% of total water volume, yet recorded biases in this scenario are multiple times higher than in scenario SW when only stormwater was removed. This difference supports the hypothesis, stated above, that due to the spatial distribution of stormwater inputs throughout the model mesh, effects on model outputs from stormwater drain inputs are limited. This result also suggests that inclusion of urban inputs may only be important for model performance during wet weather, and that priority should be given to localised water inputs over the distributed inputs when comparable amounts of water are delivered.

3.2.1.3. Scenarios 1.25Y and 0.75Y – increasing and decreasing the magnitude of the Yarra River inputs. The variation of Yarra River inflow within the uncertainty range of $\pm 25\%$ had a much larger impact on model outputs than urban stormwater inputs during both wet and dry weather. This was expected considering that the Yarra River delivers over 90% of the water to the modelled estuary reach.

The bias in terms of water level prediction at Abbotsford was 0.10 m

on average (~15% in relative terms), and decreased downstream to 0.02 m at Burnley (5% in relative terms). The variation of water level sensitivity along the estuary is a consequence of the estuarine bathymetry. At Abbotsford the estuary is approximately 20 m wide and 0.5 m deep, whereas at Burnley the width of the channel is around 50 m and depth around 4 m, therefore a change in flow rate will cause a greater change in water level at Abbotsford than at Burnley. The water level results show very little difference between increasing and decreasing the magnitude of freshwater inputs from the Yarra River. However, the effect usually was reversed between the two scenarios (i.e. artificially increasing the water flow by 25% resulted in positive level bias, while the opposite was true when decreasing the flow by 25%). Moreover, the bias values at both Abbotsford and Burnley were significantly correlated with average Yarra flow rates over the analysed periods (Spearman's $\rho = 1$, $p < 0.005$ at both sites), indicating that the effect on the water level prediction is proportional to the magnitude of flows in the Yarra River above Dight's Falls. For example, the largest bias was recorded for Period 2 when there were large wet weather flows in the Yarra River upstream of Dight's Falls ($Q_{\text{average}} = 65.1 \text{ m}^3/\text{s}$), while the smallest bias was recorded for Period 9 when the average Yarra inflow was $3.2 \text{ m}^3/\text{s}$ (Fig. 6).

The effect of varying inflow from the Yarra River upstream of Dight's Falls on surface flow velocities in the estuary was observed during both wet and dry weather – with bias being on average, around 10% of the flow velocity and never exceeding 20% (always $< 0.1 \text{ m/s}$) in any of the analysed periods. This moderate effect could be because water movement within the estuary is not only driven by the riverine inputs but also by tides (Dyer, 1997). Similar to water level, the effect seems to be of a similar magnitude but reversed between the two scenarios. The bias observed for the bottom velocities were of similar magnitude to surface velocities, while the relative bias for bottom velocities was again higher due to the extremely low velocities measured in this section of the estuary. Importantly, the 1.25Y scenario produced a larger bias than the 0.75Y and may be related to the stratified nature of the Yarra River estuary. Nevertheless, bias values were well correlated with average flow rates for both cases (1.25Y - $\rho = 0.94$, $p < 0.05$ and 0.75Y - $\rho = 1$, $p < 0.005$), indicating that the sensitivity of model predictions of velocity was proportional to the magnitude of inflows from the Yarra River during each period.

Similar to water level and velocity, salinity predictions were also more sensitive to varying inputs from the Yarra River upstream of Dight's Falls than removing flows from Gardiners Creek or the storm-water drains. In absolute terms, bias was higher at the bottom than at the top of the water column (around 4 and less than 1 on average, respectively), but due to the lower salinities at the surface the relative bias was higher at the top than at the bottom. Salinity predictions during wet and dry periods had contrasting sensitivities at the surface and bottom of the water column. In fact, some of the highest biases at the surface were obtained for dry weather periods (e.g. Periods 8 and 9), while, during wet weather periods, bias was lower. The opposite results were obtained for bottom salinity predictions. This can be explained by the entrainment of sea water from the salt-wedge during dry weather causing a higher bias at the surface, while during wet weather the salt-wedge is pushed downstream and the salinity of surface water is primarily driven by inputs from the Yarra upstream of Dight's Falls, thus the bias is lower. Conversely, salinity at the bottom is driven by the salt-wedge and only higher Yarra River inflows during wet weather periods are able to influence the position of salt-wedge and, therefore, affect the predicted bottom salinity and the resultant bias. Furthermore, the sensitivity (especially in relative terms) was higher when inflows from the Yarra River were decreased (i.e. in 0.75Y). In this scenario, the salt-wedge was able to intrude further upstream, causing higher bottom salinities and consequently higher surface salinities (due to entrainment/mixing) and increasing the overall model output sensitivity.

Finally, temperature model outputs were not sensitive to the range of Yarra River inputs tested.

These results demonstrate that it is necessary to accurately measure the large inputs of freshwater into our estuarine hydrodynamic model, as the sensitivity of model outputs increase with increasing riverine flow rates. This finding seems to be particularly important for water level, velocity and salinity predictions, but not temperature.

3.2.2. Salinity and temperature scenarios (SAL, TEMP)

All model outputs were insensitive when salinity inputs were assumed to be constant (as compared to using their more discretised values). This is most likely related to fresh water inputs having low salinities (less than 1) so that variation in this range is not important in estuarine models where much higher salinities are experienced due to salt water intrusion. Similar results were observed for temperature predictions. Water level, flow velocity and salinity predictions were insensitive to weekly-average temperature inputs as compared to using their more discretised values. The highest recorded bias was less than 1°C , rendering the model insensitive to inputs with lower temperature resolution datasets. These results indicate that significant time can be saved setting up estuarine hydrodynamic models by using the low resolution measurements to characterise salinity and temperature of inputs (e.g. grab samples). The exception is where there is a need to simulate short-term extreme flow events (Period 2 - Fig. 9) and when the model is used for predicting temperatures. These events are often caused by significant changes in weather (i.e. large storms), which are followed by changes in the air temperature and consequently changes in temperature of surface runoff. For example, there was an increase of 2.4°C in the Yarra River inflow in five hours during the storm event in Period 2 which caused the highest bias (Fig. 9). Therefore, weakly averaging of the Yarra River temperature inputs have caused the highest bias in this case.

3.2.3. Wind scenarios (0.5W, 1.5W, Vb.W)

Varying wind boundary conditions within $\pm 50\%$ of the measured magnitude had a limited effect on water surface elevation prediction, especially in the upstream reaches where water depths are lower and water surfaces are protected from winds (small reach width and high tree coverage). This result was also obtained using wind data from Viewbank (approximately 15 km to the north east of the study area) instead of Essendon Airport.

Stronger effects were evident for wind scenarios when predicting flow velocities – with a resultant bias of up to 0.05 m/s or up to 20% in terms of relative bias (but mostly $< 0.01 \text{ m/s}$ and $< 5\%$). Regardless of a relative bias of up to 50%, predicted bottom velocities are lower than surface velocities and the model was generally under-predicting the bottom velocities (as indicated in Section 3.1.2), thus a small absolute change in predicted velocity generated high relative bias values. However, some links between velocity bias and period type were observed with generally higher bias values recorded during wet weather periods.

Fig. 8 shows that salinity predictions were sensitive to variation in wind boundary conditions, particularly in the surface layer, where bias values of up to ± 4.5 and relative bias of up to $\pm 60\%$ were observed. These results could be related to mixing within the water column induced by variation of wind speed. A decrease in wind speed (0.5W) causes reduced mixing of surface water with a salt-wedge beneath, which in turn causes lower predicted salinity in the surface water, as seen by positive bias values. At the same time, due to reduced mixing, the model predicts higher salinities in the salt-wedge causing negative bias values in the bottom layer. The opposite was predicted when wind speed was increased (i.e. Figs. 8–1.5W). Furthermore, it seems that the strongest impact on surface salinity predictions occurs during dry weather, while in the case of the bottom salinity predictions higher biases are recorded during wet weather. In the absence of high inflows from the Yarra River, the effects of wind on mixing of the surface layer with the salt-wedge are much more pronounced and this is reflected in the higher bias values. On the other hand, wind effects on salinity

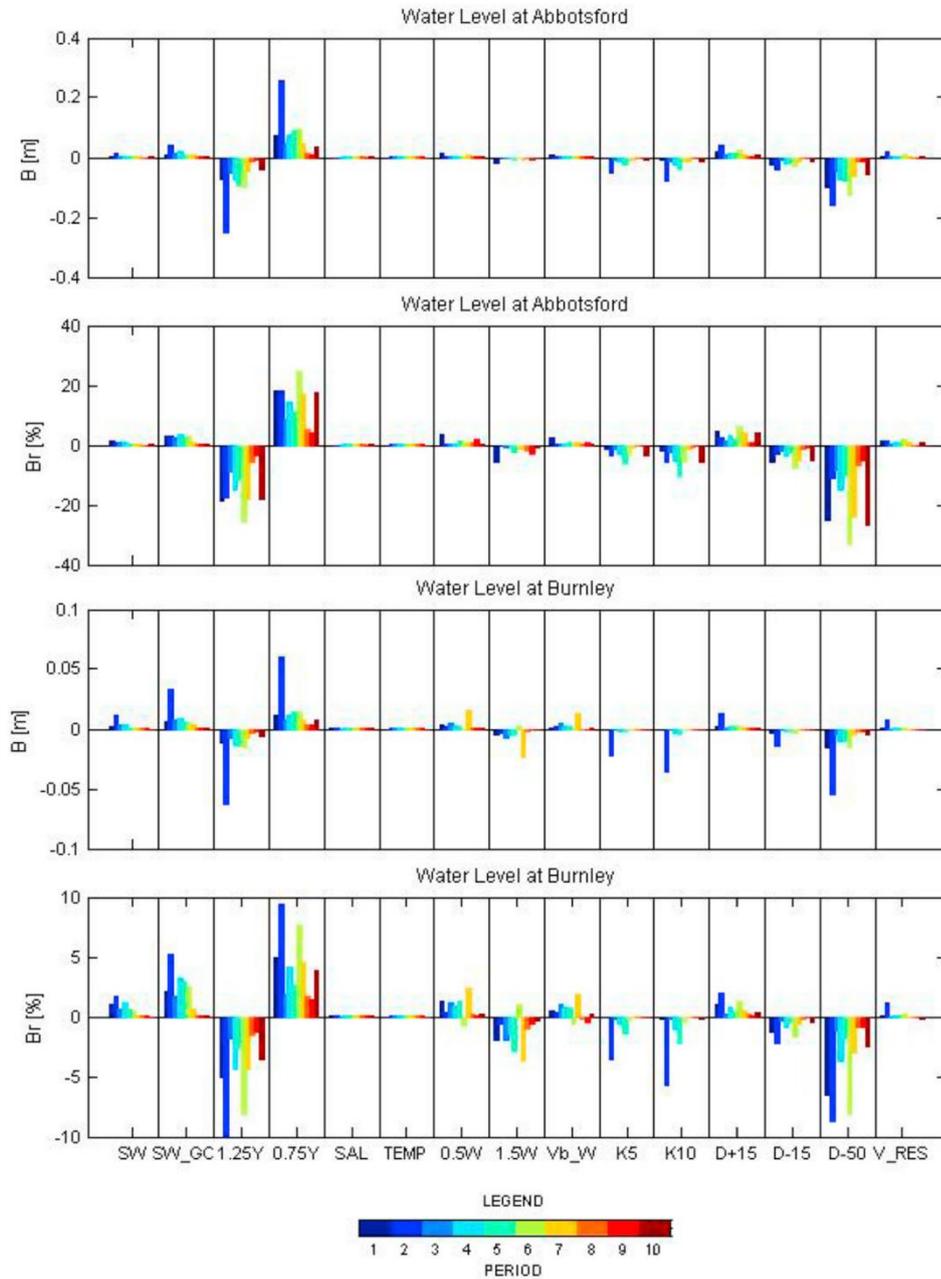


Fig. 6. Water level sensitivity at Abbotsford and Burnley (B – bias and Br – relative bias). Each graph is divided into 15 sections (i.e. the number of scenarios tested) and each of the 15 sections is further subdivided into 10 bars (i.e. each bar represents one of the 10 events). Water level sensitivity at Hawthorn and Southbank are presented in Figure S-7 in the supplementary material.

predictions at the bottom are only noticeable in conjunction with higher Yarra River inflows which are able to impact the position of the salt-wedge and thus the predicted salinity.

Temperature is also influenced by changes in the wind conditions, albeit to the lesser extent than salinity (as shown by relative bias values

- Fig. 9). The highest average absolute change in temperature was around 1 °C, rendering temperature predictions by the model insensitive to changes in wind conditions.

When the wind speed measured at Viewbank was applied (Vb_W) the bias for water level, velocity, salinity, and temperature resembles

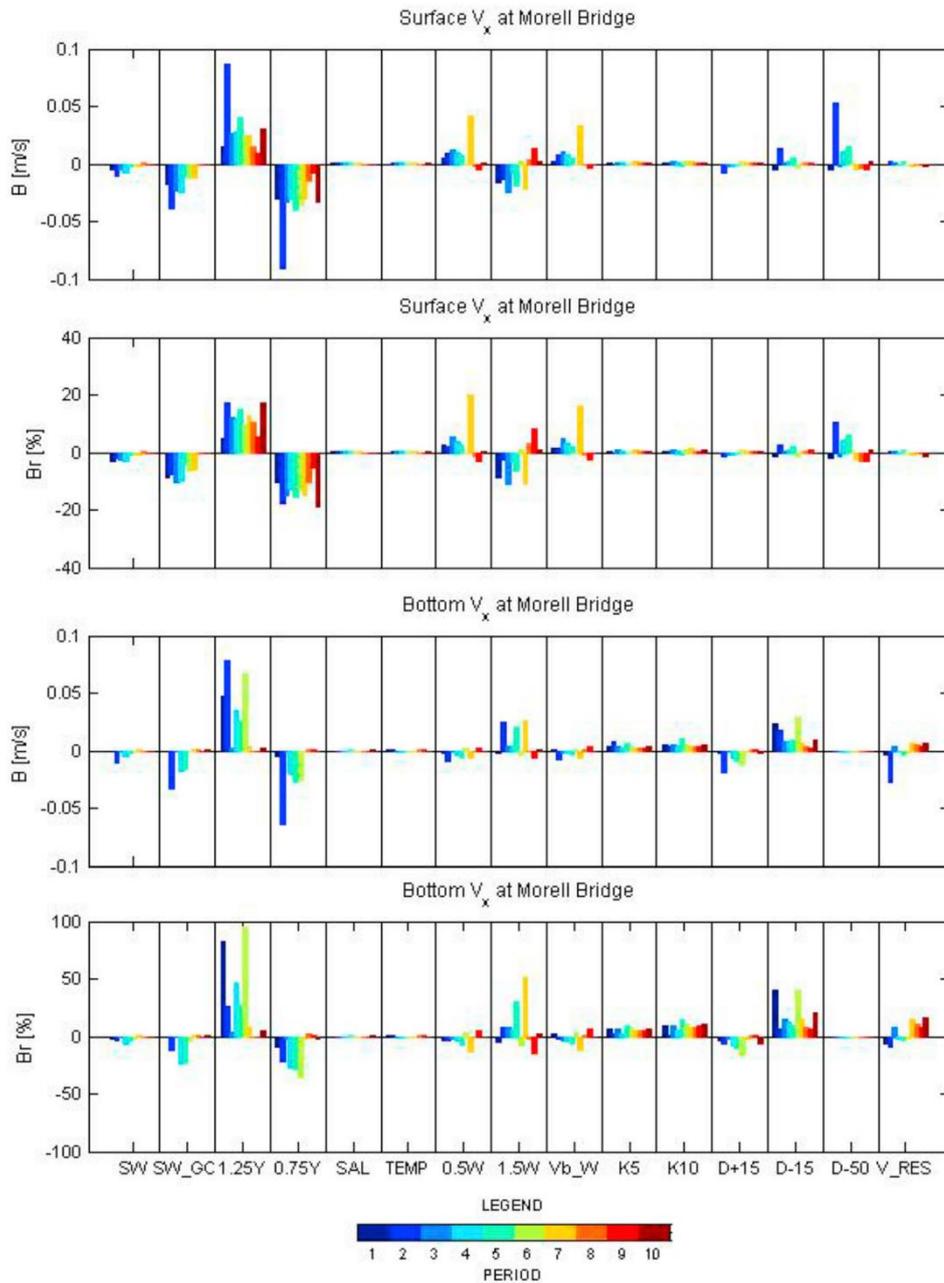


Fig. 7. Surface and bottom major flow velocity component (V_x) sensitivity at Morell Bridge (B – bias and Br – relative bias). Each graph is divided into 15 sections (i.e. the number of scenarios tested) and each of the 15 sections is further subdivided into 10 bars (i.e. each bar represents one of the 10 events). Sensitivity of surface and bottom minor flow velocity components (V_y) is presented in Figure S-8 in the supplementary material.

the 0.5W scenario (Figs. 6 and 7, Figs. 8 and 9). This can be explained by the significant linear correlations between the wind speed and direction measured at these two stations (Pearson $r = 0.72$, $p < 0.001$, $r = 0.32$, $p < 0.001$, respectively) as well as the fact that the mean absolute difference in wind speed applied in the base case scenario and the wind speed in 0.5W and W_Vb scenarios is similar (2.49 m/s and

2.15 m/s, respectively).

The model showed some sensitivity to wind inputs, particularly in terms of flow velocity and salinity outputs. Wind was particularly important for flow velocity and bottom salinity predictions during wet weather and for surface salinity prediction during dry weather conditions. In other instances, sensitivity to wind inputs was very low. This

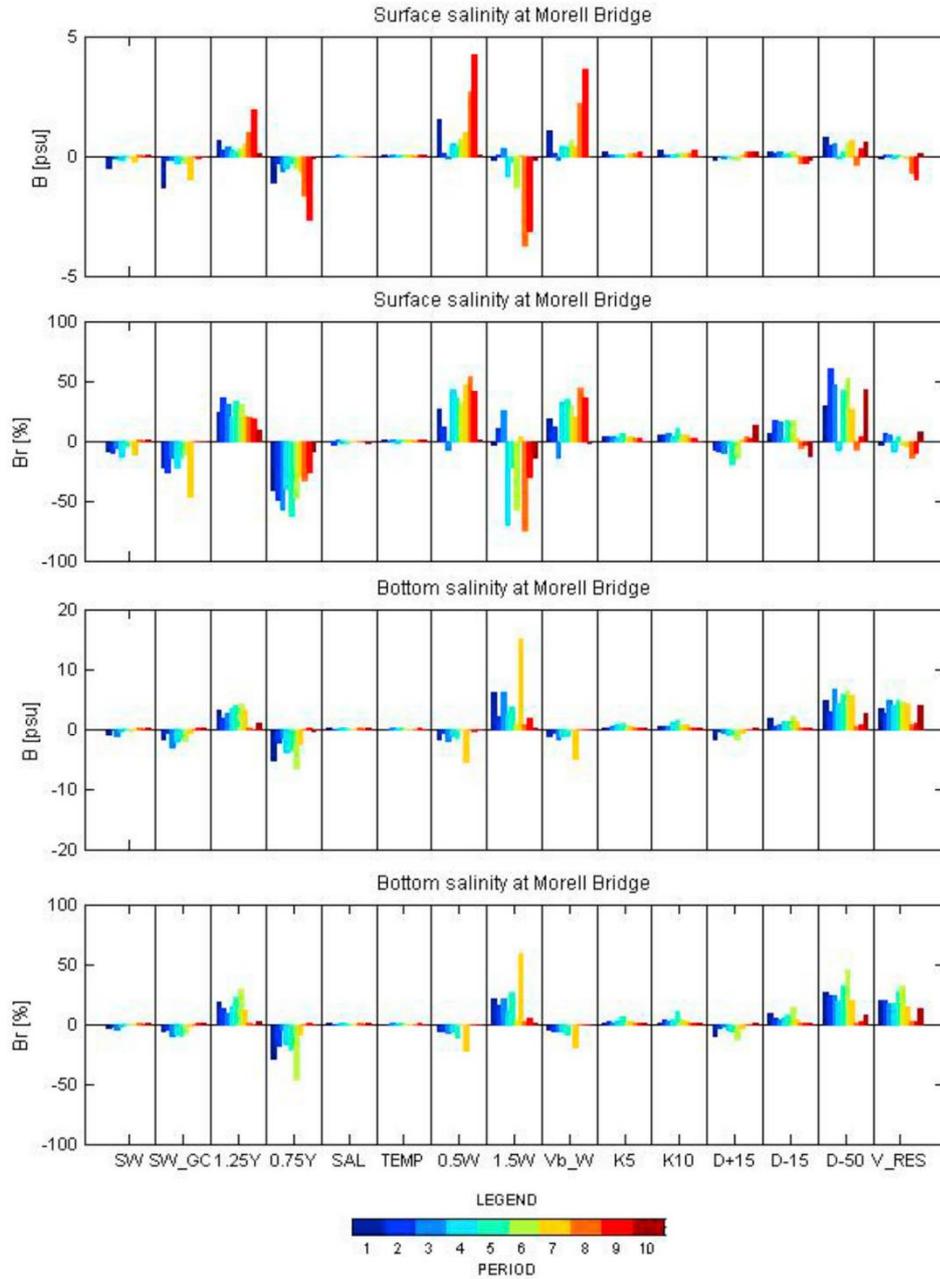


Fig. 8. Surface and bottom salinity sensitivity at Morell Bridge (B – bias and Br – relative bias). Each graph is divided into 15 sections (i.e. the number of scenarios tested) and each of the 15 sections is further subdivided into 10 bars (i.e. each bar represents one of the 10 events). Salinity sensitivity at Abbotsford is presented in Figure S-9 in the supplementary material.

suggests that if velocity prediction was the output of interest, it would be important to accurately measure wind conditions during wet weather periods. On the other hand, if salinity was the output of interest, accurate wind conditions should be obtained for the whole simulation period. These results do not support the initial hypothesis that

in case of narrow estuaries wind inputs may not be important.

3.2.4. Bed roughness scenarios (K5 and K10)

The impact of fivefold and tenfold increase of bed roughness (to 5 mm and 10 mm) was limited in regards to all model outputs. The

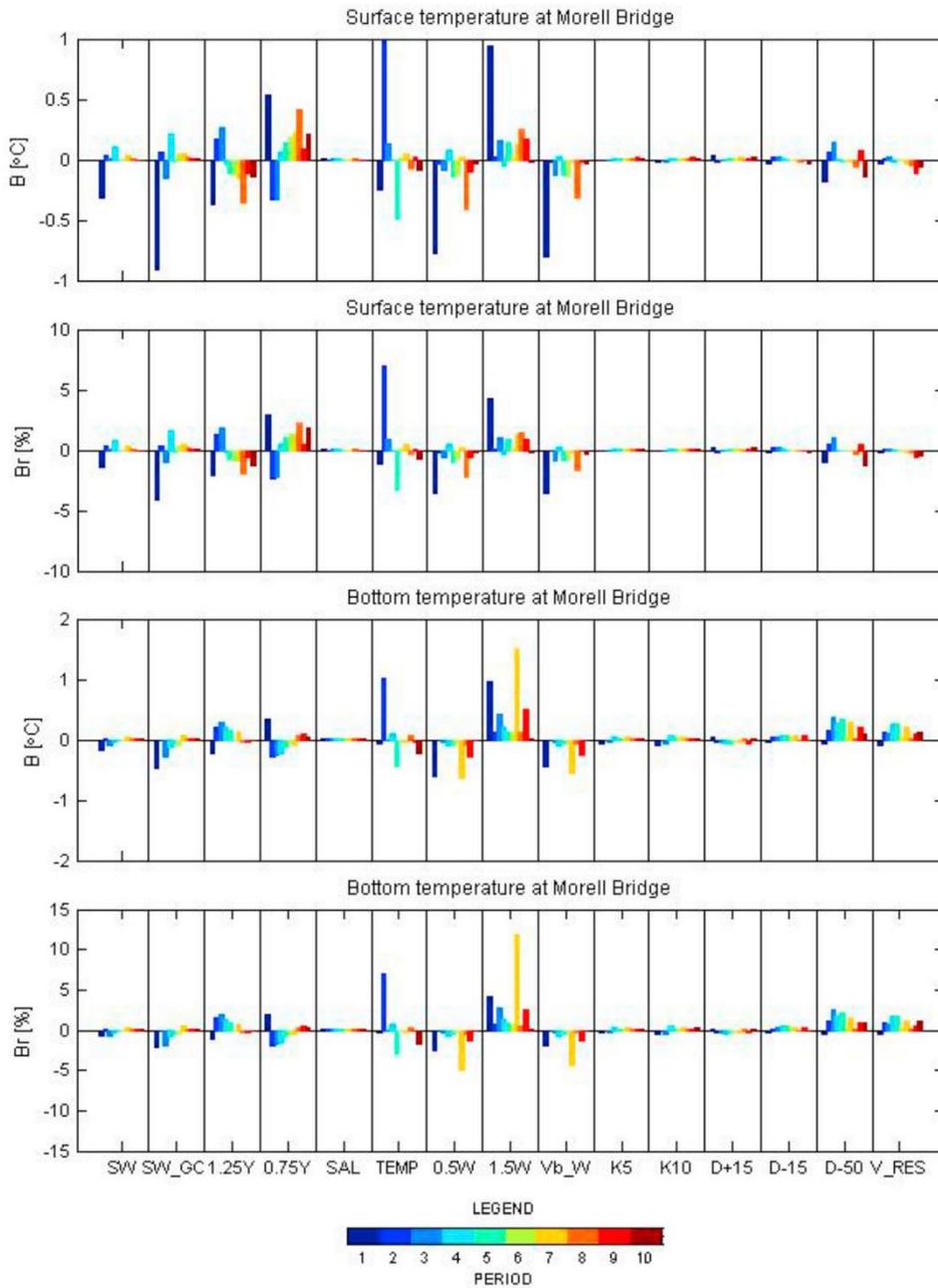


Fig. 9. Surface and bottom temperature sensitivity at Morell Bridge (B – bias and Br – relative bias). Each graph is divided into 15 sections (i.e. the number of scenarios tested) and each of the 15 sections is further subdivided into 10 bars (i.e. each bar represents one of the 10 events). Temperature sensitivity at Abbotsford is presented in Figure S-10 in the supplementary material.

change in water level prediction during dry weather periods is negligible (give range of values), while the change during wet weather periods seems to be related to the size of the event e.g. the largest change was observed during Period 2 at all sites (Fig. 6). Furthermore, the sensitivity of water level predictions seem to vary in a downstream

direction (as shown by bias and relative bias values in Fig. 6). This most likely relates to longitudinal variations in the bathymetry of the estuary.

The sensitivity of model predictions of water velocity to variation in bed roughness was limited regardless of hydrologic conditions (i.e. wet

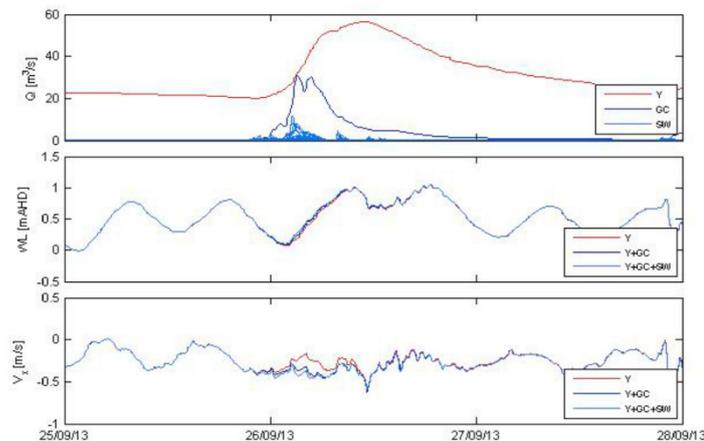


Fig. 10. Water inputs flow hydrographs (top), water level (middle) and main flow velocity component (bottom) predictions at Morell Bridge during period 5. Y – Yarra River; GC – Gardiners Creek and SW – stormwater.

or dry weather). Furthermore, predicted changes throughout the water column was also small (i.e. less than 0.01 m/s) and slightly higher at the bottom compared to the top, as expected when adjusting the bed roughness. A more accurate measurement of velocity at the sediment-water interface could have led to more demonstrable effects of adjusting this model parameter.

Salinity and temperature prediction was not substantially altered by adjusting bed roughness, with bias values less than 1 and less than 0.1 °C, respectively.

This suggests that the sensitivity of the model was limited in the range of the tested bottom roughness. Therefore, in the case of estuaries with fine sediments size composition, estimating bottom roughness is not expected to require high accuracy.

3.2.5. Bathymetry scenarios (D + 15, D-15 and D-50)

Variation of bathymetry elevation within the expected uncertainty range had a limited effect on the model predictions, particularly temperature predictions where almost no effect was observed. While an effect was observed for water level predictions, the maximum bias was less than 0.02 m at Abbotsford and decreased to less than 0.0002 m downstream at Burnley.

The prediction of surface velocity was not affected by the variation of bathymetry elevation (< 0.01 m/s, < 3%), while some change was observed in the prediction of bottom velocity. This increased when the bathymetry was shallower and decreased when the bathymetry was made deeper (< 0.03 m/s, < 40%). Interestingly, the impact of varying elevation by ± 0.15 m predicted an asymmetrical bias when either increased or decreased – (Fig. 7). This pattern was also evident for the water level and salinity predictions. Similar findings were obtained by Camacho et al. (2014) who showed that increasing mesh elevation (i.e. creating shallower conditions) had stronger effect on water levels and velocity than decreasing elevation which was suggested to relate to a stronger interaction between flow and the bottom boundary in shallow estuarine systems. In our study, deepening the mesh bathymetry may have only impacted the depth of the salt-wedge, while not influencing the surface conditions. Conversely, making the mesh bathymetry shallower is likely to have influenced surface flow properties.

These effects are more pronounced in case of the shallower D-50 scenario. The maximum sensitivity of the water level predictions from this scenario was seen at Abbotsford (bias sometimes greater than 0.10 m and 30%), while the effect decreased downstream (Burnley had a bias less than 0.06 m and 10% for all scenarios). The results observed for this scenario were similar to the 1.25Y scenario as expected. In fact,

one scenario is feeding water through a tighter cross-sectional area while the other is trying to feed more water through the same area. The sensitivity of surface velocity at Morell Bridge was marginal (< 0.01 m/s, < 5% and only higher in case of the extreme wet weather event – Period 2), however, there was no bottom velocity prediction at the deepest bin (Bin01) because the mesh bed was elevated by 0.5m (i.e. above the bin elevation). The bias of the surface salinity prediction was low (relative bias was high due to low salinities in surface waters), while the bottom salinity prediction experienced a larger change. Lower salinity concentrations were predicted near the bottom, likely due to a raised bathymetry hindering salt wedge intrusion. Surface and bottom temperature predictions remained insensitive even in the case of a 0.5 m increase in mesh elevation.

It was shown that variation in bathymetry had a limited effect on model outputs, particularly temperature predictions, where almost no effect was observed. This outcome goes against the initial hypothesis that the model outputs will be sensitive to uncertainty in bathymetry data. However, decreasing the depth (i.e. raising the bed elevation) had a stronger effect on model outputs than increasing the depth and as such, attention should be paid to obtaining accurate bathymetry inputs for shallow estuarine systems.

3.2.6. Vertical mesh discretisation scenario (V.RES)

Alteration of the vertical mesh resolution shows to have very little bias regarding model outputs, yet some variation in bottom salinity was evident during some periods i.e. bias of 5 or relative bias of 20%. The fact that these changes were seen in the prediction of bottom salinities but not in the surface salinities, indicates that the decrease in vertical mesh resolution impacts the prediction of the position of the halocline. As such, appropriate vertical resolution should be applied when simulating highly stratified environments, particularly if the aim of the modelling is to represent stratification of the estuarine system. On the other hand, if the focus of the modelling is on surface water conditions, lower vertical resolution may be sufficient.

3.3. Implications for modelling the shallow and narrow urban salt-wedge estuaries

Depending on hydrodynamic model objectives and applications, the following recommendations are made for three-dimensional hydrodynamic models used for shallow and narrow urban stratified estuaries:

- 1) Accurate prediction of the water levels (e.g. flood modelling) –

attention should be paid to obtaining accurate information for the largest water inputs (e.g. Yarra River flows upstream of Dight's Falls). Input data for minor inflows, such as ungauged stormwater inputs, are unlikely to significantly affect water level predictions, unless there are substantial errors in bathymetry estimation (i.e. D-50 scenario).

2) **Accurate prediction of the flow velocities** (e.g. modelling of sediment transport) – in addition to input data in '1' above, attention should also be paid to large localised water inputs (e.g. Gardiners Creek) and accurate measurements of wind inputs, particularly if the focus is on predicting surface water velocities. Surface water velocity predictions do not require higher than $\pm 15\%$ accuracy in bathymetry, however, it may be important for bottom velocity predictions. Salinity, temperature, bottom roughness and vertical resolution are unlikely to be important for accurate prediction of flow velocities.

3) **Accurate prediction of the salinity** (e.g. for biogeochemical modelling) – for a highly stratified estuarine system, large freshwater inputs and wind conditions during dry weather are important, especially for surface water representation. For bottom salinity (salt-wedge dynamics), the same inputs are important but mostly during wet weather. Furthermore, vertical resolution will have some effect on the prediction of bottom salinity. Equally important for both surface and bottom salinity is accurate bathymetry data, particularly if the system is shallow. Constant salinity and temperature, as well as bottom roughness and errors in bathymetry up to 15% are unlikely to influence salinity predictions.

4) **Accurate prediction of the temperature** (e.g. for biogeochemical modelling) – temperature predictions were generally the least sensitive of the input data tested (maximum bias of less than 1 °C). The greatest sensitivity was observed in relation to wind inputs, hence, effort should be made in obtaining accurate characterisation of wind conditions if accurate temperature predictions are required.

4. Conclusions

The three-dimensional hydrodynamic model of the highly stratified Yarra River estuary was able to reliably simulate complex estuarine dynamics – as demonstrated by the consistently good model fit statistics. Particularly important was the ability of the model to reproduce stratification of the estuary under various hydrodynamic conditions.

For the first time, the sensitivity analysis of shallow and narrow salt-wedge estuary model outputs (i.e. water level, flow velocity, salinity and temperature) to various input data was performed by artificially changing: flow rates, salinity, temperature, wind, bed roughness, model bathymetry and vertical mesh discretisation. In this study, the model sensitivity to ungauged stormwater inputs was also tested. Out of all model outputs, temperature showed the least sensitivity to the input data, while other model outputs showed different degrees of sensitivity depending on the test scenario. Surprisingly, uncertainty in bathymetry data did not have a significant impact on model outputs, which went against the initial hypothesis. Similarly, wind inputs were important for flow velocity, salinity and temperature predictions even though they were hypothesised to have minimal impact due to limited wind fetch. Model sensitivity was shown to be spatially variable, with a strong relationship to the bathymetry and cross sectional discretisation of the estuary. Typically, shallower regions of the estuary exhibited higher sensitivity to variation in input data. Additionally, sensitivity also varied temporally between wet and dry weather conditions.

This work has identified the importance of certain inputs, and therefore, provides guidance for future model development in other highly-stratified estuaries in respect to the model objective/intended application. For example, for sound water quality predictions, it is safe to assume that accurate water velocities are essential. It is also recommended that emphasis has to be placed on obtaining accurate measurements of the largest water inputs, as well as accurate wind

measurements. On the other hand, it is likely that less effort is required to obtain accurate bathymetry data.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecss.2018.10.022>.

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6.3 Discussion and conclusions

The model was tested robustly using the large data set of water levels, flow velocities and salinity and temperature measurements. It was shown that the model is able to reproduce well the hydrodynamics of the Yarra River estuary. Particularly important was the ability of the model to reproduce the high stratification of the estuarine water column which strongly influence the microbial water quality processes as shown with analysis of the monitoring data in Chapter 4. This was demonstrated by comparing observed and predicted salinity and temperature depth profiles during different hydrologic conditions. Therefore, the developed model proved capable of simulating complex hydrodynamics of the Yarra River estuary with high level of performance and, as such, it is fit for coupling with the microorganism model.

Due to the dynamic feedback between hydrodynamic and water quality variables (Ganju et al., 2016), modelling of water quality can be very sensitive to hydrodynamics (e.g. Allen et al. (2007)). As such, it is important to assess the sensitivity of most important hydrodynamic model outputs for modelling of *E. coli* dynamics in estuarine environment. These outputs include: 1) flow velocity, which is important for *E. coli* transport and mixing, and is a key factor for sediment resuspension/settling and; 2) salinity and temperature distribution, which is important for modelling *E. coli* survival.

Flow velocity was largely impacted by uncertainty in the major water input, i.e. the Yarra River, and to certain extent Gardiners Creek. Interestingly, exclusion of 208 stormwater drains did not have significant impact on velocity prediction, in spite of delivering similar volume of water as Gardiners Creek. This is likely related to the fact that these inputs are spatially distributed along the estuary as opposed to localised input of Gardiners Creek. Nevertheless, while not being important for velocity prediction stormwater inputs may be important as inputs of faecal contamination to the estuary. Additionally, surface velocity prediction was moderately sensitive to wind inputs, while bottom velocity prediction was somewhat sensitive to bathymetry inputs, particularly in the case when the bathymetry elevation was increased (i.e. shallower conditions).

Salinity predictions were mainly sensitive to the variation in the freshwater inflow (i.e. the Yarra River and Gardiners Creek inputs) and in the wind inputs. This was true for both the surface (i.e. freshwater layer) and the bottom (i.e. salt-wedge) salinity. Furthermore, the bottom salinity prediction were also sensitive to the vertical resolution of the mesh, likely due to the impact on the propagation of the salt-wedge along the estuary. The bias in predicted salinity of the surface layer during wet weather events remained below 2 psu and as such it's not expected that this sensitivity can cause significant changes in the prediction of the faecal microorganism concentrations. Moreover, the concentrations during

wet weather are likely driven by inputs of faecal microorganisms and microorganism-sediment dynamics rather than the die-off. However, sensitivity of the surface salinity prediction during dry weather produced bias of up to 5 psu and this could potentially cause a noticeable effect on the predicted microorganism concentrations during dry weather. The sensitivity of bottom salinity prediction produced bias of around 5 psu but in some cases over 10 psu which can cause significant difference in salinity related die-off rates. However, the majority of the *E. coli* is located in the top fresh water layer (Jovanovic et al., 2017a) and as such, the sensitivity of the bottom salinity might not have direct impact on the modelled *E. coli* concentrations.

Temperature was the least sensitive output of the model. The maximum bias in temperature prediction for any of the tested sensitivity scenarios was around 1°C. Nevertheless, because temperature is the most important environmental factor governing the survival of the faecal microorganism (Blaustein et al., 2013), the accurate prediction of temperature by the hydrodynamic model is needed. However, according to the sensitivity analyses the accurate prediction of the temperature can be achieved with the weekly averaged water temperature inputs. Particular events would require more discrete temperature inputs, however during particular storm events the temperature die-off might not be important because the microorganism concentration is likely driven by the inputs of faecal microorganisms and not by the within estuary dynamics. Furthermore, duration of the event might be too short for any significant die-off to occur.

In summary, predictions of the *E. coli* concentrations will be impacted indirectly by the sensitivity of the most important hydrodynamics model outputs. Based on the sensitivity analysis of the hydrodynamic model presented in this chapter, in the urban salt-wedge estuaries is likely the most influential inputs for modelling of the *E. coli* dynamics in the urban

Salinity, temperature, bed roughness and vertical resolution did not have significant impact on flow velocity prediction and as such are not important for modelling *E. coli* dynamics.

Chapter 7

Modelling *E. coli* dynamics in the Yarra River estuary

7.1 Introduction

While predictive microorganism models for estuaries have been developed previously, a review of the literature indicated that none of these models satisfied the requirements of an appropriate holistic estuarine microorganism model (Chapter 2). As such, there is need for the development of a more comprehensive and robust hydrodynamic-microorganism model. Additionally, limited datasets have been used for testing of existing models, hence the true performance and limitations of these models is still unknown and conclusions drawn from such models could be considered as questionable. Therefore, the aim of this chapter is to present a new hydrodynamic-microorganism model and to test its performance using the large dataset collected during this research project.

Additionally, the objective of this chapter was also to address the research questions and hypotheses presented in Chapter 2 by means of hypothesis testing using the newly developed model. It was hypothesised that the most important inputs of faecal microorganisms to the Yarra River estuary are (ordered according to highest magnitude): Yarra River upstream of Dights Falls, urban stormwater and microbes stored in bed and bank sediments (Research Question 1). Also, it was hypothesised that die-off, sediment-microorganism interaction (i.e. settling and resuspension), and hydrodynamic transport are the most important in-stream processes for microbial dynamics of *E. coli* (Research Question 2). Finally, the last research question relates to understanding the level of complexity needed for modelling of the microbial dynamics in highly stratified estuarine environments (Research Question 3). It was hypothesised that a 3D process-based model is required due to the complexity of both the microbial processes within the estuary but also the complexity of the environment itself. However, in the highly stratified estuarine environment the majority of the faecal microbes are residing in the freshwater surface layer, hence, it was also hypothesised that a simpler (conceptual) modelling approach might be suitable if modelling the microorganism concentrations in the surface layer of the water column.

Chapter 7 is comprised of two main sections. The first section is a journal publication titled “*Integrated conceptual modelling of faecal contamination in an urban estuary catchment*” published in *Water Science and Technology* in 2015 (Section 7.2). Parts of this publication related to assessment of the *E. coli* inputs into the Yarra River estuary were presented in Chapter 5. In this chapter, the whole journal publication as published in *Water Science and Technology* is presented. The work explored conceptual way of modelling faecal microorganism dynamics in the Yarra River estuary as opposed to fully process-based models. Urban (stormwater) and rural (riverine) inputs were provided through modelling and paper focused on assessing the overall importance of stormwater inputs in driving the *E. coli* dynamics

in the Yarra River estuary. This work was initially presented in form of conference paper at the 13th International Conference on Urban Drainage (ICUD) in Sarawak, Malaysia in 2014 and subsequently, after additional analysis and edits, submitted to *Water Science and Technology*. The conference paper can be found in Appendix B.1.

The second section of this chapter is a manuscript titled “*Integrated modelling of fate and transport of E. coli within an urban salt-wedge estuary*” currently under internal review for submission to Water Research (Section 7.3). The manuscript presents a new 3-dimensional model for microorganism prediction in estuarine environments. The model was tested on the extensive dataset of *E. coli* concentrations collected from the Yarra River estuary, which was described and analysed in Chapters 3 and 4. High resolution boundary conditions for the microorganism model were provided based on existing models shown in Chapter 5. The model was coupled to the calibrated hydrodynamic model of the Yarra River estuary (Chapter 6). After extensively testing the microorganism model performance, the model was used to test the hypotheses outlined above. Finally, the chapter concludes with the discussion and the summary of findings of Chapter 7 (Section 7.4).

7.2 Integrated conceptual modelling of faecal contamination in an urban estuary

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Integrated conceptual modelling of faecal contamination in an urban estuary catchment

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ABSTRACT

Urban stormwater is regarded as a key input of faecal contamination in receiving water bodies and therefore, a major concern for health risks associated with aquatic recreation. Wastewater leakages, cross connections and overflows, together with faeces washed from surfaces during rainfall events, are possible origins of faecal contamination which enter these water bodies through stormwater drains. This paper applies conceptual models to a case study of the Yarra River estuary to understand the relative importance of fluxes derived from an urban creek and the 219 urban stormwater pipes which drain directly to the estuary as compared with other inputs, such as the Yarra River itself. Existing hydrologic-microorganism models were used for the estimation of the inputs from riverine and urban stormwater fluxes. These predictions were applied as boundary conditions for a new, highly simplified, model which accounts for the transport and survival of faecal microorganisms in the estuary. All models were calibrated using a rich dataset, containing over 2,000 measured *Escherichia coli* concentrations. Mass balances from the riverine and stormwater models indicate the limited influence of urban stormwater drains on the estuary during dry weather; less than 0.05% to 10% (5th and 95th percentile; median 0.5%) of the total daily *E. coli* load entering the estuary was derived from urban stormwater drains. While wet weather contributions from stormwater drains could be more significant (2% to 50%; 5th and 95th percentile), the average contribution remained marginal (median 10%). Sensitivity testing of the estuarine microorganism model by switching off stormwater boundary conditions resulted in minimal model efficiency reduction; this may reflect the low average daily contribution from urban stormwater drains. While these results confirm previous studies which show that *E. coli* loads derived from stormwater drains are dwarfed by other inputs, it is essential to note that these results also demonstrate that some conditions reveal the opposite; high proportions from stormwater are possible when combined with low riverine inputs and high urban rainfall. Furthermore, this study focuses on the overall impacts of direct urban stormwater inputs on the faecal contamination levels within the estuary, and localized impacts would certainly require further investigation.

Key words | conceptual, *E. coli*, estuary, inputs, modelling, stormwater

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INTRODUCTION

Urban estuaries around the world are highly valued assets to the local community, as they provide aesthetics, improved microclimate and recreational opportunities (Mallin *et al.* 2000). Like many other urban estuaries, the Yarra River estuary has elevated levels of faecal contamination (Daly *et al.* 2013), which is of public health concern for recreational

users. Faecal microorganisms have been identified as the leading cause of pollution of environmental waters (Lipp *et al.* 2001; Burton & Pitt 2002; Ortega *et al.* 2009).

Urban stormwater has been recognized as an important input of faecal contamination to these waterways (Burton & Pitt 2002; McCarthy *et al.* 2011). As such, increased efforts

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have been made towards mitigating the impacts of direct stormwater inputs (i.e. the stormwater drains that discharge directly into the estuary), including the Yarra River estuary (e.g. Melbourne Water 2013). However, despite these efforts, minimal improvement in compliance figures was observed for this particular system, implying that there may be other, more significant, inputs which require mitigation.

Effective management of faecal contamination in urban estuaries requires a firm understanding of the inputs of pollution and its transport and fate within the system. Integrated modelling tools which fully account for both the input and the estuarine microorganism dynamics are absent from the literature. Indeed, most models found in the literature poorly represent the microbial dynamics, and instead (1) are calibrated and tested on a small number of measured data points (Kashefi-pour *et al.* 2002; Gao *et al.* 2011; de Brauwere *et al.* 2011), (2) represent inputs using a constant flux value, (3) predict inputs using simple relationships with flow (Garcia-Armisen *et al.* 2006; Liu & Huang 2012), and/or (4) predict inputs using a sediment-microbe correlation (Ghimire & Deng 2013). Use of these approaches might mask the true importance of a particular input, which in turn can significantly influence the results of estuarine microorganism model and misinform mitigation.

The aim of this study was to create an integrated conceptual-level *Escherichia coli* model for an estuarine catchment; we did this by linking models which already exist for riverine *E. coli* prediction (Haydon & Deletic 2006) and stormwater *E. coli* prediction (McCarthy *et al.* 2011) to a newly developed estuarine microorganism model. This integrated model was then used to assess the importance of the various inputs into the estuary. Of particular importance was whether the stormwater flow from the urban creek and the 219 urban stormwater drains, which directly enter the estuary, are a significant input of *E. coli*. In addition, there are other stormwater inputs entering the estuary indirectly through upstream river inflow. These are not assessed separately but are considered as part of riverine input. The models were calibrated on an extensive dataset, containing over 2,000 samples analysed for the most commonly used faecal indicator, *E. coli*. The major hypothesis of this work was that the importance of direct urban stormwater was minimal during dry weather periods, but increased during urban wet weather periods, especially when lower riverine flow rates were combined with higher amounts of urban rainfall. The impact of direct wet weather stormwater inputs could be important even in the case of uniformly distributed rainfall across a whole catchment, as stormwater could be entering the estuary much sooner than the riverine input due to the higher imperviousness and shorter time of concentration that characterize urbanized areas.

METHODS

The estuary and monitoring sites

The Yarra River estuary (Melbourne, Victoria, Australia) is a highly stratified, salt-wedge estuary (Beckett *et al.* 1982) and extends for about 22 km from Port Philip Bay to Dights Falls – a weir which represents the upper boundary of the estuary. Monitoring sites were selected and established for data collection (Figure 1). Two of the sites were within the estuary: Abbotsford at the very beginning of the estuarine section of the Yarra River (represents the region with little influence from the salt-wedge, but still impacted by tidal changes) and Morell Bridge, located in the lower part of the estuary (highly impacted by the salt-wedge). Both sites were equipped with refrigerated automated samplers and depth sensors and had continuous measurements of electrical conductivity (EC) and temperature (T) at 100 mm below the surface. The Morell Bridge site was also equipped with an Acoustic Doppler Current Profiler (ADCP) for 3D measurements of velocities at 1-minute intervals.

Monitoring of upstream river inputs was conducted at Kew (Figure 1), where only grab samples were taken and water levels and flow rates were measured at 6-minute intervals by Melbourne Water (the local water management authority).

Monitoring of stormwater inputs was done at Gardiners Creek, a heavily channelized creek which is the largest input of water other than the Yarra River upstream of Dights Falls. The site has been equipped with an automated sampler, EC/T sensors and a depth/velocity probe. Climate data were obtained from Australian Bureau of Meteorology and Melbourne Water for different locations in the Yarra River catchment (Figure 1). Gardiners Creek is considered to be an open channel stormwater drain because its catchment is completely developed with total impervious fraction of 47%. Furthermore, observed range of the *E. coli* concentrations (944; 6,203; 17,673 most probable number (MPN)/100mL; 5th, 50th, 95th percentile) is well within the range reported for urban stormwater (Makepeace *et al.* 1995; Burton & Pitt 2002).

Sample collection and analysis

Estuarine and riverine samples were taken approximately 100 mm below the surface, where the health exposure to recreational users is expected to be the highest. In the period from November 2012 to July 2013, 2,106 samples were collected; 1,500 during dry weather and 606 during

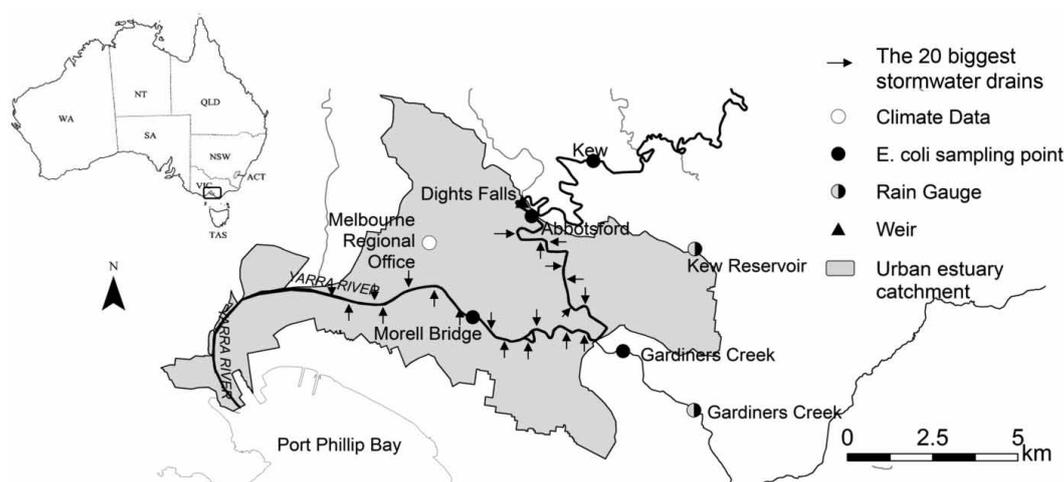


Figure 1 | Monitoring stations in the Yarra River catchment (stations: Heidelberg and Coldstream (rain data) and Viewbank and Melbourne airport (climate data) are positioned outside the figure boundary). Shaded area represents the urban estuary catchment with the biggest 20 of the 216 modelled drains shown.

wet weather conditions. All collected samples were transported to the Environmental and Public Health Microbiology (EPHM) laboratory at Monash University in coolers on ice and analysed for *E. coli* content using the Colilert method (IDEXX Laboratories 2013) within 24 h of collection. A large range of other indicators and reference pathogens were tested, but not reported here.

Riverine model

Hydrology of the upper Yarra River catchment (river inflow at Dights Falls into the estuary, Figure 1) was modelled using MUSIC - SimHyd, which is a spatially lumped catchment rain-runoff model (eWater 2012). The model was applied with some slight variations: (1) a linear-reservoir routing routine was employed (instead of MUSIC's standard Muskingum Cunge method) as it has been demonstrated previously that this simpler and more stable form of routing produces equivalent results (McCarthy 2008); (2) the model was employed using a constant 6-minute timestep (as opposed to MUSIC's standard method of daily simulation and subsequent disaggregation). This method improved the computational efficiency of the model, without compromising the results. Model inputs were areal averaged rainfall (Heidelberg, Kew, Kew Reservoir, Coldstream and Viewbank stations) and daily potential evapotranspiration, calculated using the Food and Agriculture Organization Penman-Monteith method (data from Coldstream, Viewbank and Heidelberg stations). The MUSIC-SimHyd

model was calibrated with a Monte-Carlo approach using a least squares objective function comparing the predicted flow rates with untransformed measured flow rates at Kew. The performance of the hydrologic model was assessed using the Nash-Sutcliffe coefficient of efficiency E_Q (Nash & Sutcliffe 1970). Parameter sensitivity was also explored using the Monte-Carlo results, as per others in the literature (e.g. Dotto et al. 2010).

For the prediction of riverine microbial concentrations, a modified version of the EG pathogen-hydrologic catchment model (Haydon & Deletic 2006) was applied. The main variation was that the loss of microorganisms from the subsurface store was estimated to be inversely proportional to the soil moisture instead of directly proportional, which was originally proposed by Haydon & Deletic (2006), as many studies report extended survival of faecal microorganisms at higher soil moisture contents (Desmarais et al. 2002; Schäfer et al. 1998). The model had six parameters: one parameter described build-up, two were loss coefficients and three were related to wash-off processes. Inputs to the model were time series potential evapotranspiration and flow components as calculated by MUSIC - SimHyd. The model was calibrated against Abbotsford's *E. coli* concentration dataset. Although there are obvious issues with this methodology (i.e. calibrating the upstream model to a site within the estuary), it was considered adequate for the following reasons: (1) Daly et al. (2013) showed that Kew and Abbotsford have similar distributions; (2) the correlation between the *E. coli* from the

two sites was 0.83 (Pearson correlation coefficient, $p < 0.001$); and (3) the Abbotsford dataset had many more calibration points (776 compared with 43 at Kew), which could allow a better calibrated model. The optimized parameter set for the EG model was obtained using a least squares objective function and by observing the Pareto front formed when calibrating using untransformed and log-transformed *E. coli* concentrations. Additional calibration of the model parameters was conducted using the Generalized Reduced Gradient method, without limiting the parameters and using a criterion which added the two components of the Pareto front. The model's performance was assessed by the Nash-Sutcliffe efficiency calculated using untransformed and log-transformed *E. coli* concentrations – EC and EC_{log} , respectively.

Stormwater model

Modelling of the urban stormwater input of Gardiners Creek was performed using Micro-Organism Prediction in Urban Stormwater, MOPUS (McCarthy et al. 2011), where the pervious component of the rain-runoff model was excluded. As shown previously by Dotto et al. (2011), the parameters which are used to model the pervious component are less sensitive than those used to model impervious areas, therefore demonstrating the importance of impervious areas in urbanized catchments. The rainfall-runoff module of MOPUS was calibrated against the untransformed flow rates measured at the Gardiners Creek monitoring station using the same procedure outlined above for the riverine model.

MOPUS's microorganism model has five model parameters: three which represent the build-up and die-off of microorganisms on the surface of the catchment, and two others which represent the same for the subsurface (i.e. in the stormwater drain). The inputs to the model include: time series of rainfall, relative humidity and vapour pressure. MOPUS was calibrated using the 383 *E. coli* samples taken from Gardiners Creek during dry and wet weather periods and assessed using the same procedure as the EG model.

In addition to Gardiners Creek, there are 219 stormwater drains of various sizes that drain directly into the Yarra River estuary (Figure 1 – the 20 biggest shown). MOPUS was further used to generate a time series of stormwater flow rates and microorganism concentrations for each of these stormwater inputs. This was achieved by generating 219 different parameter sets. First, the impervious area (IA) for each of the drains was estimated using an empirical relationship between impervious area and drain cross-

sectional area (McCarthy 2008). Then, due to the lack of measured data, the five microorganism model parameters were obtained by random sampling within parameter ranges defined by the optimized values from Gardiners Creek Catchment (this study) together with optimized values from literature which has used the MOPUS model on four other stormwater drains in Melbourne, Australia (McCarthy et al. 2011). Finally, the MOPUS model was executed for all 219 drains, using the relevant input data: rainfall, relative humidity and vapour pressure from Melbourne Regional Office station (Figure 1).

Simplified estuary model

The whole estuary was represented as a single reservoir where all modelled flows and microbial loads from the river, Gardiners Creek and 219 stormwater drains were linearly routed and translated through the system (Table 1 for equations). The rationale behind this approach is twofold. First, the Yarra River estuary is a salt-wedge estuary (Beckett et al. 1982), which was confirmed by measurements conducted by authors (data not shown). Essentially, this means that the fresh water layer flows over the moving sea water layer (i.e. salt-wedge), with minimal mixing between the two

Table 1 | Estuarine microorganism model (calibration parameters are in bold)

Flow
$S(t) = S(t - 1) + [Q_r(t) + Q_{sw}(t) - Q_e(t - 1)] \times \Delta t$
$Q_e(t) = S(t - \mathbf{TOC}) / \Delta t \times \mathbf{RC}$
Microbial load
$M(t) = [M(t - 1) + (N_r(t) + N_{sw}(t)) \times \Delta t] \times 10^{-k \Delta t} - N_e(t - 1) \times \Delta t$
$N_e(t) = M(t - \mathbf{TOC}) / \Delta t \times \mathbf{RC}$
Dynamic survival rate
$k = (k_{20} + 0.006 \times s) \times 1.07^{(T - 20)} + I_A / k_e H \times [1 - e^{-k_e H}]$
$s = EC / EC_{sea} \times 100$
Microorganism concentration
$C(t) = \left(1 - \frac{s}{100}\right) \times N_e / Q_e \times \varphi$

S [m^3] – inflow volume stored within estuary; Q_r [m^3/min] – river inflow; Q_{sw} [m^3/min] – stormwater inflows; Q_e [m^3/min] – discharge exiting the estuary; M [MPN] – microorganisms stored within estuary; N_r [MPN/min] – river load rate; N_{sw} [MPN/min] – stormwater load rate; N_e [MPN/min] – load rate exiting the estuary; RC [-] – routing coefficient; TOC [min] – time of concentration; Δt [min] – time step; k [1/day] – microorganism survival rate; k_{20} [1/day] – survival rate at 20°C; s [%] – percentage sea water; T [°C] – measured water temperature; I_A [MJ/m^2] – average daily solar radiation; k_e [1/m] – average light attenuation coefficient over depth; H [m] – depth of the water column; EC [mS/cm] – measured electrical conductivity at Morell Bridge; EC_{sea} [mS/cm] – electrical conductivity of sea water; C [MPN/100mL] – microorganism concentration exiting estuary; φ – unit conversion factor.

layers. Furthermore, minutely velocity measurements obtained at Morell Bridge monitoring site using an ADCP over the October 2012 to August 2013 period showed that, on average, velocity in the downstream direction was 0.16 m/s, while the upstream velocity was 0.06 m/s with only 18% of the time velocity being negative, i.e. forming upstream flow. Therefore, the estuary can be effectively regarded as a river with a moveable bottom boundary. Secondly, this model is very simple and would form a baseline level of performance achievable with minimal data input and minimal model complexity. The benefit of further increasing complexity of the model will be assessed in the future against the performance achievable with the simple microorganism model.

In addition to routing and translating microbes, the model accounts for the impact of environmental factors on the survival of microorganisms in the water column using first-order kinetics. The survival rate was modelled dynamically as a function of temperature, salinity (% sea water) and solar radiation using the expression proposed by Mancini (1978). A simple term has been added when calculating microorganism concentration to account for mixing between fresh and sea water, where sea water was assumed to be free of *E. coli*.

The estuarine microorganism model was calibrated against Morell Bridge's *E. coli* dataset (829 points), using the same methods as outlined above for the input models. Simple sensitivity testing was conducted to assess the effect of survival processes and direct stormwater inputs on the model's performance. In the first case, the model was calibrated without accounting for the survival of *E. coli* (i.e. there was no die-off). In the second case, both survival and stormwater volume and *E. coli* load were removed and the model was re-calibrated following methodology described above. Furthermore, we assessed the effect of spatial discretization on the model's performance by dividing the estuary into 33 cells of 500 m length. The model equations were applied in each cell.

Input analysis

Predicted stormwater flow rates and microorganism concentrations were used to calculate daily delivered volumes and loads to the estuary. A similar approach was taken with the riverine input, but instead of using predicted flow rates (which were substantially underestimated during base flow periods by the MUSIC model), measured data from Kew were used to achieve more realistic results. To assess the contribution of stormwater in dry and wet weather, in

terms of both daily delivered volumes and loads, a ratio of stormwater over total inputs (sum of stormwater and river inputs) was calculated. Similarly, a ratio of daily delivered stormwater volume to the average estuary volume (estimated using Geographic Information System and bathymetry data to be $4 \times 10^6 \text{ m}^3$) was also used to assess the impact of direct stormwater inputs.

RESULTS AND DISCUSSION

Input modelling

The MUSIC-SimHyd model reproduced the observed flow pattern reasonably well ($E_Q = 0.51$); however, during base flow periods there was substantial underestimation of flow rates (probably a result of the model being modified for urbanized catchments). There were also timing issues with the prediction of the peak flows. The stormwater rainfall-runoff model had quite high performance in prediction of flow rates for Gardiners Creek, with an efficiency of $E_Q = 0.81$. It performed particularly well in the region of very high flow rates ($>10 \text{ m}^3/\text{s}$), which was expected as the model was essentially developed and calibrated for the prediction of wet weather flows.

The efficiencies of the two microorganism input models were similar: $E_C \approx 0.20$ and $E_{Clog} \approx 0.40$. Although these are not high efficiencies, they agree well with the performance reported in the literature for similar microorganism models (McCarthy *et al.* 2011). The pathogen-catchment model reproduced *E. coli* patterns well, although there are certain peak prediction time issues similar to that described by Haydon & Deletic (2006). The MOPUS concentration predictions are better in the region of high concentrations, which are commonly observed during wet weather periods. Indeed, the current model structure was developed for modelling wet weather microbial dynamics in stormwater; hence it is expected to give better predictions during wet weather.

Input analysis

The relative contribution of stormwater discharging directly to the estuary during dry weather ranged from <0.5% to 10% (5th and 95th percentile), suggesting limited influence of stormwater on overall *E. coli* levels in the estuary during these periods (Figure 2(a)). As expected, wet weather stormwater proportions were higher (2% to 50%; 5th and 95th percentile), yet the average daily contribution under these conditions remained marginal (median 10%). These findings agree well with those of Daly *et al.* (2013), suggesting

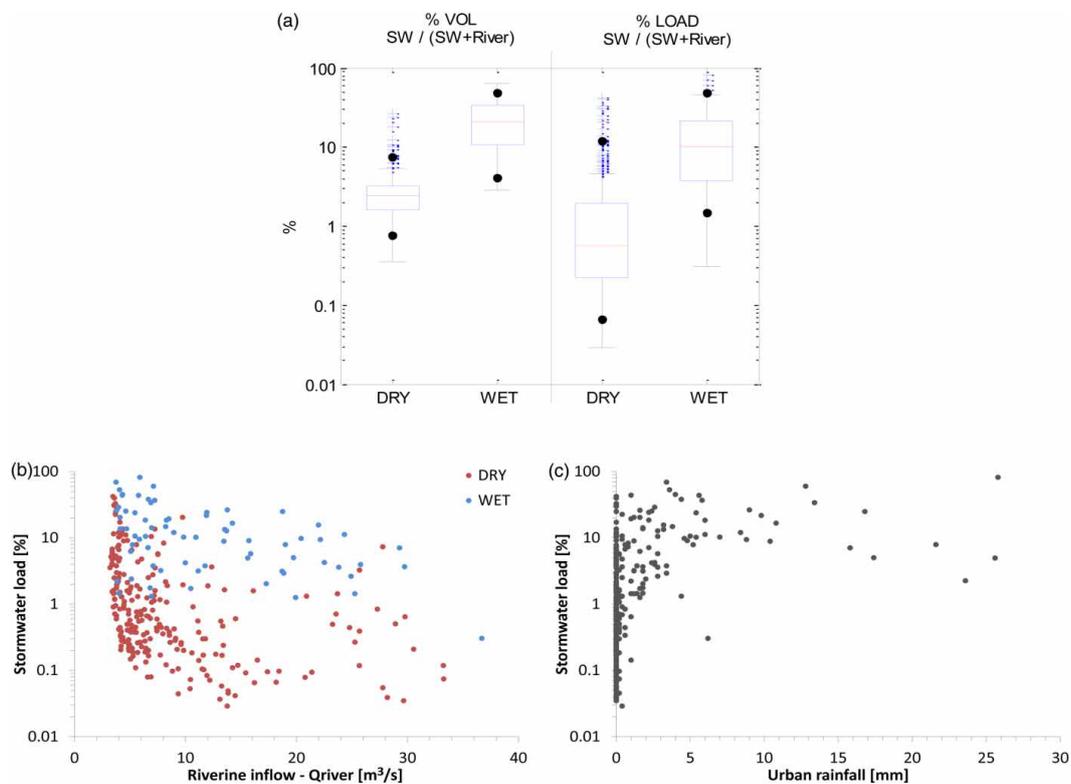


Figure 2 | (a) Modelled daily stormwater contributions during dry/wet weather conditions as a percentage of total delivered water volume (%VOL) and *E. coli* load (%LOAD) to the estuary (black dots represent 5th and 95th percentiles) for the simulated period of November 2012–August 2013; (b) the relationship between percentage daily stormwater load and riverine input flowrate during dry and wet weather; (c) the relationship between percentage daily stormwater load and urban rainfall (Melbourne Regional Office station).

the median daily *E. coli* loads coming into the estuary from the three biggest drains (two of them 3 m in diameter and one 6 × 2 m) are about 1.5 orders of magnitude lower than the riverine inputs. However, it is important to note that our results also demonstrate that some conditions can produce high stormwater contributions, especially during periods of low riverine flows and high urban rainfall amounts (see Figure 2(b) and (c)). It is also possible for urban stormwater to enter the estuary much faster than riverine inputs due to the higher imperviousness and the smaller time of concentration of urban catchments. Hence, at finer temporal scales (i.e. time step < 1 day), stormwater could have a significant impact on overall faecal contamination levels within the estuary. Furthermore, stormwater might be significantly influencing faecal microbe distribution locally around the drain outlets. All issues stated above would certainly require further investigation, which is not within the scope of this paper.

Estuarine modelling

Considering the simple approach adopted for modelling the estuary (i.e. neglecting estuarine hydrodynamic characteristics), as well as the accuracy of predicting input loads, the model performed reasonably well with E_C and E_{Clog} values of 0.37 and 0.41, respectively (Figure 3 and Table 2). Spatial discretization of the estuary into 33 cells did not have a significant effect on the model's efficiencies, which remained similar to the original model ($E_C = 0.42$ and $E_{Clog} = 0.41$). Due to its simplicity, the model's performance is very much linked to the performance of the input models, emphasizing the effect that the inputs have on the estuarine microbial dynamics and the importance of the adequate representation of these inputs.

Initial conclusions can be drawn by relying on the small amount of sensitivity testing conducted here and by exploring the optimized parameter values (Table 2). Switching off

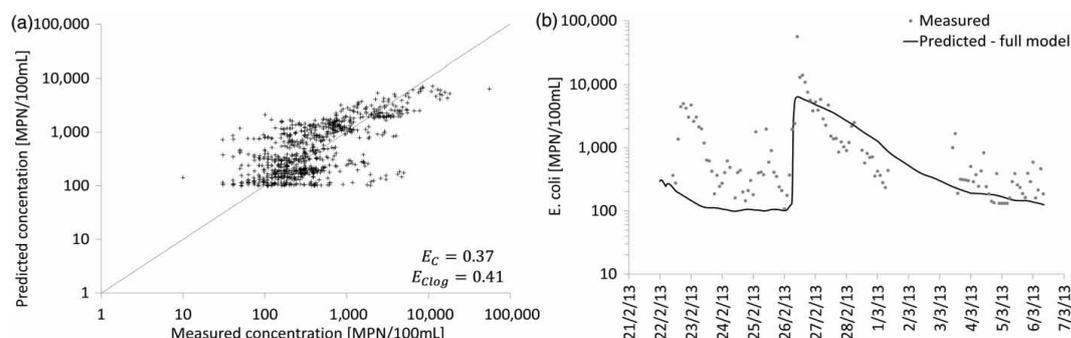


Figure 3 | Performance of the estuarine model. (a) predicted versus measured concentrations; (b) predicted versus measured pollutograph during a wet weather event.

Table 2 | Parameter ranges, distribution sampled, optimized parameter values and Nash-Sutcliffe efficiencies of the estuarine microorganism model

	Optimized calibration parameters					Model efficiency	
	RC	TOC	k_{20}	$k_e H$	EC_{sea}	E_C	E_{Clog}
Range	0.001–1	0–3,600	–1.5–1.5	1–1,000	30–60		
Distribution sampled ^a	LogU	U	U	LogU	U		
No SW/no die-off & mixing	0.009	540	–	–	–	0.32	0.34
No die-off & mixing	0.008	720	–	–	–	0.34	0.41
Full model	0.008	284	–0.3	47.7	>60	0.37	0.41

^aU – uniform distribution; LogU – log-uniform distribution; SW – stormwater.

stormwater boundary conditions resulted in minimal model efficiency reduction (as indicated by E_C and E_{Clog} with and without stormwater input), which may reflect the low average daily contribution from urban stormwater drains.

Furthermore, modelling *E. coli* die-off results in limited improvement in the model's performance. Indeed, optimized die-off calibration parameter values (Table 2) indicate that the best results are gained when there is minimal die-off. In fact, a negative k_{20} indicates that there is actually growth due to temperature fluctuations instead of die-off (k_{20} of –0.3 represents an outlier compared with literature values for *E. coli* die-off from 0.48 in fresh water to 1.09 in sea water (Hipsey *et al.* 2008)). The optimized light attenuation coefficient k_e of 11.9 1/m (calculated assuming average fully mixed depth $H = 4$ m) is more than twice as high as that reported in the literature for highly turbid estuaries (Devlin *et al.* 2008), indicating a tendency of the model to minimize die-off by reducing the detrimental effect of sunlight on microbial survival. This is also the case for the optimized EC value of sea water. The issues described above could be related to the fact that the model is very simple and does not fully account for the

hydraulic and microbial complexity of the estuarine environment. In fact, resuspension of sediments can increase microbial concentration in water systems (Pachepsky & Shelton 2011), and hence the growth observed here could be compensating for the absence of this process in the model.

CONCLUSIONS

An integrated conceptual-level model of the whole estuarine catchment was developed. Existing models for modelling faecal microorganisms in river and stormwater were linked with a new estuarine microorganism model which accounted for microbial die-off due to temperature, salinity and sunlight. The mass balance analysis using model predictions of daily faecal microorganism loads delivered to the Yarra River estuary via riverine input, Gardiners Creek and 219 stormwater drains discharging directly to the estuary revealed limited influence of urban stormwater on the estuary during dry weather. Wet weather contributions from stormwater drains were significant in some cases

(95th percentile of 50%); however, the average contribution remained marginal (median 10%). Sensitivity analysis of the new highly simplified estuarine microorganism model showed minimal change in model performance when direct stormwater inputs were removed. This may reflect the low average daily contribution from urban stormwater drains. Both input analysis and sensitivity testing confirm previous studies showing that *E. coli* loads derived stormwater drains are dwarfed by other inputs. Nevertheless, it is essential to note that these results also demonstrate that some conditions reveal the opposite; high proportions from stormwater are possible when combined with low riverine inputs and high urban rainfall amounts. This study focuses on the overall impacts of direct stormwater inputs on faecal contamination levels within the estuary, and localized impacts require further investigation.

In spite of the very simplistic modelling approach and high likelihood of missing an important process or input (as indicated by optimized parameter values), the estuarine microorganism model performed reasonably well. This is likely due to the significant effect of inputs on microbial dynamics within the estuary itself. Therefore, appropriate representation of inputs is a requirement for modelling faecal contamination in urban estuaries. Additionally, the model's performance encourages further investigation of simple conceptual ways of modelling faecal contamination in narrow river-like urban estuaries.

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7.3 Modelling *E. coli* dynamics in an urban estuary

Integrated modelling of fate and transport of *E. coli* within an urban salt-wedge estuary

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7.3.1 Abstract

Modelling of faecal microorganism dynamics in urban estuaries at a fine temporal scale is complex and requires an integrated modelling approach, with a good characterisations of the microbial dynamics in all inputs and appropriately represented in-stream microbial processes. In this study, modelling of microbial dynamics in each input was achieved using an existing model, MOPUS, while a new three-dimensional hydrodynamic-microorganism model was developed for modelling estuarine processes. The model was tested on the Yarra River estuary using extensive dataset of more than 3500 measured *E. coli* concentrations from two locations in the estuary as well as more than 80 *E. coli* depth profiles. Sensitivity analysis of the model components (i.e. microbial die-off and sediment-microorganism interaction) revealed that exclusion of these components had minimal effect on the predictive capability of the model ($E_{\text{LOG}} = 0.22$ vs. $E_{\text{LOG}} = 0.29$), suggesting that *E. coli* dynamics in the Yarra River estuary is driven by inputs and hydrodynamic transport and mixing. The importance of the Yarra River freshwater input was further confirmed by using measured datasets (instead of modelled ones) which led to a significant increase in the model's predictive performance ($E_{\text{LOG}} = 0.18$ vs. $E_{\text{LOG}} = 0.38$). To explore model structural uncertainties, the performance of a simple conceptual spatially-lumped microorganism model was tested ($E_{\text{LOG}} = 0.41$), suggesting that a simpler model could represent the

dataset as well as the new three-dimensional hydrodynamic-microorganism model. Nevertheless, process-based model outputs provide more information about the *E. coli* dynamics particularly in the spatial context.

Key words: Microbiological water quality, *Escherichia coli*, Yarra River estuary, 3D models, MOPUS, TUFLOW FV, AED2

7.3.2 Introduction

Estuaries across the world are increasingly being developed and managed for recreational purposes. However, at the same time, they are placed under environmental stressors, leading to excessive pollution and thereby limiting their benefits (Wolanski and Elliott, 2016). Majority of the adverse influences affecting estuarine health are anthropogenic (e.g. population growth, urbanization, climate change). This is not surprising considering that estuaries and continental shelf areas comprise 5.2% of the earth surface and around 60% of the global population lives along estuaries and coast (Lindeboom, 2002). The environmental stress on estuaries is likely to increase in the future because population in coastal areas is predicted to double every twenty years (Wolanski and Elliott, 2016).

Faecal microorganisms are the leading cause of pollution in urban estuaries (Burton and Pitt, 2002), and they can have significant impact on the public health. Beside health effects, medical treatment of illnesses associated with recreational waters can represent significant economic burden. For example, estimated cost for treatment of these illnesses was \$3.3 million per year for only two beaches in California, USA (Dwight et al., 2005).

For above reasons, increased effort has been placed around mitigation strategies for improvement of health of urban estuaries. However, faecal microbial dynamics in estuarine environment is very complex. It is influenced by an array of potential faecal contamination inputs such as: rivers and creeks (Martinez-Manzanares et al., 1992; Daly et al., 2013), urban stormwater (McCarthy et al., 2008; McCarthy et al., 2012), wastewater (CWP, 2000; de Brauwere et al., 2011), bed and bank sediments (Solo-Gabriele et al., 2000; Desmarais et al., 2002) and other non-host habitats and direct deposition by wildlife and humans (Weiskel et al., 1996). Moreover, faecal microorganism dynamics within estuaries is driven by their ability to survive in estuarine environment and the ability to interact with sediments (Pachepsky and Shelton, 2011). Additionally, an array of hydrological factors (e.g. flow, velocity, tide, hydrodynamic/density driven mixing) will also influence the observed complexity.

Due to the outlined complexity of microbial dynamics in urban estuaries, adequate mitigation cannot occur without a full understanding and appreciation of all the inputs and processes which occur within the system. This lends itself to the use of modelling tools, the only practical and possible way to incorporate these complex dynamics, which can then be used to explore various methods of mitigation (by means of hypothesis testing) and the influence of future externalities on the system's behaviour, such as climate change and population growth.

So far, there have been a few attempts to model microbial dynamics in estuaries. However, some limitations of existing models/modelling studies were identified. The models were developed/tested on predominantly well-mixed estuarine systems (Salomon and Pommepuy, 1990; de Brauwere et al., 2014a; Gao et al., 2015; Liu et al., 2015) and it is unknown how these models would perform in highly-stratified (salt-wedge) estuaries. There are a number of microorganisms models developed for simulating microorganism dynamics in creeks, rivers or lakes (e.g. (Wilkinson et al., 1995; Yakirevich et al., 2013; Niazi et al., 2015), However, these have not been tested in an estuarine environment. Furthermore, Hipsey et al. (2008) developed a generic microorganism model, potentially applicable to all aquatic environment, but this model also was never tested in an estuarine system.

In most of the existing models, focus was given to the water column (Salomon and Pommepuy, 1990; Kashefipour et al., 2002; Gao et al., 2015), rarely including interactions between sediments and overlaying water layer (sedimentation/re-suspension) (Gao et al., 2011b; de Brauwere et al., 2014a). However, some studies did consider settling of the microorganisms into bed sediments, albeit without accounting for resuspension of bed-stored microbes (Garcia-Armisen et al., 2006; de Brauwere et al., 2011; Liu and Huang, 2012; Liu et al., 2015). Not accounting for this resuspension could result in poor predictive performance as it is recognised that bed stores of microbes might represent an important input via resuspension of sediments and attached microbes back into the water column (Wilkinson et al., 1995; Muirhead et al., 2004; Yakirevich et al., 2013).

While some of the reviewed models accounted for die-off of microorganisms using constant die-off rates (Salomon and Pommepuy, 1990; Kashefipour et al., 2002; Garcia-Armisen et al., 2006), the majority of studies have modelled die-off rates as a function of temperature only. Surprisingly only one study incorporated die-off due to salinity (Gao et al., 2015), despite modelling faecal microorganisms in an estuarine environment.

It is hypothesised that microbial dynamics within the estuarine environment will be significantly impacted by the inputs of faecal microbes to the estuary. Yet, most of the existing models do not fully account for microbial dynamics in inputs. Indeed, the inputs are represented either with a constant

flux value, or predicted using simple relationship with flow (Garcia-Armisen et al., 2006; Liu and Huang, 2012) or with sediment concentrations (Ghimire and Deng, 2013). Such representation of input of faecal microorganisms to the estuarine model might hide the importance of the particular input, influence the results of the model and misinform mitigation strategies.

Therefore, the main aim of this study is to develop a process-based hydrodynamic-microorganism model that will account for all aspects of microbial dynamics in urban estuarine environment and its inputs. The salt-wedge Yarra River estuary was used as a case study and the reference faecal microorganism was *E. coli*. A dataset containing water level, flow velocities, salinity, temperature and *E. coli* concentrations measurements from the estuary and the main inputs was used for development and evaluation of the estuarine model. Additionally, a sensitivity analysis was performed to determine the importance of the different model components in the case study of Yarra River estuary.

7.3.3 Methods

7.3.4 Study site

The Yarra River is located in south-eastern Australia and is the major river which flows through the city of Melbourne, Australia. The total length of the Yarra River is 242 km with the catchment size of around 4000 km² composed of forested headwater reaches, predominantly rural mid-reaches and urbanised lower reaches. The last 22 km represent its estuarine section, with Port Philip Bay at the downstream end and an artificial weir, Dights Falls, at the upstream end (Figure 7 - 1). The estuary is used for secondary contact water recreation (especially rowing, kayaking, and fishing) while primary contact recreation is either restricted due to boat navigation or is not recommended due to frequently high levels of faecal indicator microbes (Department of Sustainability and Environment, 2012).

The major input of fresh water to the estuary is the Yarra River, which contributes about 70% of the total flow at the estuary mouth (Sokolov and Black, 1996). Other freshwater inputs include Gardiners Creek in the upper estuary (~7.5 km downstream of the Dights Falls) and Maribyrnong River and Moonee Ponds Creek in the lower part of the estuary. Over two hundred stormwater drains were identified along the estuary, some of which have pipe diameters greater than 3m (Daly et al., 2013; Jovanovic et al., 2015). The wastewater drainage network is separate to the stormwater and there are no wastewater treatment plants that discharge the treated effluent directly into the Yarra River estuary.

The Yarra River estuary is categorised as a highly-stratified, salt-wedge estuary (Beckett et al., 1982). According to its tidal characteristics the estuary is classified as a micro-tidal estuary (tidal water level fluctuations varying between 0.3 to 0.9 m but on average around 0.5 m), having a semi-diurnal tidal pattern with significant diurnal variations (Beckett et al., 1982). The average fluvial flow rate in the lower Yarra River is estimated to be $10 \text{ m}^3/\text{s}$ (Sokolov and Black, 1996).

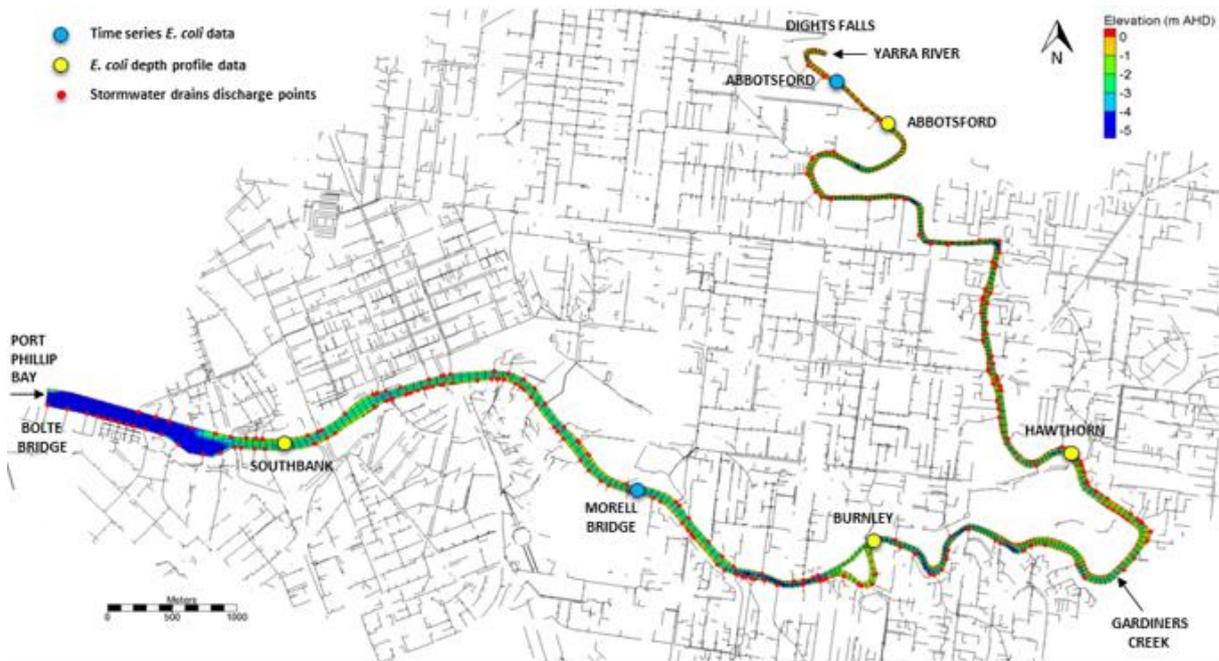


Figure 7 - 1 Yarra River estuary model mesh covering the section from Dights Falls to Bolte Bridge.

7.3.5 Model description

A coupled hydrodynamic-water quality model was applied for modelling faecal microorganism dynamics in this study. Hydrodynamics of the Yarra River estuary was modelled using a commercially-available, three-dimensional hydrodynamic modelling platform called TUFLOW FV (<https://www.tuflow.com/Tuflow%20FV.aspx>). The microorganism model was implemented within the AED2 framework (<http://aed.see.uwa.edu.au/research/models/AED/index.html>) which was coupled to the hydrodynamic model. Descriptions of the hydrodynamic model and the microorganism model are presented below.

Hydrodynamic model

TUFLOW FV is a finite volume three-dimensional numerical hydrodynamic model for modelling open surface flows in channel, rivers, estuaries, coasts and oceans. The model focuses on physical processes

in aquatic environment by solving the integral form of the non-linear shallow water equations using the finite volume numerical method on unstructured (flexible) meshes (BMT WBM, 2013). The mesh can be made of triangular or quadrilateral elements of varying size with sigma or z coordinates available for vertical mesh discretisation. The model also simulates the transport of scalar constituents, such as salinity and temperature, and includes their effect on the hydrodynamic solution through baroclinic coupling using the UNESCO equation of state (Fofonoff and Millard, 1983). The extensive description of the hydrodynamic model set up with its application to the Yarra River estuary and extensive sensitivity testing to the input data is available in Jovanovic et al. (2019). The setup of the hydrodynamic model in this study is identical as described in the aforementioned study.

Suspended sediments model

Suspended sediments can play an important role in microbial dynamics because of the known tendency of faecal microorganisms to attach to sediment particles (Pachepsky and Shelton, 2011). In addition, suspended sediments affect light penetration through the water column, which again influences microorganism survival. Therefore, in order to account for these important interactions, a simple suspended sediment transport model was incorporated within the AED2 water quality module. The model can account for a set number of different suspended sediment fractions where concentration of each fraction is influenced by hydrodynamic transport and mixing, deposition and resuspension. Deposition is parameterised as a function of settling velocity (w_s) while resuspension is a function of erosion rate (E) and critical erosion shear stress (τ_e). The full model equations are presented in Supplementary Material.

Microorganism model

The reference faecal microorganisms used for model development and evaluation was *E. coli*, a common faecal indicator organism. This microorganism was chosen because methods for its quantification are simple, efficient and cost-effective which was necessary in order to collect enough data for model development and evaluation.

The microorganism model simulates three fractions of *E. coli*: free *E. coli*, sediment-attached *E. coli* and *E. coli* deposited in the bed sediments (i.e. bed-store *E. coli*).

Free E. coli

Free *E. coli* enter the model domain by external inputs and are subject to hydrodynamic transport and mixing. Due to extremely small settling velocities of free bacteria (Garcia-Armisen and Servais, 2009), free *E. coli* are not subject to settling (Garcia-Armisen et al., 2006; de Brauwere et al., 2014a), but they are subject to die-off processes that are governed by number of environmental factors (Eq. (7 - 1)).

The total die-off rate was a sum of natural mortality (dark die-off rate) and sunlight inactivation (Eq. (7 - 2)), which were parameterised following Hipsey et al. (2008). Natural mortality was a function of water temperature and salinity (Eq. (7 - 3)). In this study, pH effects on the mortality were excluded because measured pH levels within the Yarra River estuary were near-neutral (i.e. 7 – 8; (Jovanovic et al., 2017a)) which is in the range of negligible pH impact on *E. coli* survival (Reddy et al., 1981; Hipsey et al., 2008). Sunlight inactivation was as a function of three solar bandwidths (i.e. visible, UV-A and UV-B; Eq. (7 - 4)) and again leaving out the effect of pH on sunlight inactivation for above mentioned reason.

Sediment-attached E. coli

Similarly to free *E. coli*, sediment-attached *E. coli* are subject to hydrodynamic transport and mixing and die-off. Additionally, they are also able to settle into the bed store and be resuspended back into the water column (Eq. (7 - 5)).

Sediment-attached microorganisms are subject to the environmental effects in the same way as the free microorganisms. However, due to the certain degree of protection that sediments provide to sediment-attached microorganisms, die-off rate of the attached microorganism is typically taken as a fraction of that of the free microorganisms (Jamieson et al., 2005; Garcia-Armisen et al., 2006; Russo et al., 2011; de Brauwere et al., 2014a). Therefore, a similar approach was used in this study and die-off rate of sediment-attached *E. coli* was calculated as a fraction of the die-off rate of free *E. coli* (Eq. (7 - 6)).

Sediment-attached *E. coli* settle and resuspend in the same way as the sediment particles to which they are attached. Therefore, *E. coli* deposition is a function of sediment particle settling velocity (Eq. (7 - 7)) and *E. coli* resuspension is a function of bed sediment resuspension rate (Eq. (7 - 8)).

In the current model there is no interaction between free and attached *E. coli*, because it was hypothesised that the attachment-detachment processes are slow compared to other processes (Pachepsky and Shelton, 2011). Similar approaches have been applied in several other models (Jamieson et al., 2005; Garcia-Armisen et al., 2006; Ouattara et al., 2013; de Brauwere et al., 2014a). Therefore, free microbes cannot become attached and sediment-attached microbes cannot become free. Additionally, this approach does not require knowledge of fraction of attached bacteria in the estuary, but the attached fraction becomes a diagnostic variable and an output of the model.

Bed-store E. coli

Bed-store *E. coli* is influenced by settling of sediment-attached *E. coli*, resuspension of bed-store *E. coli* and die-off (Eq. (7 - 9)). Die-off rate of microorganism in bed sediments was parameterised in a similar

way as those attached to sediment particles in water (Eq. (7 - 10)), where die-off rate is a fraction of the die-off rate for free *E. coli*. Unlike bed sediments, bed store of *E. coli* is finite and can be depleted.

Table 7 - 1 Microorganism model equations

Microorganism model equation	Parameter description	Eq. no.
Free microorganisms		
$\frac{\partial}{\partial t} C_f + \frac{\partial}{\partial x_i} (U_i C_f) = \frac{\partial}{\partial x_i} \left(\kappa_i \frac{\partial C_f}{\partial x_i} \right) - k_d^f C_f + \sum_{j=1}^n C_{f,j}$	C_f is concentration of free <i>E. coli</i> [org m ⁻³], k_d^f is die-off rate of free <i>E. coli</i> [day ⁻¹] and the last term represents free <i>E. coli</i> delivered through j-th boundary input.	(7 - 1)
Die-off rate of free microorganisms		
$k_d^f = k_m + k_l$	k_m is natural mortality die-off rate [day ⁻¹] and k_l is die-off rate due to sunlight inactivation [day ⁻¹].	(7 - 2)
Natural mortality die-off rate		
$k_m(T, S) = (k_{d_{20}} + \frac{c_{S_M} S^k}{35}) \times \theta^{T-20}$	$k_{d_{20}}$ is fresh water die-off rate of <i>E. coli</i> at 20 °C [day ⁻¹], S is salinity [psu], c_{S_M} is a constant controlling the effect of salinity on the die-off rate [day ⁻¹ psu ⁻¹], k is a parameter controlling sensitivity of k_d to salinity and θ is coefficient controlling sensitivity of die-off to temperature change [-].	(7 - 3)
Sunlight inactivation		
$k_l(I, S) = \sum_{b=1}^{N_B} \varphi(k_b + c_{S_B} S) I_b \cdot \left(\frac{DO}{K_{DO_b} + DO} \right)$	N_B is the number of discrete solar bandwidths modelled [-], b is bandwidth class [-], k_b is the freshwater die-off rate for exposure to b-th class [m ² MJ ⁻¹], c_{S_B} is a coefficient that enhances the sunlight inactivation of particular bandwidth due to salinity [m ² MJ ⁻¹ psu ⁻¹], I_b is the intensity of the b-th bandwidth [W m ⁻²], DO is dissolved oxygen concentration [mmol DO m ⁻³] and K_{DO_b} controls the sensitivity of the solar bandwidth to dissolved oxygen concentration [mmol DO m ⁻³].	(7 - 4)
Sediment-attached microorganisms		
$\frac{\partial}{\partial t} C_{att} + \frac{\partial}{\partial x_i} (U_i C_{att}) = \frac{\partial}{\partial x_i} \left(\kappa_i \frac{\partial C_{att}}{\partial x_i} \right) - k_d^{att} C_{att} - D_{att} + R_{bs} + \sum_{j=1}^n C_{att,j}$	C_{att} is concentration of the sediment-attached <i>E. coli</i> [org m ⁻³], k_d^{att} is die-off rate of sediment-attached <i>E. coli</i> [day ⁻¹], D_{att} is deposition rate of the sediment-attached <i>E. coli</i> [org m ⁻² s ⁻¹], R_{bs} is the resuspension rate of the bed-stored <i>E. coli</i> [org m ⁻² s ⁻¹] and the last term represents sediment-attached <i>E. coli</i> delivered through j-th boundary input.	(7 - 5)
Die-off rate of sediment-attached microorganisms		
$k_{att} = \gamma_{att} k_d$	γ_{att} is the scaling coefficient for the sediment-attached <i>E. coli</i> die-off rate [-].	(7 - 6)
Deposition of sediment-attached microorganisms		
$D_{att} = w_s C_{att}$	w_s is settling velocity of sediment particles to which <i>E. coli</i> are attached [m/s]	(7 - 7)
Resuspension of bed-store microorganisms		
$R_{bs} = R C_{bs}$	C_{bs} is the concentration of the bed-store <i>E. coli</i> [org g ⁻¹]	(7 - 8)
Bed-store microorganisms		
$\frac{\partial}{\partial t} (C_{bs} \rho_{sb} d_{bs}) = D_{att} - R_{bs} - k_d^{bs} (C_{bs} \rho_{sb} d_{bs})$	ρ_{sb} is bulk sediment density of estuarine sediments [kg m ⁻³], d_{bs} is depth of estuary bed where microorganisms are present [m] and k_d^{bs} is the die-off rate of bed-stored <i>E. coli</i> [day ⁻¹].	(7 - 9)
Die-off rate of bed-store microorganisms		
$k_d^{bs} = \gamma_{bs} k_d$	γ_{bs} is the scaling coefficient for the bed-store <i>E. coli</i> die-off rate [-].	(7 - 10)

7.3.6 Model set up

Model mesh

The mesh applied in this study is identical to the one described in Jovanovic et al. (2019). The mesh covers an approximately 17 km long reach of the Yarra River estuary from its head at Dights Falls to Bolte Bridge. The mesh has 1644 mostly quadrilateral elements with typically four elements used to discretise across the breadth of the estuary. Hybrid sigma-z coordinate system was applied for vertical discretisation. Above -1 m AHD (Australian Height Datum) eight sigma layers were defined and additional z layer each 0.2 m below -1 m AHD making a total of 26702 computational cells.

Model parameters

Suspended sediment model

The sediments of the Yarra River estuary are primarily composed of clay and silt (Ellaway et al., 1982). Hence, the two sediment fractions were simulated in the current model. The representative particle sizes for the two fractions were 1 μm and 10 μm with the sediment particle density of 2650 kg m^{-3} . Critical stress for deposition and erosion were 0.02 Nm^{-2} and 0.30 Nm^{-2} for clay fraction and 0.05 Nm^{-2} and 0.35 Nm^{-2} for silt fraction respectively. The erosion rate was the same for both fractions as 0.01 $\text{gm}^{-2}\text{s}^{-1}$ based on the range reported for soft natural muds by van Rijn (1993).

Microorganism model

Enteric bacteria have been shown to associate with fine grained sediment particles (< 60 μm), i.e. clay and silt (Orlob, 1956; Gannon et al., 1983; Auer and Niehaus, 1993; Wu et al., 2009; Pachepsky and Shelton, 2011). Whilst two sediment fractions representing clay and silt were simulated in sediment transport model, *E. coli* was assumed to be attached to only one fraction - clay fraction. This was based on measurements of sedimentation of *E. coli* using the water collected from the Yarra River estuary. It was determined that there was no settling of *E. coli* in the first 24h indicating *E. coli* was attached to particles of less than 1.5 μm in diameter (McCarthy et al., 2011a), which agrees well with high percentage of clay particles (< 2 μm) found in the Yarra River estuary (Ellaway et al., 1982). Furthermore, this was reinforced by the minimal settling within a six to seven day period (McCarthy et al., 2011a).

The value of fresh water mortality rate at 20 °C was set to 0.48 [day^{-1}] based on *E. coli* survival experiment in the water column of the Yarra River estuary (Schang et al., 2016b). This agrees well with the values reported in literature (e.g. 0.42 [day^{-1}] (Hipsey et al., 2008; Gao et al., 2015)). The values of other die-off parameters were adopted from Hipsey et al. (2008) and are presented Table 7 - 2. Because die-off rate due to salinity is also a function of dissolved oxygen it was necessary to estimate

dissolved oxygen concentrations in the Yarra River estuary. AED2 water quality module contains a dissolved oxygen model and the modelling of DO in the Yarra River estuary was previously conducted successfully by Bruce et al. (2014) using this model. Furthermore, Bruce et al. (2014) also used TUFLOW FV for modelling hydrodynamics of the Yarra River estuary, albeit the model mesh was simpler than the one used in this study. Therefore, the same calibrated DO model parameter values were applied in this study to simulate the DO dynamics in the Yarra River estuary.

Various scaling coefficients were found in literature for accounting for die-off of the attached microbes. For example, for simulating die-off of attached faecal coliforms Russo et al. (2011) applied die-off rate of 75% of that of the free faecal coliforms; similarly Garcia-Armisen et al. (2006) and de Brauwere et al. (2014a) applied scaling factor of 50% in their studies, Jamieson et al. (2005) even assumed that attached *E. coli* do not decay (i.e. scaling factor =0%). Since no data is available for estimating the scaling factor in the Yarra River estuary, sediment-attached *E. coli* were assumed to die-off two times slower than the free *E. coli*, hence scaling factor of 0.5 was applied (Table 7 - 2).

Reported die-off rates of *E. coli* in sediments are highly variable but on average an order of magnitude lower than those in water column (Pachepsky and Shelton, 2011). Additionally, survival of faecal microorganism in sediments was also related to sediment texture (Burton et al., 1987; Davies and Bavor, 2000; Desmarais et al., 2002; Pachepsky and Shelton, 2011). Burton et al. (1987) showed that a number of human-associated bacteria exhibited better survival in sediments with higher clay content (>25%) compared to more coarse sediments, which was interpreted by Davies and Bavor (2000) as a result of better protection from predators, which were excluded from small pores containing bacteria due to their large size. Therefore, due to the high clay content of the Yarra River sediments, no die-off of bed-stored *E. coli* was simulated in this study (i.e. $\gamma_{bs} = 0$, Table 7 - 2). Many studies have adopted similar approach for simulating die-off of microbes in bed sediments (Steets and Holden, 2003; Gao et al., 2011a; de Brauwere et al., 2014a).

Furthermore, faecal microorganisms are concentrated in the top few centimetres of the bed sediments and with generally not many microbes found below 5 cm depth (Pachepsky and Shelton, 2011). This was also true for the Yarra River estuary, where faecal microbes were abundant in the top 2 cm of the bed sediments but were often not detected below 9 cm (Schang et al., 2016b). Hence, in the current study d_{bs} parameter value was set to 0.05 m. The value of bulk sediment density was set to 200 kg m^{-3} based on the bulk density of soft muds (van Rijn, 1993).

Table 7 - 2 Microorganism model parameters, including references from which each value was obtained.

Parameter description	Symbol	Units	Value
Fresh water die-off rate of <i>E. coli</i> at 20 °C [day ⁻¹]	$k_{d_{20}}$	[day ⁻¹]	0.48 ^a
Coefficient controlling sensitivity of natural mortality to temperature change	θ	[-]	1.11 ^b
Coefficient controlling the effect of salinity on the die-off rate	c_{S_M}	[day ⁻¹ psu ⁻¹]	6.32 10 ^{-9b}
Parameter controlling sensitivity of natural mortality to salinity.	k	[-]	6.1 ^b
Freshwater die-off rate for exposure to visible light	k_{vis}	[m ² MJ ⁻¹]	0.097 ^b
Freshwater die-off rate for exposure to UVA light	k_{uva}	[m ² MJ ⁻¹]	1.16 ^b
Freshwater die-off rate for exposure to UVB light	k_{uvb}	[m ² MJ ⁻¹]	36.4 ^b
Coefficient that enhances the sunlight inactivation due to salinity for visible light	$c_{S_{vis}}$	[m ² MJ ⁻¹ psu ⁻¹]	0.0067 ^b
Coefficient that enhances the sunlight inactivation due to salinity for UVA light	$c_{S_{uva}}$	[m ² MJ ⁻¹ psu ⁻¹]	0.0067 ^b
Coefficient that enhances the sunlight inactivation due to salinity for UVB light	$c_{S_{uvb}}$	[m ² MJ ⁻¹ psu ⁻¹]	0.0067 ^b
Coefficient that controls the sensitivity of the visible light to dissolved oxygen concentration	$K_{DO_{vis}}$	[mmol DO m ⁻³]	15.6 ^b
Coefficient that controls the sensitivity of the UVA light to dissolved oxygen concentration	$K_{DO_{uva}}$	[mmol DO m ⁻³]	15.6 ^b
Coefficient that controls the sensitivity of the UVB light to dissolved oxygen concentration	$K_{DO_{uvb}}$	[mmol DO m ⁻³]	15.6 ^b
Scaling coefficient for the sediment-attached <i>E. coli</i> die-off rate	γ_{att}	[-]	0.5 ^c
Scaling coefficient for the bed-stored <i>E. coli</i> die-off rate	γ_{bs}	[-]	0.0 ^d
Bulk sediment density of estuarine sediments	ρ_{sb}	[kg m ⁻³]	200 ^e
Depth of estuary bed where microorganisms are present	d_{ps}	[m]	0.05 ^a

^a Schang et al. (2016b)^b Hipsey et al. (2008)^c Garcia-Armisen et al. (2006); de Brauwere et al. (2014a)^d Steets and Holden (2003); Gao et al. (2011a); de Brauwere et al. (2014a)^e van Rijn (1993)

Boundary conditions

The main inputs to the Yarra River estuary include the Yarra River at the upstream end of the estuary, Gardiners Creek, over 200 stormwater drains discharging directly into the estuary and returning water coming back into the modelled estuarine reach through downstream boundary driven by the upstream tidal current.

Flow rates, water temperature, salinity, TSS and DO inputs

Flow rates, water temperature and salinity inputs in this study were identical to those described in detail in Jovanovic et al. (2019), and are hence only summarised here. Measured flow rates for the Yarra River and Gardiners Creek as well as the corresponding salinity and water temperature data were supplied by Melbourne Water or collected by Monash University. Discharges from 208 stormwater drains discharging directly into the estuary within the modelled reach were estimated through a rainfall-runoff model MOPUS (McCarthy et al., 2011b) as described in Jovanovic et al. (2015) and Jovanovic et al. (2017b). Temperature and salinity data collected by Monash University from two major stormwater drains discharging into the estuary were also used to characterise stormwater inputs. Tidal

surface water elevations at Southbank supplied by Melbourne Water were used as a downstream boundary condition. Salinity at the downstream boundary was set constant to the salinity of seawater (i.e. 35psu) based on the measurements of salinity in the Port Philip Bay. The water temperature data was available from a commercial vessel navigating the bay. No diurnal variation in the water temperature of the bay was observed, thus, weekly averaged temperature was applied temperature boundary condition. The salinity and temperature boundary conditions were applied uniformly across the whole downstream boundary face because there was no data available to account for the high stratification of the water column. However, this boundary condition will only be effective during the upstream tidal flux. According to current measurements from the Yarra River estuary, upstream tidal flux occurs for around 13% of time at an average magnitude nearly three times lower than that in downstream direction. Nevertheless, small errors in prediction of the halocline and thermocline were introduced due to uniform salinity and temperature distributions across the downstream boundary face (Jovanovic et al., 2019).

Defining boundary conditions for suspended sediment model was much more difficult because much less data were available for characterising these boundary conditions. Around 170 measurements of TSS concentration from the Yarra River at Kew in period 1997 – 2016 were available for estimating TSS inputs from the Yarra River. A significant relationship was found with the flow rate ($R^2 = 0.75$, $p < 0.001$) which was used to provide the TSS boundary condition at the upstream end. Even less data were available for the Gardiners Creek, where only 55 TSS measurements in the period 2013 – 2014 were available. Nevertheless, relationship with flow was established ($R^2 = 0.72$, $p < 0.001$) and used to provide continuous TSS inputs of Gardiners Creek. The least TSS data was available for estimating TSS inputs from stormwater drains where only around 15 TSS measurements were available from one of the largest stormwater drains discharging into the estuary, Prahran Main Drain, for period 2013 – 2014. This data was used to develop relationship with flow ($R^2 = 0.32$, $p = 0.06$) and the TSS inputs from each of the stormwater drains were produced by applying this relationship with the stormwater flows estimated with the rainfall-runoff model. At the downstream boundary condition the TSS concentrations was set to zero based on the very low turbidity of salt-wedge (Jovanovic et al., 2017a).

After the TSS boundary conditions were estimated, it was necessary to partition the total TSS concentration into two simulated fractions. For the Yarra River input 70% of TSS was attributed to clay fraction and 30% to silt fraction based on the composition of the estuarine muds reported in (Ellaway et al., 1982). Gardiners Creek and stormwater drains effectively supply sediments from highly urbanised catchments, thus, the partitioning of the TSS for these inputs was based on the typical particle size distributions for urban stormwater from Melbourne (Li, 2008). 45% and 55% for Gardiners

Creek and 20% and 80% for stormwater drains were attributed to clay fraction and silt fraction respectively.

Due to the lack of measured DO concentrations, DO concentration of the Yarra River, Gardiners Creek and stormwater drains was assumed to be at saturation levels at the current temperature of the water input. This assumption seems reasonable since Yarra River enters the estuary over the weir; hence, the water is very well aerated. Similarly, Gardiners Creek and stormwater drains contribute the majority of the water during wet weather periods, when the flows are very turbulent and the oxygen levels may be assumed to be at the saturation levels. The DO levels in the salt-wedge are found to be on average around a half of that at the surface of the water column (Jovanovic et al., 2017a). Therefore, DO levels at the downstream boundary were set to 40% of the DO saturation level at a current temperature.

All flow and water level boundary conditions were defined at a 6-minute interval.

E. coli inputs

It was hypothesised that inputs will be the main drivers of the *E. coli* levels within the estuary and, thus, it was necessary to appropriately characterise these inputs. Continuous monitoring of all inputs was impossible, thus the only way to account for such a large number of inputs is by using models to characterise microbial dynamics in inputs and provide continual boundary conditions for the estuarine microorganism model.

Jovanovic et al. (2017b) demonstrated that model for microorganism prediction in urban stormwater - MOPUS (McCarthy et al., 2011b) can be effectively calibrated to predict wet weather *E. coli* concentrations even from a large catchment such as the Yarra River. Furthermore, MOPUS was also able to simulate wet weather *E. coli* concentrations in Gardiners Creek. Therefore *E. coli* boundary conditions for the Yarra River and Gardiners Creek were obtained using calibrated models described in Jovanovic et al. (2017b).

Similarly, MOPUS was also able to successfully reproduce measured wet weather *E. coli* concentrations from the three stormwater drains within the Yarra River estuary catchment (Jovanovic et al., 2017b). To provide the inputs from the other two hundred and five stormwater drains that did not have measured *E. coli* stormwater concentrations, the MOPUS model was applied in the following way: 1) an optimised model parameter set pool was created using the thousand best performing parameter sets from each of the three modelled urban catchments in Jovanovic et al. (2017b); 2) in order to diversify the produced parameter set pool, the thousand best performing parameter sets from each of the four urban catchments located in Melbourne and modelled by McCarthy et al. (2011b) were

added to existing parameter set pool; 3) Finally, the two hundred and five parameter sets were randomly withdrawn from the parameter set pool and used to produce the *E. coli* input from each of the two hundred and five remaining stormwater drains.

MOPUS was developed for predicting wet weather stormwater *E. coli* concentrations and the predictions are a function of routed rainfall intensity (McCarthy et al., 2011). As such, during dry weather, when there is no rainfall, the model was systematically under predicting the *E. coli* concentrations. To avoid this issue, *E. coli* concentrations during dry weather were estimated by sampling from a distribution of measured dry weather *E. coli* concentrations. The Yarra River and Gardiners Creek dry weather inputs were estimated using datasets collected at Dights Falls and Gardiners Creek respectively, while the dry weather inputs from stormwater drains were estimated using data set collected at Hawthorn Main Drain east and west where a substantial amount of dry weather flow monitoring was conducted. Since data were not normally distributed (Shapiro-Wilk test, $p < 0.001$), before estimating the normal distribution parameters, the data were log-transformed in attempt to increase the normality of the data. The distribution parameters are shown in Table 7 - 3. Furthermore, to avoid having large discrepancies between the values of the randomly generated dry weather *E. coli* concentrations, autocorrelation within the measured data was examined and the obtained correlation coefficients were applied to produce dry weather *E. coli* concentrations of the Yarra River, Gardiners Creek and stormwater drains as (Eq. (7 - 11)):

$$C^t = r_s C^{t-1} + (1 - r_s) 10^{[C_D^t \sim N(\mu, \sigma^2)]} \quad (7 - 11)$$

where C^t is dry weather *E. coli* concentration at time t [MPN 100mL⁻¹], r_s – Pearson’s autocorrelation coefficient [-] and C_D^t is dry weather *E. coli* concentration [log(MPN 100mL⁻¹)] at time t obtained by sampling dry weather normal distribution with median μ and standard deviation σ (Table 7 - 3).

Table 7 - 3 Medians, standard deviations and Pearson’s auto-correlation coefficients obtained for Dights falls, Gardiners Creek and Hawthorn Main Drain east dry weather *E. coli* data sets using log-transformed values.

	Median μ [log(MPN 100mL ⁻¹)]	St. dev. σ [log(MPN 100mL ⁻¹)]	r_s [-]
Dights Falls	2.24	0.27	0.61
Gardiners Creek	2.72	0.40	0.72
Hawthorn Main Drain east	3.41	0.48	0.67

The *E. coli* concentrations entering through the downstream boundary were set to zero. The water at the downstream boundary is predominantly sea water (Jovanovic et al., 2017a) which is not conducive

to survival of the *E. coli* (Šolić and Krstulović, 1992) and frequently had *E. coli* concentrations below detection limit (i.e. less than 10 MPN 100mL⁻¹). Similarly, to the discussion above, the effect of this boundary conditions is expected to be limited locally to the proximity of the downstream boundary.

Model requires explicit separation of the total pool of *E. coli* in inputs into free and sediment-attached fraction. Reported fractions of sediment-attached *E. coli* are somewhat similar across a range of different water sources. For example, 34% - 44% of *E. coli* were sediment-attached in a freshwater creek (Jamieson et al., 2005) and an average of 38% of *E. coli* were associated with sediments in the Neuse River estuary (Fries et al., 2006). In stormwater the attached fraction of *E. coli* ranged from 20% - 30% during dry weather (Characklis et al., 2005; Cizek et al., 2008) to 30% - 50% during wet weather (Schillinger and Gannon, 1985; Characklis et al., 2005; Cizek et al., 2008). Jeng et al. (2005) found percentage of *E. coli* attached to sediments in stormwater to be in a slightly lower range 22% - 30%. In the current study 40% of *E. coli* were assumed to be attached to sediment particles in all modelled inputs.

Initial conditions

Initial water level was set to 0.0 m AHD. Salinity and temperature values were set to 20 and 20°C, respectively. Suspended sediment concentrations for clay fraction was set to 30 gm⁻³ and for silt fraction to 15 gm⁻³. Concentration of DO was set to 10 mgL⁻¹ (i.e. 0.3126 mmolL⁻¹). The estuary was initially set to be free of *E. coli*, hence concentrations of free and attached fractions were set to zero. The initial concentration of the bed-stored *E. coli* was 6000 MPNg⁻¹ based on the mean measured concentrations of *E. coli* in the Yarra River estuary bed sediments (Schang et al., 2016b).

Values of all scalar constituents were set uniformly throughout the whole modelling domain. Therefore, an additional month of 'warm up' period was added to the beginning of the simulation period to allow the model to adjust to a dynamic equilibrium prior to undertaking any assessments. This ensured that model predictions during the model assessment period were not biased by the initial conditions assumption. The one month 'warm up' period was sufficient for the model to establish salt-wedge dynamics in the estuary (Jovanovic et al., 2019), thus it was considered that it will also be sufficient for establishment of estuarine *E. coli* distribution. In total, the simulation covered the period of two years, from 1st October 2012 to 1st September 2014.

7.3.7 Model validation

Due to excessively long run times of the model, a comprehensive calibration procedure of the model parameter values was not possible. Instead, all model parameter values were estimated based on literature or local experiments (see Table 7 - 2).

Measured data

In order to evaluate the microorganism model properly, *E. coli* data, which covered different hydrologic and weather conditions, was required. To obtain this data, *E. coli* concentrations were measured over the period of nearly two years from October 2012 to August 2014 at two locations in the Yarra River estuary, Abbotsford and Morell Bridge (Figure 7 - 1). Both sites were equipped with refrigerated automated samplers (Hach SD900) for the collection of water samples. The water intake to the auto-sampler at Abbotsford was fixed at approximately 40 cm above the estuary bed, while at Morell Bridge, the intake was attached to a flotation device and samples were taken from 10 cm depth (from the water surface) regardless of the tidal stage. Over 1700 samples were collected at each site. All collected samples were transported to the Environmental and Public Health Microbiology (EPHM) laboratory at Monash University in coolers on ice and analysed using Colilert method (IDEXX Laboratories, 2013) within 24h of collection. Additionally, to be able to assess the ability of the model to predict distribution of *E. coli* along the depth of the water column over 80 *E. coli* depth profiles were collected from four locations in the Yarra River estuary (Jovanovic et al., 2017a). All *E. coli* concentrations in this study were measured as Most Probable Number (MPN) per 100 ml, thus all model *E. coli* predictions were transformed to the same units for evaluation.

Model performance

The model performance was evaluated both qualitatively and quantitatively. Qualitative assessment of model performance was done visually by producing a range of plots including measured vs. modelled *E. coli* concentrations, time series plots of measured and modelled *E. coli* concentrations and depth profile plots of measured and modelled *E. coli* concentrations. Quantitative assessment of model performance was done by calculating Nash-Sutcliffe efficiency, which also enables a comparison with other microbial models in the literature.

Generally, there are two ways of calculating the efficiency of microorganism model predictions. One way is to calculate model efficiency by using raw measured and predicted microbial concentrations (McCarthy et al., 2011b; Yakirevich et al., 2013; Jovanovic et al., 2017b). The other way is to calculate model efficiency by using log-transformed measured and predicted microbial concentrations (Parajuli et al., 2009; Niazi et al., 2015). The latter is done due to high variability of microbial concentrations

found in datasets which often span a few orders of magnitude. Having a few data points with extremely high concentration values can bias the value of the model efficiency. When data is log-transformed, this issue is greatly reduced because the differences between dataset values are much smaller. In this study, Nash-Sutcliffe efficiency is calculated using log-transformed *E. coli* concentrations.

7.3.8 Sensitivity of the model to different model components

Simple ad hoc “One at a Time” (OAT) sensitivity analysis was applied to assess the importance of different model components on the model predictions. Due to extremely long run times of the model (approx. 110h), it was not possible to perform a full sensitivity analysis. Nevertheless, application of simple sensitivity testing procedures, such as OAT, can still provide valuable insights about the model structure.

Assessment of the in-stream model components (i.e. die-off and sediment-microorganism interactions) was conducted by performing model simulations where in-stream model components were successively removed and the model performance was assessed by calculating Nash-Sutcliffe efficiency.

To test the hypothesis about the importance of the inputs on the model prediction, the measured *E. coli* data available at Dights Falls was used to modify the Yarra River input (i.e. instead of modelling it). In periods when measured data were available, the Yarra River modelled *E. coli* concentrations were replaced with the measured concentrations. Since measured data were available at hourly time step, the data were linearly interpolated to produce six minutely input time series. All other *E. coli* inputs to the estuary were kept the same. It should be noted that the measured *E. coli* data at Dights Falls covered shorter period of time than the data used for model evaluation at Morell Bridge. As such, the model efficiency was calculated using only the part of the dataset at Morell Bridge that corresponded to the Dights Falls measured data period.

In order to assess the effect of increasing model complexity on model’s predictive performance, the current model was compared to a more simple conceptual model of *E. coli* dynamics in the Yarra River estuary (Jovanovic et al., 2015). A model simulation was performed for the same period and using the same inputs as described in Jovanovic et al. (2015).

7.3.9 Results and discussion

7.3.10 Model evaluation

It is important to reiterate that due to extremely long run times (more than 110 hours), this model has not been calibrated, and all parameter values have been directly adopted from literature, either from local experiments or international literature.

Measured versus predicted *E. coli* concentrations at Abbotsford and Morell Bridge are presented in Figure 7 - 2. Predicted *E. coli* concentration during three different hydrologic periods (a large wet weather event, a small wet weather event and a dry weather period) at Abbotsford and Morell Bridge are presented in Figure 7 - 3 and Figure 7 - 4 respectively.

There is an apparent discrepancy between measured and predicted concentrations at Abbotsford (Figure 7 - 3). While there is a clear dynamics in the predicted concentrations, it looks like there is a phase shift between predicted and measured concentrations. The model predictions at Abbotsford are predominantly influenced by the Yarra River input, which is confirmed by the high correlation between the Yarra River input and the model prediction at Abbotsford (Spearman rank correlation coefficient $\rho = 0.95$, $p < 0.001$ with time shift 11 time steps = 66 min to account for travel time from upstream boundary to Abbotsford). The impact of die-off on model prediction is limited because of short travel time and only a couple of small stormwater drains discharge into estuary upstream of Abbotsford, which are not expected to have significant effect on model predictions. The Yarra River input was produced by the MOPUS model, which has been reported previously to have some timing issues (i.e. misalignment between measured and predicted pollutographs) (McCarthy et al., 2011b), which may explain the observed phase shift in estuarine model predictions. Furthermore, the MOPUS model was calibrated against the measured data that covered only around one half of the period of the data used to assess the model performance at Abbotsford. This effectively means that the MOPUS model was applied outside the calibration period, which also may have influenced the estuarine model's predictions at Abbotsford.

In contrast, model was able to capture overall dynamics of *E. coli* at Morell Bridge (Figure 7 - 4). Morell Bridge is located around 12 km downstream from Abbotsford and there are a number of stormwater inputs entering along including Gardiners Creek. Whilst Yarra River is a major input of *E. coli* into the estuary (Jovanovic et al., 2015), the *E. coli* concentrations at Morell Bridge will not be influenced so strongly like at Abbotsford. This is confirmed with a significant but weaker correlation between Yarra River *E. coli* inputs and predicted *E. coli* concentrations at Morell Bridge ($\rho = 0.69$, $p < 0.001$ with time shift 213 time steps = 21.3 h).

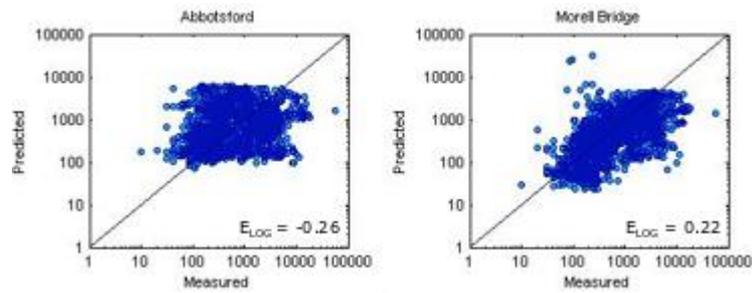


Figure 7 - 2 Predicted vs. Measured *E. coli* concentrations at Abbotsford and Morell Bridge

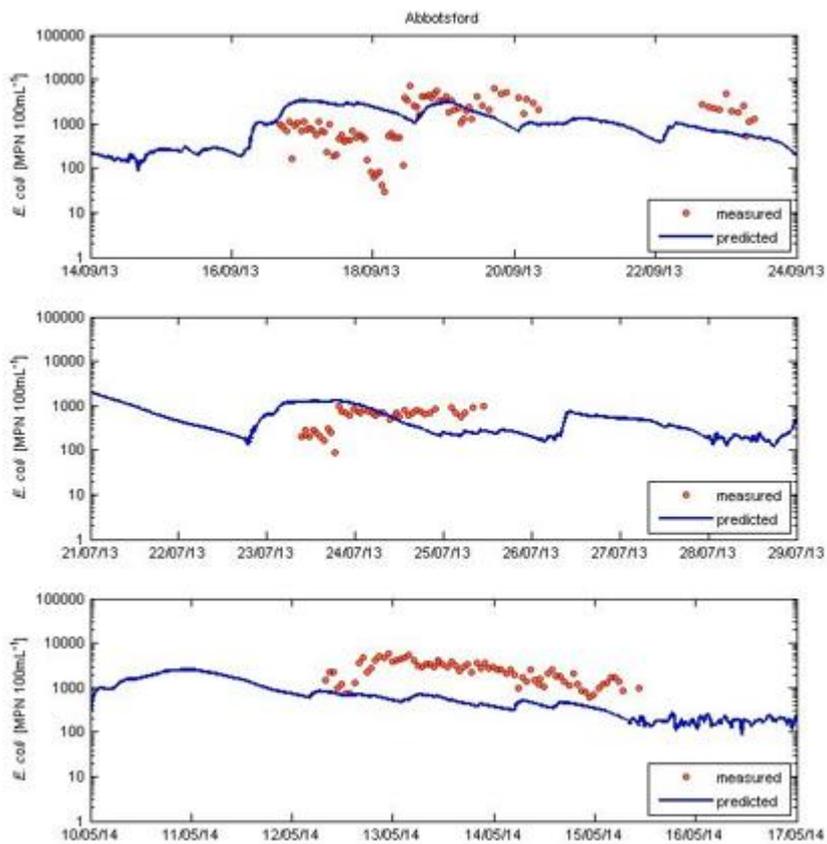


Figure 7 - 3 Measured and predicted *E. coli* concentrations at Abbotsford for three different periods including large wet weather event (top; total rainfall = 139.4 mm), small wet weather event (middle; total rainfall = 6.1 mm) and a dry weather period (bottom; total rainfall = 0.0 mm).

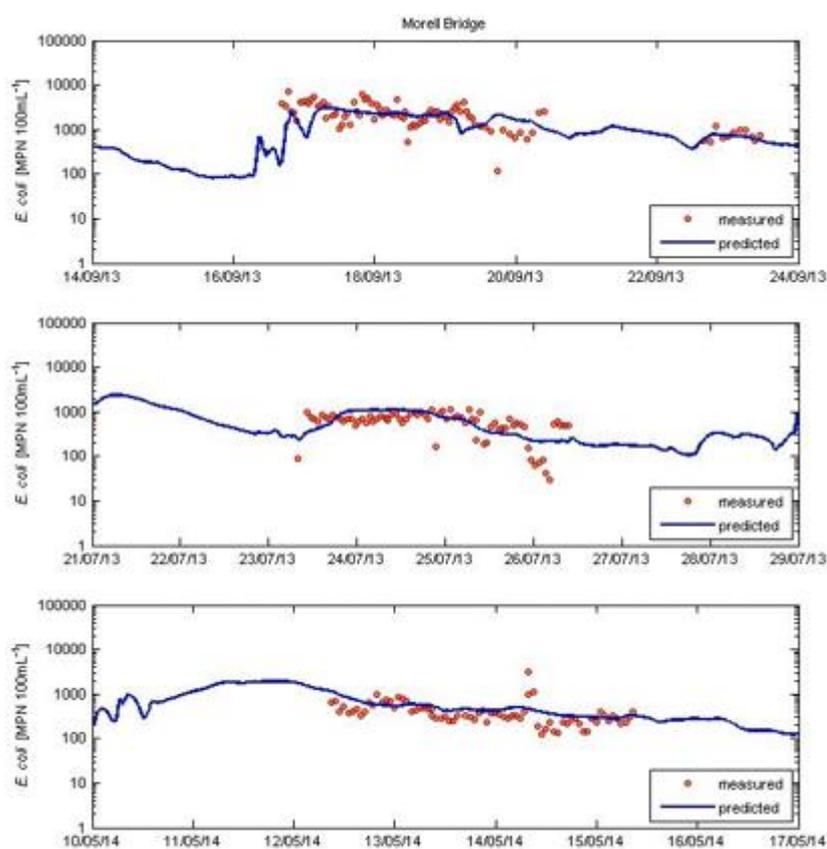


Figure 7 - 4 Measured and predicted *E. coli* concentrations at Morell Bridge for three different periods including large wet weather event (top; total rainfall = 139.4 mm), small wet weather event (middle; total rainfall = 6.1 mm) and a dry weather period (bottom; total rainfall = 0.0 mm).

Examples of measured and predicted *E. coli* depth profiles for low, mid and high flow conditions at four locations in the Yarra River estuary are presented in Figure 7 - 5. The predicted vertical distribution of *E. coli* concentrations correspond to those measured particularly at Morell Bridge and Southbank locations where strong stratification of the water column exist. However, the *E. coli* concentrations in the salt-wedge (bottom layer) seem to always be underestimated. A few possible factors may have contributed to this outcome. Firstly, the *E. coli* concentrations at the downstream boundary conditions are set to zero, which means that as the salt-wedge progresses upstream forced by tides, there is no *E. coli* entering the model domain with it. Secondly, due to difference in densities between fresh water layer and salt-wedge as well as limited tidal range in the estuary there is little mixing between the two layers (which enables the formation of salt-wedge) (Dyer, 1997). In turn, a small number of *E. coli* are able to penetrate into the salt-wedge. Finally, due to high salinity of salt-wedge, die-off rate in salt-

wedge is more pronounced than in the overlaying fresh water later so even if some *E. coli* are mixed into the salt-wedge they would quickly die-off.

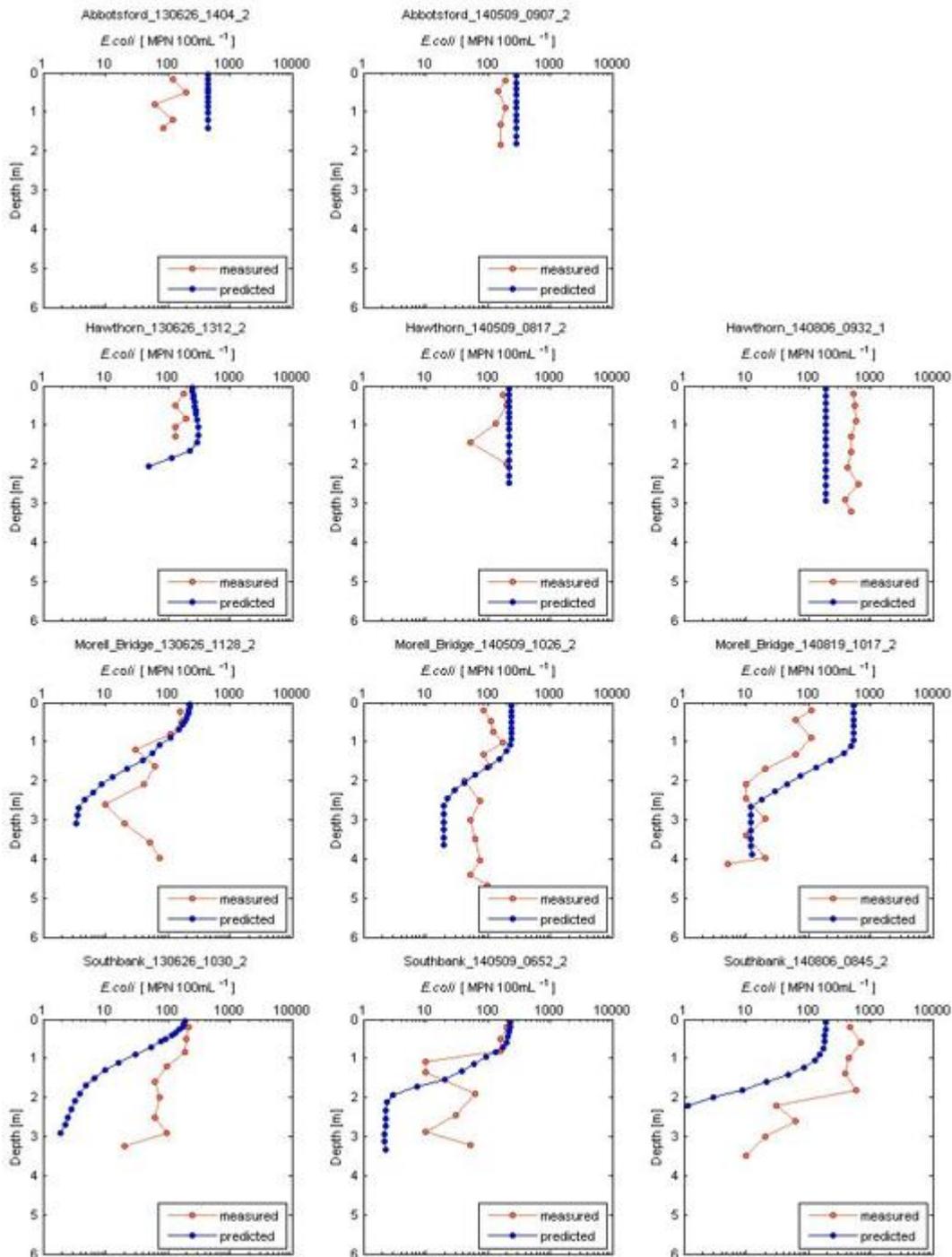


Figure 7 - 5 Measured and predicted *E. coli* depth profiles at Abbotsford (ABB - row 1), Hawthorn (HMD - row 2), Morell Bridge (MOR – row 3) and Southbank (SB – row 4) on 26th June 2013 at 11:28 am (left; average Yarra River flow rate in 24h before the depth profiling $Q_{24h} = 4.9 \text{ m}^3/\text{s}$ – representative of low flow conditions), 9th May 2014 at 10:26 am (middle; $Q_{24h} = 14.9 \text{ m}^3/\text{s}$ – representative of mean flow conditions) and 6th August 2014 at 10:20 am (right; $Q_{24h} = 32.2 \text{ m}^3/\text{s}$ – representative of high flow conditions). N.B. There was no depth profile available at Abbotsford on 6th August 2014.

Model efficiency

The performance of microorganism models is rarely assessed quantitatively by calculating model fit parameters. Most commonly, the model fit assessment is conducted visually by plotting measured versus predicted microbial concentrations typically in time series format (Garcia-Armisen et al., 2006; Hipsey et al., 2008; Bedri et al., 2011; de Brauwere et al., 2011; Gao et al., 2011b; Liu and Huang, 2012; Bedri et al., 2013; Gao et al., 2013; de Brauwere et al., 2014a). Interestingly, sometimes model fit parameters are calculated for hydrodynamic model prediction but not for microorganism model predictions within the same study. For example, Liu and Huang (2012) calculated mean average error, root mean square error and Nash-Sutcliffe Efficiency for hydrodynamic model predictions of estuarine current velocities and salinity but not for prediction of faecal coliform concentrations. Instead they relied only on visual assessment of goodness of model fit for microorganism model prediction. While qualitative assessment of model performance is important, it is hard to assess the microorganism model performance objectively. For example, visual impression of the model fit can be influenced by selecting the axis limits. In contrast, quantitative model fit assessment not only ensures objective assessment of the model performance but it also enables comparison between different models. Therefore, the performance of many microorganism models published in literature remains unknown and any comparisons with these models are impossible.

Nash Sutcliffe Efficiency criterion values for this model are presented in Table 7 - 4. The efficiencies of the model at predicting *E. coli* concentrations at Abbotsford was $E_{LOG} = -0.26$ and at Morell Bridge $E_{LOG} = 0.22$. The model efficiency values reflects well the observed model predictions in Figure 7 - 3 and Figure 7 - 4. The efficiency of the model at predicting vertical distribution of *E. coli* concentration was similar $E_{LOG} = -0.72$ but ranged from -1.42 to -0.10 at different locations. Less than zero efficiency values indicate that model predictive skills are worse than assuming the mean of measured data. Nevertheless, obtaining good model efficiency values for microorganism prediction is proven to be difficult. For example, out of a few studies that report model efficiency values, Niazi et al. (2015) reported E_{LOG} values in the range of -0.94 to 0.47 for faecal coliforms and -0.81 to 0.39 for *E. coli* for catchment scale modelling. Similarly, Parajuli et al. (2009) reported values of E_{LOG} from -2.20 to 0.38 for modelling *E. coli* at catchment scale. For stormwater microorganism modelling reported efficiency values using raw data ranged from 0.17 to 0.45 (McCarthy et al., 2011b; Jovanovic et al., 2017b).

Table 7 - 4 Model prediction performance according to Nash-Sutcliffe efficiency using log-transformed data (E_{LOG}).

	Location	E_{LOG}
TS ^a	Abbotsford	-0.26
	Morell Bridge	0.22
Depth profiles	Abbotsford	-0.62
	Hawthorn	-0.10
	Morell Bridge	-0.35
	Southbank	-1.42
	All	-0.72
^a – time series data		

Overall, the weak performance of the model at reproducing measured *E. coli* concentrations can be a consequence of a number of different reasons. Some of the reasons (in hypothesised order of importance) include:

- 1) The measured *E. coli* concentrations used for model evaluation are inherently uncertain (Harmel et al., 2016). The average total random uncertainty in estimation of *E. coli* concentrations in streams is estimated to be around 70% (but range from around 30% to 110%)(Harmel et al., 2016) and in stormwater greater than 30% (but range from 15% to 67%)(McCarthy et al., 2008).
- 2) As explained above, continuous monitoring of *E. coli* inputs to the estuary is not possible, however, continuous inputs are required as boundary conditions. To overcome this, existing microorganism models were applied to produce continuous *E. coli* inputs and provide boundary conditions to the estuary. However, as with all microbial models, the applied models were not able to fully explain the measured *E. coli* variability in inputs. Therefore, the errors in estimation of *E. coli* concentration (e.g. magnitudes, timing etc.) produced by these models are propagated through the estuarine model and impact the estuarine model predictions of *E. coli* concentrations. Furthermore, assumptions related to the downstream boundary condition might have also impacted the predicted *E. coli* concentrations.
- 3) Not all possible inputs are included in the model. For example, sewer cross connections with stormwater drains can occur which can contribute untreated sewage directly into the estuary. Additionally, there are a number of emergency relief structures (ERS) along the estuary that serve to relieve sewers in case of blockages or capacity breach. These mostly occur during wet weather due but can also occur during dry weather. In fact, 4% human wastewater was

detected at Morell Bridge using microbial source tracking techniques on one occasion within the modelling period (Henry et al., 2016).

- 4) Not all processes influencing *E. coli* concentrations are considered in the current estuarine microorganism model. For example, bank sediments are known to contain high concentrations of *E. coli*. These can be resuspended by a variety of mechanisms such as increased riverine flow, tidal action, boat traffic and recreational boating all of which occur in the Yarra River estuary. Additionally, wildlife living around and within the estuarine area can contribute to the *E. coli* concentration by direct deposition into the estuary. For example, a colony of grey-headed flying foxes located just upstream of the estuarine reach of the Yarra River was estimated to deposit up to 41 kg of faecal matter into the water column daily during winter (Henry et al., 2018). This equates to total *E. coli* load of around 10^{10} MPN day⁻¹.

7.3.11 Sensitivity of the microorganism model predictions to different model components

Surprisingly, exclusion of the die-off dynamics of *E. coli* have improved model prediction marginally (Table 7 - 5, Sim ID 1 and 1a; Figure 7 - 6). While it was expected that the model will be able to produce better prediction with inclusion of more microbial processes, and thus more model parameters, this finding suggests that model parameter values were not estimated accurately by taking values from literature or past experiments. Indeed, due to long model run times, it was not possible to calibrate the model parameters by applying some of the conventional calibration procedures. Instead, the model parameter values were estimated based on the literature and not truly calibrated to fit the observed *E. coli* concentrations. This may have resulted in worse model predictions with the simulated die-off dynamics. Nevertheless, there is likely a combination of the values of these parameters that would increase the model performance.

The exclusion of sediment-microorganism interaction did not have any effect on model performance (Table 7 - 5, Sim ID 1a and 1b; Figure 7 - 6). *E. coli* are attached to clay fraction which has very small settling velocity (around 0.06 mday⁻¹), thus, their settling is very limited. This was confirmed experimentally in the Yarra River estuary (McCarthy et al., 2011a). Similar results were obtained by Russo et al. (2011) when modelling faecal coliform sediment interaction. They showed that on average less than 2% of faecal coliforms were estimated to settle out of the water column per year. Additionally, resuspension in the Yarra River estuary is limited because of generally low bottom shear stresses. Considering the whole modelling domain, bottom shear stress was greater than the critical shear stress

needed for resuspension of clay particles (i.e. 0.30 Nm^{-2}) less than 3% of the simulation time. The average bottom shear stress value during this time was 0.62 Nm^{-2} (with 0.31 Nm^{-2} , 0.48 Nm^{-2} and 1.44 Nm^{-2} being 5th, median and 95th percentile, respectively). Using the sediment transport model parameters and the initial bed-store *E. coli* concentration the shear stress above would cause resuspension rate of $64 \text{ E. coli m}^{-2}\text{s}^{-1}$. If these are resuspended under a flow rate of $1 \text{ m}^3\text{s}^{-1}$ assuming complete mixing, the increase in concentration would be negligible, around $0.0064 \text{ MPN } 100\text{mL}^{-1}$. Therefore, resuspension is unlikely to impact the *E. coli* levels in the Yarra River estuary.

The limited effect of survival kinetics and sediment-related processes on model prediction of *E. coli* concentrations may also suggest that the inputs into the system are the main driver of *E. coli* dynamics in the estuary. This agrees well with the initial hypothesis that accurate representation of the inputs will be the most important for the simulation of microbial dynamics in the Yarra River estuary. The Yarra River is the main water input into the estuary (Beckett et al., 1982) and also the main input of *E. coli*, contributing on average more than 99% of *E. coli* load during dry weather and around 90% of *E. coli* load during wet weather (Jovanovic et al., 2015). Therefore, it was hypothesised that any improvement in the representation of the Yarra River *E. coli* input will have significant impact on the model prediction and will be reflected in the model efficiency values.

When the measured *E. coli* concentrations at Dights Falls were incorporated into the existing Yarra River *E. coli* input, the resulting model efficiency increased from 0.19 (modelled inputs; Table 7 - 5, Sim ID 2a) to 0.38 (measured inputs; Table 7 - 5, Sim ID 2b, Figure 7 - 7). Therefore, improving characterisation of the Yarra River *E. coli* input by including measured data did indeed improved the model performance.

Since it was shown that the *E. coli* dynamics in the Yarra River estuary was driven heavily by inputs into the estuarine system and hydrodynamic transport/mixing within the estuary, a comparison was made with a simpler transport model to assess if more complex hydrodynamic transport models provide significant improvement in model performance. It was shown previously that the simple routing model was able to achieve model prediction performance (E_{LOG}) of around 0.4 (Table 7 - 5, Sim ID 3) (Jovanovic et al., 2015). The 3-dimensional microorganism model (Table 7 - 5, Sim ID 3a) achieved much lower performance. The poor performance of the model was related to die-off impact on model predictions (Figure 7 - 8). When the in-stream model components were turned off, model achieved a higher model performance (Table 7 - 5, Sim ID 3c). Whilst, it may seem that there is no benefit in increasing the model complexity from conceptual to process-based, the complex model can provide outputs that can be used to gain much more insight into the microbial dynamics and at a much higher spatial resolution. Nevertheless, simple models are easy to set up, do not require as much data to run

and have short model run times, so they can be a useful tool for initial assessment and real time (online) predictions.

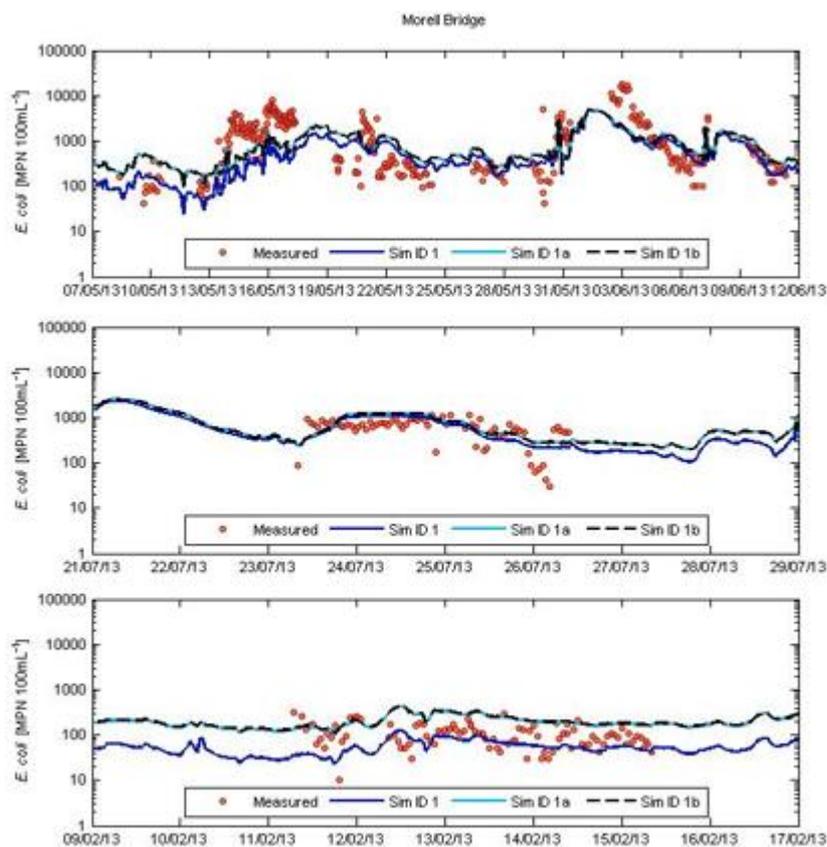


Figure 7 - 6 Sensitivity assessment of model *E. coli* concentrations predictions to in-stream microorganism model components (die-off kinetics and sediment-microorganism interaction). Measured and predicted *E. coli* concentrations from Simulation 1 and Simulation 1b (Table 7 - 5) for three periods including large wet weather event (top; total rainfall = 139.4 mm), small wet weather event (middle; total rainfall = 6.1 mm) and a dry weather period (bottom; total rainfall = 0.0 mm).

Table 7 - 5 Model prediction performance at Abbotsford and Morell Bridge for different simulations.

Simulation ID	Simulation components	<i>E. coli</i> input	Simulation period	Efficiency calculation period	Abbotsford E_{LOG}	Morell Bridge E_{LOG}
1	Transport/Mixing Die-off Resuspension/Settling	Yarra River (Jovanovic et al., 2017b) Stormwater (Jovanovic et al., 2017b)	01/10/12 – 01/09/14	01/10/12 – 01/09/14	-0.26	0.22
1a	Transport/Mixing Die-off Resuspension/Settling	Yarra River (Jovanovic et al., 2017b) Stormwater (Jovanovic et al., 2017b)	01/10/12 – 01/09/14	01/10/12 – 01/09/14	-0.25	0.29
1b	Transport/Mixing Die-off Resuspension/Settling	Yarra River (Jovanovic et al., 2017b) Stormwater (Jovanovic et al., 2017b)	01/10/12 – 01/09/14	01/10/12 – 01/09/14	-0.25	0.29
2	Transport/Mixing Die-off Resuspension/Settling	Yarra River (Jovanovic et al., 2017b) Stormwater (Jovanovic et al., 2017b)	01/10/12 – 01/09/14	16/09/13 - 01/09/14	-0.99	0.18
2a	Transport Die-off Resuspension/Settling	Yarra River (Jovanovic et al., 2017b) Stormwater (Jovanovic et al., 2017b)	01/10/12 – 01/09/14	16/09/13 - 01/09/14	-1.02	0.19
2b	Transport/Mixing Die-off Resuspension/Settling	Yarra River (Jovanovic et al., 2017b) + measured <i>E. coli</i> * Stormwater (Jovanovic et al., 2017b)	01/10/12 – 01/09/14	16/09/13 - 01/09/14	-1.39	0.38
3	Conceptual	Yarra River (Jovanovic et al., 2015) Stormwater (Jovanovic et al., 2015)	01/10/12 – 01/08/13	01/10/12 – 01/08/13		0.41
3a	Transport/Mixing Die-off Resuspension/Settling	Yarra River (Jovanovic et al., 2015) Stormwater (Jovanovic et al., 2015)	01/10/12 – 01/08/13	01/10/12 – 01/08/13	0.21	-2.13
3b	Transport/Mixing Die-off Resuspension/Settling	Yarra River (Jovanovic et al., 2015) Stormwater (Jovanovic et al., 2015)	01/10/12 – 01/08/13	01/10/12 – 01/08/13	0.27	0.27

Strikethrough simulation components are not active during the simulation.

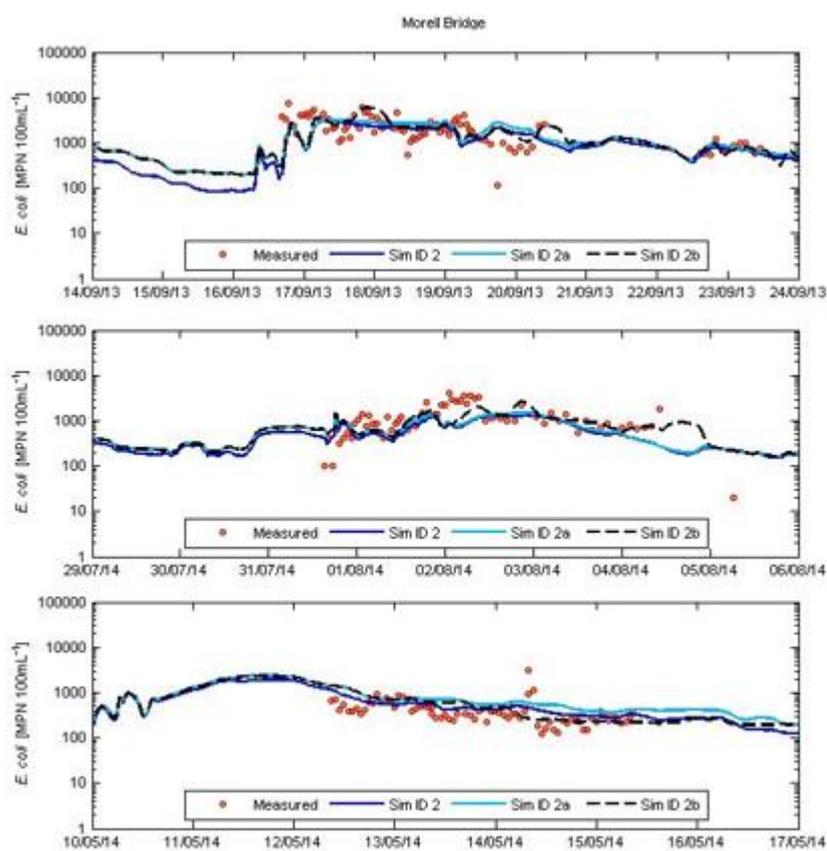


Figure 7 - 7 Sensitivity assessment of model *E. coli* concentrations predictions to Yarra River input. Measured and predicted *E. coli* concentrations from Simulation 2a and Simulation 2b (Table 7 - 5) for three periods including large wet weather event (top; total rainfall = 55.4 mm), small wet weather event (middle; total rainfall = 31.2 mm) and a dry weather period (bottom; total rainfall = 0.6 mm).

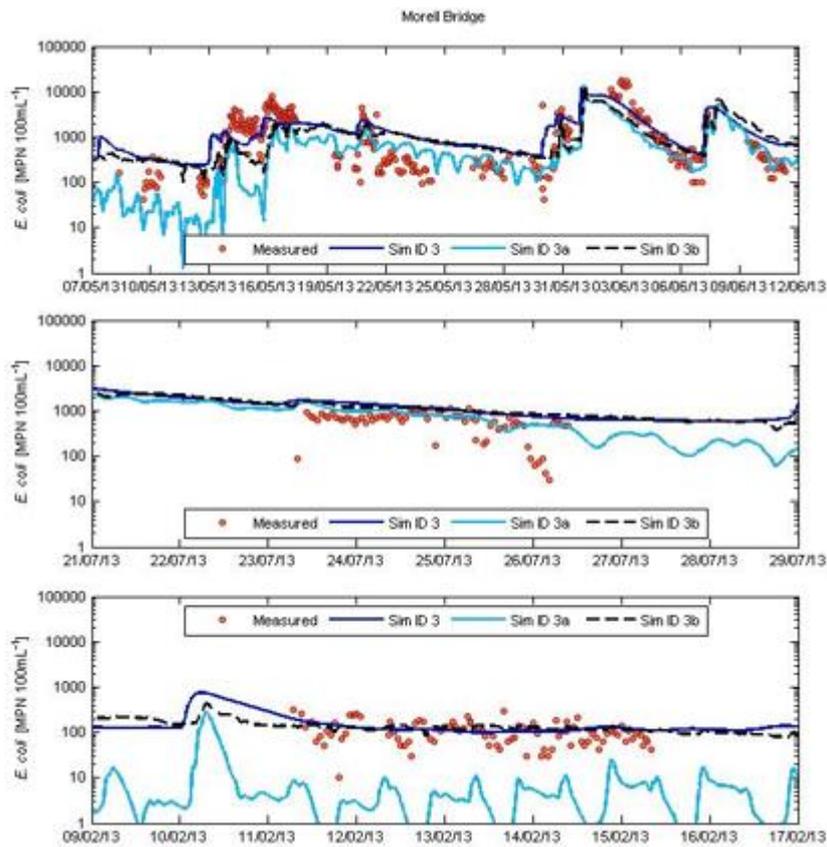


Figure 7 - 8 Comparison of *E. coli* concentration predictions from a simple conceptual model (Jovanovic et al., 2015) with the *E. coli* prediction from a three-dimensional hydrodynamic microorganism model presented in this study. Measured and predicted *E. coli* concentrations from Simulation 3 and Simulation 3a (Table 7 - 5) for three periods including large wet weather event (top; total rainfall = 139.4 mm), small wet weather event (middle; total rainfall = 6.1 mm) and a dry weather period (bottom; total rainfall = 0.0 mm).

7.3.12 Conclusions

A three-dimensional hydrodynamic microorganism model for modelling faecal microorganism fate and transport urban estuaries was presented. The model simulated die-off of faecal microorganisms dynamically as a function of temperature, salinity and sunlight. It also accounted for sediment-microorganism interactions simulating settling and resuspension of sediment attached microbes. Three fractions of faecal microorganisms were considered in the model: free fraction, sediment-attached fraction and fraction stored in bed sediments. In this study, the model was applied to simulate fate and transport of *E. coli*, a common faecal indicator organism, in the Yarra River estuary, Australia. The model was tested using an extensive dataset of more than 3500 measured *E. coli* concentrations from two locations within the estuary. Additionally, the model was also tested against more than 80 depth profiles of measured *E. coli* concentrations collected from the Yarra River estuary. This is the

first model that was tested to such an extent. The performance achieved by the model was comparable to those published in the literature for other microorganism models that were tested against significantly less data.

Exclusion of die-off and sediment-microorganism interaction marginally improved model performance. This may be the consequence of inability to calibrate model and literature-based estimation of model parameters. Additionally, limited impact of in-stream processes may also suggest that inputs and hydrodynamic transport and mixing are the major drivers determining the levels of *E. coli* in the Yarra River estuary. This was confirmed by incorporating available measured *E. coli* concentrations at the upstream boundary into the boundary condition, which improved the model performance. Therefore, accurate characterisation of the microbial levels in inputs is essential for accurate prediction of the *E. coli* levels within the estuary.

Comparison between a simple spatially-lumped conceptual microorganism model and a complex three-dimensional process-based microorganism model revealed that despite the significant increase in model complexity of the process-based model, the models achieved similar performances. However, the three-dimensional model outputs provide much more information, particularly in the spatial domain. Nevertheless, the simplicity of the conceptual model makes it very suitable for quick and initial assessments of faecal microorganism dynamics as well as real time predictions and forecasting of risks for recreational users. Therefore, each model has its value depending on the intended end use.

7.3.13 Acknowledgement

This material is based upon work supported by Melbourne Water and the Australian Research Council (Grant No. LP120100718). Our gratitude goes to these funding agencies as well as to Dr Rebekah Henry, Christelle Schang, Peter Kolotelo and Richard Williamson for the help with sampling and data collection.

7.4 Discussion and conclusions

Modelling of faecal microorganism dynamics in urban estuarine environment is a challenging and complex task. In this chapter, *E. coli* dynamics in the Yarra River estuary was modelled using an integrated modelling approach where both input *E. coli* dynamics and estuarine *E. coli* dynamics was modelled. Modelling of *E. coli* in the Yarra River estuary was conducted at two levels of model complexity by developing a simple spatially lumped conceptual model and a three-dimensional process-based model. The models were tested using one of the largest *E. coli* datasets reported in literature.

Overall, both models identified the Yarra River as a dominant driver of *E. coli* levels within the estuary and therefore the importance of it for model prediction. Contrary to common perception, analysis of inputs and sensitivity analysis of conceptual model suggested that stormwater has limited impact on faecal microorganism levels within the estuary. However further analyses are required to explore the effect of stormwater in much more detail than currently presented in this thesis. This will certainly be included as a part of future testing and exploration of the process-based microorganism model.

Some suggestion about the importance of the governing in-stream processes in the case of the Yarra River estuary was given in this chapter. Sensitivity analysis of the process-based model suggested that die-off and sediment-microorganism interaction did not have a significant impact on the model prediction. This may suggest that in the case of the Yarra River estuary the main factors controlling the *E. coli* dynamics are inputs and hydrodynamics transport and mixing. However, the process-based model was not truly calibrated but the model parameters were derived from literature or experiments, which may have been wrong or inapplicable to our case study. As such, a question remains if this would also be the case if we were able to calibrate the model parameters to the measured data. Yet, the sensitivity analysis of a conceptual model, which was calibrated to the measured *E. coli* data, revealed that exclusion of die-off dynamics did not have significant impact on model predictions. This may suggest that it is likely that in the case of the Yarra River estuary, faecal microorganism inputs and hydrodynamic transport and mixing are, indeed, that main factors influencing the *E. coli* dynamics.

This chapter attempted to answer the question related to the most appropriate methods and complexity needed for modelling faecal microorganism dynamics in urban salt-wedge estuaries (Research Question 3). This was done by comparing a simple conceptual model and a full process-based model. Both models obtained similar performance, which was a surprising finding, as one would expect that a more complex model would result in better prediction. However, it should be noted that process-based model has other benefits such as outputs that are spatially-distributed and provides

much more information about *E. coli* dynamics than a simple conceptual model. Therefore, for purposes of mitigation strategy design a process-based model will be more appropriate. Yet, if simple models can be useful if quick information about the *E. coli* levels are needed (for example, for real time forecasting and communication of risks to swimmers).

Chapter 8

Conclusions, strengths and weaknesses
of the research and future work

8.1 Introduction

Urban estuaries are currently exposed to significant environmental stress due to population growth, urbanisation and climate change which leads to increased levels of pollution. Faecal microorganism are a leading cause of this pollution and present significant management challenge for water managers. The main challenges related to microbial water quality management stem from the complexity of the faecal microbial dynamics in urban estuarine environment. Therefore, understanding faecal microorganism dynamics in urban estuarine environment is important for the assessment of health risks associated with the use of this water bodies. In order to assess the health risks, it is necessary to be able to accurately identify the levels of faecal microorganisms within estuary. A modelling tool that is able to account of the microbial dynamics in an urban estuarine environment lends itself as a practical way of addressing this pressing issue.

However, majority of the existing estuarine microorganism models are primarily focused on water column and rarely included the interaction between microorganism and sediments which has been shown as an important component of the microbial dynamics in aquatic environment. Furthermore, modelling of die-off dynamics in water column is mostly static, without functional relationship to environmental variables such as temperature, salinity, sunlight etc. Additionally, inputs of the faecal dynamics are poorly characterised, often using a constant flux value or predicted using a simple relationship with flow or TSS concentration. Lastly, the existing models were tested using scarce datasets, thus their true performance is unknown.

As such the main aim of this research was development of a more comprehensive estuarine microorganism model that will account for all important in-stream microbial processes as well as accurately characterise the microorganism levels in estuarine inputs.

The research was guided by the four major research questions:

- 1) What are the most important inputs of faecal microorganisms in an urban estuary?
- 2) What are the most important processes (including transport pathways) of faecal microorganisms in an urban estuary?
- 3) What are the most appropriate methods to model microbial dynamics in salt-wedge estuaries? What complexity is required?
- 4) What are the essential input data that need to be measured accurately in order to predict the parameters required for modelling faecal microorganisms in urban estuaries?

This chapter presents an overview and a summary of the whole research project. The key findings of this research project presented in the previous chapters of this thesis are summarised in Section 8.2. Major strengths and weaknesses of the research project are discussed in Section 8.3. Lastly, some suggestions for further investigations are given in Section 8.4.

8.2 Conclusions

In order to be able to test the microorganism model extensively, a large monitoring program was undertaken to characterise the faecal microorganism levels within the estuary. *E. coli* was chosen as a reference microorganism and the Yarra River estuary was used as a case study estuary. The time series of *E. coli* concentrations containing over 3500 data points were collected from two locations within the estuary, Abbotsford and Morell Bridge. Furthermore, due to high stratification of the water column in the estuary more than 80 depth profiles of *E. coli* concentrations at four locations in the Yarra River estuary were collected. Additionally, over 1700 *E. coli* concentrations were measured in the major inputs of faecal microorganism to the estuary, including river, creek and urban stormwater. In addition to *E. coli* concentrations a significant amount of data related to water levels, flow rates and velocities, temperature, salinity, pH, dissolved oxygen and turbidity in the estuary as well as in the main estuarine inputs were collected.

Analysis of the depth profiling data revealed that the spatial variability of *E. coli* was significantly related to the salt-wedge dynamics. At locations where salt-wedge was present *E. coli* also exhibited high stratification along depth, and in the absence of salt-wedge the vertical distribution of the *E. coli* concentrations represented a well-mixed system. The cross-sectional variability of *E. coli* was limited and within analytical measurement uncertainty. Additionally, the collected data was used to examine the relationship between *E. coli* concentrations and tidal cycle, where some confusion within the literature existed. It was shown that *E. coli* levels fluctuate over the tidal cycle and the fluctuations were related to flow velocity rather than to water level. Measured *E. coli* concentrations within the Yarra River and the two locations within the estuary identified the Yarra River as a dominant factor in determining the overall *E. coli* levels within the estuary. This was confirmed by a simple conceptual model, which showed that the Yarra River contributed on average around 99% of *E. coli* load during dry weather and around 90% of *E. coli* load during wet weather.

Therefore, faecal microorganism inputs were identified as important elements of estuarine microbial dynamics. In fact, it was hypothesised that without accurate representation of *E. coli* dynamics in inputs to the Yarra River estuary it would not be possible to accurately model the *E. coli* dynamics

within the estuary itself. Since *E. coli* levels in inputs were highly temporally variable, it was necessary to characterise *E. coli* concentrations in each input at sub-hourly time step. This was achieved by testing and modifying the existing model for microorganism prediction in urban stormwater (MOPUS) on a range of stormwater catchments, an urban creek and even the Yarra River. It was demonstrated that the model could reproduce the measured *E. coli* concentrations with certain accuracy. The model Nash-Sutcliffe efficiency (E) at urban catchments ranged from 0.17 to 0.45 and was similar to previously reported efficiency for this model (i.e. E = 0.25 - 0.41). More interestingly, the model was able to predict *E. coli* dynamics at the outlet of the large Yarra River catchment achieving efficiency of 0.64, demonstrating the MOPUS structure can be adaptable for simulating microorganism dynamics outside the stormwater domain.

Another important element of modelling microorganism dynamics in urban estuaries is accurate representation of the estuarine hydrodynamics. As mentioned above, *E. coli* fluctuations over tidal cycle were related to flow velocity and the spatial variability of *E. coli* was significantly linked with salt-wedge dynamics all of which are related to estuarine hydrodynamic. Therefore, it was an imperative to accurately simulate hydrodynamics within the Yarra River estuary. The three-dimensional hydrodynamic model of the Yarra River estuary was built using TUFLOW FV modelling platform. The model was able to reliably simulate complex estuarine hydrodynamics including salt-wedge dynamics as demonstrated by high model performance efficiency. In order to identify the most influential input data required for modelling of *E. coli* dynamics, a sensitivity analysis of the model outputs to a range of model input data was conducted. For accurate prediction of flow velocity and salinity it was necessary to obtain accurate input data on the large water inputs, such as the Yarra River and Gardiners Creek, as well as accurate measurements of wind. Surprisingly, uncertainty in bathymetry data of $\pm 15\text{cm}$ was not found to have significant effect on model predictions.

Finally, a new model for microorganism prediction in urban estuaries was developed with all important components of the microorganism dynamics in urban estuarine environment incorporated in the model structure. The model simulated free, sediment-attached and bed-store *E. coli* fractions. The instream model components accounted for die-off of *E. coli*, as a function of temperature, salinity and sunlight, and for settling and resuspension into/from bed sediments. The model was evaluated extensively using the whole dataset described above and unlike many estuarine microorganism modelling studies, which assess the model only qualitatively (i.e. visually by plotting graphs), the assessment of model efficiency in this research was also done by calculating a model fit metric – Nash-Sutcliffe Efficiency. The model efficiency at Abbotsford was -0.26 while at Morell Bridge was 0.22. The model efficiency at predicting *E. coli* depth profiles range from -1.42 to -0.10 at different locations

while it was -0.72 for the whole depth profile dataset. The model efficiencies compared well to the efficiency of other microorganism models in the literature, even though these models were tested with significantly less data. Sensitivity analysis of the model revealed a limited effect of model in-stream components on model predictions. This may have been the consequence of the fact that model parameters have been defined based on literature and may not be appropriate for the Yarra River estuary. Also, these results may suggest that the dominant components of the *E. coli* dynamics in the estuary are faecal contamination inputs and estuarine hydrodynamics. A comparison between a simple conceptual model and the process-based model of *E. coli* dynamics in the Yarra River estuary revealed that similar model performance can be achieved using different level of model complexity. This was important because these models have very different data and computational requirements and, as such, depending on the modelling problem, one or the other approach might be used without the trade-off between model complexity and performance. Finally, the developed model presents potential for being used for developing a real-time warning system for the recreational users of the estuary.

8.3 Strengths and weaknesses of the research

Data

One of the major issues with existing estuarine microorganism models is the limited availability of microbial data for model testing. Without enough data it is impossible to robustly test the model and know its true performance. The data collected in this thesis represents the largest dataset of *E. coli* concentrations collected in an estuarine environment. Additionally, a significant amount of data were also collected from a river, an urban creek and some of the largest stormwater drains that discharge into the estuary. In parallel with the microbial data, a large amount of other environmental data such as temperature, EC/salinity, pH, dissolved oxygen, turbidity, together with water level and flow data were collected from the estuary and the major inputs. The quantity of data collected as a part of this research project makes this study unique, and, thus, represents a major strength of this research.

A weakness of this research is that only one faecal indicator microorganism, *E. coli*, was monitored and the model was not tested for specific pathogens. Nevertheless, it is considered that the model structure can accommodate for simulation of faecal pathogens and that only values of the model parameters will be different. *E. coli*, as an indicator of faecal contamination, has a number of drawbacks such as inability to be used for estimation of human health risks or differentiation between human and animal sources. However, currently *E. coli* is the world standard in microbial water quality

assessment (including in this river system), and it gives an indication of the overall faecal contamination levels. Furthermore, it is easily measured which enables efficient collection of large quantities of data. As such, *E. coli* was used as a reference faecal microorganism in this research.

Analysis of the *E. coli* dynamics in an urban estuary

The collected data enabled analysis of some aspects of the *E. coli* dynamics within an urban estuarine environment that were previously limited by the lack of the available data. In particular, significant advancement was made in understanding the spatial variability of *E. coli* in a highly stratified estuary. This was considered to be a strength of this research, as the knowledge about the spatial variability of *E. coli* gained in this thesis has many practical aspects related to design of sampling strategies for monitoring and assessment of faecal contamination and/or data collection for building and testing the models of faecal microorganism dynamics in salt-wedge estuaries.

Additionally, the collected data enabled analysis of the impact of tides on *E. coli* concentrations in an estuarine environment. While other studies have reported various relationships between tidal water levels and *E. coli* concentrations, this research showed that *E. coli* concentrations do indeed fluctuate over the tidal cycle but that the fluctuations are related to flow velocity rather than water level. This was a small contribution to clarification of some disagreement in the literature, but it was regarded as a strength of the research project.

Modelling of inputs of faecal contamination to an urban estuary

Providing well characterised boundary conditions to an estuarine microorganism model is important for accurate prediction of the microbial dynamics within the estuary. This thesis has demonstrated that existing conceptual stormwater microorganism model could successfully be applied at various spatial scales of urban catchments for prediction of *E. coli* levels in an urban stormwater during wet weather. Furthermore, the model was able to predict *E. coli* concentrations in an urban creek and even in a river, which has demonstrated that the model structure can to some extent be applied outside the initially intended use.

Modelling of estuarine hydrodynamics

Hydrodynamic modelling was not the main topic of this thesis, however, modelling of hydrodynamics is a necessary first step in modelling water quality. This thesis did not only build and tested the three-dimensional hydrodynamic model of the Yarra River estuary but an additional step was undertaken to assess the sensitivity of the hydrodynamic model to a range of input data. It was identified that this was a contribution to the knowledge and, as such, it was regarded as a strength of this research.

Estuarine microorganism model development and testing

The review of the current literature identified there was a research gap in the field of modelling faecal microorganism dynamics in estuarine environment and the developed model presented in this thesis has, at least to some extent, filled this gap. The estuarine microorganism model presented in this thesis represents the most complete model from the aspect of modelling faecal microorganism dynamics in both inputs and estuary and it was evaluated using one of the largest datasets of faecal indicator organisms available. This was considered to be a major strength of this thesis.

One of the major weaknesses of the current research is that the model parameters could not be calibrated to measured data using a comprehensive calibration procedure due to long simulation run times. As such, the value of model parameters were defined using the literature and measured data was used to assess the model performance. Indeed, adopting model parameter values from the literature we were able to test the model's robustness and transferability. Overall, model was tested very conservatively, and better model performance results than those presented in this thesis would have been expected if proper calibration was performed.

Another weakness of the thesis is that impact of uncertainty of both *E. coli* input data and the model testing data (i.e. measured *E. coli* data) on the model predictions was not investigated. As such this would form a part of future work. However, 'One at a Time' (OAT) sensitivity testing was performed to gain some understanding of the model sensitivity. While the OAT sensitivity testing has certain downsides, such as inability to detect interactive effects, with proper design of sensitivity test scenarios it can provide useful insights into model sensitivity, particularly for computationally demanding models. In this thesis, it was used to examine overall effects of in-stream processes and the main input of *E. coli* on model predictions.

In this study, the estuarine microorganism model was evaluated at only one case study site – the Yarra River estuary. This is considered to be a weakness of this research. It would be beneficial to test the model on other estuarine systems that have different characteristics to those of the Yarra River estuary. This would be a good test of the current model structure and model parameterisation. However, as listed above, model testing requires significant amount of data which is often unavailable, which represents a major issue when testing microorganism models.

8.4 Future work

Calibration and testing of the model using actual pathogens and other indicators

It would be valuable to determine how the model would perform when tested using other faecal microorganisms/pathogens (e.g. *Campylobacter*, *Giardia* or enteroviruses). This task could not be undertaken in this research project because of the limitations of monitoring faecal pathogens outlined in Literature Review (Chapter 2). However, with the development of new methods for monitoring and quantifying faecal pathogens, which are more time and cost efficient, obtaining pathogen concentrations at high temporal resolution will be much easier and, hence, the model could be tested/modified/adjusted for modelling faecal pathogens. It is hypothesised that pathogen dynamics can be modelled using the existing model equations for faecal indicators, although the value of model parameters are likely to be different. Furthermore, the sensitivity the model predictions to different model components will likely be different as well. For example, settling and resuspension might be important for protozoa, which are larger and heavier microbes, but not so much important for virus which are generally the smallest microorganisms.

Sensitivity and uncertainty analyses

Due to extremely long model run times, only an ad hoc “One at a Time” sensitivity analysis was performed to explore the effect of different model components on the model predictions. In addition the previous and also due to the time constraints in this research project, estuarine microorganism model uncertainty analyses were not conducted to explore the impact of uncertainties in input data and data used for model testing on the model performance. As such, thorough sensitivity and uncertainty analyses should be conducted.

Alternative ways of providing model inputs

The inputs of faecal contaminations are shown to be important for accurate representation of dynamic of *E. coli* in an estuarine environment. Whilst this thesis presented a way of providing high resolution microorganism boundary conditions, there were still some pitfalls with the current input prediction. As such, future work could be directed at improving the way of characterising the boundary conditions of the estuarine model, which is hypothesised to improve within-estuary model predictions.

Testing of the model on other urban estuaries

The microorganism model has been tested using the Yarra River estuary as a case study. This estuary is characterised by high stratification of the water column and river-like shape. As such, future work should focus on testing of the model on other types of estuarine systems, both in terms of scale (i.e. a range of different sizes), morphology (i.e. drowned river-valley, fjordtype, lagoon-type or tectonic

estuary) and salinity structure (i.e. well-mixed, partly-mixed or highly-stratified estuaries). This would help to determine if the current model can be universally applied across different estuaries and, if not, what modifications need to be made to adjust the model to suit these different types of estuaries.

Application of the current model for estimation of human health risks

While the focus of this thesis was not on estimation of human health risks due to levels of faecal microorganism in the Yarra River estuary, the model can be applied in conjunction with a Quantitative Microbial Risk Assessment (QMRA) framework for estimation of human health risks. QMRA requires concentrations of actual pathogens, rather than faecal indicators such as *E. coli*. As such, there is a need to either calibrate the model for prediction of faecal pathogens or apply a scaling factor to *E. coli* concentration predictions, which would translate the predicted *E. coli* concentrations into concentrations of a desired pathogen.

Application of the current model for development of targeted and cost-effective mitigation strategies

Similarly to above, the exploration of the mitigation strategies for improvement of the microbial water quality of the Yarra River estuary were outside the scope of this thesis. Therefore, the developed model can be applied as a tool for hypothesis testing in order to help develop appropriate mitigation strategies. The model can be used to explore the effect of different inputs on *E. coli* levels under different conditions, test the impact of wastewater spills into the estuary (via emergency release structures), or test the impact of climate change on *E. coli* levels in the estuary.

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Appendix A
Supplementary Materials

A.1 Supplementary materials for 'Tidal fluctuations influence *E. coli* concentrations in urban estuaries' (Chapter 4)

Tidal fluctuations influence *E. coli* concentrations in urban estuaries

Dusan JOVANOVIC, Rhys COLEMAN, Ana DELETIC and David T. McCARTHY

Supplementary material

Correlation between flow velocity and water level at Morell Bridge

This section presents additional correlations between flow velocity and water level at Morell Bridge that were not included in the manuscript due to space constraints. The aim of this section is to enable further insight into relationship between flow velocity and water level over the tidal cycle in the lower estuary.

The phase shift between flow velocity and water level is 9 hours in when using both all-weather data and dry-weather data. This indicates that maximum flow velocity occurs at mid ebb tide, i.e. mid way between high tide and low tide, and vice-versa.

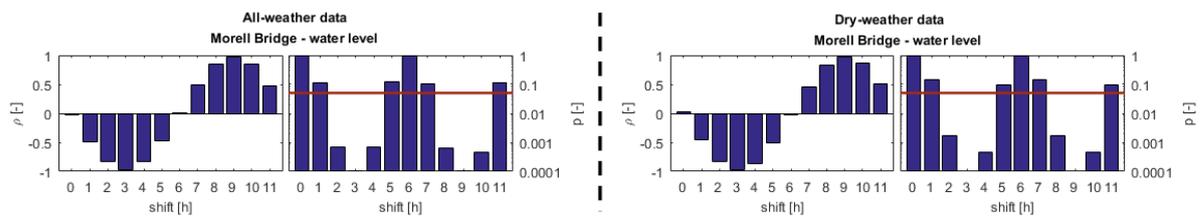


Figure A.1 - Spearman rank correlation coefficients (ρ) and corresponding p -values between flow velocity and water levels at Morell Bridge applying various shift intervals to: all-weather data (left) and dry weather data only (right). The red line indicates a p -value of 0.05.

A.2 Supplementary materials for ‘Conceptual modelling of *E. coli* in urban stormwater drains, creeks and rivers’ (Chapter 5)

Conceptual modelling of *E. coli* in urban stormwater drains, creeks and rivers

Dusan JOVANOVIC, Jon HATHAWAY, Rhys COLEMAN, Ana DELETIC and David McCARTHY

Supplementary material

Governing equations and description for the MOPUS models

List of governing equations of the MOPUS rainfall runoff model are given in Table S1, while the governing equations of the MOPUS microorganism model are given in Table S2. Exponent in equation S.3 has been changed to -4.65 and divisor in equations S.6 and S.7 to 240 compared to the equations published in McCarthy et al. (2011b) as the model has been modified for 6 minute time step calculations instead of 1 minute time step as originally proposed.

Table A.2 - 1 The governing equations of the MOPUS rainfall runoff model.

Model equation	Comment	Equation no.
Impervious surfaces		
$S_{imp}(t) = S_{imp}(t-1) + I(t) - ImpEvap(t) - Q_{imp}(t-1)$	Impervious store	(A.2 - 1)
$Q_{imp}(t) = \max(0, S_{imp}(t) - S_{impmax})$	Impervious outflow	(A.2 - 2)
$ImpEvap(t) = S_{imp}(t) \times e^{-4.65}$	Impervious store depletion	(A.2 - 3)
Pervious surfaces		
$S_{perv}(t) = S_{perv}(t-1) + I(t) - PervEvap(t) - Q_{perv}(t-1) - Q_{seep}(t)$	Pervious store	(A.2 - 4)
$Q_{perv}(t) = \max(0, S_{perv}(t) - S_{pervmax})$	Pervious outflow	(A.2 - 5)
$PervEvap(t) = \frac{\min\left[\frac{10 \times S_{perv}(t)}{S_{pervmax}}, Evap(t)\right]}{240}$	Evapotranspiration	(A.2 - 6)
$Q_{seep}(t) = \frac{0.01}{240} \times S_{perv}(t)$	Deep seepage	(A.2 - 7)
Routing routine		
$S(t) = S(t-1) + IMP \times Q_{imp}(t) + (1 - IMP) \times Q_{perv}(t) - O(t-1)$	Routing store	(A.2 - 8)
$O(t) = K \times S(t)^m$	Routed outflow	(A.2 - 9)
$R(t) = O(t - TOT) = O(t - (TOC - TOR)) = O\left(t - \left(TOC - \left[\frac{1}{K}\right]^{0.7}\right)\right)$	Translated outflow	(A.2 - 10)
<p>$S_{imp}(t)$ impervious surface store [mm], $I(t)$ rainfall depth [mm] (either gauged or weather radar-derived), $ImpEvap(t)$ amount of water removed from the impervious store [mm] due to evaporation, $Q_{imp}(t)$ outflow from the impervious store [mm], S_{impmax} capacity of the impervious store = 1 mm, $S_{perv}(t)$ pervious surface store [mm], $PervEvap(t)$ amount of water removed from the store due to actual evapotranspiration [mm], $Q_{perv}(t)$ outflow from the pervious store [mm], $Q_{seep}(t)$ amount of water lost from the store to deep seepage [mm], $S_{pervmax}$ capacity of the pervious store [mm], $S(t)$ routing store [mm], IMP effective impervious proportion, $O(t)$ amount of water removed from routing store [mm], K and m to attenuate and route flow, $R(t)$ translated outflow [mm], TOC time of concentration, TOR time of redistribution and TOT time of translation [min].</p>		

Table A.2 - 2 The governing equations of the MOPUS microorganism model.

Model equation	Comment	Equation no.
Surface component		
$Ps(t) = 10^{PsCoeff} \times \left[\frac{VP(t-1)}{\overline{VP}} \right]^{VPCoeff} \times \left[\frac{RH(t-1)}{\overline{RH}} \right]^{RHCoeff}$	Surface store	(A.2 - 11)
$Cs(t) = \frac{Ps(t) \times I_{rain}(t)^{1.293}}{I_{rain}(t)}$	Surface wash-off	(A.2 - 12)
Routed, redistributed and translated rainfall intensity		
$S_{rain}(t) = S_{rain}(t-1) + I(t) - O_{rain}(t-1)$	Routing store	(A.2 - 13)
$O_{rain}(t) = 0.2 \times S_{rain}(t)$	Routed rainfall intensity	(A.2 - 14)
$I_{rain}(t) = O_{rain}(t - (TOC - \left[\frac{1}{0.2} \right]^{0.7}))$	Translated rainfall intensity	(A.2 - 15)
Subsurface component		
$Pss(t) = 10^{PssCoeff} \times ADWP_{RI}(t)$	Subsurface store	(A.2 - 16)
$C_{ss}(t) = Pss(t) \times I_{rain}(t) \times \left[\sum_{i=A}^t I_{rain}(i) + 0.1 \right]^{-1}$	Subsurface wash-off	(A.2 - 17)
Concentration at the outlet of the catchment		
$C(t) = Cs(t) + C_{ss}(t)$	Concentration at outlet	(A.2 - 18)
<i>Ps(t)</i> microorganism levels in the surface store [orgs], <i>VP(t - 1)</i> previous day's vapour pressure [hPa], <i>RH(t - 1)</i> previous day's relative humidity [%], \overline{VP} and \overline{RH} indicate mean vapour pressure and relative humidity values, <i>PsCoeff</i> , <i>VPCoeff</i> and <i>RHCoeff</i> are calibration parameters. <i>Cs(t)</i> concentration in the outlet of the surface store [orgs/L], <i>I_{rain}(t)</i> routed, redistributed and translated rainfall intensity [mm], <i>Pss(t)</i> microorganism levels in the subsurface store [orgs], <i>ADWP_{RI}(t)</i> time since a rainfall event capable of flushing the in-pipe microorganism [days], <i>PssCoeff</i> and <i>RI</i> [mm] are calibration parameters, <i>C_{ss}(t)</i> concentration in the outlet of the subsurface store [orgs/L] and <i>C(t)</i> total concentration of microorganisms at the outlet of the catchment [orgs/L].		

Sensitivity testing of MOPUS rainfall runoff model by applying linear reservoir routing procedure

In addition to the non-linear reservoir routing technique applied by McCarthy et al. (2011b) and initially utilized in this study, the rainfall-runoff model was tested further by applying a linear routing procedure in place of the non-linear procedure. In this procedure, the outflow from the routing store is a function of only one parameter – *K* (the routing coefficient) while *m* is fixed to 1. This was tested herein to explore the possibility of reducing the number of rainfall-runoff model parameters needed to predict stormwater flow rates. In fact, previously published optimized values of the routing exponent *m* ranged from 1.00 – 1.08, giving some indication that the impact of this parameter is limited. Additionally, linear routing is desirable as it helps avoid the cross correlation between the *K* and *m* parameters. As an illustration of the cross correlation between *K* and *m*, Figure A.2 - 1 shows that the non-linear reservoir routing equation, with example values of the amount of rainfall in routing store and the store outflow, $0.1 = K \times 2^m$, is satisfied for a number of combinations of *K* and *m* parameter values.

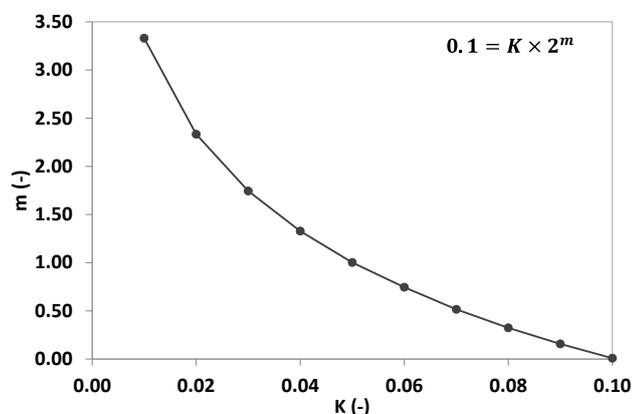


Figure A.2 - 1 Cross correlation between parameters K and m in non-linear routing routine. This example shows that the equation in the figure is satisfied for a number of different combinations of parameters K and m .

Table A.2 - 3 The optimised parameter values and the performance statistics for the rainfall-runoff model at the five urbanized catchments when linear reservoir routing was applied (i.e. $m=1$).

	Raleigh ^a		Hawthorn Main Drain east		Hawthorn Main Drain west		Pahran Main Drain		Gardiners Creek	
	RG	RADAR	RG	RADAR	RG	RADAR	RG	RADAR	RG	RADAR
<i>Optimised parameters</i>										
<i>Spervmax (mm)</i>	72	-	47	50	23	68	55	43	50	35
<i>IMP (-)</i>	0.11	-	0.39	0.32	0.44	0.39	0.20	0.19	0.21	0.22
<i>K (-)</i>	0.372	-	0.173	0.316	0.162	0.135	0.278	0.411	0.022	0.022
<i>m(-)</i>	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
<i>TOC (min)</i>	12	-	30	36	36	42	12	24	102	84
<i>Model performance</i>										
<i>E_Q</i>	0.77	-	0.71	0.35	0.67	0.16	0.90	0.75	0.93	0.88
<i>E_{EQi} min</i>	0.21	-	-0.10	-3.83	-1.11	-3.31	-2.22	-7.67	-0.32	-3.82
<i>E_{EQi} median</i>	0.74	-	0.66	0.56	0.52	0.09	-0.04	-0.41	0.63	0.35
<i>E_{EQi} max</i>	0.90	-	0.89	0.88	0.93	0.84	0.89	0.59	0.95	0.96
<i>E_V</i>	0.76	-	0.84	-0.79 ^b	0.75	0.70	0.99	0.96	0.81	0.63

^a – Only Melbourne weather radar data was used to derive rainfall intensities, hence no results are available for Raleigh catchment.

^b – The volume prediction performance was caused by one poorly predicted event. When this event was removed, the recalculated performance was 0.38.

MOPUS microorganism model results for Raleigh, Hawthorn Main Drain West and Gardiners Creek test catchments

Creek test catchments

Detailed results of MOPUS microorganism model, including two example wet weather events, predicted versus measured instantaneous *E. coli* concentrations, predicted versus measured *E. coli* event mean concentrations, predicted versus measured *E. coli* event peaks and predicted versus measured *E. coli* event loads are presented for Raleigh, Hawthorn main drain west (HMD west) and Gardiners Creek in Figure S2, Figure S3 and Figure S5, respectively. It should be noted that Prahran main drain test catchment had only measured event mean concentrations and, as such, only predicted versus measured *E. coli* event mean concentrations and predicted versus measured *E. coli* event loads are presented for this catchment in Figure S4.

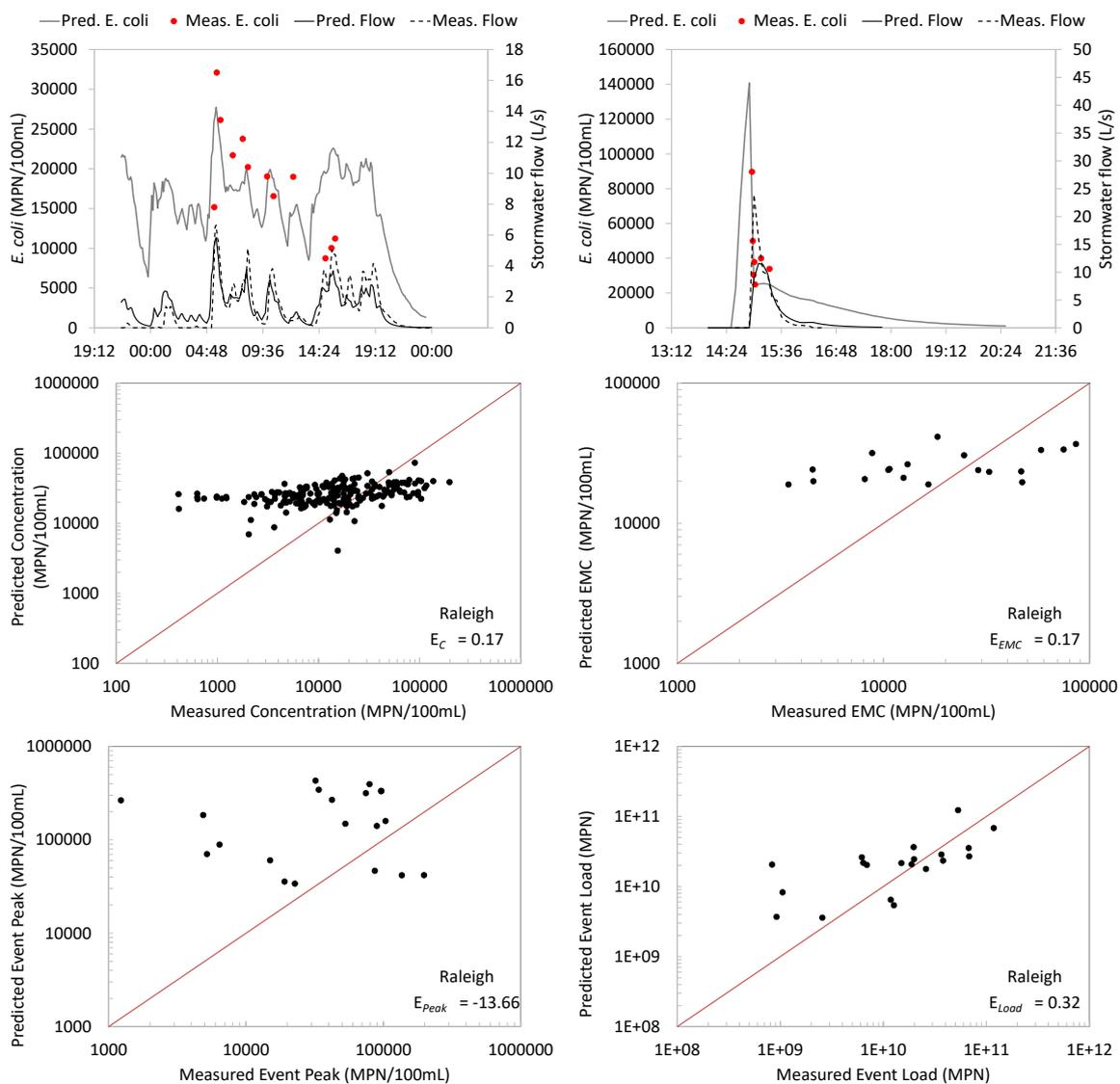


Figure A.2 - 2 Detailed results for Raleigh catchment when using gauged rainfall inputs. Top - measured and predicted *E. coli* pollutographs and hydrographs for two events, Middle Left - Predicted versus measured instantaneous *E. coli* concentrations, Middle Right - predicted versus measured *E. coli* event mean concentrations (EMCs), Bottom Left - predicted versus measured event peaks, Bottom Right - predicted versus measured event loads.

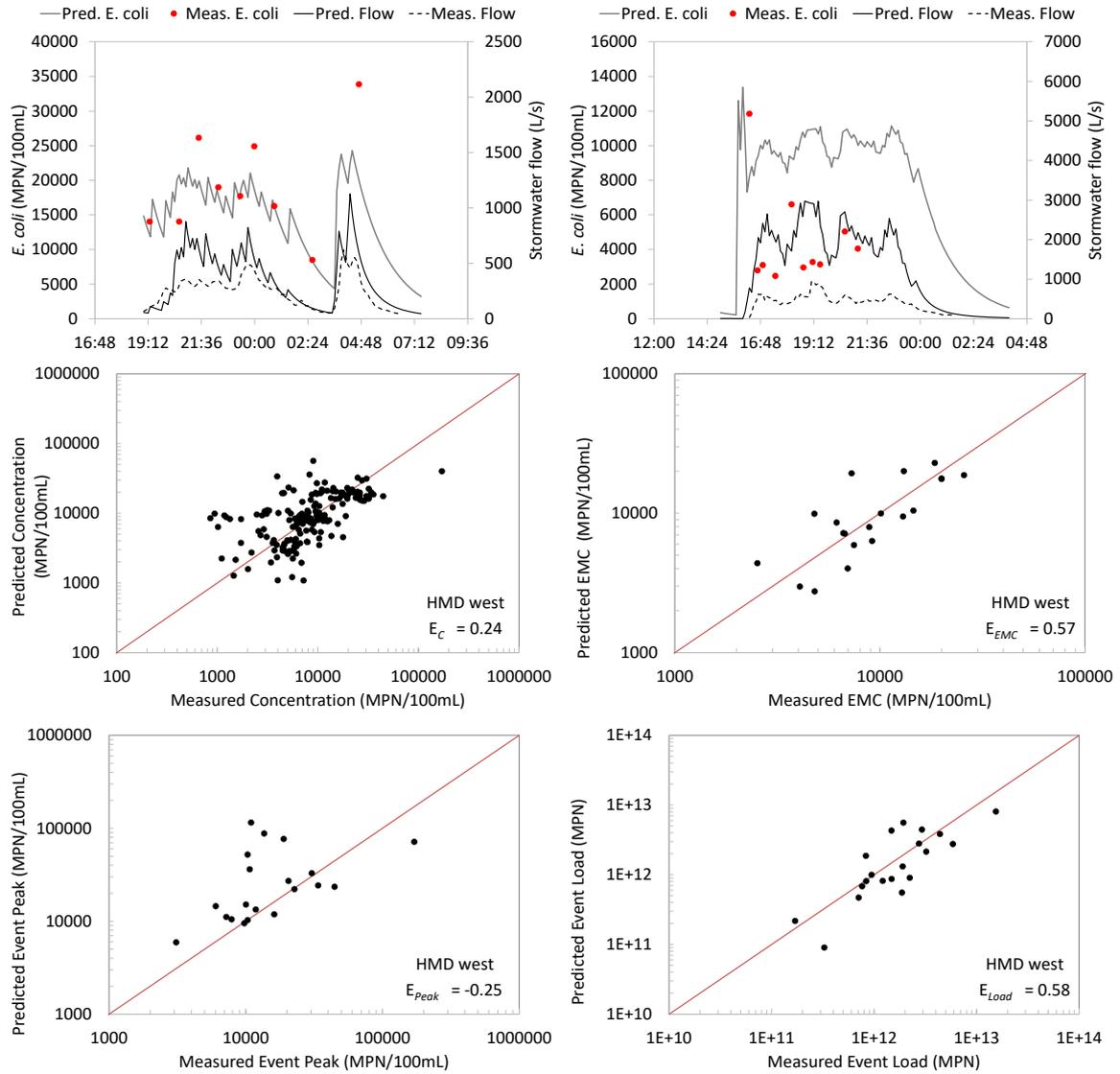


Figure A.2 - 3 Detailed results for Hawthorn Main Drain (HMD) west catchment when using gauged rainfall inputs. Top - measured and predicted *E. coli* pollutographs and hydrographs for two events, Middle Left - Predicted versus measured instantaneous *E. coli* concentrations, Middle Right - predicted versus measured *E. coli* event mean concentrations (EMCs), Bottom Left - predicted versus measured event peaks, Bottom Right - predicted versus measured event loads.

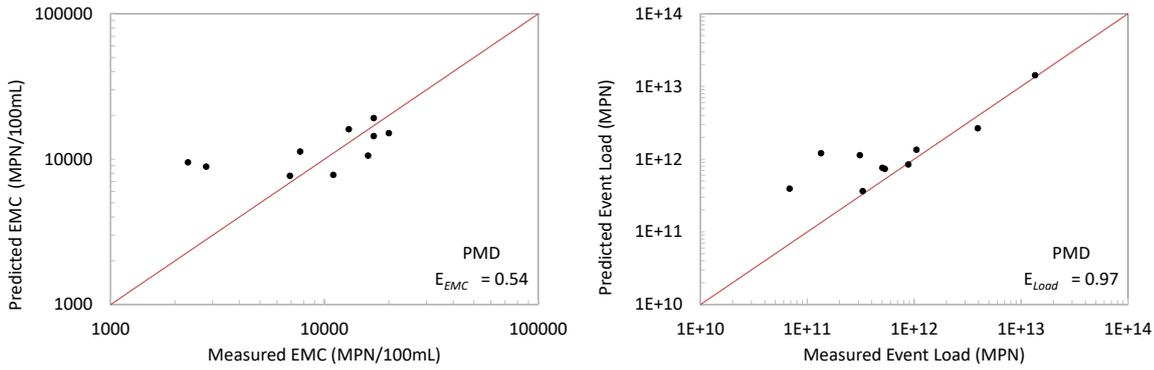


Figure A.2 - 4 Detailed results for Prahain Main Drain (PMD) catchment when using gauged rainfall inputs. Left – predicted versus measured *E.coli* event mean concentrations (EMCs) and Right – predicted versus measured event loads.

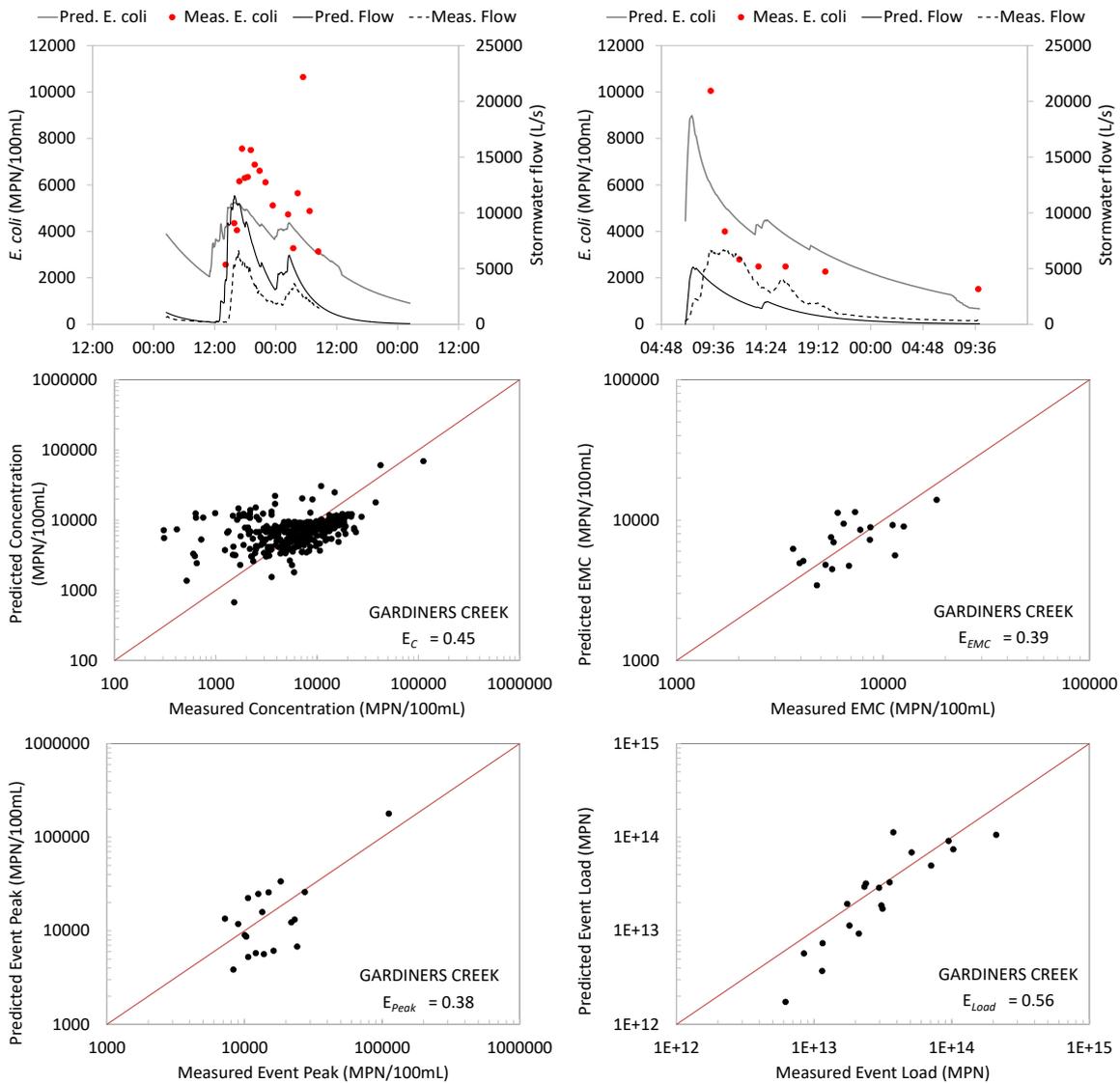


Figure A.2 - 5 Detailed results for Gardiners Creek catchment when using gauged rainfall inputs. Top - measured and predicted *E. coli* pollutographs and hydrographs for two events, Middle Left - Predicted versus measured instantaneous *E. coli* concentrations, Middle Right – predicted versus measured *E.coli* event mean concentrations (EMCs), Bottom Left - predicted versus measured event peaks, Bottom Right – predicted versus measured event loads.

A.3 Supplementary materials for ‘Modelling shallow and narrow urban salt-wedge estuaries: evaluation of model performance and sensitivity to optimise input data collection’ (Chapter 6)

Modelling shallow and narrow urban salt-wedge estuaries: evaluation of model performance and sensitivity to optimise input data collection

Dusan JOVANOVIC, Simone GELSINARI, Louise BRUCE, Mathew HIPSEY, Ian TEAKLE, Matthew BARNES, Rhys COLEMAN, Ana DELETIC and David T. McCARTHY

Supplementary material

Model performance

This section presents additional performance plots that were not included in the manuscript due to space constraints and to show assessment of model’s performance against the temperature dataset that was not presented in the manuscript. The aim of this section is to enable further insight into how well the model performed at simulating the hydrodynamics of the Yarra River estuary.

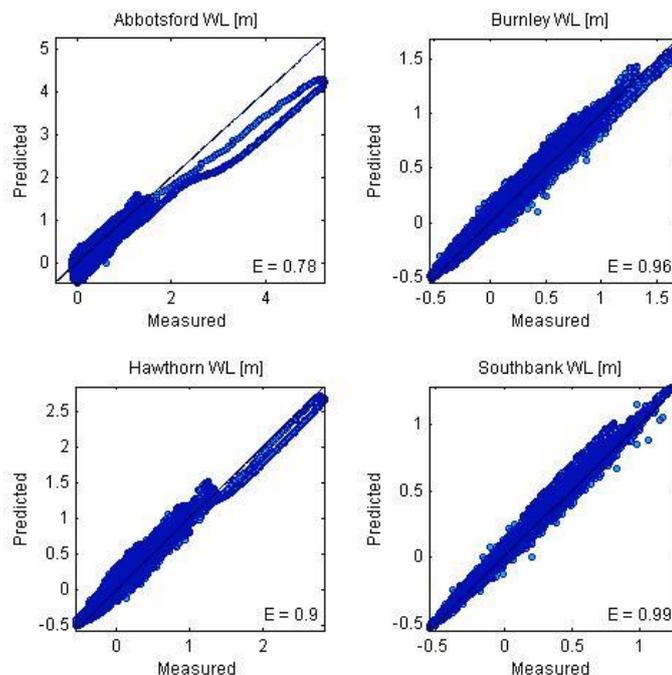


Figure A.3 - 1 Predicted versus measured water level at Abbotsford, Hawthorn, Burnley and Southbank (E - Nash-Sutcliffe efficiency).

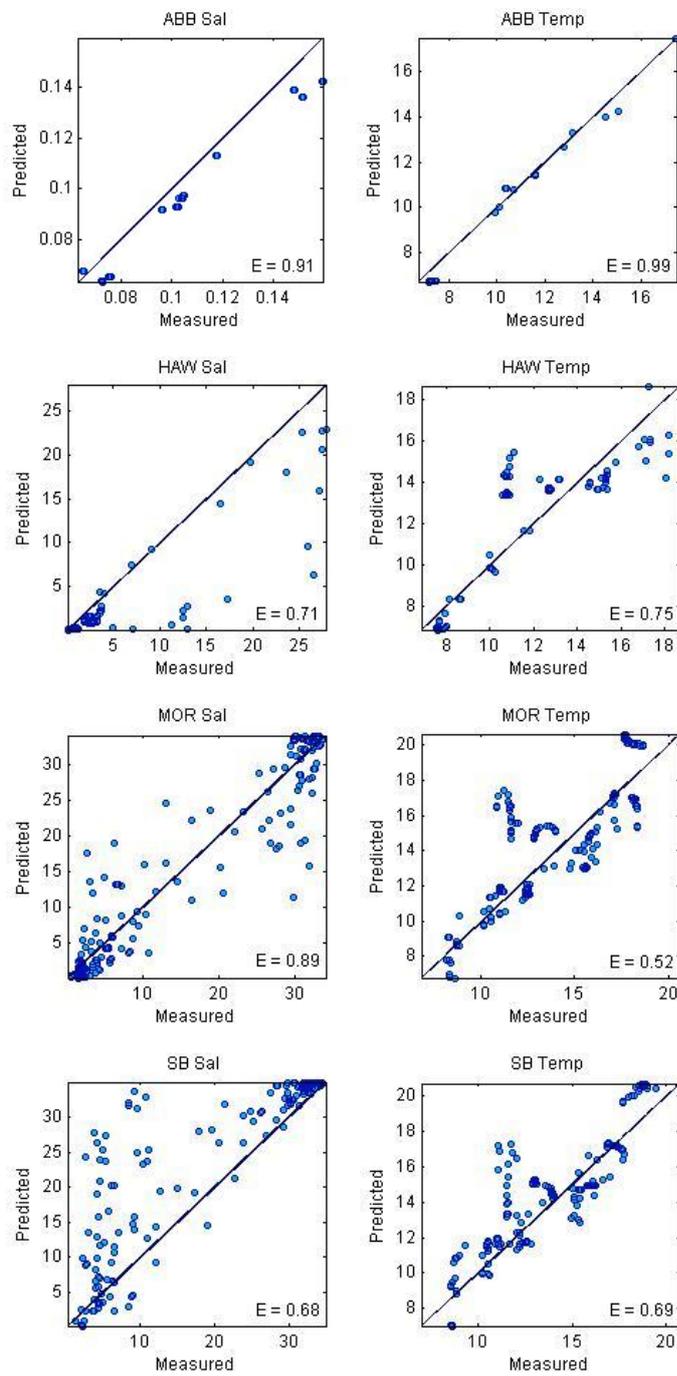


Figure A.3 - 2 Predicted versus measured salinity (left) and temperature (right) at four depth profiling sites: Abbotsford (ABB), Hawthorn (HAW), Morell Bridge (MOR) and Southbank (SB).

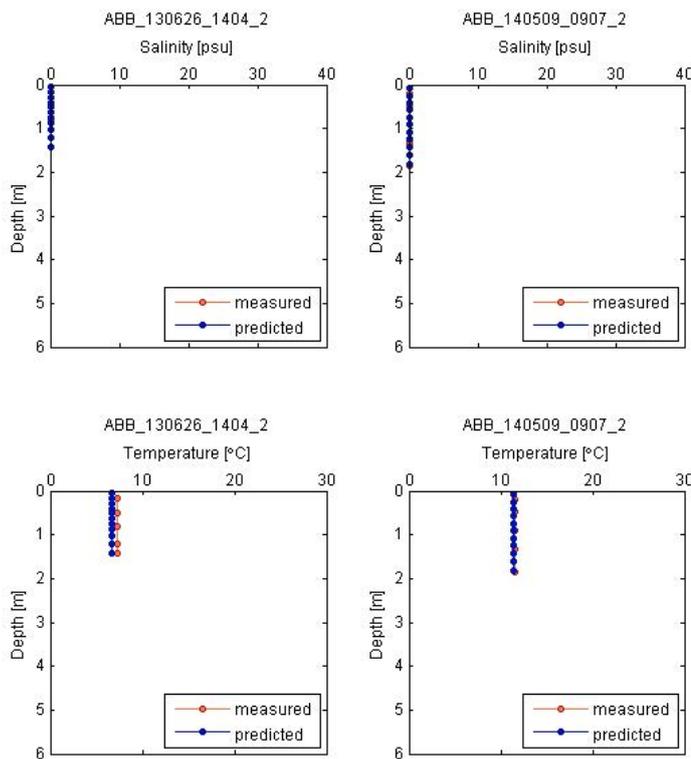


Figure A.3 - 3 Measured and predicted salinity (top) and temperature (bottom) depth profiles at Abbotsford (ABB) on 26th June 2013 at 14:04 pm (left); average Yarra River flow rate in 24h before the depth profiling $Q_{24h} = 4.9 \text{ m}^3/\text{s}$; average wind speed over 3 hours prior to depth profiling $S_{W,3h} = 0.8 \text{ m/s}$, 9th May 2014 at 09:07 am (right; $Q_{24h} = 14.9 \text{ m}^3/\text{s}$; $S_{W,3h} = 5.2 \text{ m/s}$). N.B. The measurements on 6th August 2014 were not conducted at this site.

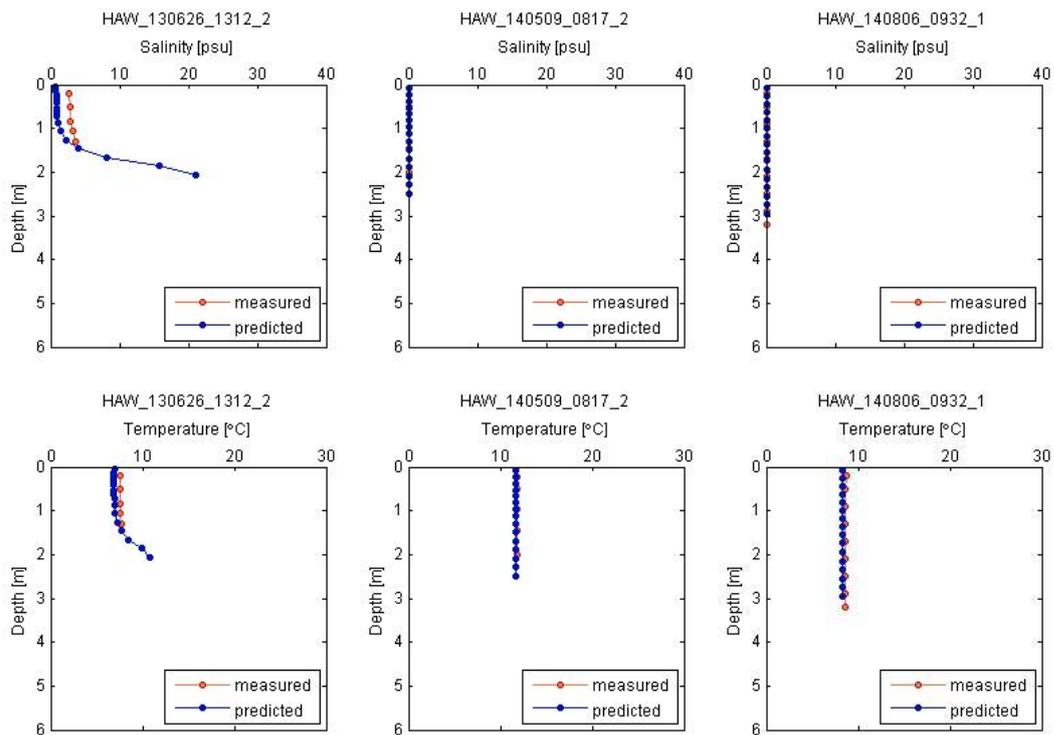


Figure A.3 - 4 Measured and predicted salinity (top) and temperature (bottom) depth profiles at Hawthorn (HAW) on 26th June 2013 at 13:12 pm (left; average Yarra River flow rate in 24h before the depth profiling $Q_{24h} = 4.9 \text{ m}^3/\text{s}$; average wind speed over 3 hours prior to depth profiling $S_{W_{3h}} = 0.8 \text{ m/s}$), 9th May 2014 at 08:17 am (middle; $Q_{24h} = 14.9 \text{ m}^3/\text{s}$; $S_{W_{3h}} = 5.2 \text{ m/s}$) and 6th August 2014 at 09:32 am (right; $Q_{24h} = 32.2 \text{ m}^3/\text{s}$; $S_{W_{3h}} = 3.4 \text{ m/s}$).

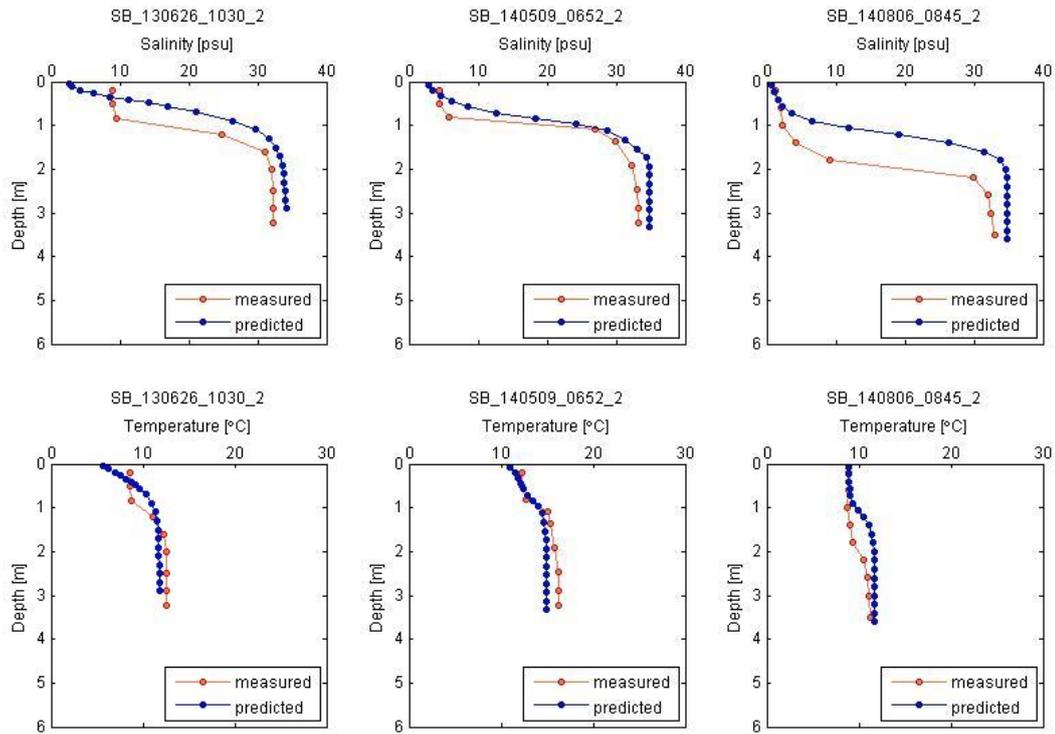


Figure A.3 - 5 Measured and predicted salinity (top) and temperature (bottom) depth profiles at Southbank (SB) on 26th June 2013 at 10:30 am (left; average Yarra River flow rate in 24h before the depth profiling $Q_{24h} = 4.9 \text{ m}^3/\text{s}$; average wind speed over 3 hours prior to depth profiling $S_{W_3h} = 0.8 \text{ m/s}$), 9th May 2014 at 06:52 am (middle; $Q_{24h} = 14.9 \text{ m}^3/\text{s}$; $S_{W_3h} = 5.2 \text{ m/s}$) and 6th August 2014 at 08:45 am (right; $Q_{24h} = 32.2 \text{ m}^3/\text{s}$; $S_{W_3h} = 3.4 \text{ m/s}$).

Assessment of temperature prediction

The assessment against the continuous temperature measurements at Abbotsford and Morell Bridge confirmed the model's ability to reproduce temperature dynamics within the Yarra River estuary. The temperature predictions were particularly good at Abbotsford (Figure A.3 - 6), with very high model performance (e.g. Nash-Sutcliffe efficiency and IOA both equal to 0.99; Table A.3 - 1). The reason for this is the proximity to the upstream model boundary (around 500m) where the measured data was used to characterise the temperature of incoming flow. The model performance at Morell Bridge was also good (Figure A.3 - 6 and Table A.3 - 1), however, slightly lower than that at Abbotsford. Moreover, the model performed better at predicting bottom than surface temperature which agrees well with the difficulties illustrated in the example of the depth profile data above.

This demonstrate that the model is capable of reproducing the temperature and salinity dynamics within the Yarra River estuary to a great extent, including the high stratification of the water column, for a variety of hydrologic conditions.

Table A.3 - 1 Model performance parameters for continuous temperature prediction at Abbotsford and Morell Bridge.

Variable	Location	B	Br	NMAE	RMSE	E	IOA	r
Temperature		[°C]	[%]	[-]	[°C]	[-]	[-]	[-]
	Abbotsford	-0.2	-1	0.03	0.48	0.99	0.99	0.99
	Morell Bridge Surface	0.8	5	0.11	2.43	0.79	0.94	0.90
	Morell Bridge Bottom	-0.6	-3	0.06	1.44	0.88	0.97	0.95

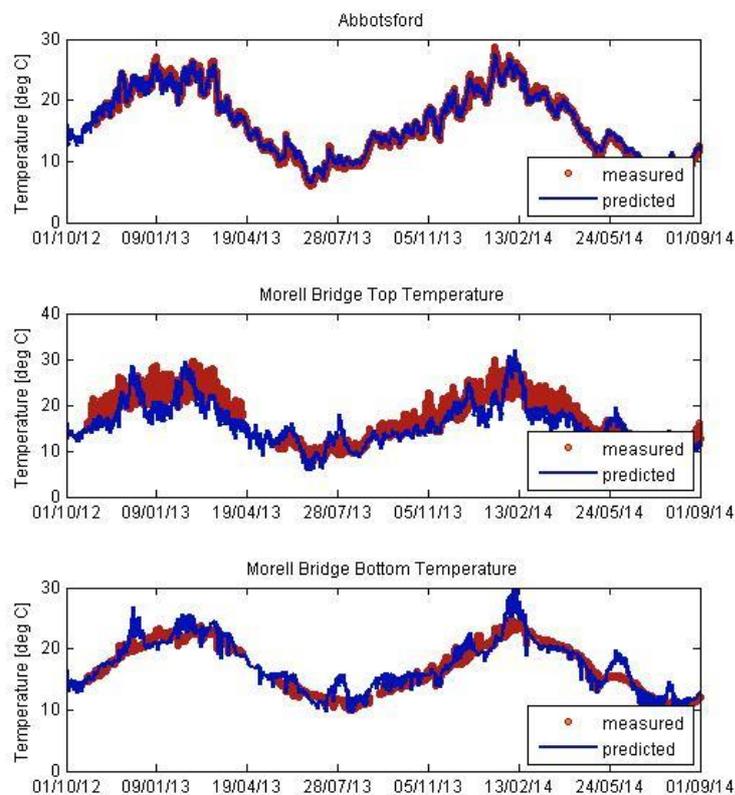


Figure A.3 - 6 Measured and predicted temperature at Abbotsford (top) and Morell Bridge Surface (middle) and Bottom (bottom).

Model sensitivity

This section presents additional sensitivity plots at sites that were not included in the paper. The following are presented here: 1) Water level sensitivity at Hawthorn and Southbank (Figure A.3 - 7); 2) Surface and bottom minor velocity component (V_y) sensitivity at Morell Bridge (Figure A.3 - 8); 3) Salinity sensitivity at Abbotsford (Figure A.3 - 9) and; 4) Temperature sensitivity at Abbotsford (Figure A.3 - 10).

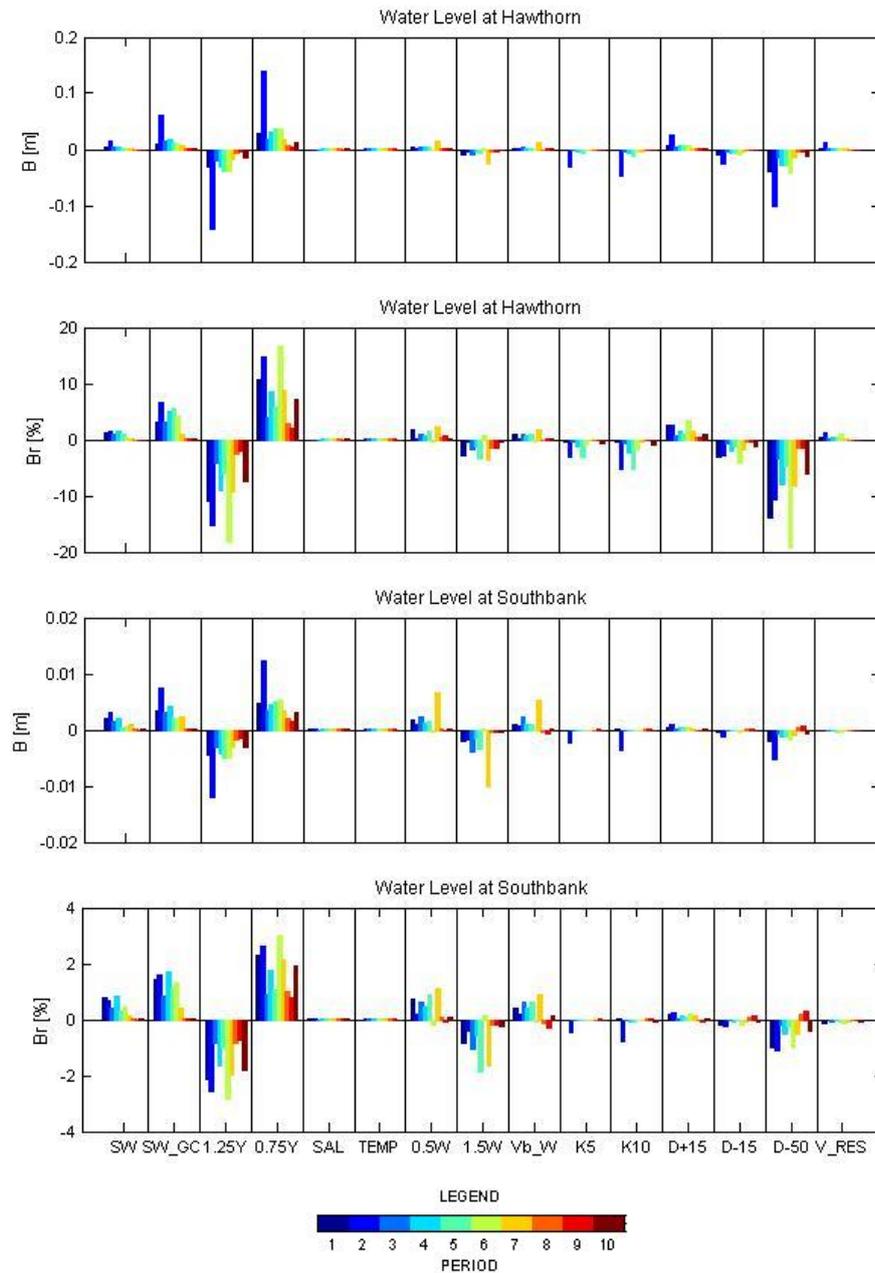


Figure A.3 - 7 Water level sensitivity at Hawthorn and Southbank (B – bias and Br – relative bias).

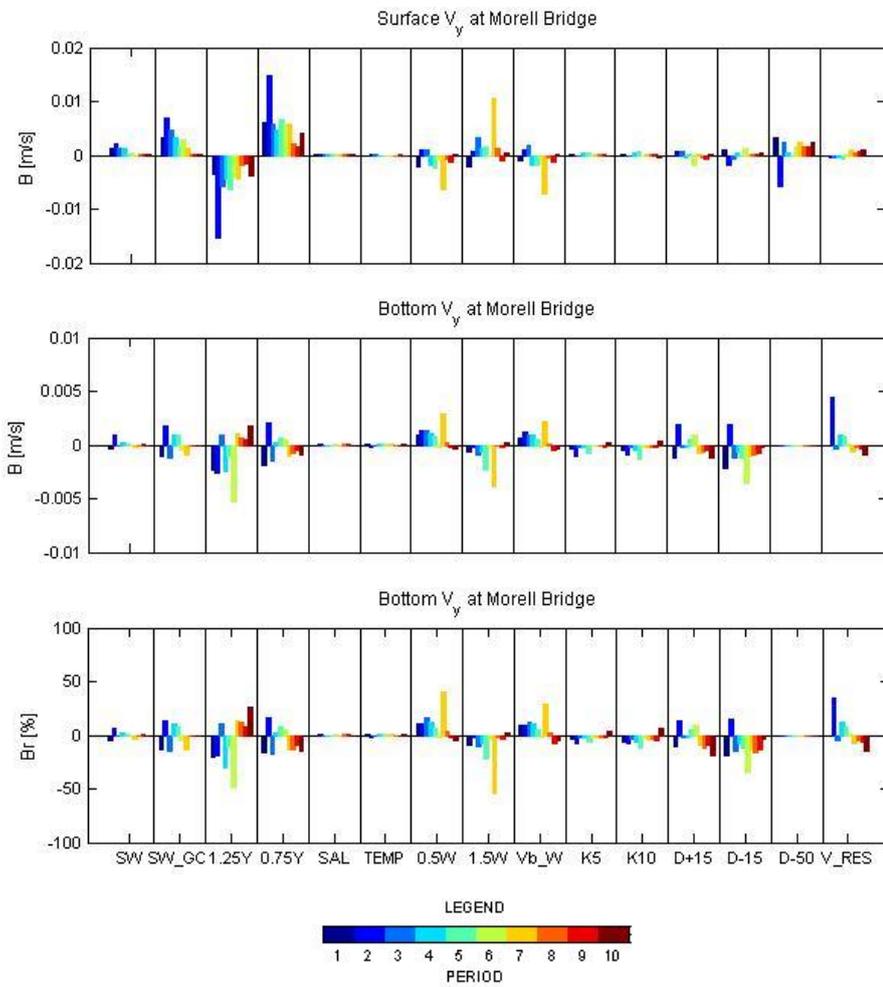


Figure A.3 - 8 Surface and bottom minor flow velocity component (V_y) sensitivity at Morell Bridge (B – bias and Br – relative bias).

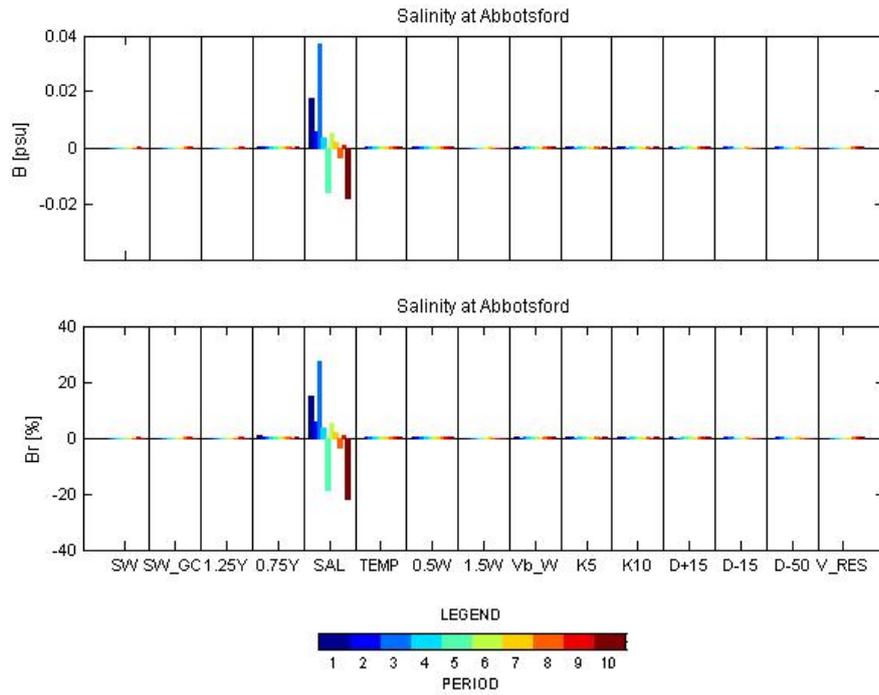


Figure A.3 - 9 Salinity sensitivity at Abbotsford (B – bias and Br – relative bias).

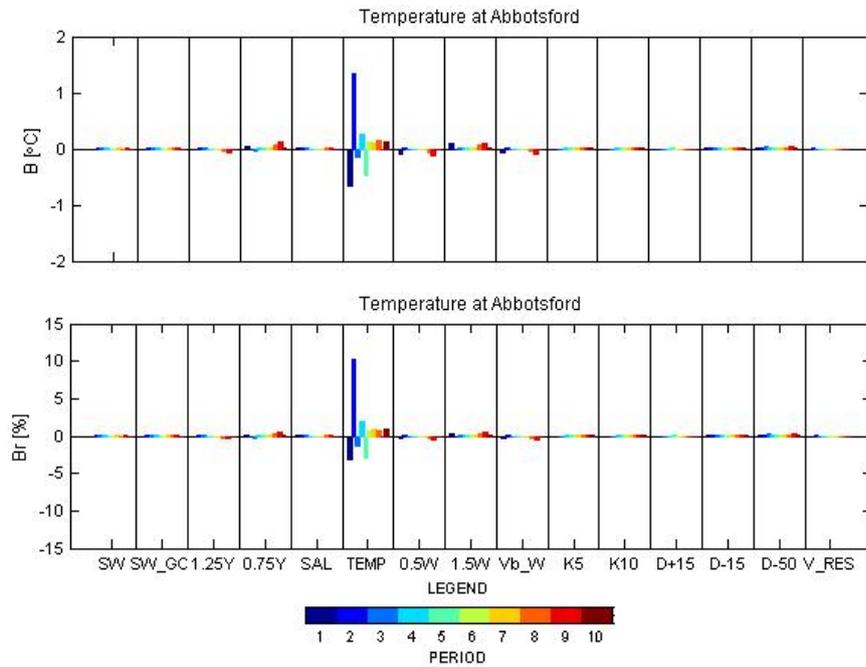


Figure A.3 - 10 Temperature sensitivity at Abbotsford (B – bias and Br – relative bias).

A.4 Supplementary materials for ‘Integrated modelling of fate and transport of *E. coli* within an urban salt-wedge estuary’ (Chapter 7)

Integrated modelling of fate and transport of *E. coli* within an urban salt-wedge estuary

Dusan JOVANOVIC, Mathew HIPSEY, Ian TEAKLE, Matthew BARNES, Rhys COLEMAN, Ana DELETIC and David T. McCARTHY

Supplementary material

Suspended sediments model

The suspended sediments model can account for a number of different fractions of sediments. Each fraction is transported by advection and diffusion and is subject to processes of settling and resuspension into/from bed sediments. The sediment transport equation is given below:

$$\frac{\partial}{\partial t} C_{SS,j} + \frac{\partial}{\partial x_i} (U_i C_{SS,j}) = \frac{\partial}{\partial x_i} \left(\kappa_i \frac{\partial C_{SS,j}}{\partial x_i} \right) - D_j + R_j \quad (\text{A.4 - 1})$$

where t is time [s], x_i is distance the i -th dimension [m], U_i is the velocity in i -th direction [m s^{-1}], κ_i is the eddy-diffusivity, $C_{SS,j}$ is suspended sediment concentration of j -th fraction [g m^{-3}], D_j is the suspended sediment deposition rate of j -th fraction [$\text{g m}^{-2}\text{s}^{-1}$] and R_j is the resuspension rate of bed sediments of j -th fraction [$\text{g m}^{-2}\text{s}^{-1}$].

The total suspended sediments concentration is a sum of concentrations of all modelled fractions:

$$C_{SS} = \sum_{j=1}^n C_{SS,j} \quad (\text{A.4 - 2})$$

where n is the number of modelled sediment fractions [-].

Deposition of suspended sediments was parameterised as a function of settling velocity and critical stress for deposition:

$$D_j = w_{S,j} \left[\frac{\tau_{d,j} - \tau}{\tau_{d,j}} \right] C_{SS,j}, \quad \tau \leq \tau_{d,j} \quad (\text{A.4 - 3})$$

$$D_j = 0, \quad \tau > \tau_{d,j} \quad (\text{A.4 - 4})$$

where w_S is settling velocity, $\tau_{d,j}$ is critical stress for deposition for j-th fraction [N m^{-2}] and τ is current shear stress provided by hydrodynamic module [N m^{-2}].

The settling velocity in the Equation A.4 - 3 is calculated according to Stoke's Law as:

$$w_{S,j}(T, S) = \frac{g d_{S,j}^2 [\rho_S - \rho_W(T, S)]}{18\mu(T)} \quad (\text{A.4 - 5})$$

where g is gravitational acceleration [m s^{-2}], $d_{S,j}$ is particle diameter of j-th fraction [m], ρ_S is sediment particle density [kg m^{-3}], ρ_W is density of water [kg m^{-3}] as function of temperature and salinity, and finally μ is the dynamic viscosity of water [$\text{kg m}^{-1} \text{s}^{-1}$] as a function of temperature.

The inclusion of critical stress for deposition in the Equation A.4 - 3 and Equation A.4 - 4 will account for the fact that deposition of sediments does not occur continually but only when hydrodynamic conditions are suitable for settling. Furthermore, since settling velocity is calculated for still water, the deposition below critical stress for deposition will be proportional to the difference between the critical stress for deposition and the current stress which reflects the movement of the water. Therefore, deposition with the calculated settling velocity will only occur if the current stress is equal to zero, i.e. in still water.

Similarly, the parameterisation of resuspension rate is based on commonly used formula where resuspension only occurs if the bottom stress is great enough to cause the resuspension of the sediment particles stored in the bed sediments:

$$R = E_j \left[\frac{\tau_b - \tau_{e,j}}{\tau_{e,j}} \right], \quad \tau_b > \tau_{e,j} \quad (\text{A.4 - 6})$$

$$R = 0, \quad \tau_b \leq \tau_{e,j} \quad (\text{A.4 - 7})$$

where E_j is erosion rate for a particular fraction [$\text{g/m}^2\text{s}$], τ_b is bottom stress supplied by hydrodynamic module [N m^{-2}] and $\tau_{e,j}$ is critical stress for resuspension of particular sediment fraction [N m^{-2}].

It is important to note that the bed sediment store is infinite, thus resuspension will be occurring as long as the bottom shear stress is higher than critical shear stress for sediment resuspension.

Appendix B
Conference papers

B.1 Modelling Impact of Stormwater on Faecal Contamination of Urban Estuaries

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Modelling Impact of Stormwater on Faecal Contamination of Urban Estuaries

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ABSTRACT

Stormwater is regarded as a key source of faecal contamination of receiving water bodies. To assess its importance on a case study of an urban estuary, the Yarra river estuary, Australia, existing models for prediction of microorganism concentrations in urban stormwater and river inputs were applied. Using the models' results, it was shown that impact of stormwater on overall levels of *Escherichia coli* in the estuary might be limited even during wet weather periods. A simple estuarine microorganism model was then developed to account for the transport and survival of *E. coli*, which was modelled as function of temperature, salinity and solar radiation. The model was used to set the baseline performance achievable using very simple conceptual approach, as well as to assess the importance that inputs have on microbial dynamics within the estuary. Simple sensitivity testing of the estuarine model confirmed limited impact of stormwater on overall levels of *E. coli* within the Yarra river estuary.

KEYWORDS

Stormwater, estuary, *E. coli*, inputs, model

INTRODUCTION

Urban estuaries around the world are highly valued assets to the local community; they provide aesthetics, improved microclimate and recreational opportunities. Like many other urban estuaries, the Yarra River estuary has elevated levels of faecal contamination (Daly et al., 2013), which poses increased public health risks for recreational users. Faecal microorganisms have been identified as leading cause of the pollution of environmental waters (Ortega et al., 2009, Lipp et al., 2001, Burton and Pitt, 2002) and stormwater has been recognized as the most important source of faecal contamination (Burton et al., 2002; McCarthy et al., 2011).

Increased efforts have been made towards mitigating impact of stormwater inputs in case of the Yarra River estuary, yet without much improvement of water quality. Therefore, understanding importance of inputs on estuarine microorganism dynamics is essential in forming a successful mitigation strategy. This is most easily achievable by using a holistic modelling tool that would fully account for both input and estuarine microorganism dynamics. However, regardless of the complexity of the microorganism models found in the literature, the microbial dynamics of inputs was not fully appreciated. Microorganism loads

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were estimated based on a small number of measured data points (Gao et al., 2011, de Brauwere et al., 2011, Kashefipour et al., 2002) and load rate was constant over time or predicted using simple correlations with flow (Liu and Huang, 2012, Garcia-Armisen et al., 2006) or sediment concentration (Ghimire and Deng, 2013). Use of these approaches might hinder the importance of particular input, which in turn can significantly influence the results of estuarine microorganism model and misinform the mitigations strategy.

The aim of this study was to assess the impact of stormwater on faecal contamination of the Yarra River estuary by developing a simple estuarine microorganism model which was integrated with existing models for prediction of microorganisms dynamics in stormwater and river inputs into the estuary.

METHODS

The estuary and monitoring sites. The Yarra River estuary is a highly stratified (salt-wedge) estuary (Beckett et al., 1982) and extends for about 22 km from Port Phillip Bay to Dights Falls, an artificially made weir which represents upper boundary of the estuary. Four monitoring sites have been carefully selected and established for data collection (Figure 1). Two of the sites are within the estuary, Abbotsford at the very beginning of the estuarine section of the Yarra River (represents the region with little influence from the salt-wedge, but still impacted by tidal changes) and Morell Bridge, located in the lower part of the estuary (highly impacted by the salt-wedge). Both sites are equipped with refrigerated automated samplers, depth sensors and have continuous measurements of Electric Conductivity (EC) and Temperature (T) at 100mm below the surface. Morell Bridge site is also equipped with an Acoustic Doppler Current Profiler (ADCP) for 3D measurements of velocities at 1 min interval.

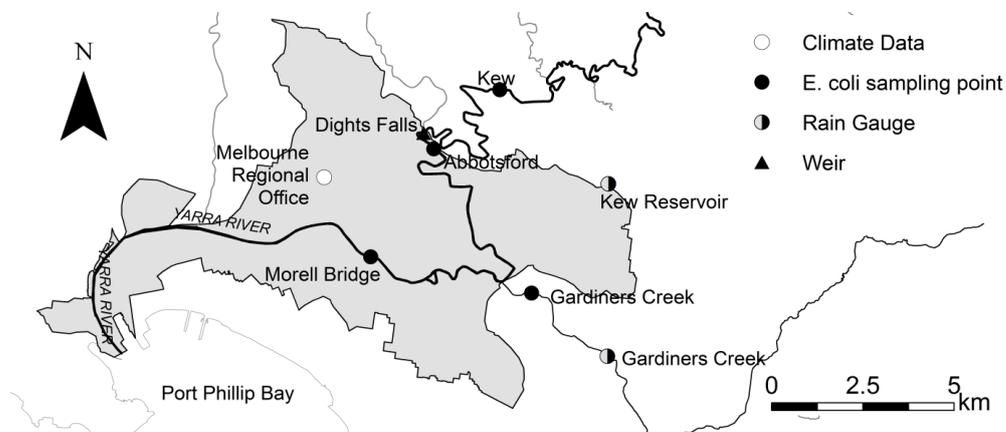


Figure 1. Monitoring stations in the Yarra River catchment (stations: Heidelberg and Coldstream (rain data) and Viewbank and Melbourne airport (climate data) are positioned outside of the figure scope).

Monitoring of upstream river inputs was conducted at Kew (Figure 1) where only grab samples were taken and water levels and flow rates were measured at 6 minute intervals by Melbourne Water (Victorian Water Agency). Monitoring of stormwater inputs was done at Gardiners creek, heavily channelized creek which is the largest source of water other than the Yarra River upstream of Dight's Falls, and is an open channel stormwater drain. The site has

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been equipped with automated sampler, EC/T sensors and depth/velocity probe. Climatic data was obtained from Australian Bureau of Meteorology and Melbourne Water for different locations in the Yarra River catchment (Figure 1).

Sample collection and analysis. Estuarine and riverine samples are taken approximately 100mm below the surface where the health risk to the recreational users is considered to be the highest. In the period of November 2012 to July 2013, 2106 samples have been collected; 1500 during dry weather and 606 during wet weather conditions. All collected samples were transported to the laboratory in coolers on ice and analyzed for *E.coli* content using Colilert method (IDEXX Laboratories, 2013) within 24h of collection. A large range of other indicators and reference pathogens were tested, but not reported here.

Input models. *River inputs.* Hydrology of the upper Yarra River catchment (river inflow at Dights Falls into the estuary, Fig. 1) was modelled using MUSIC – SimHyd which is a spatially lumped catchment rain-runoff model (eWater, 2012). The model has been applied with some slight variations: (1) a linear-reservoir routing routine was employed (instead of MUSIC’s standard Muskingum Cunge method) as it has been demonstrated previously that this simpler (and more stable) form of routing produces equivalent results (McCarthy, 2008); (2) the model was employed using a constant 6-minute timestep (as opposed to MUSIC’s standard method of daily simulation and subsequent disaggregation). This method improved the computational efficiency of the model, without compromising the results. Model inputs were areal averaged rainfall (stations: Heidelberg, Kew, Kew_r, Coldstream and Viewbank) and the daily potential evapotranspiration. For prediction of riverine microbial concentration, a modified version of the EG pathogen-hydrologic catchment model (Haydon and Deletic, 2006) was applied; the main variation was that the loss of microorganism from the subsurface store was estimated as inversely proportional to soil moisture, instead as directly proportional used in the original model.

Stormwater inputs. Modelling of urban stormwater inputs was done using Micro-Organism Prediction in Urban Stormwater, MOPUS (McCarthy et al., 2011), where the pervious component of the rain-runoff model was excluded (i.e. there was no impervious store threshold for surface runoff).

Generation of the stormwater inputs for the estuary. MOPUS generated timeseries of stormwater flow rates and microorganism concentrations only for the Gardiners Creek urban catchment, which is one of the biggest “stormwater drains” feeding into the estuary. However, there are additional 218 stormwater drains of various sizes that directly drain into the estuary. In order to produce time series of flow rates and microbial concentrations for each stormwater drain following procedure was conducted: (1) the impervious area (*IA*) for each of the drains was estimated using empirical relationship between impervious area and drain cross-sectional area (McCarthy, 2008); (2) other model parameters were obtained by random sampling within the parameter range defined by the optimized parameters values from the Gardiners Creek catchment and four other catchments located in Melbourne used for development and testing of the MOPUS model (McCarthy et al., 2011).

Input Analysis. Predicted stormwater flow rates and microorganism concentrations were used to calculate daily delivered volumes and loads to the estuary. Similar was done with river input, but instead of using predicted flow rates (which were substantially underestimated during base flow periods by the MUSIC model) measured data from Kew was used in order to get more realistic results. To assess contribution of stormwater, both in

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terms of daily delivered volumes and loads in dry and wet weather, a simple ratio of stormwater over total inputs (sum of stormwater and river inputs) was calculated. Similarly, ratio of daily delivered stormwater volume to estuary volume (estimated using GIS and bathymetry data $4 \times 10^6 \text{ m}^3$) was used to assess sole impact of stormwater inputs.

Proposed estuary microorganism model. The whole estuary was represented as a big reservoir where all flows and microbial loads were linearly routed and translated through the system (Table 1 for equations). The rationale behind this approach is twofold. Firstly, as mentioned previously the Yarra River estuary is a salt-wedge estuary, which was confirmed by measurements conducted by authors (data not shown). Essentially, this means that fresh water layer flows over the moving sea water layer (i.e. salt-wedge), with minimal mixing between the two layers. Furthermore, minutely velocity measurements obtained at Morell Bridge monitoring site using Acoustic Doppler Current Profiler (ADCP) over the Oct '12. – Aug '13 period showed that on average velocity in downstream direction was 0.16 m/s while upstream velocity was 0.06 m/s with only 18% of the time velocity being negative, i.e. forming upstream flow. Therefore, estuary can be effectively regarded as a river with moveable bottom boundary. Secondly, this model is very simple and would form a baseline level of performance achievable with minimal data input and minimal model complexity. Benefit of further increasing complexity of the model could then be assessed against performance achievable with the simple microorganism model.

In addition to routing and translating microbes, the model accounts for the impact of environmental factors on survival of the microorganisms in water column using first-order kinetics. Survival rate was modelled dynamically as function of temperature, salinity (% sea water) and solar radiation using the expression proposed by Mancini (1978). A simple term has been added when calculating microorganism concentration to account for mixing between fresh and sea water, where sea water was assumed to be free of *E. coli*.

Table 1. Estuarine microorganism model (calibration parameters are in bold)

Flow	
$S(t) = S(t-1) + [Q_r(t) + Q_{sw}(t) - Q_e(t-1)] \times \Delta t$	
$Q_e(t) = S(t - \mathbf{TOC}) / \Delta t \times \mathbf{RC}$	
Microbial Load	
$M(t) = [M(t-1) + (N_r(t) + N_{sw}(t)) \times \Delta t] \times 10^{-k\Delta t} - N_e(t-1) \times \Delta t$	
$N_e(t) = M(t - \mathbf{TOC}) / \Delta t \times \mathbf{RC}$	
Dynamic survival rate	
$k = (\mathbf{k}_{20} + 0.006 \times s) \times 1.07^{(T-20)} + I_A / \mathbf{k}_e \mathbf{H} \times [1 - e^{-\mathbf{k}_e \mathbf{H}}]$	
$s = \mathbf{EC} / \mathbf{EC}_{sea} \times 100$	
Microorganism concentration	
$C(t) = (1 - s/100) \times N_e / Q_e$	
<p>S [m³] – inflow volume stored within estuary; Q_r [m³/min] – river inflow; Q_{sw} [m³/min] – stormwater inflows; Q_e [m³/min] – discharge exiting the estuary; M [MPN] – microorganisms stored within estuary; N_r [MPN/min] – river load rate; N_{sw} [MPN/min] – stormwater load rate; N_e [MPN/min] – load rate exiting the estuary; \mathbf{RC} [-] – routing coefficient; \mathbf{TOC} [min] – time of concentration; Δt [min] – time step; k [1/day] – microorganism survival rate; k_{20} [1/day] – survival rate at 20°C; s [%] – percentage sea water; T [°C] – measured water temperature; I_A [MJ/m²] – average daily solar radiation; k_e [1/m] – average light attenuation coefficient over depth; H [m] – depth of the water column; \mathbf{EC} [mS/cm] – measured electric conductivity at Morell Bridge; \mathbf{EC}_{sea} [mS/cm] – electric conductivity of sea water; C [MPN/100ml] – microorganism concentration exiting estuary; ϕ – unit conversion factor</p>	

Calibration of the models. To explore the parameter sensitivity and calibrate the models simultaneously, a Monte-Carlo approach was utilized where the Nash-Sutcliffe coefficient of efficiency E (Nash and Sutcliffe, 1970) was the objective function. For prediction of flow

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rates E_Q was calculated with untransformed measured and predicted flow rates at 6 minutely timesteps, while the optimised parameter set for microorganism models was obtained by observing the Pareto front formed by efficiencies calculated using untransformed (E_C) and log-transformed *E. coli* concentrations (E_{Clog}). Additional calibration of the model parameters was conducted using Generalized Reduced Gradient method. Model parameters were not limited and criteria was the sum of E_C and E_{Clog} .

Music-SimHyd was calibrated against measured flow rates at Kew, while urban stormwater rain-runoff model was calibrated against flow rates at Gardiners Ck. The river microorganism model was calibrated against Abbotsford's *E. coli* concentration dataset. Although there are obvious issues with this methodology (i.e. calibrating the upstream model to a site within the estuary), it was considered adequate for the following reasons: (1) Daly et al. (2013) showed that Kew and Abbotsford have similar distributions, (2) the correlation between the *E. coli* from the two sites was 0.83 (Pearson correlation coefficient, $p < 0.001$), and (3) the Abbotsford dataset had many more calibration points (776 compared to 43 at Kew) which could allow for a better calibrated model. MOPUS was calibrated on the Gardeners creek catchment using the microbial data set with 383 calibration data points. The estuarine microorganism model was calibrated against Morell Bridge's *E. coli* dataset (829 points).

RESULTS AND DISCUSSION

Input modelling. The MUSIC-SimHyd model reproduced the observed flow pattern reasonably well ($E_Q = 0.51$); during base flow periods there was substantial underestimation of flow rates (probably a result of the fact that the model has been modified for urbanised catchments). There were certain timing issues with the prediction of the peak flows (very likely related to the routing method). Stormwater rain-runoff model had quite high performance in prediction of flow rates for Gardeners Cr, with efficiency $E_Q = 0.81$. It performed particularly well in the region of very high flow rates ($>10\text{m}^3/\text{s}$), which was expected as model was essentially developed for prediction of wet weather flows.

The efficiency of the two microorganism models was similar; $E_C \approx 0.20$ and $E_{Clog} \approx 0.40$ (Figure 2). Although these are not high efficiencies, they agree well with the performance reported in the literature for similar microorganism models (McCarthy et al., 2011). The pathogen-catchment model is reproducing *E. coli* patterns well, although there are certain peak prediction time issues similarly to Haydon and Deletic (2006) (Figure 2). The MOPUS concentration predictions are better in region of high concentrations which are commonly observed during wet weather periods. Indeed, the current model structure was developed for modelling wet weather microbial dynamics in stormwater, hence it is expected to give better prediction during wet weather.

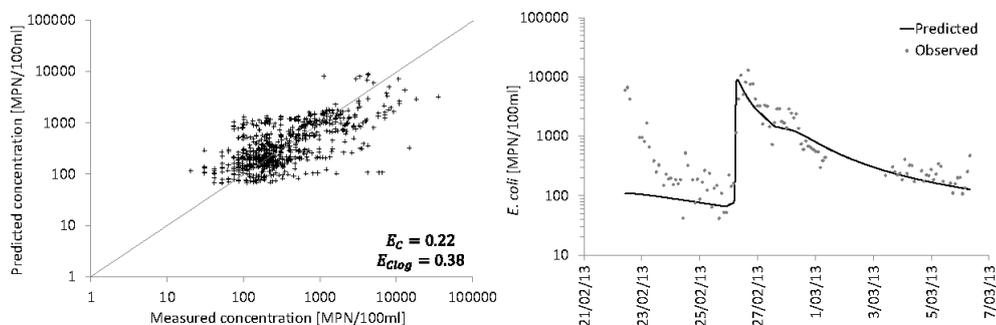


Figure 2. Results of EGpathogen-hydrologic catchment model: left - predicted vs measured concentrations; right – predicted vs measured pollutograph during a wet weather

Inputs analysis. Dry weather stormwater contribution to the estuary is very small, both in terms of delivered volume and *E. coli* loads, contributing only around 2% of total input volume and less than 1% of the total input load (Fig. 3). Wet weather stormwater flows contribute on average 20% of the total daily input volumes and 10% of the total microbial load.

Overall, the contribution from stormwater is surprisingly low and suggests that effect of stormwater on microbial dynamics within the estuary might be limited, even during wet weather periods. Indeed, this questions the importance given to the stormwater impact on the faecal pollution levels even in highly urbanised estuaries such as the Yarra River estuary. Furthermore, this finding agrees well with the findings of the fieldstudy on the Yarra River where it was estimated using measured data that median daily *E. coli* loads coming into the estuary from the three biggest drains (two of them 3m in diameter and one 6x2m) are about 1.5 orders of magnitude lower than the riverine inputs (Daly et al., 2013).

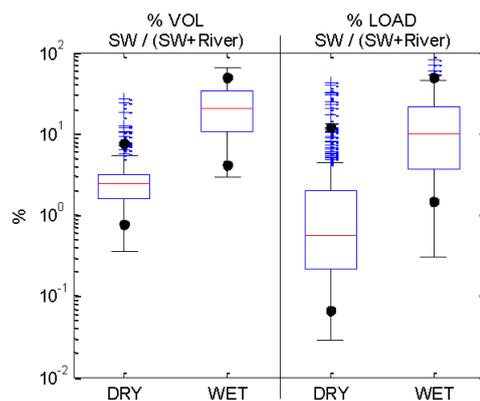


Figure 3. Modelled daily stormwater contributions during dry/wet weather conditions as percentage of total delivered water volume (%VOL) and *E. coli* load (%LOAD) to the estuary (black dots represent 5th and 95th percentiles)

Arguably, stormwater may enter the estuary much faster than riverine inputs. Furthermore rainfall can occur just within urbanized area that drains directly into the estuary, hence the impact of stormwater might be important in these cases. Even then the median daily volume of stormwater entering the estuary would be less than 5% of its total volume. Therefore it is very likely that this impact would be diminished by the estuarine buffering capacity. However, there might be localised effect around drain outlets, which is something that will be investigated in future when implementing a 3-D hydrodynamic estuarine model.

Finally from the public health risk perspective, it is not likely that the users of the estuary would conduct recreational activities during or immediately after the wet weather events but rather while after the urban wet weather event when microbial loads from upper catchment can be entering the estuary. This could be another reason to focus on mitigation of riverine rather than stormwater inputs.

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Estuarine modelling. Model's results with the example wet weather event are presented in Figure 4, while Table 2 outlines calibrated model parameters for different model set-ups. Considering simple approach used for modeling of hydrodynamics and microbial dynamics (i.e. neglecting completely characteristics of the estuarine hydrodynamics), as well as not high accuracy in prediction of input loads, the model performance is quite reasonable with E_C and E_{Clog} values of 0.37 and 0.41, respectively. Due to its simplicity the model's performance is very much linked to the performance of the input models, emphasizing the effect that inputs have on the estuarine microbial dynamics and importance of adequate representation of these inputs.

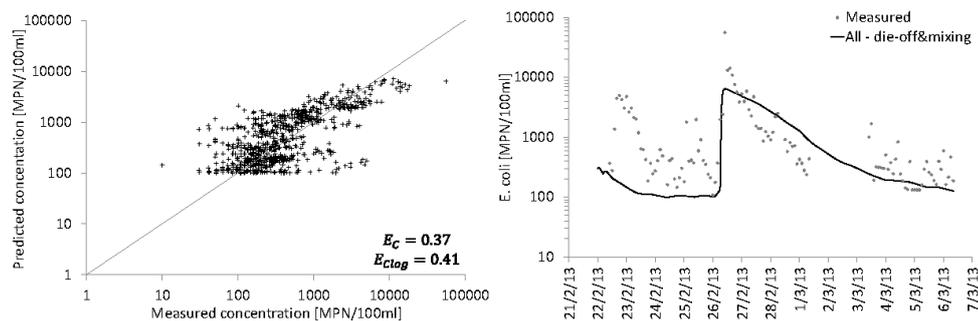


Figure 4. Performance of the estuarine model. Left – predicted versus measured concentrations; Right – predicted versus measured pollutograph during a wet weather event.

Table 2. Parameter ranges, distribution sampled, optimized parameter values and Nash-Sutcliffe efficiencies of the estuarine microorganism model

	Optimized calibration parameters					Model Efficiency	
	RC	TOC	k_{20}	k_eH	EC_{sea}	E_C	E_{Clog}
Range	0.001-1	0-3600	-1.5-1.5	1-1000	30-60		
Distribution sampled*	LogU	U	U	LogU	U		
No SW / no die-off & mixing	0.009	540	-	-	-	0.32	0.34
No die-off & mixing	0.008	720	-	-	-	0.34	0.41
Full model	0.008	284	-0.3	47.7	>60	0.37	0.41

*U – uniform distribution; LogU – log-uniform distribution

Initial conclusions can be drawn by relying on the small amount of sensitivity testing conducted here (i.e. turning on/off various inputs and processes) and by exploring the optimized parameter values (Table 2). Sensitivity analysis confirms conclusion made previously that stormwater inputs have small effect on levels of *E. coli* in the estuary (as indicated by E_C and E_{Clog} with and without stormwater input).

Furthermore, modelling of die-off also does not improve much model's performance. Moreover, optimised die-off calibration parameters values (Table 2) indicate that the best results are gained when there is no die-off or mixing (notice that EC_{sea} value is out of range because GRG calibration method was unlimited with the parameter range). In fact, negative k_{20} indicates that there is actually growth due to temperature fluctuations (instead of die-off). This could be related to the fact that model is very simple and it does not represent the estuarine environment appropriately. Additionally, it is known that resuspension of sediments

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can increase microbial levels in the water (Pachepsky and Shelton, 2011), and this process is not considered at all in the model, which could be contributing to artificial growth of *E. coli*.

CONCLUSIONS

The results of the input and estuarine microorganism modelling efforts showed that in order to explain variability of microbial dynamics within estuarine systems, accurate representation of both hydrological and microbial inputs is necessary (i.e. good input models). In the case of the Yarra River estuary, using both input model prediction and measured data, it was shown that that overall impact of stormwater on microbial levels in the estuary is limited even during wet weather periods. This was confirmed in simple sensitivity analysis of estuarine microorganism model. Results from the estuarine model encourage possibility of further exploring the use of simple microorganism models for modelling microbial dynamics in urban, narrow (river-like) estuaries. Future work will aim to confirm results showed herein using 3D hydrodynamic-microorganism model.

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B.2 3D Hydrodynamics and Vertical Mixing in a Stratified Estuary

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3D Hydrodynamics and Vertical Mixing in a Stratified Estuary

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Abstract: Estuaries are commonly classified by their flow characteristics and the extent of salt and fresh water mixing observed under normal conditions. Highly stratified, “salt-wedge” estuaries are characterised by a well-defined horizontal halocline, with a fresh surface water layer forming above the saline coastal water. Salt-wedge estuaries have large fluvial to tidal flow ratio and typically occur along microtidal coasts where the tidal range is less than 2 m. The mixing of fresh river water and saline coastal water in estuaries is primarily determined by turbulent mixing and to a much lesser extent molecular diffusion (e.g. Masselink and Hughes, 2003). Under low turbulent energy conditions the river and coastal water masses remain segregated. As turbulent mixing increases, such as during a flood event, the estuary may temporarily transition to a “partially” or “well-mixed” condition.

The hydrodynamics and vertical mixing in a stratified estuary has been explored using high-resolution datasets and numerical models. The hydrodynamics and vertical structure in the Yarra River estuary (Melbourne, Australia) was observed using a combination of ADCP (Acoustic Doppler Current Profiler) and EC/T (Electrical Conductivity and Temperature) instruments. The observed features of the estuary and position of the halocline were subsequently simulated using a 3D Non-Linear Shallow Water Equation (NLSWE) solver coupled with turbulent mixing and atmospheric exchange models. The key aspects of the numerical modelling approach required to accurately capture the vertical structure of the Yarra River estuary included:

- The inclusion of approximately 200 urban stormwater discharge inputs,
- A hybrid z-coordinate with surface sigma-layer model mesh vertical discretization, and
- Coupling of the 3D hydrodynamic model with a two-equation vertical turbulence scheme.

The coupling of the hydrodynamics with the vertical turbulence scheme was an essential component of the modelling system. Following this approach, the 1D (vertical) transport equations of momentum, salt and heat are calculated and used by NLSWE solver in the 3D circulation calculations. Efficient integration of the 3D NLSWE was achieved through a mode splitting scheme, whereby different components of the governing equations were updated using an appropriate timestep selected by taking into account physical and numerical convergence and stability considerations.

This model will ultimately form the basis for a 3D hydrodynamic-microorganism model through the coupling with the Aquatic EcoDynamics (AED²) modelling library. It is anticipated that this tool will be used by industry partners (Melbourne Water) to make scientifically-informed management decisions for improvement of water quality in the Yarra River estuary.

Keywords: *Salt wedge intrusion, water quality, Yarra River, TUFLOW FV*

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1. THE YARRA RIVER CATCHMENT AND ESTUARY

1.1. Study Site

The Yarra River is a major river in Victoria which flows westward from the southern side of the Great Dividing Range, passing through the heart of the city of Melbourne and discharging into Port Philip Bay at Hobson Bay. Total length of the Yarra River is 242 km and it drains the catchment of about 4000 km² (Sokolov and Black, 1996). The catchment can be divided in three distinct sections (Sinclair *et al.*, 1989):

- Lower part of the catchment (ca. 900 km²) is primarily urbanized and supports population of over 2 million people,
- About 1800 km² of the upper catchment is mainly forested and closed to protect the quality of water supply to metropolitan Melbourne, and
- The remainder (ca. 1300 km²) is devoted to agriculture.

The estuarine section of the Yarra River extends 22 km upstream from Hobson Bay to Dights Falls, an artificial weir which physically divides estuarine and riverine sections. This section can be further divided into two parts: an upper estuary section with depth from < 1m to 5 m, and a lower estuary section downstream from South Bank which has been heavily modified over the years and dredged to depths of 8-13 m to accommodate the needs of the Port of Melbourne (Ellaway *et al.*, 1982, Beckett *et al.*, 1982). The estuary section considered in the present study and key locations referred to throughout the paper are shown in Figure 1.

Approximately 70% of the average annual fluvial flow rate at the mouth of the Yarra River (~20 m³/s) is attributed to flows over Dights Falls (Sokolov and Black, 1996). The flow pattern of the Yarra River is very much seasonal, with lower flows recorded over summer and autumn months and higher flows over winter and spring (Beckett *et al.*, 1982). The other 30% of fresh water inputs to the estuary include: Gardiners Creek in the upper section of the estuary, about 7.6 km downstream of Dights Falls (Figure 1), the Maribyrnong River and Moonee Ponds Creek in the lower part of the estuary, about 3 and 5 km upstream of the mouth, and over 200 stormwater drains that discharge directly into the estuary.

1.2. Field Monitoring and Data Collection

Continuous high resolution water level monitoring is conducted by Melbourne Water at four monitoring stations within the estuary, namely: Abbotsford, Hawthorn, Burnley and South Bank. Velocity monitoring was conducted at the Morell Bridge monitoring station using two Acoustic Doppler Current Profiler (ADCP) devices. One device was placed in a shallower part of the cross section while the other was positioned at the deepest point of the cross section. The devices were measuring all three components of the velocity vector at minute intervals in fixed 1m cells (bins) vertically through the water column as well as in a surface dynamic cell (that adapted to the varying water level). The ADCPs were deployed from October 2012 to September 2014 and regularly serviced throughout this period.

Continuous monitoring of electrical conductivity (EC) and temperature (T) was conducted at Abbotsford and Morell Bridge monitoring stations. Abbotsford monitoring station is located at the upstream end of the estuarine section of the Yarra River, just below the Dights Falls, and it is largely free of the salt-wedge impacts while still being under tidal influences. This site was equipped with a single EC/T sensor located approximately 20cm below the water level which is assumed to be representative of the entire water column. Conversely, Morell Bridge monitoring site is under significant impacts of both tides and salt-wedge and this site was equipped with two EC/T sensors. One was located approximately 20cm below the water surface measuring the conductivity and temperature of the top freshwater layer, while the other was attached to the ADCP device and was measuring conductivity and temperature of the bottom layer, i.e. salt-wedge. Measurements were available at 6-minutely intervals. EC and T measurements were used to calculate salinity following Electrical Conductivity method (Eaton *et al.*, 2005).

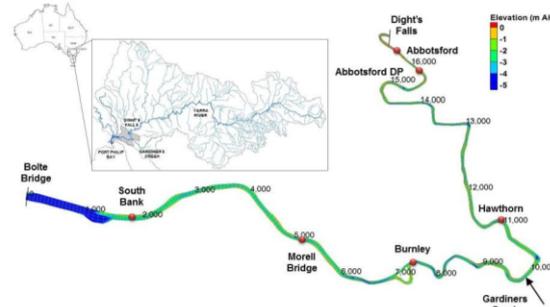


Figure 1. Yarra River catchment and upper estuary section model mesh with monitoring stations (chainage distance shown as metres from Bolte Bridge): water level – Abbotsford, Hawthorn, Burnley, South Bank; flow velocity – Morell Bridge; electrical conductivity and temperature (EC/T) – Abbotsford, Morell Bridge; salinity and temperature vertical profiles – Abbotsford DP, Hawthorn, Morell Bridge and South Bank.

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To obtain salinity and temperature distributions vertically through the water column, depth profiling monitoring campaigns were conducted. Four monitoring sites along the estuary that exhibit different extent of the salt-wedge intrusion were chosen: Abbotsford, Hawthorn, Morell Bridge and South Bank. At each site multi-parameter probe was used to measure salinity and temperature along the depth at 30 – 50 cm intervals depending on the position of the halocline/thermocline. In total 84 depth profiles of salinity and temperature were obtained over the 10 monitoring campaigns encompassing a variety of hydrological and climatic conditions. Only a selection of depth profiles obtained on 30 April 2013 is presented in this paper. The authors may be contacted for further information.

2. MODELLING THE YARRA ESTUARY PHYSICAL PROCESSES

2.1. Numerical Modelling System Overview

The hydrodynamic modelling presented in this study builds on the previous work by Bruce *et al.* (2014) using TUFLOW FV. Key aspects of the work presented here include:

- An increased horizontal and vertical resolution of the numerical model,
- Model verification using data obtained during a significant Yarra River flow event, and
- Model verification to measurements of the salinity and temperature vertical structure.

For the present study, the Yarra River estuary was resolved using an unstructured mesh comprised of predominantly quadrilateral elements. In the horizontal dimension, the model consists of 1,644 surface mesh cells with resolution varying from approximately 20-50 m. Four elements were typically used to define the Yarra estuary/river cross-section. In the vertical dimension, a hybrid z-coordinate grid configuration with eight surface “sigma” layers was adopted. The z-coordinate layers were applied at a 0.2 m resolution between an elevation of -1 m AHD and the estuary/river bed (with a variable bottom layer thickness). The eight sigma layers were applied between -1 m AHD and the water surface. The multiple surface sigma layers allows for a high resolution of the water surface boundary layer while tracking tidal water surface variations.

The model bathymetry was derived using three hydrographic surveys conducted in 2004, 2009 and 2012, supplied by Parks Victoria, Melbourne Water and Red Mapping. The first and the last survey were conducted using a vessel logging depth and position, while second survey measured 160 cross sections within the estuary. All data was used and interpolated to obtain cell elevations.

At the upstream model boundary, the freshwater flow rates were obtained from Melbourne Water gauging stations on the Yarra River at Fairfield and on Merri Creek at Northcote while electrical conductivity (used to obtain salinity) and temperature were measured locally at Dights Falls. Similar was done to describe Gardiners Creek input, where measured flow rate was obtained from Melbourne Water, while EC/T was continuously measured at the station 1 km upstream of the confluence with the Yarra River. The discharges from 208 storm water drains were also included and this boundary condition information was derived using MOPUS rainfall run off model (McCarthy *et al.*, 2011). Details on production of stormwater inputs can be found in Jovanovic *et al.* (2015). A tidally varying water level recorded at South Bank station was applied as downstream boundary condition. Salinity and temperature data for the Hobson bay was obtained from the Port of Melbourne Corporation. Although this data was recorded some distance downstream of the model boundary, depth profile measurement campaigns revealed that most of the cross section at South Bank was comprised of sea water (data not shown). Therefore, the measured data has been applied across the whole cross section.

The meteorological data was supplied by the Bureau of Meteorology and consisted of precipitation, air temperature, relative humidity and wind speed and direction. All data was acquired from the Melbourne Regional Office station, which is located within the estuary catchment except wind speed and direction measurements which were conducted at Essendon Airport, ~ 10 km away.

Salinity and temperature were simulated within the model as density-coupled scalar constituents in order to incorporate baroclinic density gradient forcing and the effect of vertical density stratification on the water column turbulent mixing. The General Ocean Turbulence Model (GOTM - Umlauf *et al.* 2003) was coupled with the hydrodynamic model through the external turbulence Application Programming Interface (API). Vertical mixing and the sensitivity to alternative models are discussed further in Section 2.3 and Section 2.4.

A bottom roughness length scale of 1mm for a single generic bed surface was represented throughout the model domain. This was assumed to be a suitable representation of the bed throughout the lower Yarra River which is nominally dominated by 70% silt and 30% fine to medium sands (Parks Victoria, 2007). Within TUFLOW FV horizontal and vertical reconstructions are performed separately. For the present study a first-order horizontal

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reconstruction (Leveque, 2002) was combined with a second-order vertical reconstruction (Fringer *et al.*, 2005). Most other model configurations and parameters adopted the “default” settings (refer BMT WBM, 2014).

2.2. Hydrodynamics

The 3D hydrodynamic model predictive skill was tested statistically with calculations of the Index of Agreement (IOA) and the Mean Absolute Error (MAE). The IOA was originally developed by Willmott (1981) and subsequently modified in Willmott *et al.* (1985):

$$IOA = 1 - \frac{\sum_{i=1}^N |O - P|^2}{\sum_{i=1}^N (|P - \bar{P}| + |O - \bar{O}|)^2} \quad \text{Equation 1}$$

Where *O* is the observed data and *P* is the model predictions over a given time period divided into *N* increments. The overbar denotes the time averaged mean of the given variable.

Following Willmott (1981) and Willmott *et al.* (1985), the IOA can vary from 0 to 1 with higher values indicating better model predictive skill. While there are no generic guidelines for the interpretation of the IOA, a value meaningfully larger 0.5 is generally considered to indicate satisfactory model performance (Willmott *et al.* 1985).

The MAE was adopted to quantify the model error in dimensional units and, as suggested by its name, provides a measure of model performance on an average sense. The MAE is computed as follows:

$$MAE = N^{-1} \sum_{i=1}^N |O - P| \quad \text{Equation 2}$$

The two-month hindcast period included a high flow event with a peak discharge close to 200 m³/s at Dights Falls on 01 June 2013 (as gauged by Melbourne Water). The model skill is particularly high with regard to water level (IOA > 0.95) and the downstream component of the current velocity (IOA > 0.80). The MAE up to 0.05 m for water level and 0.12 m/s for currents at the locations tested is due to a minor phase discrepancy between the observed and predicted variables rather than a significant difference in magnitude.

A time series comparison of the observed and predicted water level at Burnley and Hawthorn for a two-week subset of the model verification period is shown in Figure 2. The tidal anomaly recorded at Burnley (~1 m) and Hawthorn (~2 m) during the high flow event is accurately predicted by the hindcast simulation. The more typical periods of relatively low river base flow and tidally dominated water level variation are also represented accurately.

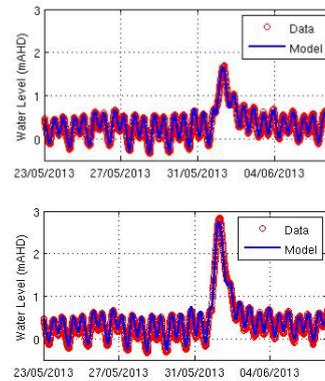


Figure 2. Comparison of Observed and Modelled Time Series of Water Level at Burnley (top) and Hawthorn (bottom).

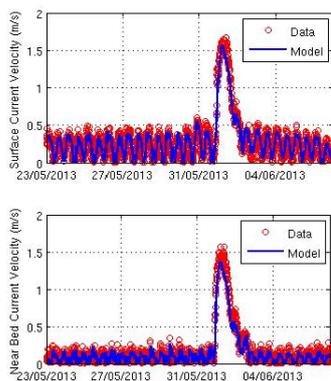


Figure 3. Comparison of Observed and Modelled Time Series of Current Velocity at Morell Bridge: Surface 1m (top) and Bottom 1m (bottom)

Figure 3 compares the observed and predicted surface and near current velocity at Morell Bridge for the shallow ADCP deployment. Similar to the water levels in the lower estuary, the current velocity is typically dominated by the semidiurnal tide and is predicted accurately by the 3D model. During these periods, the peak surface currents are up to 0.5 m/s and align in the downstream direction (approximately 280 degN at Morell Bridge). The current behavior differs significantly in the near bed layer where the peak velocities seldom exceed 0.2 m/s and align in the upstream direction (approximately 100 deg N at Morell Bridge). This represents the dense, salt-wedge intrusion driven by the tidal forcing at the river mouth.

During the high flow event the stratification at Morell Bridge temporarily breaks down and the surface and near bed currents align in the downstream direction.

Figure 3 shows good consistency between the model and observations in terms of timing and magnitude of currents during the high flow event.

2.3. Vertical Mixing

Validation of the instantaneous vertical structure predicted by the 3D model is shown in Figure 4 for salinity and temperature at Morell Bridge and Hawthorn. The observations and model results are in close agreement and show the fresh, slightly cooler surface layer and the underlying saline layer. The tapering of the salt-wedge thickness between Morell Bridge and further upstream at Hawthorn is evident in the data and hindcast predictions. The position of halocline was at a depth of 0.5-1.0 m at Morell Bridge and 1.5-2.0 m at Hawthorn.

The predicted instantaneous longitudinal salinity structure is presented in Figure 5 and shows the fresh surface layer extending to the downstream model boundary at the Bolte Bridge (chainage 0 m) and the tip of the salt-wedge located approximately 1 km downstream from Abbotsford. It is noted that the salt-wedge intrusion was not detected at Abbotsford during the monitoring campaign. The skill of the salinity prediction was also tested statistically using continuous the EC time series data and yielded the following results at Morell Bridge:

- Surface salinity IOA/MAE: 0.90 / 1.01 psu
- Near bed salinity IOA/MAE: 0.60 / 9.78 psu

It is noted that considerable scatter existed in the near bed EC dataset and that this instrument required regular servicing throughout the monitoring campaign. Furthermore, the values recorded were often inconsistent with the depth profiles obtained at this location (refer Figure 4). Consequently, there is some concern regarding the reliability of this dataset and the IOA and MAE values obtained at Morell Bridge.

2.4. Model Sensitivity

The development and sensitivity testing of the Yarra estuary model indicated that the adopted vertical discretisation and turbulent mixing model significantly influenced the predicted stratification.

Figure 6 compares the instantaneous current velocity, salinity and temperature profiles at Morell Bridge and Hawthorn for three alternative vertical discretisations (corresponding data at Morell Bridge is shown in Figure 4):

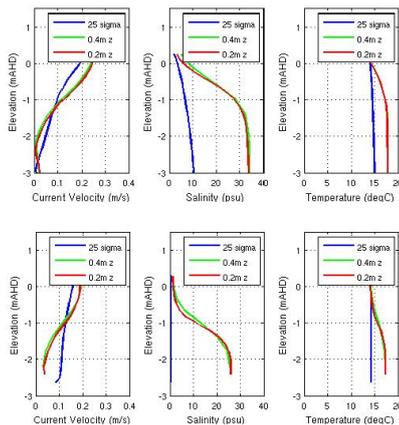


Figure 6. Model Sensitivity to Vertical Discretisation at Morell Bridge (top) and Hawthorn (bottom) 30/04/2013 08:15

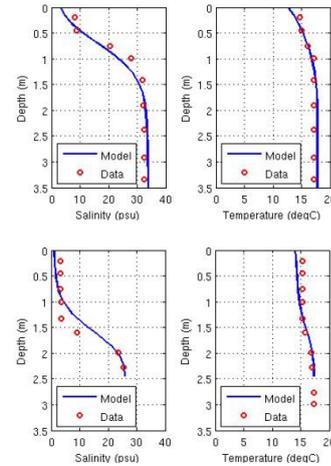


Figure 4. Comparison of Observed and Modelled Salinity and Temperature Profiles at Morell Bridge 30/04/2013 08:15 (top) and Hawthorn 30/04/2013 14:55 (bottom)

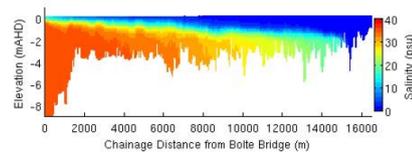


Figure 5. Modelled longitudinal salinity distribution 30/04/2013 08:15

- 25 sigma-layers from the estuary bed to water surface,
- hybrid z-coordinate grid with 0.4 m resolution layers between -1 m AHD and the estuary bed and eight sigma layers applied between -1 m AHD and the water surface, and
- hybrid z-coordinate grid with 0.2 m resolution layers between -1 m AHD and the estuary bed and eight sigma layers applied between -1 m AHD and the water surface (adopted configuration).

The hybrid z-coordinate approach is shown to be particularly suited to the highly stratified environment. The difference between the 0.4 m and 0.2 m resolution approaches is relatively minor, with the latter ultimately adopted due to the prediction of slightly steeper gradients and the associated small improvement to the predictive skill of the model when tested against the observed salinity and temperature profile datasets (including the data shown in Figure 4).

Figure 6 suggests that a sigma-layer only discretization over predicts the vertical mixing, leading to a breakdown in stratification at Hawthorn and therefore a grossly inaccurate prediction of salt-wedge intrusion. It is hypothesized that the

stretched terrain following coordinates generate additional spurious numerical mixing in comparison to the fixed z-layer approach. It is noted that model adjustments adopting a second order spatial scheme (horizontal) or reducing horizontal mixing would potentially limit the sigma-layer case numerical mixing, but these potential sensitivities were not explored further in the present study.

The model sensitivity to the vertical turbulent closure scheme was also tested, including:

- A parametric mixing model (BMT WBM, 2013) that assumes a parabolic eddy-viscosity/scalar-diffusivity distribution and includes a simple Munk and Anderson (1948) stability function,
- GOTM 2-equation k-omega model, e.g. Burchard and Baumert (1995) (with default parameters), and
- GOTM 2-equation k-epsilon model, e.g. Umlauf *et al.* (2003) (with default parameters).

Despite testing numerous configurations the simple parametric model was not able to represent the highly stratified circulation dynamics. The GOTM 2-equation models tested here were better suited to simulating the Yarra River estuary salt wedge environment and predicted virtually identical vertical structures. This suggests that either the k-omega or k-epsilon models are well suited to simulating vertical mixing in the lower Yarra River. It is noted that the results presented in Section 2.2 and Section 2.3 of this paper adopted the GOTM k-omega model.

3. DISCUSSION AND CONCLUSIONS

The value of a verified 3D hydrodynamic model for simulating the fate of water-borne constituents in the Yarra River estuary is demonstrated in Figure 7 which illustrates the predicted advection-dispersion of a plume, represented by a conservative tracer entered to the model between Morell Bridge and Burnley, during an ebb flow condition (the instantaneous salinity distribution for this time was previously shown in Figure 5). The following scenarios are considered:

- 2D-depth average hydrodynamics and plume release,
- 3D hydrodynamics with buoyant plume surface release (representative of a storm water drain/emergency relief structure (ERS) discharge), and
- 3D hydrodynamics with dense plume near-bed release (representative of a dredge-related sediment plume)

The 2D scenario assumes a depth average vertical structure. Regardless of the plume characteristics (e.g. buoyant or dense) the concentration is diluted evenly throughout the water column and is transported in the direction of the depth averaged estuarine flow. The consequence of this simplification is highlighted in the 3D scenarios shown in Figure 7. For the buoyant plume case, the 3D result clearly demonstrates that the high concentration surface accumulation is under predicted by the 2D model (by up to a factor 2). Potentially of greater significance is the difference between the 2D and 3D results for the dense plume scenario. Here it is shown that the 2D model is not an appropriate tool for predicting the accumulation of a dense plume in a stratified estuary. In contrast, the 3D model shows the dense plume accumulation remaining within the salt-wedge layer and extending approximately 10 km upstream from the location of origin.

The TUFLOW FV Yarra River estuary model will ultimately form the basis for a 3D hydrodynamic-microorganism model through the coupling with the Aquatic EcoDynamics (AED²) modelling library (e.g. Hipsey *et al.*, 2013; Bruce *et al.*, 2014). Survival of the faecal microorganisms/pathogens are known to be impacted by number of environmental factors such as: temperature, salinity, sunlight, pH and dissolved oxygen (Crane and Moore, 1985). Furthermore, it is well established that sediments, both suspended in the water column and at the estuary bed, can provide certain extent of protection from detrimental environmental effects and prolong survival of pathogens (Pachepsky and Shelton, 2011). Therefore, ability to resolve the stratified circulation dynamics, including accurate distribution of the environmental variables (i.e. salinity, temperature, total suspended solids etc.) is an essential step in describing the microbial dynamics within the Yarra River.

In addition, the demonstrated ability to simulate buoyant plume release without extensive mixing within the salt-wedge is extremely important considering presence of 218 stormwater drains that discharge directly into the

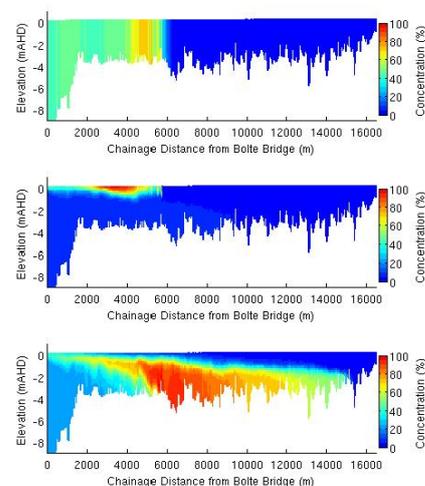


Figure 7. Modelled longitudinal tracer distribution ebbing flow: 2D-depth average plume release (top); 3D buoyant plume surface release (centre) and 3D dense plume bed release (bottom)

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estuary. During discharge events, the stormwater plume typically occupies the top of the water column where the exposure to recreational activities is the highest (therefore increasing the public health risk). A previous study, which employed a simple conceptual modelling approach, showed that on average the stormwater contribution of the total load of *Escherichia Coli* (*E. coli*, a common faecal indicator microorganism) to the estuary during wet weather remained marginal (~10%) (Jovanovic *et al.*, 2015), but was significant in some cases (around 50% of the total load). Due to the modelling limitations (e.g. box model for the estuary), this study was only able to generally assess the impact of stormwater on *E. coli* dynamics. Future research using TUFLOW FV as hydrodynamic driver will test previous findings, as well as explore stormwater impacts on much more refined temporal and spatial scale. Furthermore, the 3D hydrodynamic-microorganism model will be used to gain an improved understanding of faecal microorganism/pathogens dynamics and to scientifically-inform management decisions to improve the health of the Yarra River estuary.

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Appendix C

Co-authored journal publications

C.1 Environmental monitoring of waterborne *Campylobacter*: evaluation of the Australian standard and a hybrid extraction-free MPN-PCR method

frontiers in
MICROBIOLOGY

ORIGINAL RESEARCH ARTICLE
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Environmental monitoring of waterborne *Campylobacter*: evaluation of the Australian standard and a hybrid extraction-free MPN-PCR method

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Campylobacter is the leading agent of diarrheal disease worldwide. This study evaluates a novel culture-PCR hybrid (MPN-PCR) assay for the rapid enumeration of *Campylobacter* spp. from estuarine and wastewater systems. To first evaluate the current, culture-based, Australian standard, an inter-laboratory study was conducted on 69 subsampled water samples. The proposed Most-Probable Number (MPN)-PCR method was then evaluated, by analysing 147 estuarine samples collected over a 2 year period. Data for 14 different biological, hydrological and climatic parameters were also collated to identify pathogen-environment relationships and assess the potential for method specific bias. The results demonstrated that the intra-laboratory performance of the MPN-PCR was superior to that of AS/NZS ($\sigma = 0.7912$, $P < 0.001$; $\kappa = 0.701$, $P < 0.001$) with an overall diagnostic accuracy of ~94%. Furthermore, the analysis of both MPN-PCR and AS/NZS identified the potential for the introduction of method specific bias during assessment of the effects of environmental parameters on *Campylobacter* spp. numbers.

Keywords: *Campylobacter*, PCR, estuary, inter-laboratory, environmental interactions, culture

INTRODUCTION

Campylobacteriosis is a zoonosis spread into the environment through the release of fecal material. Current WHO figures suggest that *Campylobacter* are the leading cause of diarrheal disease in industrialized nations with annually more than 60,000 and 17,000 confirmed cases reported respectively in the United Kingdom (UK) and Australia alone (Corvisy, 2013; Hughes and Gorton, 2013). The primary route of infection is through ingestion of contaminated food products. However, environmental sources, such as water used for recreational purposes and stormwater flows, represent an often overlooked source of disease transmission (Adak et al., 1995; Pond, 2005; Arnone and Walling, 2007); 3% of confirmed cases in the UK were reported as the direct result of contact with contaminated water supplies (Anonymous, 2000). *Campylobacter* survival within non-biological settings (i.e., water and soils) (Thomas et al., 1999; Ross and Donnison, 2006; Donnison and Ross, 2009; Rodríguez and Araujo, 2012), is dependent on numerous exogenous variables. Sensitivities to seasonal variations, temperature, sunlight exposure and dissolved nutrients have been observed to directly affect concentrations of the bacterium within water sources (Jones, 2001; Boyle et al., 2008; Maal-Bared et al., 2012; Rodríguez and Araujo, 2012). Thus, variations in climatic, biological and hydrological conditions have direct implications on human health outcomes (Patz et al., 2003).

Enumeration of *Campylobacter* from complex source samples can be difficult due to the fastidiousness and fragility of the organism (Pitkänen, 2013). Furthermore, isolation from urban waters

is problematic, as they are usually present at low concentrations (Koenraad et al., 1997). Culture-based methods for the enumeration and isolation of *Campylobacter* from waters have become the international standard (Standardization ISO, 2005). The addition of concentration and pre-enrichment techniques and application of selective media has significantly improved recovery efficiencies (AS/NZS, 2001; Jokinen et al., 2012; Ugarte-Ruiz et al., 2012). However, culture-based methods are time-consuming and expensive, requiring filtration, selective enrichment, isolation and biochemical confirmation (~9 days to report).

The application of molecular tools, such as PCR, may help to circumvent some of the limitations of current methods. Assays for the detection of *Campylobacter* have been trialed and the results found to be comparable to culture-based methods (Savill et al., 2001; St-Pierre et al., 2009). It is important to note that the majority of assays were conducted on food products, primarily chicken rinses, with a limited number of environmental studies (Pitkänen, 2013). However, despite observed between-technique correlations, only three ISO methods currently utilize PCR for the detection of bacterial pathogens (IrelandNSAo, 2012; Organisation IS, 2012; Standardization ISO, 2013). One possible explanation for the lack of up-take of these methods, in water studies, is the large volume of water that needs to be filtered in order to detect low concentration microbes. Consequently, exogenous variables, such as humic acid (a principle organic component of soil and known PCR inhibitor (Schrader et al., 2012)), are also concentrated (Lübeck et al., 2003). The ability

of laboratories to remove or limit humics, and other inhibitory substances, within DNA samples may introduce inter-laboratory variability in reporting. However, with the globalization of molecular tools, such as DNA purification kits and PCR mastermixes, the variations between laboratories can be minimized and should be no different to those observed for culture-based techniques.

A further consideration is the limited ability of researchers to remove exogenous naked DNA and DNA derived from non-viable cells. Direct amplification of environmental samples can result in the over-estimation of risk if the presence of free DNA is not accounted for. The use of chemical pre-treatments, such as propidium monoazide (PMA), has been proposed for the selective removal of free and non-viable cell DNA (Nocker et al., 2006, 2007). However, the efficiency of these methods to completely remove DNA from non-viable *Campylobacter* is still under investigation (Pacholewicz et al., 2013). Prior enrichment of samples, by culture based techniques, has been demonstrated to promote detection of viable cells while limiting the presence of exogenous DNA (Abulreesh et al., 2006).

Alternative hybrid methods employing cultural enrichment and PCR confirmation to enumerate *Campylobacter* in environmental samples have been described (Savill et al., 2001; Sails et al., 2003; Nam et al., 2005; St-Pierre et al., 2009; Rodriguez and Araujo, 2010). The assays have been successfully applied to complex matrices including feces, soil, foodstuffs and some recreational waters (Hernandez et al., 1995; Savill et al., 2001; Kulkarni et al., 2002; Josefsen et al., 2004a; Khan et al., 2009; St-Pierre et al., 2009; Rodriguez and Araujo, 2010; Rodgers et al., 2012; Gharst et al., 2013; Rohonczy et al., 2013; Taboada et al., 2013), demonstrating their broad application potential. The procedures utilize the benefits of standard filtration and culture to isolate organisms in combination with PCR-assays for rapid sensitive detection. The advantage of applying such procedures is that the presence of inhibitory substances from concentrated samples can be limited or diluted to enable reproducible assay results. Furthermore, initial culture-based enrichment increases the number of viable cells for later PCR amplification procedures. However, current hybrid protocols remain overly complicated often requiring multiple enrichment steps, centrifugation and specialized DNA purification procedures (Savill et al., 2001; Sails et al., 2003; Nam et al., 2005; St-Pierre et al., 2009; Rodriguez and Araujo, 2010; Pitkanen, 2013; Rohonczy et al., 2013). For universal uptake, a successful standard procedure should require minimal specialized equipment and resources, be easily applied with good correlation across laboratories and short reporting time.

The complexity and interaction of variables within estuarine and stormwater systems has limited the use of direct culture and molecular-based methods for *Campylobacter* enumeration (Lampard et al., 2012). However, hybrid methods have not been tested directly on these systems. Here we describe and evaluate a novel, DNA-purification free, culture-PCR hybrid assay for the rapid detection and enumeration of pathogenic *Campylobacter* from estuarine and wastewater systems. Concurrently, an inter-laboratory study was conducted to evaluate the diagnostic accuracy of the current standard, AS/NZS 4276.19:2001 (AS/NZS). AS/NZS is a MPN culture-based

method requiring filtration of complex samples prior to cultivation and biochemical confirmation of bacterial genus. The study encompassed 147 samples collected over a 2 year period to evaluate the potential of the MPN-PCR method as a standard *Campylobacter* enumeration procedure for environmental waters. Environmental parameter relationships, which significantly affect *Campylobacter* concentrations and assessment of risk and human health outcomes, were also evaluated for method specific bias.

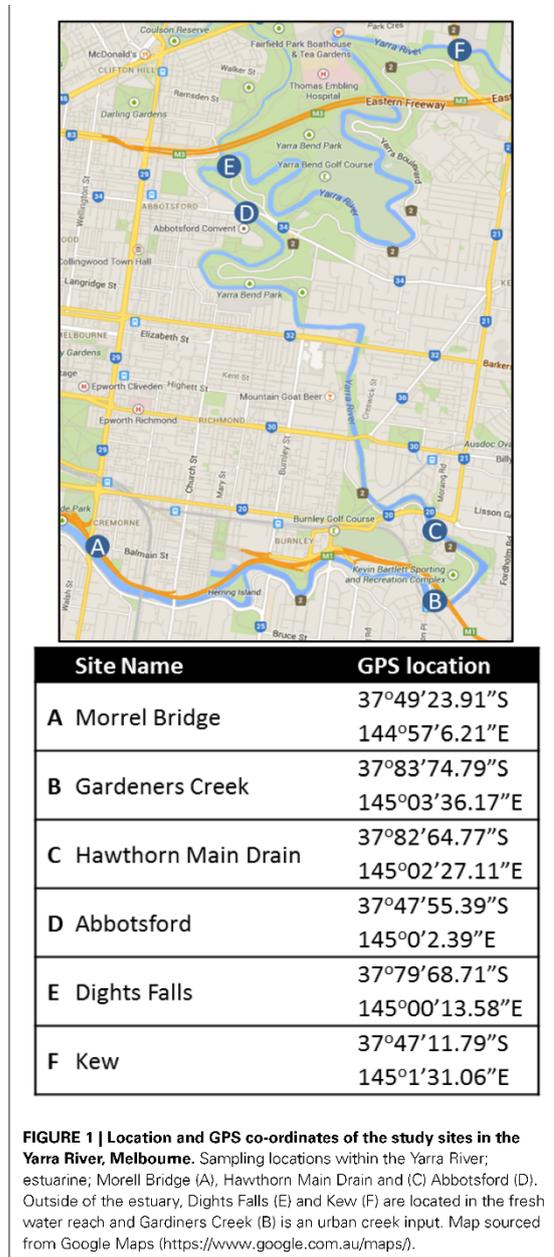
MATERIALS AND METHODS

STUDY LOCATIONS AND SAMPLE COLLECTION

Samples were collected from two systems in Victoria, Australia: the Yarra River and Monash University stormwater harvesting system.

Five sampling sites from within the Yarra River estuary (Melbourne, Australia) were selected for study from January 2012 to December 2013 (Figure 1). These consisted of two estuarine locations [Abbotsford (Abts) and Morrel Bridge (Mor)], two fresh water inputs (Kew and Dights Falls) and two urban stormwater inputs, Gardiners Ck (Gard) and Hawthorn Main stormwater drain (HMDE). Sites were selected to enable measurement of *Campylobacter* concentrations in source waters within the boundaries of the estuary. The water column within the estuary ranges from completely fresh at the riverine end (Kew/Dights Falls; 0.06–0.13 psu 5th, 95th percentile) to a salt water region at the seawater boundary. The Abbotsford site at the beginning of the estuarine section of the Yarra River was predominantly fresh (0.06–0.15 psu; 5th; 95th percentile), while Morell Bridge exhibited strong stratification driven by salt wedge (top layer salinity 0.73–9.25 psu; bottom layer 3.78–28.68 psu). The geographical positions and location descriptions for each site are presented in Table 1. Estuarine water grab samples were taken 3 m perpendicular from the bank and at an approximate depth of 0.15 m at each location. Samples were collected into 2 L polyethylene terephthalate containers that had been rinsed with a minimum of 1 L of source water prior to sample collection. The dates on which each sampling was undertaken are specified in Supplementary Material. A total of 147 estuarine samples comprising, 42 Abts, 45 Mor, 13 Kew, 16 Dights Falls, 34 Gard and 6 HMDE, were collected. Sampling days were selected to incorporate variable climatic and hydrological conditions. Rain event samples were collected using a flow-weighted strategy (McCarthy et al., 2008).

Water samples derived from a stormwater biofiltration system were included as part of the inter-laboratory evaluation of AS/NZS. The Monash University biofilters are located in Clayton, Australia, and treat stormwater from a 4500 m² multi-story carpark. The stormwater is initially fed through large basins which allow some sediment to settle (Hatt et al., 2009; Chandrasena et al., 2012). The biological filters are planted *Carex appressa* and *Melaleuca ericifolia*. As the stormwater moves through the sand-based media, the pollutants (nutrients, microbes and heavy metals) are removed through physical, chemical and biological processes. Six 30 L low complexity outflow samples, containing an estimated <10 MPN/L *C. jejuni* NCTC 11168 were collected. At the outlet, an electromagnetic flow meter (Magflow by SIEMENS) was connected to monitor flow rate. The data was stored in a Campbell CR200 data logger which triggered



a Sigma 900 autosampler every 10,000 L. A Teflon sampling tube was inserted into the outlet pipe from which ten 3 L sub samples were collected into clean polyethylene terephthalate containers.

All samples were placed on ice, divided into replicate samples and delivered to (1) Monash University, Environmental and

Public Health Laboratory (Lab-Res) and (2) ALS Environmental (Lab-Comm). ALS is a facility accredited by the National Association of Testing Authorities (NATA). Delivery and analysis occurred between 4 and 6 h of initial sample collection. Samples that underwent inter-laboratory evaluation of AS/NZS are outlined in Supplementary Material.

CULTURE-BASED MULTI-TUBE ANALYSIS OF *CAMPYLOBACTER* SPP. IN WATER SAMPLES

To calculate the inter-laboratory reproducibility of AS/NZS the procedure was conducted at two independent laboratories. Lab-Res was less than 1 h travel time from Lab-Comm; this small travel time was essential to prevent significant changes in the microbial content of samples, and the introduction of unwanted biases.

All samples underwent membrane filtration and examination for thermophilic *Campylobacter* spp. as described in the Australian/New Zealand Standard 4276.19:2001 (AS/NZS, 2001) (outlined in Figure 2) with the following modifications. Five or eleven tube MPN analyses were conducted, dependent on sample source and whether the *Campylobacter* concentrations were expected to be high. The number of tubes per sample, and the volumes filtered for each tube, are listed in Supplementary Material. Both laboratories used equivalent filtration volumes and number of tubes for each sample. For 11 tube MPN tests, two main filtrate regimes were applied: (1) 2 × 250, 3 × 100, 3 × 50, and 3 × 10 mL (2) 1 × 500, 5 × 100, and 5 × 10 mL. For 5 MPN tube tests, three main filtrate regimes were used: (1) 50, 15, 5, 1.5, and 0.5 mL, (2) 250, 100, 50, and 2 × 1 mL (3) 500, 250, 100, and 2 × 10 mL. Post-filtration onto 0.45 μM cellulose nitrate filters (Sartorius, Germany) samples were placed into 25 mL Prestons broth and resuscitated aerobically for 2 h at 37°C. *Campylobacter* selective supplement (Oxoid, United Kingdom) was added as per manufactures instructions and broths enriched for 48 h at 42°C. As outlined in AS/NZS, two 25 mL broth cultures were spiked with *Escherichia coli* strain ATCC 11775 or *C. jejuni* NCTC 11168 as negative and positive reaction controls respectively. To ensure no post-collection environmental contamination, DNA-free water, equivalent to the highest filtrate volume, was left opened to the environment for the duration of filtration and then filtered onto a 0.45 μM filter, placed into Prestons broth and enriched as described in AS/NZS. No antibiotic negative enrichment controls were included to ensure no media contamination. *Campylobacter jejuni*, *E. coli*, no antibiotic and DNA-free water contamination controls were conducted with each assay at Lab-Res, while *C. jejuni* and *E. coli* controls were conducted at the commercial lab [as outlined in (AS/NZS, 2001)].

Post-enrichment (48 h at 42°C), 2 μL of each sample was plated onto modified CCDA-Preston and incubated for 48 h at 42°C after which biochemical confirmation of *Campylobacter* using the Oxoid Biochemical Identification System (O.B.I.S) (Oxoid, United Kingdom) was conducted on isolates present under microaerophilic conditions (Figure 2).

Table 1 | Sampling site description.

Site name	Description
A Morrel bridge	High density urban developments; high watercraft usage; Queens Bridge Drain located around 950 m upstream, deep, wide channel of salt and fresh water
B Gardeners creek	Completely channelized section of Gardiners Creek; extremely receptive to rainfall events within the catchment; No tidal effect; High density industrial and residential areas upstream of site; no watercraft activity; surrounded by recreational/sporting grounds
C Hawthorn main drain	Major urban stormwater drain. Collects stormwater inputs from high density industrial and residential areas; no watercraft activity
D Abbotsford	Shallow fast-flowing riffled section; high density industrial and residential developments with recreational parklands; predominantly fresh water
E Dights falls	Site ~20 m upstream of weir, surrounded by parklands; no tidal influence; minimal watercraft; Merri creek junction just upstream; Eastern Freeway crosses Merri Creek just U/S of Merri-Yarra junction
F Kew	Low density industrial, medium level residential developments; minimal watercraft activity; no tidal affect; fresh water

MPN-PCR ANALYSIS OF *CAMPYLOBACTER* SPP. FROM ENRICHED ENVIRONMENTAL ISOLATES

Post-enrichment cultures, described above, were removed from the incubator and plated onto modified Prestons agar (AS/NZS, 2001) (Figure 2). Concurrently, a 1 uL sub-sample was taken from each 25 mL Preston enrichment (including *Campylobacter jejuni*, *E. coli*, no antibiotic and DNA-free water controls) and diluted 1:20 in DNase/RNase free water and frozen at -20°C prior to use (Figure 2). These samples were freeze-thawed (one cycle) at -20°C to fracture cells prior to PCR amplification. One cycle was assumed to be sufficient to release DNA for PCR amplification. Based on the results of Lübeck et al. (2003) the forward and reverse primer pair of OT1559 (5' CTGCTTAACACAAGTTGAGTAGG 3') (Uyttendaele et al., 1994) and 18-1 (5' TTCCTTAGGTACCGTCAGAA 3') (Uyttendaele et al., 1994) were selected for specific amplification of an ~200 bp product from *C. jejuni*, *C. coli*, *C. lari* and *C. upsaliensis*. Each 12 uL reaction consisted of 5.5 μL of SSofast Evagreen Supermix (Biorad, USA), 25 nM of each primer, 2.3 μL of DNase/RNase free water and 2 μL of lysed sample. Each sample PCR was conducted in duplicate, a no template control (NTC) was included in all assays. Amplification was performed on a Biorad CFX96 Real-Time PCR system (Biorad, USA) under the following conditions: 1 cycle of 95°C for 3 min; 40 cycles of 95°C for 5 s, 56°C for 30 s, with a plate read conducted after each cycle for fluorescence measurement. Melt curve analysis was conducted at the completion of 40 cycles of amplification and compared to the *C. jejuni* positive, NTC and *E. coli* negative controls.

ENVIRONMENTAL DATA

For Yarra River samples, rainfall (mm), temperature ($^{\circ}\text{C}$), humidity (%) and mean sea level pressure (MSLP; hPa) data was averaged from gauge measurements taken at the Melbourne Regional Office (Station ID: 86071) and available from the Bureau of Meteorology (<http://www.bom.gov.au/climate/data-services>). Average daily flow rates were available at Abbotsford, Morell Bridge and Gardiners Ck. Total nitrogen (mg/L), total phosphorus (mg/L), total suspended sediment (TSS; mg/L) were measured by the Water Study Centre (Monash University, Australia) following the procedures described in APHA-AWWA-WEF

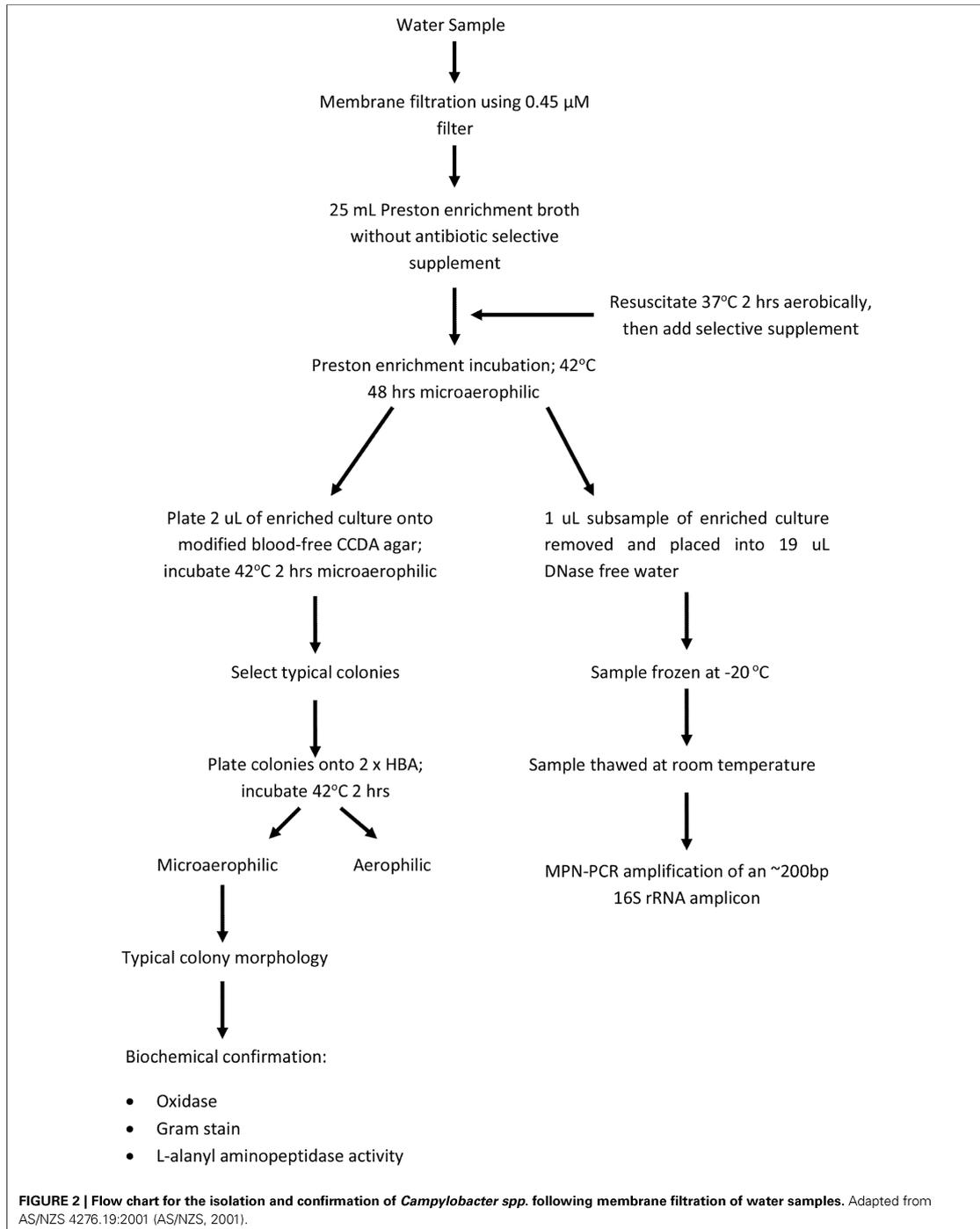
(Association APH, 2005). Electric conductivity (EC; mS/cm), dissolved oxygen (DO; mg/L), turbidity (NTU) and salinity (%) were measured *in-situ* and in the river flow, when possible, using a Horiba multi-probe (HORIBA, Japan). Hydrological and environmental parameter data was not collected for biofilter derived samples.

A 10 mL subsample of the environmental water was taken and examined for the presence of fecal coliforms and *E. coli* using the Colilert® MPN method (IDEXX, USA) outlined in AS4276.21-Method 21 (Australia S, 2005). The method was conducted by Lab-Res with results presented as MPN/100 mL (Supplementary Material). A 1:10 dilution was used for all samples except when rainfall preceding collection was >3 mm where a 1:100 dilution was applied. A negative deionized water control was included for all assays.

STATISTICAL DATA ANALYSIS

The inter- and intra-laboratory relationship between AS/NZS and MPN-PCR consisted of discrete data and were therefore assessed using the Kappa coefficient of agreement (Carletta, 1996). The Kappa coefficient measures difference based on a scale from -1 to 1 , where a value of 1 indicates complete agreement, 0 suggests a value has been obtained by chance and -1 a disagreement between results (Viera and Garrett, 2005). Summary statistics were also used to compare the AS/NZS results from the two independent laboratories. Wilcoxon matched-pairs signed rank test was conducted to compare paired concentration data from Lab-Res and Lab-Comm. Minimum and maximum values are summarized as well as the 5th and 95th percentile. Wilcoxon matched-pairs signed rank test was also applied to compare non-equivalent data derived from AS/NZS and MPN-PCR methods. Distribution patterns were plotted using box plots (Graphpad Prism 6.0, Graphpad Software Inc., USA) to demonstrate the reproducibility of the method.

The diagnostic sensitivity, specificity and likelihood ratios of the MPN-PCR method and AS/NZS were calculated globally and for each of the major filtration regimes. (Hoorfar and Cook, 2003; Šimundić, 2008). Information on true positive (TP MPN-PCR), false positive (FP MPN-PCR), true negative (TN MPN-PCR) and false negative (FN MPN-PCR) were collated for the MPN-PCR by comparison to AS/NZS. Equivalent information for AS/NZS was



collated by comparison of inter-laboratory culture-based results. For the current study, diagnostic sensitivity (TP/TP+FP) was defined as the ability of the assay to identify a positive result when *Campylobacter* were actually present (TP) (Cook et al., 2007). Diagnostic specificity (TN/TN+FN) was defined as the discriminatory ability of the assay to identify that *Campylobacter* were absent when they were truly absent (TN) (Cook et al., 2007). The likelihood ratio (LR) was defined as the likelihood that a given result would be expected in a positive tube as opposed to a negative tube (Deeks and Altman, 2004). The more distant a LR-ratio value was from a value of one, the stronger the evidence for the presence or absence of *Campylobacter* within the sample (Deeks and Altman, 2004). Positive likelihood ratios ((sensitivity/100)/1-(specificity/100)) of ≥ 10 and LR- ratios ((1-(sensitivity/100))/(specificity/100)) of < 0.1 were considered to provide strong evidence to rule-in/rule-out conclusions under most conditions tested (Deeks and Altman, 2004). Diagnostic accuracy (TP+TN/total sample number) was used to compare the performance of the MPN method to AS/NZS (Šimundić, 2008). Intra-laboratory evaluation of the diagnostic potential of the MPN-PCR was conducted as outlined in ISO 22174:2005 (Standardisation ISO, 2005). The standard presents the minimum requirements for PCR-based detection of bacteria within food and has been applied previously to *Campylobacter* assays (Josefsen et al., 2004a,b,c).

Statistical analysis was conducted using GraphPad Prism 6.0 (Graphpad Software Inc, USA) and SPSS Statistics 22 (IBM Statistics, USA). Spearman Rank correlation coefficients (Spearman, 2010) were conducted on Yarra River data to identify significant relationships for inter-laboratory and intra-laboratory method comparison data, within and between site (spatial) data and *Campylobacter* and data derived from the selected 14 environmental and biological parameters. The concentration differences between AS/NZS and MPN-PCR were also compared for each parameter to identify method specific bias. Biofilter data was included in correlative assessment of relationships between Lab-Res and Lab-Comm results. Due to the small sample size ($n = 6$) environmental parameter relationships were not assessed for these samples. For Spearman rank analysis, results below detection were taken as half the detection limit to allow comparative assessment as has been previously described for non-detect data (Helsel, 2004). Correlative analysis was not conducted between parameters where < 10 data points were available to enable confidence interval calculation (Zar, 1999).

RESULTS

INTER-LABORATORY METHOD COMPARISON

The multi-tube AS/NZS was conducted on 69 environmental samples concurrently at two laboratories (Lab-Res and Lab-Comm see Supplementary Material for details). Summary statistics of the two datasets are presented in Figure 3. The global sensitivity and specificity of AS/NZS was assessed for all inter-laboratory investigated samples and was determined to be 68.8 and 85.4% respectively. The LR+ ratio was 4.7 and LR- ratio 0.37 with the overall diagnostic accuracy of AS/NZS observed to be 76.5%. A positive correlation ($\sigma = 0.502$, $P < 0.001$) and moderate agreement ($\kappa = 0.531$; $P < 0.05$) was observed between the

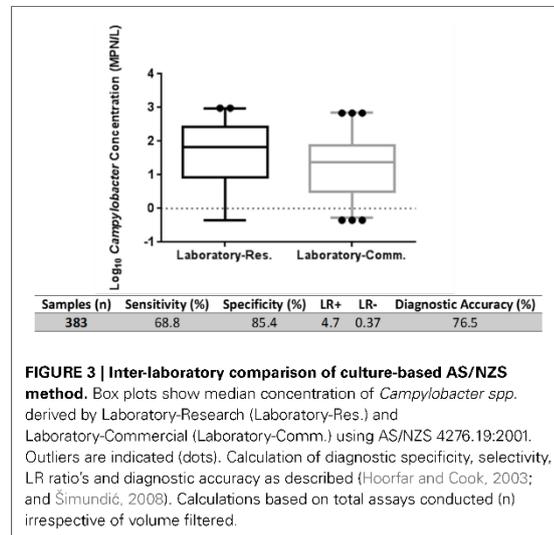


FIGURE 3 | Inter-laboratory comparison of culture-based AS/NZS method. Box plots show median concentration of *Campylobacter* spp. derived by Laboratory-Research (Laboratory-Res.) and Laboratory-Commercial (Laboratory-Comm.) using AS/NZS 4276.19:2001. Outliers are indicated (dots). Calculation of diagnostic specificity, selectivity, LR ratio's and diagnostic accuracy as described (Hoorfar and Cook, 2003; and Šimundić, 2008). Calculations based on total assays conducted (n) irrespective of volume filtered.

results of Lab-Res and Lab-Comm (Figure 4). Lab-Res results were higher than those of Lab-Comm on 34 occasions (67%); an observation that was echoed by the significant difference found between the median concentrations of the two labs ($P > 0.001$). In all assays, the control samples generated expected results.

INTRA-LABORATORY COMPARISON OF AS/NZS AND MPN-PCR

A total of 147 samples derived from the Yarra River estuary were analyzed concurrently by MPN-PCR and AS/NZS (Table 2). The strong positive correlation between the two methods was observed ($\sigma = 0.7912$, $P < 0.001$). Kappa coefficient results also supported significant agreement between the methods ($\kappa = 0.701$, $P < 0.001$) (Figure 4). However, in 41 samples, the MPN/L were not equivalent (MPN-PCR \neq AS/NZS) (Table 2). Notably, the MPN-PCR method resulted in significantly higher detected concentrations ($P < 0.01$) of *Campylobacter* spp. within 29 of the 41 non-equivalent samples (70%) with a median concentration of 82 MPN/L whereas the median concentration for AS/NZS was 24 MPN/L. All control samples behaved as expected in both the MPN-PCR and AS/NZS.

The global sensitivity and specificity of the PCR assay was assessed for the three main 5 tube MPN filtration regimes, described earlier, applied in the study (Table 3). The highest assay sensitivity (100%) was observed when 100, 50, and 1 mL filtrates were used within a single assay. The highest specificity was observed in assay volumes of 500 and 250 mL (100%), but it is important to note the relatively small number ($n = 13$) of samples investigated using this filter regime. Likelihood ratios were calculated for each filtration regime. The results indicate that the highest LR+ value, 14.2, was for assay 1 (50, 15, 5, 1.5, and 0.5 mL) while assay 2 (250, 100, 50, and 1 mL) had the lowest LR- value, 0.006. The LR values for the MPN-PCR, irrespective of filtered volume, were 9.4 (LR+) and 0.03 (LR-). The diagnostic accuracy of all regimes was high at $\sim 94\%$.

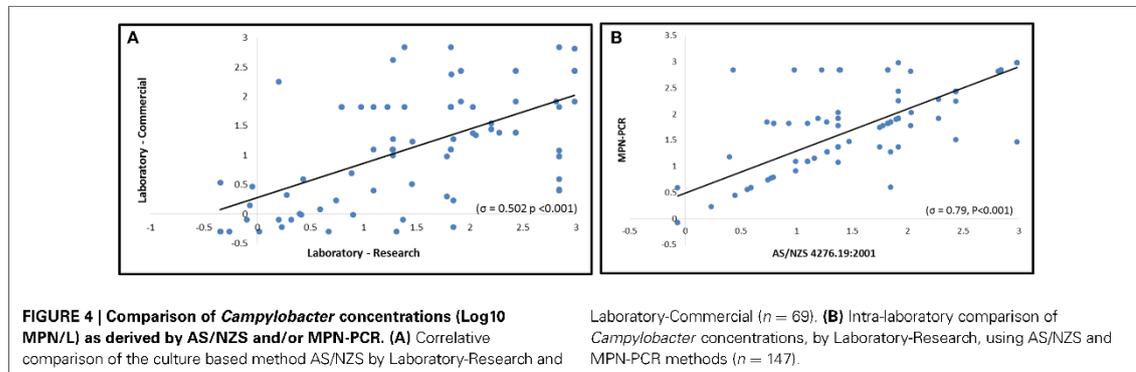


Table 2 | Yarra River *Campylobacter* spp. data included in this study.

Site	All data	MPN-PCR= AS/NZS	MPN-PCR≠ AS/NZS ^a	MPN-PCR> AS/NZS ^b
Kew	10	7 (70%)	3 (30%)	2 (66.7%)
Dights falls	16	12 (75%)	4 (25%)	3 (75%)
Abbotsford	42	28 (66.7%)	14 (33.3%)	12 (85.7%)
Morell	45	34 (75.6%)	11 (24.4%)	8 (72.7%)
Gardiners Ck	34	25 (73.5%)	9 (26.5%)	4 (44.4%)
Total	147	106 (72.1%)	41 (27.9%)	29 (70.7%)

Bracketed numbers represent % contribution to total samples from each investigated condition.

^aNumber of samples and percentage of samples where MPN-PCR *Campylobacter* concentrations (MPN/L) were not equal to (≠) that of AS/NZS.

^bNumber of samples and percentage derived from comparison of samples where MPN-PCR concentrations were greater than (>) those of AS/NZS from the MPN-PCR≠AS/NZS dataset.

SPATIAL RELATIONSHIPS BETWEEN *CAMPYLOBACTER* SPP. CONCENTRATIONS

Positive spatial correlations were observed between *Campylobacter* spp. concentrations at Abbotsford (D) and Dights Falls (E) ($\sigma = 0.53$, $p < 0.05$), using the AS/NZS method, and more significantly at Morell Bridge (A) and Dights Falls ($\sigma = 0.74$, $p < 0.01$) using the MPN-PCR method (Figure 1). An equivalent significant result could not be achieved using the MPN-PCR to establish a relationship between Abbotsford and Dights Falls, or applying the AS/NZS at Morell Bridge and Dights Falls. Positive spatial correlations were also established between Kew (F) and Gardiners Creek (B) ($\sigma = 0.69$, $P < 0.05$) as well as Gardiners and Morell Bridge ($\sigma = 0.9$, $P < 0.01$). However, these relationships were the result of comparisons between AS/NZS (Kew, Abbotsford) and MPN-PCR (Gardiners, Morell Bridge) and not due to the application of a single method. No other correlative site relationships were observed.

ENVIRONMENTAL PARAMETER AND *CAMPYLOBACTER* SPP. RELATIONSHIPS

Significant relationships are highlighted in Table 4, with all Spearman Rank data presented in Supplementary Material. For

both methods of detection (MPN-PCR and AS/NZS), significant ($p < 0.05$) positive correlations were observed between *Campylobacter* concentrations and that of daily rainfall, phosphorus levels, TSS and turbidity (Table 4). Relationships were also observed specifically between the *Campylobacter* concentrations obtained by the AS/NZS method, nitrogen, temperature, *E. coli* and DO, while relative humidity was the only relationship specific to results obtained by the MPN-PCR. It is interesting to note that all observed correlations, with the exception of temperature (-0.18 , $P < 0.05$), were positive. The results of difference analysis (i.e., AS/NZS—MPN-PCR) indicated the potential for method bias; indeed, changes in nitrogen levels and relative humidity were correlated to the differences in *Campylobacter* spp. concentrations between the two methods.

CAMPYLOBACTER SPP. WITHIN-SITE RELATIONSHIPS TO ENVIRONMENTAL PARAMETERS

As a result of the identification of putative method bias, within-site analysis was conducted independently for MPN-PCR and AS/NZS. Significant correlative relationships are outlined in Table 5. All Spearman Rank data are presented in Supplementary Material. Relationships at Abbotsford and Morell Bridge were only identified with AS/NZS, despite a significant relationship between the methods still being maintained ($\sigma = 0.72$, $P < 0.001$). Gardiners Ck had the largest number of observed significant results with rainfall (day of), EC, temperature (day of), *E. coli* and phosphorus showing significant ($P < 0.05$) correlations irrespective of the method employed. A single relationship between *Campylobacter* concentration and humidity was observed by MPN-PCR at Kew.

DISCUSSION

Campylobacter are a major cause of gastrointestinal illness, yet, many sources of disease outbreak remain unidentified. Recreational waters (rivers, lakes and estuaries), and stormwaters which are harvested for indoor or outdoor domestic uses, can represent a significant source of infection (Koenraad et al., 1997; Moore et al., 2001; Savill et al., 2001; Sidhu et al., 2012). However, isolation and enumeration from aquatic environments can be difficult due to a multitude of environmental, biological and biophysical variables (Khan et al., 2009). The current study

Table 3 | Comparison of MPN-PCR method to the culture-based AS/NZS method for three 5MPN filtration regimes.

Vol. filtered (mL)	Number of samples ^a				Samples (n) ^d	MPN-PCR ^b				
	C+P+	C+P-	C-P+	C-P-		Diagnostic sensitivity (%) ^c	Diagnostic specificity (%) ^c	LR+ ^c	LR- ^c	Diagnostic accuracy (%) ^c
50	59	3	1	2	65	95.2	66.7	2.9	0.07	93.8
15	48	1	3	13	65	97.95	81.3	5.2	0.03	93.8
5	36	1	3	25	65	97.3	89.3	9.1	0.03	93.8
1.5	17	1	1	46	65	94.4	97.9	44.4	0.06	96.9
0.5	8	2	2	53	65	80.0	96.4	22	0.21	93.8
All	168	8	10	139	325	95.5	93.3	14.2	0.05	94.5
250	53	1	7	6	67	98.1	46.2	1.8	0.04	88.1
100	56	0	2	9	67	100.0	81.8	5.5	0	97.0
50	51	0	2	14	67	100.0	87.5	8.0	0	97.0
1 (*2)	21	0	8	105	134	100.0	92.9	14.1	0	94.0
All	181	1	19	134	335	99.5	87.6	8.0	0.006	94.0
500	10	1	0	2	13	90.9	100.0	n/a	0.09	92.3
250	9	1	0	3	13	90.0	100.0	n/a	0.1	92.3
100	6	1	2	4	13	85.7	66.7	2.6	0.21	76.9
10 (*2)	10	0	3	13	26	100.0	81.3	5.3	0	88.5
All	35	3	5	22	65	92.1	81.5	4.97	0.09	87.7
Total ^d	384	12	34	295	725	96.96	89.7	9.4	0.03	93.7

^a C+ represents culture positive, C- culture negative, P+ is MPN-PCR positive and P- represents MPN-PCR negative.

^b Calculation of diagnostic accuracy based on comparison to the AS/NZS culture reference method of the same laboratory.

^c Calculation of diagnostic specificity, selectivity, LR ratio's and diagnostic accuracy as described (Hoorfar and Cook, 2003; Šimundić, 2008).

^d Calculations based on total assays conducted irrespective of volume filtered (n).

Bold values highlight the results for all samples applied to the specific filtration regime.

* Indicates where a sub-sample has been taken twice of the same volume.

Table 4 | Significant Spearman rank correlations between *Campylobacter* concentrations and environmental parameters.

Parameter	AS/NZS	MPN-PCR	Difference ^a
Rainfall, day of sampling (mm) ⁽¹⁴⁷⁾	0.18	0.18	-
Phosphorus (mg/L) ⁽⁹²⁾	0.40	0.41	-
Nitrogen (mg/L) ⁽⁹²⁾	0.30	-	0.22
Total suspended sediment (mg/L) ⁽⁴⁸⁾	0.56	0.43	-
Turbidity (NTU) ⁽⁸⁴⁾	0.51	0.46	-
Max. temperature, day of (°C) ⁽¹⁴⁷⁾	-0.18	-	-
<i>E. coli</i> (MPN/100 mL) ⁽¹⁴⁷⁾	0.22	-	-
Dissolved oxygen (mg/L) ⁽⁵²⁾	0.35	-	-
Relative humidity (%) ⁽¹⁴⁷⁾	-	0.22	-0.19

All data, from all sites, were included. Values presented have $P < 0.05$. Bold type indicates $P < 0.01$. Total number of samples in each individual analysis identified in parentheses.

^a Results derived from Spearman correlation of subtracted values from AS/NZS and MPN-PCR.

aimed to evaluate the intra-laboratory reproducibility of a novel DNA-extraction free MPN-PCR as an alternative to the current Australian Standard (AS/NZS) method. To undertake the evaluation three main factors were taken into consideration.

INTER-LABORATORY REPRODUCIBILITY OF CULTURE-BASED METHODS

Due to a dearth of data from multicenter studies, on *Campylobacter* enumeration standards, it was difficult to assess if the current results deviated from normal trends. Kappa analysis and Spearman correlations provided evidence of significant relationships between the two laboratories. Although significant, there was evidence that the two methods deviated, with some samples having differences of up to 690 MPN/L. However, a single study by Scotter et al. (1993), demonstrated that even with the use of three independent culture-based methods (two of which were international standards) on the same sample, inter-laboratory *Campylobacter* results correlated, at most, 42%. Thus, it can be assumed that the results of the sensitivity, specificity and diagnostic accuracy, achieved for AS/NZS during the current study, is indicative of the normal variation observed in culture-based studies.

The introduction of variability and uncertainty, even to standardized methods, has been recognized as unavoidable for complex samples matrices (Augustin and Carlier, 2006; Pan et al., 2010). However, it is important to highlight that these studies did not assess environmentally-derived samples, which have unique, independent source-related levels of uncertainty. Analytical methods for bacterial measurement within water

Table 5 | Significant within site Spearman Rank correlations between *Campylobacter* spp. concentration and environmental parameters.

	Abbotsford	Morell	Gardiners		Kew
	AS/NZS	AS/NZS	MPN-PCR	AS/NZS	MPN-PCR
Rainfall, day of sampling	–	–	0.55 ⁽³⁴⁾	0.57 ⁽³⁴⁾	–
Rainfall, 24 h	–0.33 ⁽⁴²⁾	–	–	–	–
TSS	0.55 ⁽¹⁴⁾	–	–	–	–
Nitrogen	0.47 ⁽²⁶⁾	0.51 ⁽²⁷⁾	–	–	–
EC	0.42 ⁽²³⁾	–	–0.57 ⁽¹⁷⁾	–0.71 ⁽¹⁷⁾	–
Humidity	–	–	–	0.36 ⁽³⁴⁾	0.67 ⁽¹⁰⁾
Turbidity	–	0.63 ⁽²⁵⁾	–	0.63 ⁽¹⁷⁾	–
Temp, day of sampling	–	–	–0.29 ⁽³⁴⁾	–0.44 ⁽³⁴⁾	–
Temp, 24 h	–	–	–	–0.46 ⁽³⁴⁾	–
<i>E. coli</i>	–	–	0.71 ⁽³⁴⁾	0.68 ⁽³⁴⁾	–
Phosphorus	–	–	0.63 ⁽²⁰⁾	0.56 ⁽²⁰⁾	–
Flow, day of sampling	–	–	–	0.50 ⁽³⁴⁾	–
Flow, 24 h	–	–	–	0.41 ⁽³⁴⁾	–

Values presented have $P < 0.05$. Bold type indicates $P < 0.01$. Total number of samples in each analysis identified in parentheses.

sources utilize sub-sampling regimes as an indication of true microbial load (Ongerth, 2013). However, microbes are not evenly distributed, spatially or temporally, thus a single sample may not be representative of actual bacterial concentrations. Recovery efficiencies for low concentration microorganisms, such as *Campylobacter*, can vary dramatically depending on water quality matrices (total suspended solids (TSS) and turbidity); which limit the volume of sample that can be processed (Pickup, 1991; Rosef et al., 2001; Ongerth, 2013). During rain events, turbidity and TSS levels within the Yarra River estuary frequently exceed 100 NTU and 100 mg/L respectively (Daly et al., 2013). Consequently, the *Campylobacter* assay filtrate volumes were reduced to ≤ 50 mL to enable filtration which may have resulted in a concurrent reduction in recovery. The efficiency of isolation may also be affected by the presence of competing organisms; the concentration of which have been shown to increase with filtrate volume (Rosef et al., 2001; Abulreesh et al., 2005). To date, only a single study has attempted to quantify some of the factors effecting inter-laboratory reproducibility of cultural isolation of *Campylobacter* from water sources (Khan et al., 2009). The researchers found that the low concentration of *Campylobacter* within water samples, as well as culture based method applied, may introduced a further level of variability between the sub-samples (Khan et al., 2009); as was observed between Lab-Res and Lab-Comm. However, unlike the current study, (Khan et al., 2009) did not account for the role of exogenous environmental factors in the introduction of variability; which is unique to this study.

It is recognized that irrespective of introduced uncertainties Lab-Res still retained higher detected concentrations of *Campylobacter* spp. in 68% of samples. A study by Augustin

and Carlier (2006) identified factors including resuscitation technique, method of plating, presence of inhibitors (chemical and biological) as well as mode and manufacturer of culture media effected inter-laboratory reproducibility of culture-based methods. Augustin and Carlier (2006) also suggested culture media (source and preparation) was a key factor in observed count differences between laboratories, and may account for some of the differences observed in the current study. Furthermore, previous studies (Williams et al., 2012) have also identified a possible culture-associated bias toward certain *Campylobacter* species. A concurrent study within the Lab-Res has identified *Campylobacter coli* as the predominant species within the Yarra River estuary (data not shown). Differences in the observed inter-laboratory concentrations could represent culture-associated bias, with one laboratory able to cultivate a subset of *Campylobacter*s that cannot, for reasons yet to be defined, be isolated within the other facility. A further investigation of this hypothesis is currently underway. However, in combination, the analysis suggests that low diagnostic accuracy between facilities is not a unique phenomenon, with small alterations in technical aspects having large impacts on final results.

INTRA-LABORATORY EVALUATION OF MPN-PCR AND AS/NZS

The current study applied ISO 22174:2005 parameters to evaluate the diagnostic potential of the proposed MPN-PCR assay (Hoorfar and Cook, 2003; Standardisation ISO, 2005). The standard, which summarizes the application of PCR based technologies for diagnosis of food-borne pathogens, has been previously applied to *Campylobacter* enumeration from food (Josefsen et al., 2004a,b,c).

ISO 22174:2005 outlines that for proposed PCR assays, pre-enrichment procedures should be equivalent to a culture-based standard to enable easy implementation into routine laboratory practices (Hoorfar and Cook, 2003; Standardisation ISO, 2005). Consequently, no variation from the outlined AS/NZS filtration and enrichment steps were undertaken. Enrichment prior to PCR enhances sensitivity by increasing the number of target cells available for amplification while reducing relative inhibitor levels (Hoorfar and Cook, 2003). Limiting the presence of inhibitory substances within complex water and soil samples is essential for accurate enumeration. However, it is important to note that *Campylobacter* culture media contains known PCR inhibitors which may affect assay outcomes (Josefsen et al., 2004c; Schrader et al., 2012). For example, Josefsen et al. (2004c) applied direct amplification from Preston enrichment culture and observed inhibition. To reduce inhibitory effects Josefsen et al. (2004c) applied a simplified DNA purification protocol. However, Josefsen et al. (2004c) did not attempt a simple dilution method, as recommended in ISO 22174:2005, which, in the current study, was found to negate any inhibitory effects introduced from the culture media.

ISO 22174:2005 also outlines that any proposed PCR assay should have a diagnostic accuracy equivalent or greater than the standard method it is replacing (Hoorfar and Cook, 2003). The results of the inter-laboratory study demonstrated a diagnostic accuracy of 76.5% for AS/NZS. However, it is important to note that calculation of the inter-laboratory diagnostic accuracy for the

culture-based method used 69 samples in comparison to the 147 applied to the intra-laboratory MPN-PCR assay.

Three sub-sampling regimes were investigated for use with estuarine waters based on sensitivity of detection (to ensure enumeration of both high and low concentrations of *Campylobacter*) and ease of filtration of the turbid water samples. To date, environmental water sampling regimes, for *Campylobacter*, often advocate the use of large sample volumes (St-Pierre et al., 2009; Lévesque et al., 2011) for enhanced diagnostic accuracy. However, this limits their application to low turbidity, low TSS waters. A study by Abulreesh et al. (2005) demonstrated that for routine diagnostics, of turbid samples, filtrate volumes below 1000 mL decreased false-negative rates by limiting co-inoculation of heterotrophic bacteria; in turn, improving the diagnostic accuracy. To date, the diagnostic accuracy, sensitivity and specificity of *Campylobacter* PCR assays incorporating low volume filtrates (<200 mL), such as those applied in the current study, have not been assessed. However, it is important to recognize that this has not prevented their use in risk assessment studies (de Man et al., 2014).

Results of the current study demonstrated that for 41 of the 147 estuarine samples (i.e., for 28% of samples), the MPN-PCR method did not achieve the same *Campylobacter* concentration as AS/NZS. Interestingly, 29 of the 41 non-matching samples had a significantly higher enumerated *Campylobacter* concentration by the MPN-PCR method. Reports by other authors have also shown enhanced sensitivity of molecular methods in comparison to culture-dependent techniques (Savill et al., 2001; Josefsen et al., 2004b; Khan et al., 2009; St-Pierre et al., 2009; Bargellini et al., 2010; Lévesque et al., 2011). Suggested reasons for observed increases in sensitivity include amplification of DNA from damaged, dead or viable but non-culturable cell forms and competition by heterotrophic bacteria inhibiting *Campylobacter* culture (Augustin and Carlier, 2006; St-Pierre et al., 2009; Lévesque et al., 2011). The future inclusion of estuarine water controls, during inter-laboratory method evaluation, will aid in determining the true-effect of contaminating DNA on assay sensitivity.

The percent sensitivity is used to indicate the ability of an assay to detect a true positive within a population (Cook et al., 2007; Šimundić, 2008). In contrast, specificity measures the capacity of a method to detect a true negative (Cook et al., 2007; Šimundić, 2008). For wet weather sampling the sensitivity and specificity of the method were observed to be 95.5 and 93.3% respectively. In contrast, the sensitivity of the dry weather regime was higher (99.5%) with a lower overall specificity (87.6%). These results suggest that performance of the MPN-PCR, in its ability to detect true negatives and true positives was greatest for the smaller volume wet weather regime. The decrease of specificity, associated with an increase in false negatives, during the dry weather regime may have been associated with the application of larger filtrate volumes. The concentration of inhibitors and heterotrophic bacterial contamination, within the broth, may have increased, resulting in inhibition of the downstream PCR assay. Lending further support to this hypothesis is the observation, that for both regimes, the specificity of the assay was lowest with the highest filtered volume and improves as filtrate volumes decrease.

Likelihood ratios (LR) determine the probability of a specific test result occurring only in positive populations to that of the probability of it occurring within negative populations (Deeks and Altman, 2004; Šimundić, 2008). LR+ ratios >10 in combination with LR- ratios <0.01 are considered to provide the strongest evidence of diagnostic accuracy (Deeks and Altman, 2004; Šimundić, 2008). Irrespective of the filtration regime applied, the MPN-PCR assay displayed ratios of LR+ 9.4 and LR- 0.03, indicating that the method has strong diagnostic accuracy under most conditions tested, and higher than that of AS/NZS (LR+ 4.7 and LR- 0.37). As was observed previously, the lowest LR+ results were achieved with the largest filtrate volumes, and may be directly associated with the presence of bacterial and environmental inhibitors. In accordance with ISO 22174:2005 the proposed PCR assay has a diagnostic accuracy, sensitivity and specificity greater than the standard method when applied to complex estuarine-derived water samples (Hoorfar and Cook, 2003).

SPATIAL AND ENVIRONMENTAL PARAMETERS RELATIONSHIPS

Previous studies on the Yarra River estuary have demonstrated spatial relationships between fecal indicator organisms and sampling locations (Daly et al., 2013). However, previous assessment of *Campylobacter* relationships utilized a small dataset, limited environmental data and a single method approach (data not shown). Thus, only limited assessment of the pathogen-factorial relationships could be conducted. In the current study, *Campylobacter* concentrations between two closely situated sites, Abbotsford and Dights Falls, correlated in 53% of samples by AS/NZS. The observed relationship was not unexpected with previous data (also conducted with AS/NZS) suggesting that the primary source of *Campylobacter*, into the estuary, is derived from agricultural inputs above Dights Falls (data not shown).

Estuaries are dynamic environments affected by a multitude of variables. Consequently, infectious disease transmission, within these systems, "should be viewed within an ecological framework" (Patz et al., 2003). The understanding of pathogen-environment relationships is essential for improved detection and the evaluation of persistence; both of which aid in prevention and lowering of disease rates (Schets et al., 2011a,b). To date, studies investigating parameter-bacteria relationships have primarily applied single method approaches (Rodríguez and Araujo, 2010; Rodríguez and Araujo, 2012). It is of significant concern that researchers often fail to recognize or evaluate the uncertainty introduced as a direct result of technique applied. Consequently, current cited relationships (reviewed in Sterk et al., 2013) may have been inaccurately identified, which may explain some of the observed between-study inconsistencies. To our knowledge, the current study is the first to assess method effect on the evaluation of environmental relationships and determine significant links between these and *Campylobacter*.

Rainfall, phosphorus, TSS and turbidity levels were observed to correlate with *Campylobacter* concentrations, across the estuary, irrespective of the method applied. Relationships between bacteria and these parameters have been previously reported (Gachter et al., 1988; McCarthy et al., 2012; Batabyal et al., 2014). However, the existence of these specific parameter associations

has not been demonstrated for *Campylobacter* spp. within estuarine settings. At Gardeners Ck within site analysis identified relationships between the pathogen, rainfall (day of sampling), conductivity, temperature (day of sampling), *E. coli* and phosphorus levels. Interestingly, this was the only site in which parameter relationships were identified by both MPN-PCR and AS/NZS. The shallow depth, low flow and difference in stormwater inputs at Gardeners Ck may have contributed to the increased number of relationships observed; as small alterations in conditions may have a larger effect on the microbial community.

Difference analysis conducted on the total Yarra River dataset identified two possible sources of method specific bias. The data demonstrated that increases in total nitrogen (TN) resulted in a concurrent increase in *Campylobacter* detected by AS/NZS, with less difference observed between concentrations derived by the two enumeration methods. TN measures all forms of nitrogen (nitrate, nitrite and ammonia) within environmental water samples. Increased concentrations of total nitrogen have been shown to support and enhance the growth of fecal indicators in a range of environments (Hirn et al., 1980; Wittman et al., 2013; Cederlund et al., 2014). *In vitro*, *Campylobacter* survival has been demonstrated to be supported by the addition of nitrate to selective agars (Sellars et al., 2002; Pittman et al., 2007). It is therefore hypothesized that the presence of exogenous nitrogen, and in particular nitrate, carried on the filter and into the enrichment culture, further promoted *Campylobacter* growth under the described experimental conditions. In contrast, it was observed that as relative % humidity increased the concentration of *Campylobacter* derived by MPN-PCR differed more significantly from those of AS/NZS; MPN-PCR having the higher detected bacterial concentration. The specific effect of humidity on the growth of *Campylobacter* within enrichment cultures remains unknown and requires further evaluation. However, previous studies have demonstrated that high humidity supports the growth of a range of non-pathogenic bacteria (Arundel et al., 1986). Increased competition from co-inoculated, endogenous bacteria would result in decreased isolation of *Campylobacter* by the culture-based method but would have limited effect on the detection of specific DNA by MPN-PCR.

In order to provide adequate assessment of risks, we must understand within-lab and between lab uncertainties. We must also focus on developing faster, cheaper and more accurate tools for quantifying potential health hazards. We contribute to the development of faster, more accurate measurement of *Campylobacter* levels in urban water systems. The MPN-PCR method presented has improved diagnostic accuracy, specificity and sensitivity in comparison to AS/NZS and is a fraction of the lab and consumable costs and time. The MPN-PCR approach may also represent a viable alternative to other culture-based international standard procedures for *Campylobacter* isolation, such as ISO 17995:2005. Inter-laboratory investigations will further define the diagnostic performance for recreational waters.

Environmental parameter relationship information is essential for accurate hazard identification, mitigation and the calculation of exposure dose response. The application of a dual-method approach to *Campylobacter* enumeration allowed method specific

effects on the identification of environment-pathogen relationships to be evaluated. The results identified the potential for method-specific bias and introduced uncertainty. Further application of dual-method approaches, such as the one implemented in this study, are required to define the total effect of method introduced-bias on evaluation of pathogen-environment interactions.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://www.frontiersin.org/journal/10.3389/fmicb.2015.00074/abstract>

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C.2 Source tracking using microbial community fingerprints: Method comparison with hydrodynamic modelling

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Source tracking using microbial community fingerprints: Method comparison with hydrodynamic modelling



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ABSTRACT

Urban estuaries around the world are experiencing contamination from diffuse and point sources, which increases risks to public health. To mitigate and manage risks posed by elevated levels of contamination in urban waterways, it is critical to identify the primary water sources of contamination within catchments. Source tracking using microbial community fingerprints is one tool that can be used to identify sources. However, results derived from this approach have not yet been evaluated using independent datasets. As such, the key objectives of this investigation were: (1) to identify the major sources of water responsible for bacterial loadings within an urban estuary using microbial source tracking (MST) using microbial communities; and (2) to evaluate this method using a 3-dimensional hydrodynamic model. The Yarra River estuary, which flows through the city of Melbourne in South-East Australia was the focus of this study. We found that the water sources contributing to the bacterial community in the Yarra River estuary varied temporally depending on the estuary's hydrodynamic conditions. The water source apportionment determined using microbial community MST correlated to those determined using a 3-dimensional hydrodynamic model of the transport and mixing of a tracer in the estuary. While there were some discrepancies between the two methods, this investigation demonstrated that MST using bacterial community fingerprints can identify the primary water sources of microorganisms in an estuarine environment. As such, with further optimization and improvements, microbial community MST has the potential to become a powerful tool that could be practically applied in the mitigation of contaminated aquatic systems.

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1. Introduction

Due to extensive population growth, urbanisation and climate change, many urban estuaries suffer from poor water quality, thereby limiting the multiple benefits that they can provide to the community (Bernhard et al., 2003; Lipp et al., 2001; Mallin et al., 2000). For example, faecal microbe contamination is the leading cause of contamination in coastal waters in the USA (Burton Jr. and Pitt, 2001) and undermines the use of these systems for

recreational and commercial activities (Gourmelon et al., 2007; Green et al., 2011; Walters and Field, 2009). Evidence-based understanding of the major sources of pollution (including faecal contamination) in aquatic systems is critical for the development of sound management strategies (Scott et al., 2002; Simpson et al., 2002).

Source apportionment is a method of identifying the sources of water and/or contamination contributing to a specific environmental system (Chen et al., 2012). It is commonly conducted using physical, chemical and biological markers (Burns et al., 2001; Christophersen et al., 1990; Hooper et al., 1990; Jiang et al., 2015, 2007; Simpson et al., 2002). However, the use of these can be problematic, especially when markers are not entirely source

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specific (e.g. Ahmed et al., 2012; Aslan and Rose, 2013; Green et al., 2014; Odagiri et al., 2015) and when multiple sources within a system have similar marker concentrations. To overcome this problem, studies have suggested using a multiple lines of evidence approach to discern the source of contamination. Hooper et al. (1990) and Kim et al. (2015) are two of the many examples where multiple simultaneous measurements are used to characterise water sources; these measurements are then fed into end-member models, or mixing models, to discern the sources of water which exist in a specified system. While the majority of end-member models have focussed on using physical or chemical measurements to characterise source waters, it is also possible to use microbial community profiles as input into these models (Bowers et al., 2011; Henry et al., 2016; Neave et al., 2014).

Microbial source tracking (MST) using microbial communities relies on (1) each source having a distinct microbial community composition, or 'fingerprint', and (2) that the source contributions to an environmental sample can be back-calculated by comparing its fingerprint to a range of source fingerprints (Korajkic et al., 2015; Unno et al., 2010). However, this method has received little attention in the literature (Henry et al., 2016; Neave et al., 2014), with no real on-ground evaluation of the technique using independent datasets. Before the outputs of microbial community MST approaches can be used to develop strategies to manage the contamination of aquatic systems, the ability of this technique to accurately determine the origins of contamination must be determined.

One independent method that can be used to evaluate the results from microbial community MST in aquatic systems is hydrodynamic modelling. For decades, hydrodynamic models have been instrumental in describing the transport of various pollutants (Bedri et al., 2013; de Brauwere et al., 2014; Hipsey et al., 2008; Hoyer et al., 2015; Liu et al., 2006; Liu and Chan, 2015; Salomon and Pommepuy, 1990). A well calibrated hydrodynamic model can be used with tracers to understand the sources of water at particular locations along the river reach. Therefore, it was hypothesized that the results of such a model could be used to predict water source loadings, just as microbial community MST can discern sources. Comparing the loadings predicted by microbial community MST and the loadings from a well-calibrated hydrodynamic model would be a first in the literature.

The overarching aim of this paper was therefore to evaluate the water source contributions of microbial contamination in an urban estuary identified using microbial community MST, by comparing them to the transport and mixing of tracers using a 3-dimensional hydrodynamic model. We do this by using bacterial community fingerprints to identify major water sources contributing to microbial contamination in an urban estuary. The Yarra River estuary, an urban, stratified salt-wedge estuary in South-East Australia that is known to have high levels of microbial contamination (Daly et al., 2013), was used as a case study. Comparison of microbial community MST for 'water source tracking' with hydrodynamic modelling provides a sound evidence base for extending microbial community MST to 'faecal source tracking' in the future.

2. Methods

2.1. Study site

The Yarra River is located in South-East Australia, in the state of Victoria (Fig. 1). The catchment is approximately 4000 km² and the river flows from the Great Dividing Range to Port Phillip Bay for approximately 240 km, through the urban centre of Melbourne (Brizga et al., 1995). The estuarine region of the river is 22 km long, extending from Dights Falls (Fig. 1) to Port Phillip Bay with the

estuary classified as a highly stratified, salt-wedge estuary (Beckett et al., 1982).

Morell Bridge (Fig. 1) was selected as the location within the estuary where the water source contributions were quantified (i.e., it is defined as the 'sink' site). The water at Morell Bridge is a mixture of sources, namely: (1) freshwater from the Yarra River upstream of Dights Falls; (2) freshwater from Gardiners Creek, a highly urbanized creek that is the second largest contributor of flows to the estuary (the Yarra River is the largest contributor); (3) stormwater and dry weather discharges from a number of drains discharging directly into the estuary; and (4) marine water from Port Phillip Bay that enters the estuary due to tidal influences. Morell Bridge is a site that is heavily used for recreational activities (e.g. rowing and boating), and as such, microbial contamination at this site could have particularly significant public health consequences.

2.2. Bacterial community profiling and MST

2.2.1. Sample collection

Between November 2012 and August 2013, water samples were collected from the Yarra River at Kew (19 samples), Gardiners Creek (14 samples), Hawthorn Main Drain (7 samples), and Port Phillip Bay, taken at the West Gate Bridge (2 samples) (Fig. 1). Due to the large number of urban stormwater drains discharging into the Yarra River (approximately 208), it was not feasible to characterise the bacterial community profiles of all urban stormwater drains. As such, Hawthorn Main Drain, one of the largest drains (as discussed in Daly et al., 2013) was used to represent the bacterial community profile of all urban stormwater drains. The samples collected below West Gate Bridge were taken to represent bacterial diversity within Port Phillip Bay. Samples taken from Kew represented bacterial contributions from the freshwater reaches of the Yarra River.

30 water samples were collected at the 'sink' location (Morell Bridge), between January 2013 and July 2013. Some of these samples were grab samples, and others were volume-weighted composite samples (sampling schedule provided in Table S1). The volume-weighted composite samples were collected using an auto-sampler triggered by an Acoustic Doppler Current Profiler (SonTek) that estimated the water fluxes using the average cross-sectional velocity and area. Volume-weighted composite samples were collected during wet weather events, the longest of which lasted for 24 h. All samples were collected in clean and sterile bottles and were taken at approximately 0.2 m depth and 2 m away from the bank. Samples were placed on ice and transported to the Monash University Environmental and Public Health Monitoring laboratory for sample processing. Samples were processed within 4–6 h of collection.

2.2.2. Sample processing

A volume of 1 L for each water sample was filtered and microbes were collected on 5 × 0.22 µm filters (Millipore). After filtration was complete, the individual filters were combined. A PowerMax DNA isolation kit (MoBio) was used to isolate the genomic DNA from the filters. The filters were stored at –80 °C for 2 h, and then crushed with sterile spatulas to form a fine powder. The powder was then transferred into the processing tubes (MoBio) and genomic DNA extraction conducted with the following modifications to manufacturer's instructions. Samples were resuspended in buffer C1 and incubated at 65 °C (which included shaking) for 45 min. Prior to elution, the captured DNA was incubated in 1.5 mL buffer C5 (1.5 mL) for 10 min at room temperature. DNA was then eluted and stored at –20 °C for less than approximately 2 weeks prior to sequencing. Pre-defined DNA extraction controls were not included at the time of processing. However, potable water controls

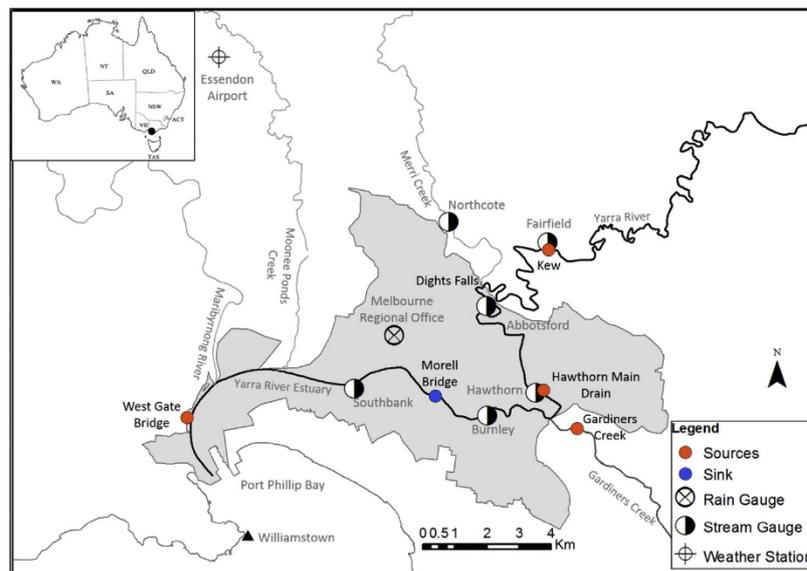


Fig. 1. Yarra River estuary showing the four main water sources (Yarra River samples taken at Kew, Hawthorn Main Drain, Gardiners Creek, and the Port Phillip Bay samples taken at West Gate Bridge) and the sink (Yarra River at Morell Bridge). Also shown are the locations of the upper extent of the estuary (Dights Falls), stream gauges, rain gauge and weather station. Insert shows the location of the Yarra River estuary in Australia. The grey shading represents the urban stormwater catchments which drain directly into the Yarra River estuary.

processed concurrently with the estuarine samples indicated no significant contamination had been introduced.

The V3-4 region of the 16S rRNA genes were amplified before sequencing. 50 μ L PCR reactions were constructed in triplicate: 5 μ L of genomic DNA (negative controls contained ultrapure water), 5 μ L 10 \times PCR buffer (Roche), 0.3 μ L of Taq polymerase (Roche), 10 μ L each of 1 μ M forward primer (5'-TCGTCGGCAGCGTCAGATGTGATAAGAGACAGCTACGGGNGGCWGCAG) and reverse primer (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC), ultrapure water to 50 μ L. The reactions were subject to cycling with initial denaturation for 2 min at 98 $^{\circ}$ C, followed by 25 cycles of denaturation (98 $^{\circ}$ C, 30 s), annealing (55 $^{\circ}$ C, 30 s) and extension (72 $^{\circ}$ C, 30 s). A final extension was carried out (72 $^{\circ}$ C, 5 min). The products of these reactions were purified using 0.6 vol of Ampure XP (Beckman Coulter) as per the manufacturer's instructions and eluted into 30 μ L of ultrapure water. Purified amplicons were subjected to a secondary PCR amplification to facilitate Illumina-compatible adapters and index sequences: 5 μ L of the forward and reverse primers from the Illumina Nextera XT DNA sample Preparation Kit (Illumina), 25 μ L of KAPA HiFi HotStart ReadyMix (KAPA), ultrapure water to 50 μ L. The triplicate reactions were combined and purified as stated previously. No amplified product could be observed within negative control reactions. Purified amplicons, with the exception of negative control reactions, were sequenced using an Illumina MiSeq with a 600c V3 Reagent Kit (Illumina) as per the manufacturer's instructions.

2.2.3. Quality filtering and OTU picking

Sequencing data were de-multiplexed using MiSeq Reporter V2.4.60 and quality trimmed and adapter filtered using Trimmomatic (Bolger et al., 2014). Reads were filtered to remove adapter sequences and trimmed to remove any terminal stretches of bases at or below Q30. Reads shorter than 180 bp were discarded. Pre-

cluster read pairs were assembled to produce single reads using PEAR (Zhang et al., 2014). The assembled reads were analysed using the QIIME 1.8.0 open-reference OTU picking workflow with UCLUST for *de novo* OTU picking and the GreenGenes 13.8 release for the reference and for taxonomic identity assignment (Caporaso et al., 2010). Taxonomic profiles for Morell Bridge samples were generated using the summarize_taxa.py and plot_taxa_summary.py scripts. β -diversity was investigated using the jackknife_beta_diversity.py script and unweighted unifrac analysis (Lozupone et al., 2011). Data for all samples is available on the Sequence Read Archive (<http://www.ncbi.nlm.nih.gov/sra/>) project reference PRJNA309092.

2.2.4. MST

OTU tables derived from quality filtering and OTU picking were applied to the SourceTracker tool as described by Knights et al. (2011). SourceTracker compares the community profiles in the 'sources' to those of the 'sink', using Bayesian methods to identify the extent of contribution of each source to the sink (as demonstrated in Fig. 2). The tool was used to identify the percentage contribution of each of the four potential sources sampled at the sink location (i.e. Morell Bridge) on 30 sampling occasions. The four sources were: the Yarra River upstream of Dights Falls (at Kew), Port Phillip Bay, Gardiners Creek and urban stormwater drains (represented by Hawthorn Main Drain). All samples, irrespective of time of collection, from each of the specified sites were used to generate the unique site specific bacterial fingerprint (i.e. all individual samples were made available to SourceTracker to create the unique site specific bacterial fingerprint). Default conditions were applied (rarefaction depth 1000, burn-in 100, restart 10, alpha (0.001) and beta (0.01) dirichlet hyperparameter) when running SourceTracker. The analysis was run three times, as per Henry et al. (2016) and the average across the three runs was calculated.

To understand the differences in microbial composition

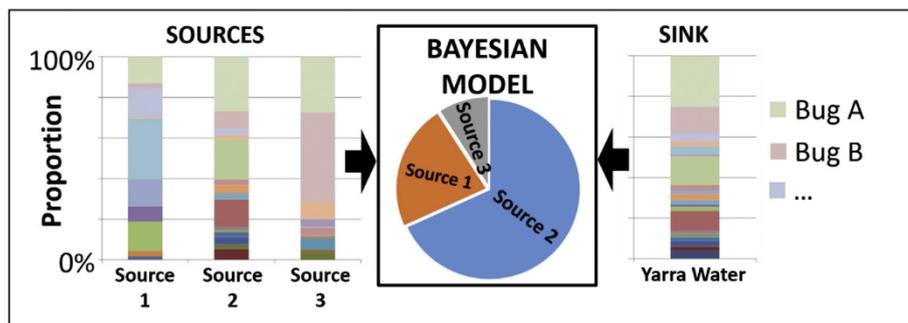


Fig. 2. Visual representation of SourceTracker (Henry et al., 2016). Step 1, community profiles are obtained for each source sample (e.g. Source 1, Source 2 and Source 3) by extracting and sequencing DNA from water samples. Step 2, community profile for a sink sample is obtained from a site where the sources of contamination is unknown by extracting and sequencing DNA from water. Step 3, the source community profiles are compared to the sink profile using a Bayesian model (e.g., Sourcetracker; Knights et al., 2011), which compares each microbe and its relative proportion to estimate sources in the sink. In this hypothetical example, the sink sample contains 20% of Source 1, 70% of Source 2 and 10% of Source 3. In this paper, we test the concept of microbial community MST using 'water source tracking'; as such, our source samples are water samples.

between sources, and to explore which bacteria were driving the source attribution, taxonomic fingerprints used by SourceTracker were extracted and presented for each source. This was conducted as follows: (1) SourceTracker was run, (2) the raw OTU counts selected by SourceTracker were recorded for each source, and for each of the 30 Morell Bridge Sink samples, (3) the raw OTU counts for each source were averaged over the 30 Morell Bridge Sink samples, and (4) averaged OTU counts were converted to relative proportions and presented as bar charts. It is noted that while only average profiles are presented here, the source fingerprints used by SourceTracker will be different for each of the Morell Bridge Sink samples; that is to say, the OTUs selected for a particular source by SourceTracker for the first Morell Bridge Sink sample will naturally be different to those of the second sample due to the random nature of the rarefaction process. Detailed data pertaining to the OTUs contained within each source fingerprint are provided in the supplementary materials. Comparison of the taxonomic composition of the source community fingerprints was conducted using the QIIME `compare_taxa_summaries.py` script using a two-sided Spearman correlation with 999 permutations for calculation of the non-parametric p -value.

2.3. 3-Dimensional hydrodynamic modelling

2.3.1. Model development

A 3-dimensional hydrodynamic model of the Yarra River estuary was developed using the Tuflow FV modelling platform (BMT WBM, 2012). The model was used to independently compare the results of microbial community MST through the simulation of the transport and mixing of tracers within the estuary. It is an updated version of the model previously presented in Bruce et al. (2014). The first update was a higher resolution mesh, which provided multiple cells across the width of the estuary (typically four, but no less than two cells) instead of just a single cell. The purpose of this was to make the model truly 3-dimensional. Secondly, the number of vertical Sigma layers above the -1 m AHD was increased from four to eight to enable better resolution of the model at the sharp halocline layer. Finally, an additional 208 cell boundary conditions were added to include the flow contributions from 208 stormwater drains that discharge urban stormwater into the estuary.

Flow rates, water levels, water temperature and salinity concentrations measured at 6 min time-steps were used as boundary conditions for the model. The upstream boundary condition was defined using measured flow rates of the Yarra River (measured at a

stream gauge at Fairfield; Fig. 1) and measured flow rates of Merri Creek (measured at a stream gauge at Northcote; Fig. 1). Water temperatures and salinity concentrations measured at the gauge at Fairfield on the Yarra River were used for both inputs. The downstream boundary condition was defined using measured water levels at a stream gauge at Southbank (Fig. 1) as well as measured water temperatures from Port Philip obtained from the Integrated Marine Observing System (measurement location shown in Fig. 1). Salinity at the downstream boundary was kept constant and was assumed to be equivalent to that of seawater (i.e. 35 psu).

Boundary conditions were also defined at points where stormwater and tributaries discharge into the estuary. For the additional inputs along the Yarra River estuary, the Gardiners Creek boundary was defined by flow rates, water temperature and salinity measured in Gardiners Creek while the discharges from the 208 stormwater drains were modelled using the rainfall-runoff model component of the model for microorganism prediction in stormwater – MOPUS (McCarthy et al., 2011). The modelling process for this is outlined in Jovanovic et al. (2015). Missing periods in temperature and salinity readings for Gardiners Creek were infilled by correlating water temperature and salinity to 2-hour moving average air temperatures ($\rho = 0.86$, $p < 0.001$) and instantaneous flow ($\rho = -0.72$, $p < 0.001$), respectively. Temperature and salinity concentrations for the 208 stormwater drains were also produced by correlating water temperature to 2-hour moving average air temperatures ($\rho = 0.86$, $p < 0.001$), and salinity to instantaneous flow rate ($\rho = 0.96$, $p < 0.001$). These correlations were developed using measurements obtained at Hawthorn Main Drain. Additional boundary conditions needed for the atmospheric module of the TUFLOW FV model included wind speed, precipitation, air temperature, relative humidity and cloud cover. The precipitation, air temperature, relative humidity and cloud cover data were collected at the Melbourne Regional Office and the wind speed data were collected at Essendon Airport by the Bureau of Meteorology (<http://www.bom.gov.au/climate/data/>).

2.3.2. Model calibration and verification

The hydrodynamic model was tested using measured data, which included: (1) water levels at Abbotsford, Hawthorn, Burnley and Southbank measured at 6-minute time-steps, (2) flow velocities measured using two Acoustic Doppler Current Profiler (ADCPs) devices deployed at Morell Bridge (one in the shallower part of the cross section and the other at the position of thalweg) and (3) 84 depth profiles of temperature and salinity (to validate the

distribution of salinity and temperature through the water column). The 6-minute time-step water level data was obtained from Melbourne Water while all other calibration data were obtained by the authors over two years of monitoring (2012–2014). The ADCPs recorded all three components of the velocity at one-minute intervals. The salinity and temperature at Abbotsford and Morell Bridge were monitored continuously using combined electrical conductivity (EC) and temperature sensors, which took measurements at 6-minute intervals. There was one EC and temperature sensor at Abbotsford, positioned at a fixed point in the water column (roughly 20 cm below the surface of the water under dry-weather conditions). At Morell Bridge, two EC and temperature sensors were deployed. One was attached to a floating device, obtaining measurements from the freshwater layer at all times (at roughly 20 cm below the water surface), and a second sensor was attached to the ADCP device in the deepest part of the cross-section, to enable measurements of EC and temperature at the bottom of water column (i.e. the salt-wedge layer). Depth profiling was conducted at Abbotsford, Hawthorn, Morell Bridge and Southbank. At each of these locations, a multi-parameter probe was used to measure the salinity and temperature at 30–50 cm intervals vertically through the water column. The Nash-Sutcliffe Coefficient, or E (Nash and Sutcliffe, 1970), was used as a model fit statistic.

2.3.3. Tracer test and source apportionment

Four tracers were included in the hydrodynamic model at the Yarra River upstream of Dights Falls (at Kew), Hawthorn Main Drain, Port Phillip Bay and Gardiners Creek to identify the contribution of these water sources to the Morell Bridge water column. Two scenarios were tested. In the first scenario, each of the four water sources had the same tracer concentration (1397 MPN/100 mL). In the second scenario, tracer concentrations were scaled to reflect differences in the bacterial density of each water source, given that bacterial densities in seawater, freshwater and stormwater are known to vary (Whitman et al., 1998; Wright and Coffin, 1983), and results of the SourceTracker analysis will be affected by these differing densities. Tracer concentrations were scaled according to the mean *E. coli* levels measured at each water source over the sampling period between November 2012 to August 2014 (measured using the Colilert MPN method outlined in AS4276.21-Method 21 (Standards Australia, 2005)). Whilst *E. coli* only represents the level of faecal contamination, it was the most appropriate measure of bacterial loadings that was available for this study. Future work should use only faecal bacteria in the community profiles as input to SourceTracker or use another parameter to scale the hydrodynamic model inputs (i.e. use molecular methods for total bacteria counts rather than *E. coli*). Scaled tracer concentrations were: 1397 MPN/100 mL for the Yarra River, 4487 MPN/100 mL for Gardiners Creek, 7789 MPN/100 mL for Hawthorn Main Drain and 734 MPN/100 mL for Port Phillip Bay. For both scenarios, the concentrations remained constant over time.

The concentration of each of the four tracers in the Yarra River at Morell Bridge (equivalent to 20 cm depth from the top of the water column) was identified at the same date and time that water samples were taken below Morell Bridge for community profiling and SourceTracker analysis between January and July 2013. The quantities of the four tracers at Morell Bridge on these occasions were used to calculate the percentage contribution of each of the four sources to the water column at Morell Bridge.

2.4. Data analysis

We explored the main factors contributing to temporal variability in the source contributions by comparing them to

instantaneous flow rates in the Yarra River at Kew (measured at the stream gauge at Fairfield; Fig. 1), Gardiners Creek and Hawthorn Main Drain, salinity concentrations at Morell Bridge and tidal fluctuations measured at Williamstown (Fig. 1). The data from Williamstown was provided by the Bureau of Meteorology (<http://www.bom.gov.au/climate/data/>). The data (both source contributions and flow rates) were checked for normality using the Shapiro-Wilk test (Shapiro and Wilk, 1965) ($\alpha = 0.05$). Due to the non-normality of the data ($p < 0.05$; Table S3), the Spearman Rank Correlation Coefficient (Spearman, 2010) ($\alpha = 0.05$) was used to identify the strength of the correlation between flow rates and the water source contributions.

Water source apportionments obtained using microbial community MST and the hydrodynamic tracer study were compared using the Spearman Rank Correlation Coefficient ($\alpha = 0.05$).

3. Results and discussion

3.1. Bacterial community profiles and fingerprints

3.1.1. Bacterial community profiles for sink

Bacterial community profiles were generated for the 30 Morell Bridge (sink) samples. Four bacterial families were found to account for the majority of reads at this location: *Comamonadaceae* (3.3%–41.5%), *Flavobacteriaceae* (1.3%–67.42%), *Actinomycetales* ACK-M1 (0.8%–28.4%) and *Rhodobacteraceae* (0.5%–29.7%) (Fig. 3). These families have been previously identified within estuarine microcosms and their abundance has been associated to changes in salinity gradients along these system (Campbell and Kirchner, 2013; Ortmann and Santos, 2016; Wei et al., 2016).

3.1.2. Bacterial fingerprints for sources

Fig. 4 presents the average source fingerprints used by SourceTracker to determine their relative contribution in each of the 30 sink samples. The fingerprints of Port Phillip Bay and the Yarra River reflect those previously reported for marine and freshwater environments, respectively (Henry et al., 2016; Kasalický et al., 2013; Mason et al., 2016; Wu and Hahn, 2006). Within Gardiners Creek and Hawthorn Main Drain, *Flavobacterium* (28.9 and 35.2% respectively) and *Comamonadaceae* (11.5% and 10.3%) constituted >10% of the total bacterial community. Both families are ubiquitous within rainwater and freshwater environments (Fisher et al., 2015; Kaushik et al., 2014; Shanks et al., 2013). Previous microbial community analyses have suggested that the presence of *Acinetobacter*, among others, may be indicative of stormwater flows and contamination (Fisher et al., 2015; Shanks et al., 2013). The presence of this genus at high levels within Gardiners Creek and Hawthorn Main Drain samples provides further support for this genera as a source specific marker. It is important to note, that there is a low correlation between the OTUs observed between Gardiners Creek and Hawthorn Main Drain ($\rho = 0.098$, $p = 0.034$), potentially indicating that adequate differentiation between these sources was possible by SourceTracker. Other than between Gardiners Creek and the Yarra River at Kew ($\rho = 0.023$, $p = 0.59$), correlations between the other sources were statistically significant ($p < 0.05$) but only weakly positive (ρ between 0.13 and 0.37).

3.2. Water source apportionment estimates using microbial community MST

There is wide variability in the water source apportionments determined by microbial community MST for the water samples taken at Morell Bridge between January and July 2013. Contributions to the bacterial community in the estuary at Morell Bridge from the freshwater reaches of the Yarra River ranged from 0.3% to

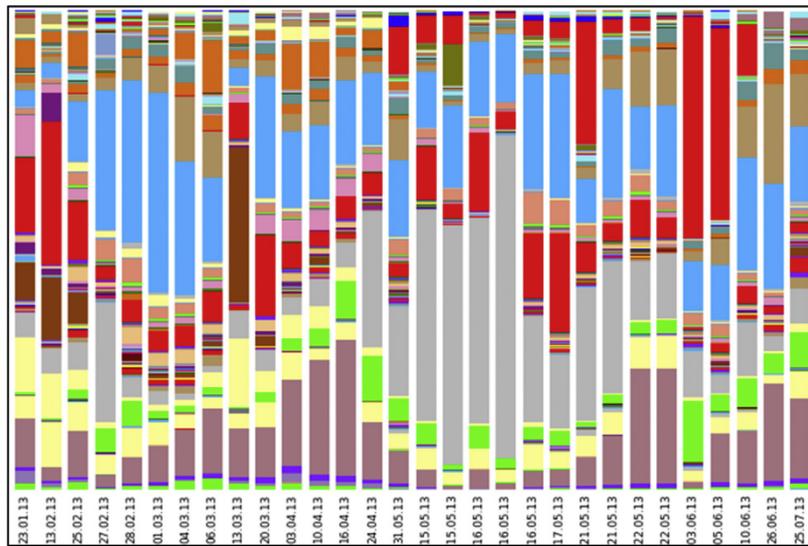


Fig. 3. Bacterial community profiles based on relative OTU abundance, at the family level, for Morell Bridge collected from January–July 2013. The full taxonomic legends for each sample are presented in the Supplementary Materials.

88.0% (RSD; relative standard deviation ranging from 0.9% to 136.4%), Gardiners Creek from 0% to 90.2% (RSD from 1.0% to 316.2%), Hawthorn Main Drain from 0.0% to 13.8% (RSD from 7.5% to 316.2%) and Port Phillip Bay from 0.0% to 64.8% (RSD from 1.1% to 16.1%). The percentage contributions and standard deviations are provided in Table S4 in the supplementary material.

SourceTracker results demonstrated a strong relationship between estuary hydrodynamics and the contribution made by each of the four water sources to the bacterial community in the estuary's water column (Fig. 5). At the beginning of the period shown in Fig. 5 (13/2/2013), microorganisms from Port Phillip Bay dominate the estuarine bacterial community. This particular sample was collected during high tide when flows in the Yarra River and Gardiners Creek were low (Fig. 1). It is likely that, at this point in time, the salt wedge had penetrated far upstream. Indeed, the salinity concentration in the top 20 cm of the Morell Bridge water column sample was 8.9 psu on this date, which is equivalent to approximately 30% seawater. Given the highly stratified nature of the Yarra River estuary, having such a high proportion of sea water in the top 20 cm of the water column indicates that there was sufficient tidal energy to mix a significant amount of the salt wedge with the overlying freshwater layer. This further suggests that large amounts of marine microorganisms were transported upstream to Morell Bridge.

The contribution of water from Port Phillip Bay to the bacterial community in the Yarra River at Morell Bridge decreased on 25/2/2013. There was a significant increase in flow in the Yarra River during this period. This would have not only led to the transport of greater bacterial loads downstream into the estuary, but concurrently would have prevented the salt-wedge (and marine microbes carried by the salt-wedge) from penetrating as far upstream as it did previously on 13/2/2013. Indeed, salinity levels were lower (approximately 5 psu) at this point in time. The appearance of Hawthorn Main Drain as a contributor to the estuarine bacterial community on 25/2/2013 could be due to a minor rainfall event that occurred between 18/2/2013 and 20/2/2013 (1.6 mm). This would have led to the discharge of stormwater into the estuary from

adjacent stormwater drains. The substantial contribution from Gardiners Creek to the bacterial community in the Yarra River at Morell Bridge on 28/2/2013 coincided with an increase in flow rate in Gardiners Creek, associated with a large amount of rainfall (approximately 34.8 mm recorded at the Melbourne Regional Office rainfall gauge by the Bureau of Meteorology; Fig. 1) within the previous 48 h (Fig. 5).

During the period between 1/3/2013 to 13/3/2013, the contribution from the freshwater reaches of Yarra River and Gardiners Creek to the estuarine bacterial community decreased. In this 12-day period, less than 1 mm of rainfall was recorded in the Yarra River catchment. Concurrently, a sharp decrease in flow rates was observed in both the Yarra River and Gardiners Creek (Fig. 5). This sharp decrease in Gardiners Creek flow, correlated with reduced contributions from Gardiners Creek to the bacterial community in the Yarra River at Morell Bridge. The decrease in the contribution of the Yarra River to the bacterial community at Morell Bridge was more gradual compared to that of Gardiners Creek during this period. This result is consistent with the hydrograph of the Yarra River upstream of the estuary, which also displayed a gradual decrease compared with the Gardiners Creek hydrograph (Fig. 5). This further emphasized the strong link between the hydrodynamics of the upstream Yarra River and its tributaries and the composition of the bacterial community in the estuary.

The strong relationship between the hydrodynamics and source contributions to the bacterial community (calculated using microbial community MST) in the estuary was evident throughout the sampling period between January and June 2013. A negative correlation was observed between instantaneous flow rates in the Yarra River at Kew ($\rho = -0.529$, $p = 0.003$), or Gardiners Creek ($\rho = -0.748$, $p < 0.001$), with the contribution from Port Phillip Bay to the estuarine bacterial community (Fig. 6). This suggests that Port Phillip Bay contributes less to the bacterial community in the Yarra River at Morell Bridge when there are higher flows in the Yarra River and its tributaries (Gardiners Creek) entering the estuary. Previous studies have shown that the movement of the salt wedge and tidal energies are largely governed by flows from the

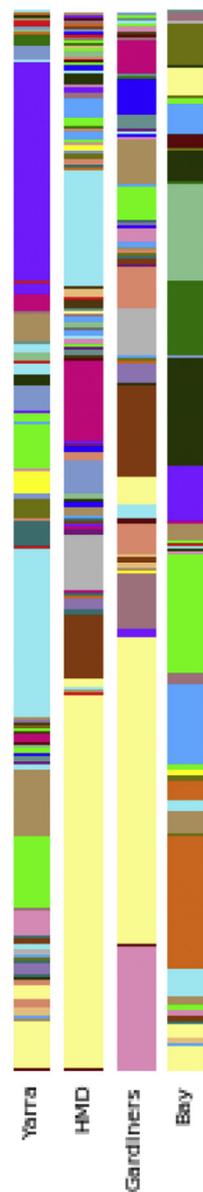


Fig. 4. Bacterial fingerprints used by SourceTracker for source attribution at Morell Bridge. Core bacterial profiles of source communities were based on averaged relative OTU abundance for fresh water contributions from the Yarra River (Yarra), salt water bay intrusions from Port Phillip Bay (Bay), Hawthorn Main Drain (HMD) and Gardiners Creek (Gardiners). The full taxonomic legend is presented in the Supplementary Materials.

upstream Yarra River (Bruce et al., 2014). There is also a strong positive correlation ($\rho = 0.827$, $p < 0.001$) between the salinity at Morell Bridge and the contribution by Port Phillip Bay to the bacterial community at Morell Bridge (Fig. 7). This emphasizes what was previously demonstrated in Fig. 5, indicating that the extent of Port Phillip Bay's contribution to the bacterial community composition in the Yarra River at Morell Bridge was strongly linked to the

movement of the salt wedge and the tidal energies, which are in turn, influenced by the magnitude of flows in the river and Gardiners Creek.

There was a positive correlation between the bacterial source contribution by Hawthorn Main Drain and the flows through this drain (Fig. 6; $\rho = 0.596$, $p = 0.012$). This was expected, as high flows in Hawthorn Main Drain, would lead to a greater amount of the bacterial community in Hawthorn Main Drain being transported downstream to Morell Bridge. There was also a positive correlation between the Hawthorn Main Drain bacterial community source contribution and flows through Gardiners Creek (Fig. 6; $\rho = 0.829$, $p < 0.001$). This result was likely due to the cross-correlation between flows in Hawthorn Main Drain and Gardiners Creek ($\rho = 0.935$, $p < 0.001$). The outlet of Hawthorn Main Drain and the confluence between Gardiners Creek and the Yarra River are less than 1.5 km away from each other. Therefore, when there are wet weather events at Gardiners Creek, flows are also observed at Hawthorn Main Drain (Henry et al., 2015).

3.3. Microbial community MST and hydrodynamic model comparisons

3.3.1. Performance of the hydrodynamic model

The hydrodynamic model accurately predicted the water levels in the Yarra River at Morell Bridge, with Nash-Sutcliffe efficiencies (E) ranging from 0.88 to 0.99. There was also good agreement between the measured and predicted flow velocity components (along the East and North directions) in the top one meter of the water column at Morell Bridge (Fig. 8). The Nash-Sutcliffe efficiency of the prediction of the velocity along the main flow direction (East) was 0.69 at the position of the shallow ADCP while it was slightly higher ($E = 0.86$) at the position of the deep ADCP. The prediction efficiency of the velocity component along the north direction (perpendicular to dominant flow direction) was slightly lower, being 0.49 and 0.69 for the shallow and deep ADCP positions, respectively).

The model's ability to predict the salinity and temperature distributions along the depth of the water column were high ($E = 0.84$ and $E = 0.72$, respectively), and these high efficiencies are also evident in Fig. 8. These temperature and salinity predictions are important because the highly stratified nature of the Yarra River estuary has a significant impact on the transport and mixing of water and pollutants within the estuary. All of the above demonstrates that the model can represent the complex hydrodynamics of the Yarra River estuary and therefore, can be used to trace the transport and mixing of various water sources entering the estuary.

There was a difference between the results of the source apportionment calculated using the hydrodynamic model depending on the boundary conditions (Fig. S1). These boundary conditions were: (1) that bacterial levels at each of the sources are equivalent and constant through time or (2) that bacterial levels at each of the sources are different but remain constant through time. These results indicated potential future improvements to our scenarios for future work; in particular, a complex model could be developed for each of the 208 drains that enter the estuary which represents the within-event and between-event variation of microorganisms common to stormwater systems (Hathaway et al., 2015; McCarthy et al., 2013, 2012, 2007).

3.3.2. Comparison of source contribution results obtained using microbial community MST and the hydrodynamic model

For most bacterial water sources (Yarra River, Gardiners Creek, Port Phillip Bay), there were statistically significant positive correlations ($p < 0.05$) between the magnitude of the water source contribution determined using microbial community MST and the

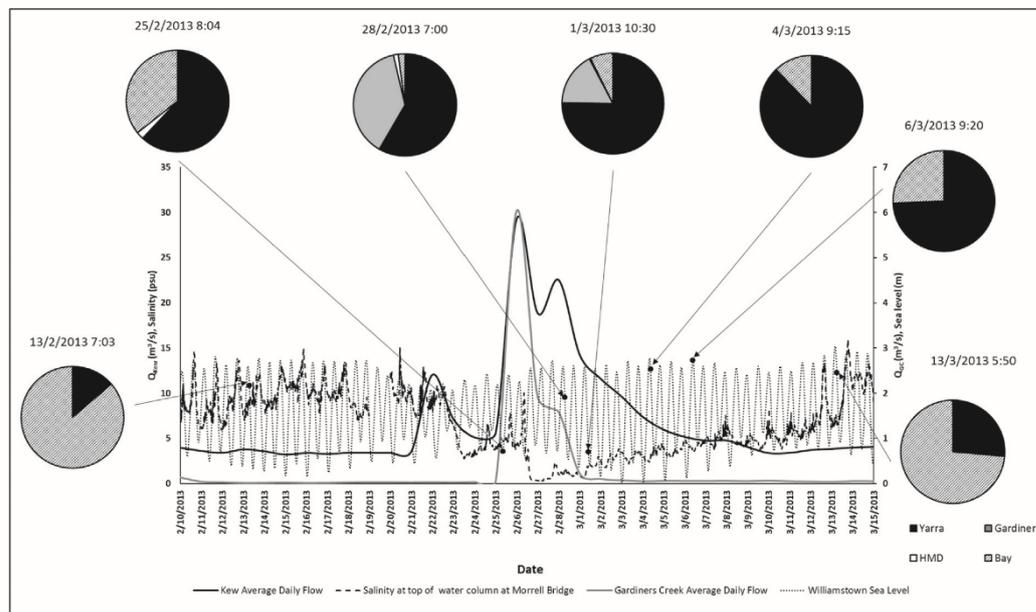


Fig. 5. Flow rates in the Yarra River at Kew (Q_{kew} ; m^3/s) and in Gardiners Creek (Q_{gcc} ; m^3/s), salinity in the top 10 cm of the water column at Morell Bridge (Salinity; psu) and sea level at Williamstown (Sea level; m) (Fig. 1) between 10/2/2013 and 15/03/2013. Morell Bridge bacterial water source apportionment quantified at eight points in time by SourceTracker shown in pie charts. Arrows reference a point on the Williamstown Sea Level curve.

calibrated hydrodynamic model (Fig. 9). This indicates that SourceTracker was successful in discriminating between the microbial fingerprints of the four sources, despite the statistically significant correlations identified between the OTUs selected by SourceTracker for Yarra River at Kew, Hawthorn Main Drain, Gardiners Creek and Port Phillip Bay. This is likely due to the low correlations between the OTUs. Correlations between the Spearman correlation coefficients range from 0.385 ($p = 0.03$) for Hawthorn Main Drain to 0.671 ($p < 0.001$) for Port Phillip Bay. The correlations become slightly more linear (i.e., closer to the 1:1 line) when it is assumed in the hydrodynamic model that bacterial densities in all four water sources differ (Fig. 9). Spearman correlation coefficients range from 0.353 ($p = 0.06$) for Hawthorn Main Drain to 0.682 ($p < 0.001$) for Port Phillip Bay.

Despite the positive correlations between the MST results and the results of the hydrodynamic modelling, there were sometimes discrepancies between the absolute numbers for source apportionment. For example, good agreement was obtained for the contributions from the freshwater reaches of the Yarra River on 26/6/2013 (88% – hydrodynamic model; 85% – microbial community MST), yet poor results were obtained on the 23/1/2013 (73% – hydrodynamic model; 22% – microbial community MST). These discrepancies could be due to uncertainties in both the microbial community MST and in the hydrodynamic modelling. These include: (1) analytical uncertainties in the processing of bacterial communities for MST; (2) sampling uncertainties; (3) temporal variability in bacterial populations in the environment; (4) source specificity and water aging; and (5) uncertainties in the hydrodynamic model. These factors are discussed in more detail below.

3.3.2.1. Analytical uncertainties. Analytical uncertainties associated with the processing, sequencing and analysis of 16S amplicon data have been previously identified and reviewed (Brooks et al., 2015; İnceoğlu et al., 2010; Luna et al., 2006; Sinclair et al., 2015;

Tatangelo et al., 2014; Wesolowska-Andersen et al., 2014). However, the extent to which each process (independently and/or in combination) will bias or alter the bacterial community profile and subsequent source allocation has had limited investigation. As such, we recognise that the bacterial community profiles for the water sources (Yarra River, Gardiners Creek, Hawthorn Main Drain and Port Phillip Bay) and the sink (Yarra River at Morell Bridge) may not be representative of the entire microbial population.

SourceTracker applies a Bayesian model to the amplicon data to generate a unique fingerprint for each source and sink community. To achieve this, the model sub-samples from the total community; represented by 1000 sequences (under default conditions). The proportion contributed by each source to the designated sink sample indicates that a unique fingerprint has been generated. Common sequences are designated and proportioned as 'unknown'. Thus, as the model is able to generate a unique profile for each sample group, through application of a small number of reads, the requirement to capture the total microbial diversity (and the effect of introduced bias) will have limited effect on the output predictions.

3.3.2.2. Sampling uncertainties. As per Harmel et al. (2016), it is well known that the total number of samples, and whether they have been taken during wet or dry weather, will have an influence on our ability to characterise lotic water sources which have high microbial variability (Daly et al., 2013; McCarthy et al., 2012). For example, it is possible that the large discrepancies in contributions from Gardiners Creek to the estuarine bacterial community by the two methods (Fig. 9b) are linked to this source of uncertainty. Indeed, we only obtained 14 samples from this water source during wet and dry weather periods. Collecting a larger number of samples may have improved the accuracy of our microbial characterisations (McCarthy et al., 2008).

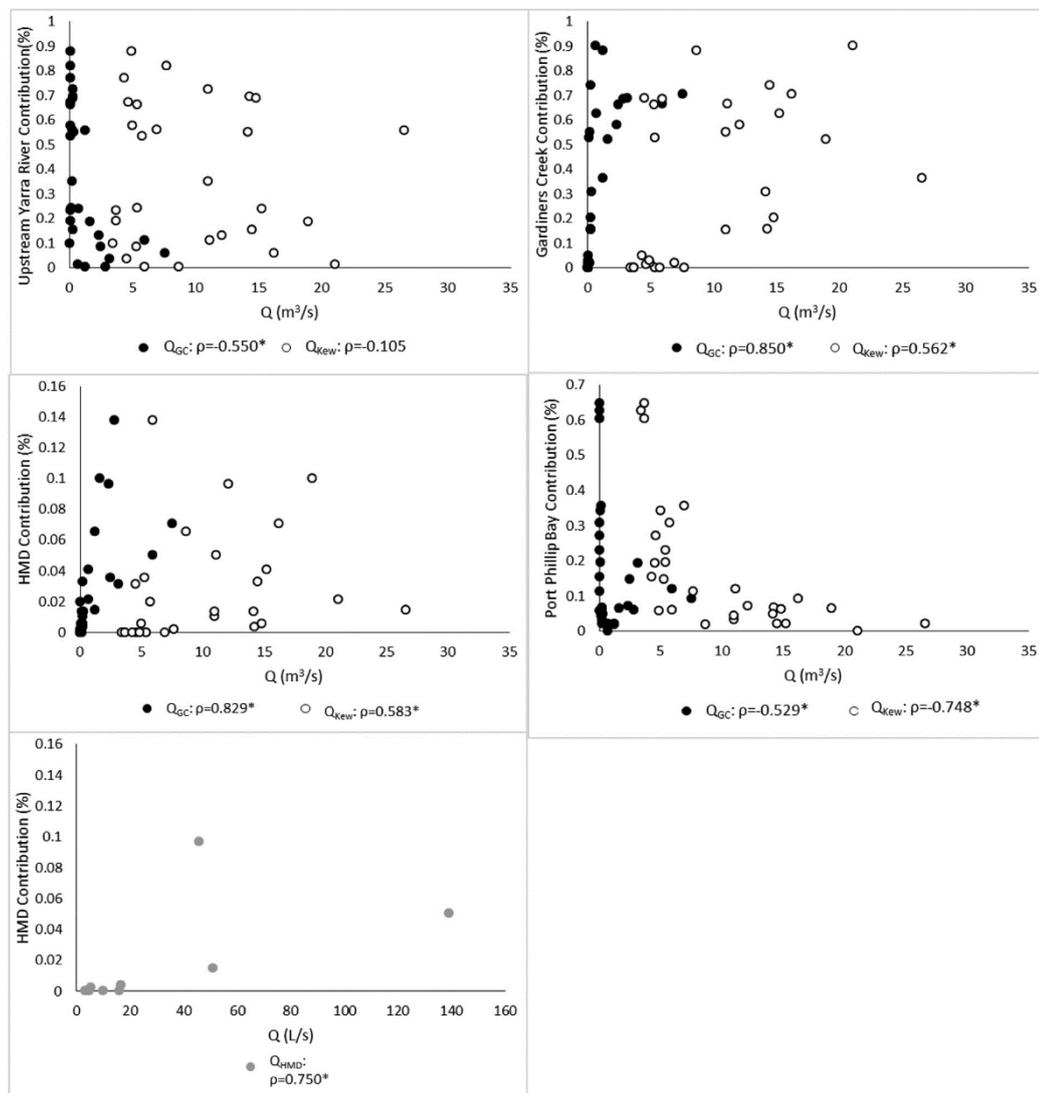


Fig. 6. Relationship between instantaneous flow in Gardiners Creek (Q_{GC}), in the Yarra River at Kew (Q_{Kew}) (upstream of Dights Falls), in Hawthorn Main Drain (Q_{HMD}) (where applicable) and the contribution of the Yarra River at Kew (Upstream Yarra), Gardiners Creek, Hawthorn Main Drain (HMD) and Port Phillip Bay to the bacterial community in the Yarra River at Morell Bridge. Spearman Correlation Coefficients (ρ) shown. Correlation Coefficients that are statistically significant ($p < 0.05$) are indicated by an asterisk.

3.3.2.3. Temporal variability. Furthermore, due to the large number of stormwater drains discharging into the Yarra River upstream of Dights Falls, it is possible that the bacterial fingerprint of the upstream Yarra River water shifts during wet weather events, and begins to resemble urban stormwater. SourceTracker may be mistakenly assigning the contribution by the freshwater reaches of the Yarra River (which has now been mixed with urban stormwater) to Gardiners Creek. As previously discussed, during wet weather events, Gardiners Creek essentially acts as a stormwater drain. This is an important finding for waterway managers.

In future studies, we could better manage this uncertainty source by using samples taken over longer periods of time and using samples taken from both wet and dry weather events, to

characterise the temporal variability in bacterial community compositions. Future work could also consider wet weather source profiles and dry weather source profiles separately, as two different sources in the SourceTracker analysis.

3.3.2.4. Source specificity and water aging. As with all MST techniques, this community-based method needs further research and testing, especially regarding source specificity (i.e., ability of this method to reliably identify between sources which have similar contamination profiles) and persistence issues (i.e., whether the source communities change with time exposed to the sink matrix). Importantly, source apportionment obtained using microbial community profiles was similar to that of a conservative

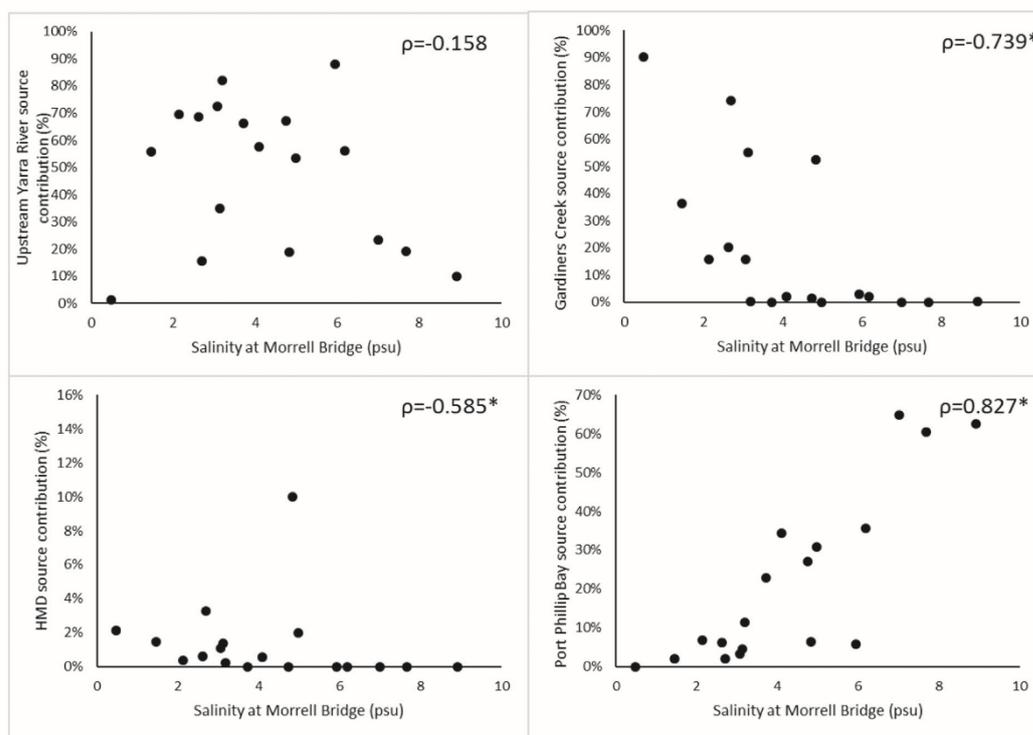


Fig. 7. Relationship between salinity at Morell Bridge and the contribution of the Yarra River at Kew (Upstream Yarra), Gardiners Creek, Hawthorn Main Drain (HMD) and Port Phillip Bay to the bacterial composition at Morell Bridge (based on the results of the microbial community MST). Spearman Correlation Coefficients (ρ) shown. Correlation Coefficients that are statistically significant ($p < 0.05$) are indicated by an asterisk.

hydrodynamic tracer model. The fact that we were able represent multiple sources using microbial community MST suggests that the method has reasonable ability to identify sources which are similar in nature (i.e. the technique could differentiate between Gardiners Creek and Hawthorn Main Drain inputs, yet the catchments of both are similarly urbanized, have similar land-uses and are located in the same city and hence climatic region). The conservative nature of the 3D hydrodynamic model shows promise that the microbial community profiles (or at least those specific source fingerprints extracted by SourceTracker) might be stable enough with time to assign sources (N.B., in this study, average travel times from each source to the sink site were as follows: Yarra River upstream: 22 h; Gardiner's Creek: 8 h; HMD: 11 h).

3.3.2.5. Uncertainties in the hydrodynamic model. Additionally, there are some limitations associated with the hydrodynamic modelling. Firstly, it was assumed that the bacterial diversity and abundance in each of the four sources remained constant over time, which is clearly not the case for these systems (Daly et al., 2013; Jovanovic et al., 2015). The inability to incorporate this large temporal variability into the boundary conditions may also be contributing to the differences between the source apportionment obtained by MST and by the hydrodynamic model.

Secondly, it is likely that the differences in bacterial densities across the four water sources were not adequately incorporated into the hydrodynamic model. The initial bacterial densities in the four water sources were determined using the average *E. coli* concentrations measured at these four locations between January 2013

and June 2013. However, it would be more effective to identify the source bacterial densities using molecular approaches which target the 16S gene (which is what we used to determine the community profiles for MST) rather than *E. coli*, which only indicates the density of one particular faecal-derived bacterium.

Thirdly, our modelling used a conservative tracer to represent the sources of water which are contaminated with microorganisms that are dynamic (e.g. can grow, die, settle, resuspend). Indeed, we would expect that as the water ages, some microorganisms contained in the water would die off, and the 'fingerprint' would change as a result (Wang et al., 2013). It is clear that advances in our ability to model microbial community dynamics and differential microbial kinetics in the aquatic environment could help improve the agreement between the source apportionments provided by microbial community MST and hydrodynamic modelling. This could be done using a particle tracking approach, and each of these particles could be configured to have similar dynamic and kinetic properties to microorganisms.

Addressing the limitations in both the microbial community MST and the hydrodynamic model would likely assist in reducing the discrepancies in the source apportionments provided by the two methods; future work should focus on reducing the uncertainties outlined above. Notwithstanding, the correlations obtained between the hydrodynamic model and the community profiling MST (Fig. 9) indicates that MST using bacterial community profiles will become a powerful tool that enables us to estimate sources based on microbial populations in waterways.

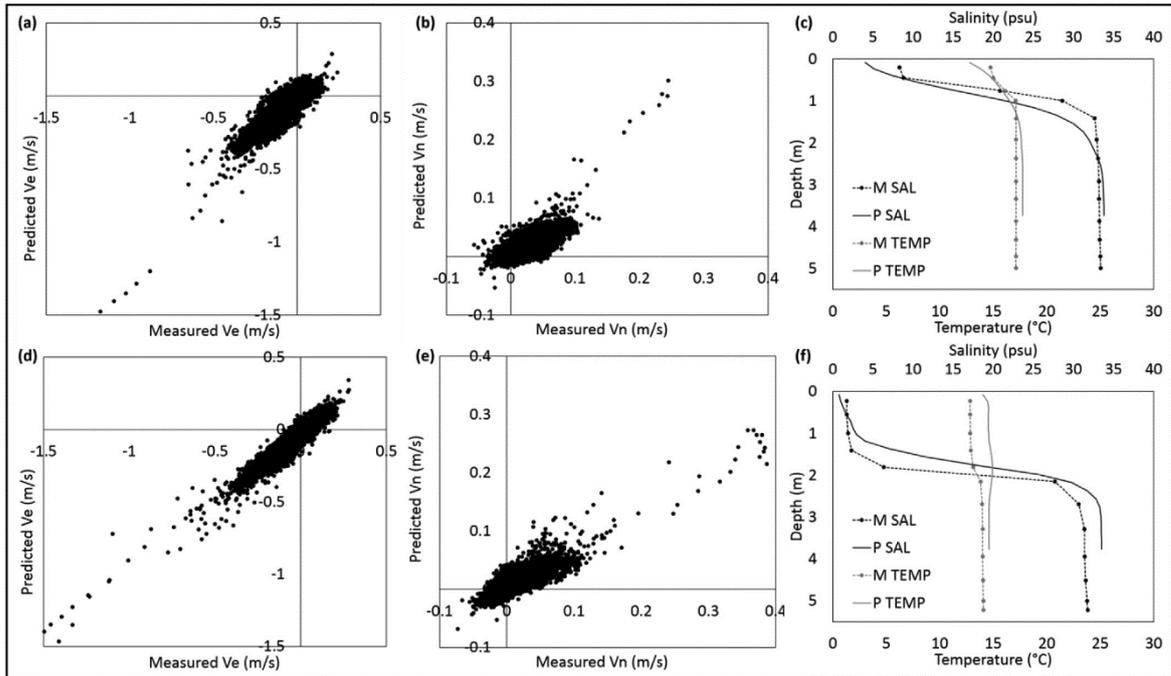


Fig. 8. Hydrodynamic model performance. Comparison between predicted and measured flow velocity components in the top 1 m of the water column at Morell Bridge: east direction – main flow direction at the shallow ADCP (a) and the deep ADCP (d); north direction at the shallow ADCP (b) and the deep ADCP (e). Predicted and measured depth profiles of temperature and salinity concentrations at Morell Bridge on 30/04/13 at 08:15 a.m. (c) and 04/06/13 at 08:40 a.m. (f).

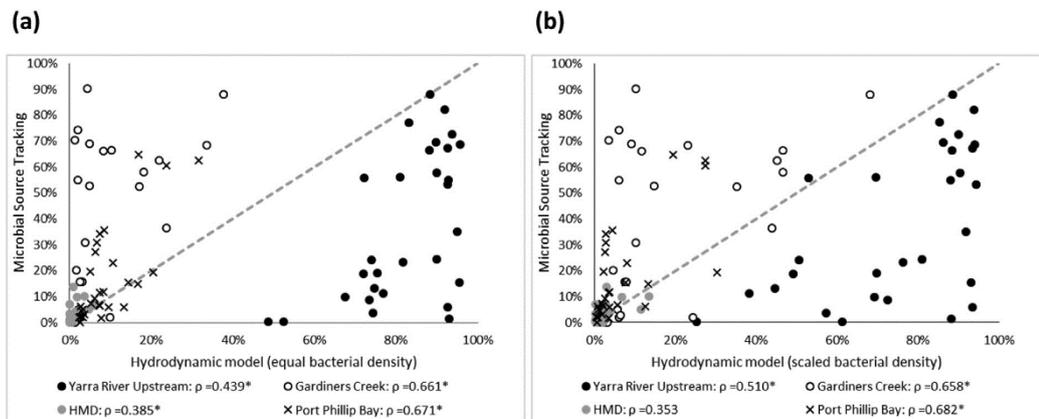


Fig. 9. Comparison of source apportionment obtained using the calibrated hydrodynamic model and MST. (a) presents the comparison when equal bacterial densities were assumed in the hydrodynamic model. (b) presents the comparisons when scaled bacterial densities were assumed in the hydrodynamic model. Spearman Correlation Coefficients (ρ) are provided and asterisks indicate statistically significant ($p < 0.05$) Spearman Correlation Coefficients. Dashed grey line represents 1:1 line.

4. Conclusion

- MST using bacterial community compositions indicated that the contribution of four water sources (Port Phillip Bay, freshwater reaches of the Yarra River, Gardiners Creek and local stormwater drains) to the bacterial composition in the Yarra River estuary can vary considerably depending on the hydrologic conditions in the estuary.

- This should be taken into account when developing strategies for the management of microbial contamination of urban estuaries. The water source contributions identified using the hydrodynamic model had a strong correlation to those determined using microbial community MST, however, there were some discrepancies in the source contributions assigned by both methods.

- Future studies are required to explore whether this error can be reduced either by: (1) understanding, and reducing, the analytical uncertainty sources when producing microbial community profiles for a water sample; (2) understanding the number of samples required to accurately characterise a site's microbial community profile, and hence using an appropriate number of samples as input to microbial community MST which accounts for the variability in bacterial community profiles in dry and wet weather periods; (3) identifying discrete communities for these two different conditions and using them both as input into SourceTracker; (4) adapting the boundary conditions of the tracer in the hydrodynamic model to fluctuate with time, and to accurately reflect bacterial densities at the water sources; and (5) to determine and account for any changes in the source 'fingerprints' as the water ages.
- This study demonstrates that bacterial community profiles can be used in MST to identify the water sources contributing to the bacterial community in an aquatic system, to better understand the origin of microorganisms to inform the management of microbial contamination for the protection of both environmental values and public health.
- There is potential for the demonstrated MST methodology to be used to validate hydrodynamic tracer models of microbial transport and mixing in aquatic environments.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.watres.2016.11.043>

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