Conducting a microbial budget – a literature review

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1 Introduction

Recently, there have been many investigations into locating and mitigating faecal pollutants delivered to the lower Yarra River, primarily from urbanised catchments. These investigations helped us to understand just some of the many problematic faecal sources which feed our receiving water bodies: leaking sanitary and stormwater sewer systems, illegal sanitary connections to the stormwater system, ineffective septic tank drainage zones and poor disposal and storage of waste organic matter.

Whilst these investigations were able to improve the quality of the system in the local vicinity of the identified faecal source through mitigation methods, there is some doubt about whether significant improvements will be seen in the Yarra River estuary, considering: the size of this water body, its large number of known and unknown sources of faecal contamination and processes which control the level of this contamination. However, it is a safe assumption that the human health risk to recreational users of the Yarra River estuary are reduced when these identified sources are located and mitigated, but the question is really "to what extent is this risk reduced?". To answer such a question for any water body, it is necessary to understand (1) the sources of faecal contamination, (2) the processes which take place within this water body and (3) the effectiveness of the mitigation options employed to control the sources which enter this water body.

This project aims at understanding the microbiological improvements (and possibly ecosystem health improvements) in receiving waters which are possible as a result of: (1) intensive microbial source tracking and associated mitigation, (2) in-stream remediation efforts and (3) installation of stormwater treatment technologies. However, to fully understand the improvements made by these options, we have to identify sources of faecal contamination, sinks and die-off processes, plus runoff treatment effectiveness. As such, to achieve the above aim, two specific objectives need to be met:

(1) calculate a mass balance of selected microorganisms for a defined flowing water body; and,
(2) experiment with treatment and mitigation options in order to identify the extent of the changes in levels of those selected microorganisms.

There are a number of hypotheses which need to be tested and examined in order to achieve this aim and associated objectives. However, until a thorough literature review has been completed there are too many hypotheses which could be made. As such, this literature review was undertaken for an array of purposes, but ultimately it will aim at increasing the overall accuracy of the project by ensuring the methodology of the project is adequate for the acceptable level of uncertainty, whilst keeping the number of experiments required for the project's success to a minimum (which will help reduce the costs of the project).

There are a number of different research questions which, if possible, need to be answered by this literature review. However, it is unlikely that this will be sufficient and, as such, it is most probable that small experiments will be required to adequately complement the answers to all of the following questions:

1. Which microorganisms should be monitored?
2. What are the sinks of microorganisms in water systems?
3. What are the sources of microorganisms in water systems?
4. What other water quality and quantity data should be collected during the monitoring?
5. How can the accuracy of the monitoring program be maximised?
6. What are some novel mitigation options which could be tested for this project?

Whilst the term "bacterial" budget was coined at the start of this project, the term "microbial budget" will be used in preference. This is mainly because the title should not restrict this literature review to just bacteria since it is believed that it would be beneficial to understand more than just a mass balance of bacteria, but also other microorganisms (including viruses).

1.1 Mass balances and budgets

Although the above questions will be answered by the literature review, one of the preliminary tasks of this review will be to search for any other studies which have attempted to conduct a similar project to the one planned. Although no study was found which was very close in methodology, some information was thought relevant to the current study and might be transferable from similar projects which have looked at using different water quality constituents.

Whilst a number of sediment budgets (e.g. Hossain and Eyre, 2002; Eyre *et al.*, 1998; Allmendinger *et al.*, 2007) and nutrient budgets (e.g. Jaworski *et al.*, 1992; McKee and Eyre, 2000; McKee *et al.*, 2000; Eyre and McKee, 2002; Witek *et al.*, 2003; Zhiliang *et al.*, 2003; Boynton *et al.*, 2008) have been reported in the literature for different water systems, only a few report on microorganism budgets or mass balances (e.g. Canale *et al.*, 1973; Urchin and Weber, 1983; Wilkinson *et al.*, 1995; Cassell *et al.*, 2002; Steets and Holden, 2003; Kim *et al.*, 2004).

Hossain and Eyre (2002) investigated the suspended sediment exchange through an estuary in sub-tropical Australia and considered the following components in their mass balance model: catchment sediment inputs, sediment exchange through estuary mouth, estuary bank erosion, suspended sediment stock (i.e. suspended sediment in water column) and sedimentation. Water quantity and quality sampling methodologies were employed to assess the sediment catchment input component. The estuarine exchange component was estimated using both water quality sampling methodologies and one-dimensional hydrodynamic models. The quantity of suspended sediment (i.e. the amount which remains suspended in the water column) was assessed using 22 sampling transects where water samples were collected along each transect using a hand pump from three different depths. These samples were assessed for suspended solid concentrations, and respective water volumes (estimated using depth probes) were estimated for each sample. Bank erosion was measured using wooden pegs set at 4m from the bank of the estuary and this distance was measured frequently during a 2 year period. Finally, assuming all other estimations were correct, the sedimentation component of the model was estimated from the difference of mass balance. Using this type of technique (where there is one unknown and one equation) cannot rule out the existence of other types of inputs or exports from such a system. For example, they found that sedimentation was responsible for 900 tonnes of sediment disappearing from the calculated budget, however there was no attempt to validate this finding with in-situ sedimentation tests, or modelling efforts. Moreover, using this type of approach does not allow an accurate assessment of the likely uncertainties in the overall microbial budget, since they are solving for a missing component.

A study conducted by Eyre *et al.* (1998) used a similar approach to that of Hossain and Eyre (2002) for another sub-tropical estuary in Australia. However, for estimations of sediment loading from a catchment, a mixed approach was used which combined monitored data and modelling procedures. Furthermore, instead of assuming the sedimentation was the difference of the mass balance, they assumed sediment deposition was equal to annual maintenance dredging records. However other aspects of their work indicate that while a mass balance of sediment was developed, there were a large number of assumptions used in the process (which led to neglecting other sources/sinks). This further illustrates the need to monitor carefully all aspects which may affect a mass balance in order to provide a check of any assumptions which may be required.

Boyton *et al.* (2008) provided an investigation into phosphorous and nitrogen budgets for an estuary in Maryland, USA. They developed a very detailed conceptual model of nutrient budgets for their

particular estuary and used this model to create a mass balance for both total phosphorous and total nitrogen. This model was comprised of a number of components: different sources (atmospheric, point, diffuse, septic), various in-system losses (sedimentation and burial, denitrification), transport processes, nutrient cycle processes and outputs to downstream systems. They used a number of techniques to quantify each of the budget's components, including: (1) direct measurements (e.g. point sources, diffuse sources and sediment characteristics were monitored using appropriate sampling campaigns), (2) assumptions based on literature values and (3) modelling results.

Both McKee and Eyre (2000) and Jaworski *et al.* (1992) also developed nitrogen and phosphorous mass balance conceptual models from which they then estimated nutrient budgets. Jaworski *et al.* (1992) had up to seven input components (including animal wastes, atmospheric deposition, wastewater effluents and groundwater imports), five output components (including crop harvest, denitrification, groundwater export, etc), some storage changes and an output to a downstream system. Again, as with Boyton *et al.* (2008) the quantification of these components was completed by numerous methods, but some critical assumptions were still made in order to estimate the nutrient budget.

Gannon *et al.* (1983) conducted a study which focused on determining the faecal coliform disappearance in a river impoundment. They monitored the inflows into the embayment, sampled the bottom sediment for coliform concentrations, and conducted experiments to determine die-off rates caused by irradiance and temperature changes. They conducted studies to determine faecal coliform disappearance rates within the water column and suggested that sedimentation and irradiance/temperature related die-off were the main factors affecting coliform disappearance. They found that while high levels of faecal coliforms were detected at the 15 inlet points around the bay, these coliform numbers were significantly reduced at the embayment's outlet point, indicating that a significant proportion of the faecal coliforms were either settling or that their survival was compromised by environmental factors. While this study did not attempt to conduct a mass balance of microorganisms, this dataset could be used to test a developed microbial budget approach. However, some components of a developed budget or mass balance would need estimating from the literature.

Not surprisingly, the data reported by Gannon *et al.* (1983) was used to create a dynamic bacterial model by Uchrin and Weber (1983). They used the conservation of mass equation to simulate lake responses to storm inputs and included the disappearance of faecal coliforms from the embayment caused by both die-off and sedimentation processes. The model showed promising results, with accurate predictions for several wet weather events. Moreover, the paper demonstrates that in order to accurately estimate a mass balance of microorganisms in water systems, it is necessary to understand the survival and settling processes.

Wilkinson *et al.* (1995) modelled faecal coliform dynamics in several streams and rivers in the United Kingdom. They monitored the level of faecal coliforms in these rivers, but instead of natural runoff events they conducted intensive sampling using controlled releases from upstream compounds. As such, there were negligible numbers of faecal coliforms in the upstream water, and all faecal coliforms found in the streams and rivers were sourced from resuspension of bottom sediments. Using this collected data, Wilkinson *et al.* (1995) developed a mass balance model to predict the number of faecal coliforms within the controlled release water. This mass balance model included many terms: I (inputs from upstream catchments), E (entrainment of organisms from the bottom sediment), S (settling from the river water column to bottom sediments), D (die-off of microorganisms) and N (the number of organisms in the channel storage). Their model produced good agreement with measured data, with coefficient of determination (R^2) values ranging from 0.55 to 0.93 at the three study sites. This paper indicates that in order to estimate a mass balance of

microorganisms in a river or stream system during wet weather flows, the survival of microorganisms in bed sediment, and their subsequent resuspension, need to be understood and represented. However, they also found that during wet weather flows, the settling component of the mass balance, together with the die-off component, could be neglected without compromising model predictions. This is because the effects of these two components are small since the time scales over which they occur are large relative to the other more dominant processes. This has major implications on estimating microorganism budgets since it shows that some components of a mass balance can be safely ignored during certain flow regimes.

Steets and Holden (2003) developed a mechanistic mass balanced based model of faecal coliform fate and transport through a coastal lagoon. They considered many components in their mass balance approach, including: dispersion, advection, die-off kinetics, settling and resuspension. For both seasonal conditions studied, the predicted water column faecal coliform concentrations were within an order of magnitude of field measurements. The model presented could help develop a microbial budget model for stream or river systems.

Recommendations for the microbial budget:
All of the above models have helped identify the main sources, processes and mechanisms which are relevant to a river/stream budget/mass balance:

- Inputs to the system from upstream areas (e.g. urban runoff containing sediment, nutrients and microorganisms)
- Losses within the water system (i.e. settling for sediments, settling and nutrient cycle processes for nutrients, settling and die-off kinetics for microorganisms)
- Sources within the water system (i.e. atmospheric deposition of sediment, nutrients and microorganisms, and resuspension of settled sediment, nutrient and microorganisms)
- Outputs to downstream systems (e.g. sediment, nutrient and microorganism outputs from a river to an estuary)

Whilst the above reviews did cover the major sources, sinks and processes which could affect a microorganism budget, only one of the reviewed budgets focussed on a river or stream mass balance (i.e. Wilkinson *et al.*, 1995). However, the emphasis in this paper was on wet weather events and did not consider dry weather processes. Since one of the major aims of this project is to determine benefits of treating dry weather flows, it is very important that these processes are included in the current project. It is evident that there is a substantial research gap in the monitoring of a stream or river reach with the focus of conducting a microbial budget. However, the literature reviewed above can provide a solid foundation to base the collection of this type of data, and has helped scope the rest of this literature review.

2 Microbial indicators – which to use, and why?

There are a number of microbial indicators which have been proposed by many different authorities for a variety of scenarios. The literature review will cover the most important of these indicator organisms and will provide insight into the advantages and disadvantages of each organism with respect to the microbial budget. Although this section covers these conventional bacterial indicators, the review also explores a number of alternative indicator organisms and directly sampled viruses and protozoa, which, if chosen, may help provide a more accurate assessment of human health risk than traditional indicators.

2.1 The purpose of microbial indicators

Watershed protection has placed its focus on water quality in recent decades. Drinking and bathing waters have been plagued with numerous outbreaks of diseases highlighting the need for more

stringent and accurate assessment strategies of microbial contamination, which can be regarded as primarily responsible for infections. In particular, pathogens originating from human sources are known to pose a greater threat to humans than animal sources as the likelihood of transmission of waterborne diseases is greater. Although the probability is minimal, the fact that microorganisms originating from animal enteric environments can sometimes cause diseases in humans must not be disregarded (Nebra *et al.*, 2003).

In assessing and mitigating the risks associated with the water body in question, indicator organisms are often used in preference to the actual pathogens, for several reasons. Pathogens can appear at low concentrations in natural waters and hence detecting and quantifying these are difficult, labour-intensive, costly and sometimes even impossible (Savichtcheva and Okabe, 2006). Several organisms respond in similar manners as pathogens to environmental stressors, occur in greater frequencies in water bodies and are more rapidly and cost effectively assayed, thus allowing them to act as surrogates (O'Toole *et al.*, 2008). Reviews show that various refined and trusted methods are available for source tracking investigations, many of which are reliant on detecting the presence of indicator organisms rather than the host-specific pathogens themselves (Sinton *et al.*, 1998; Field and Samadapour, 2007), thus providing a cheaper alternative.

Naturally, one must recognise that there are limitations to indicator organism performance in various aspects, including: low survival in the environment, the ability of some to multiply in water bodies, weakness to disinfection processes (or extreme resistance in some cases), the inability to identify pollution sources, low levels of correlation with certain pathogens and, although rarely, unrefined, costly or difficult, labour-intensive methods of enumeration. There is a general agreement that no indicator organism is ideal, but it has been recently suggested that the combined sampling of indicators may provide a better assessment of the health risks and overall water quality associated with the water body in question (Brookes *et al.*, 2005; Savichtcheva *et al.*, 2007). This section is therefore devoted to the evaluation of possible indicator organisms to study in the assessments of health risks, pollution sources and overall water quality of the freshwater body in this investigation.

In determining suitable indicators to sample, some governing selection criteria need to be established (Section 2.2). The potential microorganisms are then covered in their three respective groups, namely the conventional indicators (e.g. coliforms and faecal streptococci – Section 2.3), alternative indicators which are growing more popular in recent years (e.g. *Bifidobacteria* and bacteriophages – Section 2.4) and finally viruses and protozoa which can be directly sampled and potentially provide a more accurate human health risk assessment than indicators (Section 2.6). The evaluation of suitable microorganisms will then be investigated from a human-specific, performance, health-associated and economic perspective followed by a recommendation for this particular project (Section 2.7).

2.2 Criteria for selecting the appropriate indicator

The major question asked in the assessment of faecal contamination of water bodies is “how high is the human health risk of waterborne diseases?”, with this question having a secondary related question of “where does the source of contamination originate?”. There is general consensus on the characteristics of an ideal faecal indicator for assessing the presence of pathogens. The following list is a compilation of conditions that an indicator should meet (from Payment and Franco, 1993; Lewis *et al.* 1995; Horan, 2003; McCarthy, 2008):

- Suitable for different types of water bodies
- Of similar origin to the pathogen it is representing
- Always present when pathogens are present
- Present in high numbers, often equal to or greater than those of pathogens to allow for detection even after dilution in the water body

- Equally persistent or more persistent than the pathogens it is representing (this applies to both the environment and disinfection methods)
- Absent in the absence of contamination or pathogens
- Non-pathogenic to prevent risk to laboratory staff
- Easily, reliably and rapidly detectable in the laboratory in a cost-effective way

Furthermore, a second list of criteria for ideal faecal source tracking indicators can be drawn up after consulting reviews by Sinton *et al.* (1998), Savichtcheva and Okabe (2006), Field and Samadapour (2007) and McCarthy (2008):

- Highly host-specific, originating either from humans or animals but not both
- Excreted in high amounts to make them easily detectable
- Persistent in the environment at detectable levels
- Extensively characterised through epidemiological studies to identify behavioural traits
- Relatively easy, rapidly and cost-effectively assayed through techniques available to the researcher
- Able to grow extensively when cultured

These lists of criteria will be used to evaluate suitable indicators for the investigation in Section 2.7, and will be used as a basis of discussion in the following sections (Sections 2.3-2.6). However, it should be noted that in the literature it is frequently reported that some indicators may perform better in one water body as opposed to another (e.g. freshwater vs. seawater). In this particular case, a freshwater system was assumed to be the water body in question, therefore the criterion of applicability to different types of environments was significant here.

2.3 Conventional indicator microorganisms

2.3.1 Coliforms

Three separate groups have been conventionally used, namely total coliforms, faecal coliforms and *Escherichia coli* (abbr. *E. coli*). *E. coli*, *Klebsiella*, *Enterobacter* and *Citrobacter* all make up total coliforms in human and animal faeces, with *E. coli* having the largest percentage (96.8% and 94% in human and animal faeces, respectively) (NHMRC, 2003). These bacteria have been used widely as general indicators of microbiological quality and of faecal pollution (Horan, 2003).

As discussed by few (Savichtcheva *et al.*, 2007; McCarthy, 2008; Griffin *et al.*, 2008), total and faecal coliforms have qualities that speak against their use as indicators:

- they are able to multiply in the natural environment (see Section 4.1);
- some are of non-faecal source;
- their survival rates are significantly lower than many viral pathogens; and,
- Most of these bacteria originate from both humans and animals making them unable to distinguish between pollutant sources.

E. coli has proven to be the more popular indicator to use in the coliform group. Various Australian and international guidelines require the use of *E. coli* as an indicator for assessing overall water quality, including the Australian Drinking Water Guidelines. Reasons for this lie in the advantages of *E. coli*:

- *E. coli* have often been found to relate with pathogenic microorganisms (Savichtcheva *et al.*, 2007, Mons *et al.*, 2009);
- there is abundance in human and animal faeces;
- extensive growth is rare, except in certain conditions (Horan, 2003);
- they can be easily and cost effectively detected using either methods of multiple-tube fermentation or membrane filtration (Sinclair *et al.*, 2009); and,

- they can potentially be used in source tracking applications by using molecular markers to track *E. coli* isolates in humans (Sinton *et al.*, 1998).

Findings from various studies however show that *E. coli* does have negative attributes. Die-off was reported to be approximately ten times faster than that of oocysts in a study by Medema *et al.* (1997). Major factors which promote *E. coli* die-off are sunlight and temperature, which have been found to significantly affect *E. coli* as opposed to other indicators (Burkhardt III *et al.*, 2000) (Section 3.1.1 will discuss *E. coli* die-off in more detail). For these reasons, it has been suggested that it is unlikely *E. coli* can accurately indicate the presence of all viruses and parasites (Payment and Franco, 1993).

Despite the various downsides to this indicator, it has nevertheless been proposed that *E. coli* is a good indicator of some bacterial pathogens and of general microbial quality (Horan, 2003). Sampling for this organism, as well as an alternative indicator, would prove effective in better assessing water body health (Ogorzaly *et al.*, 2009). Many have supported the use of *E. coli* in freshwater environments (e.g. Makepeace *et al.*, 1995; NHMRC, 2004; McCarthy, 2008).

2.3.2 Faecal streptococci & enterococci

These microorganisms rarely grow in the extra-enteric environment and have higher survival rates than coliforms. Originating in the intestines, these bacteria occur in greater numbers than pathogens, but are less abundant than faecal coliforms in human faeces making their enumeration more difficult (Horan, 2003). Numerous species of both enterococci and streptococci exist with different origins. Human-specificity is not very distinguishable and most of these bacteria will occur in both humans and some animals such as cattle, pigs and birds (Horan, 2003). Furthermore, faecal streptococci are often more abundant in animals than coliforms (Sinton *et al.*, 1998).

Enterococci are perhaps the more popular choice over streptococci as various studies have found good correlations between this microorganism and: illness rates of swimmer-associated gastroenteritis, *Giardia* and *Salmonella* (Morrison *et al.*, 2008, Mons *et al.*, 2009, Touron *et al.*, 2007). Morrison *et al.* (2008) has, however, highlighted several disadvantages of enterococci as an indicator including its inability to distinguish human from animal faecal pollution, numerous similar strains that originate from environmental sources, and the possible replication in the environment if they associate with sediments and planktonic organisms. Although correlations were found to be good with *Giardia* cysts, the more rapid decay of enterococci make them unsuitable for detecting the presence of contamination (Medema *et al.*, 1997).

Another study by Touron *et al.* (2007) found correlations between enterococci and *Salmonella* at different sections of a studied estuary. At the upstream section, there were significant correlations between *Salmonella* and: thermotolerant coliforms (very similar to faecal coliforms), *E. coli* and enterococci. At the mouth of the estuary, only a relationship between *Salmonella* and enterococci was identified, and this may have been caused by the lower die-off rate for enterococci in saline conditions as opposed to *E. coli*. As indicated by Touron *et al.* (2007) and also quoted in several other studies, enterococci would be the more popular choice in saline environments due to their better tolerance than *E. coli.*

2.3.3 Clostridium perfringens

Clostridium perfringens are anaerobic, sulfite-reducing, spore-forming, Gram-positive bacteria and are common in water and soil environments (Horan, 2003). This indicator is used by detecting its spores, which are more resistant to chemical and physical parameters and persistent in environmental water bodies for longer periods of time (Wohlsen *et al.*, 2006). It has been reported that spores of *C. perfringens* are resistant against sunlight, temperature and are not affected by predators (Burkhardt III *et al.*, 2000, Savichtcheva and Okabe, 2006). Spores can be detected in

downstream locations far from the source of pollution emphasising their use in detecting remote faecal pollution. They are often present even in the absence of the less resistant coliforms indicating possible heavy industrial pollution, which may have removed other indicators (Horan, 2003; Savichtcheva and Okabe, 2006).

Many have suggested the use of *C. perfringens* as an indicator of viruses and protozoans, the latter being a popular topic of research as indicators are difficult to find for protozoan pathogens. In the case of viruses, similarities are seen in the survival rates of both microorganisms with *C. perfringens* being as robust as many viruses. Its use to assess virus removal efficiency in drinking water treatment indicates a good relationship between the two organisms (Payment and Franco, 1993; Savichtcheva and Okabe, 2006). Lucena *et al.* (1996) showed close relationships between *C. perfringens*, phages and viruses.

C. perfringens and protozoa have many similarities, which contribute to the qualities of this indicator. Both microorganisms tend to associate with small sized particles. This particle-association behaviour was observed in various studies (Brookes *et al.*, 2005; Cizek *et al.*, 2008). Cizek *et al.* (2008) highlighted similar transport behaviour between *C. perfringens, Cryptosporidium* spp. and *Giardia* spp. by observing settling velocities. Die-off rates of *C. perfringens* were lower than those of the protozoan cysts and oocysts (Medema *et al.*, 1997) and correlation analysis among various indicators with oocysts highlighted *C. perfringens* as a conservative, yet the most suitable, indicator (Touron *et al.*, 2007).

Despite the benefits of *C. perfringens* as an indicator for the investigation, the most apparent downside needs to be recognised. Because of the robustness of the spores and this indicator being of both human and animal origin, source tracking recent pollution becomes less viable. However, using this indicator in combination with a good source tracking microorganism may prove quite effective in the assessment of water body health.

2.4 Alternative indicator microorganisms

2.4.1 Bifidobacteria spp.

The potential of these microorganisms as indicators has first been noted in 1958 (Sinton *et al.*, 1998). Since then, *Bifidobacteria* have been the subject of many studies on alternative indicator microorganisms (Rhodes and Kator, 1999; Nebra *et al.*, 2003; Lamendella, 2008). They are anaerobic, Gram positive bacteria that inhabit the intestines of warm-blooded animals (Sinton *et al.*, 1998). Currently 31 different species have been discovered and documented as indicated by the most recent of studies (Lynch *et al.*, 2002). As part of the genus bifidobacterium, they are the third most prevalent bacterial genera found in the human enteric environment and outnumber coliform levels a suggested 10 – 100 times (Lynch *et al.*, 2002; Morrison *et al.*, 2008). The oxygenated environment of surface waters, temperatures below 30^{o}C throughout most of the year and rigorous nutrient requirements inhibit growth and multiplication of these bacteria, somewhat supporting their use as indicators (Lynch *et al.*, 2002; Nebra *et al.*, 2003; Bonjoch *et al.*, 2005).

Different species of *Bifidobacteria* have been found in humans and animals. This highlights the potential for use of this indicator in faecal source tracking applications. Bonjoch *et al.* (2004) reports that *B. adolescentis* and *B. dentium* were found exclusively in human sewage, while others added *B. breve*, *B. longum*, *B. catenulatum*, and *B. pseudocatenulatum* to the list (Lynch *et al.*, 2002; Matsuki *et al.*, 2004; Long *et al.*, 2005). Sinton *et al.* (1998) also indicated that *B. infantis* is predominant in humans, without disregarding the possibility of presence in some animal faeces. Choosing the suitable species to sample in this study will therefore involve rigorous consideration of the benefits and limitations of each.

Among those listed, sorbitol-fermenting *Bifidobacteria* (namely *B. adolescentis* and *B. breve*) have been the most studied options and would generally be the popular choice to sample when human faecal source tracking is required. Sorbitol is a food additive that is found exclusively in human-consumed food. The detection of sorbitol-fermenting *Bifidobacteria* would therefore suggest the likeliness of human pollution (Sinton *et al.*, 1998; Long *et al.*, 2005). In addition to this, Long *et al.* (2005) also showed that sorbitol-fermenting *Bifidobacteria* were only detected in tributaries influenced by residential areas. Several problems have, however, arisen in other studies. Rhodes and Kator (1999) showed that the two mentioned sorbitol-fermenting *Bifidobacteria* were found in pig isolates, which in turn was confirmed by Lynch *et al.* (2002) and Bonjoch *et al.* (2004), who detected *B. adolescentis* in pigs and avian bacteria, respectively. Occurrence and recovery of these species from animals is infrequent (Rhodes and Kator, 1999). Awareness of this characteristic will nevertheless help in the assessment of possible pollution sources.

Various other species have not been as widely mentioned as sorbitol-fermenting *Bifidobacteria*. A study conducted on 46 healthy human beings concluded that *B. longum* and *B. catenulatum* in addition to *B. adolescentis* were predominant in inhabiting the human gastrointestinal tract (Matsuki *et al.*, 2004). A recent investigation by Morrison *et al.* (2008), also mentions the three same species as well as *B. dentium*. Further complications arise when considering that different species are found to be excreted by humans of different age groups (Sinton *et al.*, 1998). It is therefore suggested that enumerating at least three different species of *Bifidobacteria* in the proposed study should allow for a better assessment of human faecal pollution, should this indicator be chosen. Further literature may need to be reviewed for a more in-depth coverage into the various human-specific species of *Bifidobacteria* to determine a suitable choice for sampling.

Methods for enumerating *Bifidobacteria* are not uncommon. Multiplex PCR or alternative PCR techniques can be used to detect this indicator (Savichtcheva and Okabe, 2006). Different media are also available and are used in culture-based methods, including Human Bifid Sorbitol Agar (HBSA), Beeren's and BFM Media (Rhodes and Kator, 1999; Bonjoch *et al.*, 2005). Selection of enumeration method will be dependent on cost as well as the time required, but as studies have shown, both methods are quite common.

Several disadvantages speak against the use of *Bifidobacteria* as indicator organisms. Low survival rates outside the enteric environment are characteristic of these bacteria. It has also been noted that *Bifidobacteria* are less resistant to river conditions than faecal coliforms (Long *et al.*, 2005). In addition, susceptibility to predation and the presence of other Gram-positive organisms could hinder detection (Savichtcheva and Okabe, 2006). However, seasonal variations are probably the most serious downside and once again relate to the low survival rates of these bacteria. *Bifidobacteria* have been found to survive much longer in cooler environments with temperatures below 20^{o}C. During summer months, however, detectable levels disappeared after 48 hours with decline rates of 50% every 60 hours (Rhodes and Kator, 1999; Lynch et al., 2002; Long et al., 2005). Having mentioned the various problems, it has nevertheless been suggested by Long *et al.* (2005) that *Bifidobacteria* are useful for the detection of recent faecal pollution as well as for watershed management when dealing with long-term goals.

2.4.2 Bacteroides spp.

Species of *Bacteroides* have host-specific characteristics, meaning that detecting certain types of species in water bodies will have implications on the origin. *Bacteroides* are rod-shaped, Gram-negative, anaerobes and are most abundant in human faeces. The much greater presence (about 100 times greater than *E. coli*), the anaerobic nature and enteric origin of these microorganisms are suitable qualities of an indicator. It has been reported by Sinton *et al.* (1998) that *Bacteroides* spp. have been detected only in minute amounts in animal faeces suggesting its use as a source tracking microbe. The most commonly occurring species is *B. fragilis*, although others include *B. distasonis*, *B.*

ovatus, *B. thetaiotaomicron* and *B. vulgatus*, all of which have been conveniently grouped into the general *B. fragilis* group (Sinton *et al.*, 1998).

Enumerating these *Bacteroides* can be done in two typical ways: through isolating the organisms on media, which can be cumbersome and time-consuming, or through PCR techniques involving certain rRNA genetic markers. It has been discovered that the genetic markers tend to persist more than the actual organism itself. Consequently, most investigations have been more focused on the detection of specific markers such as the 16s rRNA genetic markers of *Bacteroides* spp., believed to be human-specific (Savichtcheva and Okabe, 2006; Savichtcheva *et al.*, 2007).

Correlations between the genetic markers and various pathogens including *E. coli* O-157, *Salmonella* and toxins of *E. coli* have been discovered in one study (Savichtcheva *et al.*, 2007). However, several disadvantages for this particular indicator question the practicality of its use as an assessor of viral pollution and a source tracker. Seasonal variations have been found to occur, where rapid decrease in *Bacteroides* spp. populations occur during the summer months. Die-off rates have been measured to be greater than those of faecal coliforms and the sensitivity to sunlight and presence of organic matter suggest that survival of these bacteria in the freshwater body of this study is questionable. In addition to this, potential transfer of *Bacteroides* spp. between human and animal species has also been deemed possible (Sinton *et al.*, 1998).

Infection by bacteriophages, which leads to more rapid die-off would be another reason for the decline in numbers (Savichtcheva and Okabe, 2006). This would suggest that perhaps the predators of these microorganisms would be a more useful indicator if they can be detected, and this leads to the next possible alternate faecal indicator.

2.4.3 Bacteriophages

Bacteriophages are viruses that infect bacteria and exhibit many traits similar to human enteric viruses, therefore warranting their use as indicators. In addition to this, other justifications include: resistance to environmental stresses, abundance in wastewater and excretion in human faeces (Armon and Kott, 1995). They can be detected using simple and quick methods. Although they infect bacteria, there is no threat to humans due to the specificity of the virus. Phages can only multiply in metabolically active host cells (Grabow *et al.*, 1995), a disability to replicate outside its own environment similar to that of *Bifidobacteria.* Several studies have praised the usefulness of these indicators in assessing health risks as well as faecal source tracking (Grabow *et al.*, 1995, Sun *et al.*, 1997). Three phages are of interest in this study: phages infecting *B. fragilis*, somatic coliphages and male-specific coliphages (also known as F-specific coliphages).

Phages which infect *B. fragilis* are strictly anaerobic, meaning that they can only multiply in the gastrointestinal tract of warm-blooded animals. The most common strain detected in various studies is the *B. fragilis* HSP40 strain, which has been shown to be highly human-specific and could not be detected in closely related species of primates (Tartera *et al.*, 1989, Grabow *et al.*, 1995). Several comparative studies between *B. fragilis* phages and enteroviruses have also been performed. It was found that phages were isolated from water samples also contaminated with enteroviruses suggesting that these two share a positive correlation (Tartera *et al.*, 1989; Hot *et al.*, 2003). According to Sun *et al.* (1997), bacteriophages appear to be reliable indicators for the presence of enteroviruses. With a higher survival rate than enteroviruses, *B. fragilis* phages will certainly perform well in indicating more persistent types of viruses (Moce-Llivina *et al.*, 2005). Difficulty of recovering these organisms from waters with low levels of faecal pollution is a downside (Savichtcheva and Okabe, 2006). Sinton *et al.* (1998) adds that this indicator may not be applicable throughout the world and low counts have been found in effluents of meat industries in New Zealand laboratories.

Coliphages are viruses that attack coliforms (Griffin *et al.*, 2000) and somatic coliphages are the most abundant type of bacteriophages with a measured higher survival capability than *B. fragilis* phages (Lucena *et al.*, 1996; Moce-Llivina *et al.*, 2005) and easier detection compared to their counterpart, the male-specific coliphages (Payment and Franco, 1993). Somatic DNA phages (also known as PRD-1) have a similar representation to adenoviruses and rotaviruses, and persistence in the environment also shows some traits befitting of a potential indicator (Moce-Llivina *et al.*, 2005; O'Toole *et al.*, 2008). A potential relationship of somatic coliphages with enteroviruses was indicated by Hot *et al.* (2003). However, Formiga-Crus *et al.* (2003) showed contrary results, indicating that the microorganism was highly variable and did not correlate well with enteric viral pathogens. Grabow *et al.* (1995) report detection of these in humans, domestic animals, higher primates and seabirds, highlighting potential problems in source tracking applications. There have also been reports of replication in surface waters, which is a significant downside to the use of somatic coliphages as faecal indicators (Sun *et al.*, 1997, Tartera *et al.*, 1989, Grabow *et al.*, 1995, Formiga-Cruz *et al.*, 2003).

The second type of coliphages is the male-specific coliphage (also known as F-specific coliphage) and is generally the more preferred choice by many (Sinton *et al.*, 1998; Horan, 2003; Savichtcheva and Okabe, 2006). They are known as F-specific due to the fertility genetic factor, which their hosts possess (Grabow *et al.*, 1995). There are four genetic groups of F-specific coliphages, which are found from different sources. The two groups of interest are Groups 2 (found in pigs and humans) and 3 (reported to be found exclusively in humans), and detecting only these groups will have implications of a human pollution source (Griffin *et al.*, 2000). Distinguishing the different groups from each other is of utmost importance to prevent conclusions as found in Tartera *et al.* (1989), who did not recognise the different groups and hence did not support the use of male-specific coliphages for source tracking after detecting them in high numbers in animal faeces. As observed for somatic coliphages, both indicate potential associations with infectious enteroviruses, adding to the practicality of this indicator. Male-specific coliphages possess a single-stranded RNA genome similar to those of enteroviruses and their size and structure relate them to small RNA viruses, including polioviruses and the non-enveloped DNA adenovirus (Grabow *et al.*, 1995; O'Toole *et al.*, 2008; Ogorzaly *et al.*, 2009). A contradicting report, which should be noted, shows that correlations of male-specific phages were weaker for adeno- and entero-viruses, but stronger for Norwalk viruses (Formiga-Cruz *et al.*, 2003). This discrepancy can possibly be attributed to the different detection methods performed as this may play a specific influence as shown in Armon *et al.* (1995). Production only at temperatures above 30^{o}C inhibits growth outside the enteric environment, which was investigated with negative results. Only few disadvantages speak against the use of these indicators, including a slightly lower persistence than somatic coliphages and the difficulty of detection using various methods (Lewis, 1995).

2.5 Other less studied indicators

Four individual sources have listed some indicators that have not been widely focused upon by others. The potential of these to act as indicators may nevertheless be worth briefly mentioning. Phages infecting *B. thetaiotaomicron*, namely the G17 strain, have been found to be human-specific enabling their use in faecal source tracking. Constant proportions maintained in sewage and good persistence suggest the lack of replication and similarities to enteric viruses. Geographical distribution may however be a potential downside and further investigation is needed to assess the usefulness of these bacteriophages (Moce-Llivina *et al.*, 2005, Katharine, 2007).

The Torque teno virus has been suggested by one researcher as a potential indicator for human faecal pollution as well as the presence of viral pathogens (Griffin *et al.*, 2008). A detailed case study has yet to be found or carried out, but the proposed methodology has highlighted this virus as a potentially effective indicator. Torque teno viruses are non-enveloped DNA viruses likely to exhibit similar transport characteristics as enteric viruses. They are ubiquitous in humans, do not fluctuate

seasonally and have not been found to produce epidemic spikes. Transmission occurs via the faecal-oral route. Methods for enumerating these viruses involve rapid PCR. One can detect the virus in a wide variety of human tissue and it is suggested that they are highly resistant to environmental stressors. The lack of knowledge on this particular virus as an indicator organism would however recommend against its use in this investigation at this point in time.

Aerobic spore-forming bacteria (ASFB) have been studied as an indicator for protozoa. In an assessment with *C. perfringens*, it was found that similar high resistance and other behavioural characteristics indicate ASFB as a promising choice. The natural abundance of ASFB in surface waters due to the aerobic nature of the bacteria however does suggest otherwise (Mazoua and Chauveheid, 2005).

2.6 Directly sampled viruses & protozoa

As described earlier, some faecal indicators correlate well with pathogenic bacteria such as *Salmonella*. Direct sampling for these bacteria is also possible and has been done for the purpose of comparison (Touron *et al.*, 2007). Due to the various correlations between pathogenic bacteria and indicator organisms found in studies discussed above (e.g. Savichtcheva *et al.*, 2007, Morrison *et al.*, 2008, Mons *et al.*, 2009 and Byappanahalli *et al.*, 2009), this section will not focus on the option of using pathogenic bacteria.

However, there is some concern over the performance of indicators (especially bacterial ones) in representing the viruses and protozoa within the water body and the option to directly sample for these microorganisms has been proposed as a more reliable method. Many studies have directly sampled various viral pathogens in an effort to compare them to the chosen bacterial and viral indicators. It is generally accepted that viral assays are generally more costly than standard indicator enumeration and often just provide presence/absence results (also shown later in Section 2.7). The possibility of finding viruses that may represent the overall collection of pathogens in the water body should however be reviewed from the studies of various researchers.

Although several indicators have shown good correlations with protozoa, limitations still exist. Directly sampling these organisms may pose a serious health risk, but a review of the relevant characteristics and studies of both *Cryptosporidium* spp. and *Giardia* spp. may prove useful in assessing the quality of suggested indicators and whether the benefits of direct sampling will outweigh the high costs. This section will begin with a look at various viruses followed by a summary of the information on protozoa sampling.

2.6.1.1 Viruses

Viruses of interest in this review consist of enteroviruses, adenoviruses and the human polyomavirus. Viral assays often involve looking for the presence rather than counting the population. A rule of thumb can be adopted stating that as the amount of detail required from an assay increases, so will the cost. Real-time PCR assays have been developed to quantify various types including enteric viruses and adenoviruses. As a result, performing the assay on a representative virus is likely to give more reliable results on the pathogen distribution in the water body rather than relying on faecal indicator organisms (Katharine, 2007).

Enteroviruses have been thought to share a relationship with various bacteriophages as pointed out by Hot *et al.* (2003). A range of diseases are associated with this virus which would make it useful to quantify. Methods such as RT-PCR and a new tool known as VIRADEN (Moce-Llivina *et al.*, 2005) have allowed for easy and cost-effective enumeration. As a representative pathogen, however, various criticisms speak against its use. In particular are issues of seasonal fluctuations and sharp/epidemic spikes (Griffin *et al.*, 2008) and the potential lack of association with other

pathogens (Hot *et al.*, 2003). As a substitute, however, a suggestion has been to use the enterovirus genome, which addresses some of the disadvantages of the enterovirus (see Hot *et al.*, 2003).

Adenoviruses infect humans during childhood and remain persistent through time. These viruses are responsible for gastrointestinal, respiratory, urinary tract and eye infections (van Heerden *et al.*, 2005b). Their occurrence is abundant and along with a high resistance to UV disinfection and lesser seasonal variability than enteroviruses, these viruses can possibly represent the overall population of viral pathogens in water bodies. A recent study at recreational beaches in Lake Michigan managed to assess the risk of waterborne disease outbreaks through the use of adenoviruses (Wong *et al.*, 2009). Results from Wong *et al.* (2009) supported the use of adenoviruses as viral indicators, due to their higher resistance to inactivation as compared to enteroviruses and other animal-specific pathogens. The study, however, admitted that future research needs to be undertaken to fully understand the qualities that this potential indicator organism can offer. Two studies by Jiang *et al.* (2001; 2007) reached conclusions that support the importance of directly sampling for adenoviruses. The benefits to sample for these viruses to better assess health risks are indicated in several studies on recreational waters as well as treated drinking water (Jiang *et al.*, 2001; van Heerden *et al.*, 2005a; Wong *et al.*, 2009). Despite the support for the use of this virus, there has also been an equivalent amount of doubt cast on the potential of this virus to act as a surrogate as little correlation was found between adenoviruses and both enteroviruses and hepatitis A viruses in urban waterways (Hot *et al.*, 2003; Albinana-Gimenez *et al.*, 2006, Griffin *et al.*, 2008,).

Two types of human classified polyomaviruses are JC and BK polyomavirus and, as with adenoviruses, these strains infect the young, giving rise to persistent infections with time. The virus is widespread and excreted from more than 50% of healthy individuals, explaining their abundance in the environment (Albinana-Gimenez *et al.*, 2006; McQuaig *et al.*, 2006). The virus belongs to the polyomaviridae family and consists of a circular dsDNA genome, which has a stable presence in surface waters and lower concentrations in treated waters. Unfortunately, the use of this virus has only recently been investigated and little information is available on the distribution, persistence and seasonal stability in the environment (Albinana-Gimenez *et al.*, 2006). Nevertheless, they have been proposed as indicators for pathogenic human viruses and also as an addition to the faecal source tracking toolbox (McQuaig *et al.*, 2006).

2.6.1.2 Protozoa

The two protozoa in question, *Cryptosporidium* and *Giardia*, are intestinal microorganisms that can result in severe infections in humans upon transmission by ingestion. Affected individuals excrete large amounts of the oocysts/cysts, which find their way into environmental waters. *Giardia* is more frequently detected than *Cryptosporidum* (Mazoua and Chauveheid, 2005; Mons *et al.*, 2009). Both of these organisms have high survival rates in natural environments and disinfection processes, making them perhaps the most challenging microbiological problem to deal with, especially in the context of finding a suitable indicator. They have high resistance to chlorine disinfection (Mazoua and Chauveheid, 2005; Mons *et al.*, 2009) and are very robust against environmental stressors such as predation, UV radiation, chemical damage and starvation (Medema *et al.*, 1997). It was reported by Medema *et al.* (1997) that *Cryptosporidium* oocysts were able to survive for 6 months in membrane chambers in river water at ambient temperatures.

Horan (2003) has cast doubt on the availability of non-pathogenic organisms with similar characteristics to act as surrogates, stating that the assessment for *Cryptosporidium* and *Giardia* can only be done by sampling these organisms directly. Others support the possibility of bacterial viruses and spore forming bacteria, such as *C. perfringens* (Medema *et al.*, 1997; Mazoua and Chauveheid, 2005). Direct sampling of these organisms is very costly, and thus is not a common occurrence in urban stormwater related systems.

2.7 Evaluation of indicator performance

The final choice of indicators to use for this particular study will also depend on economic issues. It is not cheap to sample for certain indicators and an overview of the finances involved in the detection of various organisms will be presented in this section. Inquiries into prices for various organisms were made at Ecowise Environmental. Further research was conducted for comparative quotes in order to gain a better idea of price ranges within Victoria.

At present, inquiries with Ecowise Environmental assumed a 2-year sampling period with an average turnover of 20 to 40 samples a month. The costs of enumeration per sample obtained are shown below in Table 1. Where an alternative quote was obtained, a second price is listed in parentheses below Ecowise's quotation. It can be seen that a number of viruses and indicators are not included in the price list, since these were unavailable from this laboratory. Other laboratories have been considered for some of the other more specific alternate indicators, but quotations from these laboratories were not available at the time of this publication.

Table 1. Summary of enumeration costs for various indicator organisms

Organism	Enumeration Cost ($/sample)*	Comments
Escherichia coli	$30 per sample (between 20 and 70)	*Usually a 24-hour turnaround*
Enterococci	$37 per sample (between 35 and 65)	*Usually a 24-hour turnaround*
Coliphages	$160	*The possibility of detecting individual genogroups will depend on the availability of the method used – prices for genogroups are not available as yet.*
Clostridium perfringens	$70 per sample (below 100)	*Requires about 10 days, some laboratories offer a source tracking option*
Enteroviruses & Adenoviruses	$650 per sample (between 400 and 582)	*Quite labour-intensive, long turnaround period*
Cryptosporidium & *Giardia* species	$380 per sample	*Quite labour-intensive, long turnaround period*

*all tests will need to commence within 24 hours of obtaining the sample

It can be seen from the table that the enumeration of viruses and protozoa are significantly more costly than conventional faecal indicators. The prices for coliphage and *C. perfringens* enumeration are quite reasonable making them viable options for this particular project. The labour-intensive nature of enterovirus and adenovirus detection highlights the need for developing more economical detection methods if these indicators are to be used more frequently. *E. coli* and enterococci remain the cheapest options due to their inherent popularity, the availability of simple enumeration techniques and their use as standard indicators in many water quality guidelines around the world.

Recommendations for the microbial budget:

This review has covered a wide variety of possible indicator microorganisms studied in recent decades. Making the final recommendation as to what indicators should be used for this particular project can be quite difficult. Some of the microorganisms covered in this study still require significant amounts of research and refinement of detection methods to improve their viability as indicator organisms, be it for risk assessment or source tracking. While many microorganisms considered here show promise in the future, the methods are not widely implemented/developed and, as such, usually are either not readily available at commercial laboratories, or have extremely large enumeration costs. Hence, their use in this study is not recommended.

Considering the above discussions (Sections 2.3 to 2.7) and criteria identified in Section 2.2, the authors recommend three different organisms as adequate for the overall assessment of water

quality, representing bacterial, viral and protozoan contamination and source tracking. Three potential candidates are: *Escherichia coli*, F-specific coliphages and *Clostridium perfringens*. Despite their advantages in being cheap to detect, the inherent advantage in each should fulfil the requirements for this project.

E. coli will provide an overall indication of the water quality. The easy enumeration of this bacteria and abundance in human and animal faecal pollution along with available environmental guidelines will allow for a general assessment of the water body in question. The possibility of using *E. coli* as a source-tracking organism (with the help of molecular markers) can also be considered depending on additional costs. F-specific coliphages are quite robust in the environment and will act as a viral indicator, but also as the main source tracking organism if genetic groups are quantified. *C. perfringens* has a remarkable resistance to environmental stressors of the same level as protozoa and will serve as an indicator for these in addition to several other viruses. It is believed that these three organisms will provide an accurate representation of the human health risks within the selected water body, including the health risks to users of the water body derived from human origins. Also, using these indicators, it will be possible to fully understand the influence and benefits of various mitigation options on the risks of utilising the selected water body.

3 Microorganism sinks in water bodies

The most important microorganism sinks have been investigated within the following section. There are three main processes which will contribute to in-system reductions in certain microbial populations: (1) die-off, competition and predation, and (2) sedimentation. The following outlines each of these three processes, specifically with regard to the project at hand.

3.1 Die-off, competition and predation

3.1.1 Main factors influencing microbial die-off

Crane and Moore (1986) identified microorganism die-off as one of the primary factors that influence the microorganism levels in a water catchment system. Therefore, the key factors that affect microorganism die-off need to be known so that these can be taken into account when determining an accurate microbial budget. In the literature reviewed, the extent of microorganism die-off has been related to many different environmental factors, including:

- pH extremes
  (McFeters and Stuart, 1972; Reddy *et al.*, 1981; Sjogren and Gibson, 1981; Polprasert *et al.*, 1983; Crane and Moore, 1986; Solić and Krstulović, 1992; Sjogren, 1994; Scott, 2003);
- temperature extremes
  (Davenport *et al.*, 1976; Mancini, 1978; Barcina *et al.*, 1986; Crane and Moore, 1986; Flint, 1987; Solić and Krstulović, 1992; Kudva *et al.*, 1998; Scott, 2003);
- irradiance
  (McCambridge and McMeekin, 1981; Reddy *et al.*, 1981; Polprasert *et al.*, 1983; Crane and Moore, 1986; Davies and Evison, 1991; Solić and Krstulović, 1992; Scott, 2003; Chan and Killick, 2005; Kay *et al.*, 2005);
- nutrient availability
  (Dutka and Kwan, 1980; Lessard and Sieburth, 1983; Crane and Moore, 1986);
- presence of predators and competitive organisms
  (Reddy *et al.*, 1981; Barcina *et al.*, 1986; Crane and Moore, 1986; Davies *et al.*, 1995; Medema *et al.*, 1997; Scott, 2003);

- salinity; and,
  (Savage and Hanes, 1971; Mancini, 1978; Chojnowski and Mancini, 1979; Solić and Krstulović, 1992; Auer and Niehaus, 1993; Kay *et al.*, 2005);
- presence of toxicants
  (Crane and Moore, 1986).

3.1.2 Microorganism die-off

The die-off of microorganisms in the environment has been modelled using a number of different functions of time (Crane and Moore, 1986). Chick and Martin (1908, as cited by Crane and Moore, 1986) proposed the most popular model which is a first order decay function, known as Chick's Law:

$$N_t = N_0 \times 10^{-kt} \qquad \text{[org]} \qquad \text{Equation 1}$$

where N_t is the number of organisms (org) at time t, N_0 is the initial number of organisms, t is the time and k is the first order die-off rate constant.

Testing how a certain factor affects the die-off of microorganisms often requires experimentation and usually results in the reporting of how k (from Equation 1) changes with these factors. However, background die-off levels nearly always exist in these experiments as (a) not all factors can be controlled and (b) microorganisms are living organisms which have a limited lifespan. As a result, k is rarely seen to equal zero in these experiments (indicating no die-off) because there is always some external factor which is affecting the die-off. Furthermore, k often approaches a constant value which indicates that the factor is no longer having a significant effect on the die-off of the microorganism and a 'steady state' has been achieved. These issues need to be considered when making conclusions about which factors have the most impact on microorganism die-off and survival in the selected water system.

In the following sections there will be a continual reference to factors that affect microorganism die-off and to whether these factors will play a significant role in determining microorganism die-off in the selected study reach. The majority of the available literature on how microorganism die-off and survival is affected by different environmental factors focuses on bacteria, and not viruses or protozoans. This is possibly a result of the time and costs involved in protozoan and viral assays (as discussed above). As such, the following review will follow this trend and mainly focus on bacterial relationships and it is strictly noted that although these relationships *generally* hold true for various bacteria, they may differ for viruses and protozoa. However, in saying this, Ferguson *et al.* (2003) conducted a thorough review of key fate and transport processes for microorganisms in watersheds and found that the most critical factors which affect the die-off of viruses and protozoa were the same as those affecting bacteria.

pH

Acidic conditions (Cuthbert *et al.*, 1955; Kibbey *et al.*, 1978; Solić and Krstulović, 1992; Scott, 2003), together with alkaline conditions (Kovacs and Tamasi, 1979; Solić and Krstulović, 1992; Scott, 2003), have both been found to affect microorganism die-off rates. Figure 1 shows (note the logarithmic y-axis scale) the die-off rates for *E. coli* in fresh water with different pH levels (Reddy *et al.*, 1981). It is evident that pH extremes can have large effects on *E. coli* die-off, whereas in the range of pH 5 to 8 there seems to be little die-off. Similar results were found by Solić and Krstulović (1992) who noted that faecal coliform die-off in seawater samples was lowest between a pH of 6 and 7, and increased at pH extremes. Whilst it appears that bacterial (above) and protozoan (see Scott, 2003) die-off is increased by extreme pH values, this is not necessarily the case for enteric viruses which can be resistant to both low and high pH values (Ferguson *et al.*, 2003).

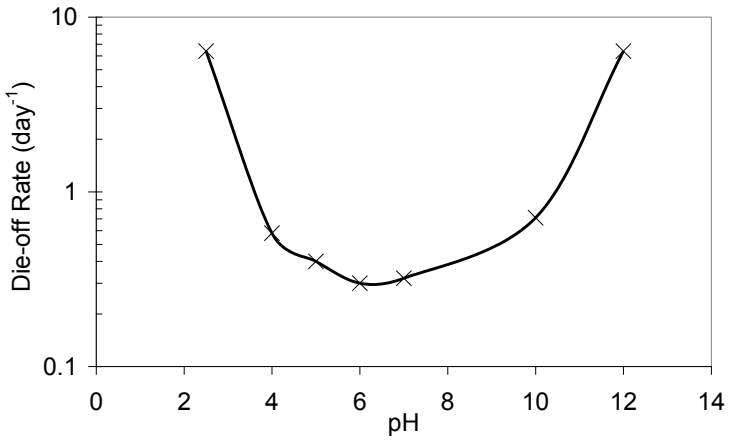


Figure 1. *E. coli* die-off at different pH values in an aquatic environment as recorded by Reddy *et al.* (1981).

Duncan (1999), in his review of urban stormwater quality, shows that the pH of wet weather flows in urban areas is generally between 6 and 8. It has also been recently shown that dry weather stormwater flows also have neutral pH values (McCarthy, *unpublished data*). Furthermore, Hatt *et al.* (2004) showed that pH in urban streams ranged from 7 to a maximum of around 8. As described above, only a minimal amount of die-off occurs within this range for bacteria and protozoa, and this, together with the knowledge that viruses are usually resistant to pH changes, implies that it is unlikely that pH will be a governing factor when determining die-off in urban stormwater or stream systems.

Temperature

Temperature changes have also been shown to adversely affect microorganism survival (Van Donsel *et al.*, 1967; Mancini, 1978; Solić and Krstulović, 1992; Chan and Killick, 1995; Thomas *et al.*, 1999; Walker *et al.*, 2001; Servais *et al.*, 2007) and temperature is often thought to be the most influential environmental factor affecting microorganism die-off (Crane and Moore, 1986). Figure 2 shows that, for a number of microorganisms, as temperature increases, the die-off coefficient also increases. This figure also shows that the die-off of *E. coli* in aquatic systems is lowest around 5°C, whilst it is highest at higher temperatures around 25°C. Similar results were found by Thomas *et al.* (1999) who showed that *Campylobacter* die-off rates in river samples were lowest at 5°C and highest at around 25-37°C. There is less agreement in the literature about how protozoan survival is affected by temperature extremes (Ferguson *et al.*, 2003). However, Walker *et al.* (2001) found that *Cryptosporidium parvum* oocyst inactivation increased proportionally with increased water sample temperatures, and this agrees with that stated by Scott (2003), that longer parasitic survival times occur at lower temperatures.

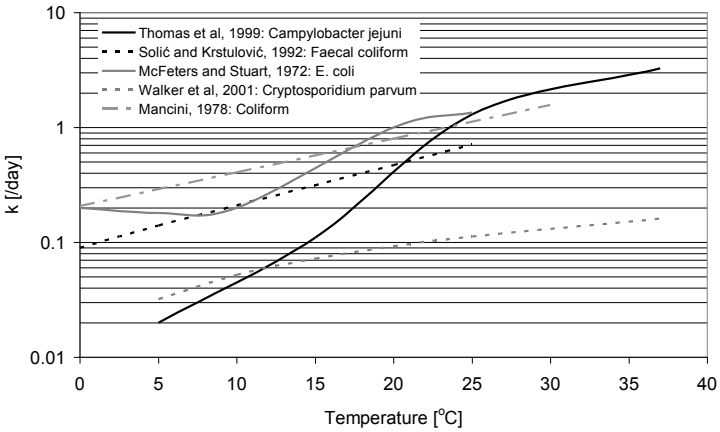


Figure 2. Dependence of the die-off coefficient *k* on water temperature for a number of different microorganisms from various sources.

Many studies have reported an increase in urban stream temperatures with an increase in imperviousness (e.g. Hatt *et al.*, 2004). Urban stream temperatures have been measured around Melbourne, Australia, and ranged between 10 and 15°C (Hatt *et al.*, 2004). Summer runoff temperatures of between 17 and 22°C were measured by Espinosa *et al.* (2001) in a catchment which has a long term average maximum ambient temperature of 24°C and an average minimum temperature of 12°C. Wisconsin (USA) stormwater runoff temperatures have been reported to be as high as 29°C (Espinosa *et al.*, 2001). Considering these likely stormwater temperature ranges, and the above results on the effects of temperature on microorganism die-off, it is expected that aquatic temperature will be important in determining the level of microorganisms in urban stormwater and stream systems.

Irradiance

There is evidence that irradiance of aquatic systems can increase bacteria, protozoa and virus die-off (e.g. Crane and Moore, 1986; Solić and Krstulović, 1992; Ferguson *et al.*, 2003; Scott, 2003; Chan and Killick, 2005; Kay *et al.*, 2005; Schultz-Fademrecht *et al.*, 2008). Figure 3 shows that as irradiance increases (i.e. radiation intensity increases) the microorganism die-off rate increases in different water samples.

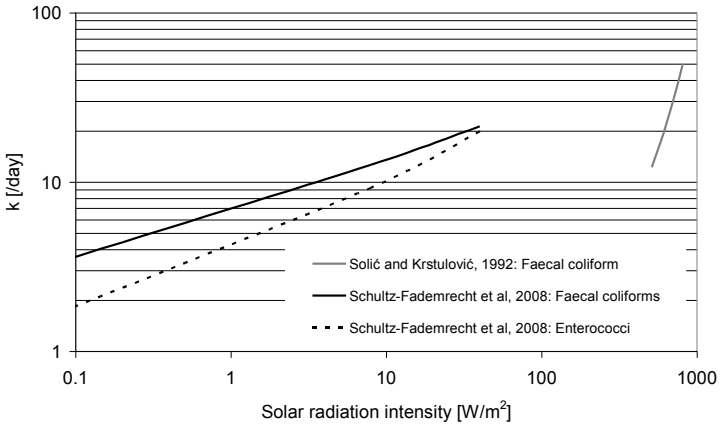


Figure 3. Dependence of the die-off coefficient *k* on water irradiance for *E. coli* and enterococci from various sources.

Considering streams are subject to light, solar radiation is proposed to occur in the selected water system and considering the increase in die-off coefficients seen in Figure 3 for indicator organisms, it is proposed that irradiation should be considered when estimating an accurate microbial budget.

Kay *et al.* (2005) found that the affects of solar radiation on enterococci in estuarine waters are highly dependent on the turbidity of these systems (see Figure 4, below). This is logical since light penetration will be reduced in highly turbid systems, thus reducing the effects of solar radiation on microorganisms (as described by Flint, 1987; Lim and Flint, 1989; Kay *et al.*, 2005). Depending on the chosen study site, the water may have differing degrees of turbidity. Furthermore, the turbidity of urban stormwater flows is often quite high, and as such turbidity should be considered for this microbial budget.

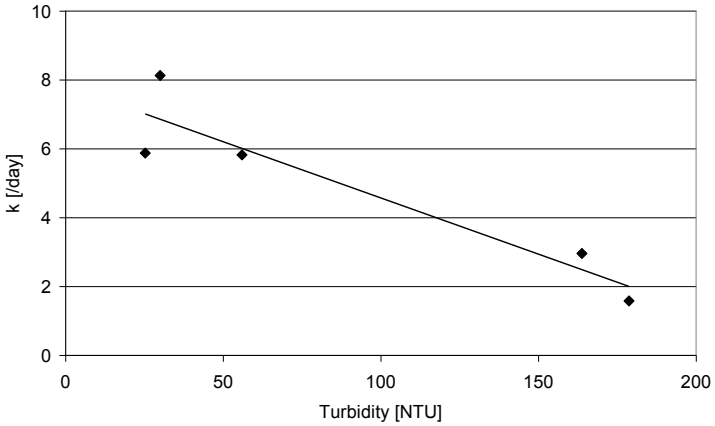


Figure 4. The relationship between the die-off rate of enterococci and turbidity in estuarine and coastal waters when subjected to a radiation source of 260W/m^2.

Nutrient levels

Studies have found relationships between microorganism survival and nutrient levels in aquatic environments (e.g. Slanetz and Bartley, 1965; Crane and Moore, 1986; Flint, 1987; Lim and Flint, 1989; Thomas *et al.*, 1999). In these environments, a marked increase in survival of faecal organisms with higher nutrient content in water samples has been observed (Dutka and Kwan, 1980; Crane and Moore, 1986). This might account for the extended bacterial survival that is found in concentrated waste storages (Crane and Moore, 1986).

Lim and Flint (1989) found that the addition of sterile synthetic wastewater (high in nutrients) to lake water samples allowed *E. coli* to increase in numbers in proportion to the amount of sewage added. They further studied this effect by conducting several independent experiments focusing on the main nutrients comprised in sewage (i.e. phosphorous, carbon and nitrogen). As such, they found that even though the sampled lake water was phosphate limited, the addition of phosphate did not significantly increase the survival of *E. coli*. The addition of carbon sources allowed the extended survival of *E. coli*, suggesting that cells might be carbon limited. Addition of nitrogen sources increased survival of *E. coli* in lake waters, more so than the addition of carbon sources. In particular, Lim and Flint (1989) showed that the survival of *E. coli* in lake water was increased with the addition of ammonium sulphate (see Figure 5, below).

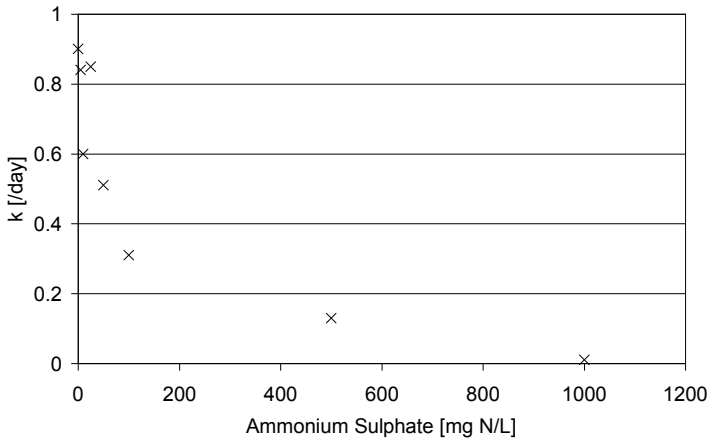


Figure 5. Die-off rate for *E. coli* in lake water with different concentrations of added ammonium sulphate (see Lim and Flint, 1989)

However, Thomas *et al.* (1999) do point out that Lim and Flint (1989), in their experimentation, used concentrations of nitrogen species which would rarely be seen in many water systems. Further, Thomas *et al.* (1999) found that the survival of *Campylobacter* spp. were not significantly impacted by nutrient levels. They then state that the relative insignificance of temperature and nutrients at levels prevailing within natural aquatic systems indicates that, within such environments, alternative parameters, such as interaction with other flora (i.e. competition or even predation), may be the primary determinants of *Campylobacter* persistence.

Predation and competition

Predation and competition of microorganisms is a contributing factor to microorganism die-off in soil and aquatic systems (e.g. Reddy *et al.*, 1981; Barcina *et al.*, 1986; Flint, 1987; Lim and Flint, 1989; Davies *et al.*, 1995; Mezrioui *et al.*, 1995; Medema *et al.*, 1997; Thomas *et al.*, 1999; Davies and Bavor, 2000). Marino and Gannon (1991) showed that faecal coliforms survived longer in samples treated with cycloheximide (a fungicide which eliminates protozoan predators) than in untreated samples. Similar results were found by Davies and Bavor (2000) who collected inlet and outlet sediment samples from a wetland and added cycloheximide to determine whether predation was occurring. It was found that thermotolerant coliform die-off rates were significantly greater (at the 95% level) in the absence of cycloheximide (i.e. coliform die-off rates are greater in the presence of predators). Figure 6 shows the die-off rates for thermotolerant coliforms with and without cycloheximide (Davies and Bavor, 2000).

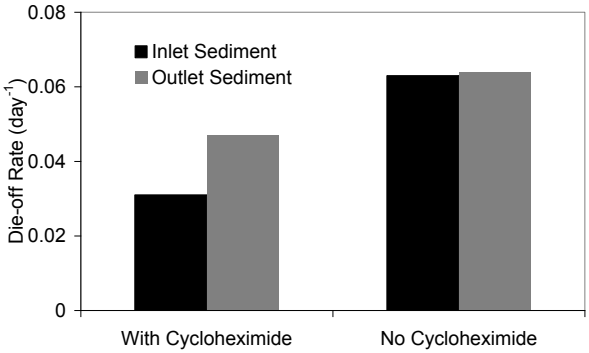


Figure 6. Die-off rates for thermotolerant coliforms in wetland inlet and outlet sediment with (no cycloheximide) and without (with cycloheximide) the presence of predators (Davies and Bavor, 2000).

However, others have found little influence of predation and Marino and Gannon (1991) found that faecal streptococci die-off rates seemed to be unaffected by the addition of cycloheximide, indicating that not all microorganisms are impaired by predators. Furthermore, Davies *et al.* (1995) found that while the addition of cycloheximide allowed faecal coliforms to survive in marine and freshwater sediments, *Clostridium perfringens* did not appear to be affected by predators. As such, depending on the microorganism, it is evident that predation can be a significant factor controlling microorganism survival in water systems.

Flint (1987) found that while predation of *E. coli* from protozoa only contributed marginally to the disappearance in river water samples, the competition between other microbes (including bacteria) for nutrients was found to be the most significant factor controlling *E. coli* disappearance (see Figure 7). He further showed that bacteriophages only slightly impacted on the survival of *E. coli* in river waters. Lim and Flint (1989) investigated these findings further. They found that, although the addition of sewage (simulating supply of nutrients) improved the survival (and in fact growth) of *E. coli* in lake water over a period of 6-10 days, if competitors/predators exist then there is a rapid decline in the viable count of *E. coli*. This was assumed to be caused by the inability of *E. coli* to compete successfully for nutrients whilst other bacteria are present. Predation was not thought to be the cause for this rapid decline since few, if any, protozoa were detected in this water.

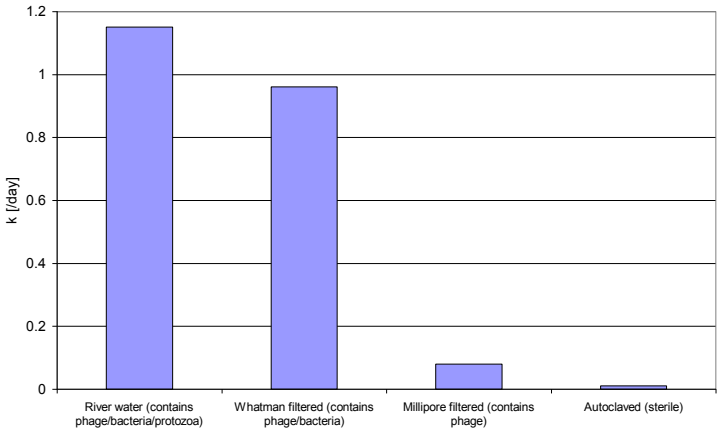


Figure 7. The die-off rate of *E. coli* in river water using different levels of filtration to reflect the presence/absence of different microbes (from Flint, 1987).

Flint (1987) also found that while *E. coli* survival was heavily impacted by temperature in raw and filtered river waters (with decay rates similar to that shown above), when using autoclaved water (i.e. with no competing or predating microorganisms) the die-off of *E. coli* was both minimal and not dependent on temperature (up to 25°C). This indicates that competition and predation may increase the microorganism die-off sensitivity to certain environmental factors.

All of the above results do indicate that competition between microorganisms can influence their survival, and moreover that this factor can be one of the most significant in controlling the resultant die-off kinetics of bacterial organisms.

Salinity

Salinity can also affect the die-off rate of microorganisms (Mancini, 1978; Crane and Moore, 1986; Solić and Krstulović, 1992; Mezrioui *et al.*, 1995; Chan and Killick, 2005; Kay *et al.*, 2005). Mancini (1978) derived a model that estimated the die-off rates for coliform bacteria in waters with different degrees of salinity. The model used a linear function to relate the percentage of sea water to the die-off rate of coliforms. The electric conductivity (a measure commonly used to estimate the salinity of aquatic systems) of seawater is usually around 50,000 µS/cm whereas the electric

conductivity of stormwater is usually between 74 μS/cm and 1,810 μS/cm (*pers. comm.* Fuchs database, 2008), at least two orders of magnitude less than seawater. McCarthy *et al.* (*unpublished work*) also showed that dry weather stormwater flows from three urban catchments in Melbourne had similar electric conductivities. Hence, it is fair to conclude that since stormwater only has less than 4% of the salinity of seawater (and at this level Mancini, 1978 showed little die-off due to salinity), the effects of salinity on die-off of microorganisms in stormwater and most stream waters is likely to be minimal. However, depending on the system which is chosen for this budget, salinity might become an important factor (i.e. in a marine or estuarine environment), hence this type of die-off should not be neglected.

Recommendations for the microbial budget:

It is evident that the survival of microorganisms in urban water systems can be impacted by a number of different factors. While some factors explained above are unlikely to be of major significance for this project (e.g. pH and salinity), most of the factors listed above are going to contribute to the microbial budget. However, from the above discussions it is also clear that certain factors are going to be more significant for this project, than are others. The following provides a list of the parameters considered important for the microbial budget (in order of decreasing significance):

1. temperature
2. predation and competition
3. irradiance
4. nutrient levels
5. salinity
6. pH

Whilst the literature review helped understand each of these factors, the kinetics derived in the literature is generally not transferable between catchments. This means that without adequate experimentation, it will be difficult to actually *quantify* how the selected microorganism is affected by each of these factors. As such, it is recommended that some experimentation be conducted to evaluate these relationships. The setup of such experiments is quite straightforward and can be completed in a timely fashion. However, the costs introduced from these experiments are expected to be considerable since replication will be required (both between and within each experiment). Furthermore, if each factor is investigated separately (e.g. temperature, predation, irradiance and nutrient levels), as well as in interaction with other factors, the number of parallel experiments would be large. However, it is possible to reduce the number of experiments required for the aim of the project. For example, although predation and competition is expected to contribute a large amount to the die-off and survival of the selected microorganism, it is a parameter which could be absorbed into background die-off conditions (i.e. as explained at the start of Section 3.1.2). This would mean that this effect is not directly quantified as such, but is included in all other estimates of k for each of the three other factors (temperature, irradiance and nutrient levels). Furthermore, Monash could appoint a PhD student to help conduct this type of research to help reduce salary costs.

Regardless of whether or not the above suggested experiments are conducted, it is highly recommended that the factors which affect microorganism survival be monitored during the project. For example, continuous probes which measure temperature, pH, turbidity and electric conductivity (as an indicator of salinity) can be easily purchased, calibrated and installed within the selected stream system. Weather stations can also be purchased and installed at the site to help monitor radiation impacts, together with turbidity readings from the in-situ probe. Finally, water quality samples could be taken on a regular basis to help monitor nutrient levels of the selected system. The only factor which could not be easily monitored would be the predators and competition

between species, however some further collection of samples and subsequent analyses could help monitor protozoan predators (however, at high costs). This provides further argument to not assess the predation/competition die-off factor separately, as discussed above. This monitored data can be used extensively in the development and testing of a predictive model which can estimate microbial budgets for other similar systems. Without this data, it will be very hard to adequately test the new model.

It should be noted that the selected stream will play a major role in determining the significance of each of the factors described above. This is because all of the above factors are highly time dependent, and, as such, each factor is only important when the exposure time of these microorganisms to these environments is significant. For example, microorganisms in a high flowing stream (say at 1m/s), from which we are only monitoring 1km, will not be as affected by environmental factors as those in a low flowing (0.1m/s), very long (10km), stream. This is because the residence time of the microorganisms in the high flowing stream is just 16 minutes, whereas for the low flowing stream it is over a day. This vast time difference will cause substantial implications on the microbial budget. Extending this example and looking at the temperature related die-off for each system assuming (a) the stream's temperature is 15°C and (b) we are investigating *E. coli* (from Figure 2 we can determine a *k* of 0.45/day). In the high flowing stream, we could expect to see a decrease in *E. coli* numbers within the monitored reach of less than 1%, but over 40% for the low flowing stream. As such, it is necessary to take this time dependence into account when considering the significance of each environmental factor for the microbial budget. This time dependence may negate the need for some of the experimentation suggested above.

3.2 Sedimentation

Sedimentation is hypothesised to be a significant factor during dry weather periods, where flow rates are below the velocity required to induce entrainment and therefore promote sedimentation of particles with attached microorganisms. However, sedimentation of microbes is also highly related to the association of microorganisms with particulate matter. As such, this section first begins with a review of the literature on the association of microbes with particles and follows with a review of the association of microbes to settleable particles.

3.2.1 Association with particles

One of the major factors that influence the way in which microorganisms are transported within urban water systems is whether they are attached to particles, exist in 'flocs' or exist freely in the environment. Many current microbial methods assume that all microorganisms exist freely in the environment and are not associated with particles (Characklis *et al.*, 2005). However, there is evidence that a proportion of microorganisms in stormwater are associated with particles. Schillinger and Gannon (1985), for example, found that around 17% of faecal coliforms in stormwater were successfully retained on a 5 μm filter, with about 12% being retained on a 30 μm filter. This indicates that a proportion (17%) of the faecal coliforms in stormwater have diameters of greater than 5 μm (i.e. they are in a 'free' phase or exist in an aggregate of organisms, or 'flocs', with diameters of greater than 5 μm) or are attached to particles with diameters of greater than 5 μm. Davies and Bavor (2000) found that thermotolerant coliforms preferentially adsorbed to fine particles of less than 2 μm in diameter and similar results were found for somatic coliphages (Davies *et al.*, 2003).

Gannon (1983), in his study of faecal coliform disappearance in a river impoundment, found that, on average, 94% of faecal coliforms were attached to particle sizes between 0.45 μm and 5 μm in diameter, whilst the remaining 6% were found to be attached to particles between 5 μm and 100 μm in diameter. Auer and Niehaus (1993), in their study of the loss of faecal bacteria in lakes, found similar results with an average of 91% of faecal coliforms being attached to particles with diameters between 0.45 μm and 10 μm, whilst the remaining 9% were attached to particles with diameters

between 10 and 102 μm. These results are not surprising considering bacteria sizes are often between 0.4 μm and 14 μm in length (Perdek *et al.*, 2003).

Information on the adsorption of protozoa to particles is limited (Ferguson *et al.*, 2003). However, Medema and Schijven (2001) showed that 75% of *Cryptosporidium* and *Giardia* oocysts/cysts attached to particles within 24 hrs in secondary wastewater effluent. A study by Cizek *et al.* (2008) showed that for dry weather flows, around 30% of both *Giardia* and *Cryptosporidium* were associated with settleable particles, whilst this increased to around 50% during wet weather flows.

Adsorption of viruses to particles/soil has been investigated by many authors and not only does the adsorption behaviour of these microorganisms vary between virus types, but it is not uncommon that different strains (of the same type) have different adsorptive capacities (Ferguson *et al.*, 2003). Gerba (1981) showed the large differences in virus adsorption rates in water, with mean adsorption rates ranging from as low as 16% for some viruses, but up to 85% for others. Davies *et al.* (2003) found that, while a significant proportion of somatic coliphages were associated with particle sizes of less than 2 μm, over half of the somatic coliphages found in wetland and pond sediment samples were associated with particles of between 2 μm and 20 μm (see Figure 8). Whilst it is apparent that adsorption of viruses to particles is variable, a proportion of viruses has been shown to be associated with particles.

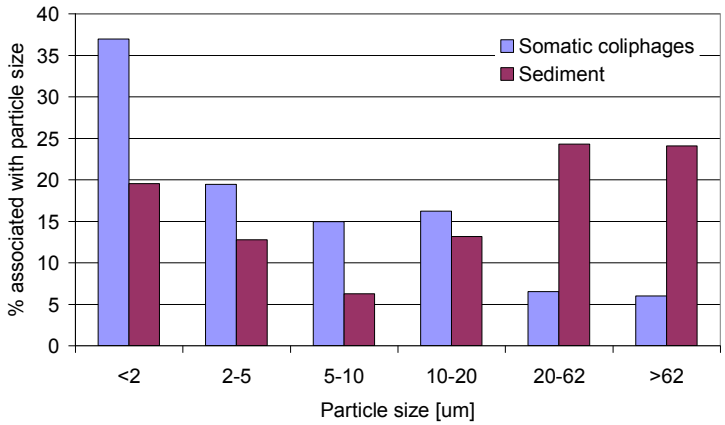


Figure 8. Average particle size associations of somatic coliphages and sediment for pond and wetland inlet stormwater samples (see Davies *et al.*, 2003).

3.2.2 Association with settleable particles and estimated sedimentation rates

Whilst the above information is very useful, it is necessary to transform these associations with particles to sedimentation rates, or at least to distinguish between settleable and non-settleable particles. Characklis *et al.* (2005) conducted a large experiment to determine the fraction of organisms which are associated with settleable particles for several different microbes, in both dry weather and wet weather flows. They found that for typical indicator organisms (faecal coliforms, *E. coli* and enterococci), the percentage attached to settleable particles range from between 20% and 35% during dry weather flows and between 30% and 55% for wet weather flows. The lower percentage found for organisms during dry weather is logical, since the microbes contained in dry weather flow are likely to be attached to smaller particle sizes due to the significantly lower flows, and hence lower acting forces. For *Clostridium perfringens* they found that the majority were associated with settleable particles during high flows (>70%), whilst a wider range were associated with these particles during dry weather flows (20%-70%). Finally, they found that total coliphages appeared to have a level of particle association similar to the traditional indicators, with dry weather associations between 30 and 50% and wet weather associations between 30 and 60%.

The above results were confirmed by a recent report by Krometis *et al.* (2007) who showed that around 40% of *E. coli*, enterococci and faecal coliforms were associated with settleable particles, while around 65% of *Clostridium perfringens* and just 13% of total coliphages were particle bound. They also note that during stormwater events the portioning behaviour remained fairly constant for each microorganism, but they showed that *Clostridium perfringens* in the rising limb of the hydrograph had very high settleable associations (80% as compared to just 45% in the recession limb). These findings indicate that the settling of indicator microbes, or their association with particles, are influenced by different sources of stormwater and different flow regimes. As such, it is essential that these changes are understood for an accurate microbial budget.

Other authors have attempted to quantify sedimentation rates for microorganisms. For example, Auer and Niehaus (1993) found that the settling loss rate for faecal coliforms was 1.38m/d by determining the association of faecal coliforms with particles of various sizes and measuring the sedimentation rate for these particles. Jeng *et al.* (2005) estimated settling loss rates for microbes in lake water, using a similar methodology as Auer and Niehaus (1993). They converted these settling loss rates to estimated exponential reduction coefficients (i.e. k_s) for faecal coliforms (k = 1.44/day), *E. coli* (k = 1.34/day) and enterococi (k = 0.864/day). However, Auer and Niehuas (1993) and Jeng *et al.* (2005) only considered these loss kinetics for a lake system, and hence different sedimentation rates would apply for systems with different storage periods (e.g. rivers/streams).

Recommendations for the microbial budget:
The importance of sedimentation in this microbial budget is once again heavily influenced by the system chosen. For example, if a system is chosen which has a very low flow during dry weather periods, then sedimentation would play a very important part in the mass balance since low flows generally induce sedimentation of even fine particles. However, at the other extreme, a very fast flowing system may have shear stresses well above those to induce sedimentation, thus making it a less significant factor.

Other characteristics of the chosen stream will also determine the influence of sedimentation in this budget, including the depth and the length of the system. For example, if a deep system was chosen, then sedimentation will take longer, and if a short stream reach was monitored then the possibly low sedimentation rate of particles with microorganisms attached, combined with a short distance for this to occur, would mean sedimentation could be negligible. The sources of microorganisms for the chosen site will also influence the significance of sedimentation. For example, if a site was selected where microorganisms entering the system are generally attached to very fine particles (e.g. influent to the system is the effluent from a biofilter), then the proportion of microbes in the system affected by sedimentation could be relatively small.

All of the above factors display the importance of the flow regime in determining the impact of sedimentation on the microbial budget. Another factor which will play a major role in determining the significance of sedimentation is the chosen monitored microorganism. It is clear from the above discussions that the association of microorganisms with sediment can vary significantly, thus this factor needs to be considered for this budget.

Experiments will be required to study the partitioning behaviour and settling behaviour of the selected microorganism in the study site, during flow regimes which need to be captured within the budget. This could be done by using various testing methods applied to samples withdrawn from the selected study site. These methods could include: the generalised pipette technique (see Palmer and Troeh, 1995; Davies and Bavor, 2000), the multiple pipette technique (Marsalek, 2008) or centrifugation (see Characklis *et al.*, 2005). It would also be interesting to assess the impact of sedimentation using in-situ field experimentation, which would help confirm settling results seen in

the laboratory. Independent of whether or not experimentation is conducted, the velocity of the stream (which is easily translated to shear stresses) should be monitored continuously during the budget. This data will help develop and validate a new predictive model of in-stream processes.

4 Microorganism sources in water bodies

The two main sources of microorganisms in water bodies considered for this budget are: (1) the growth and resuspension of microorganisms from bottom sediments during higher flow events and (2) direct faecal inputs (e.g. bird faeces entering the system). This section investigates these two sources in terms of estimating an accurate microbial budget.

4.1 Growth and resuspension

There are three processes that occur in water systems with regard to microorganisms: (1) transport of organisms by flow, (2) deposition and (3) growth of microorganisms. Typical sediment transport equations (i.e. dispersion and convection, see Graf, 1971; Yang, 1996) can be used to describe the transport of microorganisms by flow. Deposition of particles (with microorganisms attached) might occur during low flow periods through the process of sedimentation (as discussed above). The survival and growth of these deposited microorganisms, and subsequent resuspension of particles with these attached microbes during high flow periods, has been studied for decades by numerous authors (*growth* - Oliveri *et al.*, 1977; Burton *et al.*, 1987; Marino and Gannon, 1991; Makepeace *et al.*, 1995; Weiskel *et al.*, 1996; CWP, 1999; Desmarais *et al.*, 2002; *resuspension of sediment with microorganisms attached* - Schillinger and Gannon, 1985; CWP, 1999; Jamieson *et al.*, 2004; Jamieson *et al.*, 2005).

Both Burton *et al.* (1987), who tested the survival of bacteria in freshwater sediments, and Marino and Gannon (1991), who tested the survival of faecal coliforms and streptococci in separate stormwater drain sediment, reported that faecal coliform bacteria can not only survive but can also multiply in the sediments of urban streams, ditches and drains. Faecal coliform survival in street-side kerb sediments has also been reported (Bannerman *et al.*, 1996; CWP, 1999). Thus, even the stormwater/stream system itself is a source of microbial contamination when resuspension of sediment occurs (CWP, 1999). Resuspension of deposited sediment, and associated microorganisms, may occur during wet weather events and a percentage will be effectively transported to the outlet of the system.

Whilst storm drain transport has not been fully investigated for urban stormwater systems, sedimentation of microorganisms during low flow periods, and resuspension of these during high flow periods, has been investigated in detail by Kay and McDonald (1980) for a large river system in the UK. They found that, using artificial hydrographs (i.e. using controlled releases from an upstream impoundment), the release of microorganisms from channel bed sediment **was capable of producing coliform peaks in the same order of magnitude as those observed during natural flow events** (Wilkinson *et al.*, 1995). This demonstrates the ability of coliforms to either survive for long periods or multiply within the system, and indicates that the levels of microorganisms during storm events are not entirely caused by inputs into the channel, but are also highly dependent on the coliforms within the channel itself. Furthermore, experiments with repeated releases indicated that there was a finite supply of microorganisms within the channel (i.e. the channel store was depleted of available microorganisms) (Wilkinson *et al.*, 1995). Wilkinson *et al.* (1995) also showed that modelling the entrainment of faecal coliforms using a direct function of flow was adequate and the depletion of the faecal coliforms from within the channel storage could be modelled using a mass balance approach.

Recommendations for the microbial budget:
While it is apparent that microbes are capable of surviving for long periods within bed sediments, and can often grow in appropriate conditions, the subsequent resuspension of these microbes will generally only occur during wet weather periods (unless a stream is chosen which has high dry weather flows). As such, if the project aims at investigating dry weather periods only, the resuspension and growth of these microbes might not be significant enough to include in a mass balance. However, if wet weather periods are to be investigated (which is required if a complete microbial budget is necessary), then resuspension should definitely be investigated further using different experimental techniques.

For example, a number of small experiments could be devised which collect samples within a small reach of the selected stream/river which has no other inputs. Samples could be collected upstream and downstream of this reach at different flow rates (i.e. during a low, medium and high intensity wet weather event). Since negligible other sources will exist (and die-off would be minimal across a small reach length), then the difference between these samples could be attributed to either a net resuspension of microorganisms, or a net deposition of microorganisms. However, replication may have to be high in this type of experimentation to ensure adequate statistical inferences could be conducted. Sampling of bottom sediments could also be conducted to determine the survival or growth of microorganisms in these depositions.

Other experimental options exist, including ones which use the hydraulics laboratory at Monash University to simulate different flow rates applied to bed sediment collected from the study site. The bed sediment could also be tested in the laboratory to determine growth rates of microorganisms under certain environmental conditions (as discussed above).

4.2 Other in-stream inputs

Other in-stream inputs could be a source of microorganisms for the selected water body, and may include inputs from animals which reside in/around the water body. The faeces from waterfowl and other animals which live in/around stormwater ponds and wetlands have often been used to explain the high levels of microorganisms often found leaving these systems. For example, Davies *et al.* (2003) hypothesised that the reason for high somatic coliphage concentrations in a pond and a wetland in the absence of rainfall was probably from the faeces of resident waterfowl populations. Furthermore, Stenstrom and Carlander (2001) explained that the reason for high numbers of coliphages in a sedimentation pond was possibly a result of the bird-life in the area. CWP (1999) suggested that geese, gulls and ducks are a major bacterial source in USA's urban areas, especially in areas with open water bodies.

While many researchers have concluded that waterfowl do contribute large amounts to urban water systems (as described above), there have been few which have attempted to quantify this input. Shellenbarger *et al.* (2008) conducted a study specifically aimed at identifying how wildlife can affect water quality in urban areas. They measured faecal indicator bacteria concentrations, presence/absence of Sa*lmonella*, bird abundance and many other physio-chemical factors to answer this question. They conducted a correlation analysis and found a negative correlation between indicator bacteria concentrations and bird abundance, indicating that as the number of birds increased, the level of indicator bacteria decreased. However, when using a mass balance approach they found that while other factors other than bird abundance were most important for indicator loads, bird faeces still contributed to the overall number of bacterial indicators within ponds. Another study by Wither *et al.* (2005) determined the impact of bird populations on the microbiological quality of bathing waters. They concluded that despite the uncertainties within their own methodology, bird populations should be considered as potential contributors to poor bathing water quality.

Recommendations for the microbial budget:
Estimating the contribution from waterfowl and other animals into a stream is very difficult, and will vary not only between sites, but will also vary considerably within the same stream reach. The contribution will vary by population of the specific contributor (i.e. numbers of animals residing near the selected water body), their faecal excretion rates (i.e. grams per day of faeces), the contents of their faeces (i.e. abundance of specific microorganisms), and the length and width of the open water surface (i.e. if the water surface is narrow and short, or if it is wide and long).

While some of these variables listed above are site specific, there are some variables which could be obtained from the literature. For instance, there are numerous reports which document the abundance of microorganisms (usually indicators only) in animal faeces and associated typical excretion rates, and these are summarised in Table 2. These values could be used in conjunction with estimated populations near the selected site to estimate contributions to the system. However, even then, it would be difficult to enumerate these sources accurately without some type of data collection at the selected site.

Table 2. Typical faecal indicator concentrations, and excretion rates, for various animals living around open water bodies.

Animal	Density [No./g]	Excretion rate [g/day]	Load [/day]	Source
Coastal bird	EC = 7.2×10^7			Leeming *et al.* (1998)
Coastal bird	FS = 1.6×10^8			Leeming *et al.* (1998)
Duck	FC = 3.3×10^7	68	FC = 2.2×10^9	CWP (1999)
Duck	FS = 5.4×10^7	68	FS = 3.7×10^9	CWP (1999)
Duck			EC = 1.1×10^{10}	Whither *et al.* (2005)
Gull			EC = 2×10^9	Whither *et al.* (2005)
Waterfowl	FC = 3.3×10^7	120	FC = 4.0×10^9	CWP (1999)

FC – faecal coliforms, FS – faecal streptococci, EC – *E. coli*

An experiment could be conducted to help quantify the amount of faeces which enter the selected stream/river system. For example, several large trays could be positioned over the stream/river at different points within the selected reach. These trays would catch debris, including faecal material, which fall directly into the river system (e.g. faeces from birds, possums etc). These trays could be collected on a weekly basis to determine the total mass of faeces deposited into the system. Fresh faeces could also be collected from the site and enumerated for select microorganisms to determine microbial densities. This would provide a rough estimate as to the quantity of microorganisms which are being deposited into the stream. However, there would be little chance of quantifying the microorganisms introduced into the system via direct deposition (e.g. birds bathing and excreting directly in the water, possums or water rats defecating in the water, etc.).

5 Other water quality and quantity data

As described above in Sections 4 and 5, in conjunction with faecal indicators, there are benefits in collecting other stormwater quality data. It is evident that in order to adequately estimate a microbial budget, it is necessary to fully understand the impact that environmental factors have on bacterial survival. As such, it is essential that this monitoring campaign measures the following parameters:

- stream temperature – continuous monitoring probe;
- stream turbidity – continuous monitoring probe;
- radiation from sunlight – weather station (or just a probe on its own);

- nutrient content in stream – collection of water quality samples and analysed for TN, TP and some nitrogen components/species (if budget allows);

When creating a microbial budget, it is necessary to consider loads of microorganisms for specific periods of time (or events). These loads are usually estimated using a product of the sample's microorganism concentration and the corresponding flows which are represented by this sample. As such, flow rates are required to be monitored as part of this microbial budget. Not only are inflows, and outflows, from the specified stream reach required, but flow rates (and associated microorganism concentrations) are also required for any water which enters this stream reach between the inflow and outflow points (e.g. from stormwater pipes, etc.). Furthermore, in order to understand sedimentation and resuspension accurately, it is necessary to also have some estimation of velocity of the stream so that shear forces acting on particulate matter can be estimated.

The following section will address the level of uncertainty associated with the collection of both water quality and quantity data from urban stormwater and stream systems.

6 Uncertainty in flow and water quality measurements

As mentioned above, a large number of water quality data need to be collected from the system to determine an accurate microbial budget. Furthermore, measurement of flow rates is essential in developing any type of budget. The accuracy of the budget is heavily dependent on the uncertainties within the monitoring of these flows and water quality parameters. This section of the review focuses on these uncertainties and attempts to quantify some of these uncertainties using the data and research available within the literature.

6.1 Location of monitoring stations

Once the site is selected, and the number of monitoring stations required is determined (e.g. at each entry point to the stream system), their specific location needs to be established. However, even before the site is even selected, a whole range of logistical and quality control considerations come into play. The following criteria have to be considered for the choice of specific monitoring sites (US EPA, 2002; Bratieres *et al.*, *in preparation*), which will hence impact the overall site selection:

- monitoring stations (for flow and water quality sampling) should be located where access and security/safety is good (e.g. good visibility, minimal traffic hazard) and where vandalism of equipment is unlikely. Ease of access should not be overlooked, especially since the site is to be equipped with water quality monitoring equipment (e.g. a typical autosampler can take 24kgs of samples in one event).

- flow monitoring stations should be located where flows are uniform and stable for some distance upstream. While there is little literature on the estimation of uncertainty with respect to the correct positioning of flow equipment, Harmel *et al.* (2006) suggested that turbulence and pulsating flows will increase the inherent measurement uncertainty. Hence, it is important that flow measurement devices are installed in areas of low turbulence. Therefore it is recommended to avoid certain sites with, for example, steep slopes, junctions, irregular channel shapes, or locations affected by backwater and tidal conditions, or at least be sufficiently up-, or down-stream, of these locations. Monitoring locations should also be sufficiently downstream from inflows to the drainage system, particularly in wide conveyances, so that the water is well-mixed and uniform. Because of the great distance required for complete mixing, it is often useful to have composite samples taken across wide streams in order to achieve uniformity in the cross sectional area. A pilot study could also be used to identify the degrees of variation within a cross section. Guidelines do

exist for installation of flow meters in pipes, including electromagnetic flow meters which should be positioned anywhere between 5 and 10 pipe diameters downstream, and 1 to 5 pipe diameters upstream, of any obstruction (e.g. see Eisenhauer, 2008). However, for situations which are not as well defined (e.g. rivers or tributaries) it is difficult to provide specific installation guidelines without an individual site assessment.

- consideration needs to be paid to water quality equipment and how the location of this monitoring equipment can affect the monitoring program. If continuous data is required (this will be discussed in the next section) and probes which have in-built logging functions and battery supplies are used, then regular access will be required for their maintenance and downloading. Also, if autosamplers are required, then things such as pumping rates (and associated heads) need to be considered when assessing a site's feasibility.

6.2 Flow data

Flow monitoring equipment

It is paramount that flows (including velocities for shear force estimates) be monitored in order to achieve any mass balance of microorganisms within the selected water body. The following review will investigate a number of choices in terms of flow monitoring and will provide a basis as to how to choose this equipment for the different monitoring locations.

Uncertainties in flow measurements heavily depend on the type of measuring device, as well as on the flow magnitude and the quality of the installation. Harmel *et al.* (2006) reviewed the uncertainty in stream flow (i.e. open channel flow) measurements using an array of different methods and Table 3 displays a summary of these results. They found that the velocity-area flow measurement method was one of the most accurate methods available and the uncertainty sources of this type of measurement include: uncertainty in the depth estimate, uncertainty in estimating the velocity and uncertainty in estimating the channel's cross section (Bertand-Krajewski and Bardin, 2002).

Table 3. Uncertainty in streamflow measurements (adapted from Harmel *et al.*, 2006).

Type of measurement	Flow uncertainty
Velocity area – ideal conditions	± 2%
Velocity area – average conditions	± 6%
Velocity area – poor conditions	± 20%
Manning's equation – good conditions	± 15%
Manning's equation – poor conditions	± 35%
Stage-discharge relationship with flow control structure	± 6%
Stage-discharge relationship with stable channel	± 10%
Stage-discharge relationship with shifting channel	± 20%

Whilst velocity-area equipment can provide the most accurate flow measurements, it must be noted that, if this equipment is installed in poor conditions, large uncertainties can be produced (reported up to 20%, Table 3). Furthermore, for very small flows (where water heights and velocities are both low) these types of monitoring equipment can be prone to producing flow estimates with uncertainties in excess of 200% (McCarthy *et al.*, 2008). These high uncertainties are usually because either the flow meter is no longer submerged in stormwater flows (e.g. dry weather flows in stormwater pipes are often very small) or the velocity is so low that the instrument is unable to estimate flow velocities.

In a recent study conducted by Monash University, ultrasonic/Doppler flow meters were installed in the stormwater pipe inverts at three stormwater sites around Melbourne. The aim of this study was to understand the dry weather flows and loading patterns of specific pollutants. Obtaining accurate

estimates of flow rates were especially hard due to minimal flow depths. In an attempt to increase the depth of water (to submerge the probe), Monash installed several weirs downstream. However, these weirs led to two problems: (1) because the flows were low this increase in height meant that the velocities were decreased so significantly that they were below the detection limit of the instrument and hence flows could not be estimated, and (2) litter and bed sediment accumulated to such a degree that even weekly clean-outs were not sufficient to keep the probe in operational conditions. The result was that the weirs were removed and the monitoring of very small flows was not achievable in the scope of the study, with only flows which had enough depth and velocity being monitored. In future studies, Monash will design trapezoidal flumes to allow accurate flow measurements using a stage-discharge relationship, whilst still maintaining sediment transport.

In streams which have very low base-flows, ultrasonic/Doppler-based flow meters will provide accurate high flow measurements, however they may not provide accurate dry weather flow estimates. As such, stage-discharge relationships could be used in these situations. Although obtaining these stage-discharge relationships are often costly, if they are on a stable base, and an accurate survey of the channel has been conducted, then flow rates can be easily and accurately monitored using ultrasonic depth probes (Harmel *et al.*, 2006).

Flow logging and averaging intervals

The logging interval for monitoring flow can often have a significant impact on the estimated stormwater volumes, especially during wet weather flows. Figure 9 shows an example of this for a small urban carpark in Belgrade (see Deletic *et al.*, 1998 for catchment details) and it is clear that as the logging interval increases, the accuracy of the total event volume decreases and is usually underestimated (source Bratieres *et al.*, *in preparation*). This underestimation occurs because as the timestep of recording increases, the chances of missing a peak in the hydrograph also increases, thus providing an underestimate. Whilst this will vary between different catchment types (sizes and imperviousness), it is preferential (and often at no extra cost) to keep measurement logging intervals to a minimum, especially around 6 minutes (Figure 9). However, if the loggers are powered by batteries and have minimal memory, then decreasing the timestep can often require weekly visits for downloading and maintenance. Finally, using the averaging function which can be found on many current flow loggers and depth probes means that the logging timestep can be increased without losing excessive accuracy.

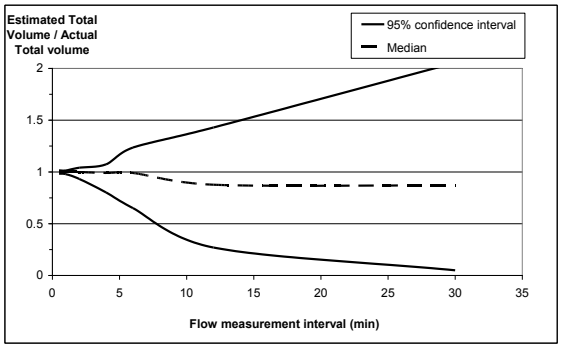


Figure 9. Flow logging intervals and their effect on the accuracy of total wet weather event volumes of a small urban carpark (source Bratieres *et al.*, *in preparation*).

Whilst the above mainly focuses on logging of wet weather flows, monitoring of dry weather systems can be slightly different. In large catchments, the monitoring of dry weather flows can be done using coarse timesteps (>6 minutes) since the flow regime is to unlikely change as dramatically as in wet weather events. However, in smaller catchments, where flows are made up from a quantifiable number of sources, the flow rate may change in magnitude quite quickly and if this variation is to be captured accurately then small timesteps should be used. However, as mentioned

above, using small timesteps has the benefit of higher accuracy and usually requires no extra cost, especially if the flow probe has mains power and a large enough memory.

Recommendations for the microbial budget.
All monitoring stations should be accessible by car, or easily assessable by foot (for downloading and maintenance activities), and in an area where vandalism is kept to a minimum. "High voltage" stickers are a useful deterrent for vandals, however will not stop tagging or graffiti. Access to power should be considered, but this is often not available and as such the majority of equipment will run from a 12 volt or 24 volt battery (although this will increase maintenance requirements). Furthermore, the site must not influence the accuracy of the dataset (i.e. it should be sufficiently downstream of any obstruction and should have a low level of turbulence).

Ultrasonic/Doppler-based devices should be used when depths and velocities are significantly above the level detectable by these instruments. This is mainly because if this type of equipment is installed correctly, the errors in calculated flow rates are minimal. However, in situations where these depths or velocities are below those required by these devices, then another measurement option is required. Flow control devices which allow sediment to pass unrestricted (e.g. trapezoidal flumes, Parshall flumes and WSC flumes) could be used in situations where space and installation of such a device permits. Flumes are accurate for low flows and are available in precast form from the USA (see Plasti-Fab.com). In places where flow control devices cannot be installed (e.g. in streams etc.) and are not suited for ultrasonic/Doppler-based equipment, then a site which has a firm base should be selected for a stage-discharge monitoring station. A stage-discharge relationship should be developed using an accurate site survey (including channel geometry) and flow measurements. In any open-channel monitoring station, it is recommended that a channel survey is conducted on a regular basis to ensure accurate measurements (Harmel *et al.*, 2006). Careful installation and regular calibration and testing should ensure that all the above recommendations will produce flow rate estimates with minimal uncertainty.

6.3 Water quality

Whilst some parameters listed in Section 6 can be monitored continuously using in-situ probes, it is not possible to continuously monitor other pollutants, such as nutrients and microorganisms (Section 2). As such, this section is divided into two separate subsections: the first describing the collection of continuous data and the second describing the collection of discrete datasets. Whilst the former can collect large amounts of information about a pollutant's variation (often in excess of what is required), the latter is limited by the fact that discrete samples must be taken from the water column, and subsequently analysed in a laboratory for select pollutants. Careful consideration must be paid to the design of the discrete sampling regime (i.e. when, where and how often samples are taken) in order to obtain adequate information.

6.3.1 Continuous data

The collection of continuous data is only available for a select number of constituents, and continuous monitoring probes for measuring microorganisms are not common. Using monitoring probes for the collection of 'easy-to-measure' physical parameters (e.g. temperature, turbidity, pH, electric conductivity, etc.) is definitely recommended for this project since these types of probes are usually quite stable. However, before employing the use of continuous probes, there are a number of aspects to consider, and many of these aspects are similar to those described in Section 6.2 for flow monitoring. Firstly, the site must be accessible and have enough water depth to cover the probe. Usually these probes have enough memory, and, as such, logging intervals can be easily set to as low as 6 minutes without any problems. However, battery consumption in these probes, especially ones with turbidity functions, is often high, and instead of memory issues it is often the battery consumption rate which will pre-determine your selected logging interval. Calibration and testing of these probes should be conducted both prior to, and during, the operational stage of the

project. The calibration requirements for these 'easy-to-measure' parameters are usually low and if calibration is conducted each time a replacement battery is required then this would be sufficient. However, stricter calibration regimes would need to be employed for continuous monitoring probes which include parameters such as ammonium.

Much of the discussion which follows in Section 6.3.2 will also relate to continuous data (such as where to position the probe) so please refer to the following section for this information.

6.3.2 Discrete data

Whilst the aim of this project is to create a microbial budget, only a certain portion of the following sections focuses on microbial water quality data collection, and the other portion focuses on the collection of more traditional water quality parameters (e.g. sediment, nutrients etc.). This was conducted in this manner for a number of reasons: (1) as listed in Section 5, these traditional parameters are also required to be monitored, and as such should be discussed, (2) providing information about the collection of these typical pollutants can help provide a reference point and help us to accurately collect microbial data and (3) the literature available on uncertainties in microbial water quality data collection is minimal. The following discussions will be structured such that the first part of each section focuses on uncertainties in collecting traditional stormwater pollutant data, and then the focus will shift to specifics on microbial water quality data collection, if available.

In order to discuss the following sections, it is necessary to provide some framework of the large number of areas which are addressed. This section will report mainly on the different sources of uncertainty which contribute to discrete water quality data. In particular, it will focus on the sources of uncertainty which apply to estimating total loads either entering, or leaving, a defined stream reach. The reason loads are used in this framework, in preference to concentrations, is because the aim of this project is to conduct a bacterial mass balance and in order to calculate a mass balance, loads need to be estimated.

Figure 10 shows the different uncertainty sources which contribute to the combined load uncertainty for any particular parameter which is measured using discrete datasets. The first source of uncertainty in estimating an event load (either a wet event, or a dry weather event) is the discrete sample uncertainty. This is the uncertainty which is involved in the collection and analysis of the sample which represents a specified column, and cross section, of water in the vicinity of the sampling area. Understanding this type of uncertainty will help determine: (1) the number of samples required to be taken within a given cross section of a water body, (2) whether storage of the samples will introduce significant uncertainties in the result and (3) whether the analytical uncertainty is within acceptable levels.

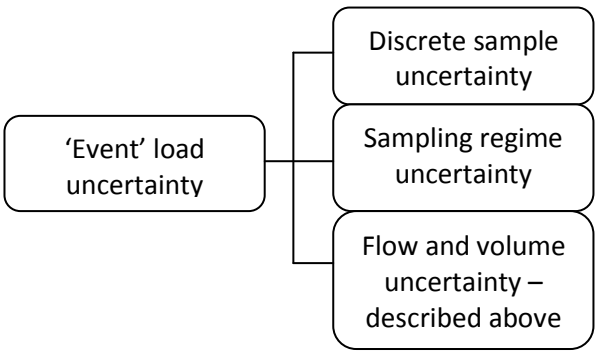


Figure 10. Showing the different uncertainty sources contributing to any pollutant load estimate.

The sampling regime uncertainty is related to the fact that the collected sample is used to represent a large amount of water which has travelled past the sampling position since the entire event volume cannot be sampled (e.g. samples have to be spaced apart in time, or by volume, since it is impossible to collect and analyse an entire stormwater event). Understanding this uncertainty will help determine the number, and spacing, of samples during wet and dry weather periods required to adequately assess the event's total load of different pollutants.

The third, and last source of uncertainty, is that related to estimating flow rates and total event volumes using the selected flow monitoring equipment (this uncertainty source was described above and will not be discussed below).

Discrete sample uncertainties

The collection of discrete data implies that a sample is withdrawn from a water body using some defined technique, this sample is then stored for a period (i.e. whilst it is transported to the laboratory and/or within an autosampler) and the sample is subsequently analysed in a laboratory (see Harmel *et al.*, 2006; McCarthy *et al.*, 2008). Each of these components has an associated uncertainty. This uncertainty will then have an influence on any further use of this data (including if this data is used to estimate loads and budgets). As such, it is very important to understand each of these associated uncertainties in order to help reduce them. The following discussion will refer to the following three uncertainties:

1. sampling uncertainty – introduced by the sampling method employed;
2. storage uncertainty – introduced by the storage of the sample before analysis; and,
3. analytical uncertainty – introduced by the laboratory analytical technique used to quantify the sample for the specified pollutant

Whilst all of these uncertainties apply to any sample taken from a water body, some of these uncertainties will differ between: (1) pollutants, (2) sampling method used (manual, automatic) and (3) dry and wet weather periods.

Sampling uncertainty. This uncertainty is related to the fact that a sample is often taken from just one position within the water cross-section (usually near the bottom of a stormwater pipe when autosamplers are used for stormwater monitoring), which is assumed to be representative of the entire water column (Harmel *et al.*, 2006). Sampling uncertainty can also be caused by a poor setup of autosamplers (e.g. long suction pipes, etc.) or incorrect procedures during manual 'grab' sampling methodologies (Harmel *et al.*, 2006; McCarthy *et al.*, 2008).

Harmel *et al.* (2006) summarised stream water sampling uncertainties when using just one intake point within the water cross section for a range of pollutants (see Table 4). Table 4 shows that as the pollutant type varies from particulate to more dissolved, the uncertainty associated with using just one intake point decreases. This is something which is reflected by Rode and Suhr (2007) who state that "compounds associated with suspended particulate matter have considerably higher sampling uncertainties than soluble concentrations". This pattern is logical since dense pollutants (such as coarse sediment), or those pollutants attached to dense material, will tend to settle out, thus creating a water quality profile within the water column. Alternatively, a more dissolved pollutant (e.g. nitrogen) will have more constant concentrations throughout the water column.

Table 4. Sampling uncertainties for different pollutants from stream water samples (adapted from Harmel *et al.*, 2006).

Constituent	Sampling uncertainty
Dissolved N (NH_3, NO_3, NO_2, NO_2+NO_3 forms)	Range of medians 0% to 4% (overall median 0%)
Total N	Range of medians 0% to 0% (overall median 0%)
Dissolved P (PO_4)	Range of medians 0% to 0% (overall median 0%)
Total P	Range of medians 0% to 17% (overall median 0%)
TSS	Range of medians 14% to 33% (overall median 20%)

Bratieres *et al.* (*in preparation*) has shown data which supports that shown in Table 4, and Figure 11 shows the difference in pollutant concentrations between stormwater samples withdrawn simultaneously from (1) the pipe's invert and (2) the top of the water column. The majority of this data (i.e. all but sample 4 in the right hand side graphs) indicates that as the pollutant type becomes more dissolved (moving from suspended solids to total nitrogen), the sampling uncertainties (or differences between the top and bottom samples) becomes smaller.

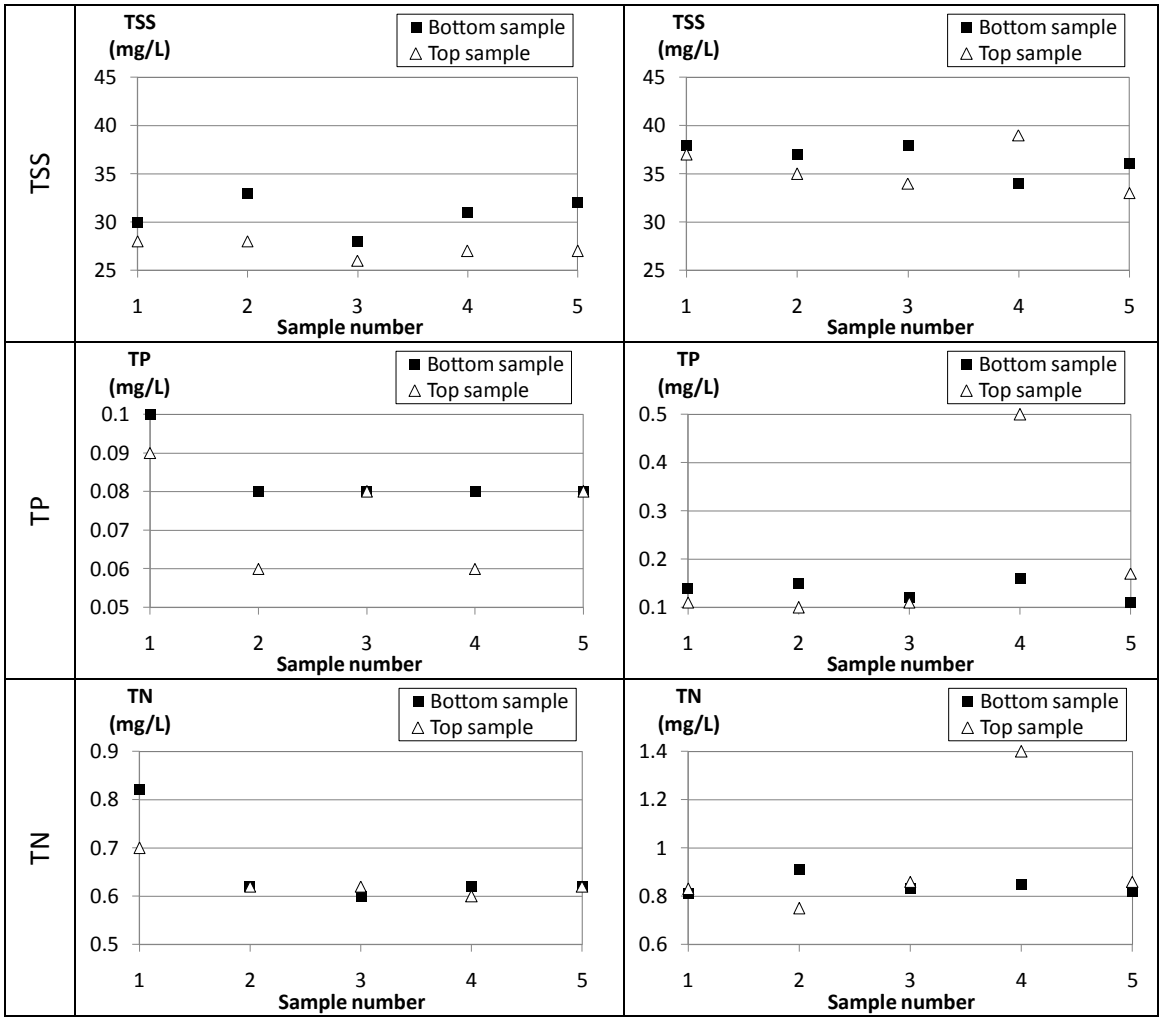


Figure 11. Pollutant concentrations in urban stormwater wet weather flows taken simultaneously from the pipe's invert and the top of the water column (source Bratieres *et al.*, *in preparation*).

Further to Harmel *et al.* (2006), there have been other studies which have estimated the sampling uncertainties for different water quality constituents. For example, Ahyerre *et al.* (1998) showed that the difference between the TSS concentrations of samples taken with two different samplers working simultaneously was around 15%. Another study by Rode and Suhr (2007) suggested that

errors of singles samples within a cross section, compared with measurements made using depth-integrated samples, leads to errors ranging from 2% to 12% for suspended sediments. Comparing this estimate to that described in Table 4 demonstrates that differences can occur between sampling uncertainty estimates, however the values still broadly agree.

Lovell *et al.* (2001) conducted a study to assess the impacts of spatial sampling along a river in South-Eastern Victoria. They collected samples from seven sites along the river. Within each of the seven sites, they collected six samples within a close proximity of one another. These samples were then analysed for TP and FRP concentrations and statistical tests were performed to assess the sampling uncertainties. They found a median sampling uncertainty of 13% and 10% for TP and FRP, respectively. Again, this indicates that the more dissolved pollutants will have lower sampling uncertainties because of better mixing within the water system. It should be noted that although spatial scale uncertainty was assessed using this approach, uncertainties associated with depths was not assessed.

Whilst there has been a significant amount of research devoted to the sampling uncertainties of typical stormwater pollutants (such as sediment and nutrients), there has been less research devoted to microbial sampling uncertainties. One paper which has touched on this aspect is that by McCarthy *et al.* (2008) who sampled two stormwater events and withdrew *E. coli* samples from the pipe's invert concurrently with samples from the top of the water column. Figure 12 shows the results of this experiment and indicates that, considering the large analytical uncertainties in assaying *E. coli* levels, the difference between the bottom and top samples is not significant.

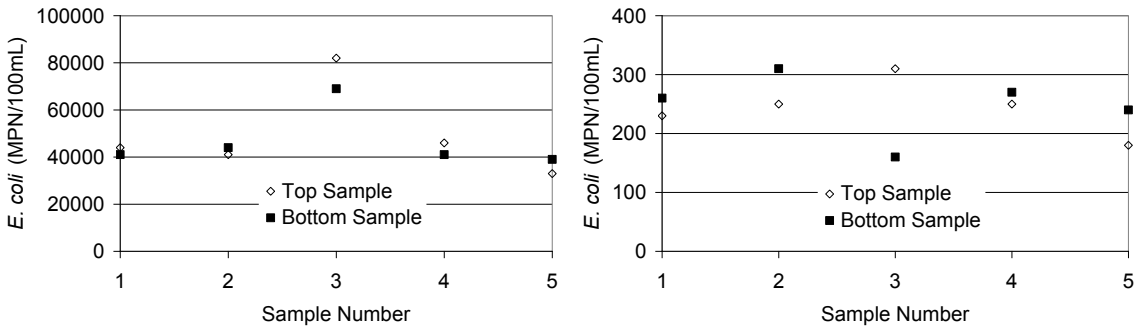


Figure 12. *E. coli* concentrations in two urban stormwater events taken simultaneously from the pipe's invert and the top of the water column (source McCarthy, 2008).

Two sample t-tests for comparison of means were conducted on the results shown in Figure 12, and the results indicate that there is no significant difference between the level of *E. coli* taken from the bottom and the top of the water column. This suggests that the amount of error introduced by only taking samples from the bottom of the water column is negligible. Furthermore, the largest relative difference between the top and bottom *E. coli* levels was found when the top sample had a higher *E. coli* level than the bottom sample. The trends shown here are logical since bacteria (and often viruses) are usually associated with fine particles (which are evenly mixed in turbulent stormwater flows) (Davies and Bavor, 2000) and, as such, sampling uncertainties for *E. coli* are usually minimal.

While the sampling uncertainties for other bacteria would be expected to follow similar trends as that for *E. coli*, it is uncertain as to whether this assumption is valid. Furthermore, it is more than probable that other types of microorganisms, such as viruses and protozoans, would behave differently to that shown for *E. coli*. Unfortunately, there is limited data in the literature as far as this is concerned.

The majority of the above discussions have been focussed around wet weather monitoring of pollutants. In the majority of stormwater pipe situations, the dry weather flow is so low that withdrawing a sample usually means sampling from the entire water column. In these types of scenarios, the sampling uncertainty would be negligible since the entire water column would be effectively sampled. In stream situations, however, where water depths are such that samples could be taken at several points, then sampling uncertainties would be present.

Recommendations for the microbial budget:
While sampling uncertainties do exist, they are often low for the more dissolved pollutants. Considering microorganisms are very small and are usually associated with fine particles, the sampling uncertainties for microorganisms are hypothesised to be small in wet weather flows where they would be reasonably well mixed. Nevertheless, it is recommended that this be tested at the chosen site using experimentation similar to that described above by McCarthy *et al.* (2008). This type of experimentation is easy to conduct and has relatively low analytical and collection costs. However, of the large number of other uncertainty sources which exist, if the budget does not allow for such experimentation, then it could be robustly argued that this uncertainty source is minimal and that funds could be more effectively used elsewhere in the project.

During dry weather flows, the sampling of stormwater pipes usually implies that sampling uncertainties do not apply because of such small water depths. However, as mentioned above, for streams during dry weather, this uncertainty can be accentuated. As such, some small experimentation is suggested to determine the existence of a concentration profile/cross section. This testing will help determine whether the samples from the stream be taken from a single point, or from multiple points across the stream's cross section. If multiple points are suggested from this experimentation, then a composite sample could be made so as to reduce analytical costs. Again, if the budget is not favourable to such experimentation, then multiple samples could be taken and combined into a composite to be safe and ensure this uncertainty is minimised. However, consideration must be paid to the extra effort required to take multiple samples and composite these samples throughout the monitoring programme, and whether a simple experiment would actually be more favourable in terms of costs.

The sampling of microorganisms should be conducted in accordance with current standards to ensure that contamination of the sample is minimised. Blank samples should be taken a regular intervals to ensure that the laboratory chosen is using proper practises and replication of samples should also be conducted regularly to ensure analytical errors are within appropriate limits (see *Analytical Uncertainty* section, below). Autoclaving of bottles which are used for the collection of microorganism samples is a necessity (see AS/NZS 2031:2001). If nutrient analyses are recommended, then cleaning of the bottles prior to use is essential, using proper reagents to ensure samples are not contaminated. Attention should also be paid to the choice of sampling bottles, since some bottles have the potential to adsorb certain pollutants and, in some situations where bottles are reused and improperly cleaned, they could leach pollutants (Harmel *et al.*, 2006).

Storage uncertainty. The storage of samples, even when refrigerated, can often affect the accuracy of the analysed result. For example, microorganisms are very sensitive to storage, and even when kept in cool temperatures the die-off over 12 hours can be significant. Storage will occur whenever a sample is collected, whether it be collected and stored by an autosampler, or manually collected and stored during transport to the laboratory. As such, it is important to assess the impact of storing water samples on the overall uncertainty of the analysed sample.

Harmel *et al.* (2006) conducted a thorough literature review on the uncertainties caused by the storage of samples for a number of different water quality pollutants (see Table 5). It is clear that

for most nutrients, it is necessary to preserve the sample, either by refrigeration or using other preservation techniques. Samples without preservation and long storage times had uncertainties averaging around 20%, with all of them underestimating the real concentration. However, samples which were refrigerated and had short storage times resulted in lower uncertainties, with an average of around 7%. One of the main factors which influenced the storage uncertainty for nutrient samples was the concentration of the nutrients, with samples that have high nutrient concentrations achieving far lower relative uncertainties than samples which have lower concentrations (Harmel *et al.*, 2006).

Table 5. Storage uncertainties for different pollutants from stream water samples (adapted from Harmel *et al.*, 2006).

Constituent	Storage uncertainty			
	Acidified, analysed within 6hrs	Iced, analysed within 6hrs	Only refrigerated, analysed within 2¼days	Unpreserved, analysed within 8days
NH_3-N	-8 %	-18 %	-16 %	-38 %
NO_3-N	-1 %	0 %	-2 %	-2 %
TKN	-1 %	3 %	-9 %	-26 %
TP		-7 %	7 %	-11 %
Filterable P		-7 %	8 %	-17 %

While there is some information about the storage uncertainty for nutrient samples, the literature review showed little information for stored microorganism samples. However, there are guidelines which suggest methods to minimise these storage uncertainties. For example, the Australian Standard for collection of microorganism samples (AS/NZ 2031:2001) suggests that all samples should be refrigerated, in the dark, between 2°C and 10°C during sample storage, but not frozen, and may be examined up to 24 hours after the time of collection.

However, in many scenarios non-refrigerated autosamplers are used to collect stormwater samples, since refrigerated samplers are very expensive and consume a lot of energy. As such, it is necessary to understand how this impacts the results of the sample, and whether significant differences are observed during storage. There is but one paper which has investigated this important factor, that of McCarthy *et al.* (2008). Their methodology and results are discussed below.

Three sites were selected for these experiments and they were repeated twice at each site. For each experiment, twenty replicate samples were withdrawn from the water column during wet weather flows. To ensure close replication, the 20 sample bottles were arranged in a circle and were filled using 20 rotations so that samples received increments of 50mL. After collection, the samples were stored in environmental conditions that were close to typical field conditions (i.e. appropriate temperature and sunlight conditions). Five samples were randomly selected at 0, 4, 8 and 24 hours after the collection time and were immediately analysed for *E. coli*. The $\log_{10}$ transformed results (transformed because the variability of *E. coli* for each site was not similar) were analysed using a two factor ANOVA (Zar, 1999) to determine whether a sample's *E. coli* level was affected by the storage time of the sample.

The results of this methodology are presented in Figures 13 and 14. Figure 13 shows the results for the six experiments (two at each site). The only experiment out of the six conducted that showed a significant difference between the 0 hour and the 24 hour sample groups was the experiment conducted at Richmond on the 10th November 2005.

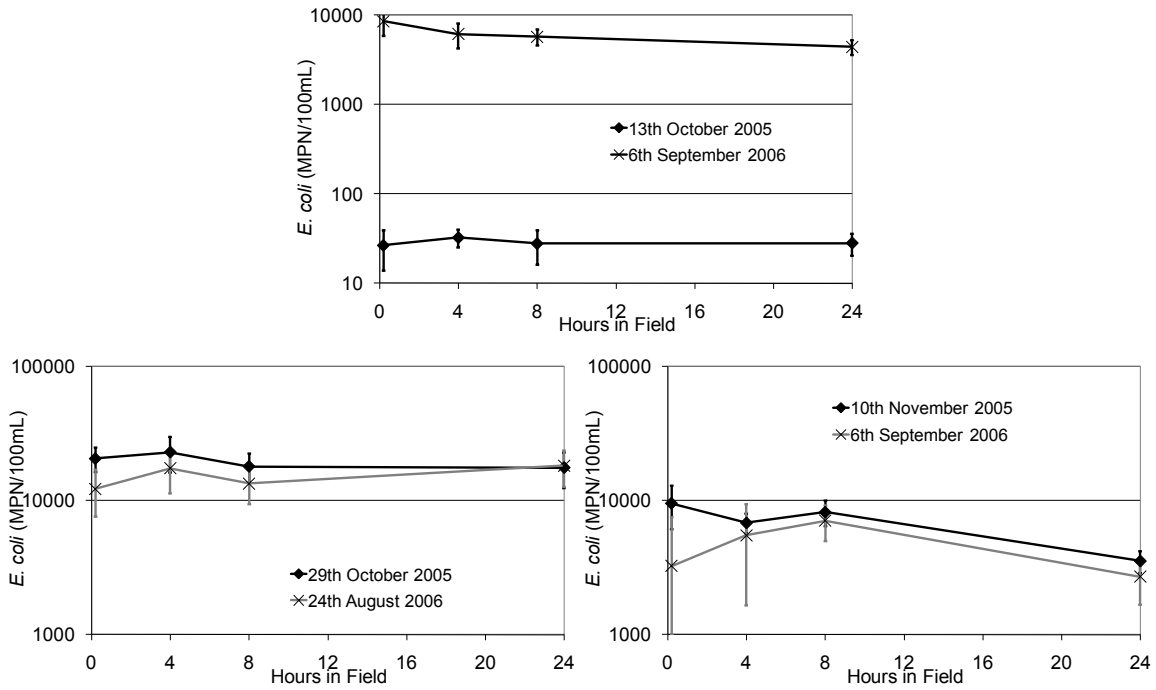


Figure 13. The impact of storage on the uncertainty in stormwater *E. coli* samples for three sites and two experiments at each site (sourced from McCarthy *et al.*, 2008). Error bars represent the 95% confidence intervals for the five replicate samples. Top – Clayton site, bottom left – Narre Warren site and bottom right – Richmond site.

To determine whether using refrigerated autosamplers could help reduce this uncertainty, a twin set of twenty replicate samples were kept in the refrigerator and these samples were analysed at 4hrs, 8hrs and 24hrs after collection (to compare refrigerated versus non refrigerated autosamplers). Figure 14 compares the *E. coli* levels in samples stored in the field with the levels of samples stored in the fridge. It shows that while these refrigerated sample groups had lower within-group variances, and varied less as storage time increased, there was still a statistically significant difference between the samples analysed at 0 hours after collection and the samples analysed after 24hours of refrigeration ($p<0.024$). While this significance was less than that for the non refrigerated sample groups ($p<0.005$), it still indicates that even with refrigeration the levels of *E. coli* in these specific samples could not be maintained. This could be attributed to a number of environmental factors which influence the survival of the *E. coli* in these samples, such as predation/competition which may not have been reduced by refrigeration.

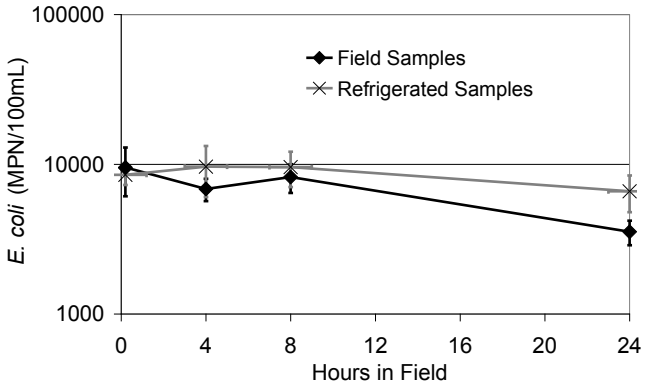


Figure 14. The difference between refrigerated samples and samples kept in the field over a 24 hour storage period (sourced from McCarthy *et al.*, 2008). Error bars represent the 95% confidence intervals for the five replicate samples.

Analysis of variance applied to the data from the six non refrigerated experiments (to determine whether *Hours in Field* significantly explained the *E. coli* levels during storage) resulted in a p-value of 0.43. This indicates that, although negative trends were seen in two of the six experiments (one at Clayton and another at Richmond), the overall result was that *Hours in Field* is not a significant factor for the *E. coli* level of stored samples up to 24 hrs. However, this is not to say that there was no uncertainty in the sample caused by storage. In fact, it was found that the uncertainty due to storage on the *E. coli* levels varied from 9% to 44%, with an average of 25% for each sample.

Comparing this with the values reported in Table 5 for storage uncertainties in nutrient samples, indicates that a higher level of uncertainty is associated with the storage of microorganisms. This is logical since so many factors influence the way in which microorganisms behave, and die-off. For example, Crane and Moore (1986) identified an array of environmental factors which affect microorganism survival, including temperature, pH, nutrient content, etc. (see Section 3.1.2, above). Although high levels of nutrients in the stormwater at Narre Warren could explain why the microorganisms survived so well at this site (see Figure 13), temperature effects were thought to be the main reason for the die-off of microorganisms during storage. In fact, storage uncertainties can be compared to the measured die-off rates for *E. coli* found in the literature. For example, Crane and Moore (1986), in their review of modelling bacterial die-off, showed that *E. coli* had a die-off rate of between 0.23 and 0.50 (average of 0.33) in waters with temperatures which range from 10 to 15°C (which is found to be typical for urban streams in Melbourne – Hatt *et al.*, 2004). These die-off rates imply a reduction of *E. coli* of between 21% and 39% (average of 28%) during a 24 hr period, and this corresponds to what is found in this section for the storage uncertainty of *E. coli*.

Recommendations for the microbial budget:

It is very clear that the storage of microorganisms, and other pollutants, in autosamplers and during transport, can contribute high uncertainties to the sample results. As such, it is necessary to either control the environmental factors which contribute to these uncertainties or at least understand them so that they can be taken into account. There needs to be a comparison between the costs of purchasing refrigerated autosamplers, and the benefits associated with these samplers, and the costs of conducting experiments which will help understand uncertainties. However, it must still be noted that it was found that storage of microorganism samples in refrigerated samplers still showed significant uncertainties. Thus the solution cannot just be "we will use refrigerated samplers", but instead we should ask the question "if we use refrigerated autosamplers, how will it reduce uncertainties?". Refrigeration will only reduce the impact of some factors affecting the survival of the microorganisms within the samples. As such, since there will be uncertainty in any technique applied (refrigeration vs. Non-refrigeration), it is necessary that some experimentation be conducted

to assess this error. This experimentation could be coupled to the experiments described at the end of Section 3.1.2 (which determine the impact of certain factors influencing microbial survival in the stream reach), thus reducing costs.

Analytical uncertainty. The last major source of uncertainty in sample collection is the error attributed to the analysis of the sample in the laboratory. For the main part of this section, the analytical uncertainty is considered the entire uncertainty in the sample after the point at which the sample was given to the laboratory for analysis. As such, the variability within a sample is considered part of analytical uncertainty (i.e. if two sub-samples from the same sample bottle yield different results, then this is part of the analytical uncertainty). Many people have reported the analytical uncertainties for different constituents, and the following provides a brief overview of this literature.

Harmel *et al.* (2006) provide a review of typical analytical uncertainties for both nutrients and sediment concentrations (see Table 6). From this table, it is evident that while analysing constituents such as TSS produces low uncertainties (because it only requires accurate weight and volume measurements), for nutrients (which require more detailed analytical procedures) these uncertainties can be much higher. Furthermore, these nutrient uncertainties are largely affected by the type of analytical procedure used.

Table 6. Analytical uncertainties for different pollutants from stream water samples (adapted from Harmel *et al.*, 2006).

Constituent	Analytical uncertainty
TSS	-9.8 % to 5.1 %
TN	up to -30 % to 30 % (depending on method)
NO_3-N	-7 % to 9 % (but up to 400 %, depending on method)
NH_4-N	-22 % to 26 % (but up to 200 %, depending on method)
TP	-24 % to 22 % (but up to 210 %, depending on method)
PO_4-P	-14 % to 22 % (but up to 400 %, depending on method)

Other studies have reported on the analytical uncertainties for similar pollutants. For example, Donohue and Irvine (2008) report on the analytical uncertainty for TN (10.4 %), dissolved inorganic nitrogen (5.3 %) and TP (7.2 %). These figures are within the ranges reported in Table 6. It is interesting to note that according to Donohue and Irvine (2008), the most important factor in the analytical uncertainty is the within-sample variability of the constituent. In fact, they suggest that only a very small proportion of the total analytical uncertainty is attributed to the analytical technique (i.e. less than a third of the figures reported above). As such, it would be expected that methods which use more of the sample volume for the analysis would yield lower associated uncertainties.

Analytical uncertainties of river water samples reported by Rode and Suhr (2007) for several pollutant types (including sediments, TP, TN, NH_4 and NO_3) are all within the ranges provided in Table 6. Furthermore, Ahyerre *et al.* (1998) report similar analytical uncertainties for TSS (10%), but also state that the uncertainties for COD and BOD are 10% and 30%, respectively. What is more, differences of around 40% for the measurement of suspended solids on a single sample between different laboratories have been reported (see Ahyerre *et al.*, 1998). Although this could possibly be caused by improper sub-sampling, at least a portion of this uncertainty is caused by variations in laboratory quality assurance/checking and laboratory methods/procedures.

Another study confirmed that concentrations of the same sample analysed at different laboratories can vary significantly (Bratieres *et al.*, *in preparation*). The results of this unpublished work are

shown in Figure 15, which shows the concentrations of TSS, TN and TP for six different locations in a wetland and for three laboratories. The variability between laboratories for TSS concentrations is quite significant, considering standard techniques were employed by each laboratory and the fact that these techniques are relatively simple to conduct. Whilst it is reasonable to assume that a portion of the variability seen in Figure 15 is caused by within sample variations, this is usually not possible for TSS analyses since the entire water sample is used for analysis. It is also possible that the variability observed here is partly caused by incorrect subsampling methods performed by the samplers. However, the consistent (somewhat systematic) overestimation of laboratory 2 for TSS concentrations, together with the consistent overestimation of laboratory 3 for TP concentrations, indicates that this is not necessarily the case and that the variability observed in Figure 15 is caused by differing laboratory conditions (e.g. incorrectly calibrated machines, incorrect procedures, etc.).

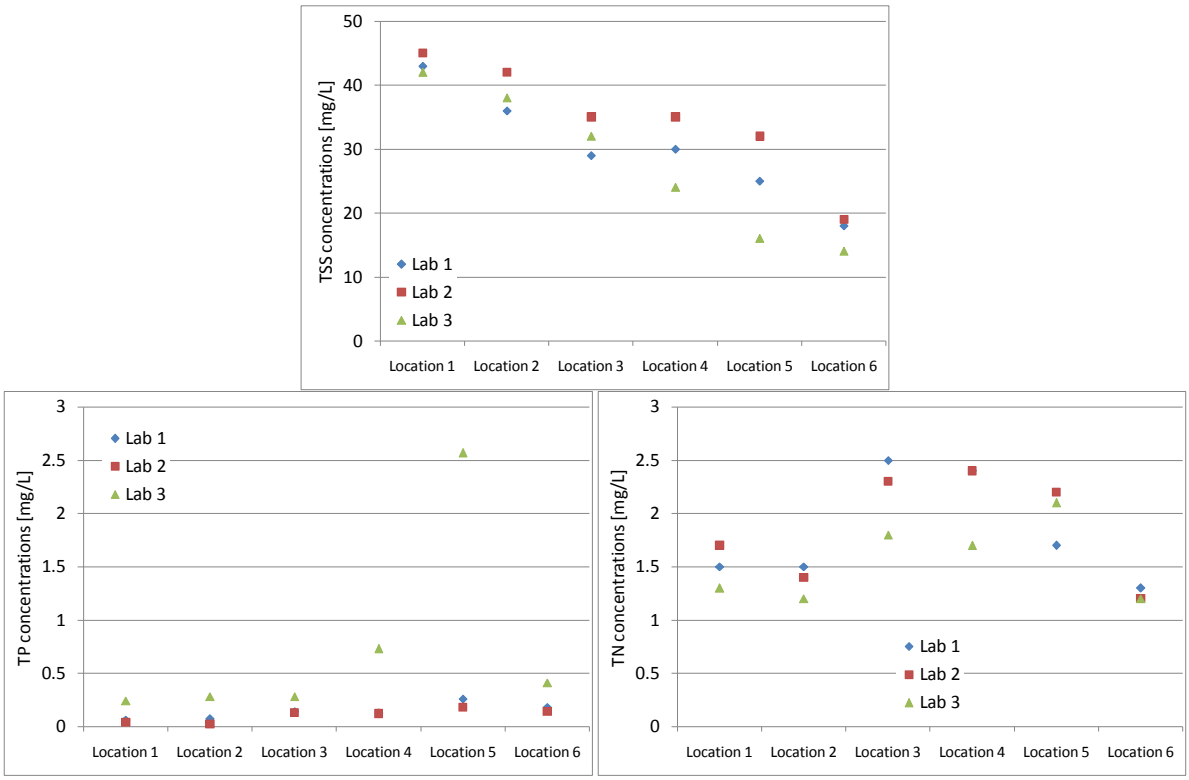


Figure 15. Variability in sample concentrations analysed between three laboratories for six different locations within a stormwater wetland (source *unpublished data*). TSS – top, TP – bottom left and TN – bottom right.

There is only a small amount of literature on the analytical uncertainty of microorganism samples. One of these is that by Roser and Ashbolt (2005) who state that replicate microbiological assays showed relative standard deviations ranging from around 10% to over 50%. Another study also assessed analytical uncertainties for a number of microorganisms and Table 7 summarises the results from an *unpublished* dataset. It is evident that while *E. coli* and enterococci have reasonable levels of uncertainty, *C. perfringens* have extremely high levels of uncertainty.

Table 7. Ranges of analytical uncertainties found for an array of microorganism concentrations (sourced from *unpublished data*).

Microorganism	Analytical uncertainty
E. coli	range from 24 % to 48 %
Enterococci	range from 5 % to 54 %
Clostridium perfringens	range from 58 % to 130 %

Another study by McCarthy *et al.* (2008) confirms the values found in Table 7 with reported *E. coli* analytical uncertainties ranging from 12% to 51%. The average found by McCarthy *et al.* (2008) (22%) compares well with the average relative analytical uncertainty provided by IDEXX Laboratories (2005), for the Colilert technique, which has a slightly greater average uncertainty of 27%. This difference in relative analytical uncertainties (22% vs. 27%) could have been because the laboratory chosen for this research used a dilution range that resulted in a smaller average analytical uncertainty.

Recommendations for the microbial budget:
The analytical uncertainty for *E. coli* has been adequately covered in the above literature, and as such conducting more extensive testing on this indicator is not necessary. However, if other indicators, or other microorganisms, are going to be used during the study, then it would be preferable to conduct some testing of the analytical accuracies for these microorganisms. Furthermore, some inter-laboratory testing could provide some robustness in the results and sporadic triplicate sample analysis during the course of the project could help ensure that the analytical uncertainty is kept within required limits. Field blanks and other quality assurance measures should be undertaken during the project.

Sampling regime uncertainty
Using data obtained from continuous monitoring probes will always produce greater accuracy than using data obtained discretely, which is assuming the probe functions correctly. This is because during discrete sampling, there are numerous opportunities to 'miss' important pollutant variations that may influence the result (Bratieres *et al.*, in preparation). As such, missing these pollutant variations may increase the uncertainty of the predictions required from the monitoring program. The section above discussed the uncertainties in the collection and the analysis of individual discrete samples which are used to characterise a pollutant's level within a certain cross section of a stream for a specific instance. This section will now describe the uncertainty that this sample represents not just that cross section of the stream at one instance, but will represent a large amount of water volume which passes that sample collection point over a certain period of time.

The structure of the sampling regime will heavily depend on the monitoring program's objectives. For instance, the sampling regime structure will differ considerably depending whether the monitoring objective is to obtain an accurate estimation of a single pollution event, or whether the objective is to obtain an estimate of the mean annual load from a catchment. While McCarthy *et al.* (2008) found that characterising a single event with, on average, 14 samples will introduce around 10% uncertainty when estimating an event's total *E. coli* load, Fletcher and Deletic (2007) found that taking just one sample from a large number of events to estimate the mean annual load had similar uncertainties. As such, the objectives of the monitoring program need to be fully understood to create an adequate sampling regime structure. For the remainder of this discussion, we will be focussing on the effects of the sampling regime structure on the estimation of loads from an 'event' (an event can be either a dry weather event of a specified period or a wet weather event).

Composite or discrete samples? Whilst it is possible to withdraw any number of samples during an event and take them all to the laboratory for analysis, this can be quite expensive and, as such, composite samples can often be used in preference without affecting the quality of the data, again depending on the project's objective. If the project's objective is to characterise the pollutant variability during wet weather events (including the assessment of peak pollutant concentrations), then composite sampling is not sufficient (Bratieres *et al.*, *in preparation*). Furthermore, if the data is to be used in model verification, then composite results will often not provide enough detail to conduct these analyses properly. The objective of this current project is to create an accurate microbial budget, and this means our aim is to accurately characterise event loads only (either during wet weather or dry weather). As such, using composite sampling is preferred. The intervals

at which we take our samples (which are then used for the composite sample) are still very important and will impact on the uncertainty of the event load estimation. As such, while the discussion below is derived mainly from the collection and analysis of discrete samples, the same sampling regime uncertainties will still apply when using these discrete samples to form a composite sample.

In general, decreasing the frequency of sampling will inevitably increase the uncertainty in most estimated values (King and Harmel, 2003; Harmel *et al.*, 2006; Bratieres *et al.*, *in preparation*). However, there are a number of other factors which also have a large influence on the magnitude of the sampling regime uncertainty. The variability of the pollutant concentrations can have a large influence on sampling regime uncertainty. For example, a pollutant with a large amount of variability will require that more samples be collected, whilst a pollutant which varies considerably less will require fewer samples to characterise the event. Furthermore, a pollutant during wet weather may vary significantly, whilst the same pollutant may vary only slightly during dry weather, thus requiring different sampling regimes for the same level of certainty.

Another factor which influences this sampling regime uncertainty is the method used to determine when to take samples. The most common methods are time-based monitoring methods (i.e. sampling at a constant time step) and flow-weighted approaches (sampling at a constant volume increment or at an interval proportional to the cumulative flow volume; Bertrand-Krajewski *et al.*, 2007). Whilst both flow-weighted and time-based sampling methods have been employed for wet weather event sampling (e.g. Leecaster *et al.*, 2002; King and Harmel, 2003; Harmel *et al.*, 2006), it is very common to use time-based methods, with large intervals, for dry weather sampling (e.g. weekly, monthly, etc.) (e.g. McCarthy *et al.*, 2008; Francey *et al.*, *in press*). If composite samples are required, then regardless of whether the samples are withdrawn using time- or flow-based methods, the discrete samples can be combined using a flow-weighted approach.

Literature on wet weather sampling strategies

Bratieres *et al.* (*in preparation*) conducted a study which looked into different sampling strategies and their impact on the resultant estimated event load of TSS for stormwater runoff from a small urbanised carpark. They found that time-based sampling produced the highest uncertainties in load estimates, and usually with the highest number of collected and analysed samples. Furthermore, they found that the number of different flow-weighted methodologies they tested all achieved similar results, indicating that, to a degree, the flow volume increment can be quite large without influencing the results significantly.

Other studies have also been conducted on stormwater sampling designs, and have concluded similarly to that discussed above. For example, King and Harmel (2003) conducted an analytical study showing that flow-based sampling methods are more accurate than time-based sampling. They also showed that, to a certain degree, increasing the flow volume between samples had little effect on the accuracy of the estimated loads. Another example is that of Leecaster *et al.* (2002), who found that using a flow-weighted approach yielded the most accuracy in TSS load estimations in urban stormwater systems, when compared with time-based and simple random-based sampling methods. They also suggested that single wet weather events are most efficiently characterised by taking 12 samples using a flow-weighted sampling regime and a flow-weighted methodology of combining these samples to estimate the total event load.

A study by McCarthy *et al.* (2008) assessed the uncertainty in the flow-weighted sampling regime employed by Monash University for TSS wet weather event loads. Their regime focussed on intensively sampling during the rising limb of the hydrograph, whilst still providing enough coverage for large events. On average, of the 48 wet weather events sampled, around 14 samples per event

were used to estimate the total event load. They found that using this sampling regime, the uncertainty which this sampling regime contributes to TSS event loads ranged from 7% to 9%, depending on the catchment type.

Literature on dry weather sampling strategies

The uncertainty in dry weather sampling strategies has received little attention in the literature. Most of the studies either entirely focus on wet weather event sampling, or focus on the periodic collection of samples (from both wet and dry weather flows) to estimate total annual loads. One good example of the latter is that by Fletcher and Deletic (2007) who conducted a study on the frequency of routine samples required to estimate long-term annual loads (including both wet and dry weather loads) for several pollutants in three different streams located in Melbourne. They found that, using lower frequencies (such as weekly or monthly) produced high uncertainties in the load estimates, with daily and three-daily sampling regimes producing uncertainties of less than 10% for long term loads.

The reason few studies focus on just dry weather sampling strategies is that wet weather events often govern the total pollution load entering a downstream system (i.e. an embayment or estuary). However, in the current project, it is hypothesised that while wet weather events are going to be important for the overall microbial budget for the selected system, the contributions from dry weather will also be significant. Furthermore, since the aim of this study is to determine the effects of mitigating dry weather discharges on downstream systems, it is necessary that we understand how dry weather flows should be sampled.

In response to the above, an in-depth analysis was conducted for this report to determine the number of samples required to accurately estimate dry weather event loads using data obtained from continuous turbidity measurements in a separate storm sewer in France (for more information on this catchment see Bertrand-Krajewski *et al.*, *accepted*). The following outlines how this data was used to determine appropriate dry weather sampling regimes. This data consisted of turbidity levels and flow rates logged at 2 minute intervals. The data was first cleaned by removing all wet weather events (since it was the aim to investigate dry weather periods only). Using this data, samples were selected using two sampling methodologies: random and systematic. These methodologies were applied to estimate the number of samples required to accurately determine: daily loads, weekly loads, monthly loads and yearly loads.

Firstly, let's use daily loads as an example. When using the random sampling method, up to 100 discrete samples were randomly selected from each day and these samples were then used to estimate the daily load using a flow-weighted approach. This was repeated for each day of the dataset (over two years). Using systematic sampling, up to 100 samples were selected with equal intervals and then used to estimate the daily load using a flow-weighted approach. The resultant 'actual' loads (calculated by using the entire continuous dataset) were then compared to the estimated load calculated using the randomly or systematically selected samples.

To present the results, ratios were calculated (estimated/'actual') and 95% confidence intervals were determined using these ratios. As such, plots were created with 'number of samples per period' on the x-axis and the ratios on the y-axis show how the estimated loads deviate from the 'actual' loads with a 95% level of confidence.

The results of the above methodology are shown in Figure 17 and Table 8. Figure 17 clearly demonstrates that in order to estimate daily loads to within 50% of their actual values (with a 95% confidence level), it is necessary to take a large number of samples per day (i.e. 21). However, as the time period of interest decreases (i.e. if we are only interested in weekly load predictions, instead of

daily), then the number of samples required per day decreases with only around 9-10 being required each day (or 67 per week). This pattern continues for both monthly and yearly loads, with the latter requiring less than one sample every second week for an estimate which has a 95% probability of being within 50% of the actual load.

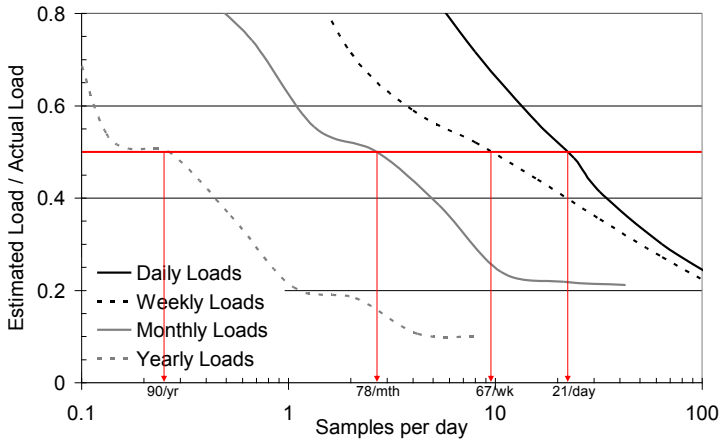


Figure 17. A graph showing the 95% deviation from the actual load as a function of the number of samples taken per day.

Table 8 compares the differences between systematically taking samples and randomly taking samples. It is evident that the number of samples required to estimate the load (to within 50% of its actual value) is decreased by at least 20% when taking samples in a systematic way (i.e. every fourth hour, etc.). This is logical since it is more likely for the sampler to capture the time period's variations when taking samples with equal intervals, as opposed to a method where all samples for a time period could be taken in a section with unusually high, or low, turbidity levels. Furthermore, it is interesting to note that as the time period of interest increases (from daily to yearly) the benefit of taking systematic samples decreases. Although there is a trend, the biggest benefit is seen for the weekly time period (not daily), and this is hypothesised to be due to the variation between days (which the weekly period needs to capture) being much greater than the variation within days (which the daily period needs to capture). In fact, when analysing the data, this is exactly what was found with an average relative standard deviation of the within-day variations in turbidity of 72% whilst the relative standard deviation of the between-day variation in turbidity was over 197%.

Table 8. The number of randomly and systematically taken samples required to estimate the load to within 50% of its actual value, with 95% confidence.

Time period	Number of samples (per time period)		Number required for random / systematic	Within / Between
	Random	Systematic		
Yearly	90	75	1.2	246 / 30
Monthly	78	62	1.3	152 / 69
Weekly	67	35	1.9	122 / 94
Daily	21	15	1.4	72 / 197

It should be noted that while the above analyses do suggest a high number of samples for the prediction of daily, weekly, monthly and yearly loads, it is possible to reduce the total number of samples analysed in the laboratory by using composite sampling methodologies. Taking daily loads as an example, while 15 samples should be taken systematically each day to achieve a load estimation to within 50% of the 'true' load, some of these samples could be combined using a flow-weighted approach. In fact, if testing for traditional pollutants, which do not experience significant

die-off or alterations during an entire day's worth of sampling, then all 15 samples could be complied into just one sample. However, since we are focusing on microorganisms, it is recommended that the samples be complied into more than one sample so that the storage of the samples is kept to a minimum. Similar reductions in samples analysed could be made for weekly sampling methodologies, however to a lesser degree because of these storage issues.

Since the focus of this project is on microorganisms, it is difficult to use the above results (which were drawn from turbidity data) to make inferences about microorganism sampling regime structures. However, many have observed positive relationships between bacterial concentrations and turbidity, including Mallin *et al.* (2000), who found that turbidity and faecal coliform levels were significantly correlated in runoff from several urbanised catchments. Furthermore, Duncan (1999), in his review of urban stormwater quality, and Davis *et al.* (1977), who investigated bacterial relationships in stormwater, found significant positive correlations between faecal coliforms and both TSS and turbidity. As such, since continuous microorganism data is not available, and it is known that microbes can significantly correlate with turbidity and TSS, it was decided that using this procedure was more preferable than not attempting to evaluate different dry weather sampling procedures.

Recommendations for the microbial budget:

For wet weather events, it is clear that the literature indicates that flow-based sampling is the preferred option to optimise the accuracy of event load estimations. Whilst the number of samples taken during each event will vary according to its size, using a flow-weighted approach ensures that the number of samples taken during an event is not too high (as compared with time-based sampling during large events). It is recommended that a flow-weighted sampling approach is adopted for the sampling of wet weather events and, to reduce costs, samples should be combined (using flow weightings) to form a single composite sample. The sampling regime structure (i.e. the selected flow intervals, etc.) should follow that already published by Monash University since it has been shown to produce reliable estimates of total event loads.

Drawing conclusions on dry weather sampling regimes would have been difficult without the analyses conducted. The main recommendation from this analysis is that a systematic method of sampling dry weather flow periods is preferable to random-based procedures, since the sampler is more likely to capture the overall variation of the pollutant. Furthermore, in order to reduce the cost of the project, composite samples could be made using the discrete samples collected, but this option becomes less viable as the time period of choice moves from daily to yearly.

The decision as to which time-period to estimate a budget should be based upon an array of factors, but obviously time, money and accuracy are the most crucial elements. Conducting a yearly budget would mean minimal labour requirements since the interval for collection is quite large (i.e. for accurate yearly load estimates from stormwater pipes, samples should be taken every two weeks). Another benefit of using such a coarse time interval is that seasonal variations in the budget will be captured, however this decision would mean that no inter-daily/weekly variations in microbial budgets would be adequately captured. Using such a large interval can also lead to some issues, especially if the project's timeline is confined by external factors which cannot be controlled.

Using a smaller time interval for the budget calculations could help resolve some of these issues, but would mean an increase in analytical and labour costs. Furthermore, unless these mass balances are calculated several times during a year (i.e. if weekly budgets are chosen, then repeating this each month) seasonal variations in the budget would be more difficult to distinguish. Again, if the project's timeline is restricted then this type of option may not be viable. However, the authors recommend that calculating a weekly budget several times over one year is the most economically

viable and accurate method for this project. This method allows repeated budgets to be estimated, hence allowing comparisons to be made between budgets (whereas when using a yearly budget, only one budget would be estimated, hence no comparisons could be made).

Monitoring duration

When describing uncertainties in estimating Site Mean Concentrations (SMCs) or annual/long-term site loads (which mainly focus on wet weather events), the number of events used to characterise this SMC is very important (see Bertrand-Krajewski *et al.*, 2002; Francey *et al.*, 2004; McCarthy *et al.*, 2008). This is because there is such a large variation between the Event Mean Concentrations (EMCs) and volumes of different events (i.e. the higher the EMC variation is, the larger the number of events that need monitoring – see Francey *et al.*, 2004). This information is usually used to assess the duration of the wet weather monitoring program (e.g. if the site and pollutant have a large variability, therefore we need to monitor the site for 40 events, which will take 2 years).

However, here we are faced with a different question, one that doesn't focus on estimating 'long-term' loads or SMCs specifically. In this project, one of the main aims is to estimate a microbial budget for a set time period (this time period was discussed above). As such, the monitoring duration will be widely influenced by this selected time period, and if it is one year, then the monitoring duration will obviously extend to at least one year. On the other hand, if the selected time period was one day, then the monitoring duration need only be one day (if no replication was required). The question that now needs to be asked is "how many of these 'one day' (or one week, month or year) time periods are required to accurately complete the project's aims?".

For example, considering temperature has a large impact on bacterial die-off in streams and on surfaces (see Crane and Moore, 1982), then it could be suggested that capturing seasonal variations is important to fully understand in-stream dynamics and their influence on the overall microbial budget. As such, in this case, a duration of a year seems to be a minimum to capture this variability. On the flipside, one could also argue that if we are determining a full microbial budget then as long as we have appropriate estimations of in-stream die-off due to seasonal changes (i.e. an experiment was conducted), then capturing this type of variability mightn't be that beneficial, especially if a model was developed and tested. However, we also need to consider other factors which are heavily seasonal and not easy to monitor (such as the amount of bird faeces deposited directly into the stream via bathing, etc.). The contribution from such a seasonally varying source means that, without monitoring the system for a full year, we may not be able to accurately determine a full microbial budget.

The previous paragraphs addressed the issue of the duration of the monitoring program for set time periods, however another issue is how many wet weather events should be monitored during this duration. This question can only be answered by addressing the key aim of the project. If the key aim of the project was to create an entire microbial budget for the selected site, which includes understanding resuspension and growth, then the monitoring of wet weather events is very important. In this situation a number of events, with differing intensities and durations, should be monitored to capture the variability which is commonly seen for microorganisms between wet weather events (e.g. McCarthy *et al.*, 2008; Kay *et al.*, *in press*). However, if the key aim is only to estimate a microbial budget for dry weather periods for the purposes of understanding how dry flow treatment improves the selected system, then detailed monitoring of wet weather events is not going to provide significant information to the budget.

One very important procedure that must be conducted to provide interesting results from this budget is the monitoring of the system prior to the application of any mitigation options (i.e. prior to treatment). Without this preliminary monitoring, it will be hard to determine the impact of the treatment systems on the selected water body. In fact, it would be very interesting to be able to

conduct such a monitoring program for an entire year, then install any mitigation options, and then monitor the system again for another year. This type of method would provide enough information to understand the full dynamics of the system (including seasonal variations) both prior to and after mitigating faecal sources.

7 Treatment technologies for faecal pollution control

This section will focus on understanding the effectiveness of some novel mitigation options which could be trialled to reduce the microorganism levels entering the selected water body. The toolbox of mitigation options available for the removal of microbial contaminants is extensive. This section will cover a wide range of treatment measures ranging from the traditional Water Sensitive Urban Design (WSUD) tools to more novel approaches adopted in recent years and finally disinfection options. Firstly, a brief overview of the scenario at hand will allow for an assessment of which tools may be feasible and which may not. This will allow for a set of evaluation criteria to be established. Subsequently, a review of various mitigation options will be carried out in the order listed above. By the end of this section, the evaluation will hopefully indicate a few feasible options that can be effectively implemented to treat microbial contaminants.

7.1 Introduction

In order to select a treatment option, a number of criteria need to be considered, especially with regard to the specific project at hand. In particular, the treatment device should:

- be easy to install and require low maintenance regimes;
- have low, or no, energy requirements since it will be installed in areas without electricity;
- only improve the selected water body's health, and not cause ill effects to the aquatic ecosystem;
- have a small footprint;
- have a flexible design, so that it can be easily used to retrofit inlets areas to treat water entering the selected water body;
- have acceptable costs, for both installation and maintenance; and,
- remove contaminants of most concern.

In the following discussions, all treatment options will be assessed against their footprint and treatment performance. Other factors (such as energy requirements, flexibility of design, costs, maintenance costs and production of harmful by-products) are also discussed, but with a lesser emphasis.

The array of tools, which will be covered in the next few sections will include: traditional Water Sensitive Urban Design (WSUD), novel technologies and disinfection methods. Another point, which needs to be mentioned in the context of this project, is that it might be desirable to use these systems to only treat dry weather flows. A lot of the available guidelines specify system performance during wet weather events and in order to interpret these results for dry weather flow, several implications may help:

- the size of the system will be comparably smaller if it only designed to treat dry weather flows;
- the system will need to be installed in such a manner that it can tolerate wet weather flow without being washed away; and,
- the system will need to have a large flow bypass to prevent inundation, which may quickly degrade its future performance.

7.2 Traditional WSUD technologies

7.2.1 Sedimentation basins & constructed wetlands

Sedimentation basins and constructed wetlands are systems of similar scale. Removal rates of microorganisms (particulate-bound and in free form) have been compared between the two systems and the general outcome supports the use of constructed wetlands over sedimentation basins (Davies and Bavor, 2000; Bavor *et al.*, 2001; Davies *et al.*, 2003). A more in-depth look at each of these technologies should however be carried out as investigations have nevertheless shown that microorganism removal rates in sedimentation basins can comply with standard requirements if certain design criteria are adopted (Mallin *et al.*, 2002). Note that the terms sedimentation basin, sedimentation pond and detention pond will be used interchangeably as they represent the same technology for the purposes of this review.

The Melbourne Water Sensitive Urban Design guidelines provide sizing criteria for both systems. Sedimentation basins are generally sized to target the settling velocities of certain particles as their primary removal mechanism is by settling (MelbourneWater, 2005) although chemical and biological processes may also occur simultaneously. Other design requirements include the retention time and the flow rate through the system. It was found that maximizing the length-to-width ratio of these basins tends to improve treatment capabilities allowing for a longer settling distance (Mallin *et al.*, 2002). The size of constructed wetlands is governed by the desired removal efficiency of various pollutants as this will influence the retention time required. Typically, these systems can occupy up to 5% of the total catchment area, are significantly shallower than sedimentation basins and use staged treatment. The inlet zone comprises a sedimentation basin where colloidal particles settle out. The macrophyte zone contains a vast amount of vegetation separated from the inlet zone, which retards the flow allowing for chemical and biological processes to take place in addition to further sedimentation of finer particles (Davies *et al.*, 2003).

Biological and chemical processes, which are found less intensively in sedimentation basins and more frequently in wetlands, include antibiosis, predation by other organisms, natural death, oxidation, adsorption to particles and exposure to toxins promoting die-off (Ottova *et al.*, 1997). Adsorption has been regarded as one of the most important mechanisms by many researchers as the association of pathogens and bacteria with particles protects them from predation, but also allows for more efficient removal by sedimentation. In three studies focusing on the same wetland and detention pond systems, it was concluded that faecal coliforms, enterococci and coliphages were all associated with finer clay sized particles (sizes of <2µm) (Davies and Bavor, 2000; Bavor *et al.*, 2001). Wetlands were found to more efficiently remove these particle sizes than sedimentation basins. The importance of adsorption has been confirmed by other studies (Ottova *et al.*, 1997; Mallin *et al.*, 2002; Greenway, 2005; Reinoso *et al.*, 2008).

Further removal of organisms is aided by the additional processes listed above. As these processes are not as predominant in sedimentation basins as in wetlands, the latter would usually be a more suitable treatment option. However, Mallin *et al.* (2002) still found reasonable reductions for faecal coliforms in detention ponds (86%), but the high length-to-width ratios used in their study may not always be feasible in treatment scenarios. The removal efficiencies of the studied sedimentation basin in Davies *et al.* (2000) was calculated as a negative percentage due to a higher concentration of thermotolerant coliforms in the effluent than in the inflow (Davies and Bavor, 2000). This net production of coliforms for this system may have been caused by bird populations living around the open water body (as mentioned in Section 4.2, animal contributions to open water systems, such as wetlands and ponds, has been proposed to be a major disadvantage in using these types of systems for microbial control). It also needs to be stressed that the coliform indicators used in these studies are most likely not sufficiently predictive of the overall pathogen demography and that the sensitivity of these coliforms to many other environmental stressors will increase the removal

efficiencies of the system, further reducing the reliability of the value in describing the behaviour of more resistant viruses and protozoa (see Section 3 for more details).

Recommendations for the microbial budget:
For more details on sedimentation pond and wetland treatment performances and footprints, please refer to Table 9, Section 7.5. It seems that wetlands are the preferred option in terms of microbial removal efficiencies, but both ponds and wetlands have significant downsides for use in this project. Firstly, their removal efficiencies for typical indicator organisms are always less than 90% (i.e. less than a log reduction). However, the major inhibitor in utilising such systems for this project is the large footprint and contact time required for adequate treatment. Whilst this footprint might be smaller if only dry weather flows are to be treated, scaling down these systems to suit this situation would still be very difficult. The main reason these types WSUD technologies were included in this report was for comparative purposes only.

7.2.2 Biofiltration systems

Biofiltration systems (or bioretention systems as they are also known) are smaller in scale as compared to constructed wetlands. Sized at approximately 2% of the catchment's impervious area, this structural WSUD tool has a significantly smaller footprint and its performance is notably influenced by presence and type of vegetation, type of filter media and filter media depth amongst other parameters (Bratieres *et al.*, 2008b). The system can easily be installed/retrofitted in suburban settings and will manage water quality improvements as well as peak flow reductions by allowing a certain ponding depth. Upon exceedence of this depth, the stormwater can overflow into a pit.

Biofiltration systems have been tested for microorganism removal efficiencies in several studies (Rusciano and Obropta, 2007; Bratieres *et al.*, 2008a; Hathaway *et al.*, 2008; Hunt *et al.*, 2008). Before looking at quoted results, it should be mentioned that Hathaway *et al.* (2008) indicates that the removal may also be influenced by external environmental factors including radiation from the sun, temperature, moisture and salinity due to the open nature of the system. The literature on bioretention systems has assessed removal rates in both laboratory conditions as well as in field sites. Laboratory tests generally employed a scaled-down version of the system in columns dosed with synthetic stormwater containing representative amounts of microorganisms found in the field (Rusciano and Obropta, 2007; Bratieres *et al.*, 2008a). Removal efficiencies of 82% for *E. coli*, >99% for *C. perfringens* and 97% for F-specific RNA coliphages were determined by Bratieres *et al.* (2008a). Rusciano and Obropta (2007) calculated mean (92%), median (99%) and a range of reduction coefficients (76% to 99.8%) for faecal coliforms. A comparison with TSS removal saw that average and median reductions above 90% were also achieved with the system (Rusciano and Obropta, 2007).

The bioretention system studied by Hunt *et al.* (2008) and Hathaway *et al.* (2008) receives runoff from a carpark that is plagued by bird activity. Removal rates of 69% and 71% for faecal coliforms and *E. coli* were determined, respectively. In comparison with other WSUD technologies, including detention ponds, wetlands and various proprietary systems, Hathaway *et al.* (2008) regarded the bioretention system as most proficient in bacteria reduction. The author, however, does highlight possible bias in the results as the carpark runoff may not have been as polluted as the runoff entering other systems. Hunt *et al.* (2008) highlights similarities in performance between the studied bioretention system and the sand filters studied by Barrett *et al.* (2003), which are covered in the next section.

Several authors have additionally considered the effects of drying on the system's performance with differing opinions. While Bratieres *et al.* (2008a) found that removal efficiencies were reduced for some bacteria, drying did not affect other bacteria removal (such as *C. perfringens*). It was found that the addition of a saturated zone at the bottom of the system may reduce the formation of

macropores (Hunt *et al.*, 2008, Bratieres *et al.*, 2008a), therefore in turn increasing the performance of such systems after longer dry periods. Knowledge gaps also require additional research to better understand the removal mechanisms of bacteria in these systems. An important aspect is the association with particles pointed out by both Rusciano and Obropta (2007) and Bratieres *et al.* (2008a).

Recommendations for the microbial budget:
In the context of this project, the use of bioretention systems as a mitigation option would definitely be feasible as there are results that show significant removal rates of different bacteria (see Table 9, section 7.5 for a summary). The amount of maintenance on these systems is often minimal. If a choice is made to reduce the footprint of the system so that it mainly treats dry weather flows (and only a very small proportion of wet weather flows), then installation of such a system must be carefully considered. For example, techniques must be employed which ensure that the system is not affected by large wet weather flows (e.g. a high flow bypass may prevent the problems of scouring and erosion of the biofilter's surface).

7.3 Novel filtration technologies & filter media

7.3.1 Natural & modified zeolite & activated carbon

Various novel filter media covered in this section can be used for treatment of stormwater runoff by either incorporating the media in traditional WSUD technologies (such as biofilters) or in a stand-alone treatment pit/trench/cartridge which has been adequately designed. The discussion below will only focus on the treatment performance (i.e. removal of contaminants) and the hydraulic conductivity of the media (which determines the footprint required to treat a certain flow regime), and will not cover how these systems will explicitly be incorporated into the specific site.

Zeolite. Zeolites are naturally occurring ion-exchange materials, which are used in the removal of ammonium ions from wastewater (Metcalf and Eddy, 2004). Several advantages of this material include its high surface area, high cation exchange capacities, superior sorption, hydraulic properties and the absence of shrinking and swelling behaviour (Bowman, 2003). Experiments have, however, shown that this material in its natural state is ineffective at removing *E. coli*, even though attachment to the material may occur (Schulze-Makuch *et al.*, 2003; Bowman, 2003).

Researchers have sought to modify the material to enhance its ion-exchange capabilities in order to remove pathogenic organisms from water. As a result, two types of modifications enhancing the removal of different organisms have emerged and have been investigated by several authors (Bowman, 2003; Schulze-Makuch *et al.*, 2003; Abbaszadegan *et al.*, 2006).

Surfactant modified zeolite involves the addition of hexadecyltrimethylammonium (HDTMA) to natural zeolite, which reverses the negatively charged surface, allowing for the attachment of microorganisms. At present, most of the research has been devoted to groundwater, and removal efficiencies of 99% for viruses and 100% for *E. coli* were reported by both Schulze-Makuch *et al.* (2003) and Bowman (2003) for a freshly installed filter pack. These values were confirmed in a number of experiments. However, virus removal efficiency declined over time becoming inefficient within five months of installation due to the degradation of the HDTMA bilayer. *E. coli* removal rates nevertheless persisted.

The inexpensive nature of the material increases its viability. A downside identified by Bowman (2003) referred to the non-selective nature of the material, absorbing pathogens as well as any other anions it comes in contact with. This disadvantage limits its use in highly saline environments, which may not be a concern for this particular project, but could play a role if various other pollutants in

the water body are attracted, thereby occupying all adsorption sites on the media. Exact knowledge about media's lifespan is currently unknown and further research is being devoted to understanding the applicability of the material in other water treatment problems.

Quaternary Ammonium Chloride (QAC) treated Zeolite is another modification, which has been tested in a pilot filter on various microorganisms including *E. coli,* bacteriophages and *Cryptosporidium parvum* (Abbaszadegan *et al.*, 2006). QAC has cationic properties, which attracts microorganisms. Results from the pilot study showed a promising range of removal rates between 2.83-log for *E. coli* and 1.19-log for *Cryptosporidium parvum*. Inactivation of the protozoan oocyst through QAC-treated zeolite was more effective than physical removal, which reportedly only achieved a 0.54-log removal (Abbaszadegan *et al.*, 2006). Refined research into this alternative modification should hopefully yield more results on the practical implementation of this filter media and the disadvantages associated with it.

Activated carbon. Activated Carbon is a popular adsorbent made by heating different organic materials up to temperatures of 700^{o}C to form a char and subsequently exposing the material to oxidizing gases including steam and CO_2 at high temperatures of 800 to 900^{o}C. A porous structure having a large surface area with high adsorption capacities is the result from this process. Any type of carbonaceous material (e.g. coconut rind, wood, etc.) can be used bearing in mind that some may contain unwanted elements, which may reduce the performance of the final product. Activated Carbon is used to remove pollutants from wastewater through adsorption processes (Metcalf and Eddy, 2004). Different types of activated carbon have been found to achieve different types of removal rates. A coconut-based carbon was not as efficient in adsorbing *E. coli* as a wood-based carbon (van der Mei *et al.*, 2008), but considerations must be paid to the fact that different types/bases of carbon may leach certain elements.

Several authors have sought to investigate removal efficiencies of modified activated carbon filters (Pal *et al.*, 2006; Bandyopadhyaya *et al.*, 2008; Kennedy *et al.*, 2008). Bandyopadhyaya *et al.* (2008) soaked the porous material in $AgNO_3$ solution and added $NaBH_4$ to reduce the compound to silver. Trials of this modified filter media on *E. coli* showed 3-log reductions for 350L of water for exposure times as short as 30 seconds. Furthermore, it was also found that the impregnation of silver particles to the activated carbon was strong enough that no leaching of silver occurred. The downside to this modification is that the media is only capable of treating very small volumes of water before its effectiveness deteriorates (Bandyopadhyaya *et al.*, 2008). These volumes are nowhere near the required scales that will be encountered in this project. A further disadvantage in the use of silver for coating highlighted by Kennedy *et al.* (2008) was the expensive preparation cost for the media.

Kennedy *et al.* (2008) proposed to modify activated carbon with copper. They conducted extensive testing on the removal of *E. coli*, as well as other bacteria, and showed significant reductions for these microorganisms. The removal rate increased with increasing copper concentration and 4-log removals with strong persistence to prevent regrowth after treatment were reported as achievable.

Pal *et al.* (2006) modified activated carbon with aluminum hydroxychloride (AHC) and subjected it to testing on *E. coli* removal. Approximately 1000L with an *E. coli* bacterial load of 10^7CFU/mL was subjected to only 30g of AHC-treated activated carbon to yield greater than a 6-log removal. The risk of leaching metals from the media was also found to be not significant (Pal *et al.*, 2006).

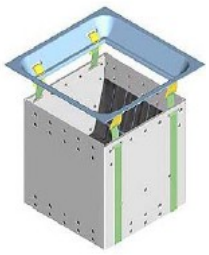
Recommendations for the microbial budget:
What the last three investigations on activated carbon do not report is the efficiency of the modifications in the removal of viruses and protozoa, and this knowledge gap does argue against the implementation of using Activated Carbon (as opposed to zeolite which has had some testing for

both protozoa and viruses). In the systems' defence, it should be mentioned that these technologies are still in their development stage and full-scale field testing has yet to be undertaken. Furthermore, many other proprietary and WSUD devices which claim good microorganism removal have also never been tested using actual pathogenic bacteria, protozoa or viruses. Easy integration of these filter media with another treatment system, such as the enviss system (in Section 7.3.4), could be attempted for this project. The filter media has shown to work under low contact times and provides adequate treatment to high hydraulic loading rates; hence this media coupled to another treatment system could have a relatively low footprint compared to other options.

7.3.2 AbTech's Smart Sponge® Plus

AbTech Industries have been developing a novel filtration system by the name of Smart Sponge® (see Figure 18) to remove hydrocarbons and oils. A recent upgrade in the technology was performed to also remove various microorganisms including *E. coli*, faecal coliforms, enterococci and *Salmonella* among others (Smart Sponge® Plus). An antimicrobial agent added to the system binds the organisms to the sponge, thereby disrupting the cell membrane rendering them obsolete. Inherent advantages of the system are the filtering capabilities, which are not hindered over time and the ability of the material to absorb five times its own weight. The replacement of sponges are usually carried out every 1 – 3 years. A further advantage of the technology is that there is neither chlorine nor heavy metals involved and disposal is made easy (AbTech Industries, 2008b, AbTech Industries, 2008c).

CO1414 for Curb Opening Drains "Inlets"



DI2020 for Drop-in Drains "Catch Basins"

Part No	Description	Dimensions (mm)	Gross Weight (approx.)		Flow Rate Q (approx.)	Trash & Debris Capacity
			With Smart Sponge	Trash & Debris Only		
Curb Opening Module:						
CO1414N	UUF, Normal size	340 x 340 x 570	9kg	2.5kg	12.6 l/s	425 cm^3
CO1414H	UUF, Half size	340 x 360 x 330	6kg	2kg	10.7 l/s	226 cm^3
Drain Insert Module:						
DI1414N	UUF, Normal size	340 x 360 x 340	9kg	2.5kg	12.6 l/s	425 cm^3
DI1414H	UUF, Half size	340 x 360 x 330	6kg.	2kg	10.7 l/s	230 cm^3
DI1420N	UUF, Normal size	355 x 490 x 340	11kg.	3kg	18.9 l/s	600 cm^3
DI1420H	UUF, Half size	355 x 490 x 340	8kg.	2.3kg	18.9 l/s	310 cm^3
DI1616N	UUF, Normal size	406 x 406 x 540	11kg	3kg	15.1 l/s	510 cm^3
DI1616H	UUF, Half size	406 x 406 x 340	8kg	2.3kg	15.1 l/s	285 cm^3
DI2020N	UUF, Normal size	490 x 490 x 340	13.6kg	3.4kg	31.5 l/s	850 cm^3
DI2020H	UUF, Half size	490 x 490 x 340	10kg	2.7kg	31.5 l/s	480 cm^3

Figure 18. Example Installations of Smart Sponge® System (AbTech Industries, 2008c).

Removal rates of microbes have been reported as being >99.99% for *E. coli* after one hour of contact. However, for the flow rates and product dimensions specified by the manufacturer (see Figure 18), the calculated contact time is only between 2.5 and 5.5 seconds. For these contact times, no removal rates have been specified. For contact times between 15 and 20 seconds, removal rates for *E. coli* are around 55% and for *Staphilococcus aureus* they are between 46 and 83% (AbTech Industries, 2008b). It is clear that since the contact time is an important design factor in the implementation of this system (AbTech Industries, 2008b), it is hard to assess the performance of the systems under these maximum flow rates.

An application of the Smart Sponge® Plus system was seen in Scarborough beach in 2003. The manufacturers clams that retrofitting the Smart Sponge® Plus to the drainage piping discharging into the beach waters resulted in successful improvement of water quality. They quote maximum removal rates of 89.4 to 99.8% for *E. coli* and 96.2 to 99.9% for enterococci (AbTech Industries, 2008a). However, they have not quoted the minimum removal rates that are very likely to occur during high flows, while we can speculate that the maximum removal rates were recorded during

very low flow rates when contact time is very long (however this is a hypothesis since no data have been provided).

Recommendations for the microbial budget:
Data on removal of protozoa or viruses, and the understanding of retention time of stormwater within the system, has yet to be fully researched. This project may offer a suitable housing for the required testing of this system since the Smart Sponge® Plus filter may certainly prove to be an efficient mitigation tool in future after adequate testing.

7.3.3 Pathex™ filter media

An antimicrobial integrated filtration system developed by AS Filtration™ by the name of Pathex™ physically ruptures microorganisms and passes the dead remains through the media to prevent clogging. No data for treatment of stormwater were available, however the manufacturer provides information for media performance when used in treatment of water used in cooling towers. The manufacturer claims that the media is able to operate at relatively high loading rates and its antimicrobial agent has shown to work at a nano-level against *E. coli*, enterococci, faecal coliforms, fungi, viruses, molds and spores. Tests on *E. coli* in treatment of cooling tower water at varying loading rates (ranging from 6 L/sec/m^2 to 20 L/sec/m^2) and influent concentrations resulted in greater than 3-log removals (AS Filtration, 2008, AS Filtration, 2009). Results reported in field tests in the same work showed slightly lower performance with removal rates ranging between 2 – 3 logs. It should also be mentioned that field tests were conducted over longer periods of time (ranging from two to three months) and that the findings were based on the total volume of water passed through the system. Data has not been found on pathogen removal other than for *Legionella,* the removal of which was not as efficient as that for *E. coli* (AS Filtration, 2009).

Recommendations for the microbial budget:
The knowledge gap in the uses of this media does not seem to warrant its use in this project. However, the possibility of testing this product in this project should not be discouraged, since further testing may reveal unforseen removal rates for some microorganisms.

7.3.4 enviss Sentinal 450

Monash University has been working on developing a modular porous pavement system with an integrated, compact treatment train for Envirostream Solutions Pty Ltd (enviss). The system (shown in Figure 19) consists of several layers from a trafficable porous pavement grate, which removes gross pollutants, to a sediment trap and fine filter media. The system will treat stormwater to non-potable standards and a recent paper has highlighted initial investigation results (Poelsma *et al.*, 2009).

Figure 19. Treatment system cross-sections & enviss box that treats stormwater

A number of filter columns, each containing varying types of media were subjected to extensive dosing with synthetic stormwater, containing significant microbial contamination as would be found

in practical conditions. The columns were dosed with 200L of water and the reduction in various water quality parameters assessed. Results show that *E. coli*, *Clostridium perfringens* and FRNA phages were effectively removed from the filter (99.9%, 99.5% and 99.8%, respectively). It should be noted that these results are just from laboratory studies, and that long term changes in the removal rates have not yet been extensively studied. It should also be mentioned that the removal rate for *E. coli* was only achieved when a disinfectant was included in the enviss system. However, similar results were obtained for *C. perfringens* and FRNA phages when using the system without disinfection.

Recommendations for the microbial budget:
The enviss system only requires a size of 0.3% of the total catchment area to treat around 90% of the annual runoff from an urban catchment. Further reductions in footprint could be achieved if the system was only designed for treatment of dry weather flows (for example, three 600mm L x 600mm W x 400mm D boxes can treat a flow rate of up to 1L/s, with this value increasing if ponding is allowed). The removal of microorganisms by this system is reasonable, even without the additional disinfection component. Further work is being conducted to determine the impacts of the disinfection component on downstream systems, with a particular emphasis on ecosystem health.

7.4 Disinfection options

Traditional disinfection techniques consist of chlorine, ozone, UV radiation, peracetic acid, heat and several other options. The role of disinfection in water treatment has seen many historical successes, but technologies still encounter their limitations due to the discovery of more waterborne pathogens for which information is unknown or scarce as highlighted in the 1993 outbreak of *cryptosporidiosis* in the US (Nwachcuku and Gerba, 2004). Concerns have also been raised over the by-products of certain disinfection methods. These disinfection by-products (DBPs) have been found to be carcinogenic and in residual amounts sufficient to pose potential risks to humans (Kuo and Yamashita, 1999; Li *et al.*, 2008). High resistance of protozoa to some disinfectants prompts for the addition of higher chemical doses and longer contact times leaving higher DBP concentrations (CWP, 1999), a counter-productive strategy that worsens the already apparent problems. Consequently, research has been devoted to discovering new disinfection techniques that may overcome these challenges. This section will first look at some traditional disinfectants followed by a list of potential new technologies, which may see large-scale application in years to come.

Chlorine disinfection is popular as it has proven its effectiveness in many investigations. Minimal amounts can achieve up to 4 log removals of total coliforms, fecal coliforms and fecal streptococci (Evans *et al.*, 1968). Obtaining the disinfectant is cost-effective and its persistence after disinfection is high, meaning that re-growth is inhibited. By-products resulting from its application are, however, toxic and cancerous if high doses are used. Several microbes are also resistant to chlorine highlighting the need for an additional alternative disinfectant (Evans III *et al.*, 1968; Morato *et al.*, 2003). It should be noted that chlorine can have adverse impacts on downstream systems, so the use of chlorine must be carefully considered.

Ozone disinfection is regarded as more efficient than free chlorine for viruses, spores and cysts (Kuo and Yamashita, 1999). Despite the advantages, cost of installing the equipment ranges at 4-5 times greater than for chlorination (Thomas *et al.*, 1990). Also, rapid decomposition in water indicates low persistence and rather than preventing re-growth, some by-products may even promote it (Morato *et al.*, 2003). The feasibility in the context of the study site is further reduced as electricity is needed for the production of ozone. UV disinfection equipment will experience the same issues in relation to this project. Despite its efficiency in pathogen removal, and the facts that it does not produce harmful DBP and is very effective against protozoa (CWP, 1999; Kuo and Yamashita, 1999), this alternative may not be useful for this project since mains power may not be available.

Nanomaterials have strong antimicrobial characteristics and are relatively inert in nature, properties which can potentially yield promising water quality results. A recent review conducted by Li *et al.* (2008) mentions that several nanomaterials incorporated in membranes have been used in water treatment with successful results of 3 to 4 log removals of pathogens. There is said to be no by-products, non-toxicity and complete retention of the nanoparticles within the treatment area. Popular alternatives, which have been studied, include silver nanoparticles (*n*Ag), photocatalytic TiO_2 and chitosan (Li *et al.*, 2008). TiO_2 is said to be more cost-effective than UV radiation and relies on the addition of H_2O_2 to Fe^{2+} salts and UV radiation, a technique referred to as photo-Fenton and heterogeneous catalysis (Blanco-Galvez *et al.*, 2007). Several commercial suppliers of nanomaterial products are available including Aquapure®, Kinetico®, QSI-Nano® and Purifics®. A detailed understanding of the capabilities and limitations of nanomaterials has yet to be gained in further research.

Recommendations for the microbial budget:

In the context of suitable mitigation measures, traditional disinfection may be the more realistic choice despite the inherent problems. The addition of chlorine is by far the most cost-effective and practical as there are inhibitions with installing equipment for generating ozone or powering UV lamps on-site. De-chlorination may be required to deal with the side-effects in order to maintain the general stream quality and ecosystem health. The use of nanomaterials would be very interesting, and using them in this project may provide some extra testing of these materials in the field, but further work needs to be conducted before these can be an easily implemented mitigation device.

7.5 Conclusive remarks

A wide range of treatment alternatives have been covered with comments on the viability of each system and its implementation as a mitigation option in the scope of this project. All treatment devices are listed in Table 9 to allow comparisons between hydraulic and treatment performances. From the understanding developed through this review, it can be seen that many novel approaches have yet to be fully developed. Most of the novel technologies covered show promising results in microorganism removal, but the absence of removal efficiency data for an extensive range of organisms makes it difficult to understand their true performance. As Section 2 noted, *E. coli* fails as an indicator for the more robust viruses and protozoa and thus, the removal efficiencies of these robust microorganisms cannot be solely assessed by looking at *E. coli* reductions. However, with increasing amounts of research being devoted to the development of more efficient treatment systems and methods for assaying different microorganisms, it is only a matter of time until viable treatment systems, which remove a broad spectrum of microbial species, become readily available.

Initial recommendations indicate that the biofiltration system's performance is more superior in microorganism removal than any of the other WSUD technologies. Its small footprint, ease of maintenance and cost-effective design also add to the list of advantages. Some of the novel technologies presented show promising results, but their use in this project will be more of a trial site to help improve the understanding of these systems. This is because most of the novel treatment systems discussed here have not been adequately tested and verified to a level where certain confidence around treatment performance can be presented. Some of the novel filter media presented has shown fantastic removal rates for some microorganisms and this media could be incorporated into either biofilters or one of the three novel treatment technologies included in this report. There is even the option of just installing small cartridges (e.g. in pipe inverts) which contain novel treatment media to help treat dry weather flows.

This project offers a unique opportunity to test a variety of stormwater treatment technologies which, to date, have not received enough attention in the literature. As such, if multiple entry points into the selected water body exist, then the treatment of this water should be done using a number of different treatment devices/media explored in this report. This is to help further understand the performance of these devices/media so that these results can be independently published for industry and research uses.

Table 9. Comparison of treatment technologies considered for this report (based upon hydraulic characteristics and treatment performance).

Technology	Hydraulic characteristics				Treatment performance	
	Hydraulic loading [mm/h]	Contact time	Footprint (% imp area)	Reference	Quoted removal efficiencies	Reference
Traditional WSUD technologies						
Detention basins	NA	5 - 40 days	2% - 5%	Melb. Water (2004)	86% - faecal coliforms -2.5% - thermotolerant coliforms 23% - enterococci 52% - somatic coliphages[4]	Mallin *et al.* (2002) Davies and Bavor (2000) Davies and Bavor (2000) Davies *et al.* (2003)
Wetlands (surface type only)	NA	72 hours	2% - 6%	Melb. Water (2004)	79% - thermotolerant coliforms 85% - enterococci 81% - somatic coliphages[4]	Davies and Bavor (2000) Davies and Bavor (2000) Davies *et al.* (2003)
Biofilters	36-180 mm/hr	3 - 17 hrs	1% - 2%	Melb. Water (2004)	82% - *E. coli* 99% - C. perfringens 97% - FRNA phages 92% - faecal coliforms 69% - faecal coliforms 71% - *E. coli*	Bratieres *et al.* (2008a) Bratieres *et al.* (2008a) Bratieres *et al.* (2008a) Rusciano and Obropta (2007) Hunt *et al.* (2008) Hathaway *et al.* (2008)
Novel Technologies						
AbTech's Smart Sponge® Plus	80-135 m/hr 60-100 m/hr	15 sec 20 sec	0.006%[3] 0.008%[3]	AbTech Industries, 2008b	55% - *E. coli* 80% - *E. coli*	AbTech Industries, 2008b AbTech Industries, 2008b
PathexTM Filter Media used in AS Filtration[TM 2]	22-72 m/hr	NA	0.01%[3]	AS Filtration, 2008	99.9% - *E. coli* (lab) 99% - *E. coli* (field)	AS Filtration, 2008
enviss Sentinal 450	2-8 m/hr	3 - 7.5 mins	0.3%[3]	Poelsma *et al.* (2009)	99.99%[*1] - *E. coli* 99.5% - *Clostridium perfringens* 99.8% - FRNA phages	Monash University (*unpublished data*)
Novel filtration media (to be implemented in Novel Technologies or WSUD Technologies)						
Zeolite with HDTMA (hexadecyltrimethylam monium)	3-3.6 m/hr 2.75 mm/h (groundwater)	2 mins 15 days	0.2%[3] NA	Schulze-Makuch *et al.* (2003) Bowman (2003)	99% - various viruses >99% for *E. coli* 99% - MS2 bacteriophage >99% for *E. coli*	Schulze-Makuch *et al.* (2003) Bowman (2003)
Zeolite with Quaternary Ammonium Chloride (QAC)	13.7 m/h	4 mins[5]	0.05%[3]	(Abbaszadegan *et al.*, 2006).	>99% - *E. Coli,* >99% - MS-2 coliphage >99% - *Klebsiella terrigena* ≈99% - PRD-1 bacteriophage ≈99% - *Chlorella vulgaris* >90% - *Cryptosporidium parvum*	(Abbaszadegan *et al.*, 2006)
GAC with $AgNO_3$ and $NaBH_4$	NA	5-15 mins	NA	Bandyopadhyaya *et al.* (2008)	99.9% - *E. coli*	Bandyopadhyaya *et al.* (2008)
GAC with copper	75 mm/hour	80 mins	2%[3]	Kennedy *et al.* (2008)	99.99% - *E. coli*	Kennedy *et al.*, 2008)
GAC with aluminum hydroxyl chloride (AHC)	12 m/h	20 sec	0.05%[3]	Pal *et al.* (2006)	99.9999% - *E. coli*	Pal *et al.*, 2006

[1]this technology uses disinfection in the media to remove *E. coli*, all other tested bugs were removed in absence of disinfection,[2]the data were not available for stormwater but only for cooling water application,[3]these figures have been estimated using a Melbourne Climate and using a filtration sizing program,[4]this data represents a time-based removal statistic, it is the percentage of time where the outlet concentrations were less than the inflow concentrations,[5]for 36inches length.

8 Summary & recommendations

Each section above has provided a conclusion section where recommendations were proposed for the current project, and hence these recommendations are not repeated in this section. However, a general overview of what has been completed is provided in this conclusion.

The above literature review has provided the necessary background information which will help develop an accurate microbial budget/mass balance for a selected water body, whilst minimising the costs of data collection. Uncertainty assessment has been included in many of the sections presented above, since it is believed that understanding these uncertainties is prevalent to the task of mass balancing microorganisms. Without a firm understanding of the uncertainties contained within monitored data, there is little chance of obtaining a physical understanding of the processes which are occurring within the selected water body.

A number of different microorganisms (including traditional indicator organisms, alternate indicators, directly sampled pathogens, etc.) were investigated in this study to determine an appropriate microorganism to monitor. It was suggested that three microorganisms be monitored to fully understand the governing processes for microorganism mass balances (*E. coli*, *C. perfringens* and F-specific coliphages). Furthermore, to fully understand the human health risk benefit to the user's of the system when mitigating influents to the selected water body, it was suggested that F-specific coliphage genetic groups were assayed to help quantify human source contamination. The high costs involved in these assays might limit the number of microorganisms which are monitored, however the use of composite sampling methodologies might help reduce the project costs.

Microorganism sinks in the selected system includes both die-off (caused by environmental conditions in the selected system or predation/competition with other microorganisms) and sedimentation. While there were a number of factors which can impact on microorganism survival, the significance of each factor was different for different microorganisms. However, it was determined that in stormwater/stream environments, the main factors influencing microorganism die-off was thought to be: temperature, predation/competition and irradiance. Whilst the literature provided some indication of how these factors are likely to impact microorganisms in the selected water body, the values provided are really not transferable between different catchments. As such, some experiments were proposed to quantify these impacts on microorganism survival (i.e. with regard to the selected microorganisms). It was also noted that the significance of these environmental factors on microorganism survival is highly dependent on the conditions at the selected water body, since these factors only become significant when the contact time with the microorganisms becomes significant.

The influence of sedimentation was determined to be very site specific as well, since sedimentation was highly related to the flow regime of the water body. Furthermore, the significance of sedimentation on microorganism levels in the water body is also dependent on the association of these organisms with particulate matter. Whilst some figures were found which estimate sedimentation rates of different microorganisms, they were usually for large lake systems which are not applicable if the study site was a river/stream (since shear forces from flowing water would help keep the organisms in suspension). Small experiments were suggested to help quantify these losses, by performing some laboratory and field testing.

Microorganism sources (other than catchment inputs) included in-stream microorganism growth and subsequent resuspension and direct faecal deposits by resident waterfowl and animals. Growth and resuspension of microorganisms will generally only be significant during high flowing waters,

where the shear forces acting on bottom sediment (with microorganisms attached) are large enough. As such, if only dry weather periods are of interest, then growth and resuspension is probably not going to be a significant factor (unless dry weather flows at the selected site are high enough to induce resuspension). Further discussions were made around how to quantify this growth in laboratory and field conditions, together with experiments which could help understand resuspension processes of microorganisms. These experiments could be conducted in conjunction with the sedimentation experiments. Direct faecal deposits as sources were also discussed, and options for quantifying this type of source were provided in the literature review.

A small section was presented on what water quality and quantity data should be collected as part of the monitoring campaign, and most variables which were suggested were highly correlated with microorganism die-off. These variables were suggested so that it would be possible to quantify the die-off of microorganisms caused by, for example, an increase in stream temperature.

A large section of this review was devoted to reviewing the literature on the uncertainty in flow and water quality measurements. Understanding the accuracy of certain monitoring equipment and regimes is essential in understanding a complete mass balance. It was suggested that, where possible, area-velocity devices should be used for flow measurements, since they were found to be most accurate.

Discrete water quality sample uncertainties were found to be sourced from three individual sources of uncertainty:

- sampling uncertainty (related to the fact that a sample is often taken from just one position within the water cross-section);
- storage uncertainty (related to the storage of the sample before analysis); and,
- analytical uncertainty (related to the laboratory analytical technique used to quantify the sample for the specified pollutant).

It was found that for microorganisms, sampling uncertainties were generally lower than for other less dissolved pollutants (but this has only been assessed for *E. coli* in just two wet weather events). Storage uncertainties for microorganisms were generally high, especially compared with most typical stormwater pollutants. This is because microorganism survival is influenced by so many variables, that storing stormwater for elongated periods of time will inevitably influence the sample concentration. Analytical uncertainties were also quite high, but still roughly comparable to other typical stormwater constituents.

Sampling regime uncertainties were also discussed (which are the uncertainties caused by the fact that a finite sample volume is withdrawn and often used to represent a volume much greater than the sample itself). It was determined that for wet weather events, there is enough information to help determine a correct sampling regime for an accurate mass balance. However, for dry weather periods, there was not a lot of information available and, as such, an analysis of continuous turbidity data was conducted. It was determined that the sampling regime required to estimate dry weather loads was dependent on the time period of interest. For example, if yearly dry weather load estimations were required, then two samples per week was sufficient, but if monthly loads estimations were required then around 20 samples per month were required. It is emphasised that this analysis was conducted using turbidity data, and not using microorganisms concentrations (because continuous data does not exist for microorganisms). One major finding was that systematically taking samples from the system would yield increased accuracy in load estimations as compared with random sampling.

Finally, the last section of the review focussed on discussing treatment devices which could be used to mitigate the faecal pollution entering the selected water body. Biofilters were found to be a potential candidate for this project, and removal capabilities could be enhanced by using some novel media which can remove microorganisms. Furthermore, there were several developed novel treatment technologies which could be implemented in this project, although all three technologies have yet to undergo a thorough analysis of their removal capabilities.

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