Evaluation of the efficacy of stormwater treatment devices for reducing water borne ecological and human health risks

Lois Jane Oulton BSc (Hons), MSc

MACQUARIE
University

Department of Environmental Sciences
Faculty of Science and Engineering
Macquarie University
NSW Australia

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Monitoring at the stormwater treatment devices within the Cooks River catchment (Sydney, Australia)

Table of Contents

List of Figures

List of Tables

Summary

Stormwater runoff is widely recognised as a primary source of pollution and cause of negative ecological effects in urban river networks. In an effort to mitigate the degradation of receiving waters, tertiary stormwater treatment devices, such as constructed wetlands and bioretention systems, are increasingly being retrofitted to urban catchments at a considerable cost as part of the Water Sensitive Urban Design (WSUD) strategy. These devices reduce the direct connection of impervious areas to receiving waterways and have the potential to combine natural biological, chemical and physical processes to treat urban stormwater runoff. However, there has been relatively limited field validation of their benefits, particularly under Australian conditions and in terms of improving ecological health, despite their increasing popularity as part of urban water policies and strategies.

The overarching aim of this research was to assess the efficacy of tertiary stormwater treatment devices retrofitted to urban catchments in Sydney, Australia, to improve water quality, and reduce potential risk of harm to ecological and human health. This was achieved by: 1) assessing the water quality improvement capacity of tertiary stormwater treatment devices; 2) assessing the toxicity of untreated and treated stormwater to freshwater biota using single-species toxicity tests in the laboratory with algae (*Pseudokirchneriella subcapitata*), a crustacean [*Ceriodaphnia dubia*] and fish embryos [*Melanotaenia duboulayi*]), and *in situ* with shrimp (*Paratya australiensis*); 3) assessing the influence of untreated and treated stormwater upon higher-levels of biological organisation (i.e. community and ecosystem responses) and 4) assessing the potential of constructed stormwater wetlands to provide a breeding ground for mosquitoes.

Chapter 2 demonstrated the ability of a constructed wetland to reduce the majority of stormwater pollutants tested across three storm events and the toxicity of stormwater to freshwater biota across two storm events. However, the potential risk of stormwater to stimulate primary production remained following treatment. In

Chapter 3, evaluation of a retrofitted bioretention basin across six storm events revealed that it had little impact on the majority of influent pollutant concentrations. Leaching of several analytes from the system occurred consistently and the toxicity of stormwater to freshwater biota was not always reduced. Chapter 4 highlighted that a larger-sized (with respect to the contributing catchment area) constructed wetland treated stormwater to a standard that enabled good improvements in ecological health at the wetland outlet, evidenced by its ability to support consistently high survival of *P. australiensis,* the presence of sensitive macroinvertebrate taxa, and reduced rates of organic decomposition. By contrast, two other wetlands that were smaller with respect to catchment area and had design constraints had a reduced capacity to achieve the same ecological improvements. Chapter 5 indicated that stormwater Zn concentrations reflective of those found in treated stormwater from a constructed wetland reinstated normal foraging behaviour in shrimp, indicating that the treatment of metal contaminants in stormwater can have discernible ecological benefits. Chapter 6 highlighted the variability of tertiary stormwater treatment systems (one bioretention basin and three constructed wetlands) to reduce indicator bacteria, particularly enterococci. Median outflow concentrations generally exceeded public health criteria for primary and secondary contact. Public health risks were further substantiated by the occurrence of immature mosquito larvae at all surface flow systems. Correlation analysis indicated that the higher nutrient concentrations in the inlet zone in comparison to the outlet zone provided conditions that were more conducive to mosquito larvae production.

The empirical evidence generated from this study has important implications for the assessment and design of future stormwater treatment systems as well as the pursuit of WSUD strategies where the primary goal is to protect and rehabilitate urban waterways. Despite some beneficial outcomes evident in the chemical and ecological analyses, the overall results presented in this thesis suggest that stormwater treatment devices are far from a panacea for the adverse impacts of urbanisation on river networks. Indeed, the data suggest that it may not be possible to treat stormwater to prescribed levels (such as those set out in national water quality guidelines). It is clear that appropriate design of treatment systems is

paramount and further research and subsequent design modifications is needed if the goal of improving the condition of urban waterways using stormwater treatment devices is to be achieved. This study has also demonstrated the importance of using a combination of chemical analysis and biological measures to provide an integrative assessment of any subsequent water quality improvements, and how this approach is necessary to quantify the benefits of tertiary stormwater treatment devices and to confirm if they function as planned.

Statement of Candidate

I certify that this thesis entitled “Evaluation of the efficacy of stormwater treatment devices for reducing water borne ecological and human health risks” has not been submitted for a higher degree to any other university or institution.

I also certify that the thesis is an original piece of research and it has been written by me. Any help and assistance that I have received in my research work and the preparation of the thesis itself have been appropriately acknowledged.

In addition, I certify that all information sources and literature used are indicated in the thesis.

The research component involving *Melanotaenia duboulayi* was conducted under Animal Research Authority project approval 2011/024 (Appendix 1).

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Lois Jane Oulton (42259509)

April 2016

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Chapter 1. General Introduction

Urban stormwater runoff

Growth of the world's population has seen an associated acceleration in global urbanization, particularly in the 20th century (Cohen, 2003). An estimated 66% of the world's population will inhabit urban areas by the year 2050 (UNPD, 2014). In Australia, approximately 89% of the population reside in urban areas, and by 2050, it is anticipated that this will rise to 93% (UNPD, 2014). Such a pervasive change in land-use resulting from urban sprawl comes at an increasing environmental cost (Alig et al., 2004; McKinney, 2006), including the degradation of urban river systems (Meyer et al., 2005; Gurnell et al., 2007).

Impervious surfaces created by urban land-use (e.g. roads and roofs) reduce the infiltration of precipitation and subsequent evapotranspiration, leading to increased overland stormwater runoff (Paul & Meyer, 2001; Lee & Heaney, 2003) (Figure 1). This alters the pathways by which runoff in a catchment reaches waterways (Walsh et al., 2004a).

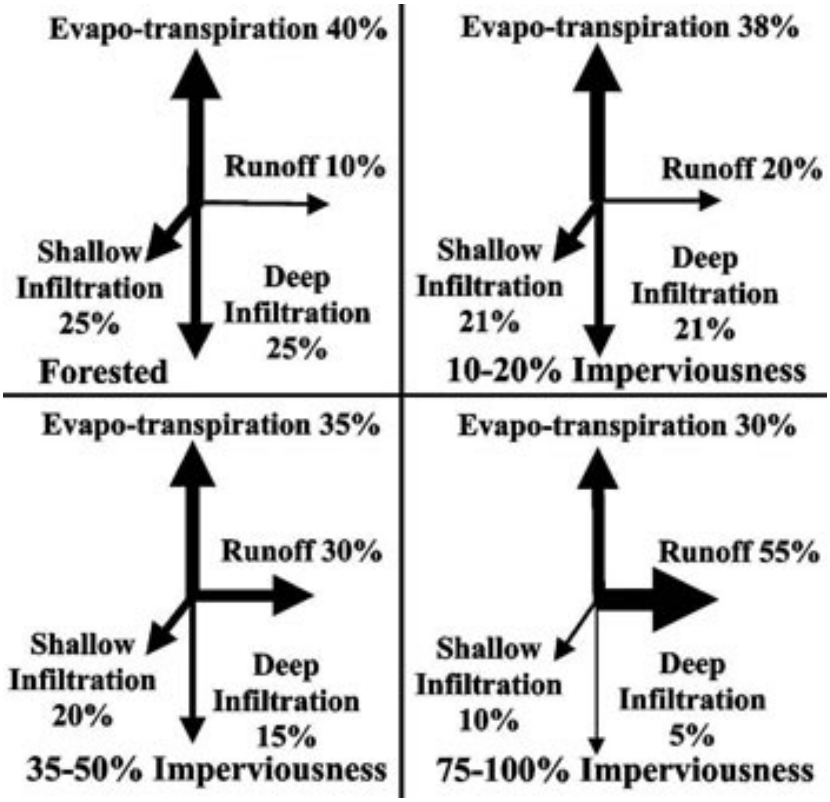


Figure 1. Example of the shift in stormwater runoff with increasing impervious surface coverage in urbanising catchments. Source: Paul & Meyer (2001).

Urban stormwater runoff has been managed traditionally for flood prevention by the rapid conveyance of runoff to nearby waterways by hydraulically efficient stormwater drainage infrastructure, with (until two decades ago) little concern given to the effects on receiving waterway health (Chapman & Horner, 2010). It is now widely recognised that this form of nonpoint source pollution from urban land use is a primary driver degrading receiving waterway health, the magnitude of which is determined primarily by catchment imperviousness and stormwater drainage infrastructure (Walsh, 2000).

Impacts on receiving waterway health

Degradation of river systems in urbanizing catchments is an increasing problem worldwide (Paul & Meyer, 2001; Findlay & Taylor, 2006). Catchment urbanization sets into motion a cascade of physical, chemical and ecological impairments to river systems that have collectively been termed the 'urban stream syndrome' (Meyer et al., 2005). These effects are often exacerbated by diverting stormwater runoff from impervious areas directly to receiving waters through drainage pipes, termed effective imperviousness (EI) (Wenger et al., 2009). EI is often a stronger correlate of stream condition than impervious cover (total imperviousness [TI]) (Hatt et al., 2004; Walsh, 2004). High EI and TI are generally associated with a number of other attributes of urban areas such as sewerage infrastructure, point source pipes and declines in riparian vegetation, all of which can add to the degradation of urban river systems (Wenger et al., 2009) (Figure 2).

Physical consequences of an increased quantity of overland stormwater runoff include an overall decrease in baseflow, and hydraulic 'flashiness' during storm events characterized by more frequent, shorter-duration and higher peak discharges (Walsh et al., 2004a; Gurnell et al., 2007). These alterations to flow regimes typically lead to channel incision, bank erosion (Paul & Meyer, 2001) and a reduction in the complexity of in-stream habitat (Walsh et al., 2005). These hydrological impacts on streams are accompanied by increases in pollutants (Hatt et al., 2004). Urbanization increases the concentrations of many pollutants in a catchment and introduces many potentially toxic substances not found in natural catchments (Walsh et al., 2004a). The types of pollutants entering urban

waterways are typically dependent on the nature of land use (residential commercial or industrial) (Hall & Anderson, 1988).

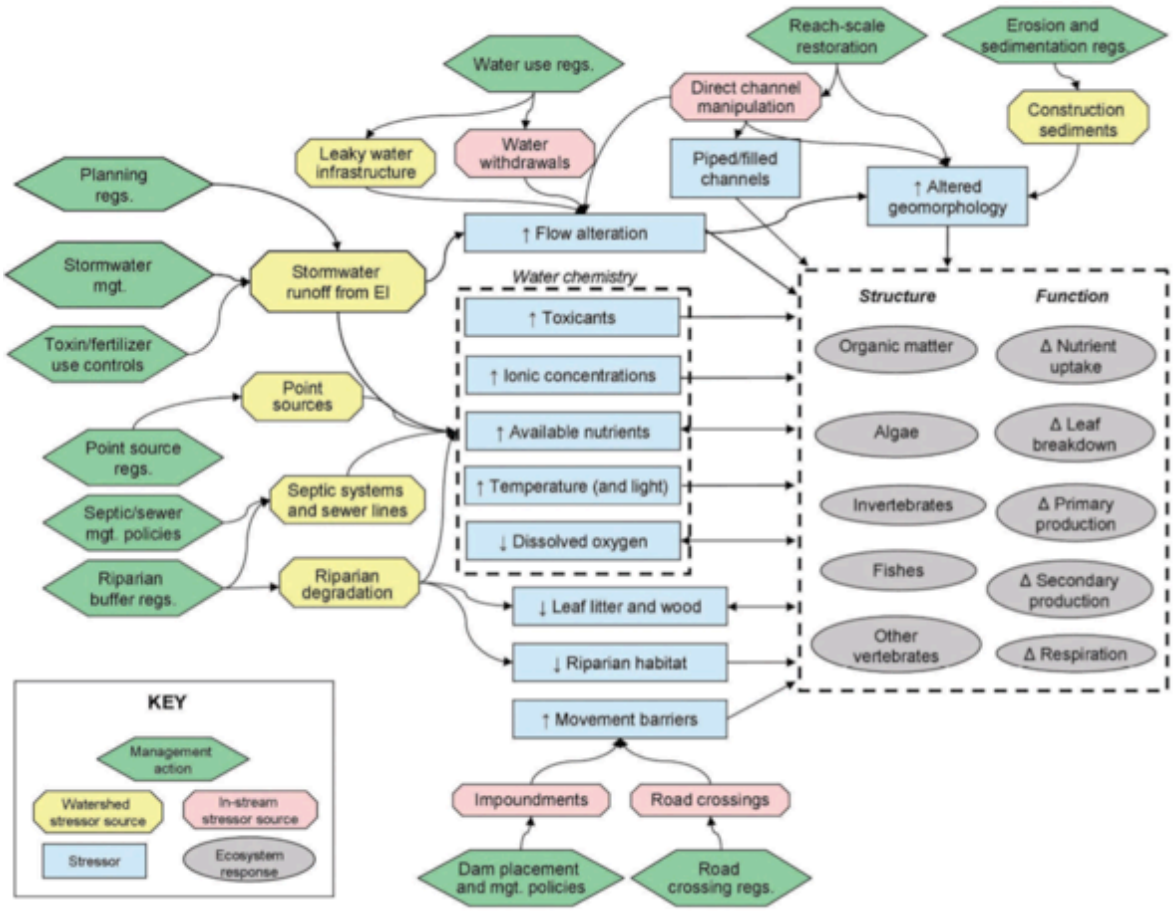


Figure 2. Conceptual model of urban impacts on streams. EI = effective imperviousness; mgt. = management; regs. = regulation. Source: Wenger et al. (2009).

Common pollutants in urban stormwater include suspended solids, nutrients, metals and faecal indicator bacteria (Birch et al., 2005; Wong, 2006; Greenway, 2010; Parker et al., 2010; Sidhu et al., 2012). As well as delivering a wide variety of pollutants, stormwater runoff can also cause alterations to thermal regimes, dissolved oxygen concentrations and pH (Walsh et al., 2004). Hatt et al. (2004) showed that concentrations of several water quality variables (e.g. total phosphorus and conductivity) in urban streams were more strongly correlated with EI than with TI during storm events.

Stormwater runoff contributes to microbial pollution in waterways and a subsequent demise in their recreational value to the public (Hathaway et al., 2011a; Hathaway & Hunt, 2012). In terms of freshwater biota, sub-lethal and lethal toxic effects of stormwater runoff have been observed *in situ* and in the laboratory (Skinner et al., 1999; Tucker & Burton, 1999; McQueen et al., 2010). Decreases in biotic richness and diversity of algae, invertebrates and fishes commonly occur with increasing urbanization, typically with the loss of sensitive species and a shift in dominance by more tolerant species (Paul & Meyer, 2001; Walsh et al., 2005). In Melbourne, Australia, macroinvertebrate and diatom assemblage composition in streams reached a threshold of degradation at EI levels of 1-5% and 6-15%, respectively (Walsh et al., 2004a). Walsh (2004) found that most sensitive macroinvertebrate taxa were absent from urban stream sites with >20% EI. Conditions in urban streams have also been shown to alter important ecosystem functions, such as organic decomposition and nutrient uptake (Meyer et al., 2005; Chadwick et al., 2006; Imberger et al., 2008), which can ultimately provide ecosystem services (Johnson et al., 2011).

Water Sensitive Urban Design (WSUD)

River networks flowing through urban areas will continue to be impacted into the future (Meyer et al., 2005), causing an increasing proportion of the population to rely on waterways degraded by the urban stream syndrome (Violin et al., 2011). Urban populations are becoming increasingly more aware of their landscape and demanding environmental improvements (Findlay & Taylor, 2006). In recent years the urban environment has become an important focal point for the implementation of Ecologically Sustainable Development (ESD) practices (Harding, 2006) and within Australia, the Council of Australian Governments endorsed the National Strategy for ESD in 1992 (Commonwealth of Australia, 1992). This has prompted increasing action to protect and rehabilitate urban waters and stream restoration projects have progressively become more common (Purcell et al., 2002). Restoration efforts have, however, focused largely on reach-scale structural (e.g. creation of riffle habitats) and bioengineering (e.g. bank stabilization using vegetation) approaches (Brown, 2000).

Assessments of reach-scale restoration efforts in urban streams have demonstrated variable improvements to physical degradation and minimal to no improvement in biological degradation (Larson et al., 2001; Purcell et al., 2002; Bond & Lake, 2003; Suren & McMurtrie, 2005; Palmer et al., 2010; Violin et al., 2011). The enhancement potential of local-scale restoration efforts are constrained by catchment-scale stressors (Walsh et al., 2006; Palmer et al., 2010) of which urban stormwater runoff is an over-arching one (Walsh, 2000). Given the strength of drainage connection with water quality and biological relationships, it has been proposed that stormwater management techniques aimed at reducing the direct connection between impervious areas and streams provides a critical pathway for more effective restoration and protection of stream health (Hatt et al., 2004; Walsh et al., 2004a; Walsh et al., 2006; Walsh et al., 2009).

In Australia, stormwater management practices have shifted away from conventional 'pipe' approaches through the adoption of Water Sensitive Urban Design (WSUD) strategies (Walsh et al., 2004a). The intergovernmental agreement on a National Water Initiative promotes WSUD, defined as the "integration of urban planning with the management, protection and conservation of the urban water cycle that ensures urban water management is sensitive to natural, hydrological and ecological processes" (NWC 2004, p. 30). Intrinsically linked with WSUD are the principles of ESD and Integrated Water Cycle Management (IWCM) (Wong et al., 2012). Stormwater treatment is an integral part of the WSUD strategy and is achieved by placing structural treatment devices along the transport pathways of stormwater runoff (e.g. in place of piped drains); this reduces the direct connection of impervious area and waterways, and aims to mitigate the impact of stormwater runoff on flows as well as the amount of stormwater pollutants entering receiving waterways (Walsh et al., 2004a; Roy-Poirier et al., 2010). Parallel principles have also been developed in the UK called Sustainable Urban Drainage Systems (SUDs) (Eriksson et al., 2007) and Low Impact Development (LID) in the USA (Hunt et al., 2008). International examples of research are considered in the thesis where relevant comparison is made to the results generated herein.

Structural stormwater treatment practices include primary treatment (sediment basins and gross pollutant devices), secondary treatment (grass/vegetated swales and sand filters) and tertiary treatment devices (bioretention systems and constructed wetlands) (UPRCT, 2004). Amongst these devices, bioretention systems and constructed wetlands have gained popularity in recent years (Knights et al., 2010; Roy-Poirier et al., 2010; WSUD, 2014a) (Figure 3) and are promoted due to their potential to meet better water quality objectives by facilitating the removal of nutrients, bacteria and heavy metals (Water by Design, 2009). Constructed wetlands are vegetated open water bodies that impede water flow and remove pollutants using enhanced sedimentation, fine filtration and biological uptake (Davies & Bavor, 2000; Water by Design, 2009). In contrast, bioretention systems operate by filtering stormwater runoff through terrestrial planted vegetation followed by vertical percolation through a filter media, where pollutants are retained via fine filtration, absorption and biological uptake (Davis et al., 2003; Water by Design, 2009). Bioretentions are subsurface vertical flow systems that do not retain surface water, whilst constructed wetlands are horizontal surface flow systems that are permanent open water bodies (Water by Design, 2009).

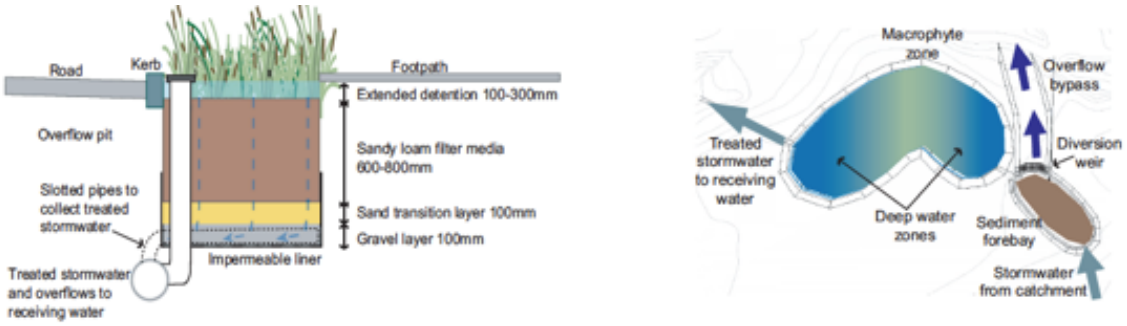


Figure 3. Schematic representation of A) cross-section of a bioretention system and B) plan view of a constructed wetland. Source: WSUD (2014a).

The effectiveness of pollutant removal in bioretention systems is influenced by the design specifications of the bioretention system including properties of the filter media, selected vegetation and the use of enhancements such as a saturated zone (Davis, 2014; Payne *et al.,* 2014). The ability of wetlands to treat stormwater is largely a function of inflow or hydraulic loading rate and storage properties (Carleton et al., 2001; Davis, 2014). Best practice design guidelines recommend

that the area required for a correctly sized bioretention system is 2-3% of the contributing catchment area and 5-7% for wetland treatment areas (Water by design, 2009).

The Australian Guidelines for Urban Stormwater Management (ANZECC/ARMCANZ, 2000), which is one of a suite of documents under the National Water Quality Management Strategy (NWQMS), recommends structural techniques as part of the management of urban stormwater. There is no current national standard that deals explicitly with the adoption of stormwater treatment devices and instead guidelines are used as a precursor. Under the *Local Government Act 1993* (NSW) councils are required to manage the environment in a way that is consistent with the principles of ESD and are responsible for preparing planning controls that improve the sustainable management of the urban water cycle (WSUD, 2014). Many councils across NSW have revised their Development Control Plans to incorporate WSUD (WSUD, 2014b), which underpin statutory Local Environmental Plans (LEPs) and related State legislation, such as the *Water Management Act 2000* (NSW). In an effort to improve stormwater quality and receiving waterway health, many Sydney (NSW, Australia) councils are increasingly retrofitting stormwater treatment devices into existing urban catchments at a high cost (Knights et al., 2010).

Thesis rationale

Although tertiary stormwater treatment devices are being promoted as potential solutions to urban stream degradation and increasingly retrofitted into existing urban catchments at a high cost, they have received relatively limited field validation of their benefits, particularly in terms of improving ecological health (Walsh et al., 2004a; Ladson et al., 2006; Moore & Hunt 2011). Neglecting to evaluate the success of restoration efforts to improve urban stream health is a common problem (Kondolf & Micheli, 1995; Davis et al., 2003; Violin et al., 2011). To aid natural resource managers in improving the resilience of waterways in the face of continuing urbanization, scientific information on the efficacy of stormwater

treatment devices to deliver improvements is needed, not only to improve the efficacy of devices, but also to justify the added costs.

Most of the published peer-reviewed literature on the efficacy of tertiary stormwater treatment devices concerns studies conducted outside of Australia, which have focused mainly on water quality improvements in terms of pollutant removal e.g. (Line & Hunt 200; Hathaway & Hunt, 2010). The ecological benefits arising from tertiary stormwater treatment devices remains largely untested, with only a few published studies worldwide quantifying the ability of stormwater treatment devices to enhance aquatic biodiversity e.g. (Greenway, 2010; Moore & Hunt 2011). In addition, there is limited evidence available as to whether any subsequent water quality improvements are sufficient to prevent toxic harm to aquatic biota and improve ecological integrity across multiple levels of biological organisation. Given that contaminant-induced stress in the aquatic environment may be manifest throughout different levels of biological organisation (Clements, 2000), it is essential to examine multiple indicators across different levels in order to demonstrate effects (Peplow & Edmonds, 2005; Clements & Kiffney, 2009). For the management of water quality in Australia, the current NWQMS approach recommends moving away from the sole reliance on chemical guidelines values through the use of integrated approach, comprising of water quality monitoring coupled with chemical-specific guidelines, direct toxicity assessment and biological monitoring (ANZECC/ARMCANZ, 2000a). Ecological evidence is essential knowledge for integration into stormwater management and to support arguments for the efficacy of stormwater treatment devices and their wider implementation.

There is also a concern that constructed wetlands may provide breeding sites for disease-bearing mosquitoes and that they become a potential human health risk for nearby human communities (Russell, 1999; Greenway, 2003). Mosquito production in water bodies is dependent on a number of factors such as water quality, predator abundance and vegetation cover (Russell, 1993). Mosquito larvae are more tolerant of pollutants than many of their potential predators such as fish and macroinvertebrates (Russell, 1993). Consequently, inlet zones of constructed stormwater wetlands containing untreated stormwater may provide nutrient rich,

predator free conditions that are conducive to larval production. Similar to evaluation of the ecological benefits of stormwater treatment systems, the inadvertent risks arising from their development has not been investigated widely. However, given the spread of vector-borne diseases, this is an important issue that requires evidence-based data to establish the public health risks if these devices are to add value to the urban landscape.

The Sydney Metropolitan Catchment Management Authority (SMCMA) coordinated the Cooks River Urban Water Initiative (CRUWI), a Federally funded project aimed at improving the quality of water flowing into the Cooks River via a series of retrofitted stormwater treatment devices. The Cooks River located in Sydney, NSW, is severely affected by urban development and is considered one of the most polluted and degraded river systems in Australia (NSW Government, 2011). This project offers an opportunity to measure the efficacy of these works. Generation of empirical data that are directly relevant to Australia's largest city, Sydney, is critical to support arguments for the wider implementation of retrofitted stormwater treatment devices and to aid future decision-making on stormwater best management practices.

Thesis aims

The overall aim of this research was to assess the efficacy of retrofitted tertiary stormwater treatment devices to improve water quality, and reduce potential risk of harm to ecological and human health. To achieve this aim, an integrative approach was taken and the following four relevant objectives were identified:

1) To assess the water quality improvement capacity of tertiary stormwater treatment devices retrofitted to an urban catchment;
2) The effect of untreated and treated stormwater to freshwater biota using single-species toxicity tests in the laboratory and *in situ*;
3) The influence of untreated and treated stormwater upon higher-levels of biological organisation (i.e. community and ecosystem responses).

4) The potential of surface flow constructed systems to provide a breeding ground for mosquitoes.

The stormwater treatment devices examined in this study were retrofitted to the Cooks River Catchment, Sydney, Australia. With the exception of one stormwater wetland, Gadigal Green, devices were installed under the CRUWI. Several potential stressors are spatially correlated in urban rivers (Townsend et al., 2009), which presents a major barrier to assessing the benefits of stormwater treatment devices on receiving waters. This problem is further exacerbated if there has not been catchment-wide application of stormwater treatment devices, as is the case in the current study area. Thus the ability to detect catchment or reach scale improvements when only a small component of a catchment is serviced by such devices is limited. To isolate the impact of treated stormwater from other factors and establish any benefits arising from the retrofitted stormwater treatment devices, monitoring was focused at the inlet (untreated stormwater) and outlet (treated stormwater) zone of each device.

Outline of the thesis

The chapters within this thesis have been written in a format ready for publication, with some additional information that adds value to the thesis. The contributions of other individuals to the work presented are acknowledged at the start of each chapter.

Chapter 2 and Chapter 3 assessed the efficacy of a retrofitted constructed wetland and bioretention basin, respectively, to remove pollutants from urban stormwater runoff that may pose a risk to freshwater biota. The inflow and outflow of the treatment devices was collected using automatic sampling procedures and analysed for filterable metals, nutrients, suspended solids, and biological and chemical oxygen demand. The collected composite samples were used to determine the toxicity of untreated and treated stormwater runoff to three freshwater species in the laboratory: *Selanastrum capricornutum*, *Ceriodaphnia dubia* and eggs of *Melanotaenia duboulayi*. The chosen test species covered a range of trophic levels and included an aquatic plant, crustacean and fish species,

as is recommended to comprehensively assess toxic impacts (USEPA, 1993; ANZECC/ARMCANZ, 2000b). It was hypothesised that, with stormwater treatment, pollutants and the toxicity to freshwater biota would be reduced. This work contributed to addressing thesis objectives 1 and 2.

Chapter 4 examined the efficacy of retrofitted stormwater wetlands to provide improvements to ecological health. This was assessed *in situ* at an organism, community and ecosystem response level by measuring toxicity to caged *Paratya australiensis*, macroinvertberate community composition, and organic matter decomposition, respectively, at the wetland inlet and outlet zone of three constructed wetlands. Physico-chemical variables were also measured at the inlet and outlet zone to assess the relationship of ecological responses with wetland treatment performance. It was hypothesised that stormwater treatment would result in better ecological health outcomes at the outlet zone compared to the inlet zone. It was anticipated that the level of change would be influenced by the size of the wetland with respect to the contributing catchment area, with the greatest effect evident in the largest constructed wetland relative to catchment area. This work contributed to addressing thesis objectives 1, 2 and 3. The stormwater Zn concentrations in this study highlighted a potential ecological risk that was further investigated in chapter 5.

Chapter 5 examined the sub-lethal effects of Zn concentrations reflective of those found in untreated and treated stormwater from constructed stormwater wetlands with respect to how they interfered with the foraging behaviour of *P. australiensis* to a chemoattractant source. It was hypothesised that abnormal foraging behaviour would be observed in shrimp exposed to Zn at untreated stormwater concentrations, but not at treated concentrations. This chapter contributed to addressing thesis objective 2.

Chapter 6 assessed the efficacy of a bioretention system and three constructed wetlands to remove indicator bacteria (faecal coliforms and enterococci) from stormwater and reduce the risk to human health. It also evaluated the potential of surface flow systems to provide a breeding ground for mosquitoes by recording the abundance and composition of immature mosquito assemblages within the

inlet and outlet zone of the wetlands. It was hypothesized that, with stormwater treatment, indicator bacteria densities would be reduced between the inlet and the outlet of treatment devices, and at the wetland sites fewer immature mosquitos would be recorded at the outlet zones in comparison to the inlet zones. This chapter contributed to addressing thesis objectives 1 and 4.

Chapter 7 provides a general discussion of the research findings and outlines suitable directions for future research.

Chapter 2. Freshwater Toxicity and Pollutant Reduction in Urban Stormwater Flowing Through a Constructed Wetland

The aim of the research presented in this chapter was to determine the efficacy of a constructed wetland to remove urban stormwater pollutants and reduce the risk to freshwater biota. This was evaluated using a combination of chemical analysis and freshwater toxicity testing. The results demonstrate the ability of the wetland to reduce the majority of stormwater pollutants tested and the toxicity of urban stormwater runoff to freshwater biota. This research contributes to data identifying the value of stormwater treatment in the urban landscape and its utility in reducing the risk of stormwater pollution to aquatic biota.

Authors

	Problem formulation	Experimental design/methodology	Statistical analysis	Results interpretation	Paper preparation
Lois Oulton[1]	90	90	97	95	90
Mark Taylor[1]	5	3	0	1	3
Culum Brown[2]	0	2	0	1	2
Grant Hose[2]	5	5	3	3	5

1 Department of Environmental Sciences, Macquarie University, NSW 2109 Australia
2 Department of Biological Sciences, Macquarie University, NSW 2109 Australia

Abstract

Stormwater runoff is a primary source of pollution and cause of negative ecological effects in urban rivers. Constructed wetlands are being retrofitted to urbanized catchments in an effort to mitigate these effects. Limited data is available on the ability of these systems to reduce the adverse impact of urban stormwater pollution on freshwater biota, especially under Australian conditions. We addressed this by measuring the efficiency of a wetland constructed in Sydney (Australia) to remove a suite of urban stormwater pollutants that may affect aquatic biota in receiving waterways (TSS, BOD, COD, nutrients and filterable metals). We also determined if any subsequent water quality improvements were sufficient to mitigate toxicity to three common freshwater species (*Pseudokirchneriella subcapitata*, *Ceriodaphnia dubia* and *Melanotaenia duboulayi*). With the exception of Fe, Mn, NH_3-N and BOD, mean positive pollutant removal efficiencies ranging from 26% to 86% were observed, with the greatest reduction for NO_x-N. Despite high removal efficiencies, mean concentrations of filterable Cu and Zn, TN and TP exiting the wetland still exceeded Australian freshwater guidelines. Significant phytotoxicity of the inflow or outflow water to *P. subcapitata* was not recorded, with growth stimulation observed following one storm event. Significant toxicity to *C. dubia* and *M. duboulayi* was observed from some inlet water samples and effects included mortality and reduced hatching success, respectively. No toxicity effects were observed in outflow water samples, demonstrating the ability of the wetland to reduce the toxicity of the influent, albeit with some analyte concentrations still in excess of guidelines. This work demonstrated that the constructed wetland studied was able to reduce both the majority of pollutant concentrations and toxicity in the input stormwater.

Introduction

Historically, stormwater runoff was regarded as an urban flood risk and diverted to local waterways. It is only since the 1970s that it has been recognized as one of the main sources of nonpoint pollution from urban land use leading to the degradation of receiving waterway health (Hall & Anderson, 1988; Walsh, 2000; Paul & Meyer, 2001). Urban stormwater runoff contains a complex mixture of pollutants often including suspended solids, nutrients and metals (Greenway, 2010) which may be directly toxic or disruptive to aquatic ecosystems (Hall & Anderson, 1988; Skinner et al., 1999; Schiff et al., 2002; Schiff et al., 2003; McCarthy et al., 2008; McQueen et al., 2010; Feist et al., 2011; Kumar et al., 2011). For example, high loadings of suspended solids can reduce light penetration for primary production, smoother habitats/benthic organisms, and increase biological oxygen demand (Wood & Armitage, 1997; Greenway, 2010). Inputs of nutrients can promote algal blooms (Anderson et al., 2002) and metals present in stormwater can have sub-lethal (Skinner et al., 1999; Sandahl et al., 2007) and lethal (Schiff et al., 2002) effects on aquatic biota.

Best management practices (BMP) for stormwater, management, also known as Stormwater Control Measures (SCMs) in the USA (Winston et al., 2012) and Water Sensitive Urban Design (WSUD) in Australia (Walsh et al. 2004), have shifted to incorporate the use of structural treatment devices such as constructed wetlands (Roy-Poirier et al., 2010). Constructed wetlands use a combination of physical, biological and chemical processes for water quality improvement (Greenway, 2010). Typically, an urban stormwater constructed wetland is configured to include: an inlet zone (detention basin); open water zone (sedimentation pond), a macrophyte zone (densely vegetated area); and an outlet zone (water level control), all of which are surrounded by a littoral zone (DLWC, 1998). Their value for wastewater treatment is widely recognized and utilized across the world, yet their applicability for stormwater management has received less attention (Carleton et al., 2000; Malaviya & Singh, 2012). Hydrologic and pollutant loadings entering stormwater wetlands are more variable than for

wastewater, making performance estimates and their design more difficult (DLWC, 1998; Carleton et al., 2001).

The ability of wetlands to treat stormwater is largely a function of inflow, or hydraulic loading rate and detention time, which are determined by storm intensity, runoff volume, and wetland size (Carleton et al., 2001). Multiple studies have demonstrated that constructed wetlands are effective at reducing metals (Scholes et al., 1998; Walker & Hurl, 2002; Ladislas et al., 2013), nutrients (Greenway, 2010) and sediments (Backstrom, 2002) in urban stormwater runoff. By contrast, there are fewer field studies that have evaluated the ability of constructed wetlands to treat a suite of urban stormwater pollutants in a single investigation that are directly relatable to freshwater biota and may present a risk (Carleton et al., 2001; Birch et al., 2004; Malaviya & Singh, 2012). In addition, there is no published peer-reviewed evidence available as to whether any subsequent water quality improvements are sufficient to prevent toxic harm to aquatic biota. Toxicity data may greatly augment chemical data by indicating the bioavailability of contaminants in untreated/treated stormwater and the potential for negative effects on aquatic biota in receiving waters. This is a critical knowledge gap in understanding if and how such treatment systems work. Closing this knowledge gap will enable the provision of specific ecological evidence to support arguments for their wider implementation.

This study focused on a constructed stormwater wetland in Sydney, Australia, and relied on a combined chemical and toxicological assessment to measure the efficacy of the structure to treat urban stormwater runoff. The overall goals of the research were to determine: (1) the efficacy of the wetland to remove a suite of pollutants from urban stormwater that may pose a risk to freshwater biota; and (2) to determine if treated stormwater was less toxic to freshwater biota than untreated stormwater. Our hypothesis was that, with stormwater treatment by the wetland system, stormwater pollutants and the toxicity to freshwater biota would be reduced.

Methods

Sampling Location

This study was conducted on Yarrowee wetland (33°53'S, 151°04'E), which was retrofitted to a residential urban area on the Cooks River catchment in the inner West of Sydney, NSW, Australia, in 2010 (Figure 4). Stormwater samples were collected from the constructed wetland during three storm events between April 2012 and October 2012.

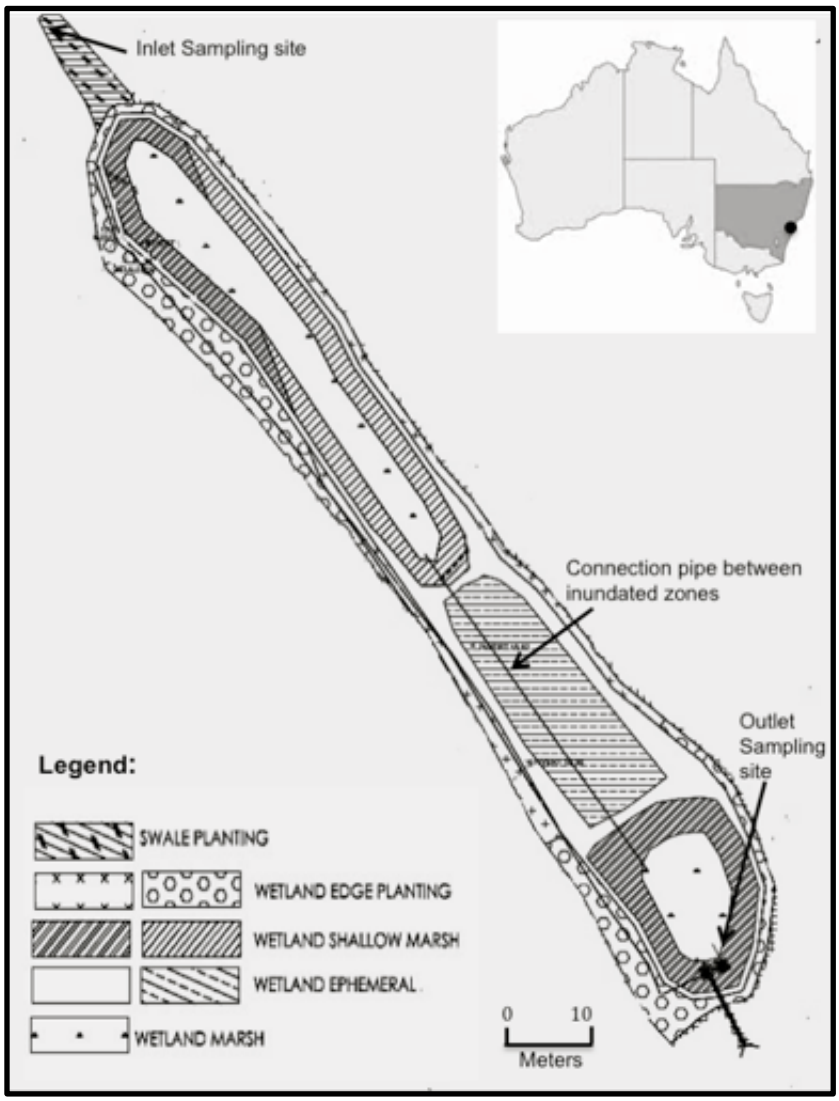


Figure 4. Diagrammatic representation of Yarrowee Wetland including sampling locations. Insert on the top right shows the site location (•) in relation to Australia. Adapted from Equatica (2009).

A 375 mm diameter stormwater pipe transports runoff to the wetland from an estimated 2.9 ha drainage area, which consists of housing (approximately 68

properties), gardens and residential roads. Street gullies leading into the wetland inlet are installed with litter baskets to screen out gross pollutants. The inlet zone is designed to function as a sediment forebay and swale area. Sandstone block walls provide scour protection and boulders provide energy dissipation at the entrance. Flows are then released from the inlet zone to wetland marsh zones, which include deep marsh, shallow marsh, and a periodically inundated zone. A connection pipe is installed between the two permanently inundated marsh zones (Figure 4). The wetland is lined with a high-density polyethylene (HDPE) liner underlain with 50 mm of sand. The surface of the system is covered with 250 mm of wetland soil and planted with grasses, sedges, rushes, shrubs and flowering plants at a density of approximately 6 plants per m^2 (a detailed list of planted species in the wetland is provided in Appendix 2; Table A1.1). The wetland has a normal operating depth of 300 mm. The site is designed with an extended detention zone (300 mm) and the maximum depth of the wetland when at full capacity is 600 mm (Equatica, 2009). At full pool the area of the wetland is approximately 0.12 ha. The wetland was designed with a hydraulic detention time of 72 h (David Knights, Environmental Engineer, Equatica, pers.comm.), however this was not confirmed with a dye tracer test in the current study. The MUSIC (Model for Urban Stormwater Improvement Conceptulisation) model for this treatment device predicts a 78%, 52% and 37% reductions in TSS, Total Nitrogen and Total Phosphorus, respectively (WSUD, 2014c). Two concrete overflow pits are located in the outlet zone, the first of which is fitted with an unplasticised polyvinyl chloride (UPVC) riser to enable water level control. Water exits the wetland and drains into a tributary of the Cooks River via a 450 mm outlet pipe connected to the overflow pit. The size of the wetland in relation to the contributing catchment area is 4.1% (calculated based on the size of the wetland and the catchment area) and therefore slightly below optimum (5-7%) for best practice design guidelines (Water by Design, 2009).

Sampling Procedure

Gamet 12M automated samplers (Gamet Equipment Pty Ltd, Armidale, Australia), were installed at the inflow and outflow drainage points of the wetland system by

Manly hydraulic laboratory (MHL, Sydney, Australia) and fitted with 24 pre-cleaned 1 L polyethylene bottles for every monitored storm event. The location of the water collection sites is indicated in Figure 4. The samplers were programmed to collect a discrete 1 L sample every 5 min for a total duration of 2 h (i.e. a total of 24 samples). Flow data for the three monitored storm events over the sampling period is provided in Appendix 2 (Figure A2.1) and demonstrates that the constructed wetland reduced the peak flow for all three storm events. This set-up allowed for events to be captured at the inlet and samples to be collected from the rising and falling limbs of the hydrograph. Samples were collected from the outlet once the system had started to discharge following the event. To obtain a more representative sample of the treated water quality in the wetland the whole of the outflow should be sampled, but in practice this is very difficult and was not logistically possible for the study due to the short sample holding times required (for the toxicity tests and some of the measured analytes) and the duration of the sampling period (finite number of sample bottles available). Nevertheless, the chosen sampling regime was deemed sufficient to evaluate whether the water leaving the wetland was of a better quality and less toxic than the influent stormwater. A Druck PDCR 1830 pressure transducer (GE Measurement & Control Solutions, USA) measured the water level over an installed sharp-crested weir (Figure 5). This data was stored by a HydroMace 2000 data logger (Mace, Dural – NSW), which activated sampling when the water level exceeded a preset limit.

A) B)

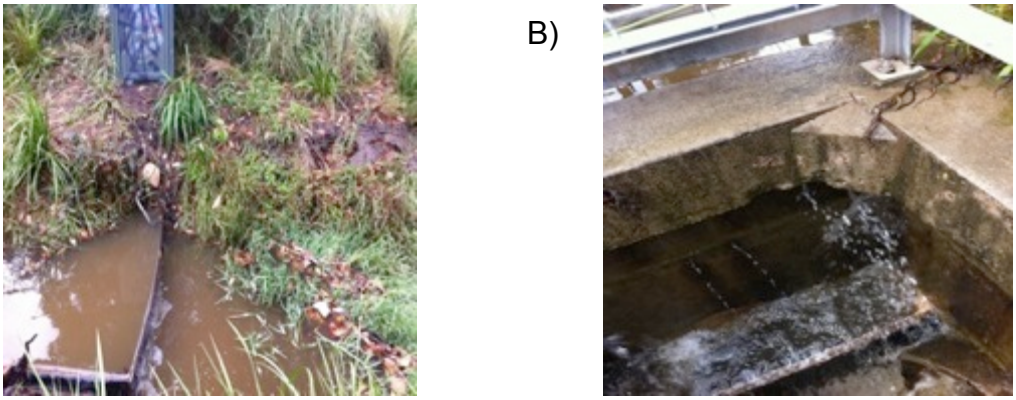


Figure 5. Side view of the weir installed at A) the wetland inlet and B) the wetland outlet overflow pit.

A telemetry system alerted us as to when sampling had commenced. At the end of the rain event, sample bottles were removed from the field site and transported on ice in portable coolers to our laboratory at Macquarie University. Flow rate data for the inflow and outflow over the sampling period was subsequently calculated in Hydstra (Kisters Pty Ltd, North America) by MHL. This data was then used to generate a flow-weighted composite sample, for both the inlet and outlet, by manually compositing the discrete samples in proportion to flow rate into a single 20 L polyethylene container (an example of how a flow-weighted sample was calculated is provided for event W1 in Appendix 2; Table A2.2). For the toxicity tests 3 L of the composite sample was transferred into 3 x 1 L polypropylene bottles with no headspace, sealed in zip-lock bags, and stored in the dark at 4°C until testing (*ca.* <72 h). The remaining composite sample was delivered into pre-conditioned sampling bottles for chemical analysis. The sample for analysis of filterable metals was filtered using a 0.45 µm Sartorius filter.

A composite sample from the inlet and outlet of the wetland for each storm event was analysed by ALS Environmental, Sydney, a National Association of Testing Authorities (NATA) accredited laboratory within 24 h of collection. Characterisation of the inflow and outflow stormwater for each monitored rain event included the determination of: total suspended solids (TSS); nutrients (total nitrogen (TN), total kjeldahl nitrogen (TKN), nitrite + nitrate as N (NO_x-N), ammonia as N (NH_3-N), total phosphorus (TP), reactive phosphorus (RP); filterable metals (aluminium (Al), cadmium (Cd), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), zinc (Zn); biological oxygen demand (BOD) and chemical oxygen demand (COD). Method references and detection limits are provided in Table 1. ALS detection limits are determined from the analysis of a specified number of real samples, blank samples, low value and higher value standards and spiked samples which are processed through the whole test method. The collected data is treated statistically using an ANOVA protocol (ALS Environmental, 2014). Laboratory quality control testing consisted of laboratory duplicates, method blanks, laboratory control spikes and matrix spikes. Relative percentage differences (RPDs) for laboratory duplicates were within the permitted range, method blanks were below detection limits, and acceptable recovery (%) of laboratory control spikes and

matrix spikes were observed (detailed QC results are given in Appendix 2; Table A2.3).

Toxicity tests

Toxicty tests were conducted using three Australian native freshwater test species that represent a range of trophic levels. The test species were a green microalga (*Pseudokirchneriella subcapitata),* a cladoceran (*Ceriodaphnia dubia)* and embryos of the crimson-spotted Rainbowfish (*Melanotaenia duboulayi).* Physicochemical measurements (temperature, pH, dissolved oxygen, and conductivity) of test samples were monitored at the beginning and end of the toxicity test using a YSI ProPlus multiparameter meter. Ranges of these values are provided in Table 2.

Table 1. Analytical methods and method detection limits.

Parameter	**Method (Reference)**	**Detection Limit (mg L^{-1})**
Filterable Metals (Al, Cd, Cu, Fe, Mn, Pb, Zn)	USEPA 6020; APHA 3125 (USEPA, 1992; APHA, 2005)	Al (0.01), Cd (0.0001), Zn (0.005), Fe (0.05), Others 0.001
Nutrients (TN, TKN, NH_3-N, NO_X-N, TP, RP)	APHA 4500 (APHA, 1999)	TKN and TN (0.1), Others 0.01
TSS	APHA 2540 D (APHA, 2005)	5
BOD	APHA 5210 B (APHA, 2005)	2
COD	APHA 5220 C (APHA, 2005)	5

Table 2. Physicochemical variables of test solutions from toxicity testing using *P. subcapitata, C. dubia* and *M. duboulayi*

Test Sample	Test Concentrations (%)	pH	Temperature (°C)	Dissolved Oxygen (%)	Conductivity (uS cm^{-1})
P. subcapitata					
C	0	7.40-7.89	24-25	nd	nd
UT	6.25, 12.5, 25, 50, 75, 100	7.39-8.90	24-25	nd	nd
T	6.25, 12.5, 25, 50, 75, 100	7.48-8.72	24-25	nd	nd
C. dubia					
C	0	8.22-8.38	22 - 23	98-101	194-198
UT	6.25, 12.5, 25, 50, 75, 100	7.24-8.32	22-23	86.2-101	77-181
T	6.25, 12.5, 25, 50, 75, 100	7.55-8.41	22-23	96-100	185-210
M. duboulayi					
C	0	7.50-7.60	25.1-26.1	81-83	235-365
UT	100	7.30-7.40	25.2-26.0	78-89	67-100
T	100	7.40-7.60	25.4-26.1	83 - 90	157-202

nd = not determined; C = Control; UT = Untreated; T = Treated

Chronic microalgal toxicity test

This bioassay measured the inhibition in cell division rate (i.e. growth rate) of the freshwater unicellular green alga *Pseudokirchneriella subcapitata*, (previously known as *Selanastrum capricornutum)*. Stock cultures in log-phase growth were maintained at CSIRO Centre for Environmental Contaminants Research (CECR, CSIRO Land and Water, Lucas Heights, Sydney) in 250 mL glass Erlenmeyer flasks containing 100 mL of USEPA culture medium (USEPA, 1994). A 72 h static, chronic toxicity test was conducted with *P. subcapitata* based on OECD Test Guideline 201 (1984) and the USEPA protocol (USEPA, 1994). For the test inoculum, exponentially growing cells from a 5-6 day old culture were centrifuged (2500 rpm) and rinsed three times and then resuspended in control medium. Test samples were filtered through a 0.45 µm cellulose acetate membrane (Sartorius, Germany) and supplemented with USEPA media nutrients, but without ethylenediaminetetraacetic acid (EDTA) to prevent alteration to the bioavailability of any metal contaminants. Where necessary, the pH of the field sample as received was adjusted with NaOH or HCL to ensure that it fell within the optimal pH test range (≥ 7 but ≤ 9).

A negative control and six treatment concentrations reflecting 6.25, 12.5, 25, 50, 75 and 100% of the test samples were prepared in triplicate. A reference toxicant, Cu (as $CuSO_4$), was run in parallel to each sample toxicity test to ensure that the batch of algae was responding to a know toxicant in a reproducible manner. Diluent and control media for the algal bioassays was the same as the culture medium, but without EDTA. Tests were conducted in 20 mL glass vials each containing 6 mL of test solution and inoculated with 1-2 x 10^4 cells mL^{-1} of the pre-washed algal suspension. Loosely capped vials were incubated in an environmental cabinet at 24 ± 1°C under continuous cool white lighting (65 ± 5 µmol photons s^{-1} m^{-2}) and randomized on a shaker platform (100 rpm). Re-randomization of vials occurred twice daily by hand. Sub-samples from each test vial were obtained at 24, 48 and 72 h for the measurement of algal cell density, which was determined using a FACSCalibur flow cytometer (BD Biosciences) using the methods described by Franklin et al. (2000). Linear regression analysis was used to fit a plot of log_{10} cell density versus time (h) for each sample and the

cell division (growth) rate per h (μ) was determined from the slope. Cell division rates per day (3.32 x μ x 24) were calculated for each replicate (CECR, CSIRO Land and Water, Lucas Heights, Sydney). Bioassays were considered acceptable if there was at least a 16-fold increase in the control biomass after 72 h and variability among the control replicates (coefficient of variation (CV)) did not exceed 20%, based on the OECD Test Guideline 201 (1984). In addition, the 72 h IC_{50} of the Cu reference toxicant had to fall within 14.9 ± 5.6 μg L^{-1} (CECR, CSIRO Land and Water, Lucas Heights, Sydney).

Acute cladoceran toxicity test

This test measures survival of *C. dubia* following exposure to the toxicant. *C. dubia* plays an important ecological role in freshwater environments by grazing on algae and bacteria and providing an important food source to fish (Thomas et al., 2008; Sornom et al., 2012; Pakrashi et al., 2013). Mass cultures of *C. dubia* were maintained at CECR using methods described by Binet *et al*. (2010). Culture water consisted of diluted mineral water (DMW; USEPA 1993), which was prepared by diluting Perrier® water in Milli-Q® (20% by volume) and supplementing with Se (final concentration 2 μg L^{-1}) and vitamin B12 (0.2 mL of a 100 mg L^{-1} stock). The prepared DMW was vigorously aerated for 24 h using an aquarium aerator prior to use. For the toxicity test, neonates (<24 h old) were isolated from mass cultures and a 48 h static acute toxicity test conducted based on the USEPA (1993) protocol. A negative control and six concentrations reflecting 6.25, 12.5, 25, 50, 75 and 100% of untreated or treated stormwater were prepared in quadruplicate. A reference toxicant, Cu (as $CuSO_4$), was run in parallel to each sample toxicity test as a positive control. DMW was used as the control media and diluent.

Tests were conducted in 20 mL of test solution in 30 mL glass vials, each containing five randomly assigned neonates that had been allowed to feed for a minimum of 2h prior to test commencement on fish food supplement and algal concentrate. Vials were loosely capped and incubated at a temperature of 25 ± 1°C and under a 16 h light: 8 h dark cycle. The test endpoint was immobilization, which was recorded at 24 and 48 h. Toxicity test results were considered acceptable if at least 90% survival was observed for the controls, based on the

USEPA (1993) protocol. In addition, the 48 h EC_{50} of the Cu reference toxicant had to fall within 7.5 ± 3.5 µg L^{-1} (CECR, CSIRO Land and Water, Lucas Heights, Sydney).

Rainbowfish embryo exposure test

This test measured hatching success of *M. duboulayi* embryos. *M. duboulayi* plays an important role in Australian freshwater food webs by regulating algal biomass and nuisance insect larvae, and serving as a food source for larger fish (Thomas et al., 2008). Five-year-old *M. duboulayi,* bred from specimens captured at Wilsons River near Lismore NSW in 1989, were used as brood stock. Fourteen adult fish (10 ♀ and 4 ♂) were housed in a 110 L glass tank furnished with gravel. Water used for fish culturing and toxicity tests was aged Sydney tap water that had been passed through the following: a mixed bed filter, activated carbon filter, and UV steriliser. Water temperature was maintained at 24 ± 1°C and photoperiod provided on a 10:14 h light-dark regime, recommended by Tappin (2010). Fish were fed twice daily with Tetramin® flake food.

One day prior to spawning, fish were conditioned by supplementing their diet with 150 mL of thawed Hikari® bloodworms and increasing the water temperature in the brood tank to 26 ± 1°C. To ensure that egg deposition took place during the spawning period six sterilised mops were provided as egg collectors the evening before spawning. Mops were constructed of bundles of green acrylic 8 ply thread fixed to polystyrene floats and distributed evenly at each end of the tank to reduce male competition for spawning sites. After 24 h the mops were removed and dipped for 30 s in Aqua Master® methylene blue solution (0.25 ml L^{-1}) to minimize fungal infection and transferred to glass incubating chambers (20 x 20 x 38 cm) containing brood tank water and vigorously aerated.

Treatment concentrations reflecting 100% untreated and treated stormwater and a negative control (fish culturing water) were prepared in triplicate. Individual eggs were harvested from the mops and any opaque (unfertilized) eggs discarded. Eggs were pooled and then ten eggs randomly allocated to 100 mL glass beakers containing 80 mL of test solution. Test vessels were then covered in plastic film,

incubated at 26 ± 1°C, and stored away from direct light to protect the developing embryos (Tappin, 2010). Each beaker was swirled daily to prevent the formation of oxygen deficient areas around the eggs. The test end point was hatching success, which was observed until all embryos could be recorded as either successfully emerged (*ca.* day 6) or dead. Mortality was defined as the absence of a heartbeat when examined under a dissection microscope at 40x magnification. Test results were considered valid if ≥ 70% hatching success was observed overall for the controls (Environment Canada, 1998).

Adult rainbowfish were housed under license and the protocol used in this study was approved by the Macquarie University Animal Ethics Committee (ARA 2011/024).

Glassware and equipment

Glassware and equipment used for sample collection, preparation of solutions and testing was cleaned prior to use by rinsing with demineralized water (3x), soaking in 10% (v/v) reagent grade HNO_3 (Merck Pty, Australia) for 24 h, followed by (5 x) rinses with demineralized water and (5 x) rinses with Mili-Q water (Millipore®). Prior to acid washing, polyethylene bottles used in the autosampler were also soaked in Merk phosphate free detergent for 24 h, followed by (3 x) rinses with demineralized water. The bottles were dried and UV sterilized in a laminar flow cabinet prior to storage in sealed plastic bags until use. For the algal bioassay all glassware was coated with a salinizing solution (Coatasil, Ajax, Australia) to reduce the adsorption of metals to vessel walls.

Data analysis

The analytical results for a measured pollutant from a single flow-weighted composite sample are equivalent to an event mean concentration (EMC) value (Li & Davis, 2009). To determine the performance of the stormwater treatment system, inflow and outflow EMC values for each pollutant were used to calculate percentage removal efficiencies (RE) on a storm-by-storm basis using Eq. (1).

$$RE = (1 - EMC\ outflow/EMC\ inflow) \times 100 \quad (1)$$

Pollutant removal efficiency values have been widely used to measure the performance of stormwater treatment devices (Carleton et al., 2000; Birch et al., 2004; Birch et al., 2005; Hunt et al., 2008). When the concentration of an analyte was below the detection limit, half of the limit was used in calculations (Dietz & Clausen, 2005, 2006; Line & Hunt, 2009). If the EMC for the inflow and outflow were both below the detection limit a RE value was not calculated. As inflow and outflow data were from the same storm event, a paired t-test in MINITAB 16 (Minitab Inc, USA) was used to compare untreated and treated pollutant concentrations (Line & Hunt, 2009). EMC data for individual pollutants that were not normally distributed were log transformed as is recommended for such data (Strecker et al., 2001; Line & Hunt, 2009). Mean inlet and outlet EMC values were compared to Australian freshwater guidelines and expressed as an enrichment factor (mean concentration of a parameter/freshwater guideline concentration; Birch et al., 2004) to determine if the wetland system could provide acceptable water quality improvements for freshwater biota. It is important to look at effluent concentrations when assessing treatment performance as it has been noted that removal efficiencies can be biased by influent (Streker et al., 2001; Hathaway et al., 2009) and background concentrations (Hatt et al., 2009). Pearson's correlation was used to determine associations between TSS removal and TN and TP.

Toxicity data were checked for normality (assessed by Shapiro-Wilk's test) and homogeneity of variance (assessed by Bartlett's test). For the algae test, the inhibitory concentration (IC) of untreated and treated stormwater to have a 10% (IC_{10}) and 50% (IC_{50}) inhibition on algal growth was calculated using linear interpolation in ToxCalc™ (Version 5.0.23, Tidepool Scientific Software, USA). Dunnett's multiple comparison test was used to determine which treatment concentrations differed significantly from the controls ($p \leq 0.05$) in order to determine the lowest observable effect concentration (LOEC; lowest concentration of stormwater to cause a statistically significant effect on the measured parameter compared with controls). For the cladoceran test, the effective concentration (EC) of untreated and treated stormwater to have a 10% (EC_{10}) and 50% (EC_{50}) effect

on cladoceran survival was calculated using maximum likelihood probit in ToxCalc^{TM}. Steel's many one rank test was used to determine which treatments differed significantly from the controls ($p \leq 0.05$) in order to estimate the lowest observable effect concentration (LOEC). An example of how EC_{10} and EC_{50} were calculated in ToxCalc^{TM} for *C. dubia* is provided in Appendix 2 for event W3 (Table A2.6). The number of fish embryos that successfully hatched was calculated as a percentage, arcsine transformed, and analysed using a one-way ANOVA followed by Tukey HSD post-hoc analysis in MINITAB 16 (Minitab Inc, USA).

Results and discussion

Pollutant Removal

Chemical analysis of water samples from the wetland system was based on collection of inflowing (untreated) and outflowing (treated) stormwater from three storm events (W1 – W3). Insufficient rainfall amounts during the 6-month study period prevented a higher number of storm events from being collected. Flow-weighted EMCs were measured for all three events and used to estimate the percentage removal efficiency of pollutants by the wetland system (Table 3). With the exception of Fe, Mn, BOD and NH_3-N, mean outflow concentrations of measured analytes were lower than inflow concentrations. In terms of positive removal efficiency overall pollutant reduction followed the order of: NO_x-N > Pb, Cd > TN, TP > Zn> TSS > TKN > RP > Cu > Al> COD. Despite the majority of pollutant concentrations being substantially reduced at the outflow, mean outflow concentrations for filterable Cu and Zn, TN and TP remained above Australian freshwater guidelines (Table 4).

Total Suspended Solids

A reduction in TSS concentrations by the wetland was observed across all three storm events, with a mean removal efficiency of 60% (range: 8% to 95%). This compares well to results published by Carleton et al. (2000) who observed a median EMC removal efficiency of 58% for a stormwater wetland constructed in a residential area in the USA and sized at 3% of the treatment catchment area. The processes responsible for TSS removal in the wetland include: sedimentation, filtration, adsorption, and flocculation (DLWC, 1998). Sedimentation in particular will be accommodated by the extended detention capacity of Yarrowee wetland. This is important as TSS reduction is frequently a primary objective in stormwater management, with the expectation that a substantial amount of organic and particulate-bound inorganic contaminants will consequently be removed (Wong, 2006). An 80% reduction in TSS is currently recommended as a best practice target for stormwater management (Queensland Government, 2009; SMCMA, 2011). Using this target and MUSIC (Model for Urban Stormwater Improvement

Conceptualization) site predictions (78% removal) as a measure of efficacy, the wetland was successful 1 out of 3 times at achieving TSS reduction targets and 2 out of 3 times at achieving site predictions. It must be noted, however, that the mean recorded TSS outflow concentration of 8.6 mg L^{-1} (range: 5 mg L^{-1} to 12 mg L^{-1}) is similar to the TSS background concentration of 6 mg L^{-1} typically adopted for wetlands (Thompson et al., 2011). Stormwater treatment systems are thought to be efficient at reducing pollutant concentrations to an equilibrium or background level (Strecker et al., 2001; Hatt et al., 2009). Further to this, all outflow concentrations of TSS (5-12 mg L^{-1}) did not exceed recommended Australian guidelines of <25 mg L^{-1} (Wong, 2006).

Despite the observed reduction in TSS concentrations, overall differences between the inlet and outlet were not significant ($t = 1.80$, $p = 0.214$) due to the variability in removal rates. Birch et al. (2004) observed variable TSS removal efficiencies (range: -98% to 46%) in a stormwater wetland (0.1% of catchment area) constructed in a Sydney residential area. Birch et al. (2004) suggested that re-suspension of sediments during high flow events may have been the cause. In the current study this was not the case and the observed variability can be attributed to the low removal efficiency of 8% obtained for event W2. This is likely to have resulted from the low inflow concentration of 13 mg L^{-1} that is close to the typical wetland background concentration of 6 mg L^{-1} (Thompson et al., 2011). Strecker et al. (2001) suggested that if extremely low influent concentrations are entering a stormwater treatment device, the use of removal efficiency as a measure of performance may not provide a true representation of whether a treatment device is well designed. This is because the treatment system can appear to not be 'efficient' for these events, yet quality of the outflowing water sample is not considerably degraded. The mean TSS inflow concentration (73.3 mg L^{-1}) is lower than the average residential runoff TSS value of 160 mg L^{-1} reported by Wong (2006), but similar to the mean TSS concentration observed in the inflow of a constructed wetland by Birch et al. (2004) (87.5 mg L^{-1}) and higher than that reported by Carleton et al. (2000) (37 mg L^{-1}).

Nitrogen Species

The nitrogen content of stormwater was reduced by the wetland in all three storm events. The removal efficiency of TN was fairly consistent (mean: 62%, range: 57% to 67%) and above best practice guidelines (45% reduction, Queensland Government 2009; 40% reduction, SMCMA 2011) and MUSIC site predictions (37% reduction). A significant positive correlation was observed between TN and TSS removal (p = 0.009), suggesting that TSS was an important carrier of particulate-bound nitrogen in the stormwater runoff. The mean removal efficiencies of TKN and NOx-N were also generally consistent at 59% (range: 55% to 64%) and 86% (range: 79% to 92%), respectively; demonstrating that organic nitrogen entering the wetland is undergoing nitrification and inorganic nitrogen is undergoing denitrification within the wetland treatment system. The highest removal efficiency was noted for NO_x-N and the consistency of treatment is apparent, with a narrow range of low outflow concentrations relative to the inflow, suggesting that nitrogen assimilation by wetland biota in the system is also highly efficient. Studies of other constructed stormwater wetlands that are smaller relative to the catchment area, have reported lower mean removal efficiencies for all three nitrogen species: TN (16% - 21.9%); TKN (-3.1% – 9%); and NO_x-N (22 - 61.7%) (Carleton et al., 2000; Birch et al., 2004). Inflowing concentrations of NH_3-N were below method detection limits. Export of NH_3 from the system was however observed, resulting in a mean negative removal efficiency of -1100% (range: -500% to -1700%). Greenway (2010) also observed an increase in ammonia in a retrofitted wetland system. A small amount of ammonia production is to be expected due to the ammonification of organic matter as part of the nitrogen cycle. Increases in the outlet concentration of NH_3-N, however, were not significant (t = -1.85, p = 0.205) and were below ANZECC/ARMCANZ (2000a) freshwater guidelines for all events.

Overall reductions in outlet concentrations were significant for TN (t = 13.38, P = 0.006), TKN (t =14.01, P = 0.005) and NO_x-N (t = 7.57, P = 0.017). Inflow concentrations of TN (1.1 – 3.5 mg L^{-1}) and NO_x-N (0.12 – 0.38 mg L^{-1}) exceeded the (ANZECC/ARMCANZ, 2000a) trigger values (TN = 0.5 mg L-1, NO_x-N = 0.04

mg L^{-1}) across all monitored storm events. Mean inflow concentrations of TN and NO_x-N were 4.1 and 6.1 times higher, respectively, than trigger values. The mean TN inflow concentration of 2.0 mg L^{-1} is close to the average residential runoff value of 2.6 mg L^{-1} reported by Wong (2006), but lower than the mean TN concentration observed in the inflow of a constructed wetland in Sydney by Birch et al. (2004) (4.38 mg L^{-1}). Following wetland treatment the mean NO_x-N concentrations at the outflow (0.04 mg L^{-1}) met the recommended trigger value, but the mean TN concentration (0.8 mg L^{-1}) still exceeded the trigger value. Birch et al. (2004) also observed TN outflow concentrations in a constructed stormwater wetland to remain above Australian freshwater guidelines. In the current study, it is interesting to note the mean TN outflow concentration is lower than the 1 mg L^{-1} background concentration typically adopted for wetland systems (DLWC, 1998; Thompson et al., 2011). Further reductions may therefore be unrealistic as the mean outlet concentration is below the background level at which wetlands are thought to be unable to reduce TN. Hathaway & Hunt (2010) observed that while inorganic nitrogen species were reduced to near zero concentrations in stormwater passing through a wetland system, organic nitrogen at the outflow persisted at an average concentration of 0.64 mg L^{-1}. The authors concluded that the presence of organic nitrogen in the wetland outflow was likely due to the plant organic matter in the wetland system providing a background source of nitrogen, supporting the concept of irreducible nitrogen concentrations for stormwater wetlands (Hathaway & Hunt, 2010). Similarly, median organic:total nitrogen ratios from stormwater wetlands located in North Carolina, USA, were found to be significantly higher at the outflow than at the inflow (Moore et al., 2011).

Phosphorus Species

A positive reduction in the phosphorus content of stormwater by the wetland was observed across all storm events. The mean removal efficiencies of TP and RP were 62% (range: 18% to 99%) and 50% (range: 43% to 78%), respectively. Studies using automatic sampling at other constructed stormwater wetlands that are smaller relative to the catchment area, have reported lower mean removal efficiencies for TP (12% - 33.3%) (Carleton et al., 2000; Birch et al., 2004) and orthophosphate (35.4%) (Carleton et al., 2000). A 60% reduction target for TP is

currently recommended for stormwater management (Queensland Government 2009; SMCMA 2011). Using this target and MUSIC site predictions (52% reduction) as a measure of efficacy, the wetland only failed to meet these targets for event W1. However, the outflow TP concentration of 0.5 mg L^{-1} for event W1 was above the 0.06 mg L^{-1} background concentration typically adopted for wetland systems (Thompson et al., 2011). Further reductions should therefore have been possible. A significant correlation between TP and TSS removal was not observed (p = 0.743), suggesting that phosphorus in the stormwater runoff was predominantly dissolved and not in particulate form. Absorption and plant uptake is considered to account for the phosphorus removal during establishment of a constructed wetland (Bavor & Adcock, 1994). More TP removal is likely to be observed over time as the formation of organic matter increases the adsorption capacity of the wetland as it matures (DLWC, 1998). Interestingly, event W3 occurred 6 months following event W1, for which a 99% removal efficiency for TP was observed.

The variability in phosphorus removal efficiency prevented the overall reduction in TP and RP from being statistically significant (TP: t = 2.79, p = 0.108, RP: t = 3.11, p = 0.089). Birch et al., (2004) also observed a large variability in the removal efficiency of TP (-14% to 39%). Mean concentrations of TP (0.41 mg L^{-1}) and RP (0.06 mg L^{-1}) in the inflowing stormwater exceeded the ANZECC/ARMCANZ (2000a) trigger values (TP = 0.05 mg L^{-1}, RP = 0.02 mg L^{-1}) by 8.2 and 3 times, respectively. The mean TP inflow concentration is the same as the average residential runoff value of 0.4 mg L^{-1} reported by Wong (2006), but higher than the mean TP concentration of 0.14 mg L^{-1} observed in the inflow of two other constructed stormwater wetlands (Carleton et al., 2000; Birch et al., 2004). Following wetland treatment the mean RP concentration at the outflow (0.02 mg L^{-1}) met the recommended trigger value, suggesting that assimilation of dissolved inorganic phosphorus by wetland biota was taking place effectively. The mean TP concentration still exceeded trigger values at the outflow by 3.9 times and therefore represents a potential risk to freshwater ecosystems. Birch et al. (2004) also observed TP outflow concentrations to remain above Australian freshwater guidelines.

In the current study, the mean outflow concentration for TP (0.197 mg L^{-1}) is above the typical wetland background concentration of 0.06 mg L^{-1}. Further reductions in TP should therefore still be possible as the mean outlet concentration is above the background level at which wetlands are unable to reduce TP. This is an important consideration as phosphorus tends to be the limiting nutrient in freshwater systems for primary production (Hecky & Kilham, 1988). Excess input can result in excessive plant growth leading to hypoxia upon plant dieback (eutrophication) (Howarth & Marino, 2006). Where inflow concentrations of phosphorus exceed wetland background levels, a longitudinal gradient of phosphorus storage in vegetation and soils typically develops alongside a gradient of decreasing water column phosphorus concentrations as water moves through the system (Walker et al., 2011). Phosphorus cycling within the wetland system can occur through plant death/decomposition and exchanges from the sediments under anaerobic (reducing) conditions (Kadlec, 1995), which may have accounted for some of the observed variability in phosphorus removal. Phosphorus cycling can be reduced through effective maintenance of the wetland system, for example, by sustaining sufficient plant cover of desirable vegetation (DLWC, 1998).

Filterable Metals

Studies of metal removal in constructed stormwater wetlands have focused largely on total metals (Walker & Hurl, 2002; Birch et al., 2004). Carleton et al. (2000) reported that metal reduction in constructed wetlands is greater for total forms than soluble forms. Filterable metal fractions are, however, more readily bioavailable (Hare, 1992). Results from this study show that outflowing concentrations of filterable metal contaminants were lower than inflowing concentrations, with the exception of Mn and Fe for which a mean negative removal efficiency of -413% (range: -1250% to 75%) and -197% (range: -17% to -460%), respectively, was observed. Birch et al. (2004) also observed elevated levels of Mn and Fe (in total form) at the outflow point of another constructed stormwater wetland in Sydney, with mean removal efficiencies of -84% and -294%, respectively. In the current study, outlet EMC values for Mn and Fe showed up to a 13-fold and 6-fold increase, respectively, suggesting that dissolution of oxide-bound Mn and Fe in sediments is taking place within the wetland and therefore demonstrates a highly

mobile behaviour. Manganese, however, is one of the least toxic metals (Wong, 2006) and outflow concentrations were below ANZECC/ARMCANZ (2000a) guidelines in all events. No ANZECC/ARMCANZ (2000a) water quality guidelines are available for Fe. Nickel was below detection limits (<0.001 mg L^{-1}) for all samples and results are therefore not presented. Mean removal efficiencies of filterable Al, Cd, Cu, Pb, and Zn by the wetland system were 36%, 75%, 40%, 75% and 61%, respectively, demonstrating a moderate to high removal for these filterable metals from stormwater. Removal efficiencies of filterable Cu and Zn in this study are higher than those reported by Carleton et al. (2000) for a slightly smaller sized wetland. Carleton et al. (2000) observed a removal efficiency of -22.2% and 11.1% for filterable Cu and Zn, respectively. Mean concentrations of Al, Cu, and Zn in the inflowing stormwater exceeded the ANZECC/ARMCANZ (2000a) freshwater guidelines by 1.2, 3.6 and 4.9 times, respectively. Following wetland treatment the mean filterable Al concentration at the outflow met the recommended guideline value, but slight enrichment of filterable Cu and Zn concentrations remained.

Despite the observed reductions in the concentration of filterable metals between the inflow and outflow, the variability in concentrations between storm events was large and consequently there was no significant difference in metal concentrations between the inlet and outlet. A larger sample size is needed to verify if significant changes in mean pollutant metal concentrations are achievable. For example, Strecker (2001) estimated that 442, 29 and 6 Cu samples were needed from a monitoring station in Portland, Oregon, to detect a 5%, 20% and 50% reduction in site mean concentration, respectively, with a 5% level of significance. In Australia, obtaining a large number of samples to be able to detect small differences is likely to be unrealistic due to the prolonged dry periods with no runoff. In addition, pollutant loading on stormwater wetlands is highly stochastic (Wong & Geiger, 1997), which is also evident in this study. Statistical assessment of reported EMC values may therefore not be appropriate if a reasonable sample size cannot be obtained.

Biological and Chemical Oxygen Demand

The mean removal efficiencies of BOD and COD were -31% (range: -200% to 83%) and 26% (range: -11% to 52%), respectively, demonstrating large variability in the reduction of these analytes in stormwater, with the overall differences between the inlet and outlet not being significant. Carleton et al. (2000) reported a negative mean removal efficiency of -21.0% for COD in a slightly smaller sized constructed stormwater wetland. The results from the current study suggest that Yarrowee wetland is not always efficient at reducing the oxygen demand required for the degradation and chemical oxidation of organic material. However, organic matter will accumulate in wetlands naturally and increasing BOD concentrations through the seasonal turnover of macrophytes (Scholz & Xu, 2002; Lee & Scholz, 2007). In the current study, a negative BOD removal efficiency was only noted for event W3. Prior to this event, the wetland outlet pond had minimal water cover due to an extended dry period. This may have resulted in the mortality of some wetland plants and their subsequent decay to an increase in organic loading. However, it must be noted that the mean inflow concentrations for BOD (5.3 mg L^{-1}) and COD (35.3 mg L^{-1}) are substantially lower than the average residential runoff values of 15 mg L^{-1} and 78 mg L^{-1} reported for BOD and COD, respectively, by Wong (2006). There are few Australian freshwater guidelines for BOD and COD, but the guideline limit for freshwater aquaculture is <15 mg L^{-1} and <40 mg L^{-1}, respectively (ANZECC/ARMCANZ, 2000c). All the outflow samples fall below the guideline limits for both BOD and COD, implying that the levels are of limited risk to aquatic biota.

Table 3. Event mean concentrations (EMC) of filterable metals (Al, Cd, Cu, Fe, Mn, Pb and Zn), nutrients (TN, TKN, NH_3, TP, RP), total suspended solids (TSS), chemical oxygen demand (COD) and biochemical oxygen demand (BOD) in stormwater runoff from the inflow and outflow of Yarrowee Wetland and the removal efficiency (RE, %) of pollutants by the system for each monitored storm event (W1 – W3).

Event	EMC (mg L^{-1})	Max Flow L s^{-1}	Al	Cd	Cu	Fe	Mn	Pb	Zn	TN	TKN	NO_X-N	NH_3-N	TP	RP	TSS	COD	BOD
W1	Inflow	35.6	0.05	bld	0.004	0.06	0.008	0.002	0.05	1.5	1.1	0.38	bld	0.61	0.07	23	27	6
	Outflow	3.1	0.04	bld	0.002	0.07	0.002	bld	0.01	0.5	0.4	0.08	0.03	0.5	0.04	5	13	bld
	RE (%)	nd	20	nd	50	-17	75	75	80	67	64	79	-500	18	43	78	52	83
W2	Inflow	12.1	0.08	0	0.003	bld	0.002	bld	0.046	1.1	1	0.12	bld	0.25	0.09	13	27	4
	Outflow	6.9	0.05	bld	0.003	0.14	0.027	bld	0.023	0.4	0.4	0.01	0.09	0.08	0.02	12	30	3
	RE (%)	nd	38	75	0	-460	-1250	nd	50	64	60	92	-1700	68	78	8	-11	25
W3	Inflow	35.7	0.06	bld	0.01	0.08	0.011	0.002	0.021	3.5	3.3	0.23	bld	0.37	0.02	185	52	4
	Outflow	3.0	0.03	bld	0.003	0.17	0.018	bld	0.01	1.5	1.5	0.03	bld	bld	0.01	9	33	12
	RE (%)	nd	50	nd	70	-113	-64	75	52	57	55	87	nd	99	50	95	37	-200
W1-W3	Mean RE (%)	nd	36	75	40	-197	-413	75	61	62	59	86	-1100	62	57	60	26	-31
	SD of RE (%)	nd	15	-	36	233	670	0	17	5	5	6	849	12	18	46	33	150

bld = below level of detection; SD = standard deviation; nd = not determined

Table 4. Descriptive statistics and enrichment above Australian freshwater guidelines for filterable metals, nutrients, TSS, BOD a COD in inlet and outlet samples from the constructed stormwater wetland.

Site		Al	Cd	Cu	Fe	Mn	Pb	Zn	TN	TKN	NO_X-N	NH_3-N	TP	RP	TSS	BOD	COD
Inlet	Min (mg L^{-1})	0.05	<0.0001	0.003	0.05	0.002	<0.001	0.02	1.1	1	0.12	0.01	0.25	0.02	13	4	27
	Max (mg L^{-1})	0.08	0.002	0.01	0.08	0.011	0.002	0.05	3.5	3.3	0.38	0.01	0.61	0.09	185	6	52
	Mean (mg L^{-1})	**0.06**	**0.0001**	**0.005**	**0.06**	**0.007**	**0.0016**	**0.039**	**2.033**	**1.8**	**0.243**	**0.01**	**0.41**	**0.06**	**73.3**	**4.6**	**35.3**
	SD (mg L^{-1})	0.01	0.0001	0.003	0.015	0.005	0.00057	0.0157	1.286	1.3	0.131	0	0.183	0.036	96.8	1.2	14.4
	n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Guidelines		0.05**	0.0002**	0.0014**	nd	1.9**	0.0034**	0.008**	0.5*	nd	0.04*	0.9**	0.05*	0.02*	<25***	15#	40#
Enrichment Factor (Mean/Guideline)		***1.2***	***0***	***3.6***	***-***	***0***	***0***	***4.9***	***4.1***	***-***	***6.1***	***0***	***8.2***	***3.0***	***2.9***	***0***	***0***
Outlet	Min (mg L^{-1})	0.03	<0.0001	0.002	0.07	0.002	<0.001	0.01	0.4	0.4	0.01	0.01	<0.01	0.01	5	1	13
	Max (mg L^{-1})	0.05	<0.0001	0.003	0.126	0.027	<0.001	0.02	1.5	1.5	0.08	0.09	0.5	0.04	12	12	33
	Mean (mg L^{-1})	**0.04**	**<0.0001**	**0.002**	**0.12**	**0.016**	**<0.001**	**0.014**	**0.8**	**0.767**	**0.04**	**0.043**	**0.197**	**0.02**	**8.7**	**5.3**	**25.3**
	SD (mg L^{-1})	0.01	0	0.0005	0.051	0.012	0	0.0075	0.608	0.635	0.036	0.024	0.265	0.02	3.5	5.9	10.8
	n	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Guidelines		0.05	0.0002	0.0014	nd	1.9	0.0034	0.008	0.5	nd	0.04	0.9	0.05	0.02	<25	15	40
Enrichment Factor (Mean/Guideline)		***0***	***0***	***1.4***	***-***	***0***	***0***	***1.8***	***1.6***	***-***	***0***	***0***	***3.9***	***0***	***0***	***0***	***0***
Site with higher concentrations		Inlet	Inlet	Inlet	Outlet	Outlet	Inlet	Inlet	Inlet	Inlet	Inlet	Outlet	Inlet	Inlet	Inlet	Outlet	Inlet
Significant difference between the inlet and outlet (p = < 0.05)		No p = 0.077	No p = 0.423	No p =0.423	No p = 0.152	No p = 0.478	No p = 0.184	No p = 0.099	Yes p = 0.006	Yes p = 0.005	Yes p = 0.017	No p = 0.205	No p = 0.108	No p = 0.089	No p = 0.214	No p = 0.878	No p = 0.272

nd = no data; SD = standard deviation; *n* = number of samples

* ANZECC/ARMCANZ (2000a) trigger values for aquatic ecosystems in lowland rivers in south east Australia. Note FRP guidelines are used for RP and NH_4^+ for NH_3

** ANZECC/ARMCANZ (2000a) trigger values for protection of 95% of freshwater biota

ANZECC/ARMCANZ (2000c) recommended guidelines for aquaculture operations

***Wong (2006) - urban freshwater guidelines

Direct Toxicity Assessment

Toxicity bioassays using untreated and treated stormwater composite samples collected from events W2 and W3 were performed with *P. subcapitata, C. dubia* and *M. duboulayi*. Quality assurance criteria were met for each test and observed responses are summarized in Table 5 (raw data is provided in Appendix 2; Table A2.4 – A2.8). *M. duboulayi* was the most sensitive species tested, as significant toxicity was evident in both untreated W2 and W3 samples. No toxicity was observed from outflow samples, demonstrating the ability of the wetland to reduce the toxicity of the influent stormwater.

Table 5. Responses of *Ceriodaphnia dubia* (48-h immobilisation), *Psuedokirchneriella subcapitata* (72-h growth rate) and *Melanotaenia duboulayi* (6-day hatching success) to inlet and outlet samples collected from the constructed stormwater wetland for two storm events (W2 and W3).

Sample	***C. dubia* 48-h Immobilisation**		***P.subcapitata* 72-h Growth Rate**		***M.duboulayi* 6-day *Hatching Success***
	EC1O[a] (%)	EC50[b] (%)	IC10[a] (%)	IC50[b] (%)	(%)
Inflow W2	>100	>100	>100	>100	33*
Outflow W2	>100	>100	>100	>100	73
Inflow W3	33	89	>100	>100	27*
Outflow W3	>100	>100	>100	>100	67

[a] Concentration to cause 10% effect (E) or 5% inhibition (I) relative to the control

[b] Concentration to cause 50% effect (E) or inhibition (I) relative to the control

*Hatching success is significantly different from the control ($p < 0.05$)

Psuedokirchneriella subcapitata

Samples were not phytotoxic to *P. subcapitata* hence the IC_{10} and IC_{50} values are reported as being greater than the highest treatment (>100% stormwater; Table 5). Exposure to W3 inflow samples reduced the cell division rate of *P. subcapitata* by 8% in the undiluted stormwater. This reduction, however, was not significantly different from the controls (Figure 6). In contrast, a significant stimulation in algal growth relative to the control of 12-13% and 9-15% was exhibited in algae

exposed to W2 inflow and outflow samples, respectively (Figure 6). This effect was observable down to a dilution of 25% untreated and treated stormwater. Scholes et al. (2007) also observed stimulated growth of *P. subcapitata* exposed to untreated stormwater. The results from storm event W2 suggest that there are sufficient nutrients in the outflow of Yarrowee wetland to contribute to eutrophication in receiving waters. The stimulation of algal growth is consistent with the high concentration of RP (0.09 mg L^{-1}). RP is considered to be chemically indicative of orthophosphate, which is the only form of phosphorus assimilated by autotrophs as it is immediately available for algal growth (Correll, 1998). Cell growth of *P. subcapitata* responds more to the addition of orthophosphate than N (Maloney et al., 1972; Chiaudani & Vighi, 1974). Chiaudani & Vighi (1974) documented that in a phosphorus limited freshwater environment eutrophication started when the orthophosphate concentration exceeded 0.010– 0.012 mg L^{-1}. This is more than 7 times lower than the W2 inflow RP concentration and similar to the level observed at the wetland outflow. The potential risk of stormwater to stimulate primary production in receiving waters can therefore remain following stormwater treatment. The concentration of RP, however, cannot be the only factor responsible for the growth stimulation observed in the treated W2 sample as it is comparable to that recorded in W3 where no algal growth stimulation was observed.

The absence of significant phytotoxicity is consistent with the chemical analysis of the stormwater. With the exception of the W3 inflow sample, Cu concentrations were below the EC_{50} values measured in reference toxicant tests conducted during this study (*ca.* 0.01 mg L^{-1}) and those observed in other studies (0.008 – 0.038 mg L^{-1}) (Chen et al., 1997; Franklin et al., 2000; Binet et al., 2010). The W3 inflow sample had a Cu concentration of 0.01 mg L^{-1} that could explain the observed reduction in cell division rate. The reduction in growth, however, was not significant and considerably less than 50%. Chen (1994) demonstrated that EC_{50} values of *P. subcapitata* in response to a metal contaminant decreased as levels of orthophosphate were reduced from 0.17 to 9.2 x 10^{-4} mg L^{-1}. It is possible that the level of orthophosphate present in the W3 inflow sample (0.02 mg L^{-1}) could have increased the tolerance of *P. subcapitata* to Cu. Concentrations of Zn, Pb,

and Cd in samples were all below the EC_{50} values of 0.178, 2.655 and 0.341 mg L^{-1}, respectively, reported by Chen et al., (1997) for *P. subcapitata*. Limited published information concerning the other metals listed in Table 3 is available. These contaminants, however, were found to be present at low concentrations.

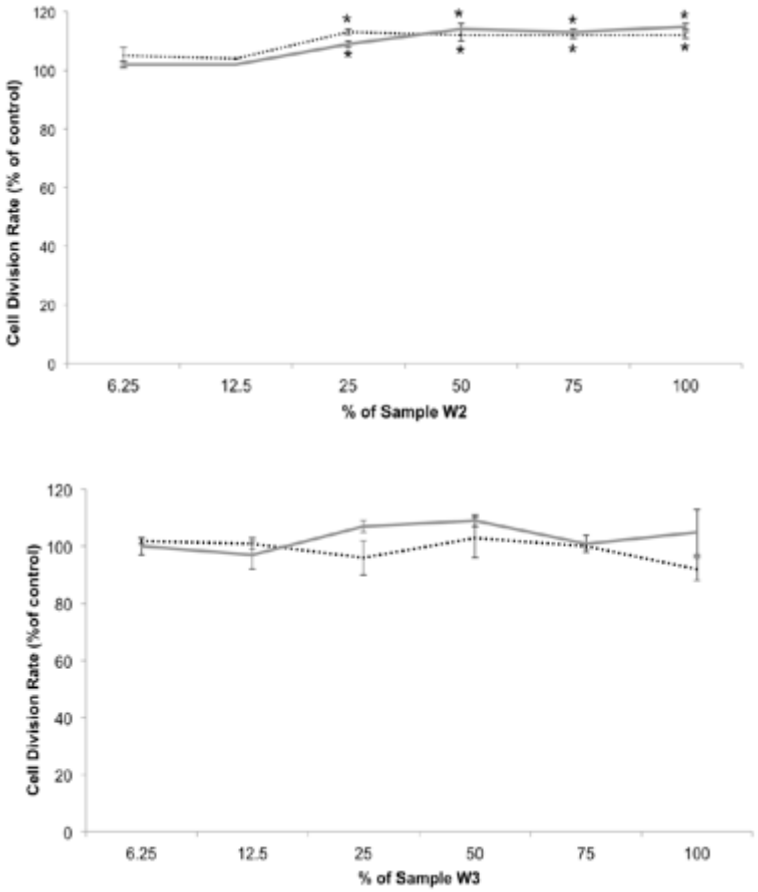


Figure 6. Percentage growth of *Pseudokirchneriella subcapitata* relative to the control when exposed to six untreated (solid line) and treated (dotted line) stormwater samples (W2 and W3) for 72 h. Data points represent the mean ± SE. *Significantly greater than growth in control ($p < 0.05$).

Ceriodaphnia dubia

Acute toxicity to *C.dubia* was only observed in one inflow sample (W3) with 10% and 50% immobilization noted at stormwater concentrations of 33% and 89%, respectively. This effect was not observable when the W3 inflow sample was diluted at 25%. The W3 outflow sample did not produce any toxicity to *C.dubia*, highlighting the ability of the wetland system to reduce the toxicity of the input stormwater. Hemming et al., (2001) also reported that survival of *C.dubia* was significantly reduced in the inflow of a wastewater treatment wetland, but not in

outflow water. The Cu concentration of the W3 inflow sample (0.01 mg L^{-1}) was above the copper EC_{50} values reported by Binet et al., (2010) (48-h EC_{50} 0.0067 – 0.0081 mg L^{-1}) and the EC_{50} values measured in reference toxicant tests conducted during the study (48 hr-EC_{50} *ca.* 0.005 mg L^{-1}). The observed toxicity to the W3 inflow sample could therefore be attributed to the Cu concentration of the sample. Cu was reduced by the wetland to concentrations below the EC_{50} for this species in the W3 outflow sample, which was consistent with the toxicity test results as *C.dubia* showed no response. In a study by Kumar et al. (2002), Cu was identified as being one of the likely metals responsible for the observed toxicity of *C.dubia* exposed to road runoff. Cu concentrations in other samples, where no toxicity was observed, were below reported 48-hr EC_{50} values. Concentrations of other metals listed in Table 3 were below the 48-hr EC_{50} values reported in the literature for Zn (0.1 – 0.36 mg L^{-1}) (Magliette et al., 1995; Kumar et al., 2002; Tsui et al., 2005), Pb (0.176 - 0.3 mg L^{-1} (Kumar et al., 2002; Tsui et al., 2005), Fe (36.69 mg L^{-1}) (Fort & Stover, 1995), Al (35.97 mg L^{-1}) (Fort & Stover, 1995), Mn (0.308 mg L^{-1}) (Hockett & Mount, 1996) and Cd (0.0545 mg L^{-1}) (Diamond et al., 1997).

Melanotaenia duboulayi

Significant differences in the hatching success of *M. duboulay*i embryos were noted for W2 and W3 samples (W2: $F_{2,6}$ = 8.60, P = 0.017; W3: $F_{2,6}$ = 7.17, p = 0.026). Tukey HSD post-hoc tests indicated that hatching success decreased significantly in relation to the control by more than 40% in untreated stormwater from event W2 (t = -3.834, p = 0.020) and W3 (t = -3.267, p = 0.039). In contrast, there was no significant difference in hatching success between the control and treated stormwater groups from events W2 (t = -0.548, p = 0.851) and W3 (t = -3.500, p = 0.874). The results, therefore, demonstrate the ability of the wetland to reduce embryotoxicity of stormwater runoff to *M. duboulayi*. This is an important factor to consider since reduced hatching success may compromise population recruitment in receiving waters.

The embryotoxicity of contaminants measured in this study to *M. duboulayi* is not documented in the literature. Kumar et al. (2002) reported no significant mortality

in juvenile specimens of *M. duboulayi* exposed to road runoff. Earlier life stages of fish, however, are generally more sensitive to pollutants (Barry et al., 1995; Williams & Holdway, 2000). Skinner et al. (1999) observed EC50s for failure of *Oryzias latipes* embryos to hatch at as low as 26% stormwater, and suggested that this might have resulted from the metal content of the stormwater. Metals are able to penetrate the chorion of fish embryos and have a number of detrimental effects on development (Jezierska et al., 2009).

In this study, W2 and W3 inflow concentrations of Zn and Cu were identified as being the most enriched metal contaminants relative to freshwater guidelines. Williams & Holdway (2000) found that hatching success of another Melanotaeniid fish, *M. fluviatilis,* decreased with increasing Zn concentration. Significant differences in percentage hatch relative to the control for 92 h-old embryos was observed at much higher Zn concentrations (3.3 mg L^{-1}) than those determined in the current study, however, only 2 h pulse-exposures were carried out. It is likely that continuous exposures would result in lower concentrations causing a lethal response. For example, Witeska (2014) observed a significant decrease in the hatching success of *Leuciscus idus* eggs continuously exposed to 0.1 mg L^{-1} of Cd, whilst Williams & Holdway (2000) noted that a 2 h pulse-exposure to 3.3 mg L^{-1} of Cd had no affect on the hatching success of *M. fluviatilis* In terms of Cu, previous research has found that continuous exposure to Cu at a concentration of 0.1 mg L^{-1} reduced the survival of fertilized embryos of *Leuciscus idus* (Witeska et al., 2014). Lower concentrations were not tested and the Cu concentrations used by Witeska et al., (2014) are higher than those observed in the current study. Given the lack of available literature on the embryotoxicity of contaminants to *M. duboulayi* it is difficult to make a definitive interpretation of the possible cause of toxicity observed to *M. duboulayi* embryos exposed to untreated stormwater during the current study. Other unknown toxic substances cannot be ruled out.

To unequivocally show that certain stormwater contaminants cause a toxic effect, toxicity identification and evaluation (TIE) procedures should be undertaken. The TIE procedure removes particular chemicals from test solutions in a step-wise process through various chemical and physical manipulations. The toxicity of the

manipulated solutions are tested at each stage to identify the probable cause of toxicity. In particular, the TIE procedure might identify specific components of stormwater causing toxicity and requiring management. This procedure has already been useful in the assessment of untreated stormwater (Kumar et al., 2002; Schiff et al., 2002)

Conclusions

This study demonstrates that the residential catchment generated stormwater runoff containing pollutant concentrations that would likely result in measurable degradation to aquatic ecosystems. Several of the stormwater analytes exceeded recommended freshwater guidelines and significant toxicity to *C. dubia* and *M. duboulayi* was observed in some inlet samples in the form of mortality and reduced hatching success, respectively. The data shows that the constructed wetland reduced the concentration of the majority of measured analytes and no toxicity was observed in outflowing samples. Despite these beneficial outcomes, the data indicate that it may not be possible to treat stormwater to receiving water quality guideline levels in all cases, particularly when pollutants concentrations at the outflow are below wetland background levels. For example, mean concentrations of filterable Cu and Zn, TN and TP exiting the wetland system still exceeded freshwater guidelines and may therefore have the potential to contribute to localised water quality problems downstream. The algal bioassay results indicated a potential for eutrophication to occur. The findings in this study align with the approaches detailed in the Australian National Water Quality Management Strategy (ANZECC/ARMCANZ, 2000), which recommend that a combination of physico-chemical and biological indicators are used as complementary tools as part of water quality management and assessment approaches. In this regard, it is recommended that toxicity testing of water exiting the wetland be periodically coupled with water quality monitoring to ensure that water quality improvements are being maintained over time. A high degree of variability was apparent in the removal of various pollutants between storm events and higher removal efficiencies may have been observed in some cases had the inflow concentration not been relatively low. Longer monitoring periods would be optimal in order to obtain a more representative characterisation of pollutant removal efficiency and to

verify significant differences between inlet and outlet pollutant concentrations. Notwithstanding the inherent limitations of field sampling and the data collected in this study, the evidence indicates that the constructed wetland demonstrated some important benefits to water quality improvement that will aid in remediating urban waterways and reducing the risk of urban stormwater runoff to aquatic biota.

Chapter 3. Freshwater Toxicity and Pollutant Reduction in Urban Stormwater Flowing Through a Field Bioretention Basin

The aim of the research presented in this chapter was to evaluate the efficacy of a retrofitted bioretention basin to remove urban stormwater pollutants and reduce the risk to freshwater biota. The assessment approach used a combination of chemical analysis and freshwater toxicity testing. The results show that the bioretention basin had little impact on the majority of measured influent pollutant concentrations and leaching of several analytes from the system occurred consistently. Reduced toxicity to freshwater biota was noted in the majority of outflow water samples relative to inflow samples despite the limited water quality improvements, suggesting the removal of an unidentified toxic substance by the bioretention system. Overall the results indicate the need for further research and subsequent design modifications to improve the performance of conventional bioretention systems in reducing the impact of urban stormwater to freshwater biota.

Authors

	Problem formulation	Experimental design	Statistical analysis	Results interpretation	Paper preparation
Lois Oulton[1]	90	90	97	95	90
Mark Taylor[1]	5	3	0	1	4
Culum Brown[2]	0	2	0	1	1
Grant Hose[2]	5	5	3	3	5

1 Department of Environmental Sciences, Macquarie University, NSW 2109 Australia
2 Department of Biological Sciences, Macquarie University, NSW 2109 Australia

Abstract

Stormwater runoff is a major source of pollution to waterways and causes undesirable ecological effects. Bioretention systems have gained considerable attention as a means to improve stormwater quality. However, the ability of these systems to reduce the risk of urban stormwater pollution on freshwater biota is not well understood. We addressed this by examining the in-field efficiency of a bioretention system constructed in Sydney (Australia) to remove a suite of urban stormwater pollutants that may affect aquatic biota in receiving waterways (TSS, BOD, COD, nutrients and filterable metals), and if any subsequent water quality improvements were sufficient to mitigate toxicity to three freshwater species across six storm events. Of the sixteen analytes measured, only TSS (75-96% removal), BOD (40-89% removal) and filterable Pb (67-90% removal) were reduced significantly by the system. Treatment of the remaining analytes was variable, ranging from increased concentrations due to leaching from the bioretention system to poor removal. The largest negative mean removal efficiency (i.e. increase in concentration) was observed for NO_x-N (-836%). Mean concentrations of filterable Al and Cu, TN, NO_x-N, TP and RP exiting the bioretention showed greater enrichment above Australian freshwater guidelines than inlet concentrations. Mean filterable Zn concentrations exiting the wetland also exceeded guidelines despite being reduced by the system. Significant toxicity to *Pseudokirchneriella subcapitata*, *Ceriodaphnia dubia* and *Melanotaenia duboulayi* was observed in some inlet samples with toxicity exposure effects including growth inhibition, mortality and reduced hatching success, respectively. Despite the bioretention system having little impact on the majority of stormwater pollutant concentrations, toxicity was not observed in all outlet samples. This suggests that other unidentified toxic substances in the influent water were reduced by the bioretention system. Although the results provide some support for the use of bioretentions as a stormwater best management practice, they indicate overall the need for further research and design modifications to improve the performance of conventional systems to reduce the risk of urban stormwater runoff to freshwater biota.

Introduction

Stormwater runoff is a product of urban land use increasing land surface imperviousness, which has lead to widespread degradation of urban waterways (Hall & Anderson, 1988; Walsh, 2000). It contributes a variety of pollutants to urban river systems such as suspended solids, nutrients, metals and pesticides, which can impair water quality and be toxic or disruptive to aquatic ecosystems (Skinner et al., 1999; Schiff et al., 2002; Walsh et al., 2004a; Meyer et al., 2005; Walsh et al., 2005; McCarthy et al., 2008; McQueen et al., 2010). For example, increased nutrient loadings can stimulate excessive plant growth leading to eutrophication, and suspended solids (sediment and organic particles) can reduce light penetration for photosynthesis, smother habitats, and increase biochemical oxygen demand (Wood & Armitage, 1997; Anderson et al., 2002; Walsh et al., 2004a; Greenway, 2010). Metals present in stormwater can cause lethal and sublethal effects to aquatic biota, such as mortality (Kumar et al., 2002), developmental anomalies (Skinner et al., 1999) and impairment to ecologically relevant behaviours (Sandahl et al., 2007; Oulton et al., 2014).

A range of structural stormwater treatment devices have been developed and deployed to address the quality of urban stormwater runoff (Birch et al., 2004; Hatt et al., 2009). Among these treatment devices, bioretention systems have gained considerable attention as a component of stormwater best management practices due to their small footprint, aesthetic benefits, ability to drain within a few hours, and tolerance of system vegetation to varying hydrologic regimes (Hatt et al., 2009; Roy-Poirier et al., 2010; Trowsdale & Simcock, 2011). Bioretention systems operate by filtering stormwater through terrestrial planted vegetation followed by vertical percolation through a porous filter media. Pollutants are retained via a number of processes including sedimentation, fine filtration, adsorption, and biological uptake (Davis et al., 2003; Hatt et al., 2009; Water by Design, 2009). Sub-surface drainage below the surface filter media drains the ‘treated’ water from the system to receiving waterways (Hatt et al., 2009). On the surface of bioretention systems particulates are captured, whilst dissolved pollutants are removed as the stormwater infiltrates through the filter media (Water by Design,

2009). The effectiveness of pollutant removal is influenced by the design specifications of the bioretention system including properties of the filter media, selected vegetation and the use of enhancements such as a saturated zone (Davis, 2014; Payne *et al.,* 2014).

Both laboratory and field studies have highlighted the potential of bioretention systems for stormwater treatment (e.g. (Davis et al., 2001; Lloyd et al., 2001; Davis et al., 2003; Hsieh & Davis, 2005; Hunt et al., 2006a; Davis, 2007; Rusciano & Obropta, 2007; Bratieres et al., 2008; Hunt et al., 2008; Hatt et al., 2009; Line & Hunt, 2009; Chapman & Horner, 2010). These studies generally report high removal of total suspended solids and heavy metals, but variable removal of nutrients, particularly the soluble forms that are of a concern due to their eutrophic effect in waterways (Taylor et al., 2005). The performance of bioretention structures to remove nutrients was shown to be influenced by vegetation selection (Bratieres et al., 2008), the phosphorus content of the fill media (Lloyd et al., 2001; Hatt et al., 2009; Hunt et al., 2006) and the potential of unsaturated bioretention media to accumulate nitrogen (Hatt et al., 2009; Line & Hunt 2009). Performance data for bioretention systems under real-weather conditions is also limited in comparison to laboratory studies and those using synthetic stormwater (Chapman & Horner, 2010; Trowsdale & Simcock, 2011). Further, while such studies have shown changes in stormwater quality from bioretention systems, the effectiveness of any subsequent water quality improvements to prevent toxic harm to freshwater biota has not been widely studied. Emerging evidence has shown how the treatment of first flush highway runoff through experimental soil bioretention columns can reduce toxicity of untreated stormwater to the early life stages of zebrafish (McIntyre et al., 2014), and juvenile salmon and invertebrates (McIntyre et al., 2015). However, the effects of stormwater treatment to aquatic species has not been tested at the field scale. Ecological evidence is essential for stormwater management to support arguments for the efficacy of stormwater treatment systems and their wider implementation, which can be costly (Knights et al., 2010).

This study provides a combined chemical and toxicological assessment of the performance of an *in situ* bioretention system under real-weather conditions in

Sydney, Australia across six storm events. The aims of the research were to determine: (1) the efficacy of the system to remove a suite of pollutants from urban stormwater that may pose a risk to freshwater biota; and (2) If treated stormwater exiting the system is less toxic than untreated stormwater to freshwater organisms from three trophic levels: a microalga (*Pseudokirchneriella subcapitata),* a cladoceran (*Ceriodaphnia dubia)* and embryos of the crimson-spotted Rainbowfish (*Melanotaenia duboulayi).* Our hypothesis was that, with stormwater treatment by the bioretention system, stormwater pollutants and the toxicity to freshwater biota would be reduced.

Methods

Sampling Location

This study was conducted at Johnston St bioretention basin (33°56' S, 151°06' E), which was retrofitted to a residential urban area on the Cooks River catchment in the inner west of Sydney, NSW, Australia in 2011 (Figure 7). Stormwater samples were collected from the bioretention basin for six storm events between November 2012 and April 2013.

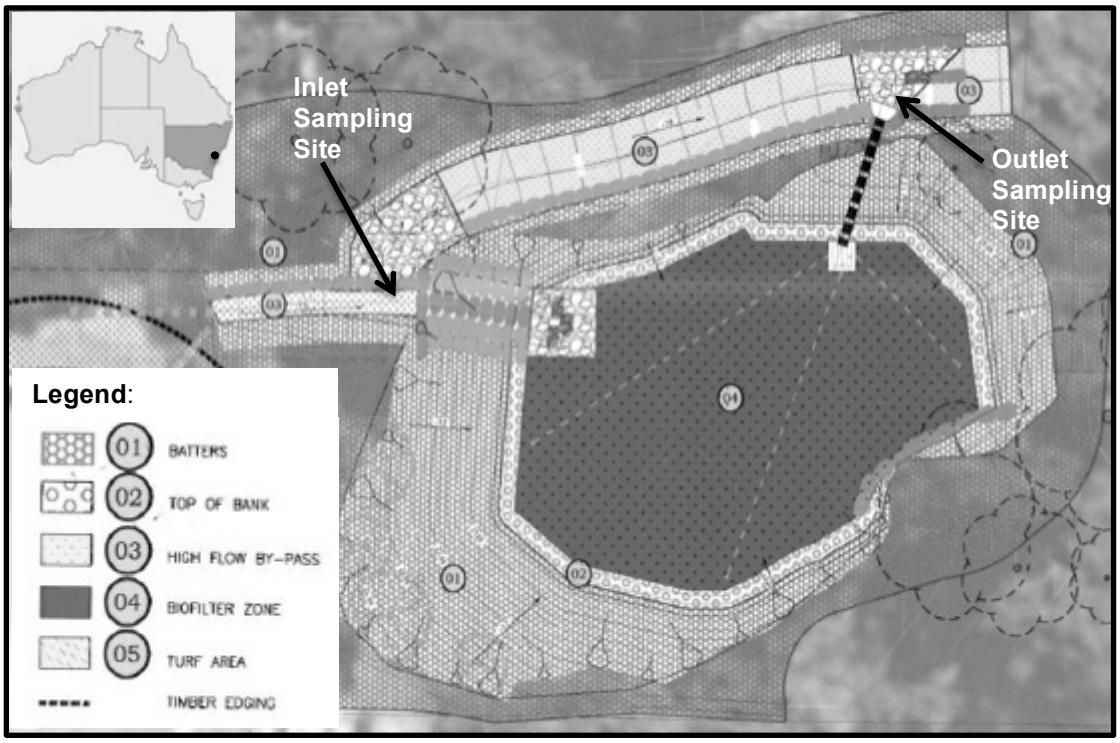


Figure 7. Diagrammatic representation of Johnston St Bioretention including sampling locations. Insert on the top right shows the site location (•) in relation to Australia. Adapted from Storm Consulting (2010).

The bioretention basin is 240 m^2 and is designed to cater for the 1 in 3 month flows, with higher flows bypassing the system via an overflow channel. The residential catchment draining into the basin is approximately 25,000 m^2 and consists of housing, gardens and residential roads. Street gullies leading into the basin are installed with litter traps to screen out gross pollutants. The size of the basin in relation to the contributing catchment area is 1% and therefore less than optimum (2-3%) for best practice design guidelines (Water by Design, 2009). The surface of the system is covered with a 50 mm mulch layer and planted with native

Australian grasses/sedges, *Carex appressa, Dianella longifolia, Gahnia malanocarpa, Ficinia nodosa, Lomandra longifolia* and *Poa labillardierei* at a density of approximately one plant per 5 m^2. Below-ground the biofilter material is made up of three layers: filter media (400 mm sandy loam, Benedict M165 Bioretention Filter Media^{TM}); a transition layer (100 mm recycled glass, Benedict Washed GlassSand^{TM}); and a drainage layer (200 mm gravel). The MUSIC (Model for Urban Stormwater Improvement Conceptualization) model for this treatment device, based on standard values attributed to this system based on its characteristics, predicts a 79%, 30% and 57% reduction in TSS, TN and TP, respectively (WSUD, 2014d). *In situ* hydraulic conductivity measurements of the filter media, which indicates the rate at which water is moving through the soil, were obtained using the single-ring infiltration method (Hatt & Le Coustumer, 2008) two years after construction, and were found to range from 234 to 408 mm h^{-1}. Perforated PVC pipes (100 mm diameter) are located in the drainage layer to collect infiltrated runoff before it is discharged to Wolli Creek, which is a main tributary of the Cooks River. Fig. 8 shows the construction of the bioretention basin.

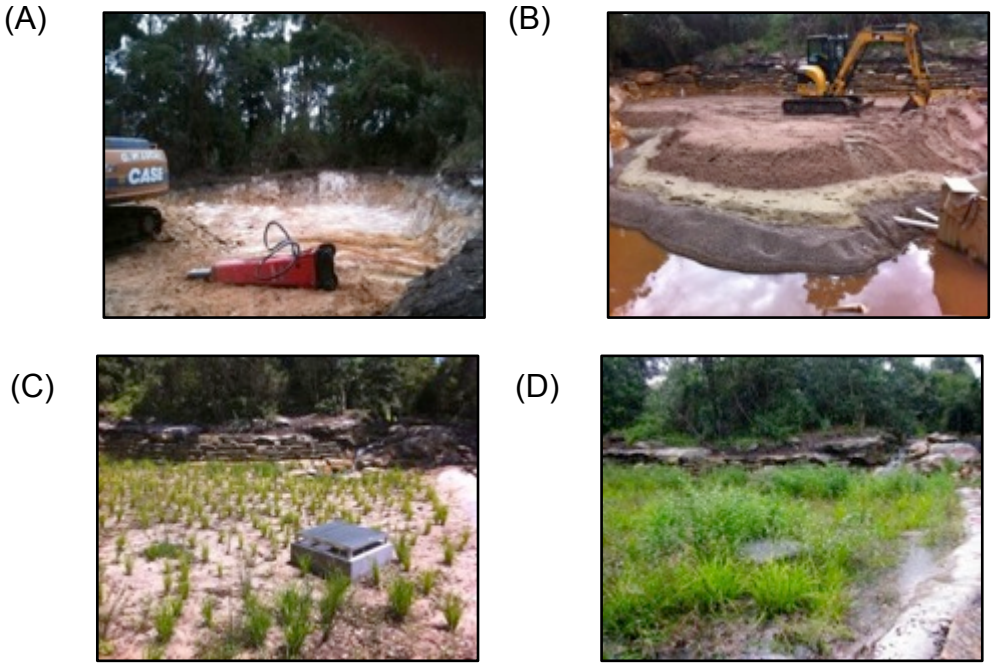


Figure 8. Johnston St bioretention basin: **A)** basin excavation; **B)** placement of underdrains and biofilter layers (top to bottom: filter media, transition layer and gravel layer) **(C)** vegetation planting **(D)** system 9 months after construction during a storm event.

Sampling Procedure

Automated samplers were installed at the inflow and outflow drainage points of the bioretention basin and flow-weighted composite samples obtained. Location of water collection is indicated in Figure 6. Full details of the sampling procedure are included in Chapter 2. Flow data for the six monitored storm events at the bioretention basin is provided in Appendix 3 (Figure A3.1) and demonstrated that the system was not always effective at reducing peak flows. This set-up allowed for both the longer and shorter duration storm events to be captured at the inlet and for a sample from the outlet to be collected once the system had started to discharge. To obtain a more representative sample of the treated water quality in the bioretention basin the whole of the outflow should be sampled, but in practice this is very difficult and was not logistically possible for the study due to the short holding times required and technical difficulties of using automatic samplers (there was only a finite number of sample bottles available). Nevertheless, the sampling regime was deemed sufficient to evaluate whether the water leaving the bioretention was of a better quality and less toxic than the influent stormwater.

Samples were analyzed by ALS Environmental, Sydney, a National Association of Testing Authorities (NATA) accredited laboratory within 24 h of collection. Full details of the water quality determinants measured and the laboratory quality control protocols employed by the laboratory are provided in Chapter 2. Detailed QC results for laboratory analysis are given in Appendix 3 (Table A3.2).

Toxicity tests

Laboratory toxicity tests were conducted on the inflow and outflow composite samples using three Australian native freshwater test species: a green microalga (*Pseudokirchneriella subcapitata),* a cladoceran (*Ceriodaphnia dubia)* and embryos of the crimson-spotted Rainbowfish (*Melanotaenia duboulayi).* Full details of the toxicity test procedures are included in Chapter 2. For event B4, 8 *M. duboulayi* eggs were randomly allocated to 100 mL glass beakers containing 80 mL of test solution instead of 10 eggs due to insufficient numbers being available.

Physicochemical measurements (temperature, pH, dissolved oxygen, and conductivity) of test samples were monitored at the beginning and end of the toxicity test using a YSI ProPlus multiparameter instrument. Ranges of these values are provided in Table 6.

Table 6. Physicochemical variables of test solutions from toxicity testing using *P. subcapitata, C. dubia* and *M. duboulayi.*

Test Sample	Test Concentrations (%)	pH	Temperature (°C)	Dissolved Oxygen (%)	Conductivity (uS cm^{-1})
P. subcapitata					
C	0	7.53-8.00	24-25	nd	nd
UT	6.25, 12.5, 25, 50, 75, 100	7.39-8.90	24-25	nd	nd
T	6.25, 12.5, 25, 50, 75, 100	7.48-8.72	24-25	nd	nd
C. dubia					
C	0	8.19-8.46	22 - 23	96-100	172-196
UT	6.25, 12.5, 25, 50, 75, 100	6.63-8.46	22-23	94-100	55-195
T	6.25, 12.5, 25, 50, 75, 100	7.21-8.37	22-23	87-100	94-193
M. duboulayi					
C	0	7.50-7.60	25.5-26.1	81-83	235-365
UT	100	6.77-7.49	25.4-26.0	78-89	58-130
T	100	7.24-7.36	25.6-26.1	83 - 90	94-145

nd = not determined; C = Control; UT = Untreated; T = Treated

Glassware and equipment

Refer to Chapter 2 for details on the cleaning of glassware and equipment.

Data analysis

Refer to Chapter 2 for details on the analysis performed on the water quality and toxicity data.

Results and discussion

Pollutant Removal

Chemical analysis of water samples from the bioretention system was based on collection of inflowing (untreated) and outflowing (treated) stormwater from six storm events (B1-B6). EMC values were measured for all six events and used to estimate the percentage removal efficiency of pollutants by the bioretention system (Table 7). Only six of the sixteen analytes measured were reduced by the bioretention system, and the percentage removal efficiency occurred in the following order: TSS > Pb > BOD > Zn > Fe > NH_3-N. Outflow concentrations of all other analytes were higher than inflow concentrations (i.e. negative removal). Mean outflow concentrations for filterable Al, Cu and Zn, TN, NO_x-N, TP and RP showed enrichment above freshwater guidelines (Table 8).

Total Suspended Solids

For TSS, removal efficiencies were high across all storm events (mean: 90%, range: 75% to 96%) and were above best practice guidelines (80% removal, (Queensland Government, 2009; SMCMA, 2011) and MUSIC site predictions (79% removal) for five out of six storm events. The lowest observed TSS removal efficiency (75%, event B2) is likely to have resulted from the inflow concentration being below the 20 mg L^{-1} background concentration typically adopted for bioretention systems (Thompson et al., 2011). Overall, the results for TSS provide strong evidence for a substantial treatment effect via physical processes (sedimentation and filtration) (Laurenson et al., 2013). Further, the results mirror previous field studies of bioretention systems (60% to 90% removal) (Hunt et al., 2008; Hatt et al., 2009; Li & Davis, 2009; Line & Hunt, 2009; Chapman & Horner, 2010; Stuart, 2011; Trowsdale & Simcock, 2011). TSS removal is important because it can contain a large amount of organic matter and particulate-bound pollutants such as heavy metals and nutrients (Roy-Poirier et al., 2010). Overall TSS outflow concentrations (3.0 mg L^{-1}) were significantly lower than inflow concentrations (38.2 mg L^{-1}) ($t = 8.91$, $p = <0.001$) and did not exceed recommended Australian guidelines of <25 mg L^{-1} (Wong, 2006).

Nitrogen and Phosphorus Species

Mean outlet concentrations of TN, TKN and NO_x-N were greater than inflowing concentrations, resulting in an overall negative removal efficiency of -106% (range: -150% to -65%), -27 (range: -100% to 32%) and -836% (range: -2362% to -164%), respectively. The system therefore failed to meet best practice guidelines for TN reduction (45%, Queensland Government, 2009; 40%, SMCMA, 2011) and MUSIC site predictions (30%). Overall increases in outlet concentrations were significant for TN ($t = 11.19$, $p = <0.001$) and NO_x-N ($t = -5.32$, $p = 0.003$), but not for TKN ($t = -0.65$, $P = 0.542$). Mean outflowing concentrations of TN and NO_x-N recorded higher enrichments above ANZECC/ARMCANZ (2000a) freshwater guidelines than inflowing concentrations, and exceeded trigger values by 4.5 and 26.8 times, respectively. The only nitrogen species to have a mean positive removal efficiency from the system was NH_3-N (mean: 5%, range: -300% to 94%). Removal however, was highly variable between events and overall differences between inlet and outlet concentrations were not significant ($t = 1.63$, $p = 0.165$). Concentrations of NH_3-N were below ANZECC/ARMCANZ (2000a) guidelines in all samples.

Laurenson et al. (2013) documented that bioretention studies thus far have shown inconsistent results in terms of nitrogen removal rates. Higher outflowing concentrations of TN are likely to have been driven by leaching of NO_x-N, which had the most negative removal efficiency of the different forms of nitrogen species. *In situ* production of NO_x-N has been noted previously in other field bioretention studies (Dietz & Clausen, 2005; Hatt et al., 2009; Li & Davis, 2009; Line & Hunt, 2009). Line & Hunt (2009) recorded a mean negative removal efficiency of -257% (range: -384% to – 57%) for NO_x-N for a bioretention in North Carolina, USA, that was larger in size in relation to the contributing catchment area (7.6%) than the system examined here. Conventional bioretention systems do not incorporate an anaerobic saturated zone (Kim et al., 2003) and it is thought that ammonification and nitrification processes take place in the aerobic conditions of the basin between storm events (Line & Hunt, 2009). Anaerobic conditions and an electron donor are required for denitrification (Hunt et al., 2006a). Consequently, NO_x-N

can accumulate in unsaturated bioretention media during non-storm periods and leach from the system during subsequent storm events (Line & Hunt, 2009; Roy-Poirier et al., 2010), particularly because it is highly mobile and has limited binding potential (Bolan et al., 2004). There is evidence of nitrification taking place within Johnston St bioretention as there is evidence of some NH_3-N removal. In some other studies of bioretention systems, NH_3-N removal was also shown to occur where NO_x-N removal was ineffective (Dietz & Clausen, 2005; Hatt et al., 2009). Re-engineering the conventional bioretention design to incorporate an anoxic overdrain zone (saturated layer) seeded with newspaper (an electron donor and carbon source) promoted significant denitrification in laboratory column studies, with nitrate mass removals of up to 80% (Kim et al., 2003). By comparison, field studies of bioretentions designed with a saturated zone have demonstrated significant reductions in NO_x-N and TN (Dietz & Clausen, 2006). Research is needed to see if modifications can be made to promote denitrification and prevent the leaching of NO_x-N in conventional bioretention systems that do not incorporate a saturated layer.

Mean TP and RP concentrations also increased from the inflow to the outflow, resulting in an overall negative removal efficiency of -170% (range: -945% to 13%) and -406% (range: -100% to > -2900%), respectively. Overall RP outflow concentrations were significantly higher than inflow concentrations ($t = -3.37$, $p = 0.020$), but due to the variability in TP removal the average increase in concentrations were not statistically significant ($t = -1.26$, $p = 0.264$). The system failed to meet best practice TP reduction targets (60%, Queensland Government, 2009; SMCMA, 2011) and MUSIC site predictions (57%). Mean outflowing concentrations of TP and RP were above ANZECC/ARMCANZ (2000a) freshwater guidelines and on average exceeded trigger values by 6.7 times and 8 times, respectively.

Higher concentrations of phosphorus in the outflow compared to the inflow are likely to have been driven, in part, by leaching of dissolved phosphorus. Similar results were reported for a field bioretention of the same size (i.e. 1% of the contributing catchment) in Melbourne, Australia (Hatt et al., 2009), where load

reductions for TP and RP were -398% and -1271%, respectively. Other field bioretention studies conducted in the USA on larger sized systems have also reported leaching of dissolved phosphorus (Chapman & Horner, 2010) and total phosphorus (Hunt et al., 2006a; Li & Davis, 2009). Possible explanations for this trend are related to soil disturbances, organic/phosphorus content of the filter media used in the bioretention device, vegetation/fauna in the system, and low influent concentrations (Dietz & Clausen, 2006; Hunt et al., 2006a; Hatt et al., 2009; Li & Davis, 2009; Chapman & Horner, 2010). According to filter media specifications, Johnston Street bioretention contained 9 mg/kg of orthophosphate and 1.5% organic matter, compared to 380 mg/kg TP and 5% organic matter in the study by Hatt et al. (2009) who also found increases in P concentrations. It is therefore unlikely that the organic/phosphorus content of the filter media contributed to the leaching of phosphorus in the current study. It is recommended that sediment sampling be undertaken at the system to determine the phosphorous index of the media.

Bioretentions are thought to be effective at reducing pollutants down to a background concentration (Hatt et al., 2009). Negative TP removal efficiencies were observed for the lowest inflow concentrations (0.09 – 0.12 mg L^{-1}). Leaching of phosphorus from field bioretentions experiencing low influent TP concentrations (mean: 0.019 - 0. 11 mg L^{-1}) has been noted in previous bioretention studies (Dietz & Clausen, 2006; Hunt et al., 2006a). Inflow concentrations of TN were also below or close to the 1.4 mg L^{-1} background concentration typically adopted for bioretention systems (Thompson et al., 2011). It may be that the observed negative removal efficiency of nutrients is in part due to the low influent concentrations. Further work to test the treatment performance of this bioretention system by performing spiking experiments with higher inflow concentrations is recommended.

FAWB (2009) recommends a best practice hydraulic conductivity target of 100 to 300 mm h^{-1} for bioretention systems in temperate climates. Design specifications for Johnston St bioretention were within this range (150 – 250 mm h^{-1}). However, *in situ* hydraulic conductivity measurements of the filter media in 2013 at Johnston

St bioretention ranged from 234 to 408 mm h^{-1}. A study by Carpenter & Hallam (2010) showed that infiltration rates in bioretention are highly influenced by construction techniques, which have been a major implementation concern (Roy-Poirier et al., 2010). Johnston St bioretention is also size-constrained, which will restrict the retention time of stormwater. Nutrient treatment may therefore have been compromised in the system by water infiltrating too quickly, reducing the contact time between the filter media/water interface and restricting the adsorption and assimilation of stormwater contaminants within the system (Laurenson et al., 2013). Lucas and Greenway (2008) suggest that rapid and reversible sorption reactions predominate as stormwater moves through the media, whilst slow and irreversible sorption reactions continue to be formed between storm events. Sufficient contact time is therefore needed for effective sorption. The leaching of nutrients could also have occurred because over a 1/3 of the system was poorly vegetated due to vandalism of the site during the monitoring period. Henderson et al. (2007) tested nutrient removal in vegetated and non-vegetated bioretention columns and observed leaching of nitrogen and phosphorus from non-vegetated systems when flushed with tap water, but retention in vegetated systems.

Filterable metals

There is a lack of data relating to the removal of dissolved metals from urban stormwater by field bioretention systems because most studies have focused on total metal removal. Dissolved metal fractions are, however, considered to have higher toxicity and bioavailability than sediment-associated metals (Hare, 1992; Santore et al., 2001). Removal of water-soluble metals from stormwater is therefore considered important for the health of aquatic ecosystems. Mean outflow concentrations of filterable metal contaminants exceeded inflow concentrations, with the exception of Mn, Pb and Zn, which had mean positive removal efficiencies of 35% (range: 0% to 81%), 80% (range: 67% to 90%) and 31% (range: 4% to 68%), respectively. Nickel was below detection limits (<0.001 mg L^{-1}) for all samples and results are not presented. Overall differences between inflow and outflow concentrations were statistically significant for Pb ($t = 8.86$, $p = <0.001$) and Al ($t = -2.61$, $p = 0.047$). Mean concentrations of Al and Cu in the outflow were higher than those in the inflow, and exceeded ANZECC/ARMCANZ (2000a) trigger

values by 1.7 and 7.1 times, respectively. Mean concentrations of Pb and Zn in inflowing stormwater exceeded ANZECC/ARMCANZ (2000a) guidelines by 1.2 and 6.2 times, respectively. Following bioretention treatment, the mean Pb concentration at the outflow was within the recommended guideline value, but enrichment of Zn concentrations above the recommended guideline remained. Trowsdale & Simcock (2011) also observed filterable Zn outflow concentrations to remain above ANZECC/ARMCANZ (2000a) guidelines following bioretention treatment.

The observed reduction of filterable Zn and leaching of filterable Cu is in agreement with a study conducted by Trowsdale & Simcock (2011) on a similar sized bioretention system. The authors suggested that Cu may be moving into solution from Cu-contaminated sediments that have accumulated in the system, due to the tendency of Cu to move or equilibrate between the particulate and dissolved phases. Metal removal is said to occur through adsorption to the surface mulch layer and to the filter media as the water infiltrates through the system (Davis et al., 2001). It is possible that the high hydraulic conductivity of Johnston St bioretention system and the fact that the system is undersized, reduced the retention time of stormwater and limited the potential for the adsorption of dissolved metals to media particles and consequently some metals passed through the media. A study conducted in Seattle, USA, on a larger bioretention system with a basin to catchment area ratio of 5% reported pollutant removal efficiencies of 58% and 72% for filterable Cu and Zn, respectively (Chapman & Horner, 2010).

Biological and Chemical Oxygen Demand

Mean changes in COD and BOD between inflow and outflow sample points were -17% (range: -55% to 21.1%) and 67% (range: 40% to 89%), respectively. Overall differences were significant for BOD ($t = 2.92$, $p = 0.043$), but not for COD ($t = -1.16$, $p = 0.298$). Hunt et al. (2008) also observed significant BOD reductions of 63% at a field bioretention basin in North Carolina. There are no published results available for COD removal in bioretention systems. The results from the current study suggest that Johnston St bioretention system is efficient at reducing the

oxygen demand required for the microbial oxidation of organic material, but not for chemical oxidation. There are few Australian guidelines for BOD and COD, but the guidelines limit for freshwater aquaculture is <15 mg L^{-1} and <40 mg L^{-1}, respectively (ANZECC/ARMCANZ, 2000c). Mean outflow concentrations of BOD and COD were below the guideline limits.

Table 7. Event mean concentrations (EMC) of filterable metals (Al, Cd, Cu, Fe, Mn, Pb and Zn), nutrients (TN, TKN, NO_x, FRP, TP), total suspended solids (TSS), chemical oxygen demand (COD) and biochemical oxygen demand (BOD) in stormwater runoff from the inflow and outflow of Johnston St bioretention and the removal efficiency (RE, %) of pollutants by the system for each monitored storm event (B1-B6).

Event	EMC (mg L^{-1})	Al	Cd	Cu	Fe	Mn	Pb	Zn	TN	TKN	NH_3-N	NO_X-N	TP	RP	TSS	COD	BOD
B1	Inflow	0.09	bld	0.01	bld	0.021	0.003	0.062	2	1.9	0.08	0.08	0.24	bld	62	26	9
	Outflow	0.24	bld	0.006	0.28	0.004	bld	0.02	3.3	1.3	bld	1.97	0.21	0.15	bld	26	bld
	RE (%)	-167	nd	40	-1020	81	83	68	-65	32	94	-2363	13	-2900	96	0	88.89
B2	Inflow	0.04	bld	0.004	bdl	0.006	0.002	0.023	0.8	0.5	bld	0.28	0.11	0.08	39	22	bld
	Outflow	0.18	bld	0.012	0.18	0.006	bld	0.022	2	1	0.02	0.98	1.15	0.35	5	34	bld
	RE (%)	-350	nd	-200	-620	0	75	4	-150	-100	-300	-250	-945	-338	87	-55	nd
B3	Inflow	0.03	0.0001	0.007	bld	0.003	0.004	0.06	1.3	0.8	0.09	0.47	0.11	0.05	10	23	3
	Outflow	0.03	0.0003	0.006	bld	0.002	0.001	0.037	2.3	1.1	0.03	1.24	0.12	0.1	bld	32	bld
	RE (%)	0	-200	14	nd	33	75	38	-77	-38	67	-164	-9	-100	75	-39	66.67
B4	Inflow	0.03	bld	0.006	bld	0.007	0.004	0.04	0.9	0.8	0.02	0.09	0.26	0.06	55	27	3
	Outflow	0.09	0.0002	0.01	0.11	0.004	bld	0.038	2	0.9	bld	1.1	0.22	0.18	bld	25	bld
	RE (%)	-200	-300	-67	-340	43	88	5	-122	-13	75	-1122	15	-200	95	7.41	66.67
B5	Inflow	0.05	bld	0.013	0.07	0.009	0.006	0.055	0.9	0.8	0.04	0.12	0.12	0.03	34	90	4
	Outflow	0.06	bld	0.022	0.06	0.009	0.002	0.04	2.00	0.9	bld	1.15	0.18	0.09	bld	71	bld
	RE (%)	-20	nd	-69	14	0	67	27	-122	-13	88	-858	-50	-200	93	21.1	75
B6	Inflow	0.05	bld	0.009	bld	0.006	0.005	0.059	0.9	0.6	bld	0.28	0.09	0.03	29	19	5
	Outflow	0.06	bld	0.007	0.06	0.003	bld	0.034	1.8	0.8	bld	1	0.13	0.09	bld	26	3
	RE (%)	-20	nd	22	-140	50	90	42	-100	-33	-	-257	-44	-200	91	-37	40
B1-B6	**Mean RE (%)**	**-126**	**-250**	**-43**	**-421**	**35**	**80**	**31**	**-106**	**-27**	**5**	**-836**	**-170**	**-406**	**90**	**-17**	**67**
	SD of RE	138	71	90	410	31	9	24	32	43	171	842	381	493	8	30.4	18

bld = below level of detection; SD = standard deviation; nd = not determined

Table 8. Descriptive statistics and enrichment above Australian freshwater guidelines for filterable metals, nutrients, TSS, BOD and COD in inlet and outlet samples from Johnston St bioretention.

Site		Al	Cd	Cu	Fe	Mn	Pb	Zn	TN	TKN	NO_X-N	NH_3-N	TP	RP	TSS	BOD	COD
Inlet	Min (mg L^{-1})	0.03	<0.0001	0.004	<0.05	0.003	0.002	0.023	0.8	0.5	0.08	<0.01	0.09	<0.01	10	<2	19
	Max (mg L^{-1})	0.09	0.0001	0.013	0.07	0.21	0.006	0.062	2	1.9	0.47	0.09	0.26	0.08	62	9	90
	Mean (mg L^{-1})	**0.04**	**0.0001**	**0.0082**	**0.053**	**0.009**	**0.004**	**0.0498**	**1.133**	**0.9**	**0.22**	**0.042**	**0.15**	**0.04**	**38.2**	**4.2**	**35**
	SD (mg L^{-1})	0.02	0	0.0032	0.008	0.006	0.00057	0.015	0.459	0.506	0.15	0.035	0.074	0.03	18.7	2.7	27
	n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	Guidelines	0.05**	0.0002**	0.0014**	nd	1.9**	0.0034**	0.008**	0.5*	nd	0.04*	0.9**	0.05*	0.02*	<25***	15#	40#
Enrichment Factor (Mean/Guideline)		***0***	***0***	***5.8***	**-**	***0***	***1.2***	***6.2***	***2.3***	**-**	***5.5***	***0***	***3***	***2.2***	***1.5***	***0***	***0***
Outlet	Min (mg L^{-1})	0.03	<0.0001	0.006	<0.05	0.002	0.001	0.02	1.8	0.8	0.15	<0.01	0.12	0.09	2.5	<2	25
	Max (mg L^{-1})	0.18	0.0003	0.022	0.28	0.009	0.002	0.04	3.3	1.3	1.97	0.03	1.15	0.35	5	3	71
	Mean (mg L^{-1})	**0.09**	**0.0002**	**0.010**	**0.123**	**0.005**	**0.001**	**0.0318**	**2.233**	**1**	**1.073**	**0.015**	**0.335**	**0.16**	**3**	**1.3**	**36**
	SD (mg L^{-1})	0.05	0.00003	0.006	0.091	0.006	0.0004	0.0086	0.547	0.1789	0.238	0.008	0.401	0.1	1	0.8	18
	n	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
	Guidelines	0.05	0.0002	0.0014	nd	1.9	0.0034	0.008	0.5	nd	0.04	0.9	0.05	0.02	<25	15	40
Enrichment Factor (Mean/Guideline)		**1.7**	***0***	***7.1***	**-**	***0***	***0***	***4.0***	***4.5***	**-**	***26.8***	***0***	***6.7***	***8***	***0***	***0***	***0***
Site with higher concentrations		Outlet	Outlet	Outlet	Outlet	Inlet	Inlet	Inlet	Outlet	Outlet	Outlet	Inlet	Outlet	Outlet	Inlet	Inlet	Outlet
Significant difference between the inlet and outlet (p = < 0.05)		Yes p = 0.047	No p = 0.178	No p = 0.450	No p = 0.050	No p = 0.078	Yes p = 0.001	No p = 0.04	Yes p = 0.001	No p = 0.236	Yes p = 0.03	No p = 0.165	No p = 0.264	Yes p = 0.020	Yes p = 0.001	Yes p = 0.043	No p = 0.298

nd = no data; SD = standard deviation; *n* = number of samples

* ANZECC/ARMCANZ (2000a) trigger values for aquatic ecosystems in lowland rivers in south east Australia. Note FRP guidelines are used for RP and NH_4^+ for NH_3-N

** ANZECC/ARMCANZ (2000a) trigger values for protection of 95% of freshwater biota

ANZECC/ARMCANZ (2000c) recommended guidelines for aquaculture operations

***Wong (2006) - urban freshwater guidelines

Direct Toxicity Assessment

Toxicity responses of *P. subcapitata, C. dubia* and *M. duboulayi* exposed to six composite samples of untreated and treated stormwater from Johnston St bioretention are summarized in Table 9 (raw data is provided in Appendix 3; Table A3.3 – A3.5). None of the test organisms responded similarly after exposure to untreated stormwater and displayed a complex pattern of responses, from no detectable toxicity to significant toxicity, highlighting the variable composition of urban stormwater quality. *P. subcapitata* was more sensitive to stormwater than *M. duboulayi* and *C. dubia*, with significant toxicity elicited in more untreated stormwater samples. Toxicity was also still observed in some outflowing samples (B4 and B5; Table 9), demonstrating the failure of the bioretention system to reduce the toxicity of the influent stormwater in all events.

Table 9. Responses of *Ceriodaphnia dubia* (48-h immobilisation), *Pseudokirchneriella subcapitata* (72-h growth rate) and *Melanotaenia duboulayi* (6-day hatching success) to inlet and outlet samples collected from the bioretention system for six storm events (B1 – B6).

Sample	***C. dubia* 48-h Immobilisation**		***P. subcapitata* 72-h *Growth Rate***		***M.duboulayi* Hatching Success**
	EC1O[a] (%)	EC50[b] (%)	IC10[a] (%)	IC50[b] (%)	(%)
Inflow B1	>100	>100	83	>100	na
Outflow B1	>100	>100	>100	>100	na
Inflow B2	>100	>100	>100	>100	87
Outflow B2	>100	>100	>100	>100	97
Inflow B3	>100	>100	>100	>100	100
Outflow B3	>100	>100	>100	>100	100
Inflow B4	>100	>100	29	93	50*
Outflow B4	>100	>100	>100	>100	58*
Inflow B5	1.6	7.8	16.9	>100	53*
Outflow B5	>100	>100	75.3	>100	77
Inflow B6	>100	>100	40.6	>100	73
Outflow B6	>100	>100	>100	>100	83

[a] Concentration to cause 10% effect (E) or inhibition (I) relative to the control

[b] Concentration to cause 50% effect (E) or inhibition (I) relative to the control

*Hatching success is signficantly different from the control ($p < 0.05$)

na = not available

Pseudokirchneriella subcapitata

Biomass and coefficient of variation among the control replicates and EC_{50} values for reference toxicant tests were all within the acceptable test range (Table A3.2 and A3.6). Four of the six untreated stormwater samples (events B1, B4, B5 and B6) were phytotoxic to *P. subcapitata* and resulted in a decrease in cell division rate relative to the control (Figure 9). The lowest concentration of untreated stormwater to produce a significant inhibition in algal growth relative to the control was 100%, 50%, 25% and 50% untreated stormwater for events B1, B4, B5 and B6, respectively (Figure 9). The response of *P. subcapitata* to untreated stormwater samples from different storm events was variable, as evidenced by the large range in IC_{10} values (16.9 to >100%). Only one untreated stormwater sample was observed to cause a > 50% reduction in algal growth (event B4) hence the IC_{50} value is reported as being greater than the highest treatment (>100%) for other storm events. Storm event B5 had the greatest effect on algal growth (IC_{10} = 16.9% untreated stormwater) and the corresponding outflowing sample remained toxic (IC_{10} = 75.3%) and reduced the cell division rate by 10%. This reduction, however, was not significantly different from the control (p > 0.05). All other outflowing samples were not phytotoxic to *P. subcapitata* hence the IC_{10} and IC_{50} values are reported as being greater than the highest treatment (i.e. >100%; Table 10) and highlight the ability of the bioretention to reduce the toxicity of the input stormwater for these events.

Urban stormwater can inhibit the growth of *P. subcapitata* (Christensen et al., 2006; Scholes et al., 2007). Inflowing stormwater samples that were toxic to *P. subcapitata* had Cu concentrations (0.006 – 0.01 mg L^{-1}) that were higher than the IC_{10} values measured in reference toxicant tests during this study (*ca.* 0.002 mg L^{-1}) and within the IC_{50} value of 0.008 ± 0.002 mg L^{-1} reported by Franklin et al. (2000). Cu concentrations measured in inflowing stormwater may therefore explain the observed reduction in cell division rate. The observed reduction in growth, however, with the exception of event B4, was considerably less than 50% and Cu concentrations in some outflow samples recorded higher concentrations yet elicited no toxicity. Chen (1994) documented that IC_{50} values of *P. subcapitata* in

response to a metal contaminant decreased consistently as orthophosphate levels were reduced from 0.17 to 9.2 x 10^{-4} mg L^{-1}. It is possible that the orthophosphate concentrations recorded in outflowing water samples (0.09 – 0.35 mg L^{-1}), which were higher than inflowing samples, increased the tolerance of *P. subcapitata* to Cu. Concentrations of Cd, Pb and Zn were below the IC_{50} values of 0.341, 2.655 and 0.178 mg L^{-1}, respectively, reported by Chen et al., (1997) for *P. subcapitata*. Limited published data concerning the toxicity of other metal analytes to *P. subcapitata* are available.

The water quality results suggest that there are sufficient nutrients in the outflow of Johnston St bioretention to contribute to eutrophication in receiving waters, yet no growth stimulation in *P. subcapitata* occurred. Chiaudani & Vighi (1974) reported that growth of *P. subcapitata* is highly sensitive to the addition of orthophosphate, and eutrophication will start in a phosphorus limited freshwater environment when the concentration exceeds 0.010 – 0.012 mg L^{-1}. Outflow concentrations of RP observed in the current study were higher (0.09 – 0.35 mg L^{-1}). Scholes et al. (2007) also observed growth stimulation in *P. subcapitata* when exposed to stormwater samples containing a lower orthophosphate concentration (0.066 mg L^{-1}) than that recorded in the outflow of Johnston St bioretention system. The unpredictability of *P. subcapitata* in the present study highlights the complexity of pollutant constituents and their interactions in urban stormwater.

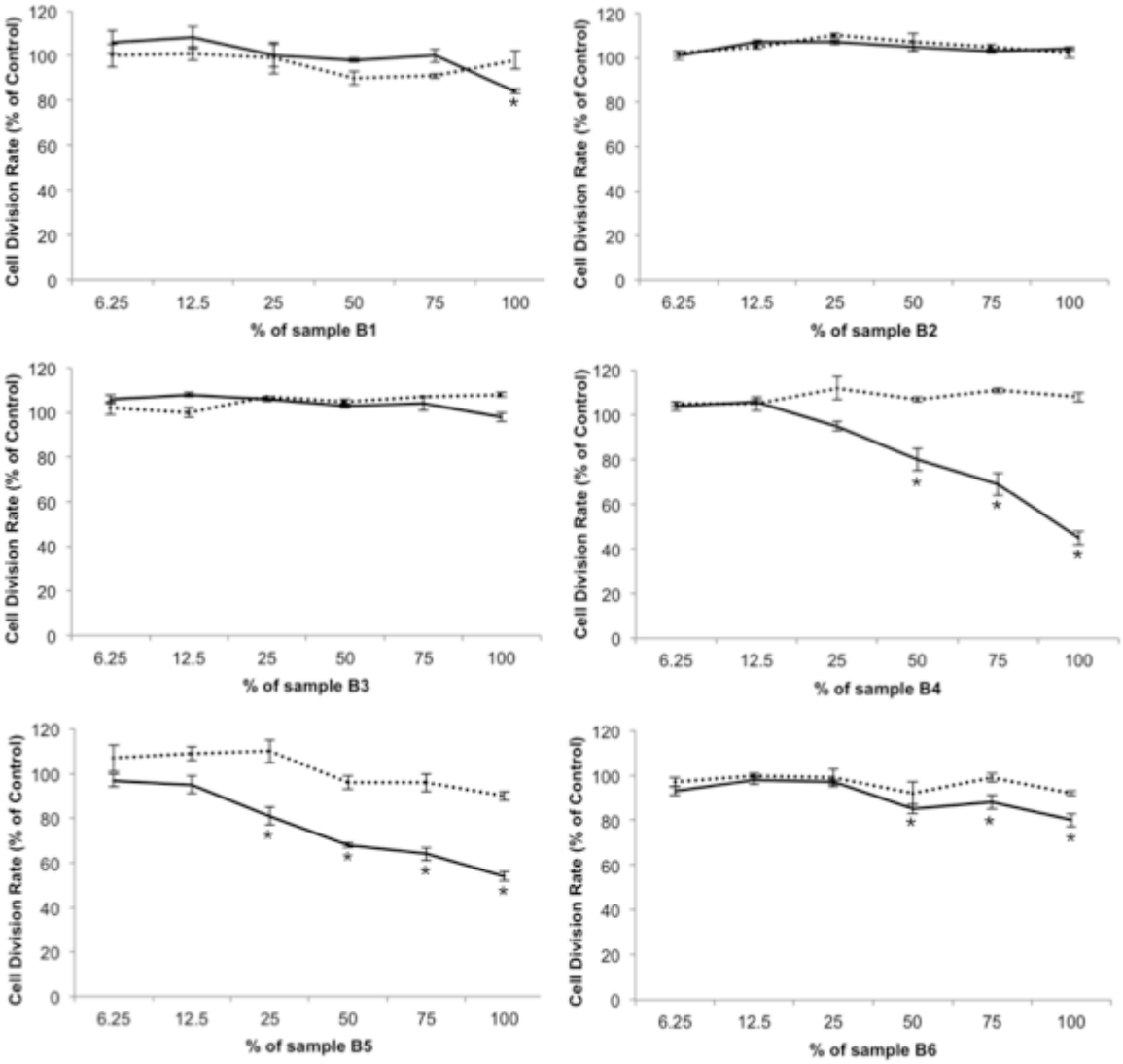


Figure 9. Percentage growth of *Pseudokirchneriella subcapitata* relative to the control when exposed to six untreated (solid line) and treated (dotted line) stormwater samples (B1-B6) for 72 h. Data points represent the mean ± SE. *Significantly greater than growth in control ($p < 0.05$).

Ceriodaphnia dubia

Survival in the control treatments and EC_{50} values for reference toxicant tests were within the acceptable test range (Table A3.4 and Table A3.6). With the exception of event B5, 100% survival occurred across all treatments. Untreated stormwater collected from event B5 was acutely toxic to *C.dubia* with an EC_{10} and EC_{50} estimate of 1.6% and 7.8% stormwater, respectively. The treated stormwater sample for this event produced no mortality, highlighting the ability of the bioretention system to reduce the cause of acute toxicity to *C. dubia*. McIntyre et al., (2015) also reported that the survival of *C.dubia* was significantly reduced in

untreated stormwater (highway runoff), but treatment of runoff through experimental soil bioretention columns prevented mortality.

A lack of acute toxicity of composite stormwater runoff samples to *C. dubia* is consistent with other studies (McQueen et al., 2010; Kumar et al., 2011). By contrast, first flush runoff samples have been shown to exhibit high acute toxicity to *C. dubia* (Kumar et al., 2010, McIntyre et al., 2015). Chronic endpoints (i.e. reproduction) have been more sensitive than acute endpoints (i.e. survival) in *C.dubia* exposed to contaminated water samples (Bailey et al., 2000; McQueen et al., 2010; McIntyre et al., 2015). Land use has also been shown to influence the acute toxicity of stormwater to Cladocera in the following order: commercial > industrial > residential > open space (Hall & Anderson, 1988). Chronic endpoints in *C. dubia* may therefore be more useful when studying the toxic effects of composite stormwater samples from residential areas where lower levels of contaminants are generally found.

The Cu concentration of the B5 inflow sample (0.013 mg L^{-1}) that resulted in acute toxicity to *C. dubia* was above the copper EC_{50} values reported by Binet et al., (2010) (48-h EC_{50} 0.0067 – 0.0081 mg L^{-1}) and the EC_{50} values measured in reference toxicant tests during the study (48 hr-EC_{50} *ca.* 0.005 mg L^{-1}). The concentration of Cu, however, cannot be the only factor responsible for the mortality observed in the untreated B5 sample as the Cu concentration in other collected samples, where no toxicity was observed, was at times within the range of reported 48-hr EC_{50} values. Concentrations of other metals listed in Table 3 were below the 48-hr EC_{50} values reported in the literature for Zn (0.25 – 0.36 mg L^{-1}) (Magliette et al., 1995; Kumar et al., 2002; Tsui et al., 2005), Pb (0.176 - 0.3 mg L^{-1} (Kumar et al., 2002; Tsui et al., 2005), Fe (36.69 mg L^{-1}) (Fort & Stover, 1995), Al (35.97 mg L^{-1}) (Fort & Stover, 1995), Mn (0.308 mg L^{-1}) (Hockett & Mount, 1996) and Cd (0.0545 mg L^{-1}) (Diamond et al., 1997). Other unidentified toxic substances in the stormwater runoff can therefore not be ruled out. A study conducted in a highly urbanized catchment in California identified organophosphate pesticides (not measured in this study) present in stormwater as being responsible for the acute toxicity observed in *C. dubia* (Schiff et al., 2002).

Chemicals in the stormwater may also be interacting and altering toxicity. For example, glyphosate, an active ingredient in herbicides, has been known to reduce the toxicity and bioavailability of metals to *C.dubia* (Tsui et al., 2005). Although not a focus of the current study, organophosphate pesticides and synthetic pyrethroids are hydrophobic (Spurlock & Lee, 2008) and will therefore largely be associated with the particulate phase. This study has shown the bioretention basin to be very effective in removing suspended solids in stormwater and it is likely that this system would be effective in removing hydrophobic pesticides via sedimentation and filtration.

Melanotaenia duboulayi

Hatching success of *M. duboulayi* in the control treatment was <70% for event B1 and results are therefore considered invalid and not presented. For all other events, hatching success in the control treatments met test acceptability criteria (Table A3.5). Rainbowfish embryos hatched well in both untreated and treated stormwater samples collected from events B2, B3 and B6, with no significant differences recorded across treatment groups. Significant differences in hatching success, however, were noted for embryos exposed to samples from events B4 and B5 (B4: $F_{2,6}$ = 31.00, p = 0.001; B5: $F_{2,6}$ = 5.47, p = 0.044). McIntyre et al., (2014) observed hatching success of the Zebrafish, *Danio rerio,* was not consistently significantly reduced in comparison to the controls when exposed to untreated stormwater across multiple events. Tukey HSD post-hoc tests indicated that hatching success significantly decreased in relation to the control by more than 30% when exposed to untreated stormwater collected from event B4 (t = -7.348, p = 0.001 and event B5 (t = -3.267, p = 0.0392). Hatching success in treated stormwater collected from event B4 (t = -6.124, p = 0.0021) was significantly reduced and more than 20% less than that in the control group, demonstrating the failure of the bioretention system to reduce the embryotoxicity of stormwater runoff on this occasion. By contrast, event B5 outflow sample had no significant effect on embryo hatching success relative to the control (t = -1.188, p = 0.5015) demonstrating the ability of the bioretention to reduce the toxicity of stormwater runoff to *M. duboulayi* for this event.

Data on the toxicity of pollutants measured in this study to *M. duboulayi* embryos is not documented in the literature. A previous study by Skinner at al. (1999) documented that stormwater runoff reduced the hatching success of the rice fish, *Oryzias latipes,* and noted that this corresponded with sample metal concentrations exceeding water quality criteria. McIntyre et al., (2014) also documented that a significant failure in hatching success of Zebrafish embryos corresponded to a sample containing high Zn concentrations. Metals can penetrate the chorion of fish embryos and cause negative effects on development (Jezierska et al., 2009). In this study there was no consistent pattern observed between metal concentrations and hatching success of *M. duboulayi* embryos, as samples with metal concentrations higher than those measured during events B4 and B5 did not produce a toxic effect. Other unknown toxic substances cannot be ruled out. For example, pesticides observed in stormwater discharges have been shown to result in significant toxicity to Chinook salmon embryos (Viant et al., 2006). To identify a specific component of the untreated/treated stormwater runoff causing a toxic response toxicity identification and evaluation (TIE) procedures should be undertaken. This procedure has proved useful in the toxic assessment of stormwater runoff (Schiff et al., 2002).

Conclusions

Stormwater in the residential catchment investigated in the current study contained enriched concentrations of filterable Cu, Pb and Zn, TN, NO_x-N, TP, RP and TSS in comparison to Australian freshwater guidelines. Significant toxicity to *P. subcapitata*, *C. dubia* and *M. duboulayi* was observed in some inlet water samples in the form of growth inhibition, mortality and reduced hatching success, respectively. The bioretention system was only effective at reducing the concentration of six of the sixteen measured analytes, with significant reductions observed for TSS (75-96% removal), BOD (40-89% removal) and filterable Pb (67-90% removal). With the exception of NH_3-N, the bioretention was ineffective at reducing nutrient concentrations and significant increases in RP, TN, and NO_x-N were observed at the outlet. The bioretention system also appeared to be a source of Al, Cd, Cu and Fe; however overall differences between the inlet and outlet were not significant due to the high degree of variability. The treated water leaving

the bioretention contained higher enriched concentrations of Al, Cu, TN, NO_x-N, TP and RP in comparison to freshwater guidelines than inlet concentrations. The results for NO_x-N are not surprising given that the system was not constructed with an anaerobic saturated zone that would promote denitrification. Higher removal efficiencies for TN and TP may have been observed had the inflow concentrations not been relatively low, and indicate that it may not always be possible to treat stormwater to receiving water quality guideline levels. The observed results may also be due to the poor vegetation cover, high hydraulic conductivity of the filter media, and small size of the system, which will have reduced the retention time of stormwater in the system and restricted the adsorption and assimilation of stormwater contaminants, particularly filterable metals and phosphorus. Longer monitoring periods would be optimal in order to obtain a more representative characterisation of pollutant removal efficiency and to verify differences between inlet and outlet pollutant concentrations. Although the bioretention had little impact on reducing most pollutant concentrations, toxicity was not observed in all of the outlet samples that were initially toxic. This suggests that other unidentified toxic substances in the stormwater runoff were reduced by the bioretention system and it would be useful to conduct toxicity identification evaluation on each species to determine the toxic constituent(s). The test organisms showed a complex pattern of responses to stormwater, varying in terms of storm event, highlighting the need to use more than one test organisms to detect a realistic toxic effect.

Chapter 4. *In Situ* Assessment of Stormwater Wetlands Using Structural and Functional Indicators of Ecological Health

The aim of the research presented in this chapter was to determine the efficacy of retrofitted stormwater wetlands to improve ecological health. Macroinvertebrate community composition, organic matter decomposition, and survival of Paratya australiensis *were assessed in situ in association with physico-chemical variables at the inlet and outlet zone of stormwater wetlands. The results demonstrate that the largest sized wetland (in relation to the contributing catchment area), treated stormwater to a standard that enabled good improvements in ecological health at the wetland outlet. In contrast, wetlands that were smaller with respect to drainage area and had design constraints had a reduced capacity to achieve the same ecological improvements.*

Authors

	Problem formulation	Experimental design	Statistical analysis	Results interpretation	Paper preparation
Lois Oulton[1]	90	90	94	95	90
Mark Taylor[1]	5	5	0	0	3
Culum Brown[2]	0	0	1	0	2
Grant Hose[2]	5	5	5	5	5

1 Department of Environmental Sciences, Macquarie University, NSW 2109 Australia
2 Department of Biological Sciences, Macquarie University, NSW 2109 Australia

Abstract

A multitude of stormwater treatment devices, including constructed wetlands, are increasingly being retrofitted into existing urban catchments in an effort to improve urban waterway health. However, relatively limited data exists on the tangible ecological benefits of these systems. This knowledge gap was addressed by evaluating the ecological efficacy of three retrofitted stormwater wetlands in the Cooks River catchment, NSW, using a range of structural and functional indicators. Measures of treatment efficacy were derived *in situ* at the inlet and outlet zone of each wetland by measuring survival of transplanted *Paratya australiensis*, macroinvertebrate community composition, and organic matter decomposition (leaf litter and cotton), in association with physico-chemical measurements. Results indicate that of the three systems evaluated, the largest constructed wetland with respect to the contributing catchment area, treated stormwater to a standard that enabled high survival rates of *P. australiensis,* supported sensitive macroinvertebrate taxa, and reduced the breakdown of cotton strips. By contrast, wetlands that were considerably undersized relative to catchment area resulted in lesser ecological improvements. The findings demonstrate that appropriate design is paramount if the goal of improved ecological outcomes from stormwater treatment devices is to be achieved. The combination of biological monitoring methods used in this study, with the exception of leaf litter decomposition, provided simple, cost effective and sensitive tools to assess the performance of retrofitted stormwater wetlands to deliver improvements in ecological health.

Introduction

Waterways draining urban areas are commonly ecologically degraded (Paul & Meyer, 2001; Walsh et al., 2005), a situation that has been termed the 'urban stream syndrome' (Meyer et al., 2005). This syndrome is largely a consequence of urban stormwater runoff (Imberger et al., 2008) and is characterized by changes to water quality, hydrology, stream channel morphology and biotic richness (Paul & Meyer, 2001; Meyer et al., 2005). Impervious surfaces in urbanized areas increase the volume of runoff mobilising deposited pollutants, such as nutrients and metals, which can serve as chemical stressors in receiving waterways (Johnson et al., 2011). Assessment of the biological impacts of urban land use on waterways have shown toxicity to aquatic biota (Tucker & Burton, 1999; Schiff et al., 2003), reductions in the diversity of freshwater biota (e.g. algae, macrophytes, invertebrates and fish) (Paul & Meyer, 2001) and alterations to stream ecosystem processes (e.g. organic matter decomposition) (Chadwick et al., 2006; Imberger et al., 2008).

In an effort to mitigate the impacts of stormwater runoff on urban waterways, a range of stormwater treatment devices are being retrofitted to urban catchments (Knights et al., 2010). Constructed wetlands are one such device, and are becoming increasingly popular (Walker & Hurl, 2002). Constructed wetlands have the potential to regulate the quantity and constituent concentrations of stormwater runoff entering receiving waters, as well as providing ancillary benefits such as the provision of aquatic habitat (Malaviya & Singh, 2012). A few studies have examined the ability of field constructed stormwater wetlands to remove various pollutants from stormwater runoff e.g. (Scholes et al., 1998; Carleton et al., 2000; Walker & Hurl, 2002; Birch et al., 2004; Greenway, 2010; Hathaway & Hunt, 2010). However, only a limited number of published studies have assessed the capacity of stormwater wetlands to enhance aquatic biodiversity (Greenway, 2010; Moore & Hunt, 2011). Thus, the ecological benefits arising from stormwater treatment devices remains largely untested (Walsh, 2004; Ladson et al., 2006; Moore et al., 2011) despite considerable implementation costs (Knights et al., 2010). Ecological evidence is essential knowledge for integration into stormwater

management and to support arguments for the efficacy of stormwater wetlands and their wider implementation. Stormwater wetland performance, in terms of pollutant removal, is influenced by the size of the treatment area with respect to the contributing catchment area (Carleton et al., 2000). Wetlland size is therfore likely to influence biological improvements and to our knowledge has not been investigated.

While chemical analysis can provide an indication of the condition of a waterbody at a point in time, it does not provide an integrative assesment of the health of a waterbody such as is possible with biological measures (Karr, 1991). Given that contaminant-induced stress in the aquatic environment may be manifest throughout different levels of biological organisation (Clements, 2000), it is essential to examine multiple indicators across different levels in order to demonstrate effects (Peplow & Edmonds, 2005; Clements & Kiffney, 2009). The most effective approach to assess ecological integrity is to use multiple structural (e.g. water quality and macroinvertebrate diversity) and functional (e.g. organic matter decomposition) indicators (Young & Collier, 2009; Imberger et al., 2010). No previously published studies have evaluated the efficacy of constructed stormwater wetlands in this way.

The aim of this study was to evaluate the efficacy of three constructed stormwater wetlands to provide improvements to ecological health. We hypothesised that treatment of stormwater within a wetland would result in better ecological health outcomes at the outlet zone compared to the inlet zone. It was anticipated that the level of change between the inlet and outlet zones would be influenced by the size of the wetland with respect to the contributing catchment area, with the greatest effect evident in the wetland that was largest relative to its catchment area. The efficacy of the wetlands was assessed *in situ* at the organism level by measuring the toxicity of stormwater to caged *Paratya australiensis* (Decapoda; Atyidae), at the community level by assessing macroinvertebrate community composition, and at the ecosystem level by measuring ecosystem function via leaf litter and cotton strip decomposition, and comparing these measurements made at the inlet and outlet zones of each wetland. Physico-chemical parameters were also measured

at the inlet and outlet zone to assess the relationship of ecological responses with wetland treatment performance.

Methods

Study area

This study was conducted at three constructed stormwater wetlands located on the Cooks River catchment in the inner West of Sydney, NSW, Australia. The study was undertaken in 2011 between May and December.

Yarrowee wetland (0.12 ha) was retrofitted to a residential urban area in a suburb of Strathfield (33°53'S, 151°04'E) in 2010. The catchment area of the wetland is 2.9 ha. The size of the wetland in relation to the contributing catchment area is 4.1%, which is slightly below optimum (5-7%) for best practice design guidelines (Water by Design, 2009). A full description of this wetland site is provided in Chapter 2.

Gadigal Green wetland (0.02 ha) was retrofitted to a residential urban area in Darlington (33°55'S, 151°08'E) in 2008 (Figure 10). The catchment draining into the wetland consists of housing, gardens, residential roads and some street parking areas. Flows from the 5.4 ha catchment area are diverted from an existing drain into a bioretention system at the same time as the wetland; we therefore assumed that the wetland was treating stormwater runoff from 2.7 ha. A sedimentation pit is located at the entrance of the wetland. The wetland has a clay liner and the surface of the system is covered with 100 – 500 mm of topsoil and has a permanent top water level of 375 mm. An overflow weir is located at the outlet. Dense vegetation cover exists throughout the system and a detailed list of species planted in the wetland is provided in Appendix 4 (Figure A4.1). The size of the wetland in relation to the contributing catchment area is 0.7% (using the above assumption) and thus significantly undersized in relation to best practice design guidelines.

Coolibah Wetland (0.04 ha) was retrofitted to a residential urban area in Turrella (33°55'S, 151°08'E) and was restored in 2010 to treat stormwater from an estimated 9.68 ha catchment area (Figure 11). The residential catchment draining into the wetland consists of housing, gardens and residential roads. A gross

pollutant trap is located at the point of entry into the wetland to remove course sediment. Sandstone blocks provide scour protection and energy dissipation at the inlet entrance. A submerged berm was created in front of the inlet to dissipate energy and to assist in directing water flow. During restoration the soil was excavated to a depth of 200 mm and the level built back up with clean fill. The wetland consists of three zones: a marsh zone (covering at least 70% of the wetland area) with an operating depth of 200 mm; a pool zone located 200 mm below the marsh zone with an operating depth of 400 mm; and a periodically inundated zone with a variable top water level in excess of 500 mm (Dragonfly Environmental, 2009). An overflow pit with water level control is installed at the outlet. The facility contains well-established vegetation and a detailed list of species planted in the wetland is provided in Appendix 4 (Figure A4.2). The size of the wetland is significantly undersized in relation to the contributing catchment area at 0.4%.

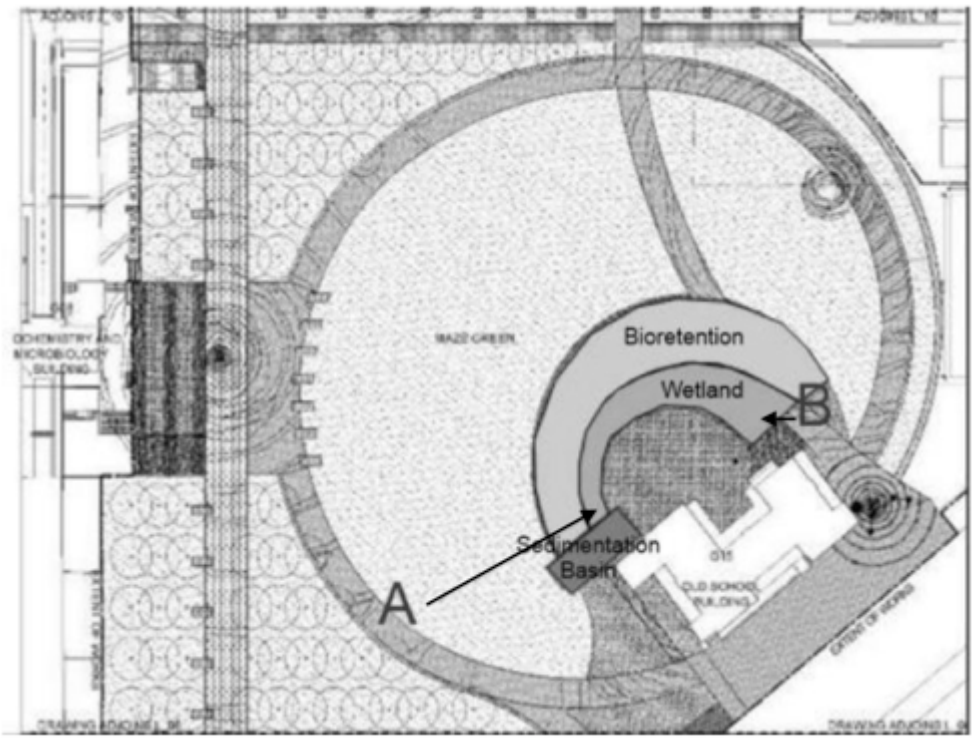


Figure 10. Gadigal Green Wetland configuration including sampling locations: A = Inlet sampling site and B = Outlet sampling site. Adapted from Thompson et al. (2011).

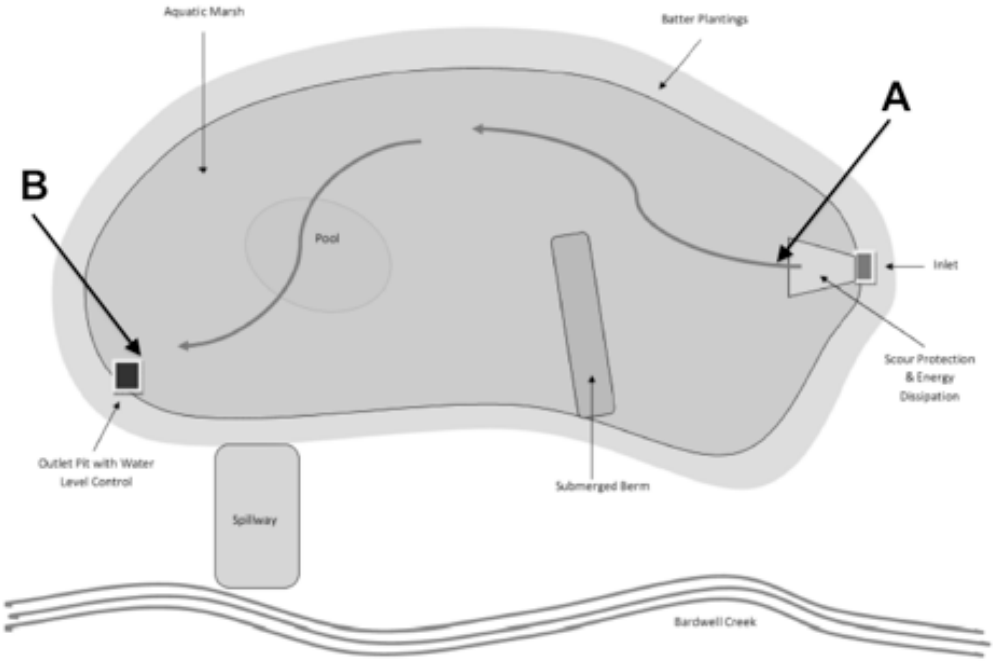


Figure 11. Schematic representation of Coolibah Wetland including sampling locations: A = Inlet sampling site and B = Outlet sampling site. Adapted from Dragonfly Environmental (2009).

Sample collection

Water and sediment samples were manually collected from the inlet and outlet zone of each wetland throughout the study period (May 2011 – December 2011). A single surface water grab sample and sediment sample was collected for analyses six times at Yarrowee wetland, three times at Gadigal Green wetland, and four times at Coolibah wetland. The samples were all collected as soon as possible after the start of a storm event, which was predicted using weather forecast and rain radar information provided by the Bureau of Meteorology (Commonwealth of Australia, 2015). Timing of grab sampling in relation to the storm event is not known; however, all samples were collected either during a storm event or when storm inflow was still occurring as per Hathaway & Hunt (2011). All of the plasticware used for sample collection was acid washed for 24 h using 10% (v/v) HNO_3 (AR grade; Chem-supply, Australia), rinsed (3x) with Milli-Q water, and dried in a laminar-flow cabinet prior to storage in sealed plastic bags until use. Water samples were collected into pre-conditioned bottles for chemical analysis, with the sample for dissolved metals filtered using a 0.45 µm Sartorius filter. A 1-L sample of sediment was scraped from the surface sediment layer of

the wetlands (~ top 2 cm) and from this, two 150-mL sub-samples were delivered into glass jars for analysis.

Australian Laboratory Services (ALS), Sydney, (National Association of Testing Authorities accredited) analysed the water samples for total nitrogen (TN) and total phosphorus (TP) using the standard method APHA 4500 (APHA, 1999), and filterable metals (Cu, Pb and Zn) by ICP-MS using methods APHA 3125 (APHA, 2005) and USEPA 6020 (USEPA, 1992). Sediment samples were analysed by ICP-AES for total particulate Cu, Zn and Pb using methods APHA 3120 (APHA, 1999) and USEPA 6010 (USEPA, 1992). Replicate samples were not analysed due to cost constraints. However, laboratory quality control testing undertaken by ALS consisted of laboratory duplicates, method blanks, laboratory control spikes and matrix spikes. Relative percentage differences (RPDs) for laboratory duplicates were within the permitted range, method blanks were below detection limits, and acceptable recovery (%) of laboratory control spikes and matrix spikes were observed (a summary of the QC results are given in Appendix 4; Table A4.1).

Measurements of temperature, conductivity, dissolved oxygen (DO) and pH were measured using a multiparameter meter (YSI ProPlus, YSI Incorporated, USA). The meter was calibrated for DO in water-saturated air and against YSI standard solutions for pH (pH 4,7 and 10 buffers) and conductivity (10,000 uS/cm solution). Turbidity was measured using a portable meter (HI 93703, Hanna Instruments®, USA) that was calibrated against three standard solutions (0, 10 and 500 NTU).

In situ toxicity testing with Paratya australiensis

The glass shrimp, *Paratya australiensis,* is common throughout southeastern Australia, where it inhabits both freshwater and estuarine environments (Walsh & Mitchell, 1995). Shrimp form a key component in Australian aquatic ecosystems by providing an important food source (Richardson et al., 2004) and processing detrital material (March et al., 2001). *Paratya australiensis* are frequently used in Australian toxicity tests (e.g. Daly et al., 1990; Hose & Wilson, 2005; Thomas et al., 2008; Kumar et al., 2010).

Adult specimens of *P. australiensis* (1.5 – 2 cm in length) were obtained from Aquablue seafoods (Pindimar, Australia). Prior to deployment, shrimp were housed for 14 days in an aquarium containing aged tap water and fed daily with Hikari® algae wafers. Photoperiod was kept constant on a 16 h light: 8 h dark cycle and the temperature maintained at 23 ± 1°C. Prior to *in situ* exposures, acclimation to lower field temperatures was initiated in a temperature-controlled room at a rate of 1°C change per hour.

In situ chambers (Figure 12A) were constructed using opaque polyvinyl chloride (PVC) tubing (9 x 10 cm). To enable water flow through the chamber, two rectangular openings (6 x 8 cm) were cut on opposing sides of the tube and covered with 1000 µm fiberglass mesh using acetic-cure silicon sealant. A PVC closure, secured with cable ties, was used to cap the top of the chamber. The base of the tube was secured with a PVC screw cap, which facilitated the addition of organisms to the chamber and the monitoring of survival during the incubation period. A hole with a diameter of 7.5 cm was cut into the screw cap and covered with fiberglass mesh (1000 µm) to ensure exposure of the organisms to the sediment and the ability to feed. In the laboratory, five individuals were randomly allocated to each of the *in situ* chambers and then transported to the field inside a portable cooler containing culture water. Three randomly selected cages were placed on the sediment surface of the inlet and outlet zone of each wetland for 96 h (Figure 12B). Surviving organisms were counted on-site every 24 h for four days and any dead shrimp removed. Exposures using *P. australiensis* were repeated six times at Yarrowee wetland, three times at Gadigal Green wetland, and four times at Coolibah wetland. Rainfall events occurred during all of the *in situ* tests and were predicted using weather forecast and rain radar information provided by the Bureau of Meteorology (Commonwealth of Australia, 2015). Total rainfall amounts during the *in situ* tests, obtained from weather stations within 5 km away from wetland sites, ranged from 5 to 149 mm for Yarrowee wetland (Strathfield Golf Club weather station), 6 to 235 mm for Coolibah wetland (Sydney Airport weather station) and 6 to 62 mm for Gadigal Green wetland (Sydney Observatory Hill weather station) (Commonwealth of Australia, 2015). Shrimp were replaced

and chambers acid washed for 24 h using 10% (v/v) HNO_3 (AR grade; Chem-Supply, Australia) prior to each exposure period.

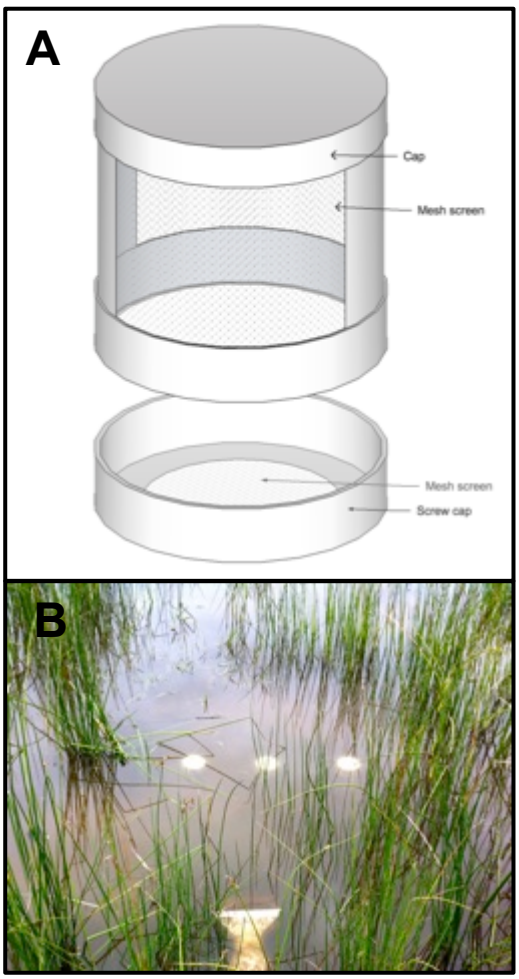


Figure 12. (A) Test chamber design (B) Chambers placed on the sediment surface.

Macroinvertebrate sampling

Two sampling surveys were undertaken in autumn 2011 and spring 2011 during dry weather conditions. Macroinvertebrate collection was based on a wetland rapid bioassessment sampling protocol (Davis et al., 1999). Sampling incorporated four sites in both the inlet and outlet zone of each wetland, which corresponded to the four sectors of the compass: north, south, east and west. In each sector, samples were collected from a minimum of two habitats (open water; emergent sedges/rushes; and submerged macrophytes) for a standardised period of time (two minutes) using a sweep net (0.12 m^2 opening; 250 μm mesh size). In open water and submerged macrophyte habitats, the sweep net was moved in a zigzag

motion from the water surface to the sediment bed. In the emergent sedges/rushes habitat the net was forced through the base of the plant to the water surface. Samples were live picked in the field on a sorting tray for 30 minutes and specimens preserved in 70% ethanol. Collected specimens were identified in the laboratory under a dissection microscope to family level with the exception of Oligochaeta (Class), Turbellaria (Order) and Chironomidae (Sub-family) (Gooderham & Tsyrlin, 2002; Madden, 2010). Family-level identification of freshwater macroinvertebrates has been found to be suitable for detecting water pollution impacts (Wright et al., 1995; Davies et al., 2010)

Organic matter decomposition

Leaf litter and cotton strip assays were used to assess organic matter decomposition, a method that has been used previously by Tiegs *et al.,* (2007).

For the leaf litter bioassay, leaves of *Eucalyptus sideroxylon* (Red Ironbark) and *Eucalyptus grandis* (Flooded Gums) were collected from an uncontaminated source and oven-dried at 50°C for 72 hours. Approximately 6 g ± 0.2 g of dried leaves were weighed and placed into PVC cages with a mesh size of 5 mm. These two species were chosen because they are both native and are representative of the natural, eucalypt-dominated vegetation in the study catchment originally and currently as street trees, and were collected on Macquarie University campus.

For the cotton strip bioassay, strips (4.5 cm wide x 30 cm long) were cut from unbleached calico (Lincraft Pty Ltd, Miranda, NSW, Australia) and encased in a 5 mm PVC mesh for support. The strips and casing were prepared in the laboratory, and sterilised at 121 °C for 15 minutes, and kept sterile until deployment.

In the field, five replicate leaf litter bags and five replicate cotton strips were submerged in the inlet and outlet zone of each wetland for 56 and 28 days, respectively. Bags were fixed at each site with PVC-coated wire. Following deployment the mesh bags containing the leaf litter and cotton strips were observed to eventually sink down to the surface of the wetland sediment. After

retrieval, each leaf litter bag and cotton strip was placed in a separate plastic bag, and stored on ice at 4°C during transport to the laboratory (2 hours).

In the laboratory, leaves and leaf fragments were carefully removed from the bags with forceps, and transferred into white trays where they were cleaned with tap water to gently wash-off any adhering debris. Cleaned leaf litter was oven-dried at 50°C for 48 h and then weighed to determine the remaining dry mass once cooled.

In the laboratory, cotton strips were removed carefully from the bags with forceps and placed into a shallow plastic tray containing several centimeters of tap water. Each side of the cotton strip was cleaned using a soft-bristled paintbrush to remove adhering debris. Cleaned cotton strips were soaked in 70% ethanol to inhibit microbial decay during storage, then air dried, and stored individually in small plastic bags until determination of tensile strength. For the determination of the extent of decomposition, each cotton strip was cut into three 1-cm wide length strips and individually extended using a Tensiometer (Instron 5542) at a rate of 50 mm min^{-1} until they broke (software: Bluehill 2). The load (mN) required to break the sub-samples was the measure of tensile strength. Pre-incubation tensile strength of the strips was determined using ten randomly selected cotton strips, which were soaked in ethanol and air dried before measurements as a procedural control.

Data analysis

Data from each wetland was analysed separately. Concentrations of measured environmental variables from the inlet and outlet were compared to ANZECC/ARMCANZ (2000a) guidelines. Principal Coordinates Analysis (PCO) was used to examine patterns in the chemical composition of water and sediment samples taken from the inlet and outlet of each wetland. This analysis was based on a Euclidean distance resemblance matrix of normalised environmental data (Clarke & Ainsworth, 1993). PCO is a metric multidimensional scaling method used to visualise dissimilarities of data by using spectral decomposition (calculation of a series of eigenvalues and eigenvectors) to approximate a matrix

of dissimilarities by the distances between a set of points in few dimensions (Gowler, 2015).

The number of surviving shrimp at the end of each 96 h exposure period was calculated as a percentage and arcsine transformed prior to statistical analysis. To explore the effects of location (i.e. inlet and outlet) on *P. australiensis* survival a paired *t*-test was performed for each exposure period. Pearson's correlation was used to determine associations between shrimp survival and water and sediment environmental variables.

To compare macroinvertebrate assemblages between the inlet and outlet of each wetland, two-dimensional non-metric multidimensional scaling (nMDS) ordinations were generated from a Bray-Curtis similarity matrix of square root transformed abundance data (Clarke & Green, 1988; Clarke, 1993). Data were grouped, by location (i.e. inlet and outlet) and compared using one-way analysis of similarity (ANOSIM) (Clarke, 1993) to test for significant differences in the macroinvertebrate assemblages among samples. The influence of particular taxa in separating the inlet zone from the outlet zone was quantified using the similarity percentage (SIMPER) procedure (Clarke, 1993). To determine if any of the water and sediment variables were related to the observed macroinvertebrate assemblage patterns, a stepwise distance based linear model (DistLM) (McArdle & Anderson, 2001) was used. This analysis was based on the Bray-Curtis similarity matrix of the square-root transformed biological data and normalised environmental data (Clarke & Ainsworth, 1993). Three biotic indices were calculated for each macroinvertebrate sample: taxonomic richness (Margalef's index) (Margalef, 1958; Rosenberg & Resh, 1993), Shannon diversity index (H) (Krebs, 1989), and SIGNAL 2 (Stream Invertebrate Grade Number Average Level) biotic index (Chessman, 2003). A paired *t*-test was used to investigate whether biotic indices differed according to location (i.e. inlet and outlet) at each wetland.

The loss of leaf litter mass was expressed as a percentage of the original mass. Exponential decay coefficients (*k*) were calculated for leaf litter mass loss (%) and

cotton strip tensile strength loss by fitting an exponential decay model, as per Tiegs et al. (2007):

$$X_t = X_0 e^{-kt}$$

Where :

t = exposure time (in days)

X_t = leaf dry weight (g) or cotton strip tensile strength (mN) when removed at time t

X_0 = initial leaf dry weight (g) or cotton strip tensile strength (mN)

K = decay rate (day $^{-1}$)

An average exponential decay rate was calculated for both leaf litter mass and cotton strip tensile strength loss by averaging the decay rate across all of the replicates. A paired *t*-test was used to test for differences between rates of decay loss at the inlet and outlet zone of each wetland for all metrics. Where significant differences existed, Pearson's correlation coefficients were used to determine associations between decomposition and surface water environmental variables.

All multivariate analysis were performed using PRIMER version 6+ (PrimerE Ltd, Plymouth, UK) and univariate analysis were done using Minitab 16 (Minitab Inc, USA). Variables that had non-normal distributions were $\log_{10}$ (x) transformed and percentage data were arcsine transformed prior to analysis. The significance level for all analysis was 0.05.

Results

Water and Sediment quality analysis

A summary of the water and sediment quality variables measured during the study at the inlet and outlet zone of Yarrowee, Coolibah and Gadigal Green wetland are presented in Table 10. A marked improvement in water and sediment quality from the inlet to the outlet zone at Yarrowee wetland was observed for all measured parameters, with lower mean pollutant concentrations observed in the outlet compared to the inlet. At the Gadigal Green wetland, concentrations of most variables were also lower at the outlet than the inlet. There were less marked improvements in water quality between the inlet and outlet zone at Coolibah wetland compared to Yarrowee and Gadigal Green wetlands (Table 10).

Despite improvements at Yarrowee wetland, mean concentrations of TP, Cu, Zn and DO in surface water were above the Australian water quality guideline trigger values at the outlet, but TN and Zn concentrations reached compliant levels. At Gadigal Green wetland mean concentrations of TN, Cu, Zn, and DO in surface water were above the Australian water quality guideline trigger values at the outlet, but TP and Pb were compliant. At Coolibah wetland mean concentrations of TN, TP, Cu, Pb, Zn and DO in surface water were above the Australian water quality guideline trigger values at the outlet. Water turbidity and conductivity values declined between the inlet and outlet of all three wetlands. The pH of surface waters remained similar across zones at Yarrowee and Gadgidal Green wetland, but decreased between the inlet and the outlet at Gadigal Green wetland. However, pH values were not at a level expected to affect aquatic biota at all sites (Table 10).

At Yarrowee wetland sediment Cu, Pb and Zn concentrations were below the recommended Australian sediment quality guidelines, with the exception of sediment Pb concentrations at the inlet. Higher concentrations of sediment Pb, Zn and Cu were recorded in the outlet zone of Coolibah wetland in comparison to the inlet and exceeded recommended Australian sediment quality guidelines for Pb and Zn. Inlet sediment concentrations of Cu, Pb and Zn were highest at Gadigal

Green wetland in comparison to other inlet zones, but were lower and below Australian sediment quality guidelines at the outlet, with the exception of Pb (Table 10).

The PCO analysis showed that there was a clear separation in water and sediment quality between the inlet and outlet zone at Yarrowee wetland and Gadigal Green wetland, with the outlet zones having greater similarity among samples compared to the inlet zone (evidenced by tighter clustering of symbols in Figure 13A,B). The separation in water and sediment quality between the inlet and outlet zone at Coolibah wetland was less clear compared to the other two wetlands (Figure 13C). The first two axes of the PCO plots explained 73.7%, 87.2 and 65.7% of the variation in the chemical composition of Yarrowee, Gadigal Green and Coolibah wetland, respectively. Separation of the wetland inlet and outlet zone at Yarrowee wetland was driven by higher concentrations of dissolved oxygen in the outlet than the inlet and the opposite trend for other pollutants in both the sediment and surface waters (Figure 13A). At Gadigal Green wetland, separation of the sites was driven by lower concentrations of all measured environmental variables in the inlet than the outlet (Figure 13B). At Coolibah wetland, separation of the sites was driven by higher concentrations of dissolved oxygen and concentrations of sediment Pb, Zn and Cu in the outlet than the inlet (Figure 13C).

Table 10. Mean, minimum and maximum of water and sediment quality variables from the inlet and outlet of Yarrowee wetland (YW) (n=6) Gadigal Green wetland (GW) (n=4) and Coolibah wetland (CW) (n=3). Variables that exceed Australian guidelines are shown in bold.

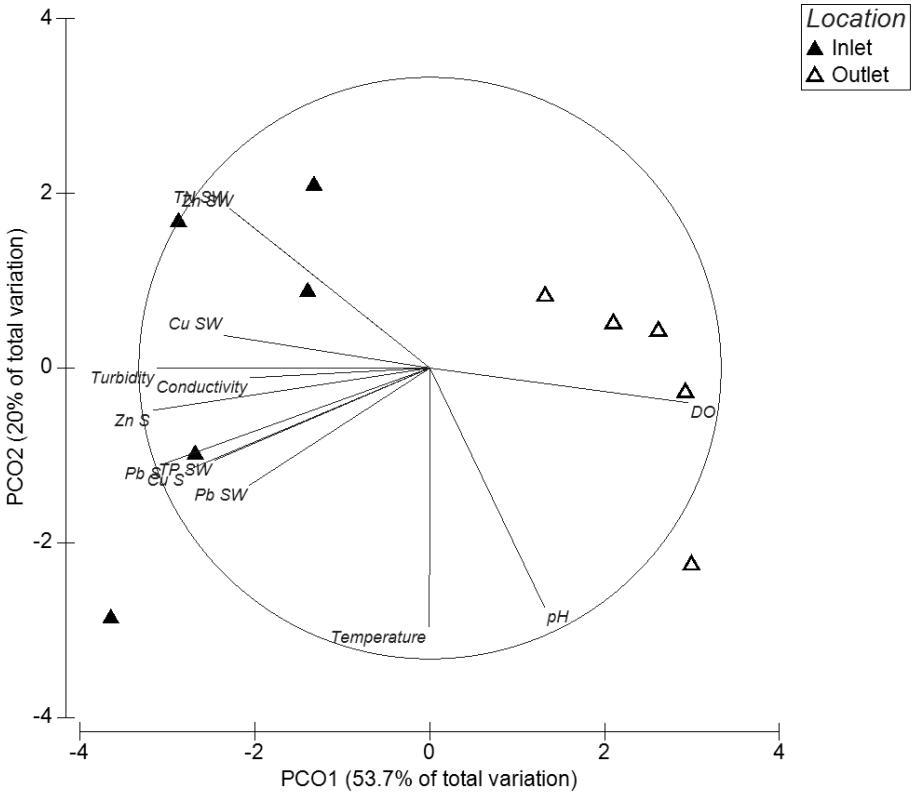
Variable	Units	Mean Concentration (Min - Max)						Australian Guidelines
		YW Inlet	YW Outlet	GW Inlet	GW Outlet	CW Inlet	CW Outlet	
TN SW	mg L^{-1}	**1.7** ± 0.3	0.5 ± 0.1	**2.5** ± 0.4	**0.6** ± 0.03	**2.2** ± 0.5	**1.1** ± 0.3	0.5[A]
TP SW	mg L^{-1}	**0.13** ± 0.04	**0.06** ± 0.02	**0.21** ± 0.07	0.05 ± 0.02	**0.21** ± 0.04	**0.20** ± 0.09	0.05[A]
Cu SW	mg L^{-1}	**0.006 ±** 0.001	**0.004** ± 0.001	**0.13** ± 0.05	**0.013** ± 0.002	**0.009** ± 0.003	**0.005** ± 0.001	0.001[B]
Pb SW	mg L^{-1}	0.003 ± 0.001	0.001 ± 0.0002	**0.005** ± 0.001	0.001 ± 0.0002	**0.004** ± 0.001	**0.005** ± 0.003	0.003[B]
Zn SW	mg L^{-1}	**0.058** ± 0.03	**0.017** ± 0.006	**0.169** ± 0.01	**0.134** ± 0.04	**0.065** ± 0.018	**0.051** ± 0.009	0.008[B]
DO	(%)	**32** ± 5	**79** ± 5	**28** ± 3	**11** ± 5	**25** ± 10	**56** ± 12	85 - 110[A]
pH		6.9 ± 0.3	7.1 ± 0.1	7.0 ± 0.08	6.7 ± 0.04	6.9 ± 0.1	6.7 ± 0.2	6.5-8.0[A]
Turbidity	NTU	13.5 ± 1.0	4.5 ± 1.0	4.8 ± 0.9	2.8 ± 0.6	9.6 ± 2.8	3.3 ± 1.2	na
Conductivity	μS/cm	776 ± 150	360 ± 31	251 ± 30	206 ± 18	242 ± 61	78 ± 22	na
Temperature	°C	16.4 ± 1.1	16.5 ± 1.3	18.7 ± 1.1	18.8 ± 1.5	15.2 ± 1.9	14.6 ± 1.7	na
Cu S	mg kg^{-1} dry weight	15 ± 4	2.1 ± 0.4	**784** ± 27	18 ± 1	39 ± 4	54 ± 3	65 - 270[C]
Pb S	mg kg^{-1} dry weight	**55** ± 12	bdl	**140** ± 6	**102** ± 3	**50.5** ± 12.4	**174** ± 10	50 - 220[C]
Zn S	mg kg^{-1} dry weight	147 ± 20	9 ± 1	**937** ± 15	112 ± 5	**219** ± 46	**366** ± 20	200 - 410[C]

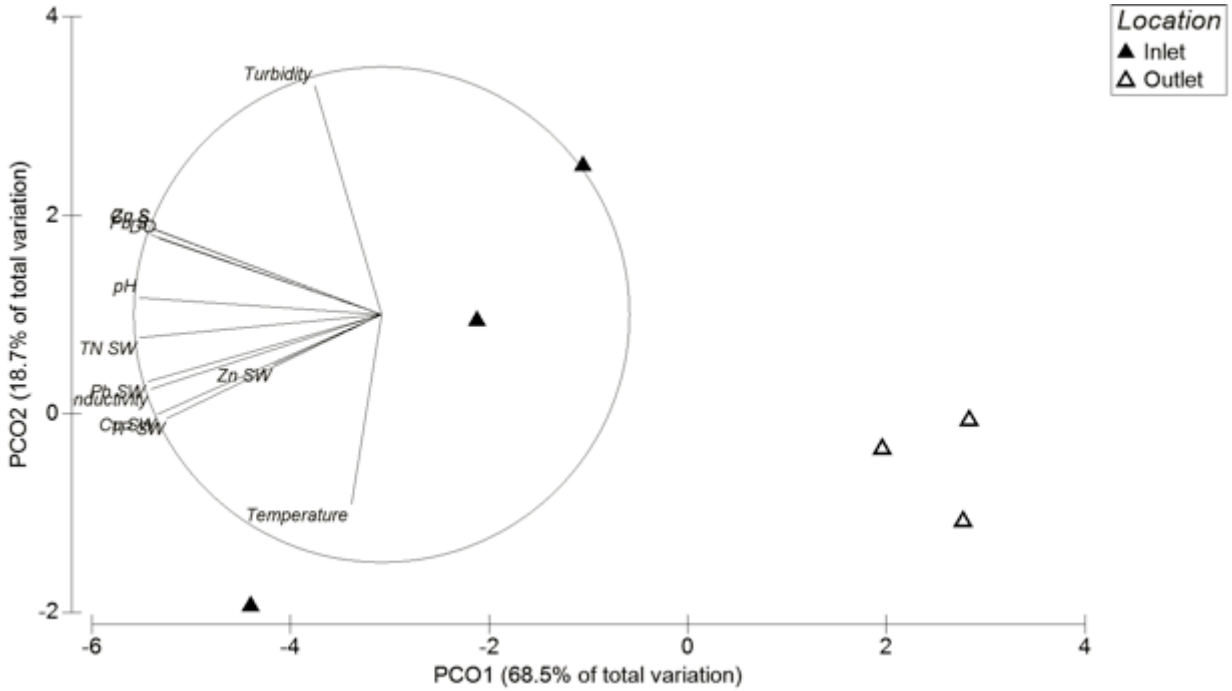
bdl = below detection limit; na = not available; SW = Surface Water; S = Sediment; YW = Yarrowee Wetland; CW = Coolibah Wetland

[A] ANZECC/ARMCANZ (2000a) Trigger values for aquatic ecosystems in lowland rivers in south-east australia

[B] ANZECC/ARMCANZ (2000a) Trigger values for protection of 95% of freshwater biota

[C] ANZECC/ARMCANC (2000a) Recommended Interim Sediment Quality Guidelines (ISQG) for aquatic ecosystems


Location
Inlet
Outlet
Tot SW
Zn SW
Cu SW
Turbidity
Conductivity
Zn S
Pb S
TP SW
Cu S
Pb SW
DO
pH
Temperature
PCO2 (20% of total variation)
PCO1 (53.7% of total variation)
4
2
0
-2
-4


Location
Inlet
Outlet
Turbidity
Zn S
Pb S
pH
TN SW
Pb SW
Zn SW
Cu SW
Temperature
PCO2 (18.7% of total variation)
PCO1 (68.5% of total variation)
4
2
0
-2
-6
-4
-2
0
2
4

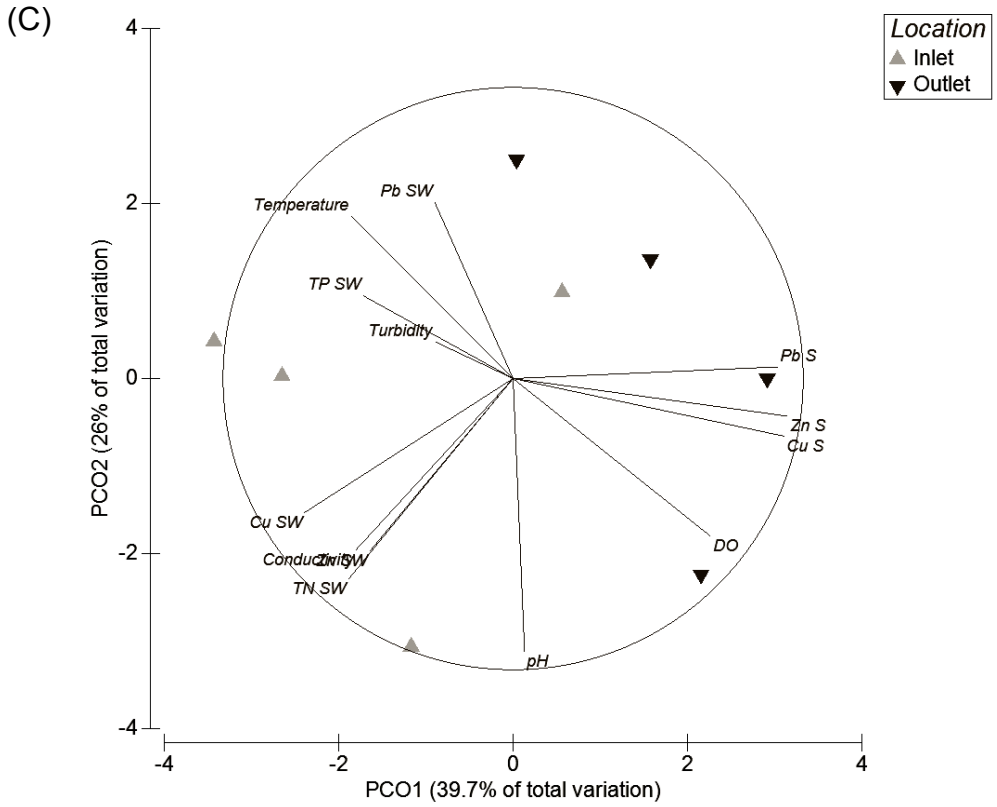


Figure 13. Principal coordinates analysis (PCO) of the inlet and outlet zone based on measured water and sediment quality variables. (A) Yarrowee wetland; (B) Gadigal Green wetland; (C) Coolibah Wetland. SW = surface water; S = sediment; DO = dissolved oxygen; TN = Total Nitrogen; TP = Total Phosphorus.

***Organism level response: In-situ exposures using* P. australiensis**

Raw survival data for *P. australiensis* is presented in Appendix 4 (Table A4.2). Survival of *P. australiensis* at Yarrowee Wetland was significantly higher at the wetland outlet compared to the wetland inlet for all six *in situ* tests ($p < 0.05$ in all cases; Figure 14A). Survival of *P. australiensis* was low in the wetland inlet, with mean 96 h survival ranging from 0 ± 0% to 27 ± 13%. In contrast, shrimp survival remained consistently high at the wetland outlet, ranging from 93 ± 7% to 100 ± 0% (Figure 14A).

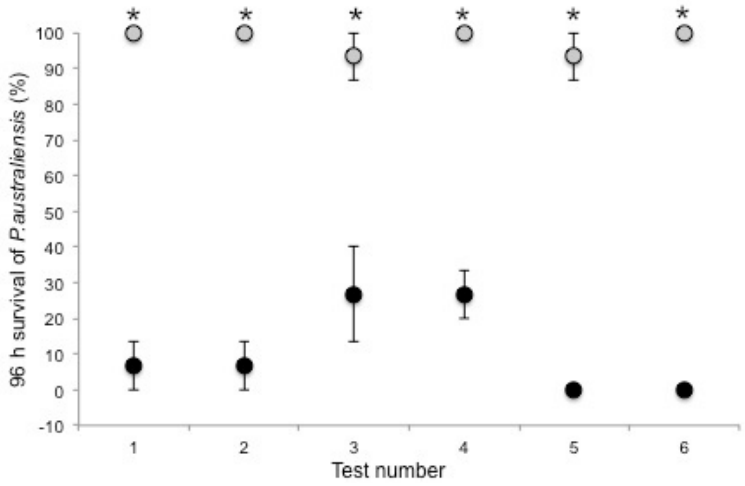
Survival of *P. australiensis* at the outlet of Gadigal Green wetland was poor, with higher survival observed at the inlet than the outlet zone on occasion. Mean 96 h survival at the wetland inlet and outlet ranged from 0 ± 0% to 53 ± 29% and 0 ±

0% to 20 ± 12%, respectively. There was no significant difference in the survival between the two sites for all three *in situ* tests (Test 1: t = -1.72, p = 0.228; Test 2: t = 1.23, p = 0.344; Test 3: t = 1.00, p = 0.424) (Figure 14B).

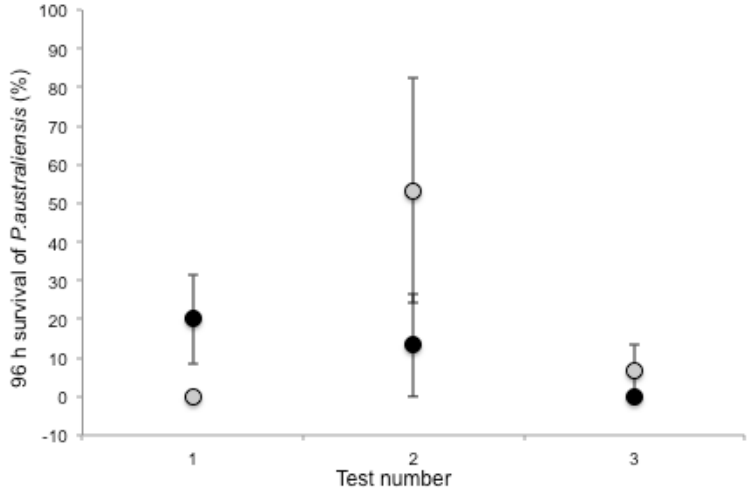
Survival of *P. australiensis* at Coolibah wetland was higher at the outlet compared to the inlet. The difference in survival between the two sites was significant for three out of the four *in situ* tests ($p < 0.05$ for exposures 1, 2 and 3). There was no significant difference in shrimp survival between the inlet and outlet for exposure period 4 (t = -2.00, p = 0.184). Mean 96 h survival of *P. australiensis* in the wetland inlet and outlet ranged from 0 ± 0% to 60 ± 0% and 13 ± 12% to 93 ± 12%, respectively; highlighting the large variability in the survival of *P. australiensis* over the different exposure periods at Coolibah wetland (Figure 14C)

Survival of *P. australiensis* after 96 h in Yarrowee wetland was significantly correlated with a number of measured variables in the inlet and outlet of the wetland (Table 11). Significant negative correlations were observed with surface water TN, TP, filterable Zn and turbidity. For the sediment, significant negative correlations were seen for Cu, Pb and Zn. A significant positive correlation was observed with surface water DO. Survival of *P. australiensis* after 96 h in Coolibah wetland and Gadigal Green wetland was negatively correlated with surface water TN, TP, filterable Cu, Pb and Zn concentrations; however these associations were not significant. Significant positive correlations with surface water DO and sediment Cu, Pb and Zn concentrations were observed at Coolibah wetland. The highest correlation at Gadigal Green wetland was noted for dissolved oxygen, however this was not significant (Table 11).

(A)



(B)



(C)

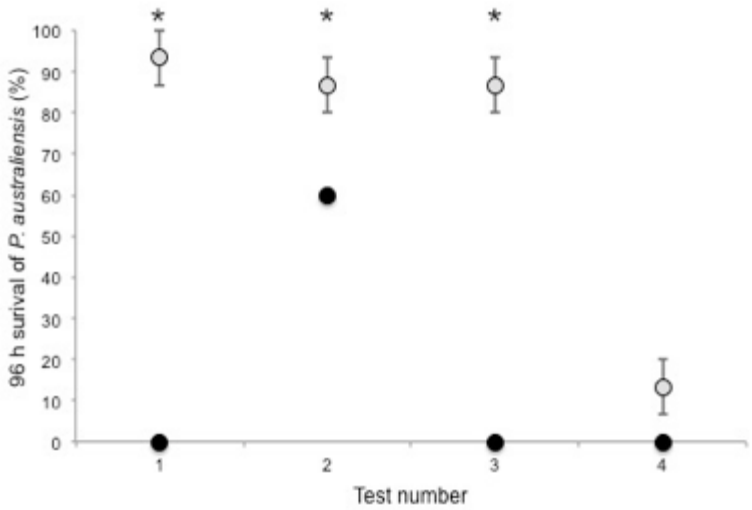


Figure 14. Mean (± SE) 96 h survival of *in situ* exposed *Paratya australiensis* in the wetland inlet (dark circles) and outlet zone (light circles) of (A) Yarrowee wetland, (B) Gadigal Green wetland and (C) Coolibah wetland. Asterisks (*) indicate significant differences in the survival between the inlet and outlet (paired *t*-test: $p < 0.05$).

Table 11. Pearson correlation coefficients (*r*) between 96 h shrimp survival and measured physical and chemical variables in Yarrowee wetland (YW), Gadigal Green wetland (GW) and Coolibah wetland (CW).

Variable	Units	Correlation with 96 h survival		
		YW *r*	GW *r*	CW *r*
TN SW	mg L^{-1}	−0.741*	−0.085	−0.356
TP SW	mg L^{-1}	−0.667*	−0.348	−0.513
Cu SW	mg L^{-1}	−0.306	−0.199	−0.294
Pb SW	mg L^{-1}	−0.369	−0.206	−0.665
Zn SW	mg L^{-1}	−0.627*	−0.001	−0.196
DO SW	(%)	0.743*	0.766	0.835*
pH SW	−	0.241	0.383	−0.086
Turbidity	NTU	−0.709*	−0.522	−0.479
Cond SW	μS/cm	−0.179	0.233	−0.552
Temperature	°C	−0.136	0.138	−0.469
Cu S	mg kg^{-1}	−0.699*	0.410	0.881*
Pb S	mg kg^{-1}	−0.83*	0.532	0.881*
Zn	mg kg^{-1}	−0.82*	0.388	0.881*

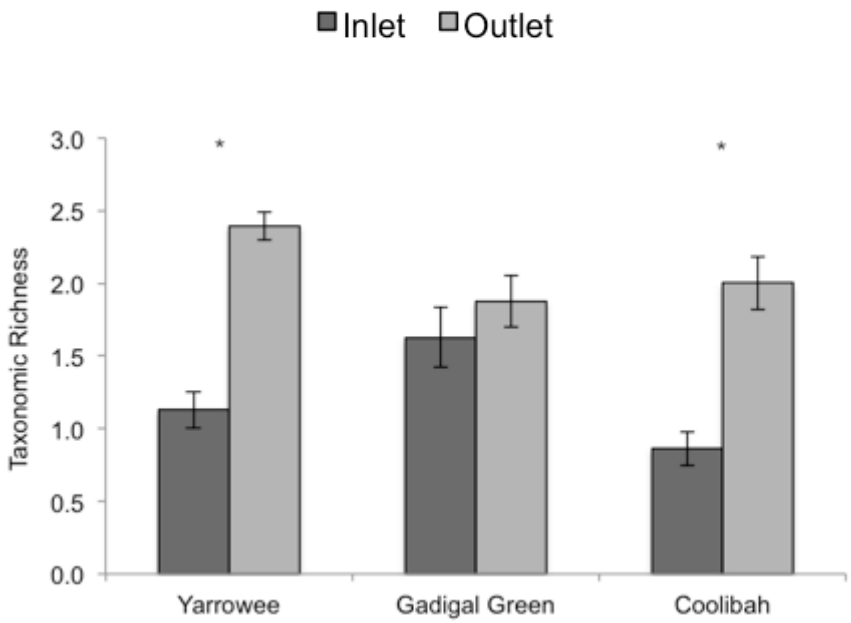
* Significant at $p < 0.05$

Community level response: Macroinvertebrates

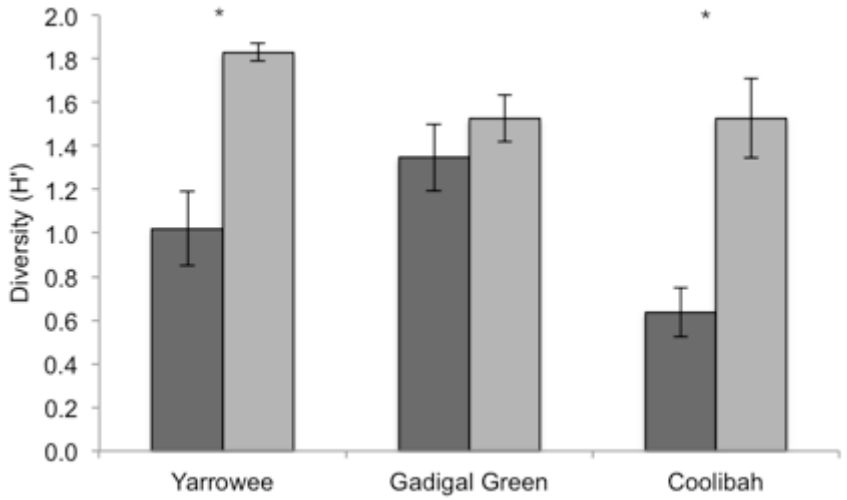
A summary of the macroinvertebrates collected in the inlet and outlet zone of all three wetlands is presented in Appendix 4 (Table A4.3 – A4.5). In total, 25, 20 and 26 taxa were recorded at Yarrowee, Gadigal Green and Coolibah wetlands, respectively, with more taxa recorded at the outlet zone in comparison to the inlet zone for all three wetlands. The taxonomic composition in the wetlands was dominated by Insecta, the majority of which were collected from the outlet zone (Yarrowee 88%; Gadigal Green: 93% Coolibah: 83%) in comparison to the inlet zone (Yarrowee 53%; Gadigal Green 67%; Coolibah 39%). Ephemeroptera (Baetidae) and Trichoptera (Leptoceridae) were only recorded at the outlet of Yarrowee wetland and were absent from the other two wetlands.

All biotic indices (taxonomic richness, Shannon Diversity index and SIGNAL 2) were higher at the outlet zone compared with the inlet zone at all three wetlands; however, differences were only significant for Yarowee wetland (richness: t = -8.06, p = <0.001; diversity: t = -4.64, p = 0.002; SIGNAL 2: t = -7.60, p = <0.001) and Coolibah wetland (richness: t = -5.27, p = <0.001; diversity: t = -4.15, p = 0.002; SIGNAL 2: t = -4.35, p = 0.001) (Figure 15).

(A)



(B)



(C)

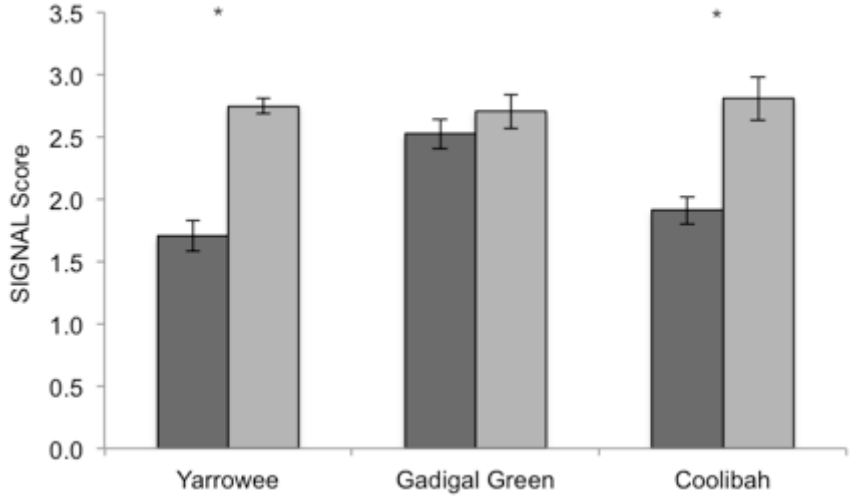


Figure 15. Biotic indices (A) taxonomic richness, (B) Shannon diversity index and (C) SIGNAL score in the inlet (dark bars) and outlet (light bars) zone of each wetland (Yarrowee, Coolibah and Gadigal Green). Values reported as mean ± SE (n = 8 for both the inlet and outlet of each wetland). Asterisks (*) indicate significant differences in the biotic index between the inlet and outlet (paired *t*-test: $p < 0.05$).

The macroinvertebrate communities at the inlet of all three wetlands were significantly different from those in the outlet zone, with the difference in community structure between the two locations varying more strongly at Yarrowee than the other wetlands (Yarrowee wetland: global $R = 1$, $p = 0.001$; Gadigal Green wetland: global $R = 0.906$, $p = 0.003$; Coolibah wetland: global R = 0.995, $p = 0.003$). These results are reflected in the nMDS ordination plots, which showed

the inlet site samples clustering separately from the outlet site samples for each of the three wetlands (Figure 16).

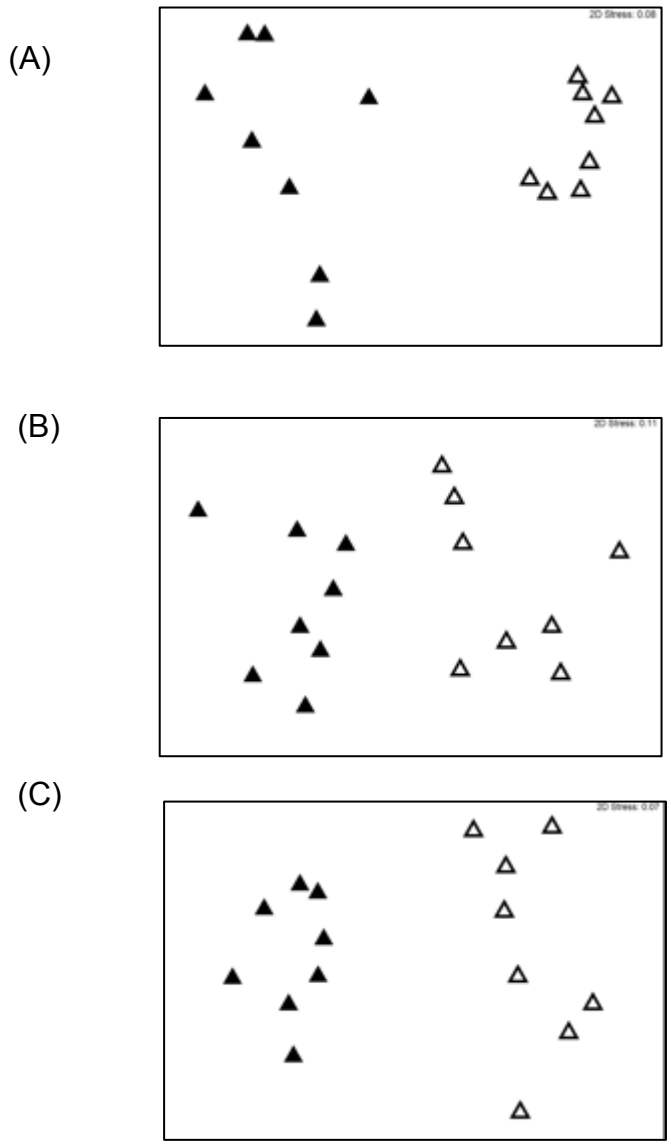


Figure 16. Two-dimensional nMDS ordinations of macroinvertebrate community samples collected from (A) Yarrowee wetland, (B) Gadigal Green wetland and (C) Coolibah wetland grouped by the inlet (black triangles) and outlet (un-shaded triangles).

Analysis using SIMPER showed that the average dissimilarity in macroinverterbate composition between the inlet and outlet at Yarrowee, Gadigal Green and Coolibah wetlands was 82%, 71% and 70%, respectively. The three taxa contributing most to the dissimilarity between the inlet and outlet zone at each wetland, in order of decreasing influence were: Protoneuridae, Culicidae and Oligochaeta at Yarrowee wetland, which together contributed 36% to the observed

dissimilarity; Oligochaeta, Culicidae and Chironominae at Gadigal Green wetland, which together contributed 43% to the dissimilarity; and Oligochaeta, Chironominae and Aeshinidae at Coolibah wetland, which together contributed 52% to the dissimilarity. All of these taxa, except Protoneuridae and Aeshnidae, were more abundant in the inlet zone than the outlet zone (Table 12).

Individually, all environmental variables were significantly correlated ($p < 0.05$) with the observed macroinvertebrate community structure except for pH and temperature at Yarrowee wetland, and surface water Cu and Zn concentrations at Coolibah wetland. Significant environmental variables each explained between 20 to 50%, 19 to 39%, and 18 to 46% of the variation in the macroinvertebrate community data at Yarrowee, Gadigal green and Coolibah wetlands, respectively (Table 13). The stepwise selection model indicated that macroinvertebrate community structure responded significantly to a few environmental variables within the inlet and outlet zone at each wetland, these were, in order of most influential: DO, sediment Pb and sediment Cu at Yarrowee wetland, which together explained 71% of the variation in the macroinvertebrate community structure; DO and surface water Zn at Gadigal Green wetland, which together explained 54% of the variation; and sediment Pb, surface water TP and sediment Zn at Coolibah wetland, which together explained 75% of the variation (Table 13).

Table 12. SIMPER results: Percent contribution and average abundance of influential taxa between the inlet and outlet of Yarrowee wetland, Gadigal Green wetland and Coolibah wetland.

	Average Abundance Inlet	Average Abundance Outlet	Contribution (%)	Cumulative (%)
Yarrowee Wetland				
Protoneuridae	0.0	7.2	14	14
Culicidae	6.6	0.5	11	25
Oligochaeta	5.9	0.5	11	36
Notonectidae	0.1	4.7	9	45
Libellulidae	0.3	3.8	7	52
Corixidae	1.1	3.6	6	59
Chironominae	4.2	2.9	5	64
Coenagrionidae	0.0	2.2	4	68
Glossiphoniidae	2.6	1.2	4	72
Tanypodinae	0.0	1.9	4	76
Baetidae	0.0	1.7	3	80
Collembola	0.6	1.3	3	82
Planorbidae	1.2	0.0	3	85
Physidae	1.2	0.7	3	88
Aeshnidae	0.0	1.2	2	90
Lymnaeidae	0.0	1.1	2	92
Gadigal Green wetland				
Oligochaeta	3.5	0.0	18	18
Culicidae	3.1	0.2	15	33
Chironominae	2.4	0.6	10	43
Libellulidae	1.0	2.5	7	51
Lymnaeidae	0.0	1.4	7	58
Coenagrionidae	2.2	2.2	7	65
Aeshnidae	1.6	0.8	7	72
Protoneuridae	0.5	0.8	4	76
Scirtidae	0.9	0.6	4	80
Hydrophilidae	0.7	0.1	4	84
Mesoveliidae	0.0	0.9	4	87
Hydraenidae	0.5	0.4	3	90
Coolibah wetland				
Oligochaeta	10.2	2.8	26	26
Chironominae	1.7	6.8	17	43
Aeshnidae	0.0	2.6	9	52
Physidae	3.1	2.0	8	60
Libellulidae	0.0	2.3	8	68
Culicidae	2.2	1.1	6	73
Protoneuridae	0.0	1.4	5	78
Turbellaria	0.3	1.0	3	81
Naucoridae	0.0	0.5	2	83
Coenagrionidae	0.0	0.6	2	85
Tanypodinae	0.0	0.5	2	87
Hydraenidae	0.1	0.3	1	89
Hydrophilidae	0.0	0.4	1	90

Table 13. Relationship between macroinvertebrate community structure and environmental variables in Yarrowee, Gadigal Green and Coolibah wetlands. Variables are listed in order of addition in the stepwise selection model. The partial r^2 and corresponding *p* values reflect the unique proportion of variation in the model accounted for by that variable once others in the list have already been fitted. SW = surface water, S = sediment.

	Marginal test		**Sequential test**		
	Individual r^2	***p***	**Partial r^2**	**Cumulative r^2**	***p***
Yarrowee Wetland					
DO	0.515	0.001	0.515	0.515	0.001
TN SW	0.352	0.001			
TP SW	0.237	0.011			
Cu SW	0.203	0.023			
Pb SW	0.229	0.006			
Zn SW	0.456	0.001			
pH	0.156	0.05			
Turbidity	0.458	0.001			
Temperature	0.114	0.133			
Conductivity	0.335	0.001			
Pb S	0.436	0.001	0.120	0.636	0.002
Cu S	0.485	0.001	0.071	0.708	0.024
Zn S	0.507	0.001			
Gadigal Green Wetland					
DO	0.396	0.002	0.396	0.396	0.01
TN SW	0.371	0.001			
TP SW	0.281	0.001			
Cu SW	0.293	0.001			
Pb SW	0.319	0.001			
Zn SW	0.184	0.021	0.148	0.544	0.01
pH	0.384	0.001			
Turbidity	0.254	0.003			
Temperature	0.194	0.010			
Conductivity	0.304	0.001			
Pb S	0.370	0.001			
Cu S	0.387	0.001			
Zn S	0.389	0.001			
Coolibah Wetland					
Pb S	0.462	0.001	0.462	0.462	0.001
TN SW	0.229	0.014			
TP SW	0.207	0.023	0.226	0.687	0.001
Cu SW	0.130	0.092			
Pb SW	0.236	0.015			
Zn SW	0.051	0.557			
pH	0.275	0.001			
Turbidity	0.453	0.001			
Temperature	0.180	0.025			
Conductivity	0.346	0.001			
DO	0.450	0.001			
Cu S	0.352	0.001			
Zn S	0.332	0.001	0.065	0.753	0.004

Organic matter decomposition

The loss of leaf litter mass was 42 to 46% in the inlet zones and 39 to 44% in the outlet zones (Figure 17). Breakdown rates ranged from 0.10 – 0.11 day^{-1} in the inlet zones and 0.09 – 0.010 day^{-1} in the outlet zones. Loss of leaf litter mass was unaffected by location (i.e. inlet and outlet) at each wetland (Yarrowee: t = 1.24, p = 0.271; Gadigal Green: t = -1.70, p = 0.150; Coolibah: t = -0.62, p = 0.562). Leaf litter decay rates were also unaffected by location at each wetland (Yarrowee: t = 0.35, p = 0.738; Gadigal Green: t = 1.39, p = 0.224; Coolibah: t = -0.45 p = 0.668).

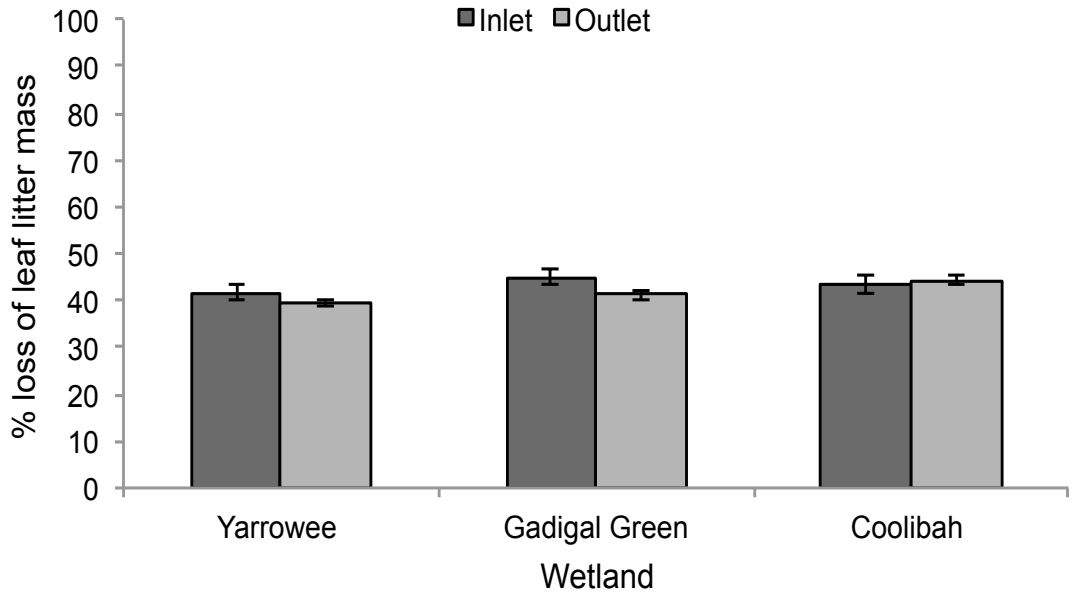


Figure 17. Percentage loss of leaf litter mass after a 56 day exposure period at the inlet and outlet zone of Yarowee, Coolibah and Gadigal Green wetland. Values reported as mean ± SE.

Tensile strength of cotton strips decreased in all three wetlands (Figure 18). Tensile strength loss (i.e. decay rate) was significantly higher at the inlet than the outlet of Yarowee wetland (inlet = 0.024 day^{-1}; outlet = 0.012 day^{-1}; t = 2.69, p = 0.031) and Gadigal green wetland (inlet = 0.028 day^{-1}; outlet = 0.010 day^{-1}; t = 8.63, p = <0.001), but not at Coolibah wetland (inlet: 0.029 day^{-1}; outlet = 0.029 day^{-1}; t = 0.000, p = 1.00) Cotton strip tensile strength was significantly lower (i.e. strips more decomposed) at the inlet than at the outlet of Yarrowee wetland (t = -2.59, p = 0.036) and Gadigal Green wetland (t = -7.98, p = <0.001), but there was

no significant difference in the tensile strength of cotton strips exposed at the inlet and outlet of Coolibah wetland (t = -0.07, p = 0.944). Significant differences in decay rates between the inlet and outlet zone at both Yarrowee and Gadigal Green wetland were significantly positively correlated with TN (Yarrowee: r = 0.689, p = 0.027; Gadigal Green: r = 0.950, p = <0.001) and TP (Yarrowee: r = 0.689, p = 0.027; Gadigal Green: r = 0.950, p = <0.001). Loss of tensile strength was significantly negatively correlated with TN (Yarrowee: r = - 0.675, p = 0.032; Gadigal Green: r = - 0.942, p <0.001) and TP (Yarrowee: r = - 0.675, p = 0.032; Gadigal Green: r = - 0.942, p <0.001).

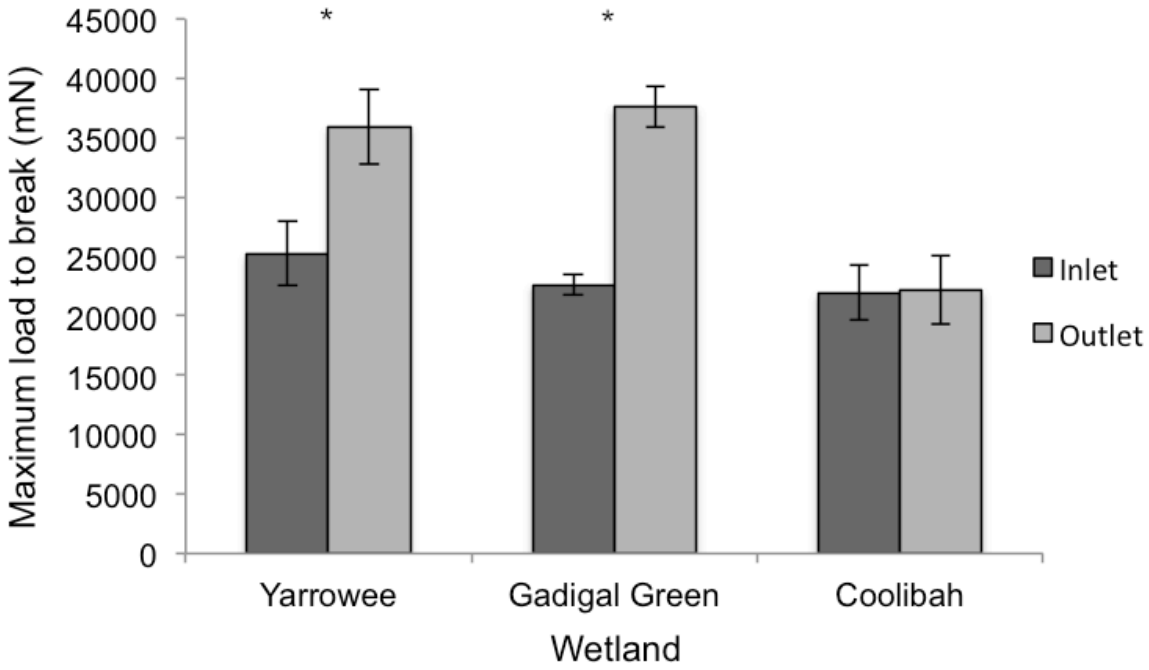


Figure 18. Tensile strength of cotton strips after the 28 day exposure period at the inlet and outlet zone of Yarrowee, Coolibah and Gadigal Green wetland. Values reported as mean ± SE. Asterisks (*) indicate significant differences in the tensile strength of cotton strips between the inlet and outlet (paired t-test : p < 0.05).

Discussion

The wetland inlet zones were ecologically degraded and reflect the 'urban stream syndrome'. Environmental responses seen at the inlet zones in comparison to outlet zones generally included: poorer water and sediment quality; lower family richness, SIGNAL and diversity scores, with significantly different macroinvertebrate assemblages; poor survival of *P. australiensis* and greater organic matter (cotton strip) decomposition. The responses in structural and functional indicators of ecological health, however, and the observed degree of change in indicators between the inlet and outlet zone were not consistent among wetland sites. The degree of change in biological responses between the inlet and outlet zone was related to the changing physico-chemical regimes within the wetlands and (potentially) better wetland design, with higher treatment performance resulting in the most marked improvements to ecological health at the outlet zone.

Water and sediment quality

A reduction in mean pollutant concentrations was generally evident between the inlet and outlet zone of the constructed wetlands, with the exception of Coolibah wetland where highest sediment metal concentrations were recorded at the outlet. The removal of pollutants in stormwater flowing through a constructed wetland, via adsorption and sedimentation processes, results in the accumulation of pollutants on the wetland substrate (DLWC, 1998). Heavy metal accumulation in sediments is influenced by the particle-size fractions of the sediment (Lin & Su, 2003). Metals are generally subject to accumulation in the fine grain fraction of sediments (Stamoulis et al., 1996), but can also be associated with the coarse grain fraction of the sediment (Qian et al., 1996; Lin & Su, 2003). In treatment facilities, more sediment is typically found close to the inlet than the outlet due to course-grained particles settling directly when entering the device (Karlsson et al., 2010), which may account for some of the observed differences in sediment quality between the inlet and outlet. To confirm this, it would be worthwhile in future studies to analyse sediment samples for particle size. Coolibah wetland was also a restored wetland and the observed differences in sediment metal concentration between the inlet

and outlet may have been caused by site contamination at the outlet. It is recommended that detailed geotechnical investigations be undertaken prior to future projects to ensure that any soil quality/contamination issues are addressed. This is important as benthic macroinvertebrate communities are affected by both water and substratum quality (Courtney & Clements, 2002; Marshall et al., 2010).

Dissolved oxygen concentrations increased between the inlet and outlet at Yarrowee and Coolibah wetland. This distribution is expected as oxygen depleting substances (e.g. organic matter) are broken down by wetland treatment and Puigagut et al. (2008) observed that the majority of organic matter was removed within the first quarter of the length of (non-stormwater) constructed wetlands. Biochemical oxygen demand, a measure of the amount of oxygen required to break down organic material, has been correlated with catchment urbanization (Walsh et al., 2001). Gadigal Green wetland, however, showed depleted oxygen concentrations at the outlet zone compared to the inlet zone despite water quality improvements. Thompson et al. (2011) conducted a water quality improvement study at Gadigal Green wetland and noted a 'dead zone' at the outlet zone and attributed this to a sub-optimal permanent pool depth, combined with unsuitable species planting for the water depth. Consequently, low dissolved oxygen at the outlet zone as a consequence of organic breakdown of plant material is likely to be responsible for some of the biological responses observed at this site.

The larger size of Yarrowee wetland with respect to the contributing catchment area, in comparison to the other two wetlands, may be the reason why it had more marked improvement in water and sediment quality between the inlet and outlet zone. Carleton et al. (2000) monitored pollutant removal at two wetlands and recorded higher efficiency at the wetland with the largest treatment area to catchment ratio and attributed this to it having a higher capacity for pollutant removal. Consequently, the water quality improvements observed at Yarrowee wetland are likely to have been responsible for the greater improvements in biological responses compared to the other two wetlands.

***Organism level response: in situ exposures using* P. australiensis**

In situ exposed *P. australiensis* were highly sensitive to the stormwater-associated stressors in the wetland inlet zone of all three wetlands, with reduced survival observed for every *in situ* test. Other studies using crustaceans as monitoring tools in streams have shown urban runoff to affect survival (Tucker & Burton, 1999) and the connection of stormwater drainage pipes to negatively correlate with their occurrence (Walsh et al., 2004b). More specifically and relevant to the current study is that Kumar *et al*. (2011) also observed poor survival of caged *P. australiensis* at the inlet site of a constructed stormwater wetland (stormwater passed through an in-stream basin and holding storage tank prior to reaching the wetland). Over a two-week exposure period, the authors noted 5% survival at the inlet site in comparison to 80% survival at the outlet site. In the current study, significantly higher survival in the wetland outlet zone in comparison to the inlet zone was consistently observed at Yarrowee wetland, demonstrating the ability of the wetland to reduce the toxicity of stormwater. The other two wetlands in this study, which had poorer treatment performance in comparison to Yarrowee wetland, did not have the same consistently high response in survival at the outlet across exposure periods.

Dissolved oxygen was a strong positive correlate with shrimp survival. Tucker & Burton (1999) observed positive associations in the survival of caged crustaceans (amphipods) with dissolved oxygen levels in urban stream sites. MacNeil et al. (2000) observed low survivorship of caged amphipods at river sites with dissolved oxygen levels of 20%. It is possible that the low dissolved oxygen concentrations at the outlet of Gadigal Green wetland (mean DO: 11%) influenced shrimp survival. Low shrimp survival recorded at Coolibah wetland during exposure period 4 was coincident with the wetland outlet recording the lowest DO concentration (34%). DO concentrations were consistently higher in the outlet of Yarrowee wetland compared to the inlet, demonstrating the ability of this site to reduce oxygen-depleting substances and support the survival of *P. australiensis.*

At Yarrowee wetland, surface water nutrients, Zn and turbidity, and sediment Cu, Pb and Zn were negatively associated with shrimp survival. With the exception of

Zn, no published studies have considered the acute toxicity of these variables on *P. australiensis.* The maximum concentration of Zn observed in surface waters during shrimp exposures was 0.280 mg L^{-1}. This concentration is much lower than Zn water concentrations previously shown to cause mortality; the acute toxicity of Zn at 15ºC and 25ºC (>80% dissolved oxygen in both cases) was assessed by Skidmore & Firth (1983) in the laboratory, who determined 96 h LC_{50} values of 5.2 and 2.1 mg L^{-1}, respectively. However, mixtures of chemicals may have additive and or synergistic effects (Walsh et al., 2004a) and it is possible that this is the case. For example, Zn toxicity to the shrimp, *Farfantepenaus paulensis*, is higher at lower salinities (Barbieri & Doi, 2011) and Skidmore & Firth (1983) demonstrated that Zn toxicity to *P. australiensis* is altered with temperature as discussed above.

Tucker & Burton (1999) observed a significant positive correlation with turbidity and survival of a caged amphipod (*Hyalella azteca*) during *in situ* exposures at urban river sites. The authors suggested that this might have been due to *H. azteca* ingesting sediment and fine particles containing particulate-bound contaminants that entered through the mesh cages, therefore increasing the potential for contaminant exposure and decreasing survival. This is a plausible explanation in the current study as *P. australiensis* is a filter-feeder and scavenger/browser on detrital material (Gemmell, 1978) and elevated turbidity levels were noted in the inlet zone in comparison the outlet zone and highest at Yarrowee wetland.

When metals enter the aquatic environment they partition between dissolved (e.g. overlying water) and particulate phases (e.g. sediment) and this speciation is a major determinant of bioavailability and toxicity of metals to aquatic biota (Eggleton & Thomas, 2004; Chapman, 2008). The major toxic effect of metals is usually caused by the dissolved fraction (ANZECC, 2000a), however, in the current study survival of *P. australiensis* at Yarrowee wetland was more strongly correlated to the metal content in the sediment than surface waters. This suggests that ingestion of metals associated with sediments maybe the main route of exposure. Kumar et al. (2011) observed 100% survival of *P. australiensis* in

surface water exposures performed under laboratory conditions using samples collected from the wetland inlet and outlet of a constructed wetland, but poor survival *in situ*, and attributed this to the shrimp being highly sensitive to the exposure of contaminated sediments at the wetland inlet. Sediments act as a sink for many pollutants (Townsend et al., 2009) and pollutants were present at much higher concentrations in the sediment than in surface waters in the current study. In the current study, sediment metal concentrations in the inlet of Yarrowee wetland are unlikely to have directly caused the mortality of *P. australiensis* as Coolibah wetland, in comparison, had higher sediment metal concentrations at the outlet, yet survival appeared relatively unaffected. However, it could be possible that flow disturbance of inflowing stormwater at the inlet zone is re-suspending contaminated sediment and increasing toxicity to *P. australiensis*. Contaminated sediment suspension has been shown to greatly increase toxicity to freshwater biota (Christensen et al., 2006).

Overall, this study indicated that the treatment of stormwater at Yarrowee wetland was sufficient to consistently support the survival of *P. australiensis*, whilst the ability of the other two wetlands to achieve the same outcome was hindered possibly by dissolved oxygen levels at the outlet.

Community level response: Macroinvertebrates

The inlet zones of two stormwater wetlands (Yarrowee and Gadigal Green) had significantly lower taxa richness, lower SIGNAL scores and lower species diversity in comparison to outlet zones. The inlet zones of all three wetlands also had dissimilar macroinvertebrate assemblages compared with outlet zones. Greenway (2010) observed macroinvertebrate richness to increase downstream of a stormwater constructed wetland in comparison to upstream and the observed pattern of degradation in macroinvertebrate assemblages between the outlet and inlet zone in the current study is consistent with that observed at urban waterways subject to an increasing gradient of urban disturbance (Pratt et al., 1981; Roy et al., 2003; Gray, 2004; Walsh, 2004, 2006; Davies et al., 2010; Marshall et al., 2010; Tippler et al., 2012). The diversity recorded at the wetland outlet was close

to the Shannon's H' of 1.66 observed in constructed stormwater wetlands in North Carolina, USA (Moore et al., 2012).

The taxa dominating the inlet zone at all wetlands were Oligochaeta, Chrionominae (Diptera) and Culicidae (Diptera), as well as Physidae (Gastropoda) at Coolibah wetland. Assemblages numerically dominated by these pollution tolerant taxa have been reported for degraded urban sites (Pratt et al., 1981; Walsh, 2004). Davies et al. (2010) also noted the presence of Physidae in urban streams, but absence from natural streams and Chessman & Williams (1999) commonly collected *Physa acuta* (Physidae), a species introduced to Australia, in urban waters. Dissolved oxygen and TP concentrations were generally strong correlates of macroinvertebrate composition at the wetlands, and were present at low and high concentrations, respectively, at the inlet zone. Tippler et al. (2012) also observed that these two factors influence macroinvertebrate assemblages in a nearby river catchment study.

Decreases in the sensitive macroinvertebrate orders – Ephemeroptera, Plecoptera and Trichoptera have been reported with increasing urbanisation (Pratt et al., 1981; Roy et al., 2003; Walsh, 2004, 2006; Gresens et al., 2007; Davies et al., 2010; Tippler et al., 2012). In the current study, Baetidae (Ephemeroptera) and Leptoceridae (Trichoptera), the latter of which were found in low numbers, were only recorded at the outlet zone of Yarrowee wetland. This finding suggests that the higher treatment performance provided by Yarrowee Wetland in comparison to the other wetlands provided better water quality to support more sensitive macroinvertebrate taxa. Ephemeroptera are highly sensitive to low oxygen conditions and lethal effects occur at DO levels <20% saturation (Connolly et al., 2004), which may explain why they were not recorded at the outlet of Gadigal Green wetland which had a mean DO concentration of 11%.

In addition to correlating with DO, macroinvertebrate responses at Gadigal Green significantly correlated with Zn surface water concentrations which were the highest observed in all three wetlands and were many times over ANZECC/ARMCANZ (2000a) guidelines. Courtney & Clements (2002) observed sensitive macroinvertebrate groups to be absent in a river with elevated Zn

concentrations. Ephemeroptera are highly sensitive to substratum quality and have been shown to be significantly more abundant on clean substratum in comparison to metal-contaminated substratum (Courtney & Clements, 2002), which could explain their absence from the outlet of Coolibah wetland which had contaminated sediment. Substantial ecological effects on macroinvertebrates e.g. alterations to taxa abundance and diversity has been associated with polluted sediments from urban streams and sediment subject to road runoff (Pettigrove et al., 2007; Marshall et al., 2010). Macroinvertebrate assemblage responses at Yarrowee wetland were significantly correlated with dissolved oxygen and heavy metal sediment concentrations, both of which markedly increased and decreased, respectively, between the wetland inlet and outlet. These factors may explain the presence of sensitive macroinvertebrate taxa at the outlet in comparison to the inlet.

SIGNAL scores in the wetlands were lower than those previously reported in Sydney, Australia for both urban and natural streams (Davies et al., 2010). However, some of the macroinvertebrate taxa that have the highest SIGNAL 2 sensitivity grades, for example stoneflies, are naturally rare in wetlands, and wetlands are therefore likely to have lower scores than streams in the same region (Chessman, 2003). It could also be due to the fact that there is not an undisturbed stream close to the stormwater wetlands which can serve as a source population of sensitive macroinvertebrate colonists (Wallace, 1990). It would be worthwhile in the future to perform community-level *in situ* toxicity tests (Courtney & Clements, 2002) to measure the direct effects of untreated and treated stormwater on macroinvertebrates collected from a reference stream. This would enable the observation of how the survival of more sensitive taxa is affected by stormwater treatment.

It is also important to note that the inlet zone of the wetlands is likely to be subject to greater flow disturbances in comparison to the outlet zone. Disturbance has been shown to influence macroinvertebrate communities, for example, by reducing invertebrate richness and density (Robinson & Minshall, 1986; Collier & Quinn, 2003, Feeley, 2012). However, sensitive macroinvertebrate taxa, including

Ephemeroptera and Plecoptera have been shown to persist in high disturbance environments (Fuller 2010; Feeley, 2012). It is likely that flow disturbance was an influential factor on the macroinvertebrate community composition observed in the current study. Nevertheless, macroinvertebrate community structure was shown to be strongly influenced by environmental variables.

Ecosystem level response: Organic matter decomposition

There were no differences in leaf litter decomposition between the inlet and outlet zone of all three stormwater wetlands. There are no other studies in the published literature examining the effect of stormwater treatment devices on leaf decomposition. Previous studies that have addressed the effects of urbanization on litter decomposition have indicated accelerated breakdown rates (Meyer et al., 2005; Chadwick et al., 2006; Paul et al., 2006; Imberger et al., 2008). The lack of difference in breakdown rates between the wetland inlet and outlet zones in this study maybe due to the type of leaf litter used. Wide ranges of decomposition rates exist across tree species due to leaf quality (Tiegs et al., 2007). Imberger et al. (2008) observed that *Pittosporum undulatum* leaves broke down faster with increasing catchment effective imperviousness, but not *Eucalyptus obliqua* leaves. Breakdown rates ranging from 0.009 to 0.022 day^{-1} were reported for *E. obliqua*, which is similar to the breakdown rates recorded for the *Eucalyptus* leaves used in the present study. Imberger et al (2008) attributed the difference in response to different leaf properties, with elevated microbial activity in urban streams (associated with higher temperatures and P concentrations) to have a greater effect on a leaf species with more labile C (i.e. *P. undulatum*) than on a leaf species with refractory C (i.e. *E. obliqua*).

As well as microbial processes, other factors, including, leaching (Meyer *et al.*, 1998), macroinvertebrate feeding and physical abrasion can affect the breakdown of leaf litter, and the mechanism of decay is likely to differ depending on the position of sites along the impairment gradient (Paul et al., 2006). It could be that different breakdown mechanisms are in action at the inlet and outlet zones. For example, leaf litter could have been broken down by greater abrasive power (associated with inflowing stormwater) and/or microbial decomposition (associated

with higher nutrient levels) at the inlet zone. Paul et al. (2006) observed faster breakdown rates in more urbanised streams and attributed this difference to physical fragmentation resulting from higher stormwater runoff. At the wetland outlet zone, the observed increased macroinvertebrate taxa richness might have accounted for the breakdown of leaf litter via shredding. Chadwick et al. (2006) observed that leaf litter breakdown rates were strongly related to invertebrate richness. However, little variation in percentage mass loss was seen between inlet and outlet zones or across sites in the current study, and it is therefore more likely that the lack of difference is due to the type of leaf litter used. Future studies should test the response with different leaf species.

The cotton strip assay was more sensitive than the leaf litter assay, which is similar to the findings of Tiegs et al. (2007). A clear biological effect using cotton strip assays was observed at two stormwater wetlands; cotton strips were significantly more decomposed in the inlet zone in comparison to the outlet zone. There are no other studies in the literature looking at the effect of stormwater treatment devices on cotton fabric decomposition. However, Young & Collier (2009) observed a significant decline in the tensile strength of cotton strips with land use stress in a river system and Imberger et al. (2010) demonstrated that cotton strip decomposition was a sensitive functional indicator of water quality and had positive correlations with nutrients (ammonium and FRP). There was a strong positive relationship between cotton strip decomposition and TN and TP concentrations in the current study. The breakdown of cellulose materials, such as cotton, is almost entirely by microbiological processes (Lategan et al., 2010). It is likely that the faster decomposition in the inlet zone was due to increased microbial activity as a result of nutrient enrichment, which is in agreement with other organic matter decomposition studies in streams (Chadwick et al., 2006; Imberger et al., 2008; Imberger et al., 2010). A reduction in decomposition rates at the outlet are of ecological benefit, as reduced microbial driven breakdown will not decrease the availability of benthic organic matter, which could ultimately impair ecosystem function (Imberger et al., 2008). The lack of change in the decomposition of cotton strips between the inlet and outlet of Coolibah wetland

may be the result of the TP concentration remaining relatively unchanged between the two zones.

Conclusions

This study is one of the first to provide a holistic ecological assessment of constructed stormwater wetlands using a range of functional and structural indicators. It is evident that untreated stormwater at the inlet zone led to poor ecological health at all of the studied wetlands. More environmental variables exceeded recommended guidelines at the inlet compared to the outlet and correlated with toxicity to *P. australiensis*, changes to macroinvertebrate assemblages and alteration to cotton strip decay rates. The degree of improvement in biological responses between the inlet and outlet zone was related to the changing physico-chemical regimes within the wetlands, with sites closest to recommended design guidelines performing better and showing the most marked improvement to ecological health at the outlet zone. The results of this study indicate that stormwater wetlands can improve ecological health and quality of stormwater, but need to be designed appropriately to provide the best ecological outcomes. The combination of biological monitoring methods used in this study, with the exception of leaf decomposition, provide simple and cost effective tools to assess the performance of retrofitted stormwater wetlands to deliver ecological improvements.

Chapter 5: Sublethal toxicity of untreated and treated stormwater Zn concentrations on the foraging behaviour of *Paratya australiensis* (Decapoda: Atyidae)

The aim of the research presented in this chapter was to determine the sub-lethal effects of Zn, a key stormwater contaminant, at untreated and treated stormwater concentrations observed in constructed wetlands. The effects of exposure were evaluated by observing how foraging behaviour of Paratya australiensis *was altered. This was achieved via laboratory experiments that monitored the ability of shrimp to perceive, approach and search for a chemoattractant source. The results demonstrate the utility of behavioural toxicology to study the effects of stormwater contaminants on aquatic biota. Furthermore, the findings indicate that treatment of metal contaminants in stormwater can have discernible ecological benefits.*

Authors

	Problem formulation	Experimental design	Statistical analysis	Results interpretation	Paper preparation
Lois Oulton[1]	92	92	92	95	90
Mark Taylor[1]	1	0	0	0	2.5
Grant Hose[2]	2	0	0	0	2.5
Culum Brown[2]	5	8	8	5	5

1 Department of Environmental Sciences, Macquarie University, NSW 2109 Australia

2 Department of Biological Sciences, Macquarie University, NSW 2109 Australia

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Abstract

Aquatic organisms use chemical cues to perform key ecological behaviours such as locating food. Anthropogenic pollutants have the potential to disrupt these behaviours by down-regulating chemoreception. Urban stormwater runoff is a major source of metal pollution, particularly Zn, and is a leading contributor to the degradation of receiving waters. Consequently, significant remedial efforts have focused on using constructed stormwater wetlands to reduce pollutant loads. However, no studies have examined the efficacy of water quality improvements on ecologically relevant behaviours in aquatic biota. We conducted controlled laboratory-based experiments to test whether Zn concentrations observed in constructed wetlands, approximating untreated (100, 400 μg L^{-1}) and treated (40 μg L^{-1}) stormwater, interfere with the foraging behaviour of the glass shrimp (*Paratya australiensis)*. The ability of shrimp to perceive, approach and search for a chemoattractant source was used to assess foraging behaviour. Abnormal foraging behaviour was observed in shrimp exposed to Zn at untreated stormwater concentrations. The strongest change relative to the control was observed for perception, which decreased by more than 80% and 60% in the 400 μg Zn L^{-1} and 100 μg Zn L^{-1} groups, respectively. The behaviour of shrimp exposed to Zn concentrations measured in treated stormwater did not differ from the controls. The results suggest that the reduction of stormwater Zn concentrations via wetland treatment can prevent abnormal contamination-induced behaviours in shrimp, leading to improved aquatic ecosystem health. This study also highlights the subtle, but biologically significant impacts arising from sublethal exposures of Zn, and emphasises the utility of behavioural toxicology. The behavioural test used here is a simple and effective approach that could be incorporated into studies assessing the efficacy of stormwater treatment.

Introduction

Organisms use sensory information to assess critical features in their environment and perform ecologically relevant behaviours such as locating food (Von Der Emde & Bleckmann, 1998), finding mates (Passos et al., 2013) and detecting predators (Petranka et al., 1987; Manassa et al., 2013). Decisions mediated by the acquisition of sensory information can have far-reaching ecological consequences at the population, community and ecosystem level (Schmidt et al., 2010). In aquatic environments organisms commonly gather sensory information from their surroundings via chemical cues (Brönmark & Hansson, 2000; Blinova & Cherkashin, 2012). Any disruption to chemosensory reception therefore has the potential to alter key behaviours and ultimately lead to restructuring of the ecological community (Turner & Chislock, 2010).

Studies examining the effects of contaminants on aquatic biota have focused largely on establishing the level of toxicant required to cause mortality, whilst sublethal effects in terms of impairment to behaviour has received less attention (Blaxter & Hallers-Tjabbes, 1992; Denoel et al., 2010). Sublethal endpoints can, however, lead to cascading secondary effects on entire ecosystems (Fleeger et al., 2003). They are also a more appropriate method for which to evaluate the low levels of contaminants observed typically in natural systems (Pestana et al., 2007) and can act as early warning signs for detecting environmental stress (Gerhardt, 1995). Recent studies have demonstrated that anthropogenic pollutants, such as trace metals, can disrupt chemoreception in aquatic organisms at non-lethal concentrations (Lurling & Scheffer, 2007). For example, environmentally relevant sub-lethal concentrations of Cu have been shown to impair olfactory function in Coho salmon to natural odours (Baldwin et al., 2003) and inhibit morphological defences in *Daphnia pulex* to predatory chemical cues (Hunter & Pyle, 2004).

A major pathway for anthropogenic pollutants to enter into the natural aquatic environment is via urban stormwater runoff (McCarthy et al., 2008). This form of nonpoint source pollution is recognised as a leading contributor to the degradation of receiving waters (Walsh, 2000). It provides a significant source of metal

pollution (Tiefenthaler et al., 2008), with Zn being a dominant component (Hickey & Pyle, 2001). Anthropogenic sources of Zn originate from the corrosion of metal objects (e.g. galvanised roofing and roadside fittings) and vehicle wear (frame, brakes and tyres) (Makepeace et al., 1995). Given the environmental effects of stormwater contaminants on receiving waters, a large emphasis has been placed on using stormwater *in situ* treatment devices to reduce pollutant loads (Birch et al., 2005). Constructed stormwater treatment wetlands are becoming widely used for the management of stormwater and combine natural biological, chemical, and physical process to treat urban runoff (Birch et al., 2004; Malaviya & Singh, 2012). Few studies, however, have examined the effect of any consequent water quality improvements on aquatic biota and there have been no published studies analysing how ecologically relevant behaviours are effected.

Crustaceans are one of the most sensitive taxa to pollution (Long et al., 2001). Sublethal concentrations of trace metals have been shown to inhibit their ability to localize prey (Sherba et al., 2000) and reduce feeding rates (Wong et al., 1993; Pestana et al., 2007). Prey localization in aquatic crustaceans depends largely upon chemoreception (Zimmerfaust, 1989). Crustaceans antennae filaments have chemoreceptors that function in the sense of olfaction and detect chemical stimuli associated with food resources (Ache & Case, 1969). Therefore, impairment of these vital chemical sensors has the potential to disrupt or disable the foraging mechanism entirely. Although the effect of metal contamination on chemoreception in crustaceans has received some attention, the relative effects of untreated and treated stormwater metal contaminants remains unstudied.

The glass shrimp (*Paratya australiensis*) is a common atyid shrimp throughout eastern Australia (Richardson et al., 2004). Given that the shrimp inhabits both freshwater and estuarine environments (Walsh & Mitchell, 1995) it provides an important food source for a range of native biota (Richardson et al., 2004). Further, the shrimp play an important role in ecosystem processes via detrital decomposition (March et al., 2001) and influence the composition of algal and benthic invertebrate communities (Pringle, 1996; March et al., 2002). Consequently, they form a key component of aquatic ecosystems and are

therefore considered a useful model to examine the effects of Zn on aquatic biota. *P. australiensis* is frequently used as an ecotoxicological test species (Daly et al., 1990; Hose & Wilson, 2005; Thomas et al., 2008), however, very little is known about the sub-lethal effects of contaminants on ecologically relevant behaviours.

In this study, Zn concentrations reflective of those found in untreated and treated stormwater from constructed wetlands were investigated with respect to how they interfere with the foraging behaviour of *P. australiensis* to a chemoattractant source. Our hypothesis was that abnormal foraging behaviour would be observed in shrimp exposed to Zn at untreated stormwater concentrations, but not at treated concentrations. The intended goal of this research was to provide a better understanding of the sublethal effects of stormwater zinc pollution on urban aquatic environments and in turn, highlight the value of stormwater treatment.

Methods

Experimental subjects

Adult specimens of *P. australiensis* (1.5 - 2 cm in length) were sourced from Aquablue seafoods (Pindimar, Australia). Following transportation to the laboratory, shrimp were acclimated for seven days in a 100-L aquarium containing aerated, aged tap water and fed daily with Hikari® algae wafers. During acclimation and testing, water temperature was maintained at 23 ± 1°C and photoperiod kept constant on a 10 h light: 14 h dark cycle.

Test Waters and Solutions

Tests were conducted in reconstituted freshwater (hardness of 80 to 90 mg $CaCO_3$ L^{-1}) which was prepared in the laboratory according to formulations provided by Marking & Dawson (1973). Each litre of water contained: 96 mg of $NaHCO_3$, 130 mg of $MgSO_4.7H_2O$, 4 mg KCL, and 60 mg $CaSO_4.2H_2O$ dissolved in Milli-Q water (Millipore®, USA) using a stirrer bar. Reconstituted freshwater was made up in 10 L batches, pH adjusted to 7.5 with 0.1 M HCL, vacuum filtered through a 0.45 µm pore membrane and stored in the dark at 4°C prior to use. The pH of stormwater based on the wetland studies we have conducted has ranged from 7.3-7.6 and is therefore very similar to the pH of the reconstituted water.

A Zn stock solution was prepared by dissolving $ZnSO_4.7H_2O$ in Milli-Q water. Test solutions were prepared through dilution of the stock solution in reconstituted freshwater to obtain nominal concentrations of 400, 100 and 35 µg Zn L^{-1}. All test solutions were made immediately prior to use. Test concentrations were chosen to reflect those observed in urban stormwater treatment wetlands. The highest dose used in this study was a total metal concentration recorded in the inflow of a Sydney stormwater wetland monitored by Birch et al. (2004), whilst 100 and 35 µg Zn L^{-1} were soluble metal concentrations observed by the authors in the inflow and outflow, respectively, of a stormwater wetland also within the Sydney metropolitan

region (Chapter 4). Soluble Zn concentrations were used in this study as the dissolved metal fraction is more readily bioavailable (Hare, 1992).

Preliminary studies showed that exposure of *P. australiensis* to 400, 100 and 35 µg Zn L^{-1} caused no mortality after 96 hours. For quality control purposes, the metal concentration of solutions were analysed according to US EPA method 6020 using ICP-MS by a National Association of Testing Authorities (NATA) accredited laboratory (Australian Laboratory Services, Sydney). Samples were filtered through a 0.45 µm Sartorius filter and acid-preserved prior to analysis. The actual metal concentrations were <10% different than nominal concentrations.

The chemoattractant used to stimulate foraging behaviour in this study consisted of an equimolar mixture of glycine and aspartic acid at a total concentration of 0.1 M (Chu & Lau, 1994). Amino acids have been shown to readily stimulate feeding responses in crustaceans (Kumar et al., 2010).

All of the reagents used in this study were of analytical grade and obtained from Chem-Supply, Australia. Glassware and equipment used for the preparation of solutions and testing was acid washed for 24 h using 10% (v/v) HNO_3 to remove any metal contaminants and rinsed (5 x) with demineralized water and (5 x) with Milli-Q.

Pre-exposure and test exposure conditions

Individual shrimp (n = 28 for each treatment group) were selected at random and pre-exposed to 800 mL of 0 µg (control – reconstituted freshwater), 400, 100 or 35 µg Zn L^{-1} in one-litre glass beakers for 72 h to emulate the typical detention time of a stormwater treatment wetland (Melbourne Water Corporation (2010). Food was withheld during this time, as previous studies have demonstrated that starvation enhances the behavioural responses of crustaceans to chemoattractants (Chu & Lau, 1994).

Behavioural tests

Following the 72 h exposure period, shrimp were transferred to fresh solutions and their chemotaxis behaviour assessed based on adapted methods established by Chu & Lau (1994) and Kumar et al. (2010). Shrimp that had molted during the exposure period (*ca*. four per treatment group) were not tested as feeding behaviour can be temporally suspended post-moult (Harpaz et al., 1987).

Each shrimp was acclimated to the behavioural set-up for 10 min after which their antennular flicking rate was recorded for 2 min to determine pre-treatment background levels. Following this, the chemoattractant was delivered via a syringe pump through tubing to a Pasteur pipette into the beaker at a rate of 2 mL min^{-1} for 10 min. The pipette was suspended in the centre of the beaker with the tip located 3 cm above the bottom. Foraging behaviour of the subject was evaluated by recording the following variables: perception (antennular flicking – recorded after the introduction of 8 and 16 mL of the chemoattractant); approach (direct movement towards the source); and searching (proportion of shrimp that initiated swimming and how long they spent active). To minimise disturbance behavioural observations were conducted behind a screen that contained several small viewing holes and recorded using behavioural scoring software (Ottoni, 2000).

Data analysis

Data were tested for normality using Anderson-Darling normality tests/normal probability plots and analysed using MINITAB 16 (Minitab Inc, USA) or SPSS 21 (IBM, USA). Some shrimp were excluded from the analysis due to technical difficulties. Change in antennular flicking of individual shrimp was calculated by subtracting the number of antennular flicks, following the introduction of both 8 and 16 mL of chemoattractant, from pre-treatment levels. The resulting data were normalised by square-root transformation and analysed using a repeated-measures ANOVA (between-subjects factor: Zn concentration, within-subjects factor: chemoattractant dose) followed by Tukey HSD post-hoc analysis. Shrimp were scored on a yes/no (1/0) basis as to whether they approached the source of

the chemoattractant and initiated swimming. To consider the effect of Zn concentration on the number of shrimp that performed these behaviours a generalized linear model with a binomial error structure was used, followed by Fisher's LSD post-hoc analysis. The time shrimp spent active during exposure to the chemoattractant was calculated as a percentage, arcsine transformed, and analysed using a one-way ANOVA to consider the effect of Zn concentration. The results were then subject to Tukey HSD post-hoc analysis. Raw data is presented in Appendix 5 (Table A5.1).

Results

Perception

Shrimp exhibited an increase in perceptive behaviour from pre-treatment levels in response to the chemoattractant. The relative change in antennular flicking rate significantly decreased in response to increasing Zn concentration ($F_{3,175}$ = 35.28, p < 0.001; Figure 19), with the concentration of chemoattractant having no significant affect ($F_{1,175}$ = 1.84, p = 0.176). The control group (reconstituted freshwater alone) showed the greatest change in antennular flicking (*ca.* increase of 16 flicks min^{-1}), whilst the group exposed to the highest concentration of 400 µg Zn L^{-1}, showed the least change (*ca.* increase of 2 flicks min^{-1}). Tukey HSD post-hoc tests indicated that an increase in antennular flicking behaviour significantly decreased in relation to the control (n = 22) by more than 80% and 60% in the 400 µg Zn L^{-1} (t = 8.441, n = 23, p < 0.001) and 100 µg Zn L^{-1} (t = 6.039, n = 24, p < 0.001) groups, respectively. By contrast, there was no significant difference between the control and 35 µg Zn L^{-1} group (t = 0.253, n = 21, p = 0.994).

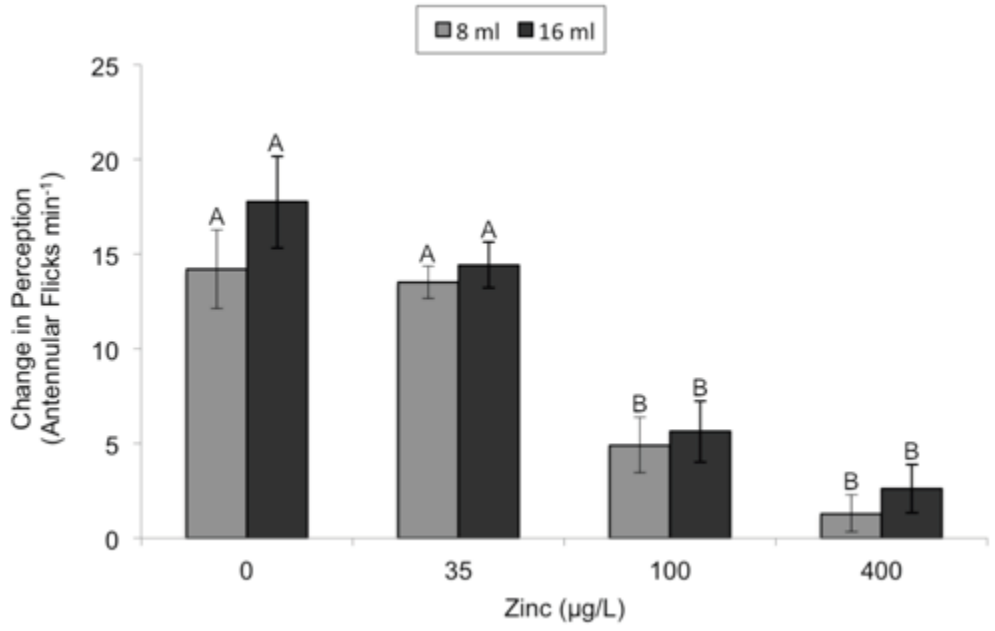

Figure 19. Mean (± SE) change in antennular flicking rate min^{-1} (perception) from pre-treatment levels following the introduction of 8 mL and 16 mL of 0.1 *M* glycine and aspartic acid (chemoattractant) in shrimp exposed to various concentrations of Zn. Means that do not share a letter are significantly different (p < 0.05).

Approach

As Zn water concentrations were increased, significantly fewer shrimp approached the mouth of the Pasteur pipette delivering the chemoattractant source (χ^2 = 24.495, p < 0.001; Figure 20). Post-hoc tests indicated that approach to source decreased significantly in relation to the control (n = 21) by more than 66% and 41% in the 400 µg (χ^2 = 16.045, n = 23, p < 0.001; 17%) and 100 µg (χ^2 = 7.572, n = 24, p = 0.006; 42%) Zn L^{-1} groups, respectively. In contrast, there was no significant difference between the control and 35 µg Zn L^{-1} group (χ^2 = 0.079, n = 23, p = 0.779) with over 80% of shrimp in both treatment groups approaching the source.

Searching

Examination of the shrimp swimming behaviour data revealed a significant effect of Zn concentration (χ^2 = 8.408, p = 0.038; Figure 20). The control and 35 µg Zn L^{-1} treatment had the highest proportion of shrimp that swam (*ca*. 95% in both groups), whilst the 400 µg Zn L^{-1} treatment group contained the least number of shrimp that initiated swimming (*ca.* 65%). Post-hoc tests indicated that the proportion of shrimp that swam decreased significantly in relation to the control (n = 21) by approximately 30% in the 400 µg Zn L^{-1} treatment group (χ^2 = 4.901, n = 23, p = 0.027). There was no significant difference between the control (95%) and the 35 µg (χ^2 = 0.004, n = 23, p = 0.948; 95%) and 100 µg (χ^2 = 1.632, n = 24, p = 0.201; 83%) Zn L^{-1} groups.

Analysis of the proportion of time that shrimp spent active showed a significant effect of Zn concentration ($F_{3, 87}$ = 5.54, p = 0.002; Figure 20). Post-hoc tests indicated that shrimp were approximately 40% less active in the 400 Zn L^{-1} treatment group compared to those in the control group (n=21) (t = 3.906, n = 23, p = 0.001; 25%). There was no significant difference in activity levels between the control (65%) and the 35 µg (t = 1.779, n = 23, p = 0.290; 53%) and 100 µg (t = 2.288, n = 24, p = 0.108; 52%) Zn L^{-1} treatments.

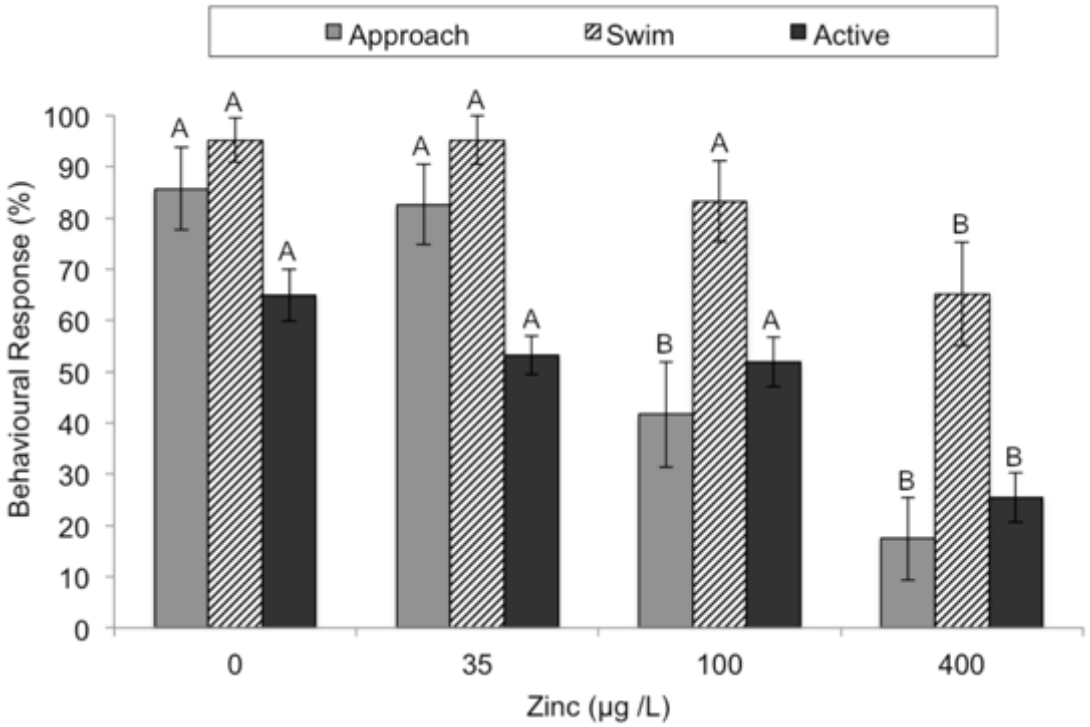

Figure 20. Foraging behaviour of shrimp in each treatment groups subjected to different concentrations of Zn. Values reported as mean ± SE. Means that do not share a letter are significantly different from the control.

Discussion

Foraging responses of *P. australiensis* were generated by addition of the chemoattractant. The foraging response was characterized by increased antennular flicking, a greater likelihood of initiating swimming and approaching the chemoattractant source, and a general increase in the time spent active. All of these responses to an alimentary stimulus have been reported for a variety of crustaceans (Hindley, 1975; Schmitt & Ache, 1979; Buskey, 1984; Chu & Lau, 1994; Kumar et al., 2010). As the concentration of Zn in the water increased, a corresponding decrease in all of these responses occurred, indicating that Zn had a strong inhibitory effect on foraging behaviour. These dose-behaviour responses were most evident in the higher Zn concentration treatments that are typical of untreated stormwater. At lower Zn water concentrations reflective of those recorded in treated stormwater, foraging behaviour was not significantly different from the control group.

Exposure to Zn at concentrations found in untreated stormwater impaired chemoreception, which, in this study, was manifested as a reduced ability of individual shrimp to perceive, approach and search for the chemoattractant. In other aquatic organisms a reduction in feeding activity has been documented as an indicator of toxic stress in response to pollutants (Butler et al., 1990; Maltby et al., 1990). When exposed to the highest Zn concentration (400 µg Zn L^{-1}), shrimp displayed a significant inhibition in foraging behaviour in relation to the control across all of the monitored behavioural responses. Pestana *et al*., (2007) also reported abnormal foraging behaviour in another atyid shrimp, *Atyaephyra desmarestii*, exposed to 400 µg Zn L^{-1} .

Crustaceans possess receptors on their antennae and leg tips which function in the sense of olfaction and gustation, respectively (Atema, 1977; Zimmerfaust, 1989). Thus, an increase in antennular flicking and initiation of swimming may serve to increase the flow of water past these receptors and enhance the ability of shrimp to perceive chemical changes in their environment. For example, antennae flicking in the spiny lobster has been shown to heighten the response of the

olfactory receptors to a stimulant (Schmitt & Ache, 1979). A contaminant-induced reduction in these behavioural responses is therefore likely to lower the probability of individuals encountering chemosensory stimuli, thereby limiting the foraging search area. This interpretation is supported by the fact that significantly fewer shrimp exposed to zinc concentrations approximating untreated stormwater approached the source of the chemoattractant and spent less time actively searching for it. Uncertainty surrounding the mechanisms responsible for causing disruption to chemical communication exists, but it is thought that contaminants may block receptor sites, interfere with signal-transduction pathways, or alter the chemical signals themselves (Lurling & Scheffer, 2007). Application of $ZnSO_4$ to olfactory epithelium has been reported to disrupt signalling transmission and produce transient anosmia in a number of organisms (Powers & Winans, 1973; Benvenuti et al., 1992; McBride et al., 2003).

Foraging behaviour has important fitness-related implications and disruption to this important sensory ability is likely to have other cascading ecosystem-wide implications. Zinc concentrations in untreated stormwater may therefore have the potential to impact on shrimp populations as changes to foraging behaviour may affect an individual's growth, reproduction and, ultimately, survival. Maltby *et al*. (1990) and Maltby & Naylor (1990) found that a reduction in the feeding activity of a crustacean, *Gammarus*, in response to Zn, correlated with reduced growth and reproduction. As shrimp occupy a keystone position in the food web of many environments (Pringle, 1996; March et al., 2001) any factors that affect populations are likely to result in secondary impacts at higher levels of biological organization (Bool et al., 2011). *P. australiensis* feeds by both filter feeding and browsing on detrital material such as leaves (Gemmell, 1978). Consequently, a reduction in feeding activity could also alter the incorporation of allochthonous-fixed organic material into the food web. Maltby (1994) for example, documented that a toxicant-induced decrease in crustacean feeding rates correlated with a reduction in community function (i.e. leaf processing). Therefore a change in the foraging behaviour of shrimp, and or a loss of shrimp from the aquatic community has the potential to cause large-scale shifts in ecosystem functioning via trophic cascades. Further studies are needed to examine how changes in the foraging

behaviour of shrimp alter fitness and what consequences this may have for higher trophic levels and the aquatic ecosystem as a whole.

To date, most studies examining the influence of pollution on aquatic biota have relied on obtaining lethal concentration values as a quantitative measure, with behavioural effects being given relatively scant consideration (Denoel et al., 2010). This study demonstrates that recording immediate mortality only provides a very blunt measure of pollution impacts and appears to underestimate the effect toxins can have on aquatic biota. This is particularly relevant for many anthropogenic pollutants that are present at sub-lethal concentrations, yet still have the potential to cause significant, adverse ecological effects (Lurling & Scheffer, 2007). This study has demonstrated that behavioural responses provide useful indicators of pollution effects on aquatic organisms, whose impacts can easily be defined and monitored under controlled conditions. *P. australiensis* appears to be a suitable test organism for use in laboratory-based behavioural toxicology. Shrimp antennular flicking and approach to the chemoattractant source were the most affected foraging responses in this study. Kumar *et al.,* (2010) also found approach to a chemoattractant source in *P. australiensis* to be significantly reduced following exposure to pesticides. Similarly, Chu & Lu (1994) found significantly fewer shrimp increased antennular flicking in response to a chemoattractant when exposed to pesticides. This study suggests that these simple behavioural responses may serve as reliable and cost-effective indicators of the sublethal effects of stormwater pollutants on crustaceans and enable the identification of stormwater contaminants of concern. In addition, attenuation of adverse foraging responses could also provide a useful indicator of effective stormwater treatment.

In the real world, stormwater delivers a complex mixture of chemicals to receiving waters (McCarthy et al., 2008) which may have additive or synergistic effects (Walsh et al., 2004a), and thus Zn is rarely encountered by aquatic organisms in isolation. For example, toxicity of Zn to the shrimp, *Farfantepenaus paulensis*, is more toxic at lower salinities (Barbieri & Doi, 2011). Metals in the aquatic environment also partition between dissolved (pore water, overlying water) and

particulate phases (sediment, suspended particulate matter and biota), the behaviour of which can be affected by different environmental conditions (Eggleton & Thomas, 2004; Beltrame et al., 2009). This partitioning behaviour means that aquatic organisms will be exposed to Zn speciation, and each form may differ in its bioavailability and toxicity (Luoma, 1983). In addition, other non-chemical stressors, such as predation, temperature fluctuations, flow alterations etc. are common aspects of natural systems (Heugens et al., 2001; Wenger et al., 2009), which may complicate or modify behavioural responses. Relying on the examination of the effects of single contaminants to assess impacts on aquatic organisms are likely to result in an underestimation of the total adverse impact of stormwater runoff on aquatic ecosystems (Walsh et al., 2004a). However, focusing on a single variable such as Zn water concentrations permits delineation of cause and effect in laboratory studies such as this. Further studies are needed that examine the effects of untreated and treated stormwater in association with other common stressors to gain a complete understanding of the cumulative effect of multiple stressors on ecologically relevant behaviours.

Conclusions

It is evident that exposure to Zn concentrations reflective of those found in untreated stormwater induced changes in the foraging behaviour of *P. australiensis.* Adverse effects on foraging responses were not evident at lower Zn concentrations, similar to those measured in treated stormwater. These results highlight the importance of stormwater treatment as part of an ecosystem protection strategy and, in particular, the need to reduce the loading of zinc and other dissolved metal toxicants in receiving aquatic environments. The findings from this study indicate the importance of examining sublethal effects of stormwater pollutants to develop a more comprehensive understanding of their potential impact on aquatic environments. *P. australiensis* appears to be a suitable species to be used in laboratory-based behavioural toxicology to assess the efficacy of stormwater treatment in Australia.

Chapter 6. Evaluation of Indicator Bacteria Reduction and Immature Mosquito Presence in Stormwater Treatment Devices

The aims of the research presented in this chapter were to determine the efficacy of stormwater treatment devices to remove indicator bacteria and evaluate the potential of surface flow systems to provide a breeding ground for mosquitoes. Four stormwater treatment devices were monitored for faecal coliforms and enterococci. Immature mosquito presence (abundance and species) was determined at the inlet and outlet zone of surface flow systems. The results highlight variability of the stormwater treatment systems to reduce indicator bacteria, particularly enterococci. Median outflow concentrations generally exceeded public health criterion for primary and secondary contact. Public health risks were further substantiated by the occurrence of immature mosquitoes at all surface flow systems. Correlation analysis indicated that the higher nutrient concentrations in the inlet zone in comparison to the outlet zone provided conditions that were more conducive to the production of immature mosquitoes. Overall the results indicate the need for further research and design improvements to eliminate potential human health risks, in particular with respect to the removal of faecal related bacteria and the presence of immature mosquitoes.

Authors

	Problem formulation	Experimental design	Statistical analysis	Results interpretation	Paper preparation
Lois Oulton[1]	90	95	97	94	90
Culum Brown[2]	0	0	0	1	2
Grant Hose[2]	5	2.5	3	3.5	5
Mark Taylor[1]	5	2.5	0	2.5	3

1 Department of Environmental Sciences, Macquarie University, NSW 2109 Australia
2 Department of Biological Sciences, Macquarie University, NSW 2109 Australia

Abstract

Waterborne pathogens and mosquitoes are a public health concern. Urban stormwater runoff contributes to elevated faecal indicator bacteria in surface waters. The link between storm events and disease outbreaks traced to faecal contamination of recreational waters is well recognised. The use of stormwater treatment devices to remove indicator bacteria, however, is not well studied. Further, little is known about the potential of surface flow stormwater wetlands to provide suitable mosquito habitat and therefore increase public health risk of exposure to vector borne diseases. This study evaluated the ability of four stormwater treatment devices (three stormwater wetlands and one bioretention system) in Sydney, Australia, to reduce faecal coliform and enterococci in stormwater. The abundance and composition of immature mosquito assemblages within the inlet and outlet zone of the wetlands was also measured. Mean faecal coliform and enterococci concentration reductions ranged from 12% to 80% and -86% to 75%, respectively. None of the stormwater treatment devices had a median outflow enterococci concentration below the Australian recommended guideline for primary contact and only one wetland site did so for faecal coliforms, but this system received low influent concentrations. A total of eight mosquito species were identified from the wetlands: *Aedes alternans, Aedomyia venustipes, Culex sitiens*, *Culex annulirostris, Culex australicus, Culex molestus, Culex globocoxitus* and *Mansia uniformis.* A significantly higher number of immature mosquitoes were recorded for inlet sites in comparison to outlet sites, the latter of which showed very low numbers or none present. Immature mosquito presence was significantly positively associated with nutrients at all three wetlands and dissolved oxygen at one wetland. The results show that the ability of stormwater treatment devices to reduce indicator bacteria in urban stormwater to below guideline values for recreational human contact was unattainable in most events. This study also highlights that a technical solution to manage water in the inlet zone and reduce mosquito larval densities may be needed to prevent stormwater wetlands from being a liability to nearby communities.

Introduction

Impervious coverage in urbanised areas reduces the infiltration of rainfall and increases the volume of stormwater runoff mobilising deposited pollutants (Davies & Bavor, 2000; Lee & Heaney, 2003). Traditionally, stormwater runoff has been rapidly collected and diverted untreated through storm drains to nearby waterways for flood prevention (Chapman & Horner, 2010). Bacterial concentrations in urban stormwater runoff are commonly above recommended guidelines for human contact (Birch et al., 2004; Hathaway et al., 2009; Krometis et al., 2009; Sidhu et al., 2012). Periods of heightened pathogen loads can negatively impact public health if receiving waters are used for recreational purposes (Gaffield et al., 2003; Sidhu et al., 2012). Contact with pathogens may cause respiratory, gastrointestinal, dermatologic, and ear, nose and throat infections (Henrickson et al., 2001). Various studies have reported links between the occurrence of storm events, bacterial inputs from untreated stormwater and proximity of swimmers to storm drain outlets with adverse public health effects (Cartwright, 1993; Haile et al., 1999; Curriero et al., 2001; Gaffield et al., 2003).

Most water-borne pathogens occur intermittently and are not easily recovered (Kashefipour et al., 2006) and instead, indicator species are generally used (Hathaway et al., 2009). Faecal (thermo-tolerant) coliforms and enterococci bacteria are common indicator species (Hose et al., 2005) and are used to monitor recreational waters in Australia (ANZECC/ARMCANZ, 2000d). While these species may not necessarily be the causative agents of illness, they originate from the faecal matter of warm-blooded animals, and their presence indicates the occurrence of pathogens of the same origin (Rusciano & Obropta, 2007). Common sources of faecal contamination in stormwater and subsequently surface waters include animal faeces (domestic and wild) and sewer overflows (human faecal pollution) (Marsalek & Rochfort, 2004). Schoonover & Lockaby (2006) showed that catchments consisting of more than 24% impervious surface had higher faecal coliform concentrations during storm conditions than non-urban catchments.

Urban populations are becoming increasingly aware of the value of their aquatic landscape and are demanding environmental improvements (Findlay & Taylor, 2006). For example, a survey conducted in NSW, Australia indicated that respondent households valued an improvement in river water quality to make it suitable for swimming (Morrison & Bennett, 2004). Over recent decades, stormwater management strategies have evolved dramatically to incorporate urban structural treatment devices, with the aim of reducing the volume of stormwater and the concentrations of pollutants therein (Roy-Poirier et al., 2010). Amongst the strategies to improve urban water quality are the installation of bioretention systems and constructed wetlands (Hathaway et al., 2009).

Wetland and bioretention systems have numerous potential mechanisms for treating allochthonous pathogenic bacteria. Bacteria in stormwater can be removed through filtration, adsorption and sedimentation processes (Rusciano & Obropta, 2007; Hathaway et al., 2009). Inactivation of immobilised bacteria is, however, controlled by abiotic and biotic factors, including: moisture conditions, ultraviolet radiation (from sun exposure), predation from other microbes, temperature, pH and organic matter content, which can all influence survival (Ferguson et al., 2003; Stevik et al., 2004). There is, however, limited peer-reviewed literature pertaining to the efficacy of wetlands and bioretention systems to remove indicator bacteria from stormwater. A small number of field studies have indicated variable performance of wetlands and bioretention systems with respect to indicator pathogen removal under storm conditions (Davies & Bavor, 2000; Birch et al., 2004; Hunt et al., 2008; Hathaway et al., 2009; Li & Davis, 2009; Passeport et al., 2009; Hathaway & Hunt, 2012). Of these studies only two examined the removal of enterococci (Davies & Bavor, 2000; Hathaway & Hunt, 2012) and only two were conducted in Australia, both at wetland sites (Davies & Bavor, 2000; Birch et al., 2004).

The proliferation of structural stormwater treatment devices has possibly increased public health risks with regard to increasing the breeding ground for mosquitoes (Russell, 1999; Metzger et al., 2002; Douglas, 2004; Hunt et al., 2006b; Metzger et al., 2008). Mosquitoes can present as a pest or vector-borne disease pathway for nearby human communities affecting adversely public health outcomes (Harding

et al., 2007). Studies have shown that traditional stormwater drains provide a suitable subterranean habitat for mosquitoes (Kay et al., 2000; Russell et al., 2002; Arana-Guardia et al., 2014). Few published studies, however, have considered mosquito production in structural stormwater treatment devices (Greenway, 2003; Gingrich et al., 2006; Hunt et al., 2006b; Kwan et al., 2008; Metzger et al., 2008), particularly for Australian conditions. This is an issue of concern due to the spread of mosquito-borne diseases in Australia, particularly Ross River virus, and the growing concern that climate change will expand the range and intensify the activity of mosquitoes and their pathogens (Russell & Dwyer, 2000).

Mosquitoes require aquatic breeding sites for the immature stages (larvae and pupae) of their life cycle (Norris, 2004). Bioretentions are a subsurface vertical flow system planted with terrestrial vegetation, that, if designed and maintained appropriately, should not retain surface water and drain within 72 h to avoid mosquito breeding (Rusciano & Obropta, 2007; Roy-Poirier et al., 2010). In contrast, surface flow constructed wetlands are permanent open water bodies (Water by Design, 2009) and therefore have the potential to provide a habitat for mosquitoes (Russell, 1999). Inlet zones of constructed stormwater wetlands containing untreated stormwater may provide conditions conducive to larval production and to our knowledge, this has not been investigated.

The objectives of the present study were to: (i) evaluate the effectiveness of three retrofitted stormwater wetlands and a bioretention system for the reduction of both faecal coliform and enterococci; and (ii) document the abundance and species composition of immature mosquitoes within the inlet and outlet zone of the three constructed wetlands. Additionally, factors thought to be associated with mosquito production were analysed in relation to mosquito abundance. Our hypothesis was that, with stormwater treatment, indicator bacteria concentrations would be reduced between the inlet and the outlet of treatment devices, and at the wetland sites fewer immature mosquitos would be recorded at the outlet zones in comparison to the inlet zones.

Methods

Study sites

This study was conducted at three constructed wetlands and a bioretention system located on the Cooks River catchment in the inner West of Sydney, NSW, Australia. The Cooks River is considered to be one of the most degraded and polluted river systems in Australia (NSW Government, 2011). The Cooks River catchment has separate sewer and stormwater systems. However, the Cooks River is heavily impacted by sewer overflows, which are surcharge points in the sewerage system that can operate in wet weather events due to stormwater infiltration into the sewer caused by cracked/leaking pipes and illegal connections to the sewer (NSW OEH, 2011).

Wetland 1 (Yarrowee Wetland; W1) was retrofitted to a residential area in Strathfield (33°53'S, 151°04'E) in 2010 to provide stormwater treatment and an aquatic habitat. A full description of this site is provided in Chapter 2. Waterfowl, particularly ducks, were frequently observed at the outlet zone during site visits.

Wetland 2 (Coolibah Wetland; W2) was retrofitted to a residential catchment in Turrella (33°55'S, 151°08'E) and restored in 2010 to provide an aquatic habitat and treat stormwater. A full description of this site is provided in Chapter 4. A deep-water pool zone was created to provide refuge for mosquito predators during low water levels (Dragonfly Environmental, 2009). The wetland was adjacent to a children's playground and children were frequently observed playing in the outlet pond during site visits.

Wetland 3 (Gadigal Green Wetland; W3) was retrofitted to a residential catchment in Darlington (33°55'S, 151°08'E) in 2008 to treat stormwater. A full description of this site is provided in Chapter 4.

A bioretention basin (Johnston St bioretention) was retrofitted to a residential catchment in Earlwood (33°53' S, 151°11' E) in 2011 to treat stormwater runoff. A full description of this site is provided in Chapter 3.

Indicator bacteria collection

Automatic water samplers (Gamet 12M, Gamet Equipment Pty Ltd, NSW) were installed at the inflow and outflow drainage points of the bioretention system and wetland W1. These automatic samplers were used to collect samples from W1 and the bioretention for three (April 2012 – October 2012) and five (January 2013 – April 2013) storm events, respectively. A flow-weighted composite sample was generated for each storm event. Full details of the automatic sampling procedure and how the flow-weighted composite sample was obtained are provided in Chapter 2. In addition to the automatic samplers, opportunistic grab samples were taken in sterile containers from both the inlet and outlet zone at W1 (4 events, May – October 2011), W2 (3 events, July – October 2011) and W3 (2 events, September – October 2011). Timing of grab sampling in relation to the storm event is not known; however, all samples were collected either during a storm event or when storm inflow was still occurring as per Hathaway & Hunt (2011). The occurrence of storm events was predicted using rain radar images provided by the Bureau of Meteorology (Commonwealth of Australia, 2015) and a water sample was collected from the wetland sites as soon as possible after the start of a rain event. The total daily rainfall amounts preceding sample collection, obtained from weather stations within 5 km away from wetland sites, ranged from 3 to 39 mm for Yarrowee wetland (Strathfield Golf Club weather station), 3 to 60 mm for Coolibah wetland (Sydney Airport weather station) and 3 to 30 mm for Gadigal Green wetland (Sydney Observatory Hill weather station) (Commonwealth of Australia, 2015). The limitations of grab sampling therefore apply as the concentrations of a given pollutant may vary during a storm event (Hathaway & Hunt, 2012). Despite these limitations, this method has been employed successively in previous studies for the sampling of indicator bacteria at stormwater treatment devices (Hunt et al., 2008; Hathaway et al., 2009; Hathaway et al., 2011a; Hathaway & Hunt, 2012).

One sample was collected from the inlet and outlet of each stormwater treatment device for each storm event for microbial analysis (faecal coliforms and enterococci). Samples were immediately placed on ice in an insulated container

for transport to a National Association of Testing Authorities (NATA) accredited laboratory (Australian Laboratory Services, Sydney). At the laboratory, samples were stored at 4°C and analysed within 24 h of collection for faecal coliforms (thermotolerant coliforms) using method AS 4276.7 (Standards Australia, 2007a) and for enterococci using method AS 4276.9 (Standards Australia, 2007b). These standards use membrane filtration and results are based on the growth of bacteria on the filter membrane being counted, which is reported as colony forming units per 100 mL (cfu/100 mL). Laboratory quality control measures in place for microbiological tests consisted of a method blank as well as a positive and negative control specific to the target organism.

Immature Mosquito collection

Mosquito sampling was undertaken as part of a macroinvertebrate survey (Chapter 4). In brief, sampling was undertaken at W1, W2 and W3 during autumn 2011 and spring 2011 in dry weather conditions. Sampling was based on a wetland rapid bioassessment protocol (Davis et al., 1999) and incorporated four sites in both the inlet and outlet pond of each wetland. In each sector, samples were collected from a minimum of two habitats for a standardised period of time (two minutes) using a sweep net (0.12 m^2 opening; 250 μm mesh size). Invertebrates were live picked in the field on a sorting tray for 30 minutes and preserved in 70% ethanol. Mosquito larvae were counted and identified in the laboratory to species level (Russell, 1993) and predatory insect invertebrates of mosquito larvae (Epiproctophora, Hemiptera and Coleoptera; Russel 1993) also counted and identified to family level (Gooderham & Tsyrlin, 2002). The number of pupae was also recorded. Samples were combined into one sample for both the inlet and outlet of each wetland to be representative of each study site. There were no fish present in the wetlands surveyed. A summary of the predatory insect invertebrates collected in the inlet and outlet zone of all three wetlands is presented in Appendix 6 (Table A6.1).

In conjunction with the mosquito sampling, dissolved oxygen concentrations were measured in the water at both the inlet and outlet using a field meter (YSI

ProPlus). Grab samples were also taken for total nitrogen (TN) and total phosphorus (TP) analysis and were delivered on ice to Australian Laboratory Services (ALS), Sydney where they were analysed for TN and TP using standard method APHA 4500 (APHA, 1999). Vegetation cover in the inlet and outlet zone was estimated visually as a percentage by the same person at each of the wetlands.

Data presentation and statistical analyses

Bacterial concentrations are reported as colony forming units (cfu) per 100 mL of sample. To evaluate the performance of each stormwater treatment device to remove influent indicator bacteria, percentage reduction efficiencies (RE) were calculated for both faecal coliforms and enterococci for each event using Eq. (1) (Hathaway et al., 2009).

$$RE = (1 - \text{Outflow concentration}/\text{Inflow concentration}) \times 100 \quad (1)$$

Median concentrations of faecal coliforms and enterococci in the outlets at each site were compared to ANZECC/ARMCANZ (2000d) recreational water quality guidelines for primary (e.g. swimming) and secondary (e.g. boating) contact.

The difference in the abundance of immature mosquitoes collected from the wetland inlet and outlet sites was evaluated using a paired *t*-test. Pearson correlation was used to determine associations between mosquito abundance and dissolved oxygen, total nitrogen (TN), total phosphorus (TP), vegetation cover and predator abundance (number of predatory insect invertebrates) in each wetland. Data were tested for normality using Anderson-Darling normality tests/normal probability plots and analysed using MINITAB 16 (Minitab Inc, USA). Variables that had non-normal distributions were $\log_{10}$ (x) transformed and percentage data arcsine transformed prior to analysis. The significance level for all analysis was 0.05.

Results

Faecal Coliforms and Enterococci

Percentage reductions in faecal coliform and enterococci concentrations for W1, W2, W3 and the bioretention system are presented in Table 14. Median inlet and outlet indicator bacteria concentrations and compliance of outlet values with respect to primary and secondary contact guidelines for recreational waters (ANZECC/ARMCANZ, 2000d) are shown in Tables 15 and 16.

With the exception of W1, the stormwater treatment devices in this study reduced the faecal coliform content of influent stormwater across all sampling occasions (Table 14). W1 showed a negative reduction on occasion, with higher faecal coliform concentrations in the outflow than the inflow. The mean concentration reduction efficiency for faecal coliforms in W1, W2, W3 and the bioretention system was 12% (range: -117 to 93%), 68% (range: 38 to 99%), 62% (range: 27 to 97%) and 80% (range: 57 to 94%), respectively; exemplifying the inter-event variation in the reduction of faecal coliforms from stormwater by the treatment devices (Table 14).

Median faecal coliform concentrations at the outlet were above ANZECC/ARMCANZ (2000d) guidelines values for both primary and secondary contact at W1, W2 and the bioretention site. Median concentrations exceeded primary contact guidelines by 13, 12, and 33 times, and secondary contact guidelines by 2, 1.8 and 5 times at W1, W2 and the bioretention system, respectively. W3 received stormwater that had a median faecal coliform concentration lower than primary contact guidelines (Table 15). Outflow concentrations of faecal coliforms for individual storm events exceeded the recommended limit for primary contact for all 6 samples taken at the bioretention system, all 7 samples from W1, all 3 samples from W2 and 0 of the 2 samples from W3. For secondary contact, outflow concentrations of faecal coliforms for individual storm events exceeded the recommended limit for all 6 samples taken at the bioretention system, 6 out of 7 samples from W1, 2 out of 3 samples from W2 and 0 of the 2 samples from W3.

A similar reduction in the enterococci content of stormwater in comparison to faecal coliforms was not observed. On occasion, outlet water samples from W1, W3 and the bioretention system had higher outflow enterococci concentrations than inflow concentrations. The mean reduction efficiency for enterococci in W1, W2, W3 and the bioretention system was -86% (range: -416 to 91%), 75% (range: 36 to 97%), 22% (range: -52 to 97%) and -29% (range: -300 to 86%), respectively; highlighting the large variability in the reduction of enterococci from stormwater by the treatment devices on an inter-event basis (Table 14).

For enterococci, none of the monitored stormwater treatment devices had median concentrations at the outlet that complied with ANZECC/ARMCANZ (2000d) target values for primary contact, although outlet levels at Wetland 2 were lower than secondary contact guidelines. Median concentrations at the outlet were above the primary contact guidelines by 60, 23, 4.8 and 114.3 at W1, W2, W3 and the bioretention system, respectively. With the exception of W3, median concentrations at the outlet were above secondary contact guidelines by 9.1, 3.5 and 17.4 for W1, W3 and the bioretention system, respectively (Table 16). Outflow concentrations of enterococci exceeded the recommended limit for primary and secondary contact for all samples taken at the bioretention, W1 and W2 and 1 out of 2 samples taken at W3 (Table 14).

Table 14. Faecal coliform and Enterococci concentrations in stormwater runoff from the inflow and outflow of the four stormwater treatment devices and the concentration reduction efficiencies (%) for each storm event.

Site	Event	Sample Method	Faecal Coliform (CFU/100 mL)			Enterococci (CFU/100 mL)		
			Inflow	*Outflow*	*Concentration Reduction (%)*	*Inflow*	*Outflow*	*Concentration Reduction (%)*
Wetland 1	1	Grab	2200	1900	14	750	450	40
	2	Grab	4700	3200	32	6100	3500	43
	3	Grab	2400	5200	-117	910	4700	-416
	4	Grab	21000	6000	71	20000	2100	90
	5	Autosampler	24000	1600	93	3600	5300	-47
	6	Autosampler	1000	2000	-100	200	1000	-400
	7	Autosampler	3000	210	93	12000	1100	91
	Event 1-7 Mean		8329	2873	12	6223	2593	-86
	Stadard deviation		9782	2070	88	7355	1923	225
Wetland 2	1	Grab	2900	1800	38	5900	3800	36
	2	Grab	7800	2600	67	11000	810	93
	3	Grab	28000	280	99	25000	700	97
	Event 1-3 Mean		12900	1560	68	13967	1770	75
	Standard deviation		13305	1178	31	9890	1759	34
Wetland 3	1	Grab	150	110	27	570	16	97
	2	Grab	91	3	97	210	320	-52
	Event 1-2 Mean		121	57	62	390	168	22
	Standard deviation		42	76	50	255	215	106
Bioretention	1	Autosampler	38000	5000	87	6000	8000	-33
	2	Autosampler	3000	1300	57	1500	1100	27
	3	Autosampler	12000	1800	85	1000	4000	-300
	4	Autosampler	30000	7000	77	21000	5300	75
	5	Autosampler	360000	20000	94	24000	3300	86
	Event 1-5 Mean		88600	7020	80	10700	4340	-29
	Standard deviation		152354	7624	14	10998	2550	159

Table 15. Median inlet and outlet Faecal Coliform concentrations (CFU/100 mL) and compliance of outlet values with primary and secondary contact guidelines.

	Faecal Coliform (CFU/100 mL)			
Site	Median Inflow	Median Outflow	Primary contact* 150 CFU /100 mL	Secondary contact* 1000 CFU/100 mL
W1	3000	2000	FAIL	FAIL
W2	7800	1800	FAIL	FAIL
W3	120.5	56.5	PASS	PASS
Bioretention	30000	5000	FAIL	FAIL

* ANZECC/ARMCANZ (2000d) Guidelines for Fresh and Marine Water Quality

Table 16. Median inlet and outlet Enterococci concentrations (CFU/100 mL) and compliance of outlet values with primary and secondary contact guidelines.

	Enterococci (CFU/100 mL)			
Site	Median Inflow	Median Outflow	Primary contact* 35 CFU/100 mL	Secondary contact* 230/100 mL
W1	3600	2100	FAIL	FAIL
W2	11000	810	FAIL	FAIL
W3	390	168	FAIL	PASS
Bioretention	6000	4000	FAIL	FAIL

* ANZECC/ARMCANZ (2000d) Guidelines for Fresh and Marine Water Quality

Immature Mosquitoes

Summary data on the abundance of immature mosquitoes and identified species are presented in Table 17. Immature mosquitoes were found at all sample sites in both autumn and spring (Table 17). The majority of mosquitoes were collected from the inlet ponds of each wetland (W1: autumn 99% and spring 99%; W2: autumn 87% and spring 70%; W3: autumn 100% and spring 98%). Overall, a significantly higher number of immature mosquitoes were recorded for inlet zone sites in comparison to outlet zone sites (t = 4.38, p = 0.007), the latter of which showed very low numbers or none present at the time of sampling. The largest difference in immature mosquito numbers between the inlet zone and outlet zone was at W1 and the smallest difference at W2. In the inlet zone at each of the three wetlands 90 – 100% of the collected immature mosquitoes were larvae and 0 – 10% were pupae (Table 17).

Eight mosquito species were identified from the wetlands. These were: *Aedes alternans, Aedomyia venustipes, Culex sitiens*, *Culex annulirostris, Culex australicus, Culex molestus, Culex globocoxitus and Mansia uniformis.* Of these, five are known pest species (i.e. bite humans) and six have been implicated as vectors/carriers of disease. Occurrence of multiple species was observed at all inlet sites with *Culex* mosquitoes dominating in all three wetlands. In spring, *C. australicus* was the most commonly encountered species occurring at all three wetlands. In autumn, *C. annulirostris* was the predominant species at W1, whilst *C. australicus* remained the most common species at site W2 and W3 (Table 18).

Nutrient concentrations were higher and predator numbers lower in the inlet zone compared to the outlet zone of all three wetlands (Table 18). The presence of immature mosquitoes was positively correlated with TN and TP and negatively correlated with predator abundance at all three wetlands; however, associations were only significant for TN and TP (p < 0.05) (Table 18). Immature mosquito abundance showed a variable response to dissolved oxygen and vegetation cover across different sites. Responses were generally negatively correlated with an increase in vegetation cover and dissolved oxygen from the inlet zone to the outlet

zone; however associations were only significant for dissolved oxygen at W1 ($p < 0.05$; Table 18).

Table 17. Number and species of immature mosquitoes collected in the inlet and outlet of three stormwater wetlands and their pest/vector status and associated pathogens.

Location		Site[a]						Pest/vector status[bc]
		W1 A	W1 S	W2 A	W2 S	W3 A	W3 S	
Inlet		**Total Number**						
	Immature Mosquitoes	100	621	13	42	8	101	
		(% of Total)						
	Aedes alternans	–	0.2	–	–	–	–	Pest; virus (RR)
	Aedomyia venustipes	2	–	–	–	–	–	Non-pest
	Culex sitiens	–	9	8	33	–	2	Pest, virus (RR)
	Culex annulirostris	63	1	–	–	–	5	Pest; virus (BF. KUN, MVE, RR, JE)
	Culex australicus	17	84	85	57	63	79	Non-pest; virus (KUN, MVE, RR)
	Culex molestus	10	2	–	–	38	4	Pest; virus (MVE)
	Culex globocoxitus	–	1	–	–	–	6	Non-pest
	Pupae (not identified)	8	2	8	10	–	4	
Outlet		**Total Number**						
	Immature Mosquitoes	1	3	2	18	0	2	
		(% of Total)						
	Culex australicus	–	–	–	89	–	–	See above
	Mansia uniformis	–	–	–	–	–	100	Pest; virus (MVE, RR)
	Culex molestus	–	–	–	6	–	–	See above
	Pupae (not identified)	100	100	100	6	–	–	

[a] Sites: W1, wetland 1; W2, wetland 2; W3, wetland 3; A, Autumn; S, Spring

[b] Complied from Russell (1993) and Russell (1999)

[c] Arboviruses: BF, Barmah Forest; KUN, Kunjin; MVE, Murray Valley encephalitis; RR, Ross River; JE, Japanese encephalitis.

Table 18. Pearson correlation coefficients between immature mosquito abundance and environmental variables measured on each sampling occasion.

Variable	***Wetland 1*** Mean ± SE		r^2	***Wetland 2*** Mean ± SE		r^2	***Wetland 3*** Mean ± SE		r^2
	Inlet	Outlet		Inlet	Outlet		Inlet	Outlet	
Dissolved Oxygen (%)	24 ± 2	74 ± 13	- 0.962*	32 ± 22	56 ± 33	- 0.893	26 ± 6	21 ± 14	0.235
Predator abundance	17 ± 13	260 ± 72	- 0.840	1 ± 1	62 ± 11	- 0.665	31 ± 7	52 ± 24	- 0.518
Vegetation cover (%)	73 ± 2.5	78 ± 2.5	0.15	28 ± 2.5	88 ± 2.5	- 0.558	77 ± 2.5	85 ± 5	- 0.865
Total nitrogen (mg L^{-1})	1.95 ± 0.45	0.65 ± 0.15	0.980*	1.8 ± 0.6	1.2 ± 0.3	0.996*	2.3 ± 0.8	0.6 ± 0	0.987*
Total Phosphorous (mg L^{-1})	0.15 ± 0.11	0.03 ± 0.02	0.982*	0.2 ± 0.1	0.1 ± 0.05	0.986*	0.2 ± 0.1	0.05 ± 0.03	0.986*

* Statistical significance ($p < 0.05$)

Discussion

Faecal Coliforms and Enterococci

The bioretention system evaluated in the current study demonstrated a moderate to high removal of faecal coliforms across all sampling events, with a high overall reduction efficiency. This is consistent with the results of other field bioretention studies conducted in North Carolina by Hathaway et al. (2009) and Passeport et al. (2009), which recorded concentration reduction efficiencies of greater than 85% for faecal coliforms. The bioretention systems monitored by Passeport et al. (2009) and Hathaway et al. (2009) had a surface area to catchment ratio of 3.2% and 6%, respectively, whilst the bioretention in the current study was undersized at 1% of the catchment. The results from this study show strong potential for the use of size-constrained bioretention systems for reducing faecal coliforms in stormwater.

The reduction efficiency of a bioretention system is partly dependent on the soil media used, as bacteria preferentially adhere to fine particles (Dale, 1974). Laboratory studies have indicated that bacteria mobility decreases, and therefore removal increases, with a higher soil clay contents in comparison to sandy soils (Huysman & Verstraete, 1993; Meschke & Sobsey, 2003). Clay particles in media enhance adsorption due to their small pore size, large surface area to volume ratio and large cation exchange capacity (Huysman & Verstraete, 1993). Hunt et al. (2008) suggested that a bioretention media containing clay and silts (fine soil particles) may therefore have more potential to reduce bacteria than a sand media. The fill media of the bioretention in the current study had a combined clay and silt content of 5.2%, which is similar to the content (6%) in the bioretention studied by Hathaway et al.(2009).

In contrast to the bioretention system, the three wetlands monitored in this study showed more variability in faecal coliform removal efficiency. Birch *et al.* (2004) also observed wide ranging faecal coliform removals (26 to 99%) at a stormwater wetland in Sydney, Australia. The overall faecal coliform reduction efficiencies reported for W2 and W3 were generally lower than those reported for stormwater wetlands (76-89%, Davis & Bavor (2000), Birch et al. (2004), Hathaway et al.

(2009)), but higher than for one particular wetland (56%) studied by Hathaway et al. (2009). The ratio of wetland size to catchment size in these previous studies ranged from 0.1 – 2%, which is within the range of W2 and W3 in this study. Hathaway et al. (2009) suggested that the wetland site experiencing the highest removal (i.e. 89%) was poorly vegetated, resulting in a larger amount of sun exposure than would be expected for wetlands and potentially leading to higher bacterial die-off (Hathaway et al., 2009). A lack of vegetation, however, is not a desirable characteristic for stormwater wetlands (Hathaway et al., 2012), as it plays an important role in impeding water flow and aiding in pollutant reduction (DLWC, 1998; Davies & Bavor, 2000).

Despite being the largest sized wetland in relation to the contributing catchment area, W1 had poor efficiency of faecal coliform removal and an increase in concentrations at the outflow was seen for some events. Hathaway et al. (2011b) observed that faecal coliform concentrations decreased through the first half of a constructed stormwater wetland before remaining relatively consistent throughout the remainder. The authors suggested that this indicated that wetlands appear to have a baseline concentration of faecal coliforms due to animal activity or internal processes such as microbial persistence. Waterfowl were frequently seen in the outlet zone at W1 and their excreta undoubtedly provided direct faecal inputs to the system. Stormwater treatment devices such as this, which are designed to also provide wildlife habitat as well as stormwater treatment, may potentially add bacteria to receiving waters by attracting animals that defecate in and around the treatment device.

The immobilisation of bacteria from the water column is facilitated by adsorption to particles and subsequent sedimentation (Davies & Bavor, 2000; Jin et al., 2005). Karim et al. (2004) observed that wet sediments in constructed wetlands provided an environment that prolongs the survival of faecal coliforms. Kibbey et al. (1978) showed that drier soils had higher and more rapid bacteria die off rates than those under moist conditions. The bioretention system rapidly drained at the surface and therefore had a low moisture-retaining capacity, which would have assisted in the inactivation of bacteria by desiccation, whilst the wetland systems retained water.

It is possible that the wet sediment conditions in the wetlands provided an environment where the faecal coliforms could survive for an extended period of time, prolonging their removal and die-off. Survival of faecal coliforms in water has been shown to be significantly longer in waters containing sediment than those without (Sherer et al., 1992). The recontamination of overlying waters with micro-organisms due to potential sediment re-suspension during storm events is a concern for stormwater wetlands (Hathaway & Hunt, 2012). Birch et al. (2004) noted that removal of faecal coliforms was substantially reduced during intense rainfall periods. This may have been a contributing factor to the inter-event variability in faecal coliform reduction at the stormwater wetlands and lower overall removal in the current study.

The reduction of enterococci was, with the exception of W2, considerably lower than for faecal coliforms. One possible explanation is that enterococci are more persistent in the environment than faecal coliforms (Noble et al., 2003; Jin et al., 2005). Enterococci can survive desiccation and regrow in rewetted sediments after up to 60 days (Hartel et al., 2005), a scenario which would regularly occur in bioretention systems. Further, the hydrophobic properties of enterococci may enable them to adsorb more efficiently to clay particles than coliforms (Davies & Bavor, 2000). Particle associated bacteria can persist longer due to protection from environmental conditions that otherwise would cause mortality e.g. predation (Roper & Marshall, 1974; Sherer et al., 1992). Wetland W2 was clay lined to ensure that it retained water, which may have accounted for the good reduction in enterococci in comparison to the other wetland sites, but this has a greater recontamination risk if enterococci are ‘stored’ in the sediment.

The reduction efficiency of enterococci by the bioretention system was variable. A study conducted by Hathaway et al. (2011), on two North Carolina bioretention systems, showed a substantial difference in enterococci removal (mean reduction of -119% and 89%, respectively) between the two systems. Soil depth, which influences hydrologic function, was identified as the most probable factor effecting the removal efficiency of the systems, with the shallower bioretention system (0.25 m) being less able to mitigate peak flows (i.e. detain stormwater) than the deeper

bioretention system (0.6 m). Shallower systems have less contact time between the stormwater and the media compared to deeper systems, thereby reducing the potential for bacterial adsorption and increasing the likelihood of stripping/passing of bacterial cells through the media (Stevik et al., 2004; Hathaway et al., 2011a). The depth of the bioretention media in the current study was 0.4 m, and the system was much smaller in relation to the contributing catchment area than was the systems studied by Hathaway et al (2011). The smaller size of the system potentially increases the hydraulic loading and limits the detention of stormwater. Due to the higher resilience of enterococci in comparison to faecal coliforms (Jin et al., 2005), there is limited potential for the reduction of enterococci by the bioretention system in the current study, given that the system has design limitations.

Davies & Bavor (2000) reported an overall 87% reduction of enterococci at a stormwater wetland in Sydney, Australia. Other stormwater wetlands monitored by Hathaway & Hunt (2012) in North Carolina showed reductions for enterococci of 69 and 41%. In the current study, the W1 and W2 wetlands had variable performance in terms of enterococci reduction. The occasional negative enterococci removal (i.e. increase in concentration) may be due to faecal inputs from waterfowl as discussed above. Sediment-bound enterococci may have also been re-suspended by storm activity. The geology of the catchment in this study is characterized by sandstone and shale, which is composed of clay and fine minerals. Fine particles are less effectively removed than medium-sized particles in stormwater (Davies & Bavor, 2000). Bacteria attached to fine shale sediments may remain suspended throughout the wetland, which may explain the low removal efficiency.

Although the bioretention and wetlands showed some potential to reduce faecal coliform concentrations, stormwater treatment devices, with the exception of W3, which had influent levels below recreational guidelines levels, had median effluent values which remained above the guideline values for both primary and secondary contact (ANZECC/ARMCANZ 2000d). Mean outlet faecal coliform concentrations recorded for the bioretention in the current study were higher than those observed in the outflow of bioretention systems in North Carolina by Hathaway et al. (2009)

(258 cfu/100 mL) and Passeport et al. (2009) (125 and 646 cfu/100 mL). For the wetlands, mean faecal coliform concentrations at the outlet were lower than those observed in the outflow of most wetland systems in North Carolina studied by Hathaway et al. (2009) (3,874 cfu/100 mL) and in Sydney, Australia by Davis & Bavor (2000) (3600 cfu/100 mL) and Birch et al. (2004) (41,369 cfu/100 mL), but higher than for one wetland in North Carolina (Hathaway et al., 2009; 184 cfu/100 mL).

For enterococci, only one stormwater treatment device had a median effluent value below the guideline levels for secondary contact and median effluent values at all sites failed to meet primary contact guidelines. Mean enterococci concentrations in the outlet were higher than those observed in the outflow of bioretention systems in North Carolina by Hathaway & Hunt (2012) (39 and 378 MPN/100 mL). At wetland sites, mean enterococci concentrations at the outlet were higher at W1 and W2 and lower at W3 than those previously reported in the outflow of a wetland in Sydney by Davis & Bavor (2000) (900 cfu/100 mL) and in North Carolina by Hathaway & Hunt (2012) (316 and 510 MPN/100 mL).

Similar studies have observed concentrations of indicator bacteria in outflows that do not meet target values for recreational human contact, despite good removal performance (Birch et al., 2004; Hathaway et al., 2009; Passeport et al., 2009; Hathaway & Hunt 2012). Therefore, levels of indicator bacteria identified in the outflowing waters in this study may still pose a risk to human exposure and public access to the stormwater wetlands should also be minimized. The source of faecal contamination in stormwater was probably a combination of sewage overflows in the catchment (human faecal pollution) (NSW OEH, 2011) and domestic animal waste from the surrounding residential areas (Young & Thackston, 1999). It is also likely that water birds contributed directly to the faecal bacteria loads in the systems, particularly at W1 where waterfowl were frequently observed. However, no single point source of faecal contamination was identified in the current study and further work is required to establish the origin of faecal pollution sources through microbial source tracking. The ability to discriminate between sources of faecal contamination is important for the potential human health risk to be

adequately understood, as faecal contamination from human sources presents a greater human health risk than faecal contamination from other sources (Domingo & Edge, 2010).

Immature Mosquitoes

This study confirms that constructed stormwater wetlands provide habitat for immature mosquitoes, which is in agreement with other studies (Gingrich et al., 2006; Metzger et al., 2008). We expand on previous research by demonstrating specifically that the inlet zone of stormwater wetlands can provide conditions more conducive to the production of immature mosquitoes compared to the outlet zone. This has important implications for mosquito management in stormwater wetlands.

The inlet zones of the stormwater wetlands were seen to be utilized primarily by *Culex* mosquito larvae, which are significant pests and vectors for disease (Russell, 1999). *Culex australicus* was the most commonly recorded species across all three wetlands in Autumn. This species feeds predominantly on birds and rabbits but not humans (Russell, 1993). The species can carry Murray Valley encephalitis (MVE) and Kunjin (KUN) viruses that can cause potentially fatal encephalitis, and Ross River (RR) virus which can cause debilitating polyarthritis (Russell, 1998; Russell & Dwyer, 2000). Water birds are principal reservoir hosts of MVE and KUN and virus activity has been linked with wetlands where the birds congregate in close association with mosquitoes (DLWC, 1998). It is thought that *C. australicus* may be involved in enzootic transmission and amplification of MVE and KUN in reservoir hosts (Russell & Dwyer, 2000). Birds frequently congregate at W1 and therefore caution should be applied when building wetlands in residential areas that are designed to provide a wildlife habitat as well as stormwater treatment. An assessment of whether mosquitoes have access to pathogen hosts should be carried out when proposing the implementation of a constructed wetland (DLWC, 1998).

C. annulirostris was recorded at two of the monitored wetlands and was the dominant species at W1 in autumn. This species is a major pest and principal vector of Barmah Forest Virus, which causes polyarthritis, Japanese encephalitis

which causes encephalitis, and MVE, KUN and RR viruses (Russell, 1999; Russell & Dwyer, 2000). Ross River virus occurs in every state of Australia and is the most common human arboviral disease in the country, with infections increasing (Russell & Dwyer, 2000). Adults of *C. annulirostris* can travel up to 12 km from breeding places (Russell, 1986). Residential housing is located only approximately 20 m away from the wetlands where this species was found, and is therefore within flight range of this species, presenting a potential pest and disease problem for nearby human communities. Consideration of residential proximity to constructed wetlands (i.e. within effective flight range) should be taken into account when assessing the risk presented by mosquitoes (Russell, 1999). Where possible, a buffer zone should be created between the flight range of local mosquitoes and residential areas and these zones should prevent harbourage of mosquitoes by being kept clear of dense vegetation and maximizing exposure to wind disturbance (Webb, 2013).

Although some of the other mosquito species identified in the wetland can be nuisance pests, there is no evidence of them causing disease in Australia. There is, however, evidence that they can carry viruses of concern (Russell, 1993). It is important to note that mosquito larvae are a natural component of aquatic food webs (Greenway, 2003) and are therefore likely to occur in the broader urban water environment in the absence of stormwater treatment devices. Prior to the construction of a stormwater wetland, surveillance of the surrounding area for the presence of any pest or vector mosquitoes should be undertaken (DLWC, 1998). We have shown that constructed wetlands can increase the potential breeding ground of mosquitoes. Pupae were recorded at all three wetlands and were found to be more abundant in inlet zone sites in comparison to outlet zone sites. Mosquitoes go through four larval instars between the egg and the pupa stage, the latter of which is the final life cycle stage before developing into the adult form (Russell, 1993). This suggests that conditions are suitable for the larvae to survive and develop into pupae at the stormwater wetlands.

It has been claimed that predators and vegetation play an important role in controlling mosquito production in constructed wetlands (Russell, 1999;

Greenway, 2003; Anderson et al., 2007). However, there is limited published material with respect to how these two factors influence mosquito numbers in stormwater wetlands. Macroinvertebrate predators, such as Dytiscidae and Notonectidae, can decrease mosquito larvae populations under experimental conditions (Ellis & Borden, 1970; Lundkvist et al., 2003). Although an inverse relationship between macroinvertebrate predator abundance and immature mosquito numbers was observed in the current study for all three wetlands, which is in agreement with the trend observed at stormwater wetlands by Gingrich et al., (2006), this association was not statistically significant. Fish were absent from the wetlands in this study, which is likely to have been a limiting factor in reducing mosquito larvae presence. Predatory fish, such as the mosquito fish *Gambusia*, have been shown to be more effective at mosquito control than any other biological agent (including invertebrates) (Russell,1993). Hunt et al. (2006) also observed a significant association between the absence of mosquito fish (*Gambusia affinis*) and the presence of mosquito larvae in constructed stormwater wetlands.

Further to this, dense vegetation in constructed wetlands is thought to support mosquito production by protecting larvae from predators and physical disturbance (Russell, 1999; Greenway, 2003). This is not the case in the current study as there were no significant associations between vegetation cover and immature mosquito numbers, and for two wetlands an inverse relationship was observed. However, in the current study only the inlet and outlet were surveyed and it is probable that the poor water quality in the inlet was more influential on mosquito production than was vegetation cover. To assist in reducing mosquito populations, the strategic removal of excessive marginal and floating vegetation and associated debris is recommended (Russel, 1999). Dense vegetation can create a refuge for mosquitoes and decaying plant material increases the suitability of the habitat for mosquitoes by increasing the organic content of the water (Webb, 2013).

The lowest number of immature mosquitoes was observed in W2, which was designed with a deep-water pool zone to provide refuge for mosquito predators during low water levels. Walton & Workman (1998) conducted a comparative study

of larval mosquitoes in two structurally different constructed wetlands receiving secondary-treated effluent: one-phase marshes, which had vegetation throughout, and 3-phase marshes, which had vegetated inlet and outlet marshes separated by a region of deeper open water. Larvae abundance in one-phase marshes was higher than in 3-phase marshes, which was attributed to greater predator abundance, particularly notonectids, which was thought to have been facilitated by habitat preference for open water. Greenway (2003) also observed that a constructed wetland with shallow marsh and deeper ponds had fewer mosquito larvae compared to other sites without this configuration. The presence of immature mosquitoes may therefore have been minimised in W2 by incorporation of the deep-water pool zone into the wetland. Incorporating deep open water zones in constructed stormwater wetlands is recommended for reducing mosquito populations, as they provide a refuge for mosquito predators and offer limited protection to mosquitoes from predators and physical disturbance (DLWC, 1998; Russell, 1999; Webb, 2013). The wetland margins should also be kept as steep and vegetation free as possible to reduce the area of shallow water and restrict the density of vegetation, minimising suitable mosquito habitat by increasing the access of predators and exposing larva to surface water movement that may disrupt survival (DLWC, 1998; Webb 2013).

Water quality is a further factor related to mosquito production in constructed wetlands (Russell, 1999). A significant and negative association between dissolved oxygen and immature mosquito abundance was observed at W1. The low dissolved oxygen content at the inlet is likely to have resulted from the decomposition of organic matter entering the wetland. Mosquito larvae can take advantage of anaerobic/low dissolved oxygen conditions as they are surface breathers (Greenway, 2003), whilst this can create unsuitable conditions for some aquatic mosquito predators that are not surface breathers (Walton, 2003). Higher numbers of immature mosquitoes were recorded in the inlet zone at W1, which unlike W2 and W3, had no form of gross pollutant trap at the point of entry into the wetland. A maintenance regime to reduce the accumulation of organic material, rubbish and sediments in the inlet zone is recommended to minimise the suitability of habitats for mosquitoes by reducing refuges for mosquitoes and increasing

exposure of larvae to increased water movement and predator access (DLWC, 1998; Russell, 1999; Webb, 2013).

Both phosphorus and nitrogen concentrations were associated significantly with immature mosquito abundance at each wetland, with inlet zones containing higher nutrient concentrations and supporting higher numbers of immature mosquitoes compared to outlet zones. Previous studies have shown that gravid female mosquitos are influenced by chemical and physical properties of the water when distinguishing between potential oviposition sites (Bentley & Day, 1989; Chen et al., 2007). Nutrient enrichment can influence mosquito oviposition preference and larval abundance by increasing microbial assemblages and microalgae that serve as a food resource for larvae (Walker et al., 1991; Johnson et al., 2010). Indeed, water enriched with nutrients enhances oviposition and larval abundance of mosquitoes in both field and laboratory-based experiments (Reiskind & Wilson, 2004; Murrell et al., 2011; Dugumna & William, 2013; Young et al., 2014). Mosquitoes may be responding to cues associated with nutrient enrichment in the wetlands and therefore showing preference for the inlet zone that contains higher nutrient concentrations. This is an important factor to consider when implementing mosquito control measures in wetland design, as the anthropogenic input of nutrients into the aquatic environment has been linked to an increased occurrence of diseases vectored by mosquitoes (Johnson et al., 2010).

Immature mosquitoes were found in low numbers or were absent from the outlet zone of the wetlands where water quality conditions were improved. Water quality of the inflowing stormwater cannot be controlled for via wetland design, as it is catchment dependent. Technical solutions to manage water in the inlet zone may need to be considered during the design phase of wetland construction. For example, implementing mechanical aeration or sprinkler systems, which disturb the water surface, causing immature mosquitoes to drown and inhibiting oviposition (DLWC, 1998; Russell, 1999). Sprinkler systems are effective in reducing the abundance of mosquitoes in wetland mesocosms (Popko & Walton, 2013) and in pond systems treating wastewater (Epipane et al., 1993). To minimize mosquitoes, constructed wetlands should be located in open areas

where wind action increases water movement and therefore disrupts larval respiration and discourages mosquito oviposition (Russell, 1999). To maximize the benefits from wind action, the long axis of the wetland should be orientated in line with the prevailing spring/summer wind direction and the shape of the wetland should be simple to facilitate good water circulation (Midge Research Group, 2007).

The mosquito survey conducted in this study has a notable limitation, as adult mosquito surveillance was not conducted at the wetland sites. The critical issue is whether the larvae survive and if adult mosquitoes emerge from pupae. Larval mosquitoes might not necessarily metamorphose or emerge due to factors such as predation (Russell, 1999). Larval mosquito monitoring, however, is important for detecting mosquito infestations and to indicate the need for possible intervention at wetland sites before a problem emerges (DLWC, 1998). For example, our study has indicated that water management options may be needed at the inlet zone of stormwater wetlands to control mosquito larvae abundance. Further studies should incorporate monitoring of emerging adult mosquito populations, which will provide data on the actual development of pest or vector borne risks.

Conclusions

The bioretention system was shown to be more effective at reducing faecal coliforms in urban stormwater than wetlands, but was less effective for controlling enterococci. A high degree of variability was apparent in the reduction of both faecal coliforms and enterococci between storm events and a longer monitoring period would therefore be beneficial to obtain a more representative characterisation of reduction efficiency and to verify significant changes between inlet and outlet concentrations. Some of the stormwater treatment devices were shown to export indicator bacteria, particularly enterococci, and therefore contribute additional bacteria contaminants to receiving waters. Despite some observed reductions in indicator bacteria, most median outflow concentrations at the stormwater treatment devices exceeded the public health criteria for primary and secondary contact, constituting a potential risk to human health. It can

therefore not be assumed that stormwater treatment devices alone will improve the recreational value of receiving waters. More research is needed to determine if both wetland and bioretention systems can be manipulated to improve treatment mechanisms and environmental conditions that will stimulate the immobilisation and inactivation of pathogenic bacteria.

Public health risks were further substantiated by the recovery of immature mosquitoes at all wetland sites monitored during this study. Data indicated that the higher nutrient concentrations in inflowing stormwater provide conditions in the inlet zone that are more conducive to the production of immature mosquitoes in comparison to the outlet zone. Incorporating water management techniques to reduce larval mosquito abundance at wetland inlet zones may therefore be needed. The data also suggested including a deep-water pool zone into the wetland design reduced the abundance of immature mosquitoes, which is consistent with other studies. With growing promotion and implementation of stormwater wetlands into urban environments to address the concern for stormwater runoff, and the added concern that mosquitoes and mosquito-borne diseases will increase with climate change, the issue of mosquito production in stormwater wetlands cannot be ignored. Further research is needed to determine the production of adult mosquitoes from these devices and what appropriate mitigation strategies can be used for mosquito abatement. This is important to minimize mosquito pest and disease risks to human residences located in close proximity to where stormwater wetlands are constructed.

Chapter 7. General Discussion

This chapter summarises the research findings and outlines suitable directions for future research.

Stormwater runoff will continue to intensify as a source of pollution for surface waters in the face of increasing urbanization, making effective mitigation strategies to protect receiving waterways increasingly important. Stormwater treatment is an integral part of Water Senitive Urban Design (WSUD), with community desires and many statutory and policy initatives driving this strategy and focussing attention on the ecologically sustainable management of water resources (see Chapter 1). However, evidence of the ability of tertiary stormwater treatment devices retrofitted to urban catchments to mitigate stormwater impacts on receiving waterways is limited, particularly for Australian conditions and in terms of ecological benefits. In light of this, the overall aim of this study was to assess the efficacy of tertiary stormwater treatment devices retrofitted to urban catchments in Sydney, Australia, to improve water quality, and reduce potential risk of harm to ecological and human health. This study has addressed this aim by determining: 1) the capacity of treatment devices to remove pollutants from urban stormwater that may pose a risk to ecological (Chapters 2-4) and human health (Chapter 6); 2) if treated stormwater was less toxic to freshwater biota than untreated stormwater (Chapter 2-5); 3) if treated stormwater led to improvements at higher-levels of biological organization (Chapter 4); and 4) if surface flow systems provided a breeding ground for mosquitoes (Chapter 6).

Summary of the research findings

Water quality

The findings of the present study showed that untreated stormwater contained pollutant concentrations that would likely result in measureable degradation to receiving waters. Concentrations of several of the stormwater analytes entering the monitored stormwater treatment devices were enriched in comparison to freshwater guidelines, particularly for TSS, filterable Cu and Zn, nutrients and faecal indicator bacteria. This is consistent with previous studies (Birch et al., 2004; Trowsdale & Simcock, 2011). The need for water quality improvement is therefore evident.

The largest retrofitted constructed wetland (relative to the catchment area) reduced the concentration of the majority of measured pollutants by orders of magnitude, but mean concentrations of filterable Cu, filterable Zn, TN, TP and indicator bacteria exiting the wetland system still exceeded freshwater guidelines and may therefore have the potential to contribute to localized water quality problems downstream (Chapter 1 and 6). This finding (i.e. exceeding freshwater guidelines at the outlet) is consistent with the changes in water quality seen for other constructed stormwater wetlands (Birch et al., 2004; Hathaway et al., 2009; Hathaway & Hunt, 2012). The other two constructed wetlands monitored in this study, which were smaller in size in relation to the contributing catchment area, showed less improvement in reducing pollutant concentrations (Chapter 4), with the exception of faecal indicator bacteria (Chapter 6). However, the poor faecal indicator removal observed at the largest wetland is likely to have resulted from faecal input from waterfowl frequently observed on-site. Carleton et al. (2000) also noted the higher capacity for pollutant removal at constructed wetlands with a larger treatment area to catchment ratio in a study conducted in the USA.

In contrast to the wetland systems, the bioretention system performed poorly (Chapter 3), with the exception of the removal of faecal coliforms (Chapter 6). This system was considerably undersized relative to best practice design guidelines. Of the sixteen analytes measured in Chapter 3, only TSS, BOD and filterable Pb were significantly reduced by the system. The treated water leaving the bioretention system contained higher concentrations of filterable Al, filterable Cu, TN, NO_x-N, TP and FRP than water at the inlet, with the largest negative removal efficiency observed for NO_x-N. The results for NO_x-N are not surprising given that the system was not constructed with an anaerobic saturated zone that would promote denitrification. Leaching of pollutants (i.e. higher outflow than inflow concentrations) has been observed in other field bioretention studies (Dietz & Clausen, 2005; Hatt et al., 2009; Li & Davis, 2009; Line & Hunt, 2009; Trowsdale & Simcock, 2011). The results indicate the need for further research and design modifications to improve the performance of conventional bioretention systems to reduce the risk of urban stormwater runoff to freshwater biota. Bioretention

systems designed with a saturated zone have demonstrated significant reductions in NO_x-N (Dietz & Clausen, 2006). Recent laboratory-based bioretention column studies conducted by Payne *et al.* (2014) have highlighted the importance of bioretention design variables, such as plant species and the presence of a saturated zone to make bioretention performance more effective.

Some of the stormwater treatment devices were exporting indicator bacteria, particularly enterococci, and therefore contributing additional bacteria to receiving waters (Chapter 6). Limited data exists on the ability of stormwater treatment devices to remove enterococci. This work indicated that enterococci might have a higher resilience than faecal coliforms in stormwater treatment devices and therefore be a more conservative and appropriate indicator of pathogen removal performance. Enterococci is more prone to failing human health standards than faecal coliforms in recreational waters (Noble et al., 2003) and is known to persist longer in aquatic environments (Griffin et al., 2001). Despite some observed reductions in indicator bacteria, most median outflow concentrations at the stormwater treatment devices exceeded the public health criterion for faecal indicator bacteria for both primary and secondary contact, demonstrating a potential risk to human health. Similar studies have observed outflowing concentrations of indicator bacteria that do not meet target values for recreational human contact (Birch et al., 2004; Hathaway et al., 2009; Passeport et al., 2009; Hathaway et al., 2012).

Based on the findings of Hathaway et al. (2011a) and the findings of the current study, it might be that the depth of the bioretention media, combined with the system being size constrained, reduced its potential to remove enterococci. Sizing of the constructed stormwater wetlands in relation to the contributing catchment area did not appear to be an important determinant of indicator bacteria reduction as the largest sized wetland had the poorest performance. Waterfowl were frequently observed in the outlet of the largest wetland system indicating that stormwater treatment devices such as this that are designed to also provide wildlife habitat as well as stormwater treatment may potentially add bacteria to receiving waters through defecation in and around the treatment device. Although

it is widely acknowledged that faecal contamination from human sources is likely to present a greater risk to human health than faecal contamination from animal sources, it is important to recognize that the latter source does not present no risk at all (Domingo & Edge, 2010). Hathaway et al., (2011b) suggested that wetlands appear to have a baseline concentration of faecal coliforms due to animal activity or internal processes such as animal microbial persistence. This should be taken into account when determining what type of stormwater treatment device should be used if the objective is to reduce bacterial pollution.

A high degree of variability was apparent in the removal of various pollutants between storm events and the water quality sampling conducted in this study has a notable limitation, as the cost of sampling, the availability of events due to an absence of rainfall in the study period and allocated times length to conduct the research all combined to limit the available field data set. A longer monitoring period would therefore be beneficial to obtain a more representative characterisation of pollutant removal efficiency by providing a better estimate of pollutant removal efficiencies. Longer monitoring would have helped verify the significant differences identified in the study between inlet and outlet pollutant concentrations. Analysing replicates of individual samples was not possible in the current study due to cost constrains and the volume of composite samples that could be generated from storm flows. In all cases analytical priority was directed towards having sufficient water for the pre-selected toxicity tests. However, taking replicates of individual samples, where water volumes were available, would have provided a better estimate of the uncertainty in the measurements obtained within the study. However, it was not always possible to monitor the inlet and outlet flows at all of stormwater treatment devices due to cost constraints. Where the cost of flow monitoring and the use of automatic samples was not feasible, grab samples were collected. The limitations of grab sampling therefore apply as the concentration of a given pollutant may vary during a storm event. The use of automatic water samplers and generating a flow-weighted composite sample at all of the stormwater treatment devices would enable a more representative sample of the treated water quality in the stormwater treatment devices to be obtained.

This would assist in providing greater detail in regard to the representation of pollutant removal efficiency and water quality improvements.

Notwithstanding the inherent limitations of field sampling, the overall results of the water quality assessment indicate that untreated stormwater contained pollutant concentrations that would likely pose a risk to ecological and human health. The findings provide some support for the use of tertiary stormwater treatment devices to improve water quality, but the data indicate that it may not be possible to treat stormwater to meet receiving water quality guidelines in all cases and treatment systems can contribute pollutants to receiving waters if they have design constraints. These outcomes have fulfilled the first objective of the study by contributing to empirical evidence on the water quality improvement capacity of retrofitted tertiary stormwater treatment devices.

Organism level responses

According to Walsh et al. (2004) it has been argued commonly that toxic effects of urban stormwater runoff are minor. The findings of the present study show clearly that there was a significant toxic impact of untreated stormwater on the selected test organisms. The single-species laboratory toxicity tests showed that untreated stormwater from the Yarrowee wetland inflow (Chapter 2) was toxic to the cladoceran (*Ceriodaphnia dubia*) and embryos of the crimson-spotted Rainbowfish (*Melanotaenia duboulayi)* on occasion, but not to a green microalga (*Pseudokirchneriella subcapitata)* which, instead of declining in growth due to toxicity, was stimulated to grow by the high nutrient concentrations. Untreated stormwater from the inflow of Johnston St bioretention basin was toxic to all three test organisms on occasion (Chapter 3). The observed toxicity of urban stormwater to micro-crustaceans and fish embryos is consistent with studies conducted in the USA (Skinner et al., 1999; McQueen et al., 2010) and both inhibition and stimulation in growth of *P. subcapitata* have been reported previously in response to urban stormwater in Europe (Scholes et al., 2007).

The ability of field bioretenion systems and constructed stormwater wetlands to reduce the toxicity of untreated stormwater is not reported in the published peer-reviewed literature. In the present study, Yarrowee wetland reduced the toxicity of stormwater runoff to test species as no toxicity was observed in outflowing samples. However, stimulation of growth of *P. subcapitata* was observed, suggesting that the potential risk of stormwater to stimulate primary producers in receiving waters can remain following stormwater treatment (Chapter 2). At Johnston St bioretention basin deleterious responses were still observed in some outflowing samples, demonstrating the failure of the bioretention system to reduce the toxicity of the influent stormwater on all occasions, which is likely to be associated with its poorer water quality improvement performance (Chapter 3). Reducing toxicity is important for the protection and enhancement of aquatic ecosystems in the receiving environment. For example, reduced hatching success of *M. duboulayi* may compromise population recruitment in receiving waters which in turn may have ecosystem-wide effects because *M. duboulayi* plays an important role in Australian freshwater food webs by regulating algal biomass and nuisance insect larvae, and serving as a food source for larger fish (Thomas et al., 2008).

Further assessment of the toxic impacts of untreated and treated stormwater involved *in situ* toxicity tests at the inlet and outlet zone of constructed stormwater wetlands (Chapter 4). This approach provided more environmentally realistic test conditions than the laboratory toxicity tests, as the effects of multiple stressor interactions on biological responses can be taken into account (Crane et al., 2007). Untreated stormwater was toxic to the freshwater shrimp (*Paratya australiensis*). Stormwater treatment at Yarrowee wetland was sufficient to consistently support high survival of *P. australiensis* at the wetland outlet, which is consistent with the findings of a study conducted at a constructed wetland in Australia by Kumar et al. (2011). The two smaller wetlands (with respect to catchment area), which also suffered from design constraints, did not perform as well as Yarrowee wetland and survival of *P. australiensis* at the outlet across exposure periods was generally lower. The sizing of constructed wetlands is therefore a critical issue to consider in order to minimize stormwater-related stresses on aquatic biota and enable effective conservation, which is in line with

the *Local Government Act 1993* (NSW) that has the protection of ecological values of waterways at the core of its objectives.

Zn concentrations in the surface water were significantly and negatively correlated with the survival of *in situ* exposed *P. australiensis* at Yarrowee wetland. Although consistently higher survival of *P. australiensis* and lower filterable Zn concentrations were observed at the wetland outlet in comparison to the inlet, concentrations of filterable Zn at the outlet remained above freshwater guidelines (Chapter 4). Controlled laboratory experiments were performed to test the sublethal toxicity of untreated and treated stormwater Zn concentrations observed in constructed wetlands on the foraging behaviour of *P. australiensis*. Abnormal foraging behaviours were observed in shrimp exposed to Zn at untreated stormwater concentrations, but the behaviour of shrimp exposed to Zn concentrations measured in treated stormwater did not differ from the controls (Chapter 5). The findings suggest that the reduction of stormwater Zn concentrations via wetland treatment can prevent abnormal pollutant-induced behaviours in *P. australiensis* and this is the first study highlighting the efficacy of water quality improvements on ecologically relevant behaviours (Oulton et al., 2014). It is important to examine sub-lethal endpoints as these can lead to cascading secondary effects on entire ecosystems (Fleeger et al., 2003).

Interestingly, the untreated Zn concentration caused no mortality to the freshwater shrimp during behavioural laboratory-based toxicity tests, but significant mortality of *P. australiensis* was observed in the inlet of Yarrowee wetland during *in situ* exposures at similar Zn concentrations. Zinc may therefore have not been the cause of mortality to *P. australiensis*. This highlights the benefit of toxicity tests being performed under realistic exposure environments, such as *in situ* tests, where the effects of multiple stressor interactions on biological responses can be taken into account (Crane et al., 2007) and the effect of other stormwater constituents is not overlooked. However, laboratory studies permit determination of causality by focussing on a single variable such as Zn water concentrations. Such focussed eco-toxicological assessments also allow the generation of direct information on the toxic impact of complex effluents such as urban stormwater

runoff. Both approaches form useful and important lines of evidence in ecological risk assesments.

The overall results of the *in situ* and laboratory based toxicity tests indicated that pollutants in untreated stormwater pose a risk to aquatic biota. Exposure produced lethal and sublethal toxic effects, such as mortality (*P. australiensis* and *C. dubia*), reduced hatching success (*M. duboulayi*), growth inhibition (*P. subcapitata*) and negative behavioural changes (*P. australiensis*). The findings suggest that stormwater treatment can reduce the toxic risk associated with urban stormwater pollution on aquatic biota, with higher benefits achieved with better treatment design. However, stimulation in growth of *P. subcapitata* was observed in response to treated stormwater from the outlet of Yarrowee wetland suggesting that the potential risk of stormwater to stimulate primary production in receiving waters can remain following treatment. It is recommended that regular toxicity testing of water exiting the wetland be carried out to ensure that water quality improvements are being maintained over time. The toxicity-based outcomes have fulfilled the second objective of the study by providing a greater understanding of the ecotoxicological impact of untreated and treated stormwater on freshwater biota.

Community and Ecosystem level responses

This is the first study that has identified the effects of untreated and treated stormwater on higher levels of biological organization in the inlet and outlet zone, respectively, of constructed wetlands. Results indicated that the constructed wetland that was the largest with respect to the contributing catchment area, treated stormwater to a standard that supported sensitive macroinvertebrate taxa and reduced organic decomposition rates. Decreases in sensitive macroinvertebrate orders have been reported with increasing urbanisation (Pratt et al., 1981; Roy et al., 2003; Walsh, 2004, 2006; Gresens et al., 2007; Davies et al., 2010; Tippler et al., 2012) and this study has shown that treatment of stormwater can support their presence. A reduction in decomposition rates at the outlet are of ecological benefit, as reduced microbial-driven breakdown will not decrease the

availability of benthic organic matter, which could ultimately impair ecosystem function (Imberger et al., 2008). Wetlands that were smaller with respect to catchment area and had design constraints did not perform as well and were not able to achieve the same ecological improvements as the larger wetland. The current work demonstrates that systems with a higher capacity for pollutant removal, which is facilitated by having a larger treatment area to catchment ratio, may have better outcomes at higher-levels of biological organization (Chapter 4). These experimental outcomes have fulfilled the third objective of the study.

Mosquito larvae were a dominant macroinvertebrate taxon and differentiated macroinvertebrate assemblages between the inlet and outlet zone of constructed wetlands (Chapter 4). Immature mosquitoes (larvae and pupae) were found in all surface flow systems (Chapter 6), confirming that constructed stormwater wetlands provide habitat for immature mosquitoes, which is in agreement with other studies (Gingrich et al., 2006; Metzger et al., 2008). This work expands on previous research by demonstrating specifically that the inlet zone of stormwater wetlands can provide conditions more conducive to the production of immature mosquitoes compared to the outlet zone. The presence of immature mosquitoes was positively correlated with TN and TP, suggesting that the higher nutrient concentrations in the inlet zone in comparison to the outlet zone were associated with an increased abundance of immature mosquitoes (Chapter 6). The anthropogenic input of nutrients into the aquatic environment has been linked to an increased occurrence of diseases vectored by mosquitoes (Johnson et al., 2010). This has important implications for mosquito management in stormwater wetlands and indicates that technical solutions to manage water in the inlet zone may need to be considered during the design phase of wetland construction. This outcome has fulfilled the fourth objective of the study.

Future directions

Further research is needed to determine if tertiary stormwater treatment devices can be manipulated to improve treatment mechanisms and environmental conditions that will stimulate the immobilization and inactivation of pathogenic

bacteria, particularly enterococci. This requires gaining a greater understanding of the processes that control indicator bacteria persistence and sequestration in stormwater treatment systems (Hathaway & Hunt, 2012), which could be experimentally evaluated using manipulated wetland/bioretention columns e.g. (Rusciano & Obropta, 2007) and pilot-scale systems e.g. (Kim et al., 2003). This research will help enhance removal of indicator bacteria and better satisfy water quality guidelines for recreational waters (cf. to the recommendations of Hathaway et al. (2012)). Reducing bacterial loads in receiving waters is an important consideration for the broader perspective of ecosystem services, which include recreational opportunities (Palmer et al., 2004; Findlay & Taylor, 2006).

Public health risks were further substantiated by evidence that the conditions in the inlet zones of stormwater wetlands resulting from untreated stormwater provide a suitable mosquito breeding habitat. Further research is needed to examine the rate and production of adult mosquitoes from these devices and if technical solutions (e.g. sprinkler systems) could mitigate these risks. Sprinkler showers used to discourage mosquito oviposition have been effective in reducing the abundance of mosquitoes in wetland mesocosms (Popko & Walton, 2013) and in pond systems treating wastewater (Epipane et al., 1993). A similar approach could be trialled in the inlet zone of constructed stormwater wetlands. Minimizing mosquito pest and disease risks to communities located in close proximity to stormwater wetlands is critical to ensure community acceptance and protect human health. Climate change is also likely to expand the range and intensify the activity of mosquitoes and their pathogens (Russell & Dwyer, 2000; Bambrick et al., 2011) making steps to reduce their presence in constructed stormwater wetlands even more important. Many councils across NSW have started to incorporate design considerations for constructed stormwater wetlands in relation to minimizing the incidence of mosquitoes into their Development Control Plans (for example see Wollongong Council (2009)). It is important that mosquito management in constructed stormwater wetlands is communicated and recognized in the adaptation of planning controls and practices to enable a more sustainable response to stormwater management.

The most negative removal efficiency (i.e. increase in concentration through the system) at the bioretention system was seen for NO_x-N. Creation of a saturated layer (anoxic zone) at the bottom of the bioretention has been recommended to promote denitfrification (Kim et al.,2003) and has proved effective in field based bioretentions for reducing NOx-N and TN (Dietz & Clausen, 2006). In the case of conventional bioretention systems, which do not incorporate a saturated layer, research is needed to see if modifications can be made to promote denitrification and prevent the leaching of NO_x-N. An internal water storage layer can be created in existing bioretentions by attaching a 90-degree PVC upturned elbow to the underdrains to force an elevated outlet and ponding of water in the bottom layer (Brown et al., 2009). Bioretention systems with greater denitrification capabilities would better satisfy the common water quality improvement goals of these systems. Indeed, it is unrealistic to expect removal of nutrients, such as NO_x-N, from a system if the design does not include suitable conditions for such processes to occur.

A lethal toxic response to untreated stormwater was not always observed in this study, particularly for *C. dubia.* Sub-lethal endpoints (e.g. reproduction) may be more sensitive than lethal endpoints (i.e. mortality) in *C. dubia* exposed to untreated stormwater runoff (McQueen et al., 2010). Low dissolved oxygen conditions were present in wetlands associated with stormwater runoff in the current study. Connolly et al. (2004) demonstrated that macroinvertebrates can persist under low dissolved oxygen concentrations, but had suppressed emergence, suggesting a sublethal stress was being placed on their development. Future research efforts should focus on examining the sublethal effects to untreated and treated stormwater, particularly because the stormwater treatment devices in the current study still had pollutant concentrations that exceeded recommended freshwater guidelines at the outflow. Laboratory analyses undertaken as part of the this thesis demonstrated that fish embryos of *M. duboulayi* are capable of predator recognition (Oulton et al., 2013) (Appendix 4). Therefore, it would be beneficial to see if untreated/treated stormwater pollutants interfere with this response as this could potentially have long-term impacts on their behaviour. Sub-lethal responses are likely to have other cascading

ecosystem-wide implications, particularly if the organism occupies a keystone position in the food web (Maltby, 1994).

It is clear that untreated and treated stormwater had a direct effect on aquatic biota and played an important role in altering macroinvertebrate community composition. However, these changes might also be influenced by biological (e.g. species and trophic) interactions occurring within an ecosystem. This could be assessed in future experiments using artificial mesocosms containing multiple trophic levels (Johnson et al., 2010), in which the direct and indirect effects of untreated and treated stormwater pollutants can be characterized and their importance in structuring ecosystems understood. The results of the current study also suggested that sediment quality in constructed stormwater wetlands influenced ecological responses. Polluted sediment from urban streams and sediments that receive road runoff can influence macroinvertebrates in field-based, microcosm experiments, for example, by altering abundance and diversity (Pettigrove et al., 2007; Marhsall et al., 2010), and causing developmental abnormalities in key taxa (Townsend et al., 2009). Sediment quality should therefore be factored in to mesocosm experiments to determine the effectiveness of stormwater treatment devices to reduce sediment toxicity (Pettigrove et al., 2007).

Concluding remarks

Untreated stormwater produced a range of deleterious water quality changes and biological responses at organism, community and ecosystem response levels, demonstrating that modification to conventional stormwater management practices is indeed justified. The results of this study indicate that retrofitted tertiary stormwater treatment devices can be effective in reducing the observed deleterious responses, but their effectiveness is limited where they are not designed appropriately. Systems that were considerably undersized relative to catchment area and had design issues showed a reduced capacity to achieve the same water quality and ecological improvements as more appropriately designed systems, and in some cases actually contributed pollutants to receiving waters.

Despite some beneficial outcomes, the data presented in this thesis indicate that it may not be possible to treat stormwater to desired levels (such as those set out in national water quality guidelines) in all cases. It can therefore not be assumed that structural measures alone will improve the health of receiving waters and they should be used in combination with non-structural measures (e.g. community-awareness programs) to manage urban stormwater quality. This is particularly true for highly urbanized catchments, as Walsh et al. (2004) indicated that effective imperviousness (impervious area that is directly connected to streams) needs to be limited to less than 5% in order to protect stream ecosystems and this may not be a realistic approach to urban stream restoration in highly urbanized catchments such as this.

The empirical evidence generated from this study will improve urban-waterway decision-making, by informing future projects and allowing environmental management authorities/organisations and government agencies to deliver better environmental outcomes for urban waterways as part of their governance and in line with strategic directions and objectives of urban water management. It is clear from this study that retrofitted tertiary stormwater treatment devices need thorough investigation to quantify their benefits and to confirm if they function as planned and that they are built to design specification to be effective. This will also allow design improvements/manipulation of treatment mechanisms to be made to make them perform better and ultimately be more reliable. The wider goals of improving the environmental condition of urban waterways, as prescribed within the Australian National Water Initiative (NWC, 2004), for example, cannot be achieved unless these issues are dealt with. It is important that stormwater treatment devices are proven to be effective to justify the added costs. Development and adoption of a national standard that deals explicitly with the adoption of stormwater treatment devices would prevent the installation of devices that are not fit for purpose. They should therefore be viewed as a developing rather than a revolutionary technology to stormwater management at this current time.

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Appendix 1. Animal Research Authority approval

MACQUARIE UNIVERSITY

ANIMAL RESEARCH AUTHORITY (ARA)

AEC Reference No.: 2011/024 **Date of Expiry:** 04 May 2012

Full Approval Duration: 05 May 2011 to 04 May 2013 (24 months)

This ARA remains in force until the Date of Expiry (unless suspended, cancelled or surrendered) **and will only be renewed upon receipt of a satisfactory Progress Report before expiry.**

Principal Investigator:
Dr Culum Brown
Dept of Biological Sciences
Macquarie University NSW 2109
0439 343 341
culum.brown@mq.edu.au

Associate Investigator:
Lois Oulton 0422 917 583

In case of emergency, please contact:
Animal Welfare Officer Dr Miriam Meek: 9850 7758 / 0439 497 383
Manager, CAHF Christine Sutter: 9850 7780 / 0428 861 163
Manager, BB&E/Fauna Park Robby Miller: 9850 4109 / 0425 213 420
or the Principal Investigator / Associate Investigator named above

The above-named are authorised by MACQUARIE UNIVERSITY ANIMAL ETHICS COMMITTEE to conduct the following research:

Title of the project: Constructed stormwater treatments in Metropolitan Sydney: their effectiveness in improving water quality and reducing the ecological risk to aquatic biota

Type of animal research and aims of project:
Research (environmental) – This project aims to measure the efficacy of stormwater treatment devices in metropolitan Sydney to improve water quality to a standard that meets relevant guidelines, and reduces the ecological risk to freshwater aquatic biota.

Surgical Procedures category: 2 (Animal Unconscious Without Recovery)

All procedures must be performed as per the AEC-approved protocol, unless stated otherwise by the AEC and/or AWO.

Maximum numbers approved (for the Full Approval Duration):

Species	Strain	Sex	Weight	Age	Total	Supplier/Source
Rainbow Fish (eggs)					5940	existing stock
				TOTAL	**5940**	

Location of research:

Location	*Full street address*
Room 159, Central Animal House Facility	Building F9A, Research Park Drive, Macquarie University NSW 2109

Amendments approved by the AEC since initial approval: N/A

Conditions of Approval:
All Permits/Licenses (to obtain and use fauna; to conduct research at interstate/overseas locations; to house animals, etc.) must be obtained prior to work commencing, and copies forwarded to the Animal Ethics Secretariat.

Being animal research carried out in accordance with the Code of Practice for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

Prof Michael Gillings (Chair, Animal Ethics Committee)

Approval Date: ***21 April 2011***

Adapted from Form C (issued under part IV of the Animal Research Act, 1985)

MACQUARIE UNIVERSITY

ANIMAL RESEARCH AUTHORITY (ARA)

AEC Reference No.: 2011/024 - 2 **Date of Expiry:** 04 May 2013

Full Approval Duration: 05 May 2011 to 04 May 2013 (24 months)

This ARA remains in force until the Date of Expiry (unless suspended, cancelled or surrendered) **and will only be renewed upon receipt of a satisfactory Progress Report before expiry.**

Principal Investigator:
Dr Culum Brown
Dept of Biological Sciences
Macquarie University NSW 2109
0439 343 341
culum.brown@mq.edu.au

Associate Investigator:
Lois Oulton 0422 917 583

In case of emergency, please contact:
the Principal Investigator / Associate Investigator named above, or
Animal Welfare Officer: 9850 7758 / 0439 497 383 ,**Manager, CAHF**: 9850 7780 / 0428 861 163
Manager, BB&E/Fauna Park: 9850 4109 / 0425 213 420

The above-named are authorised by MACQUARIE UNIVERSITY ANIMAL ETHICS COMMITTEE to conduct the following research:

Title of the project: **Constructed stormwater treatments in Metropolitan Sydney: their effectiveness in improving water quality and reducing the ecological risk to aquatic biota.**

Purpose: 9: Diagnostic Procedures

Aims of project: This project aims to measure the efficacy of stormwater treatment devices in metropolitan Sydney to improve water quality to a standard that meets relevant guidelines, and reduces the ecological risk to freshwater aquatic biota. The test endpoints will be egg hatching success at six days and post-hatch survival at ten days of rainbow fish.

Surgical Procedures category: 8: Death as an endpoint

All procedures must be performed as per the AEC-approved protocol, unless stated otherwise by the AEC and/or AWO.

Maximum numbers approved (for the Full Approval Duration):

Species	Strain	Sex	Weight	Age	Total	Supplier/Source
23: Rainbow Fish (eggs)	N/A	N/A	N/A	N/A	5940	existing stock
				TOTAL	**5940**	

Location of research:

Location	*Full street address*
Room 159, Central Animal House Facility	Building F9A, Research Park Drive, Macquarie University NSW 2109
Fish Shed, E17A	Fauna Park, 209 Culloden Rd, Macquarie University NSW 2109

Amendments approved by the AEC since initial approval: N/A

Conditions of Approval: N/A

Being animal research carried out in accordance with the Code of Practice for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence. This authority remains in force from ***4 May 2013*** unless suspended or surrendered, **and will only be renewed upon receipt of a PROGRESS REPORT annually.**

Prof Michael Gillings (Chair, Animal Ethics Committee)

Approval Date: ***15 March 2012***

Adapted from Form C (issued under part IV of the Animal Research Act, 1985)

MACQUARIE UNIVERSITY

ANIMAL RESEARCH AUTHORITY (ARA)

AEC Reference No.: 2011/024 - 3 **Date of Expiry:** 30 June 2013

Full Approval Duration: 05 May 2011 to 30 June 2013 (24 months+ extension)

This ARA remains in force until the Date of Expiry (unless suspended, cancelled or surrendered) **and will only be renewed upon receipt of a satisfactory Progress Report before expiry.**

Principal Investigator:
A/Prof Culum Brown
Dept of Biological Sciences
Macquarie University NSW 2109
0439 343 341
culum.brown@mq.edu.au

Associate Investigator:
Lois Oulton 0422 917 583

Other people participating
Vivian Haviland 0410 166 332

In case of emergency, please contact:
the Principal Investigator / Associate Investigator named above, or
Animal Welfare Officer: 9850 7758 / 0439 497 383, **Manager, CAF**: 9850 7780 / 0428 861 163
Manager, BB&E/Fauna Park: 9850 4109 / 0425 213 420

The above-named are authorised by MACQUARIE UNIVERSITY ANIMAL ETHICS COMMITTEE to conduct the following research:

Title of the project: Constructed stormwater treatments in Metropolitan Sydney: their effectiveness in improving water quality and reducing the ecological risk to aquatic biota.

Purpose: 9: Diagnostic Procedures

Aims of project: This project aims to measure the efficacy of stormwater treatment devices in metropolitan Sydney to improve water quality to a standard that meets relevant guidelines, and reduces the ecological risk to freshwater aquatic biota. The test endpoints will be egg hatching success at six days and post-hatch survival at ten days of rainbow fish.

Surgical Procedures category: 8: Death as an endpoint

All procedures must be performed as per the AEC-approved protocol, unless stated otherwise by the AEC and/or AWO.

Maximum numbers approved (for the Full Approval Duration):

Species	Strain	Sex	Weight	Age	Total	Supplier/Source
23: Rainbow Fish (eggs)	N/A	N/A	N/A	N/A	5940	existing stock
				TOTAL	**5940**	

Location of research:

Location	*Full street address*
Room 159, Central Animal Facility	Building F9A, Research Park Drive, Macquarie University NSW 2109
Fish Shed, E17A	Fauna Park, 209 Culloden Rd, Macquarie University NSW 2109

Amendments approved by the AEC since initial approval:

1. Extension of approval duration until the end of semester 1, 2013 and observation of larval heartbeat under cold lamp microscope (approved March 2013)
2. Addition of Vivian Haviland as student (approved and ratified April 2013)

Conditions of Approval:

1. Vivian Haviland to attend the next available animal ethics course

Being animal research carried out in accordance with the Code of Practice for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

Professor Mark Connor (Chair, Animal Ethics Committee) **Approval Date:** 18 April 2013

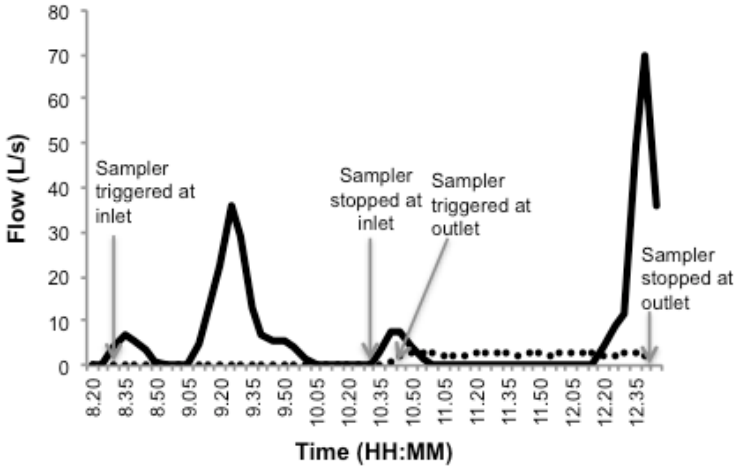
Adapted from Form C (issued under part IV of the Animal Research Act, 1985)

Appendix 2. Supporting information to Chapter 2

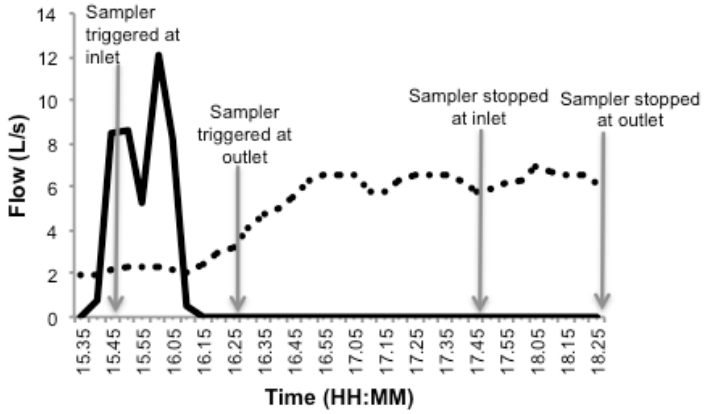
Table A1.1: Planting at Yarrowee wetland (Equatica, 2009).

Wetland Zone	Species
Swale	*Gahnia aspera*
	Imperata cylindrica
	Juncus usitatus
	Lomandra longifolia
	Leptospermum polygalifolium
	Melaleuca ericifolia
Wetland Shallow Marsh	*Eleocharis dietrichiana*
	Isolepis nodosa
	Philydrum lanuginosum
	Restio tetraphyllus
	Triglochin multifructum
Wetland Marsh	*Bolboschoenus caldwelli*
	Baumea articulata
	Cyperus difformis
	Paspalum distichum
	Phragmites australis
	Schoenoplectus validus
Wetland Ephemeral	*Bursaria spinosa*
	Carex appressa
	Callistemon rigidus
	Gahnia aspera
	Callistemon rigidus
	Imperata cylindrica
	Juncus usitatus
	Lomandra longifolia
	Microlaena stipoides
	Baumea juncea
	Carex fascicularis
	Leptospermum polygalifolium
	Persicaria decipiens

A)



B)



C)

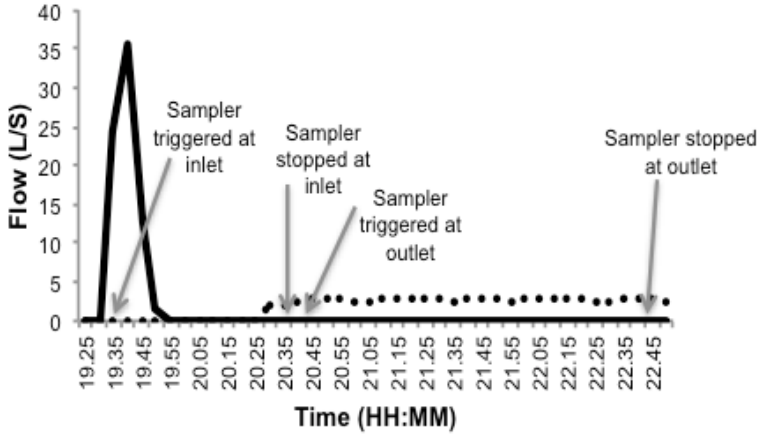


Figure A2.1: Flow data from storm events (A) W1, (B) W2 and (C) W3.

Table A2.2: Example of how a flow weighted composite sample was generated for one event (W1).

Bottle Number Inlet	Time	Flow (L/s)	Proportion of total flow (%)	mL from each sampling bottle to make 4.5 L
1	8.30	3.95	2	110
2	8.35	6.64	4	185
3	8.40	5.39	3	150
4	8.45	3.59	2	100
5	8.50	0.90	1	25
6	8.55	0	0	0
7	9.00	0	0	0
8	9.05	0	0	0
9	9.10	4.79	3	133
10	9.15	12.54	8	349
11	9.20	22.74	14	633
12	9.25	35.64	22	992
13	9.30	28.96	18	806
14	9.35	12.69	8	353
15	9.40	7.06	4	197
16	9.45	5.81	4	162
17	9.50	5.45	3	152
18	9.55	3.95	2	110
19	10.00	1.62	1	45
20	10.05	0	0	0
21	10.10	0	0	0
22	10.15	0	0	0
23	10.20	0	0	0
24	10.25	0	0	0
TOTAL		161.70		

Bottle Number Outlet	Time	Flow (L/s)	Proportion of total flow (%)	mL from each sampling bottle to make 4.5 L
1	10.45	2.5	4	176
2	10.50	2.92	5	205
3	10.55	2.78	4	195
4	11.00	2.64	4	185
5	11.05	2.5	4	176
6	11.10	2.22	3	156
7	11.50	2.36	4	166
8	11.20	2.64	4	185
9	11.25	2.78	4	195
10	11.30	2.92	5	205
11	11.35	2.78	4	195
12	11.40	2.5	4	176
13	11.45	2.64	4	185
14	11.50	2.64	4	185
15	11.55	2.5	4	176
16	12.00	2.92	5	205
17	12.05	2.92	5	205
18	12.10	2.92	5	205
19	12.15	3.06	5	215
20	12.20	2.5	4	176
21	12.25	2.5	4	176
22	12.30	2.78	4	195
23	12.35	2.64	4	185
24	12.40	2.5	4	176
TOTAL		64.06		

Table A2.3: Laboratory quality control results for analytical work for events W1-W3 (data compiled from ALS Environmental laboratory reports for this study).

Event	Parameter	LOR (mg L^{-1})	Method Blank	Laboratory duplicates		RPD (%)	Recoveries (%)		
				Sample (mg L^{-1})	Duplicate (mg L^{-1})		LCS	Acceptable recovery for LCS	Matrix spike
W1	TSS	1	<1	22	24	8.7	107	80-124	nd
	Al	0.01	<0.01	nd	nd	nd	107	92-112	nd
	Cd	0.0001	<0.0001	<0.0001	<0.0001	0	106	89-107	125
	Cu	0.001	<0.001	0.037	0.038	0	102	87-111	114
	Fe	0.05	<0.05	12.3	12.6	2.4	106	84-114	nd
	Mn	0.001	<0.001	1.48	1.43	3.8	112	87-113	119
	Pb	0.001	<0.001	<0.001	<0.001	0	104	90-110	120
	Zn	0.005	<0.005	0.012	0.011	9.5	100	85-115	119
	NH_3-N	0.01	<0.01	0.06	0.07	0	99	86-116	97
	NO_x-N	0.01	<0.01	0.26	0.26	0	103	80-130	103
	TKN	0.1	<0.1	19.4	19.4	0	96	57-123	87.3
	TN	0.1	nd	nd	nd	nd	nd	–	nd
	TP	0.01	<0.01	1.10	1.12	1.7	100	67-130	109
	RP	0.01	<0.01	0.11	0.11	0	110	85-115	113
	COD	5	<5	<5	<5	0	107	88-114	113
	BOD	2	<2	52	52	0	84	76-108	nd
W2	TSS	5	<5	10	8	33.3	101	30-150	nd
	Al	0.01	<0.01	0.34	0.34	0	104	92-112	nd
	Cd	0.0001	<0.0001	<0.0001	<0.0001	0	103	89-107	104
	Cu	0.001	<0.001	0.006	0.006	0	101	87-111	104
	Fe	0.05	<0.05	0.06	0.06	0	94	84-114	nd
	Mn	0.001	<0.001	0.006	0.006	0	98	87-113	96.5
	Pb	0.001	<0.001	0.02	0.02	0	100	90-110	106
	Zn	0.005	<0.005	0.031	0.031	0	103	85-115	107
	NH_3-N	0.01	<0.01	0.33	0.33	0	103	86-116	83.5
	NO_x-N	0.01	<0.01	20.8	20.8	0	112	80-130	nd
	TKN	0.1	<0.1	4.3	4.4	0	84	57-123	92.2
	TN	0.1	nd	nd	nd	nd	nd	–	nd
	TP	0.01	<0.01	0.98	1	2.3	84	67-130	99.5
	RP	0.01	<0.01	0.15	0.15	0	106	85-115	105
	COD	5	<5	2450	2450	0	104	88-114	104
	BOD	2	<2	178	164	8.2	98	76-108	nd
W3	TSS	5	<5	564	556	1.4	89	82-132	nd
	Al	0.01	<0.01	0.10	0.11	0	89	79-119	nd
	Cd	0.0001	<0.0001	0.0020	0.0024	17.7	92	82-114	105
	Cu	0.001	<0.001	0.001	0.001	0	91	79-115	97
	Fe	0.05	<0.05	1.52	1.58	3.4	90	78-116	nd
	Mn	0.001	<0.001	0.383	0.4	4.4	89	80-114	105
	Pb	0.001	<0.001	<0.001	<0.001	0	97	81-113	101
	Zn	0.005	<0.005	0.307	0.289	6.1	86	75-121	101
	NH_3-N	0.01	<0.001	<0.01	<0.01	0	90	89-113	nd
	NO_x-N	0.01	<0.001	2.01	2.02	0.5	107	86-124	nd
	TKN	0.1	<0.1	0.3	0.3	0	96	70-130	104
	TN	0.1	nd	nd	nd	nd	nd	nd	nd
	TP	0.01	<0.01	<0.01	<0.01	0	99	70-130	98.5
	RP	0.01	<0.01	<0.01	<0.01	0	101	86-124	103
	COD	5	<5	832	832	0	100	88-114	120
	BOD	2	<2	8680	8000	8.1	89	77-107	nd

Legend:

RPD = Relative Percentage Difference

LOR = Level of reporting

LCS = Laboratory Control Spike

nd = not determined

Acceptable RPDs on duplicates is: no limit at concentrations <10 times LOR; 0-50% at concentrations between 10 and 20 times LOR; 0-20% at concentrations greater than 20 time LOR

Acceptable recovery on matrix spikes is 70-130%

Acceptable recovery on laboratory control spikes is listed in the table

Table A2.4: Raw data for *Pseudokirchneriella subcapitata* toxicity tests and cell division (growth) rate per day for storm events (A) W2 and (B) W3. CV = coefficient of variation. Quality assurance criteria - Bioassays were considered acceptable if there was at least a 16-fold increase in the control biomass after 72 h and variability among the control replicates (CV) did not exceed 20%.

(A) **Event W2** 72-h Chronic Toxicity (*Pseudokirchneriella subcapitata*)

	Sample	Day 0	Day 1	Day 2	Day 3	Slope	Growth Rate (dbIngs/day)		Pearson	% Control	Mean %	CV (%)
Vial No.		*All cell counts in (cells/mL) by x10⁴*						Mean				
1		0.9	5.1	35.3	111.3	0.0296	2.36	2.32	99%	102%	100%	2%
2	Control	0.9	5.0	28.5	93.1	0.0283	2.26		99%	97%		
3		0.9	5.7	34.8	112.5	0.0295	2.35		99%	101%		
				Mean control rate =		***0.0292***						
Untreated Stormwater												
1		0.9	5.7	34.0	116.0	0.0296	2.36	2.44	99%	102%	105%	5%
2	6.25%	0.9	1.3	37.4	115.7	0.0324	2.59		91%	111%		
3		0.9	5.7	30.8	124.7	0.0298	2.38		100%	102%		
1		0.9	5.9	34.7	132.6	0.0303	2.42	2.42	99%	104%	104%	1%
2	12.5%	0.9	6.3	34.3	134.9	0.0303	2.41		99%	104%		
3		0.9	5.7	35.0	138.1	0.0306	2.44		100%	105%		
1		0.9	6.2	44.6	215.6	0.0333	2.65	2.63	100%	114%	113%	2%
2	25%	0.9	6.4	43.4	180.6	0.0323	2.57		99%	111%		
3		0.9	6.2	45.6	221.4	0.0335	2.67		100%	115%		
1		0.9	6.2	36.1	170.2	0.0316	2.52	2.61	100%	109%	112%	3%
2	50%	0.9	6.9	45.9	210.0	0.0330	2.63		100%	113%		
3		0.9	6.3	41.9	235.1	0.0336	2.68		100%	115%		
1		0.9	6.3	42.7	177.0	0.0321	2.56	2.61	100%	110%	112%	2%
2	75%	0.9	5.9	45.1	190.5	0.0328	2.61		100%	112%		
3		0.9	7.4	50.0	226.2	0.0335	2.67		99%	115%		
1		0.9	6.8	43.1	200.2	0.0327	2.60	2.61	100%	112%	112%	1%
2	100%	0.9	6.8	40.5	200.4	0.0326	2.59		100%	112%		
3		0.9	7.6	47.7	217.9	0.0331	2.64		99%	114%		
Treated Stormwater												
1		0.9	5.4	39.6	119.2	0.0301	2.40	2.38	99%	103%	102%	1%
2	6.25%	0.9	5.5	30.7	117.1	0.0295	2.35		100%	101%		
3		0.9	5.7	34.2	122.4	0.0299	2.38		99%	103%		
1		0.9	6.2	31.3	123.8	0.0297	2.36	2.37	99%	102%	102%	0%
2	12.5%	0.9	6.0	30.7	127.6	0.0298	2.38		100%	102%		
3		0.9	5.5	34.0	120.5	0.0299	2.38		99%	102%		
1		0.9	6.0	33.2	165.9	0.0314	2.50	2.54	100%	108%	109%	2%
2	25%	0.9	6.0	42.9	191.7	0.0327	2.60		100%	112%		
3		0.9	5.6	31.5	172.2	0.0317	2.52		100%	109%		
1		0.9	5.9	40.3	190.0	0.0325	2.59	2.64	100%	112%	114%	2%
2	50%	0.9	6.2	41.8	203.6	0.0329	2.62		100%	113%		
3		0.9	5.9	44.6	240.0	0.0340	2.71		100%	117%		
1		0.9	6.6	42.7	232.6	0.0335	2.67	2.63	100%	115%	113%	2%
2	75%	0.9	6.2	38.9	192.2	0.0324	2.59		100%	111%		
3		0.9	5.0	35.1	201.9	0.0329	2.62		100%	113%		
1		0.9	5.9	42.2	256.1	0.0343	2.73	2.68	100%	117%	115%	2%
2	100%	0.9	6.0	37.1	223.3	0.0332	2.65		100%	114%		
3		0.9	6.1	41.3	224.9	0.0334	2.66		100%	115%		

Log of Growth Rates				Below values for formula reference			
Day 0	Day 1	Day 2	Day 3				
3.954	4.710	5.547	6.046	0	24	48	72
3.954	4.700	5.455	5.969	0	24	48	72
3.954	4.754	5.542	6.051	0	24	48	72
3.954	4.758	5.531	6.064	0	24	48	72
3.954	4.114	5.573	6.063	0	24	48	72
3.954	4.757	5.488	6.096	0	24	48	72
3.954	4.771	5.540	6.123	0	24	48	72
3.954	4.801	5.535	6.130	0	24	48	72
3.954	4.757	5.544	6.140	0	24	48	72
3.954	4.791	5.649	6.334	0	24	48	72
3.954	4.805	5.637	6.257	0	24	48	72
3.954	4.789	5.659	6.345	0	24	48	72
3.954	4.794	5.558	6.231	0	24	48	72
3.954	4.836	5.662	6.322	0	24	48	72
3.954	4.800	5.622	6.371	0	24	48	72
3.954	4.798	5.630	6.248	0	24	48	72
3.954	4.769	5.654	6.280	0	24	48	72
3.954	4.869	5.699	6.354	0	24	48	72
3.954	4.831	5.634	6.302	0	24	48	72
3.954	4.835	5.608	6.302	0	24	48	72
3.954	4.883	5.679	6.338	0	24	48	72
3.954	4.733	5.598	6.076	0	24	48	72
3.954	4.738	5.486	6.069	0	24	48	72
3.954	4.758	5.534	6.088	0	24	48	72
3.954	4.790	5.495	6.093	0	24	48	72
3.954	4.781	5.487	6.106	0	24	48	72
3.954	4.740	5.532	6.081	0	24	48	72
3.954	4.778	5.521	6.220	0	24	48	72
3.954	4.778	5.632	6.283	0	24	48	72
3.954	4.744	5.498	6.236	0	24	48	72
3.954	4.772	5.605	6.279	0	24	48	72
3.954	4.791	5.621	6.309	0	24	48	72
3.954	4.772	5.650	6.380	0	24	48	72
3.954	4.818	5.630	6.367	0	24	48	72
3.954	4.790	5.590	6.284	0	24	48	72
3.954	4.701	5.545	6.305	0	24	48	72
3.954	4.767	5.625	6.408	0	24	48	72
3.954	4.781	5.570	6.349	0	24	48	72
3.954	4.784	5.616	6.352	0	24	48	72

(B)

Event W3 72-h Chronic Toxicity (*Pseudokirchneriella subcapitata*)

	Sample	Day 0	Day 1	Day 2	Day 3	Slope	Rate (dblngs/day)	Mean	Pearson	% Control	Mean %	CV (%)	Log of Growth Rates Day 0	Day 1	Day 2	Day 3	Below values for formula reference			
Vial No.		***All cell counts in (cells/mL) by x10⁴***																		
1	Control	1.4	4.9	25.3	72.3	0.0244	1.94	1.89	99%	103%	100%	2%	4.146	4.690	5.403	5.859	0	24	48	72
2		1.4	5.9	24.1	67.6	0.0236	1.88		99%	99%			4.146	4.772	5.382	5.830	0	24	48	72
3		1.4	4.9	20.1	64.6	0.0233	1.86		100%	98%			4.146	4.694	5.303	5.810	0	24	48	72
				Mean control rate =		***0.0238***														
Untreated Stormwater																				
1	6.3%	1.4	4.9	16.8	77.9	0.0241	1.92	1.93	100%	101%	102%	2%	4.146	4.690	5.226	5.892	0	24	48	72
2		1.4	5.0	18.5	71.2	0.0237	1.89		100%	100%			4.146	4.700	5.267	5.852	0	24	48	72
3		1.4	5.7	25.2	81.7	0.0248	1.97		100%	104%			4.146	4.757	5.402	5.912	0	24	48	72
1	13%	1.4	7.1	23.3	69.7	0.0234	1.86	1.92	99%	98%	101%	4%	4.146	4.853	5.367	5.843	0	24	48	72
2		1.4	5.3	19.5	74.9	0.0240	1.91		100%	101%			4.146	4.724	5.290	5.874	0	24	48	72
3		1.4	6.6	21.1	95.8	0.0251	2.00		100%	105%			4.146	4.817	5.323	5.982	0	24	48	72
1	25%	1.4	6.4	19.4	61.6	0.0226	1.80	1.82	99%	95%	96%	11%	4.146	4.803	5.288	5.790	0	24	48	72
2		1.4	6.0	20.6	100.7	0.0254	2.03		100%	107%			4.146	4.777	5.314	6.003	0	24	48	72
3		1.4	4.8	14.4	42.1	0.0204	1.63		100%	86%			4.146	4.685	5.157	5.624	0	24	48	72
1	50%	1.4	6.5	21.5	100.1	0.0253	2.02	1.96	100%	107%	103%	12%	4.146	4.811	5.332	6.000	0	24	48	72
2		1.4	6.2	29.5	122.7	0.0271	2.16		100%	114%			4.146	4.791	5.470	6.089	0	24	48	72
3		1.4	5.0	19.3	44.8	0.0212	1.69		99%	89%			4.146	4.702	5.285	5.651	0	24	48	72
1	75%	1.4	7.1	27.1	71.2	0.0238	1.89	1.90	99%	100%	100%	2%	4.146	4.848	5.433	5.853	0	24	48	72
2		1.4	5.6	18.1	72.0	0.0235	1.87		100%	99%			4.146	4.748	5.257	5.857	0	24	48	72
3		1.4	5.7	22.6	76.9	0.0243	1.93		100%	102%			4.146	4.752	5.354	5.886	0	24	48	72
1	100%	1.4	5.5	16.8	43.1	0.0206	1.64	1.74	99%	87%	92%	8%	4.146	4.743	5.225	5.634	0	24	48	72
2		1.4	5.2	25.0	67.1	0.0238	1.90		99%	100%			4.146	4.716	5.397	5.826	0	24	48	72
3		1.4	4.7	12.5	49.3	0.0211	1.68		100%	89%			4.146	4.676	5.097	5.692	0	24	48	72
Treated Stormwater																				
1	6.3%	1.4	4.5	24.9	73.5	0.0246	1.96	1.90	99%	104%	100%	5%	4.146	4.649	5.396	5.866	0	24	48	72
2		1.4	5.4	15.3	68.8	0.0230	1.83		100%	97%			4.146	4.736	5.185	5.838	0	24	48	72
3		1.4	4.5	19.2	12.3	0.0144	Removed because of outlier on day 3						4.146	4.649	5.283	5.090	0	24	48	72
1	13%	1.4	4.7	15.5	44.4	0.0209	1.67	1.83	100%	88%	97%	8%	4.146	4.674	5.191	5.648	0	24	48	72
2		1.4	4.7	16.9	83.5	0.0245	1.95		100%	103%			4.146	4.672	5.228	5.922	0	24	48	72
3		1.4	4.5	21.2	65.3	0.0236	1.88		100%	99%			4.146	4.657	5.326	5.815	0	24	48	72
1	25%	1.4	6.8	27.1	80.7	0.0245	1.95	2.03	99%	103%	107%	3%	4.146	4.833	5.432	5.907	0	24	48	72
2		1.4	4.4	22.7	98.8	0.0261	2.08		100%	110%			4.146	4.643	5.356	5.995	0	24	48	72
3		1.4	5.5	23.6	103.9	0.0260	2.07		100%	109%			4.146	4.737	5.372	6.016	0	24	48	72
1	50%	1.4	5.2	15.5	122.2	0.0262	2.09	2.06	98%	110%	109%	4%	4.146	4.719	5.189	6.087	0	24	48	72
2		1.4	5.4	16.7	92.7	0.0248	1.98		99%	104%			4.146	4.728	5.222	5.967	0	24	48	72
3		1.4	4.6	18.9	118.1	0.0266	2.12		99%	112%			4.146	4.659	5.276	6.072	0	24	48	72
1	75%	1.4	3.5	10.9	98.3	0.0251	2.00	1.90	96%	106%	101%	5%	4.146	4.549	5.037	5.993	0	24	48	72
2		1.4	4.4	19.3	69.7	0.0239	1.90		100%	100%			4.146	4.646	5.285	5.843	0	24	48	72
3		1.4	3.1	9.8	62.8	0.0227	1.81		96%	96%			4.146	4.494	4.991	5.798	0	24	48	72
1	100%	1.4	4.3	16.7	86.1	0.0248	1.98	1.99	99%	104%	105%	13%	4.146	4.632	5.222	5.935	0	24	48	72
2		1.4	5.7	15.8	53.6	0.0216	1.72		100%	91%			4.146	4.755	5.198	5.729	0	24	48	72
3		1.4	4.3	20.5	153.7	0.0283	2.26		98%	119%			4.146	4.631	5.312	6.187	0	24	48	72

Table A2.5: Raw data for *Ceriodaphnia dubia* toxicity tests for storm events (A) W2 and (B) W3. Quality assurance criteria - toxicity test results were considered acceptable if at least 90% survival was observed for the controls.

(A)

Event W2 ***C. dubia* 48-h Immobility test - Untreated and Treated Stormwater (Yarrowee Wetland)**

		Number of mobile organisms											
		Time			Mean		Prop'n mob		Mean % Control		Mean Mobile (%)		% Control
Rep		0 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 hr	48 hr
1		5	5	5			1.00	1.00					100%	100%
2	DMW	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3	Control	5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
		Mean Control + MB % Mobile =					***100%***	***100%***						
Untreated Stormwater														
1		5	5	5			1.00	1.00					100%	100%
2	6.25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	12.5%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	50%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	75%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	100%	5	5	5	5.00	5.00	1.00	1.00	100%	**100%**	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
Treated Stormwater														
1		5	5	5			1.00	1.00					100%	100%
2	6.25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	12.5%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	50%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	75%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	100%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%

(B)

Event W3 ***C.dubia* 48-h Immobility test - Untreated and Treated Stormwater (Yarrowee Wetland)**

		Number of mobile organisms											
		Time			Mean		Prop'n mob		Mean % Control		Mean Mobile (%)		% Control	
Rep		0 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 hr	48 hr
1		5	5	5			1.00	1.00					100%	100%
2	DMW	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3	Control	5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
	Mean Control + MB % Mobile =						***100%***	***100%***						
Untreated Stormwater														
1		5	5	5			1.00	1.00					100%	100%
2	6.25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	12.5%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	25%	5	5	4	5.00	4.50	1.00	0.80	100%	***90%***	100%	***90%***	100%	80%
3		5	5	4			1.00	0.80					100%	80%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	4			1.00	0.80					100%	80%
2	50%	5	5	4	4.75	4.00	1.00	0.80	95%	***80%***	95%	***80%***	100%	80%
3		5	5	4			1.00	0.80					100%	80%
4		5	4	4			0.80	0.80					80%	80%
1		5	4	4			0.80	0.80					80%	80%
2	75%	5	5	3	4.50	3.75	1.00	0.60	90%	***75%***	90%	***75%***	100%	60%
3		5	5	4			1.00	0.80					100%	80%
4		5	4	4			0.80	0.80					80%	80%
1		5	4	2			0.8	0.4					80%	40%
2	100%	5	4	2	4.25	1.5	0.8	0.4	85%	**30%**	85%	***30%***	80%	40%
3		5	5	1			1	0.2					100%	20%
4		5	4	1			0.8	0.2					80%	20%
Treated stormwater														
1		5	5	5			1.00	1.00					100%	100%
2	6.25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	12.5%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	50%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	75%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	100%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%

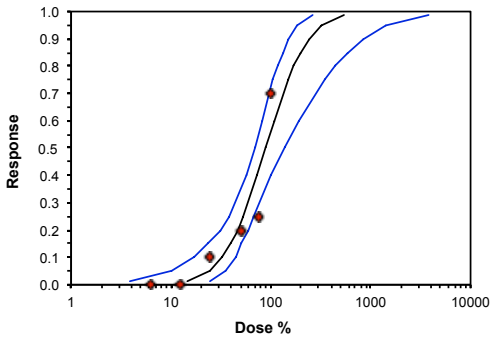
Table A2.6: ToxCalc output for 48-h *Ceriodaphnia dubia* survival following exposure to untreated stormwater from event W3.

Ceriodaphnia Dubia 48 Hr Survival				
Conc-%	**1**	**2**	**3**	**4**
Control	1.0000	1.0000	1.0000	1.0000
6.25	1.0000	1.0000	1.0000	1.0000
12.5	1.0000	1.0000	1.0000	1.0000
25	1.0000	0.8000	0.8000	1.0000
50	0.8000	0.8000	0.8000	0.8000
75	0.8000	0.6000	0.8000	0.8000
100	0.4000	0.4000	0.2000	0.2000

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	25	50	35.3553	4

Maximum Likelihood-Probit											
Parameter	**Value**	**SE**	**95% Fiducial Limits**		**Control**	**Chi-Sq**	**Critical**	**P-value**	**Mu**	**Sigma**	**Iter**
Slope	2.96923	0.71438	1.56905	4.36942	0	4.99428	9.48773	0.29	1.94883	0.33679	4
Intercept	-0.7865	1.29275	-3.3203	1.74726							
TSCR											

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	14.6334	3.84411	24.2654
EC05	3.355	24.8235	10.2328	35.4888
EC10	3.718	32.9018	17.0499	43.9596
EC15	3.964	39.7898	23.804	51.3387
EC20	4.158	46.2788	30.6672	58.7713
EC25	4.326	52.6829	37.5868	66.9224
EC40	4.747	73.0306	57.4146	101.479
EC50	5.000	88.8851	69.739	138.474
EC60	5.253	108.182	82.527	193.952
EC75	5.674	149.965	106.271	348.873
EC80	5.842	170.717	116.935	442.491
EC85	6.036	198.558	130.475	584.881
EC90	6.282	240.126	149.459	832.515
EC95	6.645	318.269	182.302	1408.68
EC99	7.326	539.9	263.332	3796.67



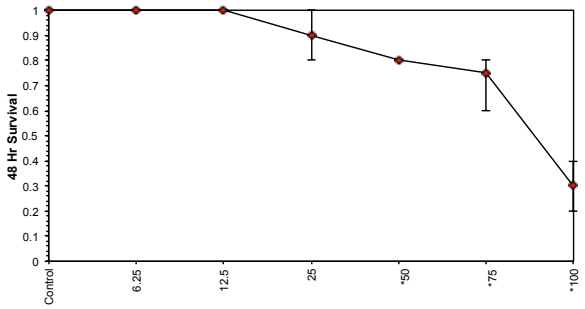


Table A2.7: Raw data for hatching success of *Melanotaenia duboulay*i eggs exposed to untreated and treated stormwater from events W2 and W3. Quality assurance criteria - test results were considered valid if ≥ 70% hatching success was observed overall for the controls.

Event	Replicate	Treatment	Eggs hatched	Hatching success (%)	Mean hatching success (%)
W2	1	Fish Culturing Water (Control)	7	70	80
	2		10	100	
	3		7	70	
	1	100% Untreated	4	40	33
	2		4	40	
	3		2	20	
	1	100% Treated	8	80	73
	2		8	80	
	3		6	60	
W3	1	Fish Culturing Water (Control)	7	70	73
	2		6	60	
	3		9	90	
	1	100% Untreated	2	20	27
	2		2	20	
	3		4	40	
	1	100% Treated	6	60	67
	2		6	60	
	3		8	80	

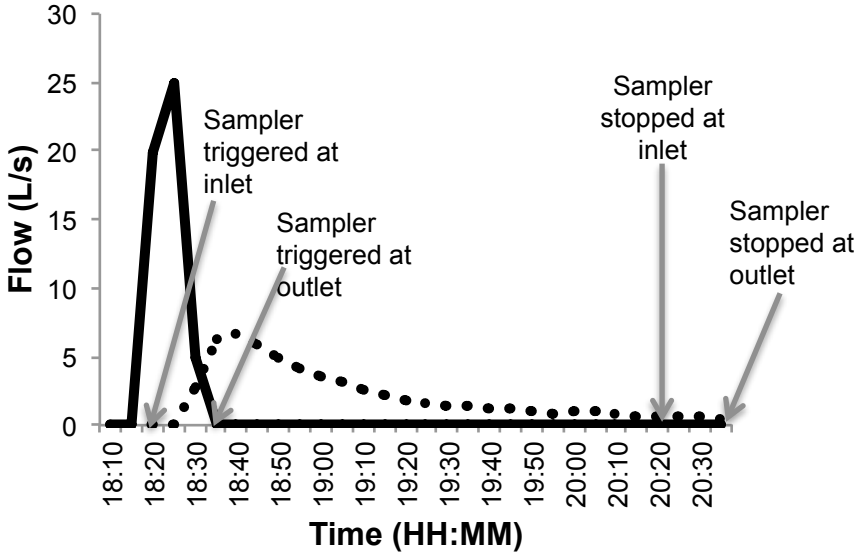
Table A2.8: Quality assurance analysis for a) *Pseudokirchneriella subcapitata* (IC_{50} of Cu reference toxicant) and b) *Ceriodaphnia dubia* (EC_{50} of Cu reference toxicant) toxicity tests. Quality assurance criteria - 72 h IC_{50} of the Cu reference toxicant for *P. subcapitata* had to fall within 14.9 ± 5.6 µg $L^{-}1$. 48 h EC_{50} of the Cu reference toxicant for *C. dubia* had to fall within 7.5 ± 3.5 µg L^{-1}.

a) *Pseudokirchneriella subcapitata*	72 h EC_{50} (µg L^{-1})
Event	
W2	13.2
W3	15.1

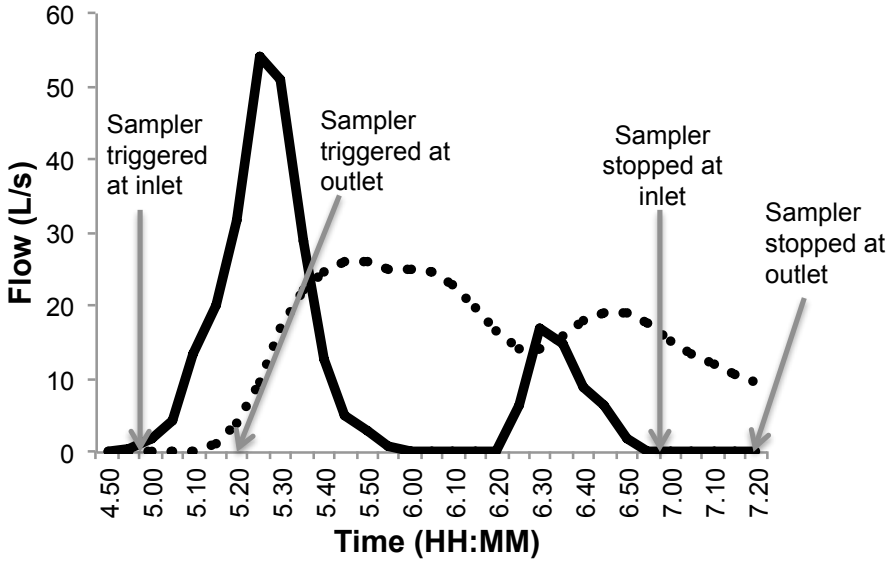
b) *Ceriodaphnia dubia*	48 h IC_{50} (µg L^{-1})
Event	
W2	5.2
W3	5.4

Appendix 3. Supporting information to Chapter 3

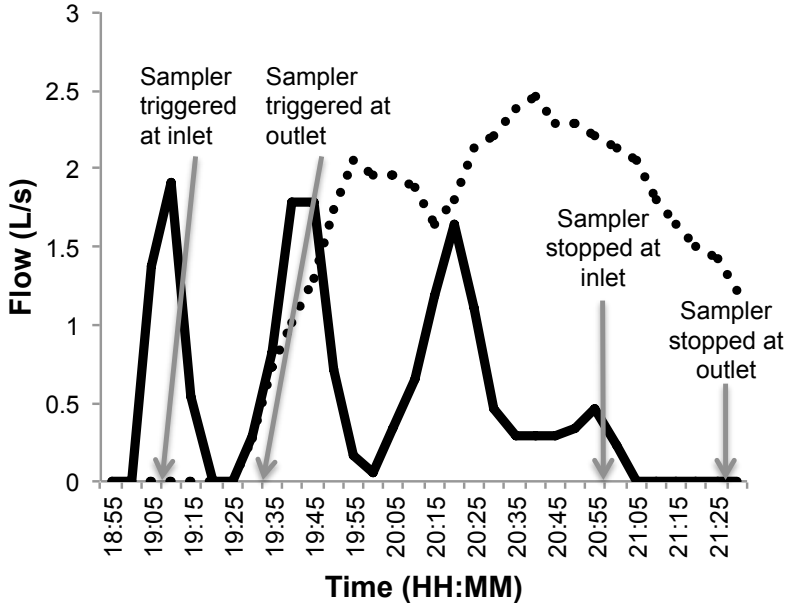
(A)


Flow (L/s)
30
25
20
15
10
5
0
Sampler triggered at inlet
Sampler triggered at outlet
Sampler stopped at inlet
Sampler stopped at outlet
18:10
18:20
18:30
18:40
18:50
19:00
19:10
19:20
19:30
19:40
19:50
20:00
20:10
20:20
20:30
Time (HH:MM)

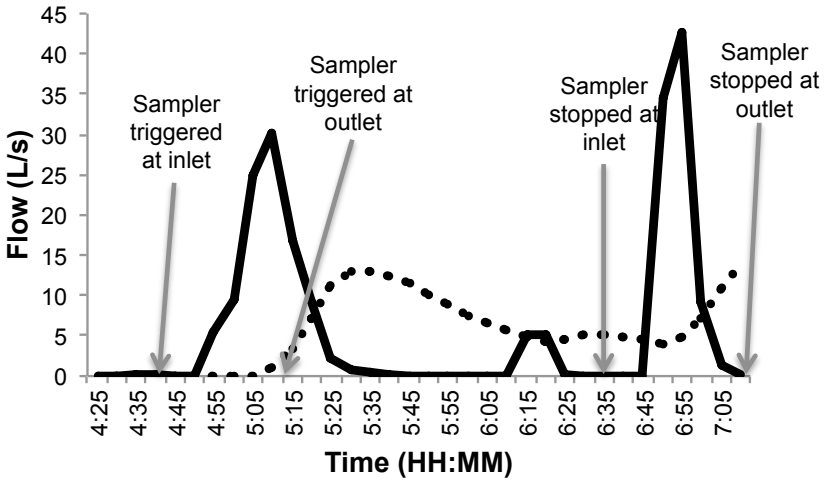
(B)


Flow (L/s)
60
50
40
30
20
10
0
Sampler triggered at inlet
Sampler triggered at outlet
Sampler stopped at inlet
Sampler stopped at outlet
4.50
5.00
5.10
5.20
5.30
5.40
5.50
6.00
6.10
6.20
6.30
6.40
6.50
7.00
7.10
7.20
Time (HH:MM)

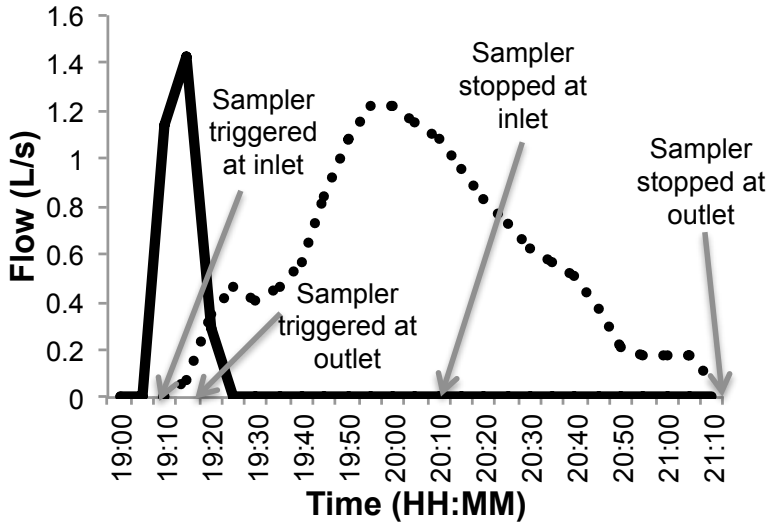
(C)


Flow (L/s)
3
2.5
2
1.5
1
0.5
0
Sampler triggered at inlet
Sampler triggered at outlet
Sampler stopped at inlet
Sampler stopped at outlet
18:55
19:05
19:15
19:25
19:35
19:45
19:55
20:05
20:15
20:25
20:35
20:45
20:55
21:05
21:15
21:25
Time (HH:MM)

(D)



(E)



(F)

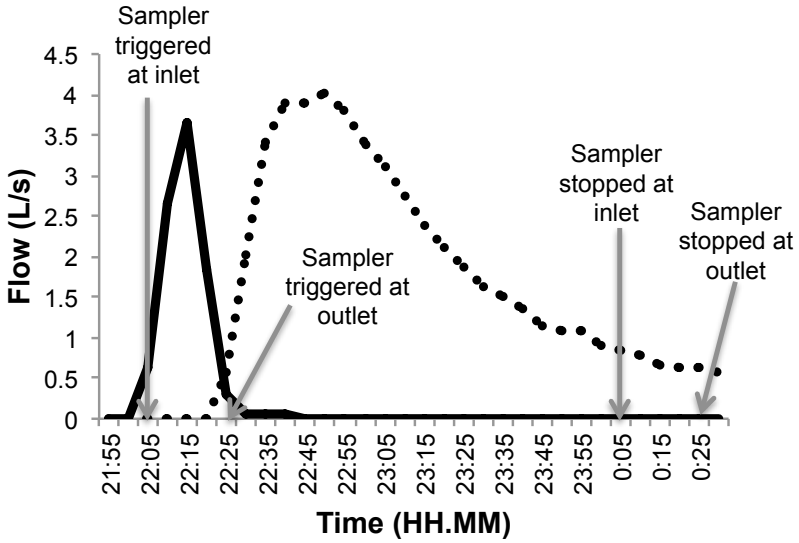


Figure A3.1: Flow data over the sampling period for storm events (A) B1, (B) B2, (C) B3, (D) B4, (E) B5, (F) B6.

Table A3.2: Laboratory quality control results for analytical work for events B1-B6 (data compiled from ALS Environmental laboratory reports for this study).

Event	Parameter	LOR (mg L^{-1})	Method Blank (mg L^{-1})	Laboratory duplicates		RPD (%)	Recoveries (%)		
				Sample (mg L^{-1})	Duplicate (mg L^{-1})		LCS	Acceptable recovery for LCS	Matrix spike
B1	TSS	5	<5	62	68	9.2	96	82-132	nd
	Al	0.01	<0.01	<0.01	<0.01	0	97	79-119	nd
	Cd	0.0001	<0.0001	<0.0001	<0.0001	0	99	82-114	102
	Cu	0.001	<0.0002	0.013	0.014	8.7	106	79-115	98.2
	Fe	0.05	<0.05	<0.05	<0.05	0	98	78-116	92.3
	Mn	0.001	<0.001	0.204	0.201	1.2	100	80-114	95.5
	Pb	0.001	<0.001	<0.001	<0.001	0	96	81-113	92.3
	Zn	0.005	<0.005	0.008	0.008	0	109	75-121	107
	NH_3-N	0.01	<0.01	0.05	0.04	0	99	89-113	70.5
	NO_x-N	0.01	<0.01	0.02	0.02	0	105	86-124	95
	TKN	0.1	<0.1	1.1	1.5	33.5	110	70-130	105
	TN	0.1	nd	nd	nd	nd	nd	nd	nd
	TP	0.01	<0.01	<0.01	<0.01	0	106	70-130	111
	RP	0.01	<0.01	0.08	0.08	0	108	86124	102
	COD	5	<5	18000	16700	7.7	98	88-114	101
	BOD	2	<2	4	5	22.2	93	77-107	nd
B2	TSS	5	<5	119	100	17.4	99	82-132	nd
	Al	0.01	<0.01	0.37	0.32	16	107	79-119	nd
	Cd	0.0001	<0.0001	0.0001	0.0002	0	94	82-114	91.1
	Cu	0.001	<0.001	0.002	0.002	0	96	79-115	100
	Fe	0.05	<0.05	0.35	0.39	9.9	106	78-116	nd
	Mn	0.001	<0.001	0.412	0.427	3.5	102	80-114	95.7
	Pb	0.001	<0.001	<0.001	<0.001	0	100	81-113	95.2
	Zn	0.005	<0.005	0.052	0.056	7.4	95	75-121	98.6
	NH_3-N	0.01	<0.01	0.21	0.2	0	94	89-113	79.4
	NO_x-N	0.01	<0.01	6.66	6.72	0.8	101	86-124	nd
	TKN	0.1	<0.1	34.5	36.8	6.4	84	66-119	74.7
	TN	0.1	<0.1	nd	nd	nd	nd	nd	nd
	TP	0.01	<0.01	4.38	5.04	13.8	77	69-117	70.4
	RP	0.01	<0.01	9.4	9.65	2.6	97	86-124	nd
	COD	5	<5	104	112	7.4	99	88-114	126
	BOD	2	<2	4	5	0	96	77-107	nd
B3	TSS	5	<5	18	20	10.2	99	82-132	nd
	Al	0.01	<0.01	0.02	0.02	0	95	79-119	nd
	Cd	0.0001	<0.0001	<0.0010	<0.0001	0	91	82-114	104
	Cu	0.001	<0.001	<0.001	<0.001	0	96	79-115	106
	Fe	0.05	<0.05	<0.05	<0.05	0	89	78-116	nd
	Mn	0.001	<0.001	0.002	0.002	0	91	80-114	104
	Pb	0.001	<0.001	<0.001	<0.001	0	89	81-113	104
	Zn	0.005	<0.005	<0.005	<0.005	0	89	78-116	111
	NH_3-N	0.01	<0.01	0.09	0.09	0	98	89-113	83.9
	NO_x-N	0.01	<0.01	0.1	0.11	0	101	86-124	nd
	TKN	0.1	<0.1	8.1	8.6	5.4	106	66-119	101
	TN	0.1	<0.1	nd	nd	nd	nd	nd	nd
	TP	0.01	<0.01	32.7	34.9	6.5	111	69-117	101
	RP	0.01	<0.01	0.09	0.08	0	91	86-124	85.8
	COD	5	<5	326	326	0	96	88-114	119
	BOD	2	<2	3	5	50	100	77-107	nd
B4	TSS	5	<5	26	26	0	98	82-132	nd
	Al	0.01	<0.01	41.2	41.3	0.2	98	79-119	nd
	Cd	0.0001	<0.0001	<0.0001	<0.0001	0	94	82-114	122
	Cu	0.001	<0.001	0.006	0.006	0	96	79-115	128
	Fe	0.05	<0.05	<0.05	<0.05	0	98	78-116	nd
	Mn	0.001	<0.001	0.007	0.007	0	100	80-114	105
	Pb	0.001	<0.001	0.004	0.004	0	93	81-113	122
	Zn	0.005	<0.005	0.04	0.046	15.5	97	75-121	129
	NH_3-N	0.01	<0.01	0.04	0.04	0	98	89-113	114
	NO_x-N	0.01	<0.01	0.27	0.26	0	104	86-124	81.1
	TKN	0.1	<0.1	1.2	1.3	9.7	90	66-119	87.4
	TN	0.1	<0.1	nd	nd	nd	nd	nd	nd
	TP	0.01	<0.01	1.43	1.31	8.7	97	69-117	81.5
	RP	0.01	<0.01	0.02	0.02	0	88	86-124	89
	COD	5	<5	1290	1310	1	98	88-124	103
	BOD	2	<2	3	3	0	96	77-107	nd
B5	TSS	5	<5	6	8	37	93	82-132	nd
	Al	0.01	<0.01	0.05	0.06	0	97	79-119	nd
	Cd	0.0001	<0.0001	<0.0001	<0.0001	0	107	82-114	80.1
	Cu	0.001	<0.001	0.013	0.013	0	97	79-115	86.4
	Fe	0.05	<0.05	<0.50	<0.50	0	113	78-116	nd
	Mn	0.001	<0.001	0.009	0.009	0	100	80-114	80.7
	Pb	0.001	<0.001	0.006	0.006	0	100	81-113	76.9
	Zn	0.005	<0.005	0.055	0.055	0	104	75-121	91.5
	NH_3-N	0.01	<0.01	<0.01	<0.01	0	100	89-113	84.6
	NO_x-N	0.01	<0.01	<0.01	0.03	100	103	86-124	90.3
	TKN	0.1	<0.1	71.4	82	13.8	82	66-119	91.8
	TN	0.1	<0.1	nd	nd	nd	nd	nd	nd
	TP	0.01	<0.01	8.78	9.58	8.7	80	69-117	90.7
	RP	0.01	<0.01	1.62	1.62	0	100	86-124	88.6
	COD	5	<5	924	924	0	99	88-114	nd
	BOD	2	<2	<2	<2	0	89	77-107	nd
B6	TSS	5	<5	<5	<5	0	96	82-132	nd
	Al	0.01	<0.01	0.02	0.02	0	90	79-119	nd
	Cd	0.0001	<0.0001	<0.0001	<0.0001	0	107	82-114	109
	Cu	0.001	<0.001	0.001	<0.001	0	98	79-115	101
	Fe	0.05	<0.05	<0.05	<0.05	0	90	78-116	nd
	Mn	0.001	<0.001	0.014	0.013	0	98	80-114	103
	Pb	0.001	<0.001	<0.001	<0.001	0	96	81-113	98.5
	Zn	0.005	<0.005	0.034	0.032	8	97	75-121	114
	NH_3-N	0.01	<0.01	0.03	0.03	0	99	89-113	nd
	NO_x-N	0.01	<0.01	0.08	0.07	0	99	86-124	nd
	TKN	0.1	<0.1	2.9	2.9	0	95	66-119	93.4
	TN	0.1	<0.1	nd	nd	nd	nd	nd	nd
	TP	0.01	<0.01	0.13	0.16	20.7	90	69-117	91
	RP	0.01	<0.01	<0.01	<0.01	0	96	86-124	92.5
	COD	5	<5	38	38	0	93	88-114	106
	BOD	2	<2	<2	<2	0	80	77-107	nd

Legend:
RPD = Relative Percentage Difference
LOR = Level of reporting
LCS = Laboratory Control Spike
nd = not determined
Acceptable RPDs on duplicates is: no limit at concentrations <10 times LOR; 0-50% at concentrations between 10 and 20 times LOR; 0-20% at concentrations greater than 20 time LOR
Acceptable recovery on matrix spikes is 70-130%
Acceptable recovery on laboratory control spikes is listed in the table

Table A3.3: Raw data for *Pseudokirchneriella subcapitata* toxicity tests and cell division (growth) rate per day calculations for storm events (A) B1, (B) B2, (C) B3, (D) B4, (E) B5 and (F) B6. CV = coefficient of variation. Quality assurance criteria - Bioassays were considered acceptable if there was at least a 16-fold increase in the control biomass after 72 h and variability among the control replicates (CV) did not exceed 20%.

(A) **Event B1** **72-h Chronic Toxicity of Untreted and Treated stormwater (Johnston St bioretention basin to *Pseudokirchneriella subcapitata*)**

	Sample	Day 0	Day 1	Day 2	Day 3	Slope	Growth Rate (dblngs/day)	Mean	Pearson	% Control	Mean %	CV (%)			Log of Growth Rates				Below values for formula ref only			
Vial No.		*All cell counts in (cells/mL) by x10⁴*													Day 0	Day 1	Day 2	Day 3				
1		1.2	4.1	18.6	58.1	0.02403	1.91	1.90	100%	101%	100%	4%			4.060698	4.612784	5.270213	5.764101	0	24	48	72
2	Control	1.2	5.6	20.3	68.7	0.02454	1.96		100%	103%					4.060698	4.745855	5.306854	5.836767	0	24	48	72
3		1.2	4.2	15.6	50.3	0.02288	1.82		100%	96%					4.060698	4.625312	5.192567	5.701654	0	24	48	72
				Mean control rate =		***0.02382***																
Untreated storm water													**STDEV**	**SE**								
1		1.2	5.0	25.3	93.4	0.02680	2.14	2.01	100%	113%	106%	8%	8%	5%	4.060698	4.699838	5.403292	5.970161	0	24	48	72
2	6.25%	1.2	5.1	19.8	51.6	0.02310	1.84		99%	97%					4.060698	4.710117	5.296884	5.712902	0	24	48	72
3		1.2	5.0	19.9	81.8	0.02565	2.04		100%	108%					4.060698	4.700704	5.299507	5.912753	0	24	48	72
1		1.2	4.2	15.6	55.7	0.02343	1.87	2.05	100%	98%	108%	8%	9%	5%	4.060698	4.623249	5.192846	5.745543	0	24	48	72
2	12.5%	1.2	6.6	25.1	95.8	0.02642	2.10		100%	111%					4.060698	4.820201	5.398808	5.981184	0	24	48	72
3		1.2	2.9	17.0	97.0	0.02730	2.18		98%	115%					4.060698	4.456366	5.229682	5.986906	0	24	48	72
1		1.2	5.8	20.5	90.0	0.02595	2.07	1.90	100%	109%	100%	8%	8%	5%	4.060698	4.764176	5.311754	5.954146	0	24	48	72
2	25%	1.2	4.7	14.6	47.6	0.02226	1.77		100%	93%					4.060698	4.672098	5.163758	5.677789	0	24	48	72
3		1.2	6.6	17.6	59.3	0.02317	1.85		99%	97%					4.060698	4.821514	5.246006	5.772981	0	24	48	72
1		1.2	4.9	19.6	56.1	0.02364	1.88	1.86	100%	99%	98%	2%	2%	1%	4.060698	4.685742	5.292478	5.749272	0	24	48	72
2	50%	1.2	6.6	21.1	50.6	0.02263	1.80		98%	95%					4.060698	4.821514	5.323871	5.703893	0	24	48	72
3		1.2	5.7	22.4	56.2	0.02359	1.88		99%	99%					4.060698	4.755875	5.350829	5.749427	0	24	48	72
1		1.2	6.1	17.1	66.6	0.02391	1.91	1.91	99%	100%	100%	5%	5%	3%	4.060698	4.782473	5.232488	5.82367	0	24	48	72
2	75%	1.2	6.0	17.8	52.7	0.02273	1.81		99%	95%					4.060698	4.778151	5.250664	5.721975	0	24	48	72
3		1.2	6.1	27.4	71.4	0.02512	2.00		99%	105%					4.060698	4.786041	5.437116	5.853455	0	24	48	72
1		1.2	4.3	11.5	31.3	0.01971	1.57	1.59	99%	83%	84%	1%	1%	1%	4.060698	4.632457	5.06032	5.49485	0	24	48	72
2	100%	1.2	5.2	14.0	34.0	0.02019	1.61		98%	85%					4.060698	4.711807	5.145818	5.531607	0	24	48	72
3		1.2	5.5	11.4	35.5	0.01995	1.59		98%	84%					4.060698	4.737193	5.05576	5.550595	0	24	48	72
Treated storm water																						
1		1.2	5.3	22.4	85.3	0.02600	2.07	1.90	100%	109%	100%	8%	8%	5%	4.060698	4.720986	5.350248	5.930898	0	24	48	72
2	6.25%	1.2	4.9	19.7	45.5	0.02248	1.79		99%	94%					4.060698	4.689309	5.293363	5.657534	0	24	48	72
3		1.2	6.3	16.2	58.9	0.02308	1.84		99%	97%					4.060698	4.796574	5.209515	5.769746	0	24	48	72
1		1.2	5.3	22.0	79.4	0.02557	2.04	1.91	100%	107%	101%	6%	6%	3%	4.060698	4.722634	5.343212	5.899821	0	24	48	72
2	12.5%	1.2	4.9	15.3	57.2	0.02326	1.85		100%	98%					4.060698	4.691081	5.183555	5.75732	0	24	48	72
3		1.2	4.5	15.8	52.4	0.02300	1.83		100%	97%					4.060698	4.652246	5.197832	5.719083	0	24	48	72
1		1.2	3.0	15.0	71.2	0.02531	2.02	1.88	99%	106%	99%	13%	12%	7%	4.060698	4.477121	5.176959	5.852541	0	24	48	72
2	25%	1.2	3.7	13.0	30.8	0.02012	1.60		99%	85%					4.060698	4.568202	5.114611	5.488551	0	24	48	72
3		1.2	3.8	17.0	72.6	0.02522	2.01		100%	106%					4.060698	4.579784	5.231215	5.860817	0	24	48	72
1		1.2	5.3	19.6	45.2	0.02231	1.78	1.71	98%	94%	90%	5%	4%	3%	4.060698	4.721811	5.291369	5.655523	0	24	48	72
2	50%	1.2	3.9	11.2	34.2	0.02033	1.62		100%	85%					4.060698	4.58995	5.048442	5.534407	0	24	48	72
3		1.2	3.2	9.3	46.0	0.02193	1.75		99%	92%					4.060698	4.510545	4.96708	5.662947	0	24	48	72
1		1.2	6.1	18.0	41.1	0.02139	1.70	1.73	98%	90%	91%	2%	2%	1%	4.060698	4.782473	5.254548	5.614264	0	24	48	72
2	75%	1.2	4.6	14.1	42.6	0.02163	1.72		100%	91%					4.060698	4.664642	5.148603	5.629613	0	24	48	72
3		1.2	4.7	16.6	44.8	0.02218	1.77		99%	93%					4.060698	4.668386	5.220631	5.651278	0	24	48	72
1		1.2	3.9	18.3	53.3	0.02363	1.88	1.86	100%	99%	98%	7%	7%	4%	4.060698	4.588832	5.261739	5.726727	0	24	48	72
2	100%	1.2	4.7	13.4	79.6	0.02488	1.98		99%	104%					4.060698	4.675778	5.127753	5.900749	0	24	48	72
3		1.2	3.2	10.0	42.2	0.02164	1.72		99%	91%					4.060698	4.498311	4.999131	5.624798	0	24	48	72

(B)

Event B2 72-h Chronic Toxicity of Untreted and Treated stormwater (Johnston St bioretention basin to *Pseudokirchneriella subcapitata*)

	Sample	Day 0	Day 1	Day 2	Day 3	Slope	Growth Rate (dblngs/day)	Mean	Pearson	% Control	Mean %	CV (%)			Log of Growth Rates				Below values for formula reference only			
Vial No.		*All cell counts in (cells/mL) by x10^4*													Day 0	Day 1	Day 2	Day 3				
1		1.2	5.3	37.9	146.5	0.02987	2.38	2.41	100%	99%	100%	2%			4.060698	4.725095	5.57841	6.165778	0	24	48	72
2	Control	1.2	5.9	54.2	163.8	0.03092	2.46		99%	102%					4.060698	4.773055	5.734079	6.214234	0	24	48	72
3		1.2	6.2	39.7	154.2	0.02996	2.39		100%	99%					4.060698	4.789581	5.598791	6.187944	0	24	48	72
				Mean control rate =		***0.03025***																
Untreated storm water													STDEV	SE								
1		1.2	7.7	44.9	165.2	0.03015	2.40	2.43	99%	100%	101%	2%	2%	1%	4.060698	4.887054	5.65215	6.218036	0	24	48	72
2	6.25%	1.2	7.2	49.0	155.3	0.03011	2.40		99%	100%					4.060698	4.856124	5.690019	6.191199	0	24	48	72
3		1.2	6.1	40.8	189.0	0.03113	2.48		100%	103%					4.060698	4.788168	5.611086	6.276393	0	24	48	72
1		1.2	6.1	51.0	234.7	0.03270	2.61	2.58	100%	108%	107%	1%	1%	1%	4.060698	4.788168	5.707229	6.370476	0	24	48	72
2	12.5%	1.2	6.1	44.3	238.8	0.03254	2.59		100%	108%					4.060698	4.788168	5.64611	6.378107	0	24	48	72
3		1.2	7.0	39.0	231.3	0.03190	2.54		100%	105%					4.060698	4.845098	5.590842	6.364101	0	24	48	72
1		1.2	6.7	54.3	239.8	0.03279	2.61	2.58	100%	108%	107%	1%	1%	1%	4.060698	4.823474	5.7348	6.379849	0	24	48	72
2	25%	1.2	6.7	49.7	212.1	0.03195	2.55		100%	106%					4.060698	4.825426	5.696706	6.326623	0	24	48	72
3		1.2	6.5	48.2	225.7	0.03229	2.57		100%	107%					4.060698	4.810904	5.682596	6.353493	0	24	48	72
1		1.2	5.9	40.9	165.9	0.03048	2.43	2.54	100%	101%	105%	6%	7%	4%	4.060698	4.773055	5.612148	6.219742	0	24	48	72
2	50%	1.2	7.2	54.0	319.7	0.03420	2.73		100%	113%					4.060698	4.856124	5.732635	6.504688	0	24	48	72
3		1.2	5.7	50.0	167.4	0.03096	2.47		99%	102%					4.060698	4.758912	5.69897	6.223833	0	24	48	72
1		1.2	6.4	46.0	207.5	0.03176	2.53	2.49	100%	105%	103%	3%	3%	1%	4.060698	4.808886	5.662286	6.317039	0	24	48	72
2	75%	1.2	6.9	37.3	174.1	0.03032	2.42		100%	100%					4.060698	4.835691	5.571942	6.240699	0	24	48	72
3		1.2	6.1	40.3	205.7	0.03158	2.52		100%	104%					4.060698	4.783189	5.604982	6.31315	0	24	48	72
1		1.2	6.9	40.9	211.7	0.03154	2.51	2.51	100%	104%	104%	3%	3%	2%	4.060698	4.836957	5.611192	6.32568	0	24	48	72
2	100%	1.2	6.4	40.7	170.5	0.03048	2.43		100%	101%					4.060698	4.807535	5.610021	6.231622	0	24	48	72
3		1.2	6.7	49.2	233.2	0.03244	2.58		100%	107%					4.060698	4.826723	5.691612	6.367766	0	24	48	72
treated storm water																						
1		1.2	5.9	41.8	213.6	0.03191	2.54	2.47	100%	105%	102%	4%	4%	2%	4.060698	4.770852	5.621592	6.329622	0	24	48	72
2	6.25%	1.2	5.8	43.4	193.1	0.03145	2.51		100%	104%					4.060698	4.764176	5.63749	6.285827	0	24	48	72
3		1.2	6.1	40.6	140.0	0.02950	2.35		99%	98%					4.060698	4.786041	5.60874	6.146252	0	24	48	72
1		1.2	6.8	39.9	218.3	0.03167	2.52	2.53	100%	105%	105%	2%	2%	1%	4.060698	4.834421	5.600537	6.338954	0	24	48	72
2	12.5%	1.2	5.6	43.3	174.5	0.03098	2.47		99%	102%					4.060698	4.744293	5.636889	6.241671	0	24	48	72
3		1.2	6.0	43.9	234.1	0.03246	2.59		100%	107%					4.060698	4.777427	5.642168	6.369401	0	24	48	72
1		1.2	6.2	51.4	257.2	0.03320	2.65	2.64	100%	110%	110%	2%	2%	1%	4.060698	4.792392	5.711217	6.410203	0	24	48	72
2	25%	1.2	5.9	55.3	271.0	0.03371	2.69		100%	111%					4.060698	4.767898	5.742647	6.432953	0	24	48	72
3		1.2	6.3	44.4	244.6	0.03264	2.60		100%	108%					4.060698	4.796574	5.647187	6.388439	0	24	48	72
1		1.2	6.2	49.4	275.4	0.03350	2.67	2.59	100%	111%	107%	3%	3%	2%	4.060698	4.791691	5.693903	6.440027	0	24	48	72
2	50%	1.2	6.1	43.7	232.6	0.03239	2.58		100%	107%					4.060698	4.784617	5.640382	6.366554	0	24	48	72
3		1.2	5.1	42.6	191.5	0.03161	2.52		99%	104%					4.060698	4.708421	5.62941	6.282123	0	24	48	72
1		1.2	6.3	44.3	207.2	0.03173	2.53	2.54	100%	105%	105%	1%	1%	1%	4.060698	4.797268	5.646502	6.316285	0	24	48	72
2	75%	1.2	5.1	44.4	217.7	0.03240	2.58		99%	107%					4.060698	4.703291	5.647383	6.337819	0	24	48	72
3		1.2	5.6	39.2	202.8	0.03160	2.52		100%	104%					4.060698	4.748188	5.593508	6.307132	0	24	48	72
1		1.2	5.3	39.9	187.4	0.03132	2.50	2.47	100%	104%	102%	1%	1%	1%	4.060698	4.720159	5.601082	6.27277	0	24	48	72
2	100%	1.2	5.9	41.5	178.3	0.03092	2.46		100%	102%					4.060698	4.767898	5.617839	6.251103	0	24	48	72
3		1.2	7.3	47.4	178.7	0.03077	2.45		99%	102%					4.060698	4.865104	5.675503	6.252173	0	24	48	72

(C)

Event B3 72-h Chronic Toxicity of Untreted and Treated stormwater (Johnston St bioretention basin to *Pseudokirchneriella subcapitata*)

	Sample	Day 0	Day 1	Day 2	Day 3	Slope	Growth Rate (dblngs/day)	Mean	Pearson	% Control	Mean %	CV (%)			Log of Growth Rates				Below values for formula reference			
Vial No.		*All cell counts in (cells/mL) by x10^4*													Day 0	Day 1	Day 2	Day 3				
1	Control	1.2	4.0	21.8	174.5	0.03033	2.42	2.45	99%	99%	100%	1%			4.060698	4.603144	5.338855	6.241671	0	24	48	72
2		1.2	4.4	34.3	173.7	0.03095	2.47		99%	101%					4.060698	4.643453	5.534914	6.2398	0	24	48	72
3		1.2	4.6	26.4	188.5	0.03085	2.46		99%	100%					4.060698	4.661813	5.421439	6.275219	0	24	48	72
				Mean control rate =		***0.03071***																
Untreated storm water													STDEV	SE								
1	6.25%	1.2	4.6	42.1	250.3	0.03322	2.65	2.59	99%	108%	106%	4%	4%	2%	4.060698	4.664642	5.624179	6.398513	0	24	48	72
2		1.2	4.8	42.5	253.9	0.03326	2.65		99%	108%					4.060698	4.676694	5.627878	6.404731	0	24	48	72
3		1.2	5.4	43.5	175.5	0.03107	2.48		99%	101%					4.060698	4.731589	5.63799	6.244203	0	24	48	72
1	12.5%	1.2	4.7	40.6	271.3	0.03357	2.68	2.63	99%	109%	108%	1%	1%	1%	4.060698	4.669317	5.60874	6.433482	0	24	48	72
2		1.2	4.6	35.4	241.1	0.03271	2.61		99%	107%					4.060698	4.662758	5.549003	6.382233	0	24	48	72
3		1.2	5.0	44.6	238.1	0.03292	2.62		99%	107%					4.060698	4.696356	5.649237	6.376759	0	24	48	72
1	25%	1.2	4.0	40.2	228.8	0.03290	2.62	2.60	99%	107%	106%	2%	2%	1%	4.060698	4.604226	5.60455	6.359437	0	24	48	72
2		1.2	4.9	38.6	249.7	0.03294	2.62		100%	107%					4.060698	4.690196	5.586587	6.397419	0	24	48	72
3		1.2	4.9	30.7	221.0	0.03187	2.54		100%	104%					4.060698	4.690196	5.487138	6.344392	0	24	48	72
1	50%	1.2	5.1	36.2	206.6	0.03173	2.53	2.52	100%	103%	103%	2%	2%	1%	4.060698	4.70757	5.558709	6.31513	0	24	48	72
2		1.2	5.2	38.9	224.4	0.03227	2.57		100%	105%					4.060698	4.716003	5.58995	6.351023	0	24	48	72
3		1.2	4.7	30.5	184.3	0.03094	2.47		100%	101%					4.060698	4.672098	5.4843	6.265525	0	24	48	72
1	75%	1.2	4.7	31.7	195.4	0.03133	2.50	2.54	100%	102%	104%	5%	5%	3%	4.060698	4.672098	5.501059	6.290925	0	24	48	72
2		1.2	4.7	34.8	295.1	0.03374	2.69		99%	110%					4.060698	4.672098	5.541579	6.469969	0	24	48	72
3		1.2	4.2	28.0	176.2	0.03075	2.45		99%	100%					4.060698	4.623249	5.447158	6.246006	0	24	48	72
1	100%	1.2	4.7	30.9	163.9	0.03035	2.42	2.41	100%	99%	98%	3%	3%	2%	4.060698	4.668386	5.489958	6.214579	0	24	48	72
2		1.2	5.3	32.6	192.6	0.03108	2.48		100%	101%					4.060698	4.725912	5.513218	6.284544	0	24	48	72
3		1.2	4.8	27.1	140.7	0.02924	2.33		100%	95%					4.060698	4.677607	5.432167	6.148263	0	24	48	72
treated storm water																						
1	6.25%	1.2	4.8	34.6	259.0	0.03297	2.63	2.51	99%	107%	102%	5%	5%	3%	4.060698	4.684845	5.539327	6.4133	0	24	48	72
2		1.2	5.1	35.0	154.5	0.03008	2.40		100%	98%					4.060698	4.708421	5.54382	6.188985	0	24	48	72
3		1.2	4.6	35.7	187.0	0.03135	2.50		100%	102%					4.060698	4.661813	5.552911	6.271795	0	24	48	72
1	12.5%	1.2	5.8	33.9	152.2	0.02971	2.37	2.45	100%	97%	100%	3%	3%	2%	4.060698	4.765669	5.529815	6.182415	0	24	48	72
2		1.2	3.8	27.5	174.3	0.03084	2.46		99%	100%					4.060698	4.579784	5.439491	6.241372	0	24	48	72
3		1.2	4.8	34.7	200.9	0.03160	2.52		100%	103%					4.060698	4.681241	5.539829	6.302893	0	24	48	72
1	25%	1.2	5.1	34.9	250.1	0.03269	2.60	2.62	100%	106%	107%	1%	1%	0%	4.060698	4.710117	5.543074	6.398096	0	24	48	72
2		1.2	4.9	37.6	261.1	0.03313	2.64		100%	108%					4.060698	4.692847	5.575419	6.416724	0	24	48	72
3		1.2	5.1	36.3	260.0	0.03299	2.63		100%	107%					4.060698	4.705008	5.559548	6.414973	0	24	48	72
1	50%	1.2	5.2	31.1	240.7	0.03225	2.57	2.58	100%	105%	105%	2%	2%	1%	4.060698	4.715167	5.492201	6.38144	0	24	48	72
2		1.2	5.3	38.6	250.9	0.03284	2.62		100%	107%					4.060698	4.720159	5.586137	6.399431	0	24	48	72
3		1.2	4.6	33.4	210.3	0.03188	2.54		99%	104%					4.060698	4.659916	5.523876	6.322819	0	24	48	72
1	75%	1.2	4.4	34.7	249.2	0.03293	2.62	2.62	99%	107%	107%	0%	0%	0%	4.060698	4.643453	5.540329	6.396478	0	24	48	72
2		1.2	5.1	43.6	235.9	0.03279	2.61		100%	107%					4.060698	4.706718	5.639586	6.372654	0	24	48	72
3		1.2	4.3	35.9	236.5	0.03276	2.61		99%	107%					4.060698	4.632457	5.555578	6.373739	0	24	48	72
1	100%	1.2	5.0	38.7	236.7	0.03263	2.60	2.64	100%	106%	108%	1%	1%	1%	4.060698	4.698101	5.588047	6.374125	0	24	48	72
2		1.2	5.0	39.1	268.9	0.03333	2.66		100%	109%					4.060698	4.698101	5.591732	6.429558	0	24	48	72
3		1.2	5.2	36.6	286.5	0.03348	2.67		100%	109%					4.060698	4.716838	5.563244	6.457064	0	24	48	72

(D)

Event B4 72-h Chronic Toxicity of Untreted and Treated stormwater (Johnston St bioretention basin to *Pseudokirchneriella subcapitata*)

	Sample	Day 0	Day 1	Day 2	Day 3	Slope	Growth Rate (dblngs/day)	Mean	Pearson	% Control	Mean %	CV (%)			Log of Growth Rates				Below values for formula			
Vial No.		*All cell counts in (cells/mL) by x10⁴*													Day 0	Day 1	Day 2	Day 3				
1	Control	1.2	4.4	29.4	107.9	0.02810	2.24	2.29	99%	98%	100%	2%			4.060698	4.641474	5.46879	6.033142	0	24	48	72
2		1.2	4.6	29.9	133.6	0.02919	2.33		100%	102%					4.060698	4.666518	5.475962	6.125709	0	24	48	72
3		1.2	4.2	28.0	122.8	0.02880	2.29		100%	100%					4.060698	4.621176	5.447778	6.089128	0	24	48	72
				Mean control rate =		***0.02870***																
Untreated storm water													STDEV	SE								
1	6.25%	1.2	5.4	34.1	175.7	0.03065	2.44	2.38	100%	107%	104%	3%	3%	2%	4.060698	4.728354	5.532245	6.244871	0	24	48	72
2		1.2	4.7	30.6	127.4	0.02893	2.31		100%	101%					4.060698	4.675778	5.485863	6.105101	0	24	48	72
3		1.2	5.1	33.5	151.1	0.02988	2.38		100%	104%					4.060698	4.70927	5.524785	6.179178	0	24	48	72
1	12.5%	1.2	5.7	29.6	190.5	0.03073	2.45	2.41	100%	107%	106%	2%	2%	1%	4.060698	4.753583	5.471145	6.279781	0	24	48	72
2		1.2	4.6	40.2	133.4	0.02973	2.37		99%	104%					4.060698	4.662758	5.603794	6.125091	0	24	48	72
3		1.2	4.6	33.9	160.9	0.03043	2.42		100%	106%					4.060698	4.662758	5.529687	6.206448	0	24	48	72
1	25%	1.2	3.3	17.5	83.9	0.02629	2.10	2.18	99%	92%	95%	4%	4%	2%	4.060698	4.519828	5.241795	5.923607	0	24	48	72
2		1.2	4.9	26.7	127.2	0.02863	2.28		100%	100%					4.060698	4.687529	5.426186	6.104487	0	24	48	72
3		1.2	3.8	24.4	92.3	0.02720	2.17		99%	95%					4.060698	4.574031	5.38739	5.965296	0	24	48	72
1	50%	1.2	3.1	11.9	76.4	0.02520	2.01	1.83	98%	88%	80%	11%	9%	5%	4.060698	4.494155	5.075547	5.88298	0	24	48	72
2		1.2	3.0	11.3	56.8	0.02359	1.88		99%	82%					4.060698	4.471292	5.052309	5.754119	0	24	48	72
3		1.2	2.7	7.9	32.6	0.02010	1.60		99%	70%					4.060698	4.428135	4.896526	5.512951	0	24	48	72
1	75%	1.2	1.9	9.1	31.4	0.02075	1.65	1.57	97%	72%	69%	7%	9%	5%	4.060698	4.285557	4.959518	5.496376	0	24	48	72
2		1.2	2.0	8.9	28.8	0.02024	1.61		97%	71%					4.060698	4.290035	4.951338	5.459845	0	24	48	72
3		1.2	7.6	7.7	32.6	0.01818	1.45		89%	63%					4.060698	4.881955	4.888741	5.513084	0	24	48	72
1	100%	1.2	1.6	2.9	3.1	0.00659	0.52	1.03	93%	23%	45%	8%	3%	2%	4.060698	4.190332	4.462398	4.49693	0	24	48	72
2		1.2	1.3	6.9	14.4	0.01680	1.34		91%	59%					4.060698	4.09691	4.840106	5.156852	0	24	48	72
3		1.2	1.2	5.8	11.7	0.01540	1.23		90%	54%					4.060698	4.08636	4.762679	5.067071	0	24	48	72
treated storm water																						
1	6.25%	1.2	4.0	37.9	126.9	0.02960	2.36	2.40	98%	103%	105%	2%	2%	1%	4.060698	4.60206	5.578066	6.103462	0	24	48	72
2		1.2	4.9	40.6	169.2	0.03094	2.47		99%	108%					4.060698	4.685742	5.608633	6.228426	0	24	48	72
3		1.2	4.4	36.8	140.3	0.02992	2.38		99%	104%					4.060698	4.644439	5.566084	6.147027	0	24	48	72
1	12.5%	1.2	4.8	39.1	107.1	0.02840	2.26	2.40	98%	99%	105%	5%	5%	3%	4.060698	4.683047	5.592066	6.029911	0	24	48	72
2		1.2	4.9	37.6	189.7	0.03139	2.50		100%	109%					4.060698	4.692847	5.575188	6.278113	0	24	48	72
3		1.2	5.1	40.6	161.8	0.03061	2.44		99%	107%					4.060698	4.70757	5.608954	6.208979	0	24	48	72
1	25%	1.2	5.0	39.4	186.3	0.03137	2.50	2.57	100%	109%	112%	10%	11%	7%	4.060698	4.695482	5.595055	6.270166	0	24	48	72
2		1.2	7.1	53.7	437.5	0.03591	2.86		100%	125%					4.060698	4.853698	5.730217	6.640968	0	24	48	72
3		1.2	4.8	34.3	138.1	0.02954	2.35		100%	103%					4.060698	4.683947	5.534914	6.140225	0	24	48	72
1	50%	1.2	5.3	37.4	190.3	0.03128	2.49	2.44	100%	109%	107%	2%	3%	1%	4.060698	4.722634	5.572523	6.279439	0	24	48	72
2		1.2	5.0	34.3	175.8	0.03077	2.45		100%	107%					4.060698	4.702431	5.535294	6.244969	0	24	48	72
3		1.2	4.9	31.5	151.0	0.02983	2.38		100%	104%					4.060698	4.692847	5.498311	6.178919	0	24	48	72
1	75%	1.2	4.7	38.1	180.5	0.03123	2.49	2.53	99%	109%	111%	2%	2%	1%	4.060698	4.672098	5.580925	6.256357	0	24	48	72
2		1.2	5.2	40.7	206.1	0.03189	2.54		100%	111%					4.060698	4.716003	5.609808	6.314015	0	24	48	72
3		1.2	4.3	36.5	215.7	0.03226	2.57		99%	112%					4.060698	4.63749	5.561698	6.33381	0	24	48	72
1	100%	1.2	4.2	33.6	165.6	0.03073	2.45	2.46	99%	107%	108%	3%	3%	2%	4.060698	4.62634	5.525951	6.219139	0	24	48	72
2		1.2	5.0	40.2	145.8	0.03006	2.40		99%	105%					4.060698	4.699838	5.60455	6.163758	0	24	48	72
3		1.2	4.6	32.7	210.5	0.03183	2.54		99%	111%					4.060698	4.663701	5.514813	6.32317	0	24	48	72

(E)

Event B5 72-h Chronic Toxicity of Untreted and Treated stormwater (Johnston St bioretention basin to *Pseudokirchneriella subcapitata*)

	Sample	Day 0	Day 1	Day 2	Day 3	Slope	Growth Rate (dblngs/day)	Mean	Pearson	% Control	Mean %	CV (%)			Log of Growth Rates				Below values for formula			
Vial No.		*All cell counts in (cells/mL) by x10⁴*													Day 0	Day 1	Day 2	Day 3				
1	Control	1.16	2.69	10.25	34.43	0.02083	1.66	1.71	99%	97%	100%	3%			4.064458	4.429752	5.010724	5.536937	0	24	48	72
2		1.16	3.14	10.08	39.39	0.02125	1.69		100%	99%					4.064458	4.49693	5.003461	5.595386	0	24	48	72
3		1.16	3.61	10.16	49.4	0.02224	1.77		99%	104%					4.064458	4.557507	5.006894	5.693727	0	24	48	72
				Mean control rate =		***0.02144***																
Untreated storm water													STDEV	SE								
1	6.25%	1.16	1.9	6.39	27.59	0.01940	1.55	1.65	96%	90%	97%	6%	5%	3%	4.064458	4.278754	4.805501	5.440752	0	24	48	72
2		1.16	2.24	10.75	33.17	0.02104	1.68		98%	98%					4.064458	4.350248	5.031408	5.520745	0	24	48	72
3		1.16	2.67	10.86	39.04	0.02163	1.72		99%	101%					4.064458	4.426511	5.03583	5.59151	0	24	48	72
1	12.5%	1.16	2.62	10.05	35.84	0.02106	1.68	1.61	99%	98%	95%	8%	7%	4%	4.064458	4.418301	5.002166	5.554368	0	24	48	72
2		1.16	2.74	9.68	37.99	0.02122	1.69		99%	99%					4.064458	4.437751	4.985875	5.579669	0	24	48	72
3		1.16	2.35	6.91	24.5	0.01851	1.47		98%	86%					4.064458	4.371068	4.839478	5.389166	0	24	48	72
1	25%	1.16	2.46	6.44	14.5	0.01545	1.23	1.38	100%	72%	81%	9%	7%	4%	4.064458	4.390935	4.808886	5.161368	0	24	48	72
2		1.16	2.71	8.6	23.24	0.01836	1.46		100%	86%					4.064458	4.432969	4.934498	5.366236	0	24	48	72
3		1.16	2.48	7.75	21.71	0.01796	1.43		99%	84%					4.064458	4.394452	4.889302	5.33666	0	24	48	72
1	50%	1.16	1.83	4.39	11.99	0.01426	1.14	1.16	98%	67%	68%	4%	3%	1%	4.064458	4.262451	4.642465	5.078819	0	24	48	72
2		1.16	2.2	4.2	15.45	0.01523	1.21		96%	71%					4.064458	4.342423	4.623249	5.188928	0	24	48	72
3		1.16	2.33	4.17	13.3	0.01430	1.14		98%	67%					4.064458	4.367356	4.620136	5.123852	0	24	48	72
1	75%	1.16	1.8	3.71	12.08	0.01403	1.12	1.09	96%	65%	64%	8%	5%	3%	4.064458	4.255273	4.569374	5.082067	0	24	48	72
2		1.16	1.83	3.27	9.47	0.01245	0.99		96%	58%					4.064458	4.262451	4.514548	4.97635	0	24	48	72
3		1.16	1.77	4.12	12.94	0.01462	1.17		96%	68%					4.064458	4.247973	4.614897	5.111934	0	24	48	72
1	100%	1.16	1.64	3.94	8.43	0.01235	0.98	0.93	97%	58%	54%	8%	4%	2%	4.064458	4.214844	4.595496	4.925828	0	24	48	72
2		1.16	1.53	3.47	6.31	0.01068	0.85		97%	50%					4.064458	4.184691	4.540329	4.800029	0	24	48	72
3		1.16	1.53	3.65	7.85	0.01195	0.95		96%	56%					4.064458	4.184691	4.562293	4.89487	0	24	48	72
treated storm water																						
1	6.25%	1.16	2.68	9.66	31.55	0.02025	1.61	1.82	99%	94%	107%	10%	11%	6%	4.064458	4.428135	4.984977	5.498999	0	24	48	72
2		1.16	2.66	13.37	61.82	0.02451	1.95		98%	114%					4.064458	4.424882	5.126131	5.791129	0	24	48	72
3		1.16	2.76	12.57	56.28	0.02382	1.90		99%	111%					4.064458	4.440909	5.099335	5.750354	0	24	48	72
1	12.5%	1.16	2.88	15.03	63.68	0.02473	1.97	1.85	99%	115%	109%	5%	6%	3%	4.064458	4.459392	5.176959	5.804003	0	24	48	72
2		1.16	2.54	9.79	47.04	0.02254	1.80		98%	105%					4.064458	4.404834	4.990783	5.672467	0	24	48	72
3		1.16	2.72	12.03	44.95	0.02254	1.80		99%	105%					4.064458	4.434569	5.080266	5.65273	0	24	48	72
1	25%	1.16	3.77	17.37	64.72	0.02460	1.96	1.88	100%	115%	110%	9%	9%	5%	4.064458	4.576341	5.2398	5.811039	0	24	48	72
2		1.16	3.23	14.7	68.2	0.02486	1.98		99%	116%					4.064458	4.509203	5.167317	5.833784	0	24	48	72
3		1.16	3.19	9.37	40.57	0.02125	1.69		99%	99%					4.064458	4.503791	4.97174	5.608205	0	24	48	72
1	50%	1.16	2.42	8.67	26.31	0.01925	1.53	1.64	99%	90%	96%	6%	5%	3%	4.064458	4.383815	4.938019	5.420121	0	24	48	72
2		1.16	2.64	10.46	36.59	0.02123	1.69		99%	99%					4.064458	4.421604	5.019532	5.563362	0	24	48	72
3		1.16	2.9	11.3	37.59	0.02134	1.70		99%	100%					4.064458	4.462398	5.053078	5.575072	0	24	48	72
1	75%	1.16	2.22	6.68	34.9	0.02047	1.63	1.64	96%	96%	96%	7%	7%	4%	4.064458	4.346353	4.824776	5.542825	0	24	48	72
2		1.16	2.57	7.36	27.79	0.01915	1.53		99%	89%					4.064458	4.409933	4.866878	5.443889	0	24	48	72
3		1.16	2.59	11.03	40.81	0.02195	1.75		99%	102%					4.064458	4.4133	5.042576	5.610767	0	24	48	72
1	100%	1.16	2.31	6.69	26.07	0.01882	1.50	1.54	98%	88%	90%	4%	4%	2%	4.064458	4.363612	4.825426	5.416141	0	24	48	72
2		1.16	2.09	5.49	35.57	0.02033	1.62		94%	95%					4.064458	4.320146	4.739572	5.551084	0	24	48	72
3		1.16	2.47	6.1	28.01	0.01892	1.51		97%	88%					4.064458	4.392697	4.78533	5.447313	0	24	48	72

(F)

Event B6 72-h Chronic Toxicity of Untreted and Treated stormwater (Johnston St bioretention basin to *Pseudokirchneriella subcapitata*)

	Sample	Day 0	Day 1	Day 2	Day 3	Slope	Growth Rate (dbIngs/day)	Mean	Pearson	% Control	Mean %	CV (%)			Log of Growth Rates				Below values for formula reference			
Vial No.		*All cell counts in (cells/mL) by x10^4*													Day 0	Day 1	Day 2	Day 3				
1	Control	1.17	4.38	26.72	69.56	0.02545	2.03	2.19	99%	93%	100%	8%			4.068186	4.641474	5.426836	5.84236	0	24	48	72
2		1.17	12.39	49.77	180.9	0.02988	2.38		98%	109%					4.068186	5.093071	5.696968	6.257439	0	24	48	72
3		1.17	6.07	27.7	104.6	0.02714	2.16		100%	99%					4.068186	4.783189	5.44248	6.019532	0	24	48	72
				Mean control rate =		***0.02749***																
Untreated storm water													STDEV	SE								
1	6.25%	1.17	4.88	22.29	92.22	0.02646	2.11	2.04	100%	96%	93%	3%	3%	2%	4.068186	4.68842	5.34811	5.964825	0	24	48	72
2		1.17	4.86	22.1	67.15	0.02473	1.97		100%	90%					4.068186	4.686636	5.344392	5.827046	0	24	48	72
3		1.17	6.22	24.43	85.87	0.02580	2.06		100%	94%					4.068186	4.79379	5.387923	5.933841	0	24	48	72
1	12.5%	1.17	4.72	23.23	113.62	0.02772	2.21	2.15	100%	101%	98%	3%	3%	2%	4.068186	4.673942	5.366049	6.055455	0	24	48	72
2		1.17	5.69	28.72	101.99	0.02718	2.17		100%	99%					4.068186	4.755112	5.458184	6.008558	0	24	48	72
3		1.17	4.49	19.86	87.34	0.02610	2.08		100%	95%					4.068186	4.652246	5.297979	5.941213	0	24	48	72
1	25%	1.17	5.24	23.94	94.29	0.02658	2.12	2.11	100%	97%	97%	1%	1%	1%	4.068186	4.719331	5.379124	5.974466	0	24	48	72
2		1.17	5.74	21	96.07	0.02628	2.09		100%	96%					4.068186	4.758912	5.322219	5.982588	0	24	48	72
3		1.17	5.33	23.95	98.31	0.02677	2.13		100%	97%					4.068186	4.726727	5.379306	5.992598	0	24	48	72
1	50%	1.17	5.47	15.72	50.52	0.02235	1.78	1.86	99%	81%	85%	4%	4%	2%	4.068186	4.737987	5.196453	5.703463	0	24	48	72
2		1.17	5.07	16.12	56.36	0.02313	1.84		100%	84%					4.068186	4.705008	5.207365	5.750971	0	24	48	72
3		1.17	4.79	18.23	67.1	0.02440	1.94		100%	89%					4.068186	4.680336	5.260787	5.826723	0	24	48	72
1	75%	1.17	4.17	12.41	53.64	0.02274	1.81	1.93	100%	83%	88%	6%	5%	3%	4.068186	4.620136	5.093772	5.729489	0	24	48	72
2		1.17	5.21	19.1	82.23	0.02544	2.03		100%	93%					4.068186	4.716838	5.281033	5.91503	0	24	48	72
3		1.17	4.69	20.13	64.38	0.02439	1.94		100%	89%					4.068186	4.671173	5.303844	5.808751	0	24	48	72
1	100%	1.17	4.25	11.94	37.7	0.02072	1.65	1.75	100%	75%	80%	5%	4%	3%	4.068186	4.628389	5.077004	5.576341	0	24	48	72
2		1.17	4.93	16.68	55.01	0.02311	1.84		100%	84%					4.068186	4.692847	5.222196	5.740442	0	24	48	72
3		1.17	4.5	14.77	46.31	0.02212	1.76		100%	80%					4.068186	4.653213	5.16938	5.665675	0	24	48	72
treated storm water																						
1	6.25%	1.17	5.93	26.4	92.8	0.02644	2.11	2.11	100%	96%	97%	4%	4%	2%	4.068186	4.773055	5.421604	5.967548	0	24	48	72
2		1.17	4.68	22.96	76.29	0.02556	2.04		100%	93%					4.068186	4.670246	5.360972	5.882468	0	24	48	72
3		1.17	5.36	29.16	107.13	0.02759	2.20		100%	100%					4.068186	4.729165	5.464788	6.029911	0	24	48	72
1	12.5%	1.17	5.45	26.54	96.42	0.02681	2.14	2.18	100%	98%	100%	2%	2%	1%	4.068186	4.736397	5.423901	5.984167	0	24	48	72
2		1.17	5.34	27.49	115.38	0.02789	2.22		100%	101%					4.068186	4.727541	5.439175	6.062131	0	24	48	72
3		1.17	5.5	23.71	114.86	0.02754	2.19		100%	100%					4.068186	4.740363	5.374932	6.060169	0	24	48	72
1	25%	1.17	4.25	27.69	123.75	0.02870	2.29	2.16	100%	104%	99%	7%	7%	4%	4.068186	4.628389	5.442323	6.092545	0	24	48	72
2		1.17	5.58	28.19	110.26	0.02761	2.20		100%	100%					4.068186	4.746634	5.450095	6.042418	0	24	48	72
3		1.17	4.85	15.77	78.7	0.02498	1.99		100%	91%					4.068186	4.685742	5.197832	5.895975	0	24	48	72
1	50%	1.17	4.53	26.77	94.26	0.02704	2.15	2.00	100%	98%	92%	9%	8%	5%	4.068186	4.656098	5.427648	5.974327	0	24	48	72
2		1.17	4.44	25.17	74.22	0.02567	2.05		99%	93%					4.068186	4.647383	5.400883	5.870521	0	24	48	72
3		1.17	2.94	12.52	47.92	0.02278	1.81		99%	83%					4.068186	4.468347	5.097604	5.680517	0	24	48	72
1	75%	1.17	5.27	30.15	109.74	0.02781	2.22	2.16	100%	101%	99%	3%	3%	2%	4.068186	4.721811	5.479287	6.040365	0	24	48	72
2		1.17	3.87	24.73	101.93	0.02761	2.20		99%	100%					4.068186	4.587711	5.393224	6.008302	0	24	48	72
3		1.17	4.43	21.16	84.63	0.02607	2.08		100%	95%					4.068186	4.646404	5.325516	5.927524	0	24	48	72
1	100%	1.17	3.63	20.67	75.32	0.02576	2.05	2.01	99%	94%	92%	2%	2%	1%	4.068186	4.559907	5.31534	5.87691	0	24	48	72
2		1.17	3.93	22.58	68.34	0.02524	2.01		99%	92%					4.068186	4.594393	5.353724	5.834675	0	24	48	72
3		1.17	3.62	19.62	63	0.02470	1.97		99%	90%					4.068186	4.558709	5.292699	5.799341	0	24	48	72

Table A3.4: Raw data for *Ceriodaphnia dubia* toxicity tests for storm events (A) B1, (B) B2, (C) B3, (D) B4, (E) B5 and (F) B6. Quality assurance criteria - toxicity test results were considered acceptable if at least 90% survival was observed for the controls.

(A)

Event B1 ***Ceriodaphnia dubia* 48-h Immobility test - Untreated and Treated Stormwater (Johnston St Bioretention)**

		Number of mobile organisms											
		Time			Mean		Prop'n mob		Mean % Control		Mean Mobile (%)		% Control	
Rep		0 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 hr	48 hr
1		5	5	5			1.00	1.00					100%	100%
2	DMW	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3	Control	5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
	Mean Control + MB % Mobile =						***100%***	***100%***						
Untreated Stormwater														
1		5	5	5			1.00	1.00					100%	100%
2	6.25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	12.5%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	50%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	75%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	100%	5	5	5	5.00	5.00	1.00	1.00	100%	**100%**	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
Treated Stormwater														
1		5	5	5			1.00	1.00					100%	100%
2	6.25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	12.5%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	50%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	75%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	100%	5	5	5	5.00	5.00	1.00	1.00	100%	**100%**	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%

(B)

Event B2 ***Ceriodaphnia dubia* 48-h Immobility test - Untreated and Treated Stormwater (Johnston St Bioretention)**

		Number of mobile organisms											
		Time			Mean		Prop'n mob		Mean % Control		Mean Mobile (%)		% Control	
Rep		0 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 hr	48 hr
1		5	5	5			1.00	1.00					100%	100%
2	DMW	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3	Control	5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
		Mean Control + MB % Mobile =					***100%***	***100%***						
Untreated Stormwater														
1		5	5	5			1.00	1.00					100%	100%
2	6.25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	12.5%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	50%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	75%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	100%	5	5	5	5.00	5.00	1.00	1.00	100%	**100%**	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
Treated Stormwater														
1		5	5	5			1.00	1.00					100%	100%
2	6.25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	12.5%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	50%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	75%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	4	4			0.80	0.80					80%	80%
2	100%	5	5	5	4.75	4.75	1.00	1.00	95%	**95%**	95%	***95%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%

(C)

Event B3 ***Ceriodaphnia dubia* 48-h Immobility test - Untreated and Treated Stormwater (Johnston St Bioretention)**

		Number of mobile organisms											
		Time			Mean		Prop'n mob		Mean % Control		Mean Mobile (%)		% Control	
Rep		0 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 hr	48 hr
1		5	5	5			1.00	1.00					100%	100%
2	DMW	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3	Control	5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
		Mean Control + MB % Mobile =					***100%***	***100%***						
Untreated Stormwater														
1		5	5	5			1.00	1.00					100%	100%
2	6.25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	12.5%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	50%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	75%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	4			1.00	0.80					100%	80%
2	100%	5	5	5	5.00	4.25	1.00	1.00	100%	**85%**	100%	***85%***	100%	100%
3		5	5	4			1.00	0.80					100%	80%
4		5	5	4			1.00	0.80					100%	80%
Treated Stormwater														
1		5	5	5			1.00	1.00					100%	100%
2	6.25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	12.5%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	50%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	75%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	100%	5	5	5	5.00	5.00	1.00	1.00	100%	**100%**	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%

(D)

Event B4 ***Ceriodaphnia dubia*** **48-h Immobility test - Untreated and Treated Stormwater (Johnston St Bioretention)**

		Number of mobile organisms											
		Time			Mean		Prop'n mob		Mean % Control		Mean Mobile (%)		% Control
Rep		0 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 hr / 48 hr
1	DMW Control	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100% / 100%
2		5	5	5			1.00	1.00					100% / 100%
3		5	5	5			1.00	1.00					100% / 100%
4		5	5	5			1.00	1.00					100% / 100%
		Mean Control + MB % Mobile =					***100%***	***100%***					
Untreated Stormwater													
1	6.25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100% / 100%
2		5	5	5			1.00	1.00					100% / 100%
3		5	5	5			1.00	1.00					100% / 100%
4		5	5	5			1.00	1.00					100% / 100%
1	12.5%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100% / 100%
2		5	5	5			1.00	1.00					100% / 100%
3		5	5	5			1.00	1.00					100% / 100%
4		5	5	5			1.00	1.00					100% / 100%
1	25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100% / 100%
2		5	5	5			1.00	1.00					100% / 100%
3		5	5	5			1.00	1.00					100% / 100%
4		5	5	5			1.00	1.00					100% / 100%
1	50%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100% / 100%
2		5	5	5			1.00	1.00					100% / 100%
3		5	5	5			1.00	1.00					100% / 100%
4		5	5	5			1.00	1.00					100% / 100%
1	75%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100% / 100%
2		5	5	5			1.00	1.00					100% / 100%
3		5	5	5			1.00	1.00					100% / 100%
4		5	5	5			1.00	1.00					100% / 100%
1	100%	5	5	5	5.00	5.00	1.00	1.00	100%	**100%**	100%	***100%***	100% / 100%
2		5	5	5			1.00	1.00					100% / 100%
3		5	5	5			1.00	1.00					100% / 100%
4		5	5	5			1.00	1.00					100% / 100%
Treated Stormwater													
1	6.25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100% / 100%
2		5	5	5			1.00	1.00					100% / 100%
3		5	5	5			1.00	1.00					100% / 100%
4		5	5	5			1.00	1.00					100% / 100%
1	12.5%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100% / 100%
2		5	5	5			1.00	1.00					100% / 100%
3		5	5	5			1.00	1.00					100% / 100%
4		5	5	5			1.00	1.00					100% / 100%
1	25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100% / 100%
2		5	5	5			1.00	1.00					100% / 100%
3		5	5	5			1.00	1.00					100% / 100%
4		5	5	5			1.00	1.00					100% / 100%
1	50%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100% / 100%
2		5	5	5			1.00	1.00					100% / 100%
3		5	5	5			1.00	1.00					100% / 100%
4		5	5	5			1.00	1.00					100% / 100%
1	75%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100% / 100%
2		5	5	5			1.00	1.00					100% / 100%
3		5	5	5			1.00	1.00					100% / 100%
4		5	5	5			1.00	1.00					100% / 100%
1	100%	5	5	5	5.00	5.00	1.00	1.00	100%	**100%**	100%	***100%***	100% / 100%
2		5	5	5			1.00	1.00					100% / 100%
3		5	5	5			1.00	1.00					100% / 100%
4		5	5	5			1.00	1.00					100% / 100%

(E)

Event B5 ***Ceriodaphnia dubia* 48-h Immobility test - Untreated and Treated Stormwater (Johnston St Bioretention)**

		Number of mobile organisms											
		Time			Mean		Prop'n mob		Mean % Control		Mean Mobile (%)		% Control	
Rep		0 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 hr	48 hr
1		5	5	5			1.00	1.00					100%	100%
2	DMW	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3	Control	5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
				Mean Control + MB % Mobile =			***100%***	***100%***						
Untreated Stormwater														
1		5	5	2			1.00	0.40					100%	40%
2	6.25%	5	5	3	5.00	2.75	1.00	0.60	100%	***55%***	100%	***55%***	100%	60%
3		5	5	3			1.00	0.60					100%	60%
4		5	5	3			1.00	0.60					100%	60%
1		5	5	2			1.00	0.40					100%	40%
2	12.5%	5	5	2	5.00	2.00	1.00	0.40	100%	***40%***	100%	***40%***	100%	40%
3		5	5	2			1.00	0.40					100%	40%
4		5	5	2			1.00	0.40					100%	40%
1		5	5	0			1.00	0.00					100%	0%
2	25%	5	5	0	5.00	0.50	1.00	0.00	100%	***10%***	100%	***10%***	100%	0%
3		5	5	2			1.00	0.40					100%	40%
4		5	5	0			1.00	0.00					100%	0%
1		5	5	0			1.00	0.00					100%	0%
2	50%	5	5	0	5.00	0.50	1.00	0.00	100%	***10%***	100%	***10%***	100%	0%
3		5	5	2			1.00	0.40					100%	40%
4		5	5	0			1.00	0.00					100%	0%
1		5	5	0			1.00	0.00					100%	0%
2	75%	5	5	1	5.00	0.25	1.00	0.20	100%	***5%***	100%	***5%***	100%	20%
3		5	5	0			1.00	0.00					100%	0%
4		5	5	0			1.00	0.00					100%	0%
1		5	5	0			1.00	0.00					100%	0%
2	100%	5	5	0	5.00	0.00	1.00	0.00	100%	**0%**	100%	***0%***	100%	0%
3		5	5	0			1.00	0.00					100%	0%
4		5	5	0			1.00	0.00					100%	0%
Treated Stormwater														
1		5	5	5			1.00	1.00					100%	100%
2	6.25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	12.5%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	50%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	75%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	100%	5	5	5	5.00	5.00	1.00	1.00	100%	**100%**	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%

(F)

Event B6 ***Ceriodaphnia dubia* 48-h Immobility test - Untreated and Treated Stormwater (Johnston St Bioretention)**

		Number of mobile organisms											
		Time			Mean		Prop'n mob		Mean % Control		Mean Mobile (%)		% Control	
Rep		0 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 hr	48 hr
1		5	5	5			1.00	1.00					100%	100%
2	DMW	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3	Control	5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
		Mean Control + MB % Mobile =					***100%***	***100%***						
Untreated Stormwater														
1		5	5	5			1.00	1.00					100%	100%
2	6.25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	12.5%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	50%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	75%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	100%	5	5	5	5.00	5.00	1.00	1.00	100%	**100%**	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
Treated Stormwater														
1		5	5	5			1.00	1.00					100%	100%
2	6.25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	12.5%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	25%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	50%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	75%	5	5	5	5.00	5.00	1.00	1.00	100%	***100%***	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%
1		5	5	5			1.00	1.00					100%	100%
2	100%	5	5	5	5.00	5.00	1.00	1.00	100%	**100%**	100%	***100%***	100%	100%
3		5	5	5			1.00	1.00					100%	100%
4		5	5	5			1.00	1.00					100%	100%

Table A3.5 Raw data for hatching success of *Melanotaenia duboulayi* eggs exposed to untreated and treated stormwater from events B2-B6. For event B4, 8 eggs were randomly allocated to each beaker instead of 10 eggs due to insufficient numbers being available. Quality assurance criteria - test results were considered valid if ≥ 70% hatching success was observed overall for the controls.

Event	Replicate	Treatment	Eggs hatched	Hatching success (%)	Mean hatching success (%)
B2	1	Fish Culturing Water (Control)	10	100	100
	2		10	100	
	3		10	100	
	1	100% Untreated	10	100	87
	2		9	90	
	3		7	70	
	1	100% Treated	10	100	97
	2		9	90	
	3		10	100	
B3	1	Fish Culturing Water (Control)	10	100	100
	2		10	100	
	3		10	100	
	1	100% Untreated	10	100	100
	2		10	100	
	3		10	100	
	1	100% Treated	10	100	100
	2		10	100	
	3		10	100	
B4	1	Fish Culturing Water (Control)	8	80	80
	2		8	80	
	3		8	80	
	1	100% Untreated	4	50	50
	2		3	37.5	
	3		5	62.5	
	1	100% Treated	4	50	58
	2		5	62.5	
	3		5	62.5	
B5	1	Fish Culturing Water (Control)	9	90	90
	2		8	80	
	3		10	100	
	1	100% Untreated	5	50	53
	2		5	50	
	3		6	60	
	1	100% Treated	10	100	77
	2		6	60	
	3		7	70	
B6	1	Fish Culturing Water (Control)	10	100	87
	2		8	80	
	3		8	80	
	1	100% Untreated	5	50	73
	2		8	80	
	3		9	90	
	1	100% Treated	9	90	83
	2		7	70	
	3		9	90	

Table A3.6 Quality assurance analysis for a) *Pseudokirchneriella subcapitata* (IC_{50} of Cu reference toxicant) and b) *Ceriodaphnia dubia* (EC_{50} of Cu reference toxicant) toxicity tests. Quality assurance criteria - 72 h IC_{50} of the Cu reference toxicant for *P. subcapitata* had to fall within 14.9 ± 5.6 µg $L^{-}1$. 48 h EC_{50} of the Cu reference toxicant for *C. dubia* had to fall within 7.5 ± 3.5 µg L^{-1}.

a) *Pseudokirchneriella subcapitata*	72 h EC_{50} (µg L^{-1})
Event	
B1	12.8
B2	11.9
B3	11.3
B4	9.5
B5	10.1
B6	10.5

b) *Ceriodaphnia dubia*	48 h IC_{50} (µg L^{-1})
Event	
B1	4.5
B2	5.3
B3	5.2
B4	5.2
B5	5.3
B6	5.0

Table A3.7: ToxCalc output for 48-h *Ceriodaphnia dubia* survival following exposure to untreated stormwater from event B5.

Ceriodaphnia Dubia 48 Hr Survival

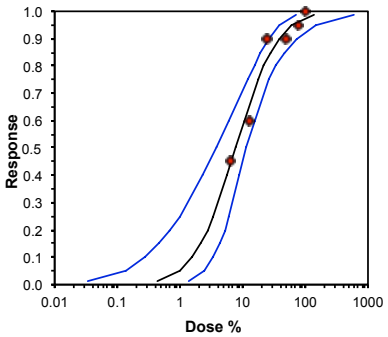
Conc-%	1	2	3	4
Control	1.0000	1.0000	1.0000	1.0000
6.25	0.4000	0.6000	0.6000	0.6000
12.5	0.4000	0.4000	0.4000	0.4000
25	0.0000	0.0000	0.4000	0.0000
50	0.0000	0.0000	0.4000	0.0000
75	0.0000	0.2000	0.0000	0.0000
100	0.0000	0.0000	0.0000	0.0000

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	<6.3	6.3		

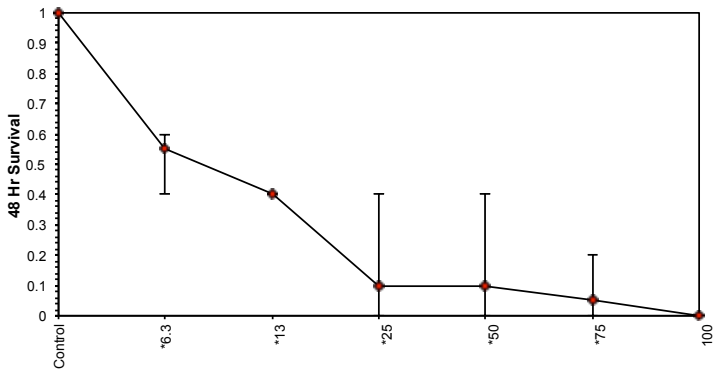
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	1.86436	0.38603	1.10774	2.62098	0	2.03187	9.48773	0.73	0.89178	0.53638	3
Intercept	3.3374	0.508	2.34173	4.33307							
TSCR											

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	0.44053	0.03335	1.34416
EC05	3.355	1.02216	0.13585	2.47589
EC10	3.718	1.60098	0.2863	3.43992
EC15	3.964	2.16701	0.47244	4.3035
EC20	4.158	2.75647	0.70219	5.1511
EC25	4.326	3.38843	0.98485	6.02044
EC40	4.747	5.70021	2.28367	9.02048
EC50	5.000	7.79436	3.73547	11.665
EC60	5.253	10.6579	5.98836	15.3919
EC75	5.674	17.9292	12.0475	26.5806
EC80	5.842	22.0398	15.2506	34.4205
EC85	6.036	28.035	19.5036	47.883
EC90	6.282	37.9468	25.7258	74.9422
EC95	6.645	59.4351	37.3079	151.313
EC99	7.326	137.905	71.0972	595.73



Dose-Response Plot



Appendix 4: Supporting information to Chapter 4

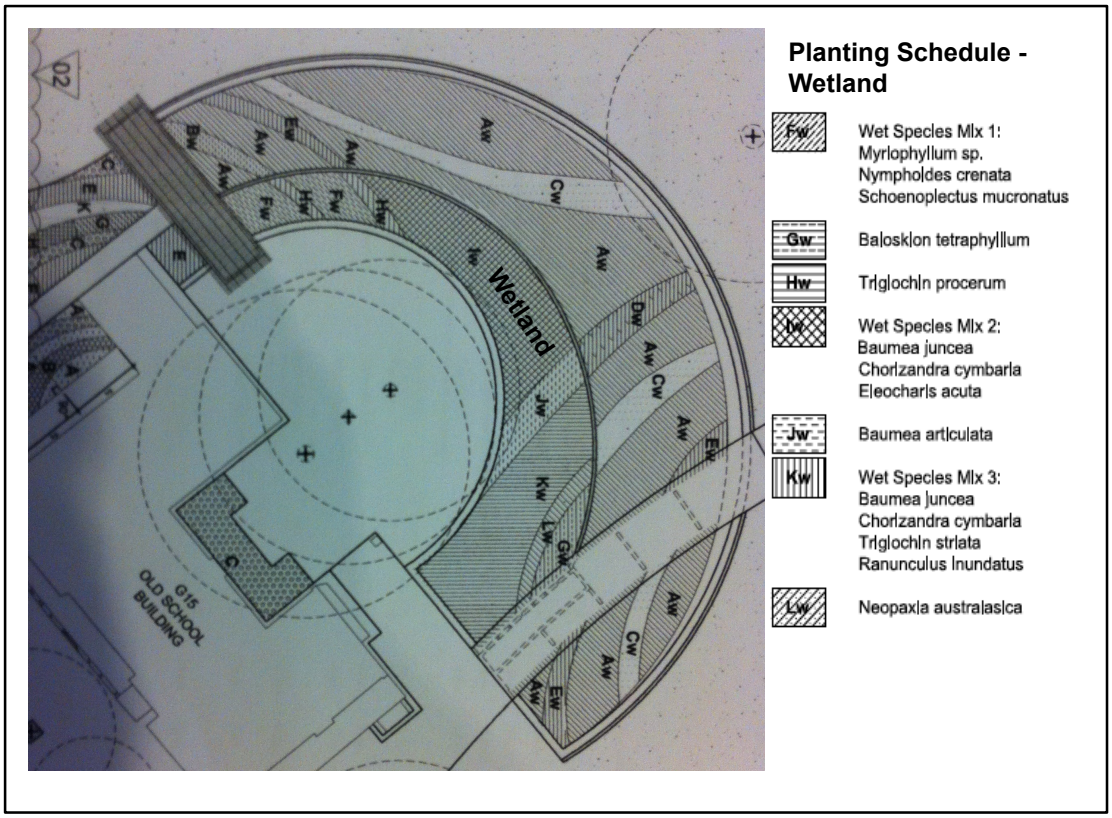


Figure A4.1: Planting plan at Gadigal Green Wetland. Adapted from Taylor Thomson Whitting (2006).

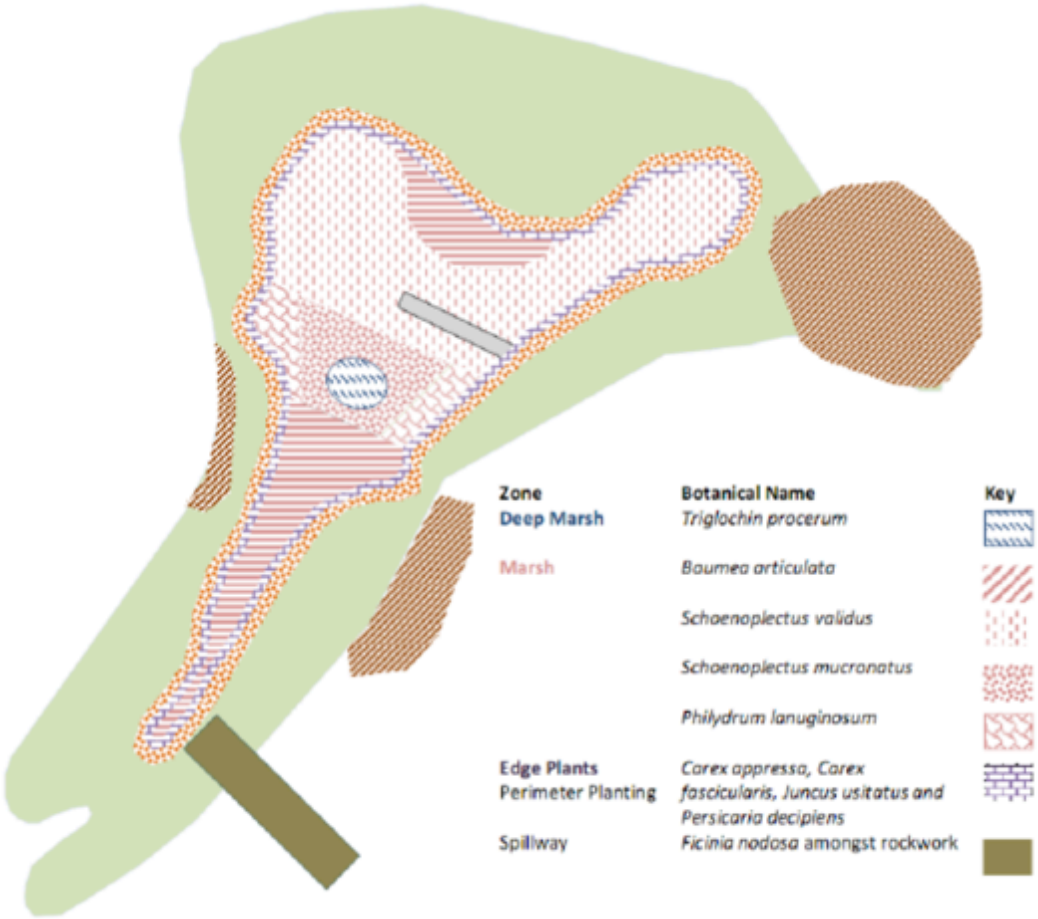


Figure A4.2: Planting plan at Coolibah wetland **(**Dragonfly Environmental, 2009).

Table A4.1: Summary of laboratory quality control results for analytical work (data compiled from ALS Environmental reports for this study). SW = Surface Water; S = Sediment.

Parameter	LOR (SW = mg L^{-1}, S = mg/kg)	Method Blank (SW = mg L^{-1}, S = mg/kg)	Laboratory duplicate RPD (%)	Recoveries (%)		
				LCS	Acceptable recovery for LCS	Matrix spike
TN SW	0.1	nd	nd	nd	nd	nd
TP SW	0.01	<0.01	0-28	90 - 96	67-109	86 - 126
Cu SW	0.001	<0.001	0	91-103	87-111	91 - 116
Pb SW	0.001	<0.001	0	99-103	90-110	85 - 117
Zn SW	0.005	<0.005	0 -13	86-103	85-115	88.8 113
Cu S	5	<5	0- 21	93-98	90-114	97 - 102
Pb S	5	<5	0-2.3	93-102	85-111	90-100
Zn S	5	<5	0	98-99	89 - 112	89 -94

Legend:

RPD = Relative Percentage Difference

LOR = Level of reporting

LCS = Laboratory Control Spike

nd = not determined

Acceptable recovery on matrix spikes is 70-130%

Acceptable recovery on laboratory control spikes is listed in the table

Acceptable RPDs on duplicates is: no limit at concentrations <10 times LOR

Table A4.2: Raw data for *Paratya australiensis* toxicity tests at (A) Yarrowee Wetland (B) Gadigal Green Wetland and (C) Coolibah Wetland.

(A)

Inlet	0 hrs		24 hrs		48 hrs		72		96 hrs	
	Shrimp	%	Shrimp	%	Shrimp	%	Shrimp	%	Shrimp	%
Test 1	5	100	5	100	1	20	1	20	1	20
	5	100	5	100	0	0	0	0	0	0
	5	100	5	100	0	0	0	0	0	0
Test 2	5	100	5	100	5	100	2	40	0	0
	5	100	5	100	5	100	4	80	1	20
	5	100	5	100	5	100	3	60	0	0
Test 3	5	100	5	100	4	80	3	60	0	0
	5	100	5	100	4	80	2	40	2	40
	5	100	5	100	4	80	4	80	2	40
Test 4	5	100	3	60	3	60	3	60	1	20
	5	100	5	100	3	60	3	60	1	20
	5	100	5	100	5	100	4	80	2	40
Test 5	5	100	0	0	0	0	0	0	0	0
	5	100	0	0	0	0	0	0	0	0
	5	100	1	20	1	20	0	0	0	0
Test 6	5	100	0	0	0	0	0	0	0	0
	5	100	0	0	0	0	0	0	0	0
	5	100	1	20	0	0	0	0	0	0
Outlet	**0 hrs**		**24 hrs**		**48 hrs**		**72**		**96 hrs**	
	Shrimp	%	Shrimp	%	Shrimp	%	Shrimp	%	Shrimp	%
Test 1	5	100	5	100	5	100	5	100	5	100
	5	100	5	100	5	100	5	100	5	100
	5	100	5	100	5	100	5	100	5	100
Test 2	5	100	5	100	5	100	5	100	5	100
	5	100	5	100	5	100	5	100	5	100
	5	100	5	100	5	100	5	100	5	100
Test 3	5	100	4	80	4	80	4	80	4	80
	5	100	5	100	5	100	5	100	5	100
	5	100	5	100	5	100	5	100	5	100
Test 4	5	100	5	100	5	100	5	100	5	100
	5	100	5	100	5	100	5	100	5	100
	5	100	5	100	5	100	5	100	5	100
Test 5	5	100	5	100	5	100	5	100	5	100
	5	100	5	100	5	100	5	100	5	100
	5	100	4	80	4	80	4	80	4	80
Test 6	5	100	5	100	5	100	5	100	5	100
	5	100	5	100	5	100	5	100	5	100
	5	100	5	100	5	100	5	100	5	100

(B)

Inlet	0 hrs		24 hrs		48 hrs		72		96 hrs	
	Shrimp	%	Shrimp	%	Shrimp	%	Shrimp	%	Shrimp	%
Test 1	5	100	1	20	0	0	0	0	0	0
	5	100	1	20	1	20	1	20	0	0
	5	100	1	20	0	0	0	0	0	0
Test 2	5	100	5	100	5	100	5	100	5	100
	5	100	3	60	3	60	1	20	0	0
	5	100	3	60	3	60	3	60	3	60
Test 3	5	100	1	20	1	20	1	20	1	20
	5	100	1	20	0	0	0	0	0	0
	5	100	0	0	0	0	0	0	0	0
Outlet	**0 hrs**		**24 hrs**		**48 hrs**		**72**		**96 hrs**	
	Shrimp	%	Shrimp	%	Shrimp	%	Shrimp	%	Shrimp	%
Test 1	5	100	2	40	2	40	1	20	1	20
	5	100	3	60	2	40	2	40	2	40
	5	100	0	0	0	0	0	0	0	0
Test 2	5	100	1	20	0	0	0	0	0	0
	5	100	1	20	0	0	0	0	0	0
	5	100	5	100	5	100	4	80	2	40
Test 3	5	100	1	20	0	0	0	0	0	0
	5	100	1	20	0	0	0	0	0	0
	5	100	0	0	0	0	0	0	0	0

(C)

Inlet	0 hrs		24 hrs		48 hrs		72		96 hrs	
	Shrimp	%	Shrimp	%	Shrimp	%	Shrimp	%	Shrimp	%
Test 1	5	100	0	0	0	0	0	0	0	0
	5	100	2	40	2	40	0	0	0	0
	5	100	2	40	2	40	0	0	0	0
Test 2	5	100	3	60	3	60	3	60	3	60
	5	100	4	80	3	60	3	60	3	60
	5	100	4	80	4	80	3	60	3	60
Test 3	5	100	0	0	0	0	0	0	0	0
	5	100	0	0	0	0	0	0	0	0
	5	100	1	20	1	20	0	0	0	0
Test 4	5	100	1	20	0	0	0	0	0	0
	5	100	0	0	0	0	0	0	0	0
	5	100	0	0	0	0	0	0	0	0
Outlet	**0 hrs**		**24 hrs**		**48 hrs**		**72**		**96 hrs**	
	Shrimp	%	Shrimp	%	Shrimp	%	Shrimp	%	Shrimp	%
Test 1	5	100	5	100	5	100	5	100	5	100
	5	100	5	100	5	100	5	100	5	100
	5	100	5	100	5	100	5	100	4	80
Test 2	5	100	5	80	4	80	4	80	4	80
	5	100	5	100	5	100	5	100	5	100
	5	100	5	100	5	100	5	80	4	80
Test 3	5	100	5	100	5	100	5	100	5	100
	5	100	5	100	5	100	5	100	4	80
	5	100	5	100	5	100	5	100	4	80
Test 4	5	100	3	60	2	40	1	20	1	20
	5	100	2	40	1	20	1	20	1	20
	5	100	2	40	1	20	0	0	0	0

Table A4.3: Macroinvertebrate Taxa recorded at Yarowee Wetland

	Inlet Zone		Outlet Zone	
Taxa	**Total count**	**% of total abundance**	**Total count**	**% of total abundance**
Oligochaeta	433	27.15	6	0.47
Turbellaria	61	3.82	0	0.00
Collembola	9	0.56	40	3.13
Gastropoda/Planorbidae	20	1.25	0	0.00
Gastropoda/Lymnaeidae	0	0.00	18	1.41
Gastropoda/Physidae	28	1.76	9	0.70
Hirudinea/Glossiphoniidae	99	6.21	16	1.25
Diptera/Culicidae	719	45.08	4	0.31
Diptera/Chironomidae/Chironominae	189	11.85	82	6.41
Diptera/Chironomidae/Orthocladiinae	0	0.00	8	0.63
Diptera/Chironomidae/Tanypodinae	0	0.00	43	3.36
Diptera/Ceratopognidae	0	0.00	1	0.08
Odonata/Libellulidae	3	0.19	126	9.85
Odonata/Aeshnidae	0	0.00	17	1.33
Odonata/Protoneuridae	0	0.00	437	34.17
Odanata/Coenagrionidae	0	0.00	60	4.69
Hemiptera/Notonectidae	1	0.06	183	14.31
Hempitera/Corixidae	25	1.57	184	14.39
Coleoptera/Hydrophilidae	1	0.06	9	0.70
Coleoptera/Dytiscisdae	4	0.25	1	0.08
Coleoptera/Hydraenidae	1	0.06	0	0.00
Coleoptera/Hydrochidae	2	0.13	0	0.00
Ephemeroptera/Baetidae	0	0.00	26	2.03
Trichoptera/Leptoceridae	0	0.00	2	0.16
Acarina/Tetragnathidae	0	0.00	7	0.55

Table A4.4: Macroinvertebrate Taxa recorded at Gadigal Green Wetland

	Inlet Zone		Outlet Zone	
Taxa	**Total count**	**% of total abundance**	**Total count**	**% of total abundance**
Oligochaeta	144	30.84	0	0
Colembola	1	0.21	0	0
Isopoda	1	0.21	0	0
Hirudinea/Glossiphoniidae	0	0	4	2.05
Diptera/Culicidae	110	23.55	2	1.03
Diptera/Chironomidae/Chironominae	93	19.91	7	3.59
Diptera/Stratiomyidae	0	0	1	0.51
Diptera/Ceratopognidae	1	0.21	0	0
Diptera/Sciomyzidae	0	0	1	0.51
Odonata/Libellulidae	11	2.36	59	30.26
Odonata/Aeshnidae	30	6.42	10	5.13
Odonata/Protoneuridae	9	1.93	12	6.15
Odonata/Coenagrionidae	42	8.99	58	29.74
Hemiptera/Notonectidae	13	2.78	4	2.05
Hempitera/Naucoridae	0	0	2	1.03
Hemiptera/Veliidae	0	0	20	10.26
Hemiptera/MesoVellidae	0	0	1	0.51
Coleoptera/Dytiscisdae	8	1.71	7	3.59
Coleoptera/Scirtidae	4	0.86	5	2.56
Acarina/Tetragnathidae	0	0	2	1.03

Table A4.5: Macroinvertebrate Taxa recorded at Coolibah Wetland

	Inlet Zone		Outlet Zone	
Taxa	**Total count**	**% of total abundance**	**Total count**	**% of total abundance**
Oligochaeta	914	79.34	77	9.13
Turbellaria	6	0.52	17	2.02
Gastropoda/Lymnaeidae	0	0	3	0.36
Gastropoda/Physidae	138	11.98	48	5.69
Dipetera/Culicidae	54	4.69	19	2.25
Diptera/Chironomidae/Chironominae	29	2.52	490	58.13
Diptera/Chironomidae/Orthocladinae	1	0.09	1	0.12
Diptera/Chironomidae/Tanypodinae	0	0	7	0.83
Diptera/Ceratopognidae	2	0.17	0	0
Diptera/Sciomyzidae	1	0.09	0	0
Odonata/Libellulidae	0	0	50	5.93
Odonata/Aeshnidae	0	0	56	6.64
Odonata/Protoneuridae	0	0	39	4.63
Odonata/Coenagrionidae	0	0	8	0.95
Hemiptera/Notonectidae	0	0	1	0.12
Hemiptera/Corixidae	0	0	4	0.47
Hemiptera/Naucoridae	0	0	7	0.83
Hemiptera/Belostomatidae	0	0	1	0.12
Coleoptera/Hydrophilidae	0	0	4	0.47
Coleoptera/Dytiscisdae	1	0.09	0	0
Coleoptera/Scritidae	0	0	2	0.24
Coleoptera/Hydraenidae	1	0.09	3	0.36
Colembola	0	0	5	0.59
Acarina/Tetragnathidae	0	0	0	0
Isopoda	2	0.17	1	0.12
Amphipoda/Talitridae	3	0.26	0	0

Appendix 5: Supporting information to Chapter 5

Table A5.1: Raw data for antennular flicking and foraging behaviour of shrimp exposed to Zn at (A) 0 μgL^{-1} (B) 35 μgL^{-1} (C) 100 μgL^{-1} and (D) 400 μgL^{-1}.

(A)

Flicks min^{-1}			Direct movement to source (Yes=1, No=0)	Swam (Yes=1, No=0)	Time spent active i.e. walking or swimming (seconds)
Pre-treatment	8 mL of chemoattractant	16 mL of chemoattractant			
4	17	24.5	1	1	354
2	30	30	1	1	540
4	27.5	20	1	1	396
5	28	31	1	1	359
4	29	30.5	1	1	372
5	15	27	1	1	343
6.5	31.5	34	1	1	454
0	12	35	1	1	343
0	10	40	0	1	512
0	13	30	1	1	348
0	18	12	0	1	306
0	13	18	1	1	258
11	0	26	1	1	340
2	10	10	1	1	339
4	15	15	0	0	265
0	20	16	1	1	407
8	10	11	1	1	463
9	20	12	1	1	355
9	36	17	1	1	436
14	19	16	1	1	449
15	17	13	1	1	501
3	27	28	nd	nd	nd

nd = not determined

(B)

Flicks min^{-1}			Direct movement to source (Yes=1, No=0)	Swam (Yes=1, No=0)	Time spent active i.e. walking or swimming (seconds)
Pre-treatment	8 mL of chemoattractant	16 mL of chemoattractant			
5	11	8	0	1	149
10	20	23	1	1	325
0	7	12	1	1	273
0	15	15	1	1	348
8	18	19	1	1	318
7	19	25	1	1	290
3	20	16	1	1	459
0	14	23	1	1	306
2	15	21	1	1	250
7	16	10	0	1	100
0	13	9	1	1	297
0	12	24	1	1	342
2	18	20	1	0	260
1	21	16	1	1	408
4	21	14	0	1	328
5	18	18	0	1	406
5	15	17	1	1	217
0	18	17	1	1	548
2	21	19	1	1	314
3	18	21	1	1	341
5	23	25	1	1	474
nd	nd	nd	1	1	410
nd	nd	nd	1	1	127

nd = not determined

(C)

Flicks min^{-1}			Direct movement to source (Yes=1, No=0)	Swam (Yes=1, No=0)	Time spent active i.e. walking or swimming (seconds)
Pre-treatment	8 mL of chemoattractant	16 mL of chemoattractant			
10	13	11	0	1	333
8	7	9	1	1	240
0	14	9	1	0	248
7	14	6	0	1	506
1	6	5	1	1	286
7	12	13	1	1	494
5	13	5	0	1	539
10	15	20	1	1	424
2	9	13	1	1	305
8	10	7	1	1	335
5	7	6	0	1	475
1	3	5	0	1	119
12	24	16	1	1	320
1	3	3	0	0	103
15	16	20	1	1	307
6	4	7	0	0	37
5	9	11	0	1	263
1	1	4	0	0	0
3	6	9	0	1	339
1	0	7	0	1	205
7	9	11	0	1	351
20	26	24	0	1	384
1	0	11	1	1	375
7	40	46	0	1	434

(D)

Flicks min^{-1}			Direct movement to source (Yes=1, No=0)	Swam (Yes=1, No=0)	Time spent active i.e. walking or swimming (seconds)
Pre-treatment	8 mL of chemoattractant	16 mL of chemoattractant			
12	10	4	1	0	40
0	1	10	0	1	193
6	8	15	0	1	186
13	16	9	1	1	279
1	3	11	0	1	198
8	10	11	0	1	137
4	14	18	1	1	137
0	0	10	0	0	90
0	14	14	1	1	78
14	11	10	0	0	310
11	11	9	0	0	169
20	18	21	0	0	182
8	0	1	0	1	77
0	0	6	0	1	131
0	0	0	0	0	0
2	4	4	0	1	231
9	4	10	0	1	186
1	9	5	0	1	281
0	4	0	0	1	102
0	0	0	0	0	31
0	2	2	0	0	110
3	2	3	0	1	159
2	3	1	0	1	204

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Sublethal toxicity of untreated and treated stormwater Zn concentrations on the foraging behaviour of *Paratya australiensis* (Decapoda: Atyidae)

Lois Jane Oulton · Mark P. Taylor · Grant C. Hose · Culum Brown

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Abstract Aquatic organisms use chemical cues to perform key ecological behaviours such as locating food. Anthropogenic pollutants have the potential to disrupt these behaviours by down-regulating chemoreception. Urban stormwater runoff is a major source of metal pollution, particularly Zn, and is a leading contributor to the degradation of receiving waters. Consequently, significant remedial efforts have focused on using constructed stormwater wetlands to reduce pollutant loads. However, no studies have examined the efficacy of water quality improvements on ecologically relevant behaviours in aquatic biota. We conducted controlled laboratory experiments to test whether untreated (100 and 400 μg L^{-1}) and treated (40 μg L^{-1}) stormwater Zn concentrations observed in constructed wetlands interfere with the foraging behaviour of the glass shrimp (*Paratya australiensis*). The ability of shrimp to perceive, approach and search for a chemoattractant source was used to assess foraging behaviour. Abnormal foraging behaviour was observed in shrimp exposed to Zn at untreated stormwater concentrations. The strongest change relative to the control was observed for perception, which decreased by more than 80 and 60 % in the 400 μg Zn L^{-1} and 100 μg Zn L^{-1} groups, respectively. The behaviour of shrimp exposed to Zn concentrations measured in treated stormwater did not differ from the controls. The results suggest that the reduction of stormwater Zn concentrations via wetland treatment can prevent abnormal contamination-induced behaviours in shrimp, leading to improved aquatic ecosystem health. This study also highlights the subtle, but biologically significant impacts arising from sublethal exposures of Zn, and emphasise the utility of behavioural toxicology. The behavioural test used here is a simple and effective approach that could be incorporated into studies assessing the efficacy of stormwater treatment.

Keywords Atyidae · Stormwater Zn · Sublethal toxicity · Foraging behaviour · Stormwater treatment

L. J. Oulton (✉) · M. P. Taylor
Environmental Science, Department of Environment and Geography, Macquarie University, North Ryde, Sydney, NSW 2109, Australia
e-mail: lois.oulton@mq.edu.au

G. C. Hose · C. Brown
Department of Biological Sciences, Macquarie University, North Ryde, Sydney, NSW 2109, Australia

Introduction

Organisms use sensory information to assess critical features in their environment and perform ecologically relevant behaviours such as locating food (Von Der Emde and Bleckmann 1998), finding mates (Passos et al. 2013) and detecting predators (Petranka et al. 1987; Manassa et al. 2013). Decisions mediated by the acquisition of sensory information can have far-reaching ecological consequences at the population, community and ecosystem level (Schmidt et al. 2010). In aquatic environments organisms commonly gather sensory information from their surroundings via chemical cues (Brönmark and Hansson 2000; Blinova and Cherkashin 2012). Any disruption to chemosensory reception therefore has the potential to alter key behaviours and ultimately lead to restructuring of the ecological community (Turner and Chislock 2010).

Studies examining the effects of contaminants on aquatic biota have focused largely on establishing the level of toxicant required to cause mortality, whilst sublethal effects in terms of impairment to behaviour has received less attention (Blaxter and Hallers-Tjabbes 1992; Denoel

et al. 2010). Sublethal endpoints can, however, lead to cascading secondary effects on entire ecosystems (Fleeger et al. 2003). They are also a more appropriate method for which to evaluate the low levels of contaminants observed typically in natural systems (Pestana et al. 2007) and can act as early warning signs for detecting environmental stress (Gerhardt 1995). Recent studies have demonstrated that anthropogenic pollutants, such as trace metals, can disrupt chemoreception in aquatic organisms at non-lethal concentrations (Lurling and Scheffer 2007). For example, environmentally relevant sub-lethal concentrations of Cu have been shown to impair olfactory function in Coho salmon to natural odours (Baldwin et al. 2003) and inhibit morphological defences in *Daphnia pulex* to predatory chemical cues (Hunter and Pyle 2004).

A major pathway for anthropogenic pollutants to enter into the natural aquatic environment is via urban stormwater runoff (McCarthy et al. 2008). This form of nonpoint source pollution is recognised as a leading contributor to the degradation of receiving waters (Walsh 2000). It provides a significant source of metal pollution (Tiefenthaler et al. 2008), with Zn being a dominant component (Hickey and Pyle 2001). Anthropogenic sources of Zn originate from the corrosion of metal objects (e.g. galvanised roofing and roadside fittings) and vehicle wear (frame, brakes and tyres) (Makepeace et al. 1995). Given the environmental effects of stormwater contaminants on receiving waters, a large emphasis has been placed on using stormwater in situ treatment devices to reduce pollutant loads (Birch et al. 2005). Constructed stormwater treatment wetlands are becoming widely used for the management of stormwater and combine natural biological, chemical, and physical process to treat urban runoff (Birch et al. 2004; Malaviya and Singh 2012). Few studies, however, have examined the effect of any consequent water quality improvements on aquatic biota and there have been no published studies analysing how ecologically relevant behaviours are effected.

Crustaceans are one of the most sensitive taxa to pollution (Long et al. 2001). Sublethal concentrations of trace metals have been shown to inhibit their ability to localize prey (Sherba et al. 2000) and reduce feeding rates (Pestana et al. 2007; Wong et al. 1993). Prey localization in aquatic crustaceans depends largely upon chemoreception (Zimmerfaust 1989). Crustaceans antennae filaments have chemoreceptors that function in the sense of olfaction and detect chemical stimuli associated with food resources (Ache and Case 1969). Therefore, impairment of these vital chemical sensors has the potential to disrupt or disable the foraging mechanism entirely. Although the effect of metal contamination on chemoreception in crustaceans has received some attention, the relative effects of untreated and treated stormwater metal contaminants remains unstudied.

The glass shrimp (*Paratya australiensis*) is a common atyid shrimp throughout eastern Australia (Richardson et al. 2004). Given that the shrimp inhabits both freshwater and estuarine environments (Walsh and Mitchell 1995) it provides an important food source for a range of native biota (Richardson et al. 2004). Further, the shrimp play an important role in ecosystem processes via detrital decomposition (March et al. 2001) and influence the composition of algal and benthic invertebrate communities (Pringle 1996; March et al. 2002). Consequently, they form a key component of aquatic ecosystems and are therefore considered a useful model to examine the effects of Zn on aquatic biota. *P. australiensis* is frequently used as an ecotoxicological test species (Hose and Wilson 2005; Thomas et al. 2008; Daly et al. 1990), however, very little is known about the sub-lethal effects of contaminants on ecologically relevant behaviours.

In this study, Zn concentrations reflective of those found in untreated and treated stormwater from constructed wetlands were investigated with respect to how they interfere with the foraging behaviour of *P. australiensis* to a chemoattractant source. Our hypothesis was that abnormal foraging behaviour would be observed in shrimp exposed to Zn at untreated stormwater concentrations, but not at treated concentrations. The intended goal of this research was to provide a better understanding of the sublethal effects of stormwater zinc pollution on urban aquatic environments and in turn, highlight the value of stormwater treatment.

Materials and methods

Experimental subjects

Adult specimens of *P. australiensis* (1.5–2 cm in length) were sourced from Aquablue seafoods (Pindimar, Australia). Following transportation to the laboratory, shrimp were acclimated for 7 days in a 100-L aquarium containing aerated, aged tap water and fed daily with Hikari® algae wafers. During acclimation and testing, water temperature was maintained at 23 ± 1 °C and photoperiod kept constant on a 10 h light: 14 h dark cycle.

Test waters and solutions

Tests were conducted in reconstituted freshwater (hardness of 80 to 90 mg $CaCO_3$ L^{-1}) which was prepared in the laboratory according to formulations provided by Marking and Dawson (1973). Each litre of water contained: 96 mg of $NaHCO_3$, 130 mg of $MgSO_4{\cdot}7H_2O$, 4 mg KCL, and 60 mg $CaSO_4{\cdot}2H_2O$ dissolved in Milli-Q water (Millipore®, USA) using a stirrer bar. Reconstituted freshwater was made up in

10 L batches, pH adjusted to 7.5 with 0.1 M HCL, vacuum filtered through a 0.45 µm pore membrane and stored in the dark at 4 °C prior to use. The pH of stormwater based on the wetland studies we have conducted has ranged from 7.3–7.6 and is therefore very similar to the pH of the reconstituted water.

A Zn stock solution was prepared by dissolving $ZnSO_4 \cdot 7H_2O$ in Milli-Q water. Test solutions were prepared through dilution of the stock solution in reconstituted freshwater to obtain nominal concentrations of 400, 100 and 35 µg Zn L^{-1}. All test solutions were made immediately prior to use. Test concentrations were chosen to reflect those observed in urban stormwater treatment wetlands. The highest dose used in this study was a total metal concentration recorded in the inflow of a Sydney stormwater wetland monitored by Birch et al. (2004), whilst 100 and 35 µg Zn L^{-1} were soluble metal concentrations observed by the authors in the inflow and outflow, respectively, of a stormwater wetland also within the Sydney metropolitan region (Oulton et al. unpublished data). Soluble Zn concentrations were used in this study as the dissolved metal fraction is more readily bioavailable (Hare 1992).

Preliminary studies showed that exposure of *P. australiensis* to 400, 100 and 35 µg Zn L^{-1} caused no mortality after 96 h. For quality control purposes, the metal concentration of solutions were analysed according to US EPA method 6020 using ICP-MS by a National Association of Testing Authorities (NATA) accredited laboratory (Australian Laboratory Services, Sydney). Samples were filtered through a 0.45 µm Sartorius filter and acid-preserved prior to analysis. The actual metal concentrations were <10 % different than nominal concentrations.

The chemoattractant used to stimulate foraging behaviour in this study consisted of an equimolar mixture of glycine and aspartic acid at a total concentration of 0.1 M (Chu and Lau 1994). Amino acids have been shown to readily stimulate feeding responses in crustaceans (Kumar et al. 2010).

All of the reagents used in this study were of analytical grade and obtained from Chem-Supply, Australia. Glassware and equipment used for the preparation of solutions and testing was acid washed for 24 h using 10 % (v/v) HNO_3 to remove any metal contaminants and rinsed (5×) with demineralized water and (5×) with Milli-Q.

Pre-exposure and test exposure conditions

Individual shrimp ($n = 28$ for each treatment group) were selected at random and pre-exposed to 800 mL of 0 µg (control—reconstituted freshwater), 400, 100 or 35 µg Zn L^{-1} in one-litre glass beakers for 72 h to emulate the typical detention time of a stormwater treatment wetland (Melbourne Water Corporation 2010). Food was withheld during this time, as previous studies have demonstrated that starvation enhances the behavioural responses of crustaceans to chemoattractants (Chu and Lau 1994).

Behavioural tests

Following the 72 h exposure period, shrimp were transferred to fresh solutions and their chemotaxis behaviour assessed based on adapted methods established by Chu and Lau (1994) and Kumar et al. (2010). Shrimp that had molted during the exposure period (ca. four per treatment group) were not tested as feeding behaviour can be temporally suspended post-moult (Harpaz et al. 1987).

Each shrimp was acclimated to the behavioural set-up for 10 min after which their antennular flicking rate was recorded for 2 min to determine pre-treatment background levels. Following this, the chemoattractant was delivered via a syringe pump through tubing to a Pasteur pipette into the beaker at a rate of 2 mL min^{-1} for 10 min. The pipette was suspended in the centre of the beaker with the tip located 3 cm above the bottom. Foraging behaviour of the subject was evaluated by recording the following variables: perception (antennular flicking—recorded after the introduction of 8 and 16 mL of the chemoattractant); approach (direct movement towards the source); and searching (proportion of shrimp that initiated swimming and how long they spent active). To minimise disturbance behavioural observations were conducted behind a screen that contained several small viewing holes and recorded using behavioural scoring software (Ottoni 2000).

Data analysis

Data were tested for normality using Anderson–Darling normality tests/normal probability plots and analysed using MINITAB 16 (Minitab Inc, USA) or SPSS 21 (IBM, USA). Change in antennular flicking of individual shrimp was calculated by subtracting the number of antennular flicks, following the introduction of both 8 and 16 mL of chemoattractant, from pre-treatment levels. The resulting data were normalised by square-root transformation and analysed using a repeated-measures ANOVA (between-subjects factor: Zn concentration, within-subjects factor: chemoattractant dose) followed by Tukey HSD post hoc analysis. Shrimp were scored on a yes/no (1/0) basis as to whether they approached the source of the chemoattractant and initiated swimming. To consider the effect of Zn concentration on the number of shrimp that performed these behaviours a generalized linear model with a binomial error structure was used, followed by Fisher's LSD post hoc analysis. The time shrimp spent active during exposure to the chemoattractant was calculated as a percentage, arcsine transformed, and analysed using a one-way ANOVA to consider the effect of Zn concentration. The results were then subject to Tukey HSD post hoc analysis.

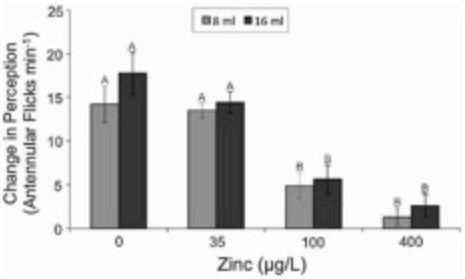

Fig. 1 Mean (±SE) change in antennular flicking rate min^{-1} (perception) from pre-treatment levels following the introduction of 8 and 16 mL of 0.1 M glycine and aspartic acid (chemoattractant) in shrimp exposed to various concentrations of Zn. Means that do not share a *letter* are significantly different, $n = 24$ for each treatment group

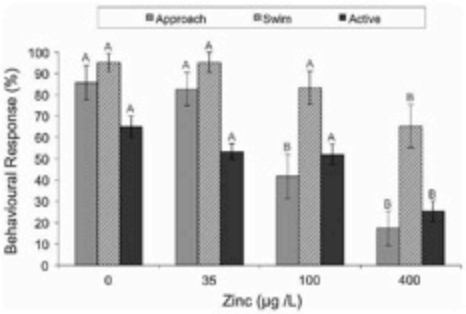

Fig. 2 Foraging behaviour of shrimp in each treatment groups subjected to different concentrations of Zn. Values reported as mean ± SE, $n = 24$ for each treatment group. Means that do not share a *letter* are significantly different from the control

Results

Perception

Shrimp exhibited an increase in perceptive behaviour from pre-treatment levels in response to the chemoattractant. The relative change in antennular flicking rate significantly decreased in response to increasing Zn concentration ($F_{3,175} = 35.28$, $P < 0.001$; Fig. 1), with the concentration of chemoattractant having no significant affect ($F_{1,175} = 1.84$, $P = 0.176$). The control group (reconstituted freshwater alone) showed the greatest change in antennular flicking (ca. increase of 16 flicks min^{-1}), whilst the group exposed to the highest concentration of 400 µg Zn L^{-1}, showed the least change (ca. increase of 2 flicks min^{-1}). Tukey HSD post hoc tests indicated that an increase in antennular flicking behaviour significantly decreased in relation to the control by more than 80 and 60 % in the 400 µg Zn L^{-1} ($t = 8.441$, $P < 0.001$) and 100 µg Zn L^{-1} ($t = 6.039$, $P < 0.001$) groups, respectively. By contrast, there was no significant difference between the control and 35 µg Zn L^{-1} group ($t = 0.253$, $P = 0.994$).

Approach

As Zn water concentrations were increased, significantly fewer shrimp approached the mouth of the Pasteur pipette delivering the chemoattractant source ($\chi^2 = 24.495$, $P < 0.001$; Fig. 2). Post hoc tests indicated that approach to source decreased significantly in relation to the control by more than 66 and 41 % in the 400 µg ($\chi^2 = 16.045$, $P < 0.001$; 17 %) and 100 µg ($\chi^2 = 7.572$, $P = 0.006$; 42 %) Zn L^{-1} groups, respectively. In contrast, there was no significant difference between the control and 35 µg Zn L^{-1} group ($\chi^2 = 0.079$, $P = 0.779$) with over 80 % of shrimp in both treatment groups approaching the source.

Searching

Examination of the shrimp swimming behaviour data revealed a significant effect of Zn concentration ($\chi^2 = 8.408$, $P = 0.038$; Fig. 2). The control and 35 µg Zn L^{-1} treatment had the highest proportion of shrimp that swam (ca. 95 % in both groups), whilst the 400 µg Zn L^{-1} treatment group contained the least number of shrimp that initiated swimming (ca. 65 %). Post-hoc tests indicated that the proportion of shrimp that swam decreased significantly in relation to the control by approximately 30 % in the 400 µg Zn L^{-1} treatment group ($\chi^2 = 4.901$, $P = 0.027$). There was no significant difference between the control (95 %) and the 35 µg ($\chi^2 = 0.004$, $P = 0.948$; 95 %) and 100 µg ($\chi^2 = 1.632$, $P = 0.201$; 83 %) Zn L^{-1} groups.

Analysis of the proportion of time that shrimp spent active showed a significant effect of Zn concentration ($F_{3,87} = 5.54$, $P = 0.002$; Fig. 2). Post-hoc tests indicated that shrimp were approximately 40 % less active in the 400 Zn L^{-1} treatment group compared to those in the control group ($t = 3.906$, $P = 0.001$; 25 %). There was no significant difference in activity levels between the control (65 %) and the 35 µg ($t = 1.779$, $P = 0.290$; 53 %) and 100 µg ($t = 2.288$, $P = 0.108$; 52 %) Zn L^{-1} treatments.

Discussion

Foraging responses of *P. australiensis* were generated by addition of the chemoattractant. The foraging response was characterized by increased antennular flicking, a greater likelihood of initiating swimming and approaching the chemoattractant source, and a general increase in the time spent active. All of these responses to an alimentary stimulus have been reported for a variety of crustaceans

(Buskey 1984; Chu and Lau 1994; Kumar et al. 2010; Hindley 1975; Schmitt and Ache 1979). As the concentration of Zn in the water increased, a corresponding decrease in all of these responses occurred, indicating that Zn had a strong inhibitory effect on foraging behaviour. These dose-behaviour responses were most evident in the higher Zn concentration treatments that are typical of untreated stormwater. At lower Zn water concentrations reflective of those recorded in treated stormwater, foraging behaviour was not significantly different from the control group.

Exposure to Zn at concentrations found in untreated stormwater impaired chemoreception, which, in this study, was manifested as a reduced ability of individual shrimp to perceive, approach and search for the chemoattractant. In other aquatic organisms a reduction in feeding activity has been documented as an indicator of toxic stress in response to pollutants (Butler et al. 1990; Maltby et al. 1990). When exposed to the highest Zn concentration (400 μg Zn L^{-1}), shrimp displayed a significant inhibition in foraging behaviour in relation to the control across all of the monitored behavioural responses. Pestana et al. (2007) also reported abnormal foraging behaviour in another atyid shrimp, *Atyaephyra desmarestii*, exposed to 400 μg Zn L^{-1}.

Crustaceans possess receptors on their antennae and leg tips which function in the sense of olfaction and gustation, respectively (Zimmerfaust 1989; Atema 1977). Thus, an increase in antennular flicking and initiation of swimming may serve to increase the flow of water past these receptors and enhance the ability of shrimp to perceive chemical changes in their environment. For example, antennae flicking in the spiny lobster has been shown to heighten the response of the olfactory receptors to a stimulant (Schmitt and Ache 1979). A contaminant-induced reduction in these behavioural responses is therefore likely to lower the probability of individuals encountering chemosensory stimuli, thereby limiting the foraging search area. This interpretation is supported by the fact that significantly fewer shrimp exposed to untreated stormwater zinc concentrations approached the source of the chemoattractant and spent less time actively searching for it. Uncertainty surrounding the mechanisms responsible for causing disruption to chemical communication exists, but it is thought that contaminants may block receptor sites, interfere with signal-transduction pathways, or alter the chemical signals themselves (Lurling and Scheffer 2007). Application of $ZnSO_4$ to olfactory epithelium has been reported to disrupt signalling transmission and produce transient anosmia in a number of organisms (Benvenuti et al. 1992; McBride et al. 2003; Powers and Winans 1973).

Foraging behaviour has important fitness-related implications and disruption to this important sensory ability is likely to have other cascading ecosystem-wide implications. Zinc concentrations in untreated stormwater may therefore have the potential to impact on shrimp populations as changes to foraging behaviour may affect an individual's growth, reproduction and, ultimately, survival. Maltby et al. (1990) and Maltby and Naylor (1990) found that a reduction in the feeding activity of a crustacean, *Gammarus*, in response to Zn, correlated with reduced growth and reproduction. As shrimp occupy a keystone position in the food web of many environments (Pringle 1996; March et al. 2001) any factors that affect populations are likely to result in secondary impacts at higher levels of biological organization (Bool et al. 2011). *P. australiensis* feeds by both filter feeding and browsing on detrital material such as leaves (Gemmell 1978). Consequently, a reduction in feeding activity could also alter the incorporation of allochthonous-fixed organic material into the food web. Maltby (1994) for example, documented that a toxicant-induced decrease in crustacean feeding rates correlated with a reduction in community function (i.e. leaf processing). Therefore a change in the foraging behaviour of shrimp, and or a loss of shrimp from the aquatic community has the potential to cause large-scale shifts in ecosystem functioning via trophic cascades. Further studies are needed to examine how changes in the foraging behaviour of shrimp alter fitness and what consequences this may have for higher trophic levels and the aquatic ecosystem as a whole.

To date, most studies examining the influence of pollution on aquatic biota have relied on obtaining lethal concentration values as a quantitative measure, with behavioural effects being given relatively scant consideration (Denoel et al. 2010). This study demonstrates that recording immediate mortality only provides a very blunt measure of pollution impacts and appears to underestimate the effect toxins can have on aquatic biota. This is particularly relevant for many anthropogenic pollutants that are present at sub-lethal concentrations, yet still have the potential to cause significant, adverse ecological effects (Lurling and Scheffer 2007). This study has demonstrated that behavioural responses provide useful indicators of pollution effects on aquatic organisms, whose impacts can easily be defined and monitored under controlled conditions. *P. australiensis* appears to be a suitable test organism for use in laboratory-based behavioural toxicology. Shrimp antennular flicking and approach to the chemoattractant source were the most affected foraging responses in this study. Kumar et al. (2010) also found approach to a chemoattractant source in *P. australiensis* to be significantly reduced following exposure to pesticides. Similarly, Chu and Lau (1994) found significantly fewer shrimp increased antennular flicking in response to a chemoattractant when exposed to pesticides. This study suggests that these simple

behavioural responses may serve as reliable and cost-effective indicators of the sublethal effects of stormwater pollutants on crustaceans and enable the identification of stormwater contaminants of concern. In addition, attenuation of adverse foraging responses could also provide a useful indicator of effective stormwater treatment.

In the real world, stormwater delivers a complex mixture of chemicals to receiving waters (McCarthy et al. 2008) which may have additive or synergistic effects (Walsh et al. 2004), and thus Zn is rarely encountered by aquatic organisms in isolation. For example, toxicity of Zn to the shrimp, *Farfantepenaus paulensis*, is more toxic at lower salinities (Barbieri and Doi 2011). In addition, other non-chemical stressors, such as predation, temperature fluctuations, flow alterations etc. are common aspects of natural systems (Heugens et al. 2001; Wenger et al. 2009), which may complicate or modify behavioural responses. Relying on the examination of the effects of single contaminants to assess impacts on aquatic organisms are likely to result in an underestimation of the total adverse impact of stormwater runoff on aquatic ecosystems (Walsh et al. 2004). However, focusing on a single variable such as Zn water concentrations permits delineation of cause and effect in laboratory studies such as this. Further studies are needed that examine the effects of untreated and treated stormwater in association with other common stressors to gain a complete understanding of the cumulative effect of multiple stressors on ecologically relevant behaviours.

Conclusions

It is evident that exposure to Zn concentrations reflective of those found in untreated stormwater induced changes in the foraging behaviour of *P. australiensis*. Adverse effects on foraging responses were not evident at lower Zn concentrations, similar to those measured in treated stormwater. These results highlight the importance of stormwater treatment as part of an ecosystem protection strategy and, in particular, the need to reduce the loading of zinc and other dissolved metal toxicants in receiving aquatic environments. The findings from this study indicate the importance of examining sublethal effects of stormwater pollutants to develop a more comprehensive understanding of their potential impact on aquatic environments. *P. australiensis* appears to be a suitable species to be used in laboratory-based behavioural toxicology to assess the efficacy of stormwater treatment in Australia.

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Conflict of interest The authors declare that they have no conflict of interest.

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Appendix 6. Supporting information to Chapter 6

Table A6.1: Predatory taxa recorded at the inlet and outlet of three stormwater wetlands.

	Inlet Zone Sites[a]						Outlet Zone Sites[a]					
Predatory Taxa	**W1 S**	**W1 A**	**W2 S**	**W2 A**	**W3 S**	**W3 A**	**W1 S**	**W1 A**	**W2 S**	**W2 A**	**W3 S**	**W3 A**
Odonata/Libellulidae	3	0	0	0	5	6	39	87	24	26	17	42
Odonata/Aeshnidae	0	0	0	0	14	16	15	2	22	34	6	4
Hemiptera/Notonectidae	1	0	0	0	0	13	94	89	0	1	3	1
Hempitera/Corixidae	0	25	0	0	0	0	176	8	1	3	0	0
Hemiptera/Naucoridae	0	0	0	0	0	0	0	0	0	7	2	0
Hemiptera/Belostomatidae	0	0	0	0	0	0	0	0	1	0	0	0
Hemiptera/Veliidae	0	0	0	0	0	0	0	0	0	0	0	20
Hemiptera/Mesoveliidae	0	0	0	0	0	0	0	0	0	0	0	1
Coleoptera/Dytiscidae	0	4	1	0	5	3	1	0	0	0	0	7
Coleoptera/Hydrophilidae	0	1	0	0	0	0	7	2	3	1	0	0

[a] = Sites: W1, wetland 1; W2, wetland 2; W3, wetland 3; A, Autumn; S, Spring

Appendix 7: Supporting information to Chapter 7

OPEN ACCESS Freely available online

PLOS ONE

Predator Recognition in Rainbowfish, *Melanotaenia duboulayi*, Embryos

Lois Jane Oulton, Vivian Haviland, Culum Brown*

Department of Biological Sciences, Macquarie University, Sydney, Australia

Abstract

Exposure to olfactory cues during embryonic development can have long term impacts on birds and amphibians behaviour. Despite the vast literature on predator recognition and responses in fishes, few researchers have determined how fish embryos respond to predator cues. Here we exposed four-day-old rainbowfish (*Melanotaenia duboulayi*) embryos to cues emanating from a novel predator, a native predator and injured conspecifics. Their response was assessed by monitoring heart rate and hatch time. Results showed that embryos have an innate capacity to differentiate between cues as illustrated by faster heart rates relative to controls. The greatest increase in heart rate occurred in response to native predator odour. While we found no significant change in the time taken for eggs to hatch, all treatments experienced slight delays as expected if embryos are attempting to reduce exposure to larval predators.

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* E-mail: Culum.Brown@mq.edu.au

Introduction

Predation exerts one of the greatest selective pressures on prey organisms particularly during the vulnerable early juvenile growth phases [1]. In the presence of this selective force, it should not be surprising to discover that many organisms display innate anti-predator responses to visual or olfactory predator cues. In many circumstances, such innate responses are then finely honed following exposure to predators either directly (individual learning; [2]) or via the observation of attacks on conspecifics (social learning; [3]). In aquatic ecosystems the presence of predators is often signalled by chemosensory cues that may take a number of forms. In the simplest form, prey may be able to detect odours emanating directly from the predator. Some chemical cues, however, may indicate the threat of predation indirectly. Alarm substances released from damaged conspecifics, for example, can also signal that a predation event has taken place. Numerous papers have shown that the presence of such cues and their relative concentration, signal that a predator is in the vicinity, and the cues can be used to predict future predator attack [4,5]. Consequently prey show anti-predator responses such as hiding or schooling when they detect these cues.

Innate responses to predator cues have been shown in a number of organisms. Even after 15 generations of isolation from predators, steelhead trout, *Oncorhynchus mykiss*, still responded to the odour emanating from their natural predators [6]. Predator recognition may also be indicated by subtle observation of fish behaviour and or numerous neurophysiological variables associated with the flight or fight response. Both naïve Atlantic salmon, Salmo salar, and Nile tilapia, *Oreochromis niloticus*, increase opercular beat rates in the presence of predator cues [7,8]. Similarly, heart rate is significantly elevated following predator detection [9]. Appropriate physiological responses to predatory cues that differ from exposure to control cues suggest that animals can differentiate between these cues and thus recognize them. Whether the recognition system is cognitive or an innate reflex is often difficult to determine. Where graded responses are illicited to cues that vary in threat content, however, it is likely that cognitive processes are involved as the animal refers to innate or learned templates during the recognition process [1].

It has been suggested that animals may be able to detect and respond to chemical cues during early embryonic stages. Salmonids, for example, may begin to imprint on the chemical signature of their home stream in the final stages of embryogenesis [10]. Chickens exposed to certain odours whilst still in the egg, later show preferences for such odours post-hatching [11]. Moreover, both salamander and frogs exposed to predator cues as embryos show appropriate anti-predator responses as tadpoles upon encountering the cues again [12].

Detection of predator cues by embryos can also effect the timing of hatching. Detection of potential egg predators speeds up development and causes early hatching in amphibians [13], while detection of potential larvae predators causes a delay in hatching [14,15]. To date, however, few studies have examined predator detection by fish embryos despite the fact that the egg membrane is highly permeable [16] and early detection of predators may significantly enhance survival. Here we exposed four-day-old rainbowfish embryos (*Melanotaenia duboulayi*) to a host of predator cues and examined their response by observing changes in heart rate and hatch time.

Methods

Ethics Statement

Fish embryos are not covered by animal ethics legislation in Australia, but the adult stock and the entire protocol was approved by the Macquarie University Animal Ethics Committee (ARA 2011/024).

Brood stock and culture

M.duboulayi eggs were obtained from brood stock originating from a wild population captured at Wilsons River near Lismore NSW in 1989. 14 adult fish were maintained in an isolated, 110 L glass aquarium containing aged tap water. The aquarium was furnished with river gravel and a filter. Temperature was maintained at 26±1°C and photoperiod kept constant on a 10 h light: 14 h dark cycle.

Two days prior to spawning, fish were fed to satiation with commercial flake fish food twice daily supplemented with 150 ml of thawed bloodworms at midday. Six sterilized spawning mops, consisting of bundles of green acrylic 8 ply thread suspended in the water column with polystyrene floats, were placed into the broodstock tank. Mops remained in place for 48 h during which time spawning occurred.

Following egg deposition, the mops were removed, treated in a Methylene blue solution (0.25 ml L^{-1}) for 30 s to minimize fungal infections and transferred to isolated egg incubating chambers (20×38×20 cm). The aged water in the chambers was aerated to enhance oxygenation. Four-days post fertilization, individual eggs were gently teased from the mops and their hear rate monitored as outlined below. This time-point was chosen as heart chamber development and blood pigmentation in a closely related species (*M. fluviatilis*) is readily observed at this stage of development [17].

Test Water

Tests were conducted in petri dishes containing 14 ml of synthetic water (hardness: 80 to 90 mg $CaCO_3$ L^{-1}), which was prepared in the laboratory according to Marking & Dawson [18]. Each litre of water contained: 96 mg of $NaHCO_3$, 130 mg of $MgSO_4.7H_2O$, 4 mg KCL, and 60 mg $CaSO_4.2H_2O$ dissolved in Milli-Q water (Millipore, USA) using a stirrer bar. The pH adjusted to 7.5 with 0.1 M HCL, vacuum filtered through a 0.45 µm pore membrane and stored in the dark at 4°C prior to use.

Stimulus preparation

Stimulus preparation was based on that outlined elsewhere [19]. Briefly, a single spangled perch (120 mm standard length (SL)) and goldfish (140 mm SL) were established in 110 L aquaria and the filters turned off for 24hrs. Scented water was then extracted and frozen (−20°C) in 1 ml aliquots. Conspecific extract was created by killing an adult rainbowfish by decapitation and immediately removing the skin (1 cm^2). The skin was placed on ice, crushed in 1 ml of synthetic water and passed through filter paper (6 µm, Advantec). The final solution was increased to 10 ml using synthetic water and stored in 1 ml aliquots in a freezer. Both the rainbowfish and the goldfish had been fed commercial flake food (Tetramin for tropical fish) while the spangled perch were fed frozen prawns supplemented with flake. Thus the diet of the fish was unlikely to influence the behaviour of the embryos.

Experimental protocol

Harvested eggs were placed in 10 ml of synthetic water in small plastic petri-dishes. Eggs were examined in batches of five and assigned to one of four chemical cue treatments: 1) control, 32 µl of synthetic water; 2) Conspecific extract, 32 µl of rainbowfish odour; 3) Predator, 32 µl of spangled perch (*Leiopotherapon unicolor*) odour; 4) Novel predator, 32 µl of goldfish (*Carassius auratus auratus*) odour. The odour introduced to each petri dish was psuedor-andomised to control for time of day and stress induced by repeatedly harvesting eggs.

The time taken for 100 heartbeats to occur in each egg was observed using a dissection microscope at 40× magnification (see Movie S1). Observation of each egg was repeated and the two counts averaged and converted to beats min^{-1}. A total of 20 eggs were examined for each treatment. Data were normally distrib-uted and analysed using ANCOVA with treatment as the fixed effect, petri-dish number as a covariate and heart rate as the dependent variable.

Following observations of heart rate, the eggs were left in their solutions so that hatch time could be recorded. In addition, a further sample of 20 eggs per treatment were harvested and placed directly into petri dishes to determine if our observations induced changes in hatch time and hatching success due to handling stress. The incidence of hatching was recorded daily until all embryos could be recorded as either hatched or dead. Hatch data was analysed using ANOVA.

Results

Analysis of the heart-rate data showed a highly significant effect of cue (ANCOVA: $F_{3,\ 75} = 14.989$, $P<0.001$; Fig. 1). Post-hoc analysis revealed significant differences between all treatments (Fisher's PLSD: $P<0.03$ in all cases) with the exception of conspecific extract and goldfish odour (Fisher's PLSD: $P>0.05$). All odours elicited a faster embryonic heart rate relative to the control (synthetic water), with the native predator (silver perch) odour producing the greatest increase in heart rate. Heart rate significantly increased with petri-dish number indicating that the eggs became increasingly stressed as we repeatedly sampled different eggs from the mops over the course of the day (ANCOVA: $F_{1,\ 75} = 20.548$, $P<0.001$).

Examination of the hatch time data showed no differences between cues (ANOVA: $F_{3,\ 84} = 0.592$, $P = 0.622$) nor did handling influence hatching time (ANOVA: $F_{1,\ 84} = 0.084$, $P = 0.773$). In general, however, eggs exposed to predator cues tended to hatch slightly later than controls. Post-hoc analysis using a one-tailed t-tests based on the assumption that embryos should delay hatching when detecting larval predators suggested that the hatch date of eggs exposed to conspecific extract showed a

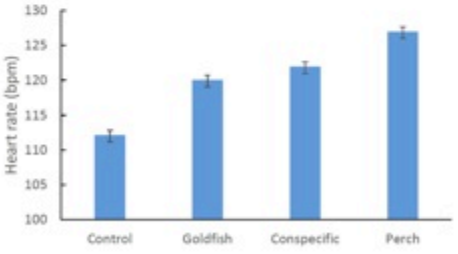

Figure 1. Mean (±SE) heart rate (beats per minute) of rainbowfish embryos exposed to a range of chemical cues. All cues induced a significant increase in heart rate relative to the control (distilled water).
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marginally delayed hatching relative to the control eggs ($t = 1.556$, $P = 0.063$) and while both the other treatments showed similar trends they were not significant (goldfish: $t = 1.302$, $P = 0.099$; perch: $t = 0.85$, $P = 0.199$). Hatching success did not differ between treatments (ANOVA: $F_{3, 24} = 0.929$, $P = 0.442$; average 42.5%), however those eggs that were handled were less likely to hatch than those that were not (ANOVA: $F_{1, 24} = 13.636$, $P = 0.001$).

Discussion

Rainbowfish embryos can distinguish between chemical cues emanating from various potential predators and from alarms substances released from damage inflicted on conspecifics. While a substantial increase in heart rate was observed in response to a novel predator (goldfish) relative to control levels, the greatest response was to the native predator (spangled perch). The response to the conspecific extract was indistinguishable to that of the goldfish. Quite clearly these embryos have had no prior exposure to predators given that they were raised in isolated aquaria thus the recognition system must be entirely innate. What is more surprising is the fish that the eggs were derived from have been in captivity for multiple generations [20]. Similar observations have been made in juvenile steelhead trout that have been isolated from predators for 15 generations [6]. While previous comparative studies of the anti-predator behaviour of rainbowfish have shown that isolation from predators over geological time scales can result in naiveté [21,22], evidently innate predator recognition systems can be relatively long lived even in the absence of direct selection.

While we observed no significant shift in hatch day in response to predator cues, there was a tendency for all treatments to delay hatching relative to controls. The difficulty we face, however, is that hatching success is relatively low (ca 42%) and the embryonic stage is very short (just 7 days at 26°C), thus in order to detect significant delays in hatch date we undoubtedly require more power. Such low hatching success is typical of rainbowfish where individual females spawn hundreds of eggs per week, but this was further exacerbated by handling the eggs during the experiment. Most of them succumb to fungal infections in the lab but the hatch rate in the wild is likely to be significantly lower. It is interesting to note, however, that the eggs tended to delay hatching after exposure to the predator cues which is what would be expected if the cues were emanating from larval rather than egg predators [13,14,15]. Larval rainbowfish are only 4–5 mm long when they hatch and they undoubtedly fall prey to a wide range of predators including small fish and invertebrates. Juvenile spangled perch and goldfish are both well known for their broad dietary niche and both attack and consume larval fishes when they encounter them.

While we have clearly shown that rainbowfish larvae can detect and differentiate between predator cues, it remains unknown what the longer-term effects of this early exposure might be. Research in amphibians suggest that exposure to predator cues during embryogenesis can lead to appropriate avoidance behaviour as larvae [12]. No such response was observed in Atlantic salmon fry when exposed to pike odours between 27 and 1 day pre-hatch [23]. However, there are bound to be a number of other physiological and behavioural costs associated with accelerating or decelerating development. Not least of which is the potential for developmental instability. Clearly heart rate increases in fish embryos during exposure to predator cues, perhaps an indication of underlying stress, which may affect hatching success and larval behaviour. Studies on Atalantic salmon have shown that maternal stress has great impact on key larval characteristics including reduced body size, yolk sac volume, and an increase in morphological malformations [24]. Future studies will need to pay close attention to these potential long-term impacts of predator exposure during embryogenesis.

Supporting Information

Movie S1 A movie showing the heart beat and circulation of a rainbowfish embryo.
(AVI)

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Author Contributions

Conceived and designed the experiments: LO CB. Performed the experiments: VH. Analyzed the data: LO CB. Contributed reagents/materials/analysis tools: LO CB. Wrote the paper: CB.

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