



Functional ecological processes in upland swamps and chain of ponds systems in the Blue Mountains and Southern Highlands of eastern New South Wales, Australia



Lorraine J Hardwick

B.Sc, M.App.Sci



Department of Biological Sciences, Macquarie University

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Summary

Globally, biodiversity is declining, leading to concerns of permanent loss of ecosystems, central to which is the increasing strength of evidence for the inextricable linkage between biodiversity and ecosystem function. There is an urgent research need to identify the crucial relationships between biodiversity and the functions they require and provide. Many Australian aquatic ecosystems are still poorly studied, including remote and atypical streams, rare and groundwater ecosystems. Furthermore, even in systems where biodiversity is known, ecological functions have rarely been examined.

By investigating functional attributes alongside biodiversity measures it was possible, through a sequence of studies to more fully understand dynamics of two rare and at-risk wetland systems west of the Sydney Basin in eastern NSW, Australia. The aquatic ecology and function of Blue Mountains upland peat swamps as part of the THPSS (Temperate Highland Peat Swamps on Sandstone) and Mulwaree ‘chain-of-ponds’ are little known. They represent fundamentally different ecosystems, but both face growing anthropogenic pressure.

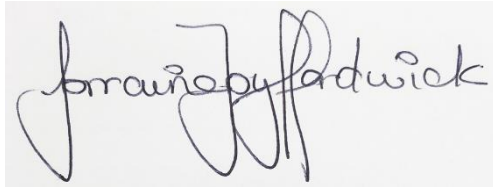
These studies highlighted the importance of including functional indicators into biodiversity studies as a way of gaining better information for restoration and management. The THPSS swamps were porous to pollutants, with groundwater and streams downstream carrying stormwater nutrients. Leaf litter and cotton strip decay rates were very low, invertebrate abundances were low, but urban stormwater increased both. Release of excavated naturally occurring ironstone, combined with nutrients and high groundwater, led to proliferation of iron bacteria, complex redox reactions, anoxia and reduced productivity.

The Mulwaree chain-of-ponds are geomorphic anomalies that act as mesotrophic monomictic lakes once disconnected from river flows. The ponds maintain significant aquatic macrophyte biodiversity, which maintains unusual clear water conditions. The majority of macrophytes are perennial rhizomatous species, but some, such as Water Ribbons (*Cycnogeton procerum*) are at risk due to water borne dispersal. Water ribbons play a pivotal role in maintaining macrophyte dominance and in carbon cycling within the ponds.

Statement of Originality

This work has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

(Signed)

A handwritten signature in black ink, appearing to read 'Fraunhofer', written on a light grey rectangular background.

Date: _ _5 February 2019_

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Chapter 1

Introduction



Lorraine Hardwick

Department of Biological Sciences

Introduction

Traditionally, biodiversity metrics have been used to characterize aquatic ecosystem biodiversity and health, both in Australia (Chessman & Hardwick, 2014; Growns *et al.*, 1995) and elsewhere (Junk, 2006). This is understandable, given that freshwater ecosystems are highly biodiverse and support an extraordinarily high proportion of the world's biodiversity (Abell *et al.*, 2008).

Freshwater ecosystems support nearly 6% or at least 126,000 of all described species, with up to 1 million undescribed species (IUCN, 2019), despite occupying only 0.8% of the Earth's surface (Dudgeon *et al.*, 2006).

Western scientific knowledge of Australia's diverse aquatic ecosystems is steadily improving. Before European colonization, merely 240 years ago, the continent previously supported a low Indigenous human population understanding and managing biodiversity with a small ecological footprint in a large and remote landmass with large swathes of highly variable geological and biological diversity. Since then, Australian ecosystems have been sporadically investigated and still reveal surprising new diversity. Some river systems, such as the Murray Darling Basin rivers and associated wetlands have been investigated extensively. Urban streams have been studied well in some areas (Walsh & Kunapo, 2009; Walsh *et al.*, 2004; Wright *et al.*, 2011), as have upland streams (Boulton & Lake, 1992; Doeg *et al.*, 1989), some tropical northern rivers (Mackay *et al.*, 2003; Pusey *et al.*, 2004), arid zone rivers (Balcombe *et al.*, 2011; Puckridge, 2010; Sheldon *et al.*, 2010) and wetlands of the Swan Coastal Plain in Western Australia (Davis & Froend, 1999; Horwitz *et al.*, 2009). Groundwater systems are more recently becoming known, mostly in the arid west of Australia (Halse, 2002; Humphreys, 2008), deep aquifers (Hose, 2015; Humphreys, 2006; Korbel *et al.*, 2013) and hyporheic zones along regulated and unregulated rivers (Boulton, 2008; Hancock *et al.*, 2005).

Many aquatic ecosystems have been poorly investigated and there is a need to identify which should be the target of future attention, an exercise performed recently for terrestrial vegetation (Haque *et al.*, 2017). Furthermore, there is a lack of taxonomic knowledge of Australian aquatic fauna, with many undescribed species. For example, systematic searching of a remote location in North Western Australia recently yielded surprising biodiversity, including 20 new species of fish

(Shelley, 2016) thereby increasing the total known Australian fish fauna by 10%. For the same part of Australia, in the emerging field of groundwater ecology, the 750 species recently catalogued at one location comprised 22% of the then known global fauna (Humphreys, 2008). The extraordinary endemism and historical significance included living species previously only known from the Cretaceous fossil record and unique taxa of stygal Dytiscidae (Coleoptera - 83 species) and Candonidae (Cladocera - 84 species) (Humphreys, 2008). These examples suggest that Australian aquatic biodiversity is extensive, endemic and under-represented in the global inventory (Abell *et al.*, 2008; Cresswell, 2016; Humphreys, 2008).

Many such studies have identified that Australian and global freshwater biodiversity is in serious decline (Cresswell, 2016) and facing unprecedented levels of threat (IUCN, 2019). There is evidence that widespread biodiversity decline in most global ecosystems may be as high as any extinction events known from the fossil record (Barnosky *et al.*, 2011), and despite research and management efforts, that decline is ongoing (Turnbull *et al.*, 2013). Importantly, biodiversity declines in freshwater outstrip those in terrestrial systems and may be higher in less studied regions and faunas (Vaughn, 2010), such as Australia, with its comparatively poorly known and highly endemic freshwater environment (Abell *et al.*, 2008). As such, there is an urgent need to learn more about Australia's unknown aquatic biodiversity resources and understand the threats they face. Rather than just understanding structural diversity, however, it is important to comprehend drivers of system function to recognize the processes critical for ecosystem maintenance and resilience. There is recent and increasing concern amongst the scientific community that, along with biological diversity, functional diversity is equally important and also under threat (Cadotte, 2011a). Therefore, widespread decline in both species and functional diversity requires new investigative approaches (Cadotte, 2011a; Vaughn, 2010). Recent evidence related to biodiversity and function suggests that functional diversity, such as ecological traits, is more relevant to function and under greater threat from human activities rather than is species richness (Cadotte, 2011b). With this comes a need to more formally define what we mean by 'biodiversity' and 'function' and more fully understand the drivers of both.

What is Biodiversity?

Biological diversity is a broad concept, encompassing metrics such as genetic variation, species diversity, taxa richness, species evenness, abundance and community structure (Hooper *et al.*, 2005). Biodiversity is usually measured by how many genotypes, species, taxa or ecosystems, evenness in community structure and distribution, differences in their functional traits and interactions both within and between groups. There is a distinction between biodiversity, which includes measures of evenness, and aspects of biodiversity such as taxa richness, abundance, presence/absence of particular species and community composition, which are relatively simple measures (Hooper *et al.*, 2005). Both derived biodiversity that is calculated as a biodiversity index, and the various aspects of biodiversity are used when reporting ecosystem function (Balvanera *et al.*, 2006). These definitions of biodiversity may also be organized by spatial scale. Alpha diversity (α) is the diversity within each site, Beta diversity (β) is the difference in taxa composition between sites and Gamma diversity (γ) it the diversity across the entire landscape.

What is Function?

Within the construct of functional ecology there must be continued development of understanding the definition of function in all its forms. There is a need to identify useful indicators of functional integrity that may or may not involve ‘structural biodiversity’ per se. A useful endpoint of any ecological study should be an understanding of how that system ‘works’, it’s biodiversity and functional elements that are affected by disturbance, and management options to address ecosystem function as well as structure.

Research of ecological function is increasing, but there is often confusion about the catch-all term. Ecological function has multiple meanings, from plant functional traits to phytoplankton productivity as a functional process. Jax (2005) described the four primary meanings of ‘function’ in ecology, which assist in clarifying and classifying the term.

1. Function as interaction, being the interaction between two organisms, such as the predation of one species for example chironomids by another e.g. odonates, or nutrient assimilation by phytoplankton. These examples are purely descriptive.

The interactions can be further described as cause-effect, adding a temporal element;

2. A broadening of these simple interactions or processes introduces a systems element, including how organisms interact with each other but also with their environment. So, questions asked include how the system functions, what current state and trajectories are important and what contributes to sustenance of the system;
3. A further extension attaches roles to how organisms interact within their systems. So that, for example, macrophytes (e.g. *Cycnogeton procerum*) are considered primary producers. There is a distinction between the function, for example primary production, and the carrier; for example, macrophytes or phytoplankton are both primary producers but interact within their ecosystem in entirely different ways. Role-based groupings can be clustered in a number of ways, including by traits (Lavorel & Garnier, 2002), by functional groupings such as functional feeding groups (FFGs) (Cummins, 1974), or by the niches that an organism fills within a system (more recently termed “functional effect groups and “functional response groups” (Catovsky, 1998); and
4. More broadly again, is the inclusion of humans into ecological systems, so that function is related to its use by humans. This anthropogenic view of function includes the concept of ecosystems services (Costanza *et al.*, 1997), which has become commonplace in valuing ecosystems of all types. These include the services provided by groundwater (Griebler & Avramov, 2015; Tuinstra & van Wensem, 2014), wetland resources (Zedler & Kercher, 2005), leaf traits affecting litter decomposition (Dias *et al.*, 2013), invertebrates (Prather *et al.*, 2013) and in landscape and natural resource management (Cork, 2007; Liu *et al.*, 2010; Turner *et al.*, 2010).
5. A further, more recent inclusion of the function concept is ‘human wellbeing’ (Naeem, 2009), being how human wellbeing is affected in the Anthropocene, where biodiversity and functions of ecosystems have been immeasurably and irreversibly altered.

Therefore ‘function’ is a multifaceted concept, from simple interactions between two organisms through to complex relationships involving human derived ecosystem services. In the scientific context, function is a more recent player in identifying internal ecosystem relationships.

The application of these concepts to wetland research can be illustrated by organic material movement through an aquatic ecosystem. Organic matter enters freshwater systems through two processes: allochthonously, whereby organic matter enters streams from litter fall or from upstream flow, or autochthonously, whereby algae in phytoplankton or periphyton undertake photosynthesis (Boulton, 2014) (Figure 1). Allochthonous carbon inputs occur more commonly in upland and shaded waterbodies, whereas autochthonous productivity becomes more important in larger, less shaded waterbodies where phytoplankton and periphyton can dominate. Measuring biomass of invertebrate detritivores that are involved in litter decomposition or chlorophyll A in the water column can provide simple measures of function (the first of Jax’s functional levels). Rates of leaf litter decomposition under different environmental conditions, or gross and net primary productivity at various depths exemplify the second level of functionality. Functional feeding groups involved in litter decomposition, their interaction with decomposing microbes and how they vary across different habitats exemplifies the third level of functionality. The fourth level of functionality, ecosystem services, is provided by these processes including the purification of air and water; generation preservation and renewal of soils; detoxification and decomposition of wastes; cycling and movement of nutrients; maintenance of biodiversity; partial stabilization of climate and provision of aesthetic beauty (Daily, 2003). Finally, once these services are identified it is possible to extrapolate their relationships to human wellbeing. This final level of ecosystem functionality allows for the quantification of impacts on air and drinking water quality, loss of aesthetics, the need for waste treatment, vegetation and soil restoration. Once these values and roles are understood, it becomes possible to better protect these aquatic systems.

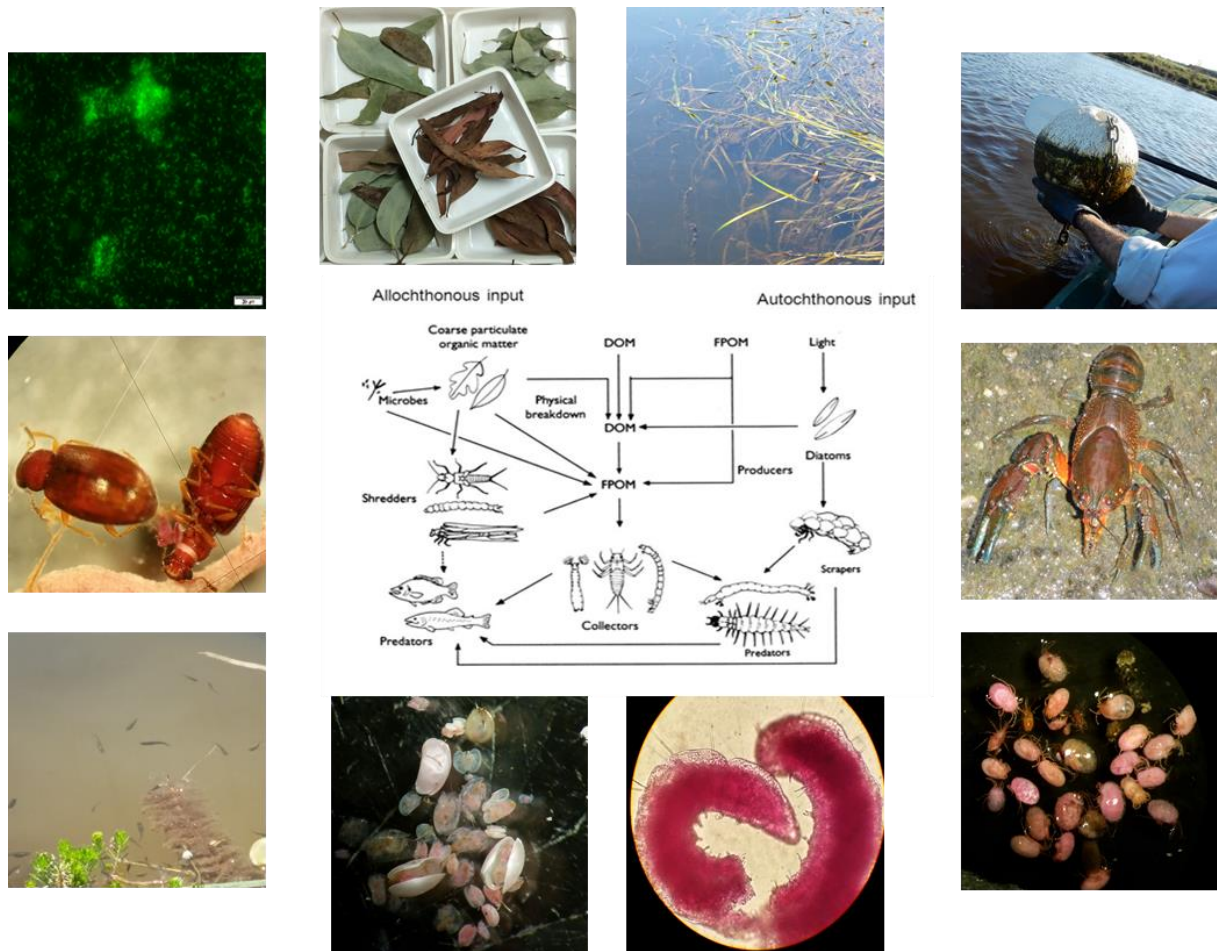


Figure 1. The interplay between the autochthonous and the allochthonous aquatic food chain (modified from Cummins (1973)). (Photos taken during thesis, Lorraine Hardwick).

Abiotic Drivers

Abiotic factors are instrumental in driving biological communities and are therefore potentially important drivers of ecosystem function (Truchy *et al.*, 2015). Perhaps the most important of these is physical habitat or the geomorphic template, within which aquatic systems are structured (Brierley, 2008). The geomorphic context is often overlooked, but is integral to ecosystem functioning (Brierley *et al.*, 2010) and resilience (Fuller *et al.*, 2019).

Morphology of aquatic systems is influenced by multiple variables, operating over many scales (Thomson, 2001). Effective patch size can vary and impacts may operate at variable intensity across an aquatic environment, depending on its heterogeneity (Frainer *et al.*, 2018). Therefore, an understanding of natural and impacted or disturbed ecosystems requires an understanding of geomorphology.

The most important water quality driver is thought to be temperature, which has an overriding influence on metabolic processes and is the primary regulator of the chemical reactions that are the basis of biological functioning in freshwater. Light and nutrients are also highly influential (Bott, 2006; Hauer, 2006), driving primary production in ecosystems, with nutrients being important to heterotrophic decomposition processes (Gulis, 2006). Other abiotic drivers that may affect ecological function in freshwater systems include suspended sediments (Bunn & Davies, 2000), electrical conductivity (Boulton, 2007; Roache *et al.*, 2006) and acidity (Andr n & Jarlman, 2008; Wright *et al.*, 2011). Furthermore, hydrology usually plays a defining role in driving biotic structure (Bunn, 2002) and function (Lake, 2000; Stanley *et al.*, 2010), particularly in wetlands. These abiotic factors may work together synergistically or antagonistically to create the abiotic diversity from which biological structure and function stem (Truchy *et al.*, 2015). There are other abiotic drivers that are important but are difficult to measure (e.g. natural conditions and anthropogenic impact), but other measurements may still be helpful surrogates of these (e.g. distance to stormwater infrastructure, temperature variability, flow variability, water clarity).

Biodiversity and Ecosystem Function

The links between biodiversity (genetic, species, assemblage or trait) and function have received increasing attention in the past two decades (Gamfeldt & Hillebrand, 2008) as ecologists attempt to understand the role that biodiversity plays in aquatic ecosystem functioning. Ecologists now conclude, for example, that high biodiversity implies more highly functional or more resilient ecosystems (Cardinale *et al.*, 2011; Hooper *et al.*, 2005; Tilman *et al.*, 2014) and conversely, that species functional characteristics influence the ecosystems they inhabit (Hooper *et al.*, 2005). This paradigm arises because higher species richness leads to increased ecological function as a result of greater functional trait complexity; more species fill available ecological niches more

effectively, reducing niche gaps that may destabilize an ecosystem (Vaughn, 2010). The corollary is that at low levels of biodiversity, the associated niche gaps reduce the fitness and resilience of an ecosystem. A difficulty with this paradigm is our lack of understanding of the multiple traits an individual species might use to fill various niches within an ecosystem (Poff, 2006). Furthermore, many aquatic ecosystems are dominated by few species but exhibit high productivity, such as sphagnum peat mires and salt marshes (Cardinale *et al.*, 2006), and there is emerging evidence for complex and multivariate relationships between diversity and productivity (Cardinale *et al.*, 2009).

There are many similar conflicts in the paradigm of diversity and function or productivity in aquatic ecosystems. These include the relative importance of taxonomic versus functional diversity; diversity effects on productivity (Hooper *et al.*, 2005); the dominance of biotic versus abiotic factors; and whether diversity does actually enhance stability or not (Gamfeldt *et al.*, 2008). These opposing views have led to growing integration of community ecology (biotic description and interactions) and ecosystem ecology (with a more holistic view that includes material and energy flow). There has been a rapid growth in knowledge in the field of biodiversity and ecosystem functioning in the last two decades. Initially, in the late 1990s, acceptance of the importance of biodiversity-ecosystem function relationships took time, followed by a period of exploration and testing of new ideas (Naeem, 2009.). In the past decade, substantial scientific endeavor has been spent on more clearly identifying and quantifying those relationships.

Evidence now suggests that biodiversity enhances the efficiency by which ecological communities capture resources and produce biomass (Cardinale *et al.*, 2012), and the stability of ecosystem processes in changing environments (Loreau & de Mazancourt, 2013). Knowledge of other aspects of these relationships are continuing to develop. Areas of continuing research include the importance of environmental conditions, of trophic interactions, of rare versus common species, and of the role of ecosystem connectivity.

This new direction in ecosystem science has been termed ‘biodiversity and ecosystem functioning’, or BEF (Daam *et al.*, 2019). As the science has matured, a greater understanding has developed of predictive and non-linear effects on biodiversity and ecosystem functions

caused by global changes. In this context, the ability to predict ecosystem features and biodiversity-ecosystem function may be complicated by a range of factors, (Vaughn, 2010) including:

- spatial and temporal patterns within ecosystems;
- relationships between abiotic and biotic drivers;
- the effect of biodiversity loss on food webs;
- the instrumental but variable effect of species traits on ecosystem functions;
- the variable ecological effect of functional grouping affiliations; and
- the decline in abundance of common species affecting biodiversity overall.

This means that various empirical methods across a range of scales will be required to successfully predict relationships into the future.

As aquatic ecosystems are affected by an increasing number and intensity of stressors, the need to more fully understand the drivers and outcomes of what is an increasing rate of change will become even more important (Dow *et al.*, 2013). Anthropogenic drivers including urbanization and agricultural impact combined with shifting climate norms will continue to act on aquatic ecosystems. Some effects are already evident, and outcomes can more easily be predicted with some certainty. This means that some threats and risks can already be managed to some extent. However, functionality and biological diversity of many rare and unexplored ecosystems remain unknown. The threats to these ecosystems may not be managed effectively, and they are therefore at considerably greater risk of loss. Despite considerable progress in research over the past decades, difficulties remain in incorporating scientific outcomes into “user friendly” resources for natural resource management, the multitude of functions supported by ecosystems, functional distinctiveness of rare species, multitrophic interactions and spatial-temporal scales (Daam et al 2019).

Much of the BEF literature has recommended using traits rather than taxa richness per sé, to link to ecosystem function (Lavorel & Garnier, 2002; Schmera *et al.*, 2017), however, more recent theoretical modelling suggests linking α and β diversity relationships to function (Thompson *et al.*, 2018). Furthermore, species richness does not include community structure, species loss and gains within an ecosystem. More recent developments of BEF include integration of community

structure under the banner of community analysis and functional ecosystems (CAFE) outlined by Leibold *et al.* (2017) and further developed by Bannar-Martin *et al.* (2018). There is now growing acceptance that biodiversity generally only explains a fraction of the variation in ecosystem functioning at local scales (Thompson *et al.*, 2018). Many other factors, including temperature and other abiotic drivers also impinge on functioning but not necessarily biodiversity. There is therefore a need for more knowledge around abiotic drivers, biological diversity, ecological traits and ecological function.

In Australia, there is a challenge to identify the abiotic and anthropogenic drivers that affect biodiversity and ecosystem function simultaneously, particularly in less well-known aquatic environments that may support substantial endemism. There is a need for a greater emphasis on both developing functional and biotic knowledge in Australian surface and groundwaters, which can then feed into effective ecosystem management,

Thesis Aims

This thesis aims to identify the aquatic ecology and function of two rare and poorly known wetlands, relate that to abiotic drivers altered by human influences and attempt to explain how changes in ecological function can be used to understand anthropogenic impacts and ecosystem response. This understanding can assist in providing guidance to management.

The thesis explores the ecology of two important wetland systems through four studies to illustrate the benefits of integrating biotic and functional measures and relating them to abiotic drivers in aquatic ecosystem science. The contrasting rare and endangered aquatic-terrestrial ecotonal habitats, both vegetated and under intense anthropogenic pressure, provide an opportunity to more fully understand ecosystem processes in rare geomorphic settings. The importance of abiotic factors, such as temperature, are used to attempt to understand how anthropogenic driven change, such as climate change, may impact ecological functions within rare aquatic systems.

There has been recent extensive research undertaken of the geomorphology and hydrology of the two wetland systems to be investigated (Cowley, 2017; Williams, 2018). These works set the

geomorphic framework within which this ecological study sits. This thesis references that work but does not duplicate it, instead the view is that this work sits as a sister document to those already existing.

The Blue Mountains upland swamps are a common feature on the sandstone escarpment west of Sydney (Fryirs, 2016), but are of such natural value that they form part of the endangered ecological community the Temperate Highland Peat Swamps (THPSS) listed under the Commonwealth Environment protection and Biodiversity Conservation Act 1999 (EPBC Act) and the NSW Threatened Species Conservation Act (2005). The THPSS swamps dominate the valleys on the flat escarpment of the Blue Mountains, west of Sydney and to the north and south (Fryirs, 2018). These Holocene sedimentary wetlands support a diverse and highly endemic flora and fauna that has led to their legal protection as threatened ecosystems. One of the characteristic features of peat mires is their slow productivity and decomposition rates. They store enormous quantities of carbon, much of it recalcitrant. Carbon in Blue Mountains swamps is stored in sedimentary layers that is bioavailable in the surface organic fines, but decreasingly so at depths in the alternating organic sands, stored as recalcitrant, mineralized peat (Cowley, 2016). The swamps are complex hydrologically (Cowley *et al.*, 2018), with naturally persistent groundwater that leaves the swamps as surface water. Eroded swamps may exhibit surface water throughout their length. They can therefore be described as ecotonal aquatic-terrestrial ecosystems, with hyporheic elements. Two studies investigating the groundwater/hyporheic and the surface water components of these interesting systems are performed as part of this thesis.

The aims of the studies in these wetlands are:

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To identify the effect of urbanization on groundwater invertebrate community structure and function within upland swamps. Abiotic drivers of rainfall response and groundwater depth, alteration to temperature, nutrients (as indicators of urban impact), physical water quality, natural geographic features and indicators of urban proximity are used to set a template to which groundwater faunal metrics and carbon decomposition can be related. This study investigates how hydrology of natural and urbanized water affect swamp groundwater invertebrates (stygofauna), their abundance and functional groupings. Organic matter decomposition as an

indicator of water productivity are used to investigate how that productivity relates to stygofaunal communities

To investigate processes of eucalypt litter decomposition in streams emanating from upland peat swamps by measuring microbial, detrital and total decay, related to urban proximity and influence. Abiotic drivers including temperature, nutrients, physical water quality and indicators of urban proximity are used to identify urban impact on functional aspects of decomposition. This study investigates how heterotrophic processes of natural occurring eucalypt leaf decay supports microbes, detritivores and larger shredding invertebrates; and how that process is affected by urbanized water running through the swamps.

Mulwaree chain-of-ponds, on the southern highlands/Monaro plains region of New South Wales are not protected by legislation, but are rare and unusual geomorphic features at a continental scale, vulnerable to threats and are important for evolutionary history under Australian Federal Government High Ecological Value Aquatic Ecosystems (HEVAE) (Aquatic Ecosystems Task Group, 2012). The Mulwaree River is a geomorphic remnant river system (Williams, 2018), unique and somewhere between local ‘instream wetlands’ (Zierholz, 2001) and swampy meadows (Mactaggart *et al.*, 2008). Mulwaree-chain-of-ponds represents an apparent highly productive and readily available source of carbon, with high primary productivity based on aquatic macrophytes and phytoplankton. Our understanding of these ponds is not known but based on knowledge of ponds globally (Boix *et al.*, 2012; Céréghino, 2014; Downing, 2010). The system is likely to be active productively. Carbon is likely to be sequestered seasonally to created macrophyte and phytoplankton biomass, that is then decomposed and released, either to the atmosphere or to the base of the ponds where it is flushed downstream with connected flow. We hope to show that adequate nutrients enable rapid integration and flow enables rapid dispersal of readily available carbon and nutrients for productive purposes downstream. The ponds are thought to act seasonally on annual carbon budgets.

The aims of the two studies in this system are:

To investigate Mulwaree Pond aquatic productivity during connected flow and disconnected non-flow periods, to understand gross primary productivity, net ecosystems productivity and microbial respiration as measured by 24-hour light and dark bottle incubation in situ in four ponds within the chain-of-ponds system. Waterbody abiotic drivers of temperature, water clarity, nutrients, and physical water quality impact on these measures. Planktonic respiration is hypothesized to be consistent through depth, while phytoplanktonic activity is greater mid-pond compared to in the fringing macrophyte beds and also during periods of disconnected flow. This study investigates the importance of temperature as a biotic driver in ecosystem function in a pond system.

To investigate the relative seasonal importance of macrophyte and phytoplankton dominance in the ponds, related to abiotic drivers, such as temperature and flow. To identify, map and describe functional groups of the macrophyte resources. Further, to investigate decomposition processes of *Cyanogeton procerum*, a common but water dispersed and thus at-risk aquatic macrophyte, by identifying the importance of microbial contribution to total decomposition rates that includes herbivory by shredders. This study will investigate the relative seasonal importance of macrophyte and phytoplankton dominance in the ponds in order to more fully understand processes of stability and alternative stable states within the ponds.

The two divergent wetland ecosystems provide an opportunity to investigate and integrate ecological functions, including productivity and decomposition, with abiotic factors and anthropogenic impacts on ecosystem structure. A further objective of the respective studies will be to develop management tools that so that conservation and rehabilitation measures for these endangered ecosystems can be more effectively designed and implemented.

These two systems require basic scientific knowledge, in order to document their taxa richness and their abiotic drivers, such as hydrology, water quality and human impacts. However, it is necessary to measure ecosystem functioning simultaneously. As aquatic ecosystems operate at relatively small spatial scales, a challenge will be to provide some broadscale biological and functional information while being predictive at relevant ecological scales.

The thesis will be set out as:

- Chapter 1: Introduction chapter outlining the theoretical basis for the thesis
- Chapter 2: a background chapter on physical and ecological structure of the wetland systems to be used and that will inform the data chapters to follow.
- Chapters 3-6: a sequence of four following scientific investigation chapters that integrate the regional setting of the wetlands and report ecological aspects of biodiversity and function studies outlined above and represented in Figure 2.
- Chapter 7: a discussion chapter which will focus the outcomes of these studies, how they relate to current ecological theory, knowledge gaps and a brief discussion on how restoration ecology theory can be used to guide future management for both wetland systems.

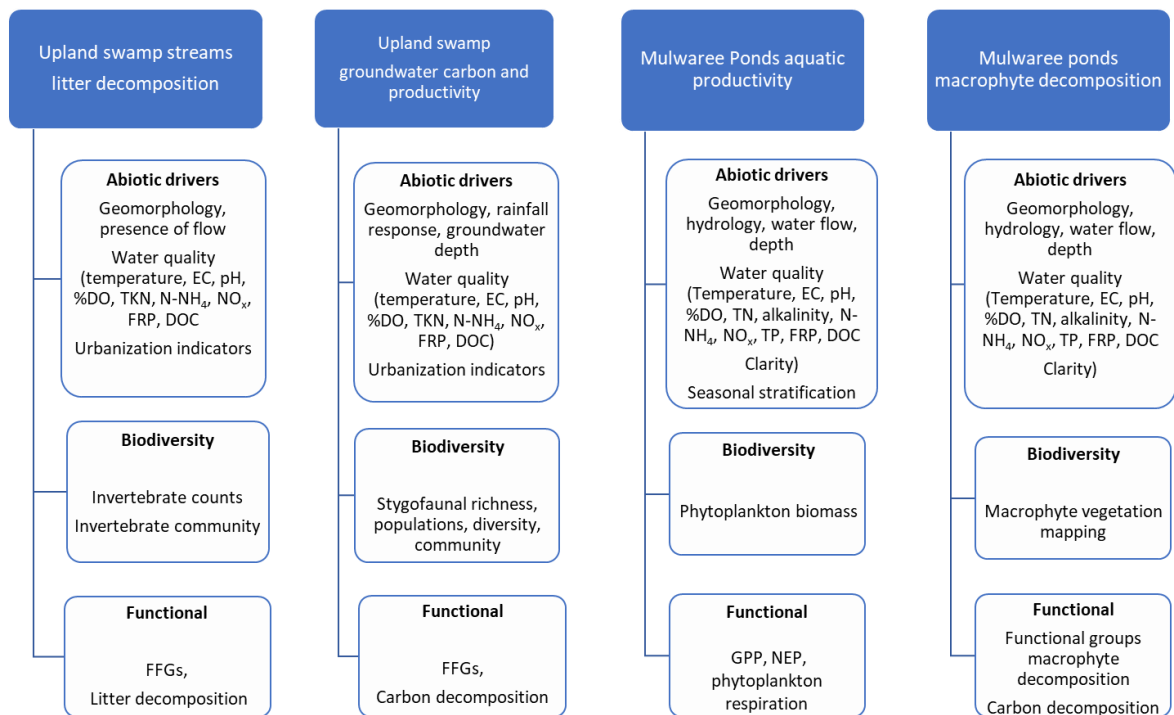


Figure 2. Conceptual diagram of planned structural and functional indicators, including abiotic drivers, biodiversity metrics and functional aspects of the four case studies.

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Chapter 2

Features of peat swamps and chain-of-ponds: Blue Mountains upland swamps
(THPSS) and the Mulwaree chain-of-ponds



Lorraine Hardwick

Department of Biological Sciences

Introduction

This chapter introduces the two wetland environments to be used for the thesis. Both the Blue Mountains upland peat swamps as part of the Temperate Highland Peat Swamps on Sandstone (THPSS) and the Mulwaree chain-of-ponds are unusual aquatic systems. Neither rivers or acknowledged as wetlands, they nevertheless should be described and understood to enable protection and management. While the Blue Mountains swamps are listed under both Federal and State legislation for protection, they continue to be at risk. Mulwaree chain-of-ponds are not protected. The aim of this chapter is to provide literature review and context for the scientific studies to follow.

Blue Mountains Peat Swamps

The Blue Mountains swamps are a common feature on the sandstone escarpment west of Sydney (Fryirs, 2014a) but are of such natural value that they form part of the endangered ecological community, the Temperate Highland Peat Swamps on Sandstone (THPSS) listed under the Commonwealth of Australia's Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act) and the NSW Threatened Species Conservation Act (2005) (Figure 1). The terrestrial diversity of the swamps are highly biodiverse, supporting many threatened and vulnerable endemic biota, including the plants *Dillwynia stipulifera*, *Boronia deanei* subsp. *deanei*, the giant dragonfly - *Petalura gigantea* and the Blue Mountains water skink – *Eulamprus leuraensis* (Benson, 2012). Formed during the Holocene as valley fill topogenous swamps, they comprise layers of sandy peaty deposits atop a sandstone ironstone base (Fryirs, 2014a). Common vegetation comprises xeromorphic shrubs, ferns, grasses and rushes such as *Grevillea acanthifolia*, *Gleichenia dicarpa*, *Poa labillardieri* and *Schoenus apogon* described by Keith (2004) as montane bogs.

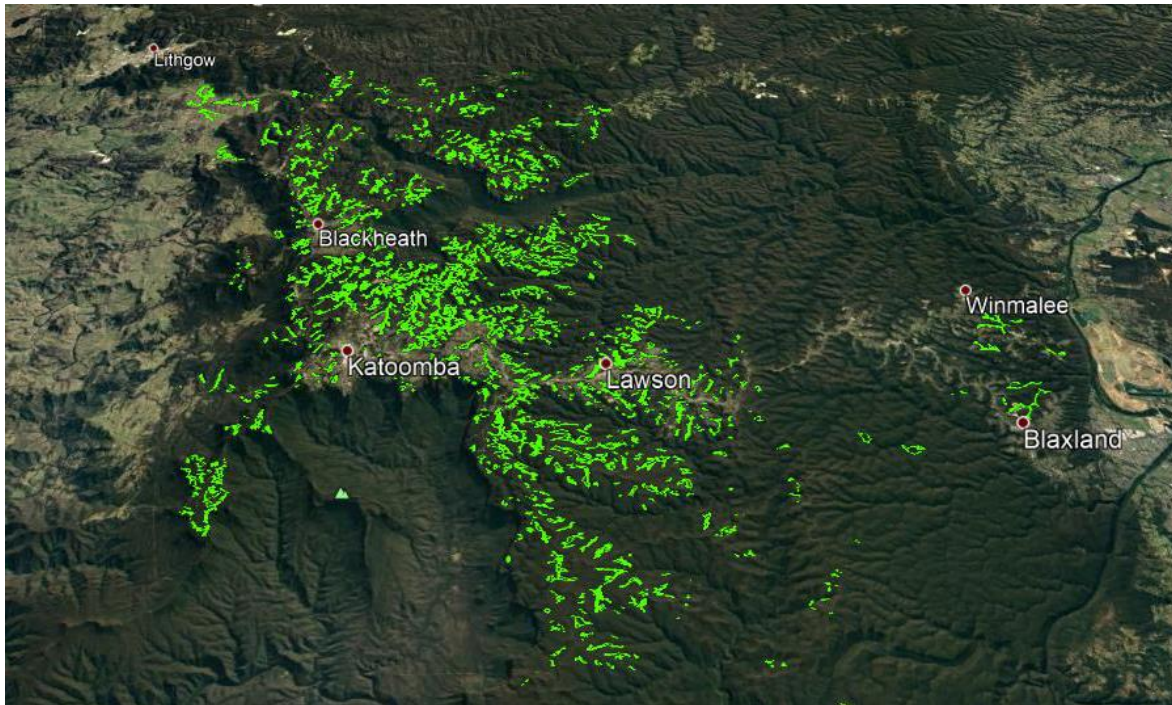


Figure 1. Distribution of Blue Mountains Temperate Highland Peat Swamps on Sandstone.

Source: Google Earth (2018 Google DigitalGlobe CNES/Airbus.) available at

<https://datasets.seed.nsw.gov.au>.. Areas delineated as bright green are swamp locations

These swamp soils are naturally nutrient poor, especially lacking in phosphorus (Keith & Myerscough, 1993). The complexity of sedimentary layers within the swamps, their variability in slope, morphology, aspect and size combined with groundwater or rainfall derivation, naturally ensure high intrinsic variability in groundwater level and vegetation. Depth of the swamp strata rarely exceeds 3 metres (Cowley, 2017). Superimposed on these natural features, continued urban development on the escarpment above and within the swamps has altered catchment permeability and consequent hydrology (Cowley, 2016).

Conditions in the swamps are geomorphically heterogeneous at relatively small scales (Fryirs, 2014a). The age of these topogenous swamps is understood to be mostly Holocene in origin, having commenced following the last Glacial Maximum (Nanson and Cohen 2014). Warming temperature and increasing rainfall at the start of the Holocene is thought to have created suitable conditions that led to the accumulation of peat. In the Blue Mountains, erosion of the sandstone escarpment into gullies of low profile, combined with vegetation dominated by sedges and other Restionaceae and humid conditions, enabled rapid development of peat fens. (Hope, 2002).

Differences in vegetation, aspect, slope altitude, fire and geological conditions are thought to be influential in determining the variation in current day peat swamps on Sydney sandstone. They are commonly found between 600 and 1100 m a.s.l (Fryirs, 2014a). Due to their geological base, many of the swamps comprise layers of sand and alternating peat, so are more commonly peaty sands rather than continuous peat (Fryirs, 2018). The surfaces are mostly comprised of surface organic fines.

The Blue Mountains swamps are generally classified as porous aquifers (Gibert, 2009) with extensive peaty sand deposits (Fryirs, 2014a). Depths of groundwater are limited by depth of the sedimentary layers, at the surface by terrestrial vegetation below by underlying basal sandstone (Figure 2). These depths, mostly less than 3 metres and with high slopes that mitigate against long term storage, with complex sedimentation and hydrology (Cowley, 2016; Fryirs, 2014a) are likely to provide variable habitat that is unlike both surface and ground water aquatic systems.

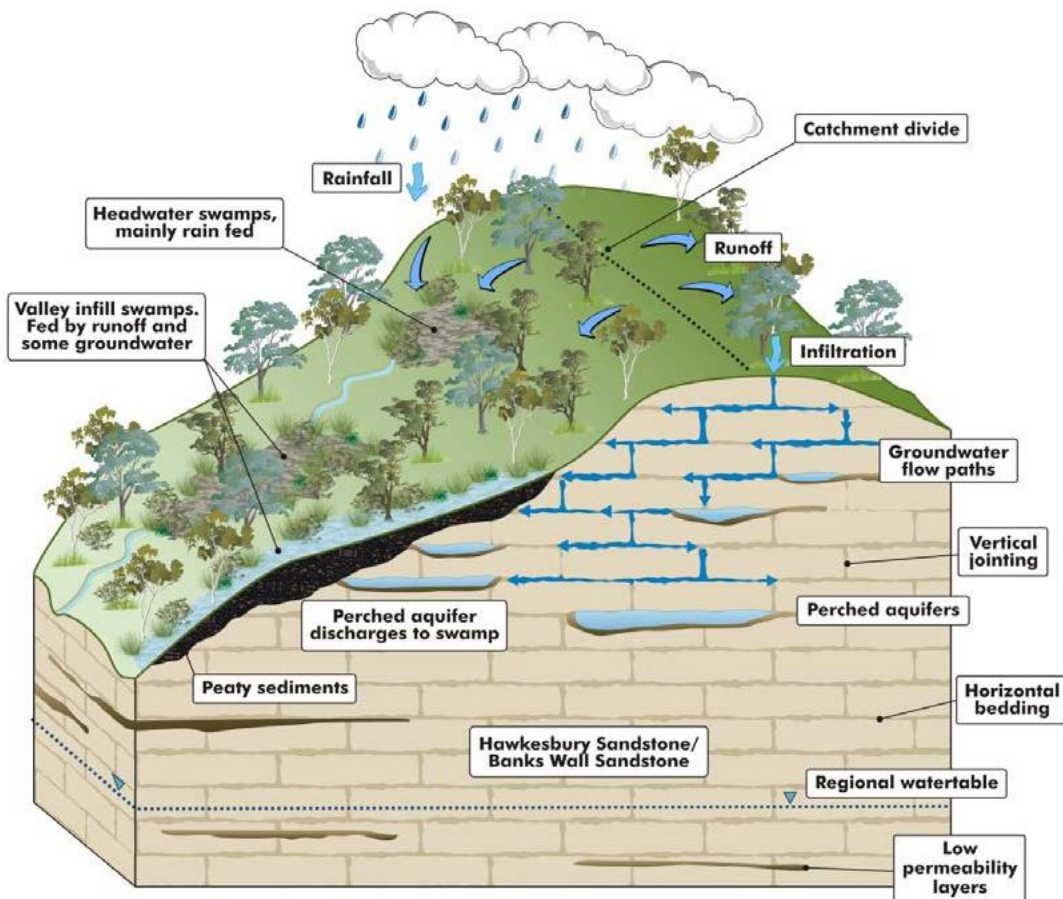


Figure 2. Conceptual block diagram for the occurrence of perched aquifers and vertical connectivity enhanced by vertical jointing in the Hawkesbury Sandstone (Source: Jacobs-SKM. (2014).

The habitat itself can best be described as shallow subterranean habitat (Culver & Pipan, 2011). Shallow subterranean habitats (SSH) exhibit features including groundwater-fed habitats that are less than 10 metres deep, are common across the landscape, experience daily temperature variation, are rich in organic matter compared to other groundwater habitats and connected closely to surface conditions. Examples given by Culver and Pipan, (2011), include seepage springs, epikarst and hyporheic areas around river channels but the peaty sand swamps of the Blue Mountains are a rare and complex example of SSH's that have been poorly studied and understood.

Features of Peat Mires or Swamps

Peat formation

Peat swamps form in conditions where organic matter has deposited over time, forming most rapidly under warm and wet conditions. They commonly comprise up to 90% water (Pemberton 2005). Peat formation has more commonly formed in areas with higher latitude, through northern Europe and in the southern latitudes of greater than around 40 degrees Shotyk (1988). Conditions in functioning peat swamps may be harsh, with low dissolved oxygen and nutrient levels, with high acidity (Pemberton 2005). In Australia, peat mires are mostly poorly formed, only found more extensively in Tasmania (Whinam *et al.*, 2003); (Pemberton 2005). In south eastern Australia, peat formation has occurred in many small headwater catchments, where sediment has formed in valley fills (Nanson & Cohen, 2014). Accumulation of peat and sandy sediment in such small narrow valleys may be due to intrinsic factors such as threshold valley slopes, or extrinsic factors such as climate (Nanson and Cohen 2014).

Common in higher latitude peat, *Sphagnum* forms a relatively rare component of most peat mires in south eastern Australia, which are instead composed of terrestrial derived peat from surrounding and local vegetation (Pemberton, 2005). Generally, geochemistry of peats is a function of their plant origin, with bogs dominated by *Sphagnum* moss, fens by *Carex* and

swamps by woody plant detritus, at least in Northern Hemisphere studies (Shotyk 1988). They are often acidic but may be moderated by surrounding soils and sediments. Storage of carbon in peat is considerable, with carbon dynamics related to storage and loss of major importance in global carbon cycling (Grover & Baldock, 2010).

As peats are laid down sequentially and under variable climate conditions, they contain not only records of past climatic and environmental conditions but ecosystem responses to climate change (Bigler, 2001). Deposited remains of aquatic and terrestrial organisms such as diatoms, chironomids and other insects, pollen and plant macrofossils, provide a record of Holocene environments (Bigler 2001). Furthermore, they are internally and externally variable, biologically diverse and highly endemic (Coronel *et al.*, 2007; Warner & Asada, 2006). Variability in altitude, aspect and catchment character provides a template that provides a diversity of opportunities for biological succession. In addition, climatic history and fire provides further mosaics. This variability manifests itself in strong spatial heterogeneity of vegetation (Kato *et al.*, 2010). Continued peatland vegetation development leads to ‘hummock’ and ‘hollow’ characteristics. Generally, hummocks may be above the water table and support shrubs and peat mosses, while hollows occur below the water table and include water logging tolerant bryophytes and other plants (Kato *et al.*, 2010).

Peatlands (Gore, 1983; Pemberton, 2005) are classified according to vegetation, water chemistry, water source and hydrology: bogs have complex vegetation including mosses and shrubs, rainwater fed, mineral poor, low pH (pH<5.0); fens support grasses, sedges and rushes are groundwater fed with less acid (pH>6.0), higher nutrients and higher mineral content; moors have open sedge-shrubland on shallow organic peats and soils (Hope, 2002; Hunter & Bell, 2007; Pemberton, 2005). More complex classifications based on floristics have been provided for *Sphagnum* communities (Whinam *et al.*, 2003), morphology, water chemistry and soils (Pemberton, 2005). Rates of formation and loss are also highly variable, with anthropogenic loss extensive and rates of formation complicated and related to water availability and thickness of the aerobic surface layer (acrotelm) (Belyea & Clymo, 2001).

The diplotelmic mire hypothesis suggests that there is a strong difference between the highly organic aerobic acrotelm and the more dense anaerobic layers exhibiting lower decomposition

rates (Ingram, 1978). These conditions appear to be relevant to THPSS (Cowley, 2017), particularly for carbon dynamics although the system is complicated by significant sand deposits.

The hummock and hollow nature of peat swamps discussed above (Kato *et al.*, 2010) mean that peat mires provide extremely heterogeneous patches of vegetation habitat, hydrology (Belyea & Baird, 2006) and consequent patchy distribution of small, immobile animals. As ecotonal ecosystems, both terrestrial and aquatic invertebrates comprise the faunal biodiversity at relatively small scales, leading to heterogeneity in invertebrate communities within individual wetlands (Batzer & Wissinger, 1996). These communities comprise both invaders from surrounding aquatic or terrestrial systems or are specific to peatlands. Channels exhibit distinct longitudinal aquatic connectivity, with colonization by stream dwelling invertebrates and dispersal by drift and active movement (Bilton *et al.*, 2001), whereas swamps where water movement is entirely hyporheic, restrict dispersal processes (Dole-Olivier, 2009). Some species, such as groundwater isopods and amphipods may employ active dispersal, however, obligate groundwater species exhibit low fecundity, lack of larval dispersal and long developmental stages, which limits their dispersal ability (Dole-Olivier, 2009).

Aquatic invertebrate communities are strongly associated with their habitat, in particular vegetated habitat (Clapcott *et al.*, 2012; Duggan *et al.*, 2001; Kato *et al.*, 2010; Whatley *et al.*, 2014a) and the variability in size, shape, altitude, aspect, stratification, vegetation, and water quality differences in swamps may produce very diverse and variable invertebrates communities at quite small scales. Generally, aquatic invertebrates are thought to be more successful in dispersing long distances than terrestrial invertebrate taxa, and may tend to be k-strategists, produce less eggs and invest more in 'parenting' than terrestrial invertebrates (Kappes *et al.*, 2014). Some species, such as groundwater isopods and amphipods may employ active dispersal, however, obligate groundwater species exhibit low fecundity, lack of larval dispersal and long developmental stages, which limits their dispersal ability (Dole-Olivier, 2009). Therefore, environmental predictability in aquatic habitats would be expected to be higher than for terrestrial habitats (Kappes *et al.*, 2014), but in Australia, with one of the more unpredictable aquatic systems in the world, this would have to be tested. It is likely that aquatic invertebrates in peat swamp systems in Australia, where variability is at a premium within small spatial scales, need to adopt more flexible and adaptive life histories and to be more generalist than specialist.

Elsewhere, peat bog food webs reflect high specificity between single plant species and detritivores, however stable isotope analysis indicated that terrestrial predators may forage on both aquatic and terrestrial prey in peat swamps (Kato *et al.*, 2010), thus linking the aquatic and terrestrial food webs.

Hydrology

Hydrology is complex, sourced by both rainfall and groundwater (Pemberton, 2005). They may naturally be channelized or entirely groundwater based. While anthropogenic activities may create drainage lines through peat mires, if the groundwater layer (catotelm) is intact, these may have only a small impact on hydrological processes within the mires (Nanson *et al.*, 2014). Furthermore, peat mires without channels may transfer water as groundwater at highly variable rates, depending on slope, composition of the peat layers, vegetation and water content (Fryirs, 2014b; Grover & Baldock, 2013). Peat swamp vegetation is variable, in rainfall derived (ombrotrophic) or groundwater derived (minerotrophic) (Figure 3) habitat across and between swamps (Charman, 2002).

Depending on morphology of the individual peat mires, flows may range from low hydraulic conductivity within intact peat to flashy spate conditions. Erosion may increase sediment transport in peat streams and low dissolved oxygen is common, leading to perceptions that peat mires support few stygofaunal communities (O'Driscoll *et al.*, 2014).

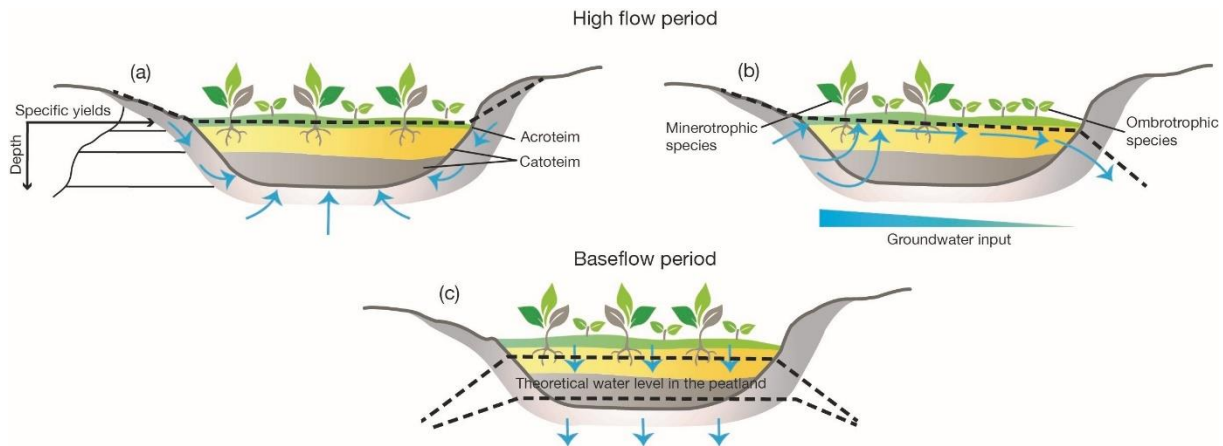


Figure 3. Conceptual diagrams for hydrologic patterns in the peat-interface fed by groundwater. (a) High flow period for a homogenous groundwater-fed peatland. (b) High flow period for a system locally fed by groundwater; water-table level will vary as a function of hydrological conditions but also as a function of peat material hydrodynamic properties. (c.) Baseflow period. The high specific yield of peat mat layers may sustain wet conditions and water availability near the surface. Source: Bertrand et al. (2011), redrawn Worthington (2019).

In the Blue Mountains upland swamps, varied geomorphology has set a template that has had profound impact on their hydrology (Fryirs et al.) leading to highly variable and individual hydrological conditions. Mostly valley fill swamps in elongated and relatively steep catchments, they are fed by a combination of rainfall, surface flow and groundwater seepage from surrounding sedimentary rock strata (Cowley 2018). Layered strata of peat and sands. As well as high slope naturally lead to rapid rainfall response and stable baseflow within the swamps. Channelization, altered morphometrics and vegetation and other urban impact effects have altered hydrology, reducing groundwater levels, resulting in dewatering (Cowley 2018).

These swamps vary from other swamps globally in being mostly groundwater in nature. Where streams exist within the swamps, they are often fast running on a sandstone base due to relatively high slope. Swamps of low relief only rarely exhibit the open ponds that are common elsewhere (O'Driscoll et al., 2014; Warner & Asada, 2006; Yule, 2010).

Global loss of peatlands

Globally, peatlands have been significantly lost, due to continued drainage and clearing for urbanization and agriculture (Mazerolle *et al.*, 2006), harvesting for fuel and horticultural use. In Australia, mechanisms include draining and clearing for agriculture, impacts of longwall mining, coal seam gas exploration, climate change and bushfires (Booth *et al.*, 1998; Grover *et al.*, 2012; Hennessy, 2004b, 2004a; Keith, 2006). Habitat loss and fragmentation are key to the broadscale extinctions now evident in all aquatic ecosystems (Baguette *et al.*, 2013). Apart from direct biodiversity and ecosystems function losses as a result of these cumulative impacts, there is strong evidence that secondary effects of peatland loss include increased carbon dioxide emissions as the large store of peat carbon is oxidized (Jaenicke *et al.*, 2010; Strack *et al.*, 2011).

Aquatic invertebrates, their habitat and peat swamps

Freshwater species comprise nearly 6% of all described species, despite freshwater covering only around 1% of land area. Furthermore, the extinction rates of these fauna are estimated to be up to 5 times greater than for terrestrial taxa so freshwater habitats and species are considered some of the most threatened ecosystems in the world (Dudgeon *et al.*, 2006). In particular, Australian freshwater ecosystems are highly endemic and biologically significant, but are not well protected (Stewart, 2011). Aquatic invertebrate communities are usually strongly associated with their habitat, in particular vegetated habitat (Clapcott *et al.*, 2012; Duggan *et al.*, 2001; Kato *et al.*, 2010; Whatley *et al.*, 2014b) and the variability in size, shape, altitude, aspect, stratification, vegetation, and water quality differences are likely to produce very diverse and variable communities at quite small scales.

Furthermore, aquatic invertebrate communities in spring or groundwater fed habitats, while a comparatively small aquatic ecosystem type worldwide, are particularly biodiverse, highly endemic and are at high risk (Bogan *et al.*, 2014). Small aquatic habitats such as pools or ponds are thought to be important in aquatic biodiversity conservation (Coronel *et al.*, 2007). They may act as aquatic refuges or islands, maintaining high γ diversity, while supporting high aquatic variability within individual ponds (Coronel *et al.*, 2007). It is likely that aquatic invertebrates in peat mire systems in Australia, where variability is at a premium within small spatial scales, may need to adopt more flexible and adaptive life histories and to be more generalist than specialist.

Elsewhere, peat bog food webs reflect high specificity between single plant species and detritivores. A recent Japanese study used stable isotope analysis to show that terrestrial predators may forage on both aquatic and terrestrial prey in peat mires (Kato *et al.*, 2010), thus linking the aquatic and terrestrial food webs.

Growing knowledge of stygofauna and why groundwater is important

The invertebrate fauna of groundwater systems (stygofauna) are slow in gaining attention and recognition long focussed on surface waters. This is despite aquifers containing 97% of global unfrozen freshwater (Danielopol *et al.*, 2003) and significant groundwater use by humans. More than two billion people rely on groundwater as their primary water source (Famiglietti, 2014), an average of 43% of irrigation is groundwater derived (Siebert *et al.*, 2010) and the groundwater footprint (the area required to sustain groundwater use and groundwater-dependent ecosystem services) is currently 3.5 times the available aquifer area (Gleeson *et al.*, 2012). Groundwaters are poorly understood, despite the fact that they may contain considerable biodiversity (Gibert, 1994; Griebler *et al.*, 2014) and greater emphasis on increased knowledge may yield a greater understanding of groundwater quality and provide substantial ecosystem services (Sorensen *et al.*, 2013). And within groundwater studies, bias towards more accessible groundwater (karstic springs, subterranean rivers and caves), lack of taxonomic knowledge of groundwater fauna and the high number of cryptic species, mean that porous aquifers such as upland peat swamps have been barely investigated. Advancing knowledge of groundwater systems is beginning to lead towards ecological recognition, development of assessment frameworks and an understanding of groundwater ecosystem services, but the pace is slow (Griebler & Avramov, 2015; Griebler *et al.*, 2010; Murray *et al.*, 2006; Tomlinson & Boulton, 2010)

The aquatic invertebrates of Australian peat mires have been poorly studied, with little known of their community structure, α , β and γ diversity, dispersal abilities and endemism. Furthermore, the environmental services they provide, under changing environments, is unknown (Prather *et al.*, 2013). In general, studies have concentrated mostly on terrestrial or open water macroinvertebrate fauna, (Spitzer, 2005; Whatley *et al.*, 2014a; Yule, 2010). Significant temporal and spatial patterns have been found in macroinvertebrate communities in some streams

draining peat mires in Ireland (O'Driscoll *et al.*, 2014) and high diversity in others in England (Ramchunder *et al.*, 2011). While invertebrate metrics may not change, succession in community structure and relative abundance of macroinvertebrates with stream size is known to differ (Ramchunder *et al.*, 2011). But meiofauna (those smaller than 500 μm), a common component of groundwater, continue to be unrepresented in aquatic studies generally, despite being ubiquitous in all freshwater environments.

The combination of specialized aquatic conditions existing in both peat swamps and in groundwater creates a habitat where a complicated functional community that may not identify with either may be distinctive in its individuality. Peatland invertebrates may therefore be highly specialized depending on their affinity with bogs; occurring only in bogs (tyrphobiontic), typical of bogs (tyrphophilous), facultative inhabitants (tyrphoneutral) or accidental immigrants (tyrphoxenous) (Spitzer, 2005). Common invertebrates in northern hemisphere peat swamps include chironomids (Brown *et al.*, 2016), ants and carabid coleopterans (Brigić *et al.*, 2017), an ecotonal community of aquatic and terrestrial fauna. In Australian peat swamps groundwater fauna are dominated by microcrustaceans (copepods, syncarids, ostracods and other groups), Nematoda, Oligochaeta, Acarina and chironomids (Bailey, 2010; Hose, 2017) and terrestrial invertebrates including chironomid adults, Collembola, ants, spiders and Thysanoptera (Pusey & Bradshaw, 1996).

Invertebrates inhabiting the swamps selectively choose microhabitats that suit them (Hose, 2017) and may be relatively adaptable to changing conditions, but also form heterogeneous communities at small scales as well. Because peat swamps include groundwater systems, aquatic invertebrates may also be termed stygofauna or groundwater fauna. Variable anthropogenic influences, ecotonal groundwater conditions and complex sedimentary layers may create novel stygofaunal communities and enhance proportions of stygoxenes (accidental stygofauna) and stygophiles (stygofauna inhabit groundwater preferentially but not obligately) relative to obligate groundwater fauna (stygobionts) (Hose, 2015).

High biodiversity in groundwater

Recent discoveries of the unexpected high biodiversity of groundwaters, including most of the taxonomic groups found in surface water habitats, mean that groundwater is an important biodiverse environment worthy of investigation and broad scale protection (Gibert, 2009). Stygobionts, (obligatory groundwater-dwelling species) constitute only 5% of freshwater fauna known to date, but up to 43% of the known Crustacea (Gibert, 2009). Furthermore, due to difficulties in studying groundwater ecosystems, biodiversity is likely to be highly underestimated.

Knowledge of Australian subterranean fauna is increasing with emphasis on hyporheic zones in alluvial aquifers (Hancock *et al.*, 2005; Hancock, 2008) and karstic caves in arid Western Australia (Eberhard *et al.*, 2009; Halse, 2002; Humphreys, 2008), which are now recognized as extremely rich biodiversity hotspots for stygobiotic fauna. More recently still, a growing understanding of the impact of environmental factors on groundwater fauna has enabled the development of methods to identify groundwater health based on stygobiotic indicators in alluvial aquifers (Hose, 2015; Korbel & Hose, 2011, 2015).

Lack of study in groundwater systems in Australia

However, many groundwater systems in Australia have not been studied at all, leaving vast subterranean areas likely to maintain significant biodiversity that hasn't been discovered. Further, identification of Australian subterranean fauna is in its infancy, so that species level identification is fraught. Temperate south-eastern Australian limestone caves have revealed significant biodiversity, but there is a need for additional sampling effort in non-limestone environments in the region (Guzik, 2011). The aquatic invertebrates of Australian peat mires have been poorly studied, with little known of their community structure, α , β and γ diversity, dispersal abilities and endemism. Furthermore, the environmental services they provide, both positive and negative and under changing environments, is unknown (Prather *et al.*, 2013).

Features of Chain of Pond Ecosystems

Geomorphic background and identity - Mulwaree Ponds

Mulwaree chain-of-ponds is a geomorphic and hydrological anomaly that has created an unusual aquatic ecosystem. Currently mostly relictual, the upper Mulwaree River runs largely along the Mulwaree Faultline to the South of Goulburn NSW, Australia (Thomas, 2013). The river, once much larger, became disconnected from its headwaters in the Quaternary by capture from incision to the Shoalhaven River, followed by alluvial deposits that created a groundwater source for Lake Bathurst (Abell, 1995). Lowering of the Wollondilly River maintained flow of the river to the north east. The base of Lake Bathurst (666 m.A.S.L) sits below Mulwaree Creek (675 m A.S.L), only contributing to the river during flood conditions, when a depth of 12 metres enables overflow to Mulwaree Creek across the western sill at 678.s m ASL (Abell, 1995).

Formation during Holocene

Hydrological disconnection occurred in the Holocene during conditions of low sediment supply, lack of continuous channels and presence of densely vegetated swampy meadows (Mould & Fryirs, 2017; Prosser *et al.*, 1994). These conditions were instrumental in formation of the current chain-of-ponds system. The current river flows seasonally as a meandering shallow channel within a well-developed palaeochannel floodplain (Williams, 2018). The ephemeral ponds cut into deep and coarse palaeochannel sediments (Williams, 2016), are a rare remnant of a river geomorphology now largely extinct. Fine surface sediments and a wide floodplain limit lateral surface flows and a bedrock control downstream near the confluence with the Wollondilly River limit longitudinal stream energy (Williams, 2018). These ponds can be distinguished from the swampy meadows described by MacTaggart (Mactaggart *et al.*, 2008) by having defined pools, interspersed by vegetated swampy meadows, with stable geomorphology in infilled valleys of low slope and a downstream geomorphic control.

Structurally, Mulwaree Ponds are best described as a chain-of-ponds or a stream with low flow regime (Mactaggart *et al.*, 2008) and as a river, intermittent (Datry *et al.*, 2017). Steep sided

scour pools, interspersed by sedimented and vegetated channels, developed since the Holocene, are well documented along the western slopes of southeastern Australia (Cohen & Nanson, 2007; Eyles, 1977a; Hazell *et al.*, 2003; Mould *et al.*, 2017). Widespread clearing and agricultural development post European colonization has decimated most of these chain-of-pond systems, leaving virtually no examples (Eyles, 1977b). Once ubiquitous, (MacTaggart *et al.*, 2007), most chain-of-ponds systems on the south west slopes of NSW converted into eroded streams from the period of European settlement in the 1820s to the 1950s (Cook, 2018; Eyles, 1977; Hazell *et al.*, 2003), but which have since seen signs of landscape recovery (Rustomji & Pietsch, 2007). These systems underwent substantial gully erosion, controlled more by thresholds of incision into the vegetated valley floors, than by changes to upstream sediment supply (Prosser *et al.*, 1994). Once these discontinuous pond systems were altered to more continuous channels, they then had increasing capacity to transport flow, sediment and other materials (Brierley *et al.*, 1999). Agricultural practices in the heads of catchments led to increased stream power, thus converting these small discontinuous channels carrying fine sands deposited locally to large, incised, and bedload dominated channels with low hydraulic and corresponding ecological diversity (Brierley *et al.*, 1999; Hazell *et al.*, 2003).

Altered streams have become more permanent, turbid and with active gully erosion, supporting phytoplankton and microbenthic food webs (Deegan & Ganf, 2008) rather than periphyton and macrophytes (Boulton, 2014). Impacted hydrology has variously increased groundwater levels and liberated ancient deposited salinity (Eberbach, 2003), combined with European farming practices that have increased eutrophication (Davis & Koop, 2006). These changes have altered carbon dynamics in streams in south eastern Australia (Sheldon & Walker, 1997) and represent a significant loss of productivity to downstream aquatic ecosystems, as nutrients and carbon once accumulated for later release are continually released as different forms. Furthermore, loss of riparian and upstream vegetation that would have fueled food webs originally, no longer do so (Reid, 2008)

Furthermore, climate change projections that may substantially alter rainfall seasonality, with less in winter and spring and more during autumn (NSW Government, 2018), may alter pond stratification dynamics and affect productivity. But it is increased variability that may create

more intense flows and greater disturbance that is likely to impact pond ecology the most (Lake, 2000). All of these stressors are likely to threaten this unusual aquatic system.

Riparian conditions

Riparian conditions in the Mulwaree catchment are highly altered. Once open woodlands of *Eucalyptus melliodora*-*E. blakelyi* on the low slopes and plains, with *Acacia mearnsii*, *Themeda australis* and *Danthonia pallida* have been replaced by *Panicum effusum*, *Poa* spp., *Stipa falcata* and exotic weeds. The Mulwaree River system exhibits poor water quality in places and high electrical conductivity, total nitrogen and total phosphorus and is mostly cleared of vegetation (EnvironmentACT, 2004; GHD, 2013). Floodplain riparian ecosystems are highly disturbed (EnvironmentACT, 2004).

Major land uses in the Mulwaree catchment include grazing cattle and fine wool sheep and cropping. The majority of the catchment is cleared, increasing runoff and erosion (GHD, 2013). The streambanks are largely cleared of woodland vegetation, replaced with exotic woody shrubs.

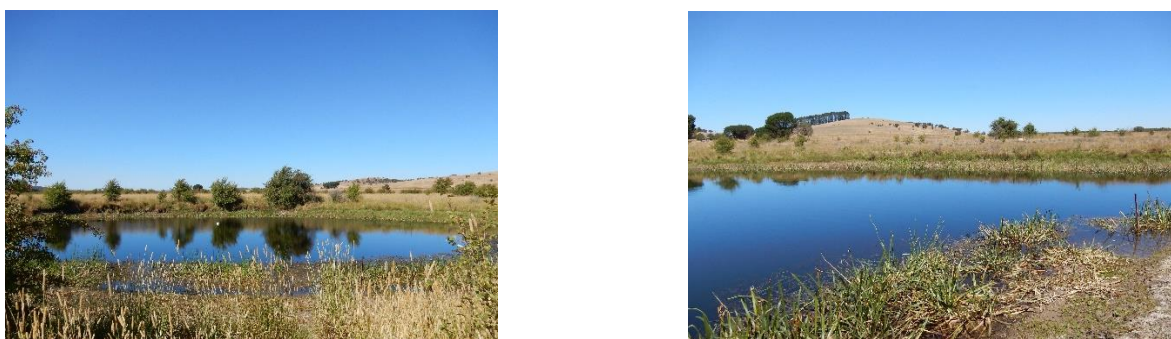


Figure 4. Mulwaree chain-of-ponds during disconnection, January 2017 (Photos: Lorraine Hardwick)

Aquatic macrophytes including *Cyanogeton procera*, *Typha domingensis*, *Eleocharis sphacelata*, and *Myriophyllum propinquum* form a dense margin of aquatic vegetation around the ponds. Aquatic plant diversity is high. Recruitment into the ponds is presumably restricted by water depth, however low turbidity and high recorded nutrients (GHD, 2013) indicate that the ponds are highly productive. Observed aquatic plant gradation appears predictable from the edge of the

ponds to the extent of growth, limited by depth. Gradation from *Carex* spp., *Cyperus* spp., *Eleocharis acuta* R.Br and *Phragmites australis* (Cav.) Trin ex, Steud (to water level) gradating to *Myriophyllum verrucosum* Lindl., *Ludwigia peploides* subsp. *montevidensis*, (to 500 mm depth) *Cyanogeton* sp., (to 1.5 m depth) extends to *Vallisneria australis* S.W.L.Jacobs & Les APNI. (to 3 m depth). Other species are scattered along the littoral zone contributing to high biodiversity within and between ponds.

Hydrological connectivity

The Mulwaree chain of pond system is closely connected by longitudinal connectivity and flows at least for part of the year (Williams, 2018). This system is margin and 'link' vegetated, surrounded by riparian or fringing vegetation. As fringing riparian vegetation changes seasonally and oxygen, temperature and conductivity stratification vary vertically, patch conditions related to pool productivity (Fairchild *et al.*, 2005) are important ecologically.

Furthermore, connectivity within pools may also vary as longitudinal flow changes. Seasonal disconnection of the stream system into separate ponds and drying conditions enables study of effects of ephemerality on productivity. They may have active connection to groundwater, being situated in flat terrain and often in the middle of swampy meadows (Mactaggart *et al.*, 2008) so there may be substantial movement between the surface waters and hyporheos. However, more recent analysis provides uncertainty on groundwater connection (Williams, 2018). The ponds provide an opportunity to study seasonal change. Coupled with predation, pond metacommunity dynamics would be expected to be complex and interesting (Howeth & Leibold, 2013).

Differences between Upland Swamps and Chain-of-Ponds

Montane peat swamps and chain-of-ponds systems represent spatially disparate ecosystems, with individual aquatic systems more or less isolated from others geographically, but also functionally. Mulwaree-chain-of -ponds represents a highly productive and readily available source of carbon, with high primary productivity based on aquatic macrophytes and phytoplankton. The system is active productively. Carbon is sequestered seasonally to created macrophyte and phytoplankton biomass, that is then decomposed and released, either to the atmosphere or to the base of the ponds where it is flushed downstream with connected flow. Adequate nutrients enable rapid

integration and flow enables rapid dispersal of readily available carbon and nutrients for productive purposes downstream. The ponds act on annual budgets.

On the other hand, one of the characteristic features of peat mires is their slow productivity and decomposition rates. They store enormous quantities of carbon, much of it recalcitrant. Carbon in Blue Mountains swamps is stored as sedimentary layers more available in the surface organic fines but increasingly unavailable at depths in the alternating organic sands, stored as recalcitrant, mineralized peat (Cowley, 2016). Peat ecosystems are important to protect as a means of maintaining sequestered carbon, but it also means that management of highly productive systems such as Mulwaree chain-of-ponds will require management to maximize carbon sequestration and maintenance of biodiversity. Finding common defining elements and patterns with which to measure natural character, effects of degradation and impact, is exceedingly difficult.

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Chapter 3

Productivity and stygofaunal assemblages; structural and functional aspects related to urban development in Blue Mountains peat swamps



Lorraine Hardwick

Department of Biological Sciences, Macquarie University

Abstract

The upland swamps in the Blue Mountains are under threat from urbanization. Biodiverse and highly endemic, they sit on the sandstone escarpment just west of Australia's largest city, Sydney. Formed of peat and sand since the Holocene, these swamps are important, not only for biodiversity, but also for water and carbon storage. As groundwater systems, they have been poorly studied, and the stygofauna are little known. In this paper, we investigated the groundwater meiofauna of twelve upland swamps with varying urban impact: from pristine to highly connected to their urban landscape. We found that anthropogenic abiotic drivers have altered hydrology and increased pollutants into the swamps. Less urbanized swamps exhibited lower EC (29 $\mu\text{S}/\text{cm}$) and alkalinity (5.8 mg/L) compared to urbanized swamps (65-125 $\mu\text{S}/\text{cm}$ and 18-38 mg/L). Decomposition of cotton strips were also greater, suggesting that swamp productivity is increased by altered urban impact. The environmental variable most strongly correlated with the stygofauna communities was temperature, with a global correlation of 0.410. Urbanization effects have thus been shown to impact swamp ecological status and function.

Introduction

The Anthropocene continues to be a period of unparalleled ecological change brought about by anthropogenic impacts on the planet (Crutzen & Stoermer, 2000). As this new epoch progresses, and global change becomes increasingly evident, the need to understand, predict and mitigate further changes intensifies. While global climate change models relating increasing temperature to biological response are continually evolving (Crutzen *et al.*, 2000), predictive models for other more local impacts are also gaining traction. The challenge of scale in predicting effects in these models however, is very real.

Global models of change often focus on predicting general patterns at regional scales, but are not designed to address local dynamics (Verburg *et al.*, 2016). Conversely, models at local scale may not translate across spatial boundaries. Thus, there is a need to test and integrate local studies globally to develop better predictive ability and to move conceptual models forward.

One model that is receiving attention from aquatic ecologists worldwide is the “urban stream syndrome” (Meyer *et al.*, 2005; Walsh *et al.*, 2005), which predicts the effects of catchment urbanisation on aquatic systems. These effects include elevated concentrations of nutrients and contaminants, flashier hydrographs, alterations to channel morphology, reduced biotic richness and increasing dominance of tolerant aquatic species. However the impacts of urbanization vary across stream systems (Gwinn *et al.*, 2018; Kollaus *et al.*, 2015; Roy *et al.*, 2009), and there is growing recognition that stream and other aquatic ecosystems vary considerably in their response to anthropogenic impacts, and so a more nuanced approach to management is needed (Booth *et al.*, 2016; Parr *et al.*, 2016) along with greater knowledge across a range of aquatic ecosystem types.

Groundwater dependent ecosystems are particularly poorly represented in the urban stream syndrome literature, despite them supporting many rare and endemic species and providing important ecosystem services, including water storage, supply and purification (Aldous & Bach, 2014). Groundwater dependent ecosystems span a range of surface and subsurface ecosystems (Eamus *et al.* 2006), but include groundwater fed swamps and peat bogs. The need for greater knowledge of groundwater fed swamps and peat bogs systems is particularly urgent because of

the many imminent threats. This is no more evident than in the Blue Mountains of NSW, Australia, where upland peat swamps are threatened by urbanization (Cowley, 2016).

The Blue Mountains upland peat swamps are a unique geomorphic system, situated on the sandstone escarpment west of Sydney, NSW Australia and north and south along the eastern seaboard. The swamps are a common feature (Fryirs, 2015) in that region, but are of such natural value that they are listed under state and federal legislation as an endangered ecological community (Commonwealth Environment Protection and Biodiversity Conservation Act 1999; NSW Threatened Species Conservation Act 2005).

Urban development across the Blue Mountains region, particularly in the escarpment and plateau areas above the swamps, has altered catchment permeability and consequently, hydrology of the swamps (Cowley, 2016). The change to hydrology and introduction of extraneous sediments has been linked with a loss of structural integrity, change to the sedimentary profile of the swamps and lower water content retention (Cowley, 2016). It is likely that as a consequence of these changes to the physical aspects of the swamps, the aquatic fauna and ecosystem function of swamps in urbanized catchments may also differ from those in relatively undisturbed catchments. The urban stream syndrome has proved useful in understanding upland swamp streams (Belmer *et al.*, 2018; St.Lawrence, 2014), and Chapter 4 has shown that rates of ecosystem function (e.g. leaf litter decomposition) in streams emanating from swamps vary with the degree of catchment urbanisation. It is uncertain if and how these effects impact on the ecology of groundwater ecosystems within the peat swamps themselves; if and how aquatic biological communities and ecosystem function are affected.

The saturated sediments of the peat swamps form perched aquifers that are fed, in part, by local groundwater (Cowley et al 2019). The depth of the swamp sediments rarely exceeds 3 metres (Cowley, 2017), creating a habitat best described as shallow subterranean habitat (Culver & Pipan, 2011). Like hyporheic environments where surface water and groundwater meet (Hose, 2005; Tomlinson & Boulton, 2010), swamps may be viewed similarly as an ecotone between surface water and groundwater, in which the diversity of invertebrates would be expected to be high.

Invertebrates inhabiting natural swamps in catchments nearby are known to selectively choose microhabitats within the sedimentary layers (Hose, 2017). These communities include a mix of invertebrate fauna typical of both terrestrial or soil environments, surface waters in the region, and a suite of fauna (termed stygobionts) that are morphologically adapted to a life in the subsurface (for example, lacking eyes and colouration).

In general, studies have concentrated mostly on terrestrial or open water macroinvertebrate fauna, (Spitzer, 2005; Whatley *et al.*, 2014; Yule, 2010). Groundwater meiofauna (those smaller than 500 μm) in peat swamps continue to be unrepresented in aquatic studies and are poorly known. The combination of specialized aquatic conditions existing in both peat swamps and in groundwater creates a habitat where a complicated functional community, that may not identify with either, may be distinctive in its individuality.

Aims

The aim of this study was to examine the influence of catchment urbanization on the structure and function of swamp groundwater ecosystems. It was hypothesized that: there is a negative relationship between urban impact (catchment imperviousness and distance to urban infrastructure) and the stygofaunal community structure and ecological function of the swamps. It was hypothesized that these catchment impacts affected decomposition processes which in turn correlated with changes in stygofaunal communities. To achieve this, the site and catchment characteristics of 12 swamps across the Blue Mountains region were used to quantify the relative extent of urban impact at each site. The groundwater invertebrate communities and organic matter decomposition were also measured and related to the site and catchment characteristics.

Methods

Study Sites




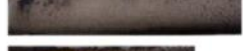


Twelve upland peat swamps were chosen between Wentworth Falls and Mt. Victoria in the Blue Mountains region of New South Wales (Figure 1). Described by Keith (2004) as montane bogs, the swamps sit atop the sandstone escarpment of the Great Dividing Range west of Sydney. The swamps can be classified as porous aquifers (Gibert, 2009a), comprising extensive peaty sand deposits, typically to a depth of up to 3 m. (Table 1, (Fryirs, 2014)). The sedimentary sequence is relatively consistent across the swamps of the region (Fryirs, 2014).

Formed during the Holocene as valley fill topogenous swamps, they comprise layers of sandy peaty deposits atop a sandstone ironstone base (Fryirs, 2014). The swamps form in depressions in the landscape in which vegetative organic matter, which, when saturated by ground and surface water inputs, decomposes slowly, leading to the formation of organic-rich, peat-like sediments. Organic content of the sediments typically decreases, and bulk density increases with depth. Depth of sediment layers vary between and within individual swamps, creating mosaics of habitat at small spatial scale (Fryirs, 2014; Hose *et al.*, 2017).

The swamps are highly biodiverse, supporting many threatened and vulnerable endemic biota, including the plants *Dillwynia stipulifera*, *Boronia deanei subsp. Deanei*, the giant dragonfly – *Petalura gigantea* and the Blue Mountains water skink – *Eulamprus leuraensis* (Benson, 2012). Common vegetation comprises xeromorphic shrubs such as *Grevillea acanthifolia*, *Epacris breviflora*, *Hakea microcarpa* and *Leptospermum obovatum*. Common grass like plants include *Gymnoschoenus sphaerocephalus*, *Lepidosperma filiforme* and *Poa labillardieri*, with *Gleichenia dicarpa* the most common fern in most natural swamps (Keith, 2004).

Swamps used in this study were chosen from swamps with existing groundwater piezometers, using a stratified design. Piezometers were installed both by Blue Mountains City Council for community Landcare/Swampcare/Bushcare monitoring (PP, MS, AS, WFL, PG) and for Macquarie University research purposes (MH, GC, WC, TG, ME, KFR, PS). Swamps spanned the range of catchment urbanization derived from Fryirs (2016), included both channelized and intact systems and provided a spatial coverage across the region.

Table 1. Photograph of a sediment core showing characteristics s of sediment classes found in swamps (Reproduced from Fryirs et al., 2014)

Sediment Class	Typical thickness (cm)	Bulk density (g/cm ³)	Organic content(%) Averaged across sites	Photograph
Surface organic fines	20-100	0.45	31.7	
Alternating organic sand	5-55	0.87	13.1	
Fine cohesive sand	5-35	1.11	7.6	
Basal sand and gravel	5-45	1.32	2.6	
Saprolite		1.22	2.6	
Contemporary sand	Variable	1.17	2.4	

Site Mapping

Catchment areas were manually calculated using SIX (Spatial Information Exchange) maps. The NSW topographic base map (Spatial Services, 2017) with a combination of Digital Topographic Database (DTDB), Geocoded Urban and Rural Addressing System (GURAS) database and the Digital Cadastral Database (DCDB) file identifier (018E63C0-56D2-4298-94D8-9BA17E26D347) were accessed.

Contour based mapping of swamp area and length was manually derived, using this remote sensed resource. Verification was performed with high resolution satellite imagery sourced from DSFI Spatial Services NSW. The layer depicts an imagery map of NSW showing a selection of SPOT5® satellite imagery, standard 50 cm orthorectified imageries, high resolution 10 cm Town Imageries, revised 17/7/2017.

Quality control was performed by duplicate manual mapping to verify method, with <10% difference between derived area. Altitude and slope were derived from Google Earth Pro (Google Inc, 2015). Where possible, pre-existing data for total percent impervious cover in the catchments for the swamps were obtained (Fryirs, 2015) where catchment boundaries were derived from 2002 SPOT raster imagery imported to ARCGIS. Vegetated colourations were then

removed and impervious area defined by two colour values, then converted to vector polygons. These were then intersected with the catchment area layer and impervious area calculated followed by derived percentages. These values were validated manually for several swamps using Google Earth Pro distance tool (Google Inc. 2015) by manually measuring cover of impervious units within the catchment and subtracting that value from catchment area. Distance to stormwater and sewerage infrastructure were also calculated manually using Google Earth Pro distance tool (Google Inc. 2015) overlain with Blue Mountains City Council (BMCC) infrastructure spatial layers.

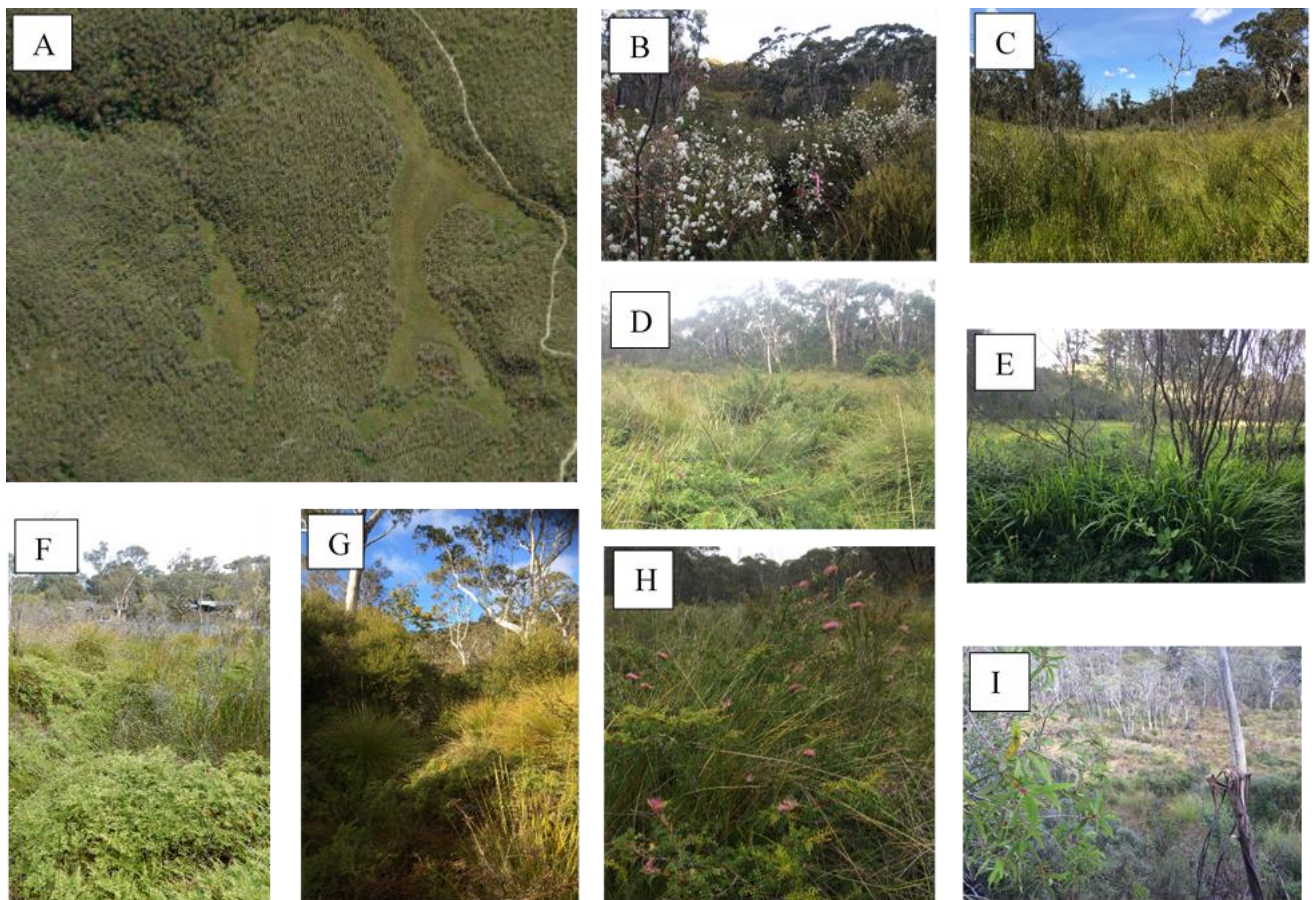


Figure 1. Upland swamps in the Blue Mountains. A. Upland peat swamp from above, near North Hazelbrook, NSW (maps.six.nsw.gov.au) B. Michael Eade Reserve, C. Asgard swamp, D. Marmion rd., Yosemite swamp, E. Pitt Park swamp, F Fifth Ave. swamp, G. Mt. Hay swamp, H. Grand Canyon swamp, I. Mt. Hay swamp.(Photos: Lorraine Hardwick)

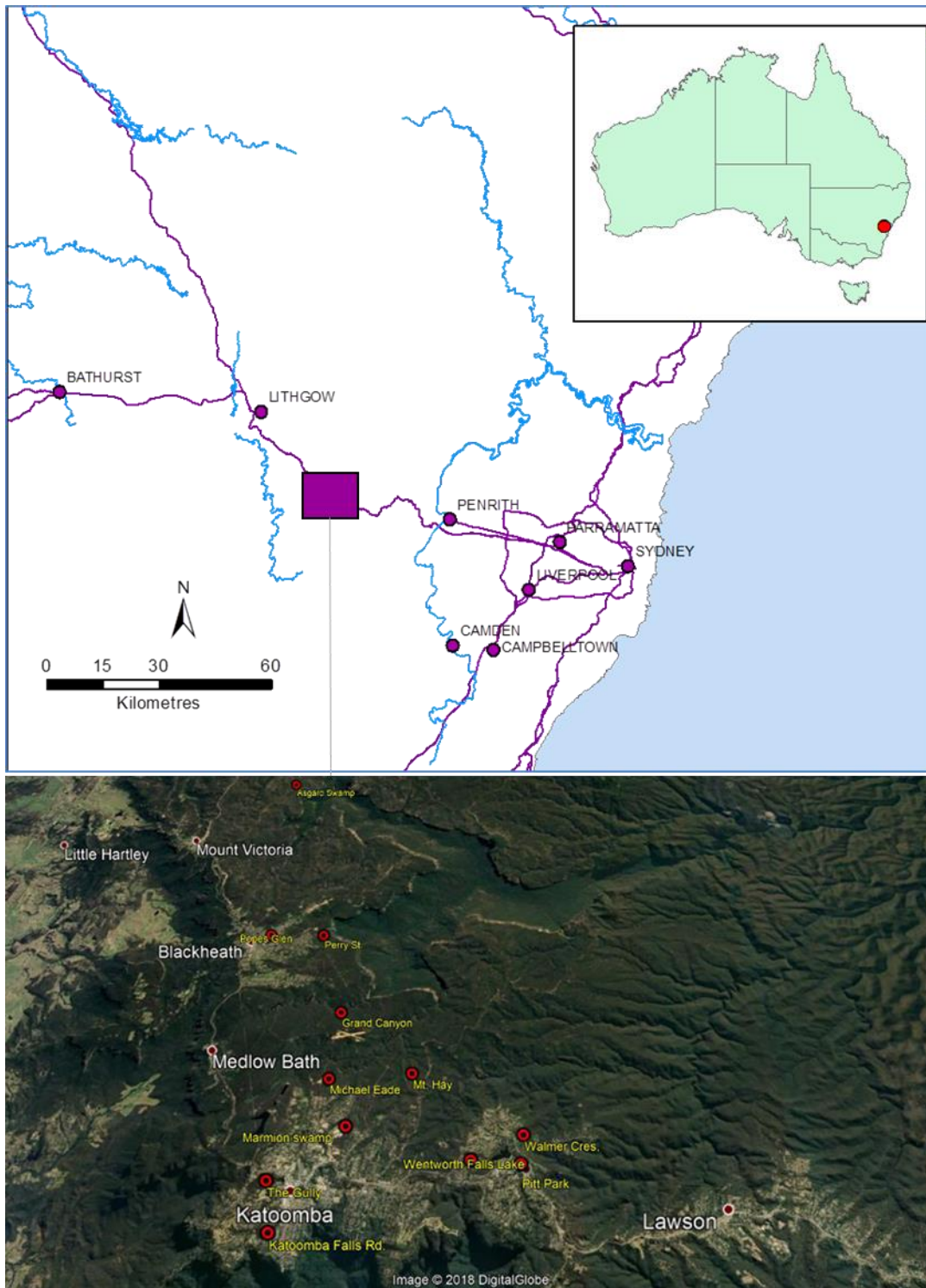


Figure 2. Swamp sites with installed piezometers. Map above, prepared in ARCGIS Version 10.6.1 2018 Esri. Map below source: Google Earth Pro 7.3.2.5491 (64-bit), 2018 Google LLC.

Hydrology Methods

Piezometers had been previously installed in the central line of the swamps (Cowley, 2016) using a Russian D corer and auger. The piezometers were constructed of 50-mm diameter slotted PVC pipe and sealed with end caps and well (top) caps (Thermofisher Scientific - www.thermofisher.com.au). The pipe was slotted (1-mm width) over its entire depth. The piezometers extended through the sedimentary alluvium to sandstone bedrock and thus varied in depth (Table 2). One piezometer in each of the swamps was surveyed (Table 2). Static water levels and time to refill were collected at each visit to calibrate groundwater level loggers. Depth loggers (Levellogger Junior, Model 3001, Solinst Canada) were installed in each piezometer at 20 cm above the base of the piezometer. Water levels were recorded at 30-minute intervals between June 2016 and August 2018. Barometric pressure compensation was achieved using a barometric logger (Barologger Edge Solinst, Canada, Model 3001) installed above water level at Mt. Hay. This site was within the accepted geographical range (300 m altitude and 20 km distance) of all other loggers (Solinst.com). Data was downloaded using Solinst Levellogger software 4 (version 4.2.0).

Rainfall data were derived from rainfall observations for September 2016 to August 2018 at Faulconbridge (station no.63081), Katoomba (station no. 063039) and Blackheath (station no. 63009) (Bureau of Meteorology, 2017).

Table 2. Blue Mountains upland swamp details. Data from Cowley (2015) was used to identify suitable swamps, validate measurements and provide data for the geomorphic assessment. All other data was derived manually. Piezometer depth was constant, mean phreatic/vadose depths varied over 8 sampling events.

Swamp name	Walmer Cres.	Pitt Park	Wentworth Falls Lake	Mount Hay rd.	Marmion rd./Yosemite swamp	Michael Eade reserve	Katoomba Falls Rd.	The Gully, Katoomba	Grand Canyon Rd.	Perry St.	Popes Glen Reserve	Asgard Swamp #2
Location	Wentworth Falls	Wentworth Falls	Wentworth Falls	Leura	Katoomba	Katoomba	Katoomba	Katoomba	Medlow Bath	Blackheath	Blackheath	Mt. Victoria
Acronym	WC	PP	WFL	MH	MS	ME	KFR	TG	GC	PS	PG	AS
Swamp ID	1	2	3	4	5	6	7	8	9	10	11	12
Swamp ID (Cowley 2015)	334	300	368	460	533	119	382	226	375	480	485	NA
Mean phreatic/vadose depth (cm)	181/8.3	81/11.3	56/0.25	142/77	61/57	148.5/20	114.6/10	157/18.7	115.9/4.4	125/1.7	134/17.1	112/28
Piezometer depth (cm)	188	90	54	221	115	167	135	175	119	125	140	139
Latitude	-33.7007	-33.7086	-33.7071	-33.6823	-33.69817	-33.6842	-33.7257	-33.7124	-33.6626	-33.6338	-33.6335	-33.5607
Longitude	150.3757	150.3747	150.3607	150.3477	150.3249	150.3184	150.3078	150.3048	150.3195	150.3085	150.2929	150.2896
Elevation (m)	877	872	904	901	960	946	949	983	946	976	1023	897
Geomorphic assessment	Moderate	Poor	Poor	Poor	Moderate	Good	Moderate	Poor	Good	Moderate	Poor	Good
Catchment size at site (ha)	8.29	221.4	37.27	19.5	45.83	31.91	6.7	50.6	39.16	23.32	72.78	67.34
Aspect (decimal degrees)	22	195	124	328	359	29	276	224	54	64	67	44
Average swamp slope %	12	1.6	3.9	7.1	4.1	6.6	10.3	2.4	8	8.6	0.6	3.4
Adjacent swamp slope %	11.8	0.8	7.8	7.8	4.9	6.8	10.5	1.8	5.6	3.3	0	5.8
Swamp area ($m^2 \times 10^3$)	6.629	1.597	10.85	1.95	4.899	2.643	3.062	3.454	1.264	13.27	0.849	2.156
Swamp length (m)	627	267	664	323	794	471	241	409	308	257	190	524
Impervious cover (%)	20.5	32	48.1	1.3	54.6	13.1	52	39.6	4.6	23.9	38.5	5
Coefficient of variation of groundwater depth	4	17.4	32.85	14.98	26.71	6.72	22.48	13.37	4.25	13.63	6.3	4.2
Coefficient of variation of piezometer tube temperature	10.25	17.87	27.63	15.2	13.4	16.27	20.15	17.18	17.14	19.4	25.26	15.13
No. stormwater pits	4	41	38	0	81	15	15	36	0	18	130	0
No. stormwater lines	3	41	4	0	15	1	5	11	0	3	19	0
Distance to stormwater (m)	124	30	0	1667	247	354	10	175	1290	233	0	4398
Channelised (1) or intact (0)	0	1	1	1	1	0	1	1	0	0	1	0
Distance to urbanization (m)	48	54	12.6	41	106	432	15	54	475	165	50	4398

Cotton Strip Decomposition

Cotton strips were deployed on four occasions; 8 November 2015, 17 August 2016, 4 December 2016, 24 February 2017. Cotton strips were left in situ for 6 weeks before being retrieved, with the exception of August 2016 (winter) when strips were deployed for a longer period after earlier results showed slow rates of decomposition.

Cotton strips were constructed from a single batch of fine calico (spotlightstores.com.au). They were prepared by washing twice in a washing machine with no detergent, followed by drying, cutting to a 35 x 450 mm strips, tied at one end with a length of nylon fishing line, surrounded by PVC mesh and weighed with a stainless steel nut. These were then wrapped in aluminium foil and autoclaved at 121°C for 15 minutes. At each sampling, the cotton strip was inserted into the piezometer below the water level. These were removed after 6 weeks (for one event 12 weeks) and immediately frozen at -4°C. Control strips were similarly prepared, then placed in sterile deionized water in sterile glass capped laboratory jars (Schott Duran) and placed at constant temperature (20 °C) for the same incubation period. Before measuring cellulose decomposition, strips were thawed and gently washed to remove sediment. They were then air dried and cut into three 25 x 120 mm subsamples. Each subsample was inspected under a dissecting microscope and threads removed so that each subsample was 60 threads wide x 120 mm long. Relative cellulose decomposition was measured as the loss in tensile strength of the cotton strip, as measured by maximum load at break using an Inston 5540 series benchtop electromechanical testing system with 25 mm grips and load frame, and Bluehill Extended System and Software (M18-14443-EN, Revision A 2004). The maximum load at break derived from three subsamples per sample were averaged and compared to a mean value of tensile strength of 10 control cotton strips to derive the relative loss of tensile strength.

Water Quality and Stygofauna

Groundwater water quality and stygofauna sampling were performed four times; on 8 November 2015, 17 August 2016, 4 December 2016, 24 February 2017. Water was removed from the piezometer using a PVC bailer (www.thermofisher.com.au) until the piezometer was empty (Figure 3). The water removed from the piezometer was sieved (63-µm mesh) and the sieve contents retained and preserved in 100% ethanol. Sample size was corrected to a per 4 litre value.

The piezometer was allowed to refill and was again emptied, with the water removed being analysed for physic-chemical parameters using a handheld meter (YSI Professional Plus multiparameter water quality meter, xylemanalytics.com.au) (Figure 3).

The piezometer was allowed to refill and be emptied a third time, with the water removed in the final collection used for water quality analysis (Figure 3).



Figure 3. Method for collection of water quality and stygofauna samples.

All water analysis was performed at a NATA registered laboratory by Standard Methods (APHA., 2005) for Total Phosphorus (TP 4500BF), Total Kjeldahl Nitrogen (N_{org} 4500B), (determined titrimetrically in accordance with APHA latest edition 2320-B), Dissolved Organic Carbon (2320B), Ammonia, Nitrite and Nitrate (determined colourimetrically based on APHA NH_3 4500G , 4500- NO_2^- B, 4500- NO_3^-) and Phosphate (4500F). Results below detection limits were substituted to $LOD/\sqrt{2}$ (Croghan, 2003; Ogden, 2010).

Stygofaunal samples were processed by flotation and decantation adapted from Korbel et al. (2007) and Hose (2012). Samples were rinsed into a 63 μm mesh sieve before dewatering and decanting into 50 mL Falcon tubes with Ludox® colloidal silica solution (Ludox® HS-40, 1.3 g/mL, Sigma-Aldrich Pty. Ltd., Castle Hill, Australia). Samples were then shaken and allowed to settle for 20 min before the supernatant was tipped back into the sieve. This process was repeated twice for each sample and the floated sample rinsed in tap water and stored in 100% ethanol with several drops of 1% Rose Bengal solution (www.chemsupply.com.au) until

identification. Stygofauna were sorted under 40X magnification (Olympus SZX9) before identification at 400x magnification (Olympus BHC) to the highest taxonomic resolution using relevant keys.

Data Analysis

Logger depth data collected at 30 minutes intervals were compensated for barometric pressure (BP) by converting BP (kPa) to height equivalent using the ratio 0.10229 (as per Solinst.com) and subtracting resultant BP (m) from depth logger results. These depth values were used directly to calculate the coefficient of variation (Minitab ® 18.1) in height data and were compensated to groundwater depth below ground for hydrological analysis. A simple empirical rainfall runoff regression model (Vaze, 2012), was developed as the best option given the data available. Daily rainfall data (measured to 9:00 a.m.) were regressed against water depth changes for each swamp for offsets of -1 to +3 days to calculate water table response for all swamps. WC, PP, WFL and MH were regressed against mean records for Faulconbridge and Katoomba stations combined, MS, ME, KFR, TG against Katoomba station and GC, PS, PG and AS against mean records of Katoomba and Blackheath combined stations. Missing values in the records precluded them being used alone. Regression outputs for each day were graphed to identify time to peak R^2 . Water temperature variability was similarly analyzed to calculate the coefficient of variation for each swamp.

Principal Components Analysis (PCA) was used to identify similarities in site and catchment characteristics among swamps and identify groups of sites having similar relative urban impacts. Environmental and water quality data were normalized, and draftsman's plots and Pearson's Correlation Analysis were used to identify correlated variables ($R > 0.90$), with one of the correlated variables removed prior to analysis.

Water quality data and relative loss of tensile strength were analyzed using basic univariate statistical methods and general linear modelling, using environmental data groupings (from PCA) and sampling event as fixed factors. Spearman's correlation was used to identify correlations between relative loss of tensile strength and water quality parameters.

The BEST procedure in PRIMER-E was used to identify the environmental variable(s) most strongly correlated with (were 'best' at explaining) changes in the stygofauna assemblages. Stygofaunal samples were corrected to a standard per 4 litre quantity. Stygofauna data were square root transformed and analyzed with normalized environmental data using the BEST procedure in Primer. BEST compares resemblance outputs for both data sets, using Euclidean distance measures for environmental data and Bray Curtis dissimilarity measures for stygofaunal data. Further, stygofauna were also analyzed at order or family level resolution due to high numbers and richness of rare taxa, such as the Acarina, with 238 individuals in 51 samples. Non-metric multidimensional scaling (nMDS) of root transformed stygofaunal data was used to graphically depict similarity within and between sites, between pre-identified urbanized site groupings and across times. Similarity of percentages (SIMPER) analysis was used to identify indicator taxa within and between sites. Analysis of similarities (ANOSIM) was used to further investigate nMDS graphical output to identify similarities between sites and sampling events. PCA, nMDS, BEST, ANOSIM and SIMPER were done using PRIMER-e software v7 (www.primer-e.com).

Simpson's dominance and diversity of the full data set were calculated, with Buzas and Gibsons evenness and Shannons index (unbiased entropy) calculated as the most appropriate index (Jost, 2007) to investigate α diversity using the software PAST (Hammer, 2001). Results for α diversity for each invertebrate sample were then regressed against water quality and catchment variables. Common approaches to identifying β diversity involve identifying γ diversity and partitioning by subtraction of α diversity (Clough, 2007; Jost, 2007). This was not possible here because there is no definitive known value for γ diversity, so little of the Blue Mountains upland peat swamps have been investigated. Whittaker's (1960) beta diversity ($S/\bar{\alpha}-1$) measured as the proportion by which the regional species richness exceeds local species richness was calculated on binary transformed data. This result was standardised between 0 and 1 by using $\beta-1/N-1$ (Sorensons Index of Dissimilarity) (Baselga, 2015; Harrison *et al.*, 1992). Although biodiversity indices are seen as promising for groundwater studies (Korbel & Hose, 2011), due to high numbers of rare and cryptic taxa, taxonomic difficulties and known high regional biodiversity generally, beta diversity results should be interpreted with caution.

Functional analysis was performed on derived functional feeding groups from available keys (Centre for Freshwater Ecosystems, 2018) and stygofauna functional groups (Hose, 2015b). Stygofauna groups were estimated based on knowledge of invertebrate groups (Appendix 3). Meiofauna such as Microcrustacea, Rotifera and Acari – Hydracarina, known to be stygobiotic (Boulton, 2008; Hose *et al.*, 2017; Wiecek, 2012) were allocated as such, most Insecta were assessed as stygophiles with several taxa that appeared to be stream associated fauna, which were assessed as stygoxenes. The most common group, the Oribatidae, were all assessed as stygoxenes for their known association with terrestrial vegetation (Déchéne & Buddle, 2009; Osler & Murphy, 2005) as were any other clearly terrestrial taxa. Known life histories were incorporated into stygogroup determination (Centre for Freshwater Ecosystems, 2018; Gooderham, 2002). Correlations (Pearsons) between functional invertebrate groups (FFGs and stygofaunal functional groups) and relative loss of tensile strength in cotton strips was used as an indicator of system productivity.

Correlations of disturbance indicator metrics including mean abundance and taxa richness of oligochaetes (<10% of total) and crustacea (> 50% of total) were measured (Korbel *et al.*, 2011). Both numbers of individuals and taxa were analyzed using a general linear model (GLM) following outlier analysis, using sites as a random factor and sampling event (time) as a covariate (Minitab ® 18.1). The significance level (α) for all statistical tests was $P < 0.05$.

Results

Hydrology

Water table depth varied from above ground level for Wentworth Falls Lake (WFL) where the piezometer is in the stormwater outflow flume, to more than 60 cm below ground level for Mount Hay (MH), where the piezometer is located beside the eroded channel (Figure 4).

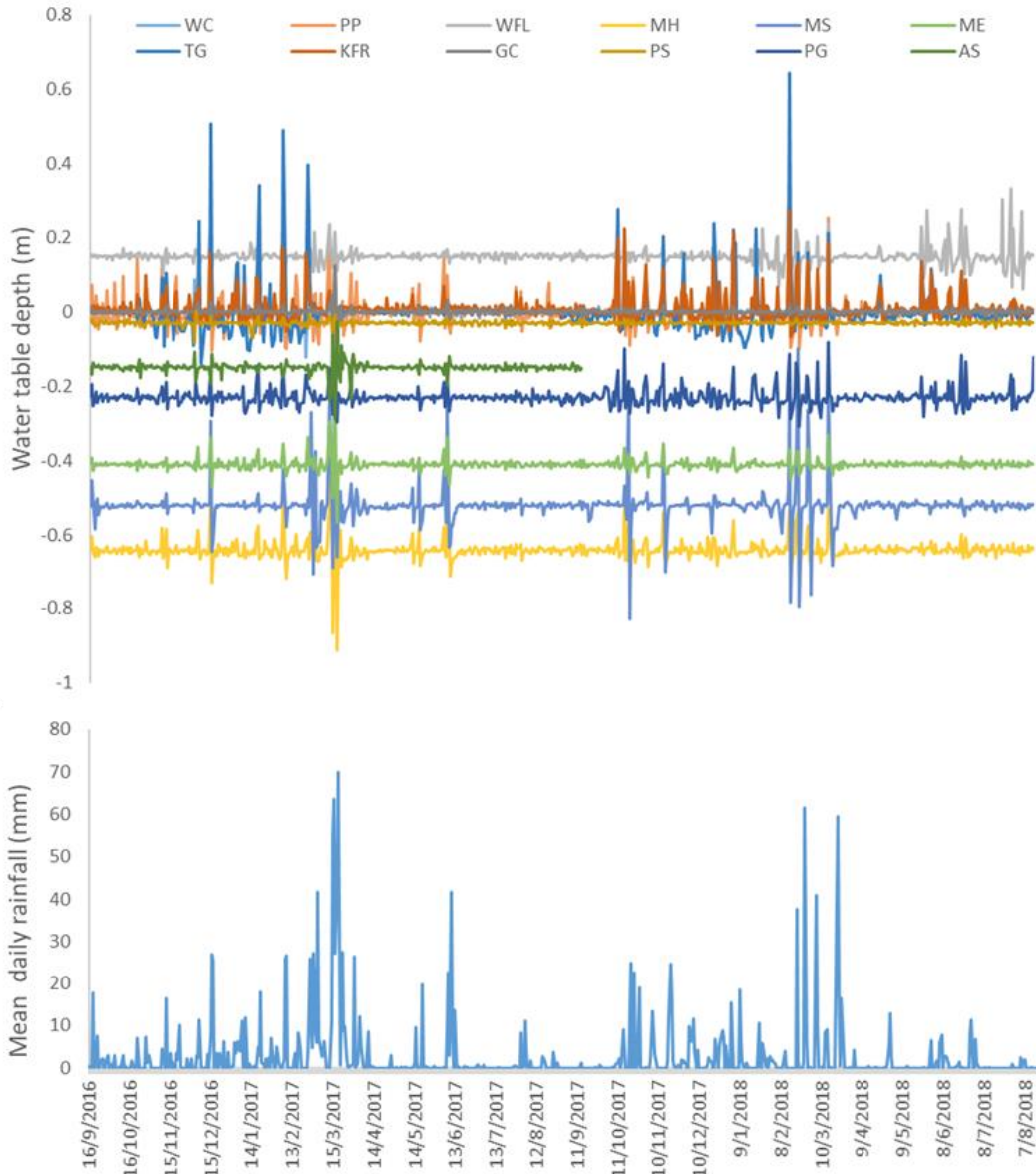


Figure 4. Water table depth (m) for 12 upland swamps (top panel) and associated mean daily rainfall (mm) for three stations Faulconbridge, Katoomba and Blackheath (bottom panel). Asgard swamp has only partial data due to logger malfunction.

Water table depth remained around a stable level in all swamps for the period September 2016 to August 2018. During periods of rainfall, water level exhibited an enhanced variability around that level. TG, PP and KFR water levels were affected by surface flow following rainfall events, with post-rainfall water levels up to 0.6 m above the surface. WFL displayed increased variability in water levels following rerouting of stormwater flow paths upstream in May 2018 (Figure 4).

Peak water table responses varied between sites but generally occurred 1 to 2 days after rain, with the maximum R^2 not exceeding 0.25 (Figure 5). Water levels in MS and TG responded most quickly to rainfall. Small responses followed by larger peaks 2 days later were evident in water levels at ME, AS, WC, GC, PS, PG and WFL, presumably due to rainfall directly entering the piezometers. Smaller secondary peaks in water level were found at swamps that are close to stormwater: PG, WFL and PS. Levels in both PP and MH responded to rainfall with a single large peak, which suggested there was gradual percolation from the adjacent channel.

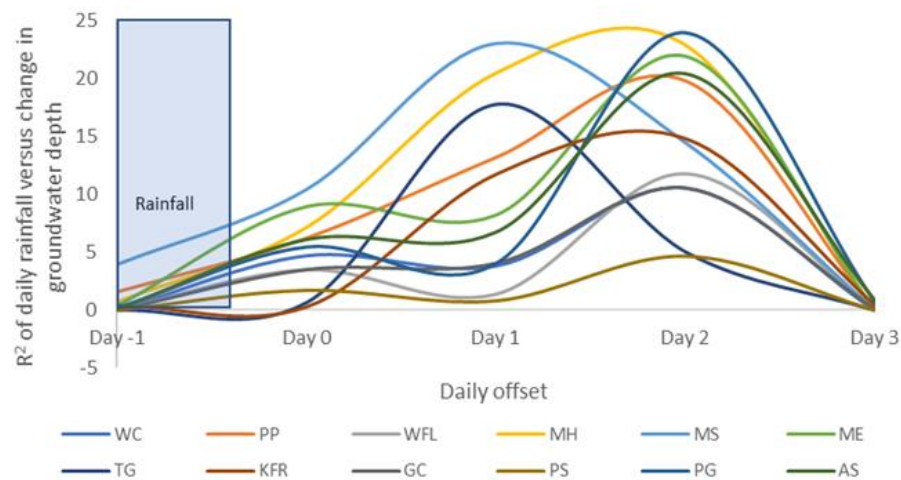


Figure 5. Rainfall response in swamps, regression of rainfall versus water table depth for 1 day antecedent, to 3 days following rainfall events. Rainfall records are mean daily to 9 am, requiring day-1 for rainfall response

Environmental Data

Principal components analysis based on environmental data (Figure 6) highlighted three distinct site groups; the first group of a ‘less urbanized’ or ‘natural’ group consisting of GC, AS, ME, WC, PS and MH (Table 2). Sites in this group generally were further from distance to stormwater (>230 m) low values of catchment imperviousness (< 25%), temperature variability (<15°C) and temperature, and little channelization or stormwater pits present at the site. A second group of ‘urban’ sites, which included WFL, MS, KFR and TG, was characterized by sites with impervious area (39-55%), stormwater pit density (15-81/catchment), and variation in groundwater depth (13.4-32.9) and temperature variability (13.4-27.6 °C) and the presence of channels. Two other swamps, PP and PG formed a group of highly disturbed sites, which were characterized by pH (>6.2), high EC (98,152 $\mu\text{S}/\text{cm}$ respectively), DOC (6-7.6 mg/L), and stormwater lines in the catchment (41,19 respectively).

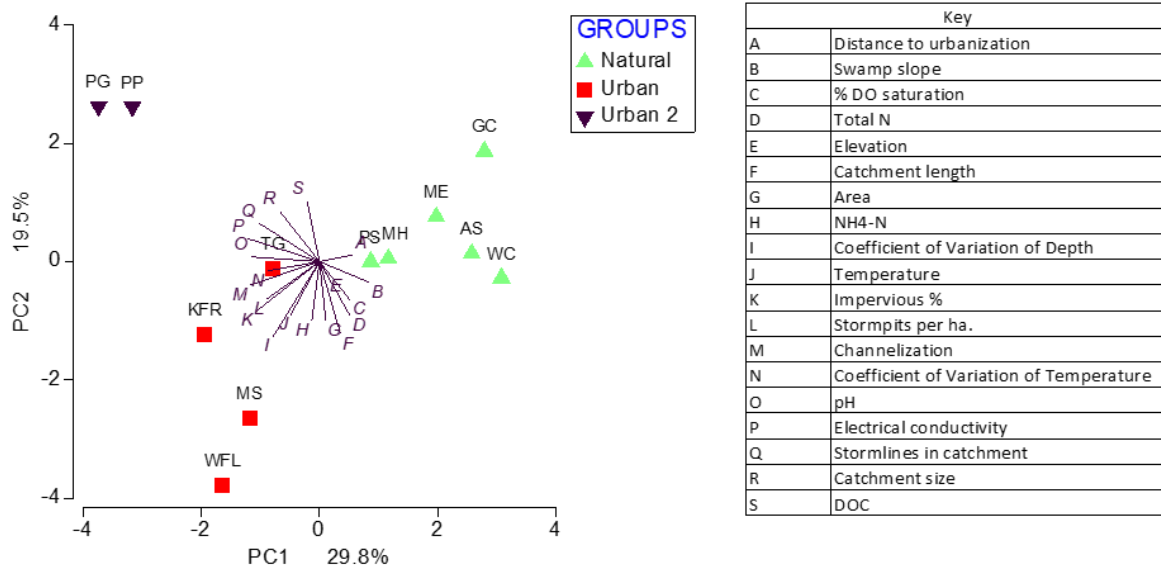


Figure 6. Principal Components Analysis (PCA), of 19 normalized environmental criteria for 12 upland swamps of varying urban impact. Criteria for analysis included geomorphic features of each swamp including slope, catchment size, swamp area and length. Further criteria related to urban pressure, such as % impervious area in catchment and stormwater pits per hectare, were included. Water quality data and hydrological variability metrics were also included. Correlated variables with values >0.9 were deleted.

Water Quality

Groundwater quality varied between swamps, with temperature variability (30-minute logging from September 2016 to August 2018), pH, electrical conductivity and total alkalinity higher at more disturbed sites (Table 2 and Appendix 2, Table 2). Nitrogen concentrations, DOC and temperature were generally greater in groundwater of more urbanized swamps, and dissolved oxygen generally less in more disturbed sites.

Water temperature differed significantly over sampling events. Across the urbanization site groupings from the PCA, mean values of pH, electrical conductivity (EC), total alkalinity (T.alk) and % dissolved oxygen (%DO) were significantly different between the groups ($P < 0.05$). While the other water quality parameters were not different, variability in water quality increased with urbanization across all sites and events for water temperature, EC, T.alk and decreased for % DO.

Table 3. Water quality for the swamp groups identified by Principal Components Analysis.

Natural group – AS, GC, ME, MH, PS, WC. Urban group – MS, WFL, KFR, TG. Urban 2 group – PG, PP.

		Water Temperature (°C)	pH	EC (µS/cm)	DO (% Sat)	NH4-N (mg/L)	Total Alk (mg/L)	Total N (mg/L)	DOC (mg/L)
<i>Natural</i>									
Mean		13.1 +/- 0.5	5.91 +/- 0.59	28.8 +/- 28.8	46.6 +/- 2.7	0.13 +/- 0.03	5.9 +/- 0.5	4.75 +/- 0.39	5.2 +/- 0.9
Min		8.6	4.37	4.6	24.0	0.01	3.0	0.87	0.5
Median		13.5	5.42	28.3	46.0	0.07	5.0	4.50	3.0
Max		16.6	6.62	48.4	81.4	0.45	12.0	9.60	17.0
<i>Urban</i>									
Mean		13.5 +/- 0.6	5.90 +/- 0.11	62.4 +/- 4.19	39.3 +/- 2.5	0.20 +/- 0.02	18.3 +/- 1.2	6.07 +/- 0.64	5.1 +/- 0.9
Min		7.2	4.89	4.5	18.7	0.03	9.0	2.20	1.5
Median		13.9	5.80	62.0	37.2	0.22	18.0	5.90	4.0
Max		17.9	7.20	98.2	68.0	0.55	34.0	16.00	23.0
<i>Urban 2</i>									
Mean		13.4 +/- 1.0	6.19 +/- 0.19	125.2 +/- 15.5	34.0 +/- 3.1	0.06 +/- 0.11	38.1 +/- 3.2	1.83 +/- 0.19	6.8 +/- 1.7
Min		7.1	5.26	63.8	21.1	0.02	26.0	0.90	2.5
Median		14.4	6.32	114.4	31.9	0.05	36.0	1.73	3.8
Max		16.8	6.94	205.9	46.7	0.12	57.0	2.60	19.0
GLM results									
Group	F-value	0.9	27.62	112.79	7.85	0.31	190.34	3.04	0.89
	P	0.347	<0.001	<0.001	<0.01	0.583	<0.001	0.087	0.351
Sampling	F-value	4.37	1.03	0.64	0.19	0.16	0.6	0.11	0.84
	P	<0.005	0.40	0.636	0.942	0.959	0.666	0.977	0.505
Group:sampling	F-value	0.81	1.55	1.96	0.04	0.14	1.05	0.06	1.58
	P	0.524	0.20	0.119	0.997	0.967	0.392	0.993	0.195

There were small to moderate and significant correlations between EC and temperature (0.255, $P < 0.05$) pH (0.385, $P < 0.05$), T.alk (0.825, $P < 0.001$), TN (-0.317, $P < 0.05$); pH and T.alk (0.438, $P < 0.001$) and temperature and DOC (0.274, $P < 0.05$) (Table 3 and Appendix 2, Table 1).

Cotton Strip Decomposition

Relative decomposition of cotton strips were significantly greater for urbanized site groups (Urban and Urban 2), than Natural group, ($F_{(2,57)}$, 11.87, $P < 0.001$) and for sites ($F_{(11,44)}$, 7.13, $P < 0.001$). (Figure 7). There was a significant difference over time ($F_{(4,44)}$, 8.22, $P < 0.001$), with the sample in February 2017 (in-situ. 12 weeks), different to November 2015 (in-situ. 6 weeks).

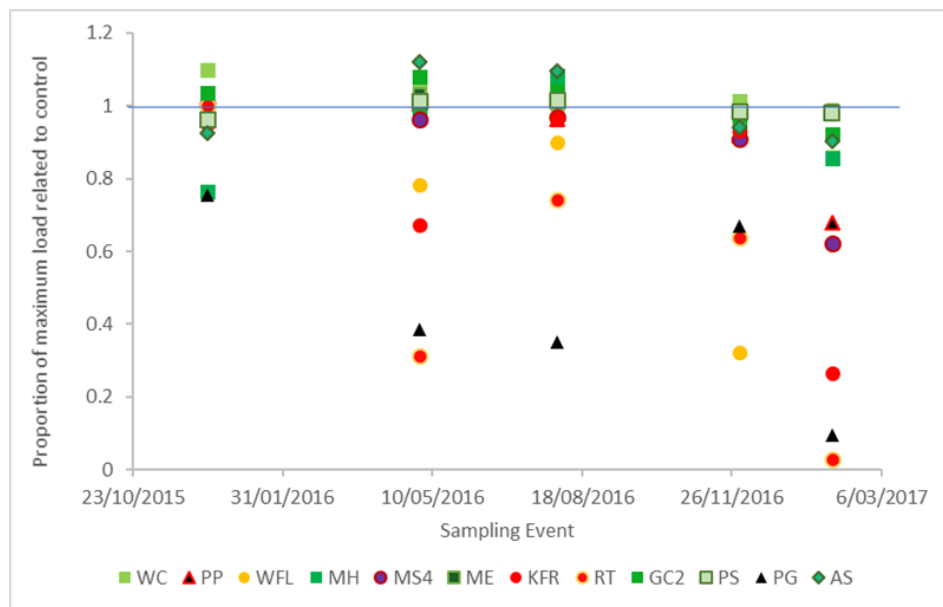


Figure 7. Proportion of relative cotton strip maximum load tension break compared to control strips for five sampling occasions. Strips were left in situ for 6 weeks for events 1-4 and for 12 weeks for the final event. Colours to match PCA of environmental data. Natural (green), urban (yellow to red), urban 2 (black).

Both relative cottons strip decomposition and variability increased with decreasing proximity to stormwater and urbanization; and groundwater levels near the surface (Figure 8).



Figure 8. Results from Tukeys comparisons for relative cotton strip decomposition, for proportion of loss of tensile strength measured as maximum load to break (Newtons) related to control cotton strips. Significant differences ($P < 0.05$) are shown above the graphs, from A to D. Columns sharing common letters above are not significantly different.

There were significant negative correlations between relative cotton strip decomposition and abiotic factors including temperature ($r = -0.427$, $P < 0.05$), pH ($r = -0.447$, $P < 0.001$), electrical conductivity ($r = -0.475$, $P < 0.001$) and total alkalinity ($r = -0.382$, $P < 0.005$), and a positive correlation between cotton strip decomposition and dissolved oxygen ($r = 0.254$, $P = 0.05$). Therefore, cotton strip decomposition increased with increasing alteration to water quality

Stygofauna

One hundred and fourteen taxa were collected from the 12 swamps over four sampling times. Identification to species was not possible as identification keys were not available and the taxa often atypical. The most speciose taxa group were the Acari (aquatic and semi-terrestrial mites) with 48 taxa, the most numerous taxa were the cyclopoid (6041 individuals) and harpacticoid copepods (3280 individuals), nematodes (2146 individuals) and nemertean (4260 individuals) (Appendix 3). The greatest taxon richness was found at WFL (21 taxa) and GC (20 taxa) in August 2016 and the highest count at MS (4139 individuals) (Figure 9). Variability within sites

was high, but generally more natural swamps supported fewer stygofauna than more urbanized ones (Figure 9).

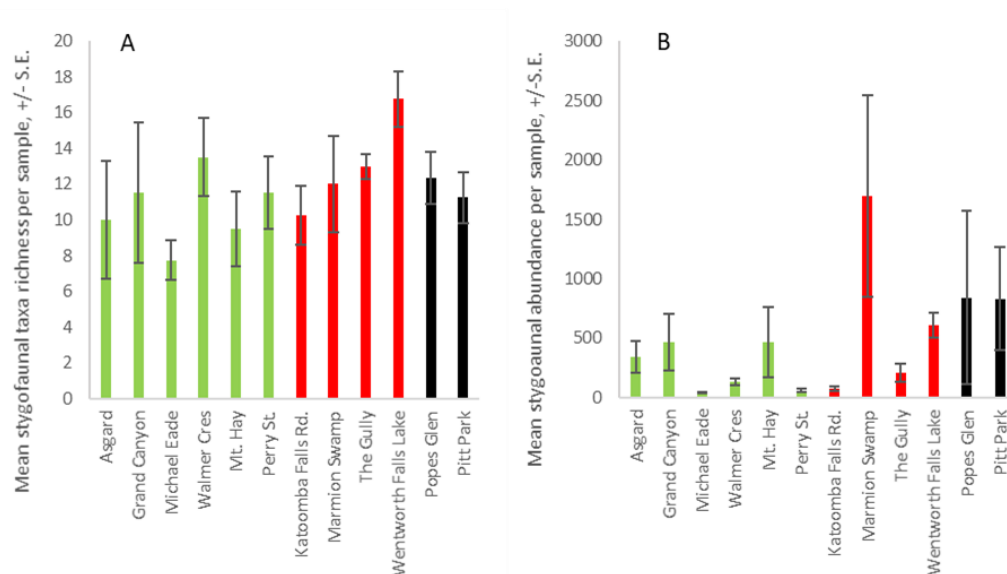


Figure 9. Mean stygofaunal taxa richness (A) and abundance (B) per sample, +/- S.E. for 12 upland swamps, coloured to match PCA groups of natural (green), urban (red) and urban 2 (black).

One outlier was removed prior to analysis (Grubbs test $G_{4.86}$, $P < 0.001$). There was a significant difference between events in the abundance ($F_{(3,31)}$, 3.39, $P < 0.05$) and richness ($F_{(3,31)}$, 7.40, $P < 0.005$) of stygofauna. For abundance, sampling 1 (November 2015) (162 +/- 52.9) was less than February 2017 (703 +/- 214). Sampling 1 (November 2015) mean taxa richness (7.91 +/- 1.28 S.E.) were significantly less than August 2016 (14.50 +/- 1.33 S.E.) and February 2017 (13.46 +/- 0.767 S.E.). There were no significant differences in abundance or taxa richness across sites.

Once sites were grouped into the PCA groupings, mean stygofaunal abundance within natural sites (251 +/- 70.1) were significantly less than highly urbanized sites (836 +/- 360), but neither were different to urban sites (649 +/- 253), ($F_{(2,44)}$, 379, $P < 0.005$).

Predators were the dominant functional feeding group with 44 taxa, with 26 gatherers and scrapers, 12 filter feeders and 5 shredders. The most common ecological group were the stygophiles with 53 taxa, 38 stygoxenes and 23 stygobionts. The number of stygobiont taxa may

be inflated as many of these taxa also inhabit surface waters, including rotifers, microcrustaceans and Hydracarina. However, due to their small size and limited mobility through sediments, these taxa may be very likely adapted specifically to groundwater in swamps. Shannons α diversity for sites varied between 0.741 for GC to 1.892 for WFL, indicating high diversity (Table 4).

Dominance was relatively low for most sites, except for MH and GC. Whittakers β diversity was calculated at 3.0714, standardized to Sorensons index of dissimilarity was 0.279, suggesting that dissimilarities overall within the swamps were relatively low

Table 4. Stygo fauna α diversity as measured by Shannons- H_U entropy (unbiased) using population and taxa data (minimum of 0 for single taxon communities to maximum of many taxa per community), Simpsons Dominance (0 (all taxa are equally present) to 1 (one taxon dominates the community completely), Simpsons Diversity, (1-Dominance) and Buzas and Gibsons Evenness, e^H/S ($\exp(H)$ divided by number of taxa) measuring the evenness with which individuals are divided among the taxa present).

Swamp	WC	PP	WF	MH	MS	ME	KF	TG	GC	PS	PG	AS
Simpsons Dominance (0-1)	0.253	0.489	0.226	0.625	0.562	0.239	0.271	0.360	0.655	0.323	0.510	0.538
Simpsons Diversity, 1-Dominance (0-1)	0.747	0.511	0.774	0.375	0.438	0.761	0.729	0.640	0.346	0.677	0.490	0.462
Shannon_H Diversity Index (0 - max)	1.868	1.182	1.892	0.743	1.006	1.569	1.631	1.492	0.741	1.565	1.091	0.975
Buzas and Gibson's Evenness (e^H/S)	0.511	0.326	0.405	0.270	0.252	0.679	0.538	0.357	0.256	0.451	0.292	0.380

Regressions of α diversity against environmental conditions were significant for stormpits per ha ($F_{(3,11)}, 10.25$, $P < 0.05$), distance to urban areas ($F_{(3,11)}, 20.24$, $P < 0.05$), and distance to stormwater lines ($F_{(3,11)}, 15.56$, $P < 0.05$) and to stormwater ($F_{(3,11)}, 25.06$, $P < 0.015$). Potential benchmarks for groundwater assessments (Figure 10) exhibited a slight pattern for mean abundance of Crustacea and mean taxa richness for Oligochaeta.

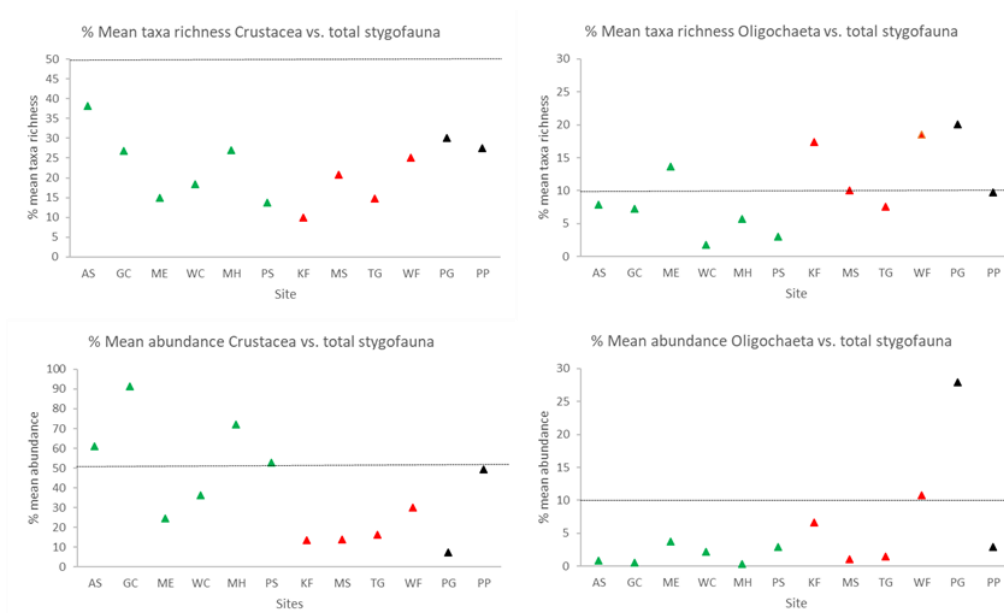


Figure 10. Groundwater organizational assessment metrics (Korbel et al., 2011) percent of Crustacea (>50%) and Oligochaeta (<10%), coloured to match PCA groups of natural (green), urban (red) and urban 2 (black).

Stygofauna communities at most sites varied little over time (Figure 11A) (with a sample statistic R: 0.023 and significance of 21.3%, compared to differences between sites (with a sample statistic R: 0.429 and significance of 0.1% (ANOSIM). Differences between sites were masked by the large number of sites and similarities between them. Once the groupings identified by the environmental data PCA were applied to the stygofaunal ordination plot however, a separation between natural and most urbanized sites was evident (Figure 11B).

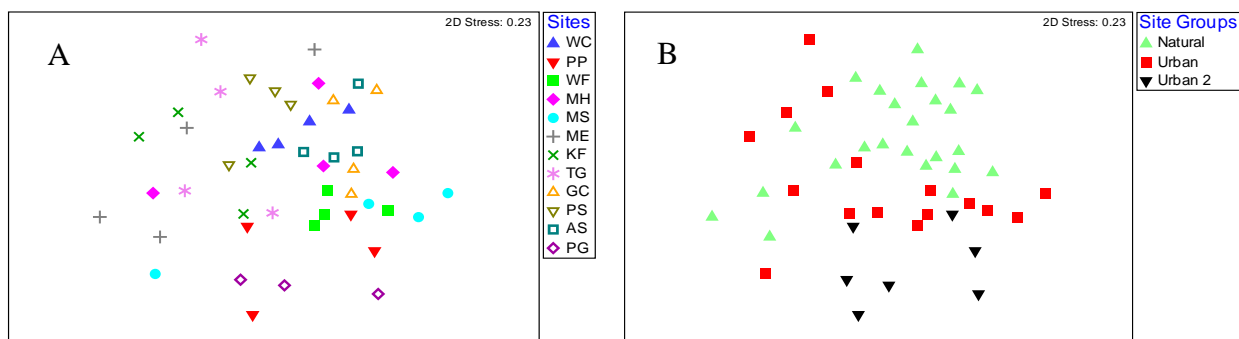


Figure 11 A. NMDS plot for square root transformed stygofaunal data for 12 upland swamps. One missing data point at PG (December 2015). Figure 11B. NMDS plot for square root transformed stygofaunal data for 12 upland swamps, displayed as groups identified by PCA of environmental data (Figure 6).

The environmental variable most strongly correlated with the stygofauna communities was temperature, with a global correlation of 0.410 (Table 5). The suite of variables that together were most influential included the number of stormwater lines, EC, catchment length and channelization in the catchment (Table 5).

Table 5. BEST analysis of correlations between 21 normalized environmental variables and stygofaunal communities. Sample statistic (Rho): 0.41, Significance 61%.

No. of variables	Correlation	Selections
1	0.410	temperature
2	0.394	stormlines, temperature
3	0.403	stormlines, electrical conductivity (EC), temperature
4	0.397	stormlines, length, EC, temperature
5	0.403	stormlines, channelization, length, EC, temperature

SIMPER analysis (Appendix 3) showed that common taxa dominated similarities within and between sites, with only a difference in populations identifying the sites from each other. Correlations between functional groups of invertebrates and cotton strip decomposition were not highly significant for most groups, but were significant but weakly correlated for number of scrapers (R^2 , -0.402, $P=0.0059$) and for stygophiles (R^2 , -0.327, $P=0.025$) (Table 6).

Table 6. Pearsons correlation between invertebrate functional groups (functional feeding groups and stygofaunal groups) and cotton strip decomposition

<i>Parameter</i>	<i>Cotton strip decomposition</i>		<i>Parameter</i>	<i>Cotton strip decomposition</i>
<i>Gatherer</i>	R, -0.240, P=0.104		<i>Stygoxene</i>	R, -0.146, P=0.329
<i>Predator</i>	R, -0.255, P=0.084		<i>Stygophile</i>	R, -0.327, P<0.05 *
<i>Scraper</i>	R, -0.402, P<0.01 *		<i>Stygobiont</i>	R, 0.000, P=0.999
<i>Shredder</i>	R, -0.087, P=0.562		<i># Individuals</i>	R, -0.178, P=0.231
<i>Filterers</i>	R, -0.004, P=0.977		<i># taxa</i>	R, -0.279, P=0.057

Discussion

This study has provided an insight into functional features of upland swamps in the Blue Mountains and the complex nature of stygofaunal communities within them. Shifts in the structure and function of the swamp ecosystems were correlated with key variables linked to urbanization.

Diversity, Distinctiveness and Populations of Swamp Stygofauna

This study has shown that the stygofauna communities in the swamps were highly variable, with several very populous groups and multiple rare taxa, a common feature of groundwater communities (Hahn, 2009; Martin, 2009). The community was dominated by the Copepoda; Cyclopidae and Harpacticoida, Dugesidae (Turbellaria), Nematoda and Nemertea (Appendix 3). The domination of copepods mirrors unpublished data in studies in upland swamps (Hose, 2015b), however our study found few Ostracoda and Syncaridae that were common in those studies and others from elsewhere in Australia (Glanville *et al.*, 2016). Instead, nematodes, nemerteans and turbellarians; indicators elsewhere of poor water conditions, were common, particularly in more urbanized catchments. SIMPER analysis reinforced the importance of common taxa in typifying sites and identifying differences between them (Appendix 3). The most common taxa listed above, drove site differences. Several water mites, the tanypod chironomid *Paramerina* sp., oligochaete taxa and larval Scirtidae (Coleoptera), distinguished between sites, also were more common in sites closer to stormwater and with higher urbanization pressure (KF, TG and MS).

While scirtid larvae are known from bog habitats (Bailey, 2010; Centre for Freshwater Ecosystems, 2018) and springs (Halse, 2002; Wood *et al.*, 2005), they are filter feeding atmospheric oxygen breathers, which makes them unusual inhabitants for groundwater. Genetic analysis of existing scirtid taxa suggests that some may be relictual and the phylogeny uncertain (Cooper *et al.*, 2014), indicating a need for further work on this group.

Interestingly, predators dominated the functional community, mostly due to high numbers of Acarina, which were widespread and speciose. The most numerous, Oribatidae, are often strongly associated with specific vegetation or soil types, including liverworts, terrestrial plants,

mosses, creating distinct communities within small microhabitats (Colloff & Halliday, 1998; O'DOWD *et al.*, 1991). They are therefore a mainly terrestrial fauna (Déchéne *et al.*, 2009; Osler *et al.*, 2005), as are the Mesostigmata (Proctor, 2009) and both groups may have entered the samples from overhanging vegetation, been present in soil or the groundwater proper. Halacarids (Halacaridae and Pezidae) were also common. This group, mainly marine in origin, have invaded freshwaters over time (Bartsch, 1996), so their phylogeny may be interesting. The Hydracarina (Hydrachnidae) are also usually aquatic, comprising an important community in a range of habitats, including groundwater (Goldschmidt, 2016). Several taxa in the samples were obviously stygobionts, lacking eyes and colouration.

Also interesting were the relatively high number of insect taxa compared to Crustacea. Commonly, insects dominate surface water, but are rare in groundwater systems in Australian and European subterranean environments (Deharveng, 2009; Watts, 2003), where crustacean diversity and populations dominate the stygofauna. Groundwater fauna are generally dominated by Crustacea such as Copepoda, Ostracoda, Syncarida and Amphipoda (Hancock, 2008; Hose, 2015a; Humphreys, 2008). In this study, insects were common, suggesting aquatic ecosystems with close affinity to surface water. Most of the insect taxa appeared to be stygophiles, based on their morphology, lacking stygofaunal traits of lack of pigmentation, eyes, elongated and small and simplification of limbs (Hose, 2015a; Tomlinson *et al.*, 2007). However, several taxa were reliably present in a number of swamps, suggesting a strong association. These included *Paramerina sp.*, and Scirtidae, which were more common in more urbanised sites but still present in lower numbers in some undisturbed swamps (Appendix 3). Others, such as leptophlebiids (Ephemeroptera) and carabids (Coleoptera) are usually rare in groundwater. Unusually, common stygofauna found in other local swamp studies, including Bathynellidae and Parabathynellidae syncarids, Neoniphargidae amphipods, Ostracoda and Isopoda were absent or rare (Hose, 2017).

Australian stygofauna generally represent a phylogenetic assemblage of diverse Gondwanan and Pangaeian origin (Humphreys, 2006; Thurgate, 2001). The upland swamps are relatively recent in geological terms, originating in the Holocene (Fryirs, 2014), so that evolutionary pressures creating specialized groundwater fauna are likely to be secondary to movement of stygobionts from neighboring fractured rock aquifers and adaptation of existing stygophiles. Local bedrock groundwater is a potential source for stygofauna. Using Radon mixing models, Cowley *et al.*

(2019) found that between 19% and 79% of swamp water derives from bedrock. Overland flow of rainwater within the swamps may also be a contributor that may explain the high numbers of stygophiles and stygoxenes.

The lack of research in peat swamp groundwater worldwide means that it is difficult to compare natural and disturbed peat stygofaunal meiofauna. What is known about peat swamp invertebrates is mostly derived from macroinvertebrate communities inhabiting surface ponds within the peat (Oyague Passuni, 2015; Verberk *et al.*, 2006). The diverse hump and hollow architecture within swamps (Kato *et al.*, 2010; Spitzer & Danks, 2006) creates a variety of aquatic habitat within peat (Batzer & Wissinger, 1996) saturated peat, small pools, large pools and streams through the swamps, that is available for stygofauna. Further, the ecotonal nature of peat swamps means that both terrestrial and aquatic invertebrates comprise the faunal biodiversity at relatively small scales within individual wetlands (Batzer *et al.*, 1996). These communities comprise both invaders from surrounding aquatic or terrestrial systems or are specific to peatlands. In this study, the high proportion of stygoxenes and stygophiles emphasized the ecotonal aspect of the study area, but also, a lack of taxonomy may have hindered differentiation between swamps with varying urban impact.

This study suggests a gradual change in stygofaunal communities, rather than threshold-based responses to environmental conditions. Populations became larger with increasing urbanization impact. High taxa richness was not confined to more undisturbed swamps, but also at sites such as Wentworth Falls that has a strong connection to stormwater. Site differences in abundance and taxa richness were not significant, however once sites were grouped in the PCA derived urbanization gradient, individuals per sample increased with impact.

This suggests that urbanization affects productivity by increasing density of invertebrates and some taxa richness within peat swamps. A general pattern of low invertebrate taxa richness in the most undisturbed swamps (MH and GC) may suggest that naturally the swamps are species poor, with a similar finding in nearby undisturbed swamps (Hose, 2017). It is not surprising that water quality with higher nutrients and dissolved oxygen would provide conditions for a greater diversity of invertebrates (Chapter 4). In conditions of non-limiting carbon, both nutrients and

dissolved oxygen are integral to aquatic food webs (Boulton, 2014; Sigeo, 2005) so elevated levels of both as a result of more urban swamps would stimulate biological productivity and diversity. However, the stygofaunal community structure correlated with infrastructure, water temperature and EC, so there were obvious urban effects on stygofauna. These patterns have been observed elsewhere with Hydracarina distribution correlated strongly to EC and temperature (Wiecek, 2012). The use of groundwater assessment metrics (Korbel *et al.*, 2011) showed some promise, however required further testing in a variety of groundwater conditions. High numbers of stygoxenes and stygophiles in the ecotonal upland swamps may have complicated results, with greater numbers of insects and a large number of rare taxa.

The general patterns observed for stygofauna communities globally are low local diversity relative to regional diversity, high levels of endemism, simplified food webs with few obligate predators and relictual fauna. Stygofaunal taxa richness increases over distance and β diversity is as or more important than α diversity across spatial scales (Gibert, 2009b). The results of this study suggest low α diversity in least and most disturbed swamps, with relatively high α diversity in more ‘intermediate’ urban swamps. Therefore, there appeared to be a unimodal α diversity pattern; swamps had relatively low diversity (β diversity) for stygofauna, with the main impact of urban development affecting productivity, rather than taxon richness. Diversity measures are complicated, with small aquatic habitats such as pools or ponds thought to be important in aquatic biodiversity conservation (Coronel *et al.*, 2007). They may act as aquatic refuges or islands, maintaining high γ diversity, while supporting high aquatic variability within individual ponds (Coronel *et al.*, 2007). This study is unable to make any prediction on these observations, except that more replication and sampling intensity could improve predictability of biodiversity relationships within, between and across upland swamps.

Differences in Swamp Hydrology, Groundwater Quality and Decomposition Processes

The results indicate that anthropogenic influences alter Blue Mountains swamp characteristics, by altering hydrology, water quality, invertebrate communities, functional groupings and decomposition processes.

Hydrology

Water levels in swamps responded quickly to rainfall, peaking within 1 to 2 days, with the response generally greater and faster in catchments with greater impervious cover. However, regression responses were only 25%, which may have suggested that there is a body of water retained within the swamps. These findings are borne out by Cowley (Cowley, 2017) who suggested residence times of between 4 and 13 months for Blue Mountains swamps.

Generally, boreal peat swamps in temperate regions have long been conceptualized as a two layered diplotelmic system, with an upper aerobic sediment layer (acrotelm) above the water table that has high organic decay rates, and an anaerobic layer (catotelm) below the water table, where decomposition rates and groundwater flow are lower (Ingram, 1978). Under these conditions, groundwater residence time within the swamps would be greatest at depth and increased by the presence of chemically inactive inorganic carbon fractions that reduce decay rates.

This conceptualization was borne out of the structure of Northern Hemisphere peat swamps. More recent analysis of more peat swamps globally has led to a call for greater flexibility in classification of biogeochemical cycling in peat swamps (Morris *et al.*, 2011b). Newer classifications still lean towards a surface layer with higher geochemical cycling rates and water flow under which increasing organic matter under compression and lower cycling and flow rates exist (Morris & Waddington, 2011a). These rules do not apply in THPSS swamps because of sedimentary layers, the presence of sand, deeper layers of basal sand and gravel and an impermeable bedrock base. In these swamps, the uppermost sediment layer consists of surficial organic fines (SOF), originating from decomposing litter and xerophytic plant roots. Further layers below contain sand with lower concentrations of organic material and gravimetric moisture content but also capable of significant groundwater flow rates (Cowley, 2016). Furthermore, there is evidence of significant complexity within individual swamps, including well defined tunnels and narrow fissures within the swamps (Fryirs, 2014).

Intact and deep layers of SOF and organic material in the alternating organic sands (AOS) (Cowley *et al.*, 2016) may therefore maintain residence times and provide important

environmental services of water and carbon storage (Griebler & Avramov, 2015). There appears a close link between surface and groundwaters with residual water either remaining within the swamps or simply percolating to the base, entering eroded streams and leaving the swamps. When swamps are eroded by anthropogenic factors, retention is lost, leading to drying of the organic surface layers and rapid runoff (Cowley, 2017). Furthermore, one of the defining impacts of the urban stream syndrome (Walsh *et al.*, 2004) is hydrological alteration, including increased frequency of erosive and overland flows, increased ‘flashiness’ of stream flows and reduced lag time to peak flows. Hydraulics within swamps therefore may have played an important role in maintaining accidental and occasional stygofaunal communities, with a possibility of higher proportions of stygoxenes and stygophiles than other groundwater and peat swamps.

Productivity

A notable effect of proximity to urbanized infrastructure and catchment imperviousness was an increase in productivity, with both higher but uncorrelated stygofaunal populations and cellulose decomposition. Cotton strip decomposition rates increased with increasing urban development, such that more urbanized peat swamps had higher productivity. Decomposition of cotton was slow related to other studies (Clapcott *et al.*, 2010; Lategan *et al.*, 2010), in retrospect requiring at least 3 months exposure in situ, but there was a clear pattern of increasing decomposition rates in more urbanized catchment groundwater. This response has implications for productivity in upland swamps as increasing urbanization may alter speed of biogeochemical processes within the swamps. Traditionally, low rates of organic matter decomposition were attributed to anoxia, low nutrients, temperature and pH. There is now evidence that microbial activity is inhibited in peat swamps by the build-up of phenolic compounds under anoxic conditions (Fenner & Freeman, 2011). Phenol oxidases capable of degrading phenolics only act under aerobic conditions, releasing and available carbon and nutrients. Changes to groundwater levels in peat swamps in more urban swamps, as a result of channelization (Cowley, 2017), with elevated groundwater variability due to stormwater runoff may enable greater phenol degradation through this process. This has repercussions for THPSS, which in addition to urbanization, are expected to be subject to increasing drought and rewetting which increases carbon release through this process (Fenner *et al.*, 2011).

Correlations between functional invertebrate groups and cotton strip decomposition were mostly weak, however, both scrapers and stygophiles were significantly and negatively correlated with decomposition rates (and urbanization). This suggests that peat bog scrapers, such as turbellarians and oribatid mites and stygophiles such as nematodes, dugesids and oligochaetes may be negatively affected by altered water quality.

Furthermore, sites with more human impact also exhibit lower stygofaunal community evenness and corresponding higher dominance. However, the results are not definitive in this limited study. Further analysis, more data or greater knowledge of taxa functional group allocation, and other traits including body size or life cycle length, provide a possible way forward. It is recognized that there needs to be a much greater knowledge of trait and function relationships before there is a more complete understanding that would enable more predictive outcomes (Daam *et al.*, 2019).

Water quality

The upland swamps have evolved to low nutrient, pH, EC, DO and alkalinity environments, with highly adapted sclerophyllous vegetation (Keith & Myerscough, 1993). Alteration to these attributes with increasing urban effect was correlated with swamp internal integrity.

Several effects of urbanization are well known. The urban stream syndrome (Roy *et al.*, 2009; Walsh *et al.*, 2005), discussed in the previous chapter, has predictable impacts on stream water quality. These effects include increased temperature, electrical conductivity, toxicants and suspended sediments (Walsh & Kunapo, 2009). In the Blue Mountains, this has also included increased pH and other geochemical pollution as a result of alkaline runoff from concrete infrastructure (Wright *et al.*, 2011). Other toxic components of urban stormwater, such as heavy metals, plastics and insecticides may act synergistically or antagonistically to affect swamp ecology (de Zwart *et al.*, 2018). In Chapter 4, leaf litter decomposition was hindered by historic disturbance created by excavation. Naturally occurring ironstone has led to flocs of iron reducing bacteria and associated toxic conditions within the outflowing streams of PP and TG, which were

also used for this study. Interestingly, carbon decomposition as observed for cotton strips (Figure 9) was greatest in these swamps. Conflicting responses such as these require more research.

Urban effects have also likely increased nutrients into runoff, that has then infiltrated swamp groundwater. Groundwater nutrients such as phosphorus, which is quickly adsorbed to particles was not measurable within groundwater or surface water associated with the swamps. This suggests that it is utilized by ecosystems before it gets into the swamps. Nitrogen increased with urbanization (Table 3) but fell again with greater levels of human impact. These results mirror those in Chapter 4 for surface water, suggesting that there is a unimodal pattern for swamps where nutrients increase. However, higher impact associated with low slope, previous excavation (chapter 4), and the presence of contemporary sands (Cowley *et al.*, 2016) has led to reduction in nitrogen, possibly through anaerobic denitrification or scavenging for plant biomass.

The absence of influences separated out more pristine swamps, such that distance to urban development and lack of anthropogenic drivers discriminated natural from urban swamps. The results mirror those for streams associated with upland swamps (Chapter 4), where anthropogenic impacts led to elevated nutrients, pH, electrical conductivity and associated alkalinity. There was clearly a link between urbanization and groundwater quality, suggesting altered functionality within the swamps. The current natural function of low nutrient, pH, DO swamp systems are being altered by increasing pollutants that are not removed by passage through the groundwater and were able to typify the most impacted sites. Our results support that assertion, with generally higher productivity with increasing urbanization and its inherent stormwater quality (Chapter 4).

Conclusion

Urbanization in the catchments of upland swamps in the Blue Mountains is correlated with changes in ecosystem structure and function. Impervious cover in catchments was linked to faster and greater increases in groundwater levels in the swamps following rain. Combined with channelization of urban swamps, storage in more urbanized swamps was generally less. Response time to groundwater level generally was short, between 1-3 days. Urbanization therefore had repercussions for long term water storage within the swamps. Water from urbanized catchments retained nutrients and other pollutants through the swamp. In turn, as pollutants including EC, alkalinity, temperature and stormwater infrastructure increased, stygofaunal communities responded by increasing biodiversity and populations. It cannot be concluded that more normal pH as a result of concrete pollution and nutrients were implicated in altering stygofaunal communities. More importantly though, decomposition rates increased with catchment urbanization. Higher decomposition rates, in concert with expected further water temperature intensification due to climate change may result in organic matter losses and decreased organic matter accumulation (Belyea & Malmer, 2004). In turn, as the ratio of sands to organic matter increase as with increasing urbanization (Freeman *et al.*, 2001), water flow may increase, reducing residence time and water quality (Cowley *et al.*, 2016; Dinsmore *et al.*, 2013).

In conclusion, the functioning of upland peat swamps in the Blue Mountains is already being affected by urbanization, evidenced by altering hydrology, water quality, increasing microbial and invertebrate productivity and alterations to stygofauna assemblages. The further indication of there being negative relationships (albeit a weak association) between decomposition aspects of productivity and abundance of scrapers and stygophiles provides scope for further work. Given the importance of peat swamps, both locally and globally in carbon storage (Chapter 2), and the current lack of knowledge of their aquatic and terrestrial invertebrate fauna, further studies are merited (Spitzer, 2005). A key recommendation for management is to reduce and prevent the entry of stormwater from urban areas into the swamps. While the hydrological and sediment consequences of stormwater influx can be mitigated through physical barriers, chemical and toxicant pollution are more difficult to achieve.

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Appendices

Appendix 1 Water Quality Results

Appendix 1 Table 1 Spearmans Rho for water quality correlations across all sites and times. Decomposition increased at a negative rate related to temperature, pH , EC and alkalinity. It increased positively with increasing DO%

Spearmans Rho	Proportional Tension Cotton Strip	Temperature °C	pH	Electrical Conductivity $\mu\text{S/cm}$	DO % Saturation	NH4-Nitrogen (mg/L)	Total alkalinity (mg/L)	Total Nitrogen (mg/L)
Temperature	-0.427							
p	0.001*							
pH	-0.447	0.243						
p	0*	0.061						
EC	-0.475	0.255	0.385					
p	0*	0.049*	0.002*					
DO % Sat	0.254	0.159	-0.11	-0.239				
p	0.05*	0.225	0.404	0.066				
NH4-N	-0.144	0.007	0.038	0.022	-0.087			
p	0.272	0.96	0.775	0.867	0.511			
T.ALK	-0.382	0.11	0.438	0.825	-0.247	0.069		
p	0.003*	0.403	0*	0*	0.057	0.6		
Total N	0.09	0.101	-0.127	-0.317	0.112	0.129	-0.227	
p	0.494	0.443	0.333	0.014*	0.394	0.326	0.081	
DOC	-0.209	0.274	0.09	0.057	-0.061	-0.297	0.176	0.143
p	0.109	0.034*	0.493	0.663	0.643	0.021*	0.178	0.275

Table 2. Mean Water Quality Data for 12 swamps, $n=5$, +/- S.E.

Site	Temperature °C	Temperature Variability °C	pH	Electrical Conductivity $\mu\text{S/cm}$	Dissolved oxygen % Sat	Ammoniacal Nitrogen (mg/L)	Total Alkalinity mg/L	Total Nitrogen mg/L	Dissolved Organic Carbon mg/L
WC	12.56+/- 0.76	10.25	4.99+/- 0.15	39.72+/- 2.31	45.38+/- 1.28	0.05+/- 0.02	4.20+/- 0.37	3.29+/- 0.85	1.70+/- 0.51
PP	13.66+/- 1.26	17.87	6.22+/- 0.31	98.02+/- 13.95	42.73+/- 3.23	0.05+/- 0.01	31.40+/- 2.09	2.00+/- 0.29	6.00+/- 1.98
WF	15.40+/- 1.44	27.63	5.95+/- 0.17	60.36+/- 3.24	57.88+/- 3.04	0.16+/- 0.06	18.20+/- 1.69	6.99+/- 2.35	3.80+/- 0.8
MH	13.42+/- 1.11	15.2	5.42+/- 0.17	19.13+/- 4.43	36.65+/- 3.19	0.28+/- 0.04	6.00+/- 1.34	3.20+/- 0.53	5.10+/- 2.23
MS	13.76+/- 1.11	13.4	5.51+/- 0.21	78.78+/- 5.11	40.20+/- 6.20	0.23+/- 0.03	25.00+/- 3.21	6.65+/- 1.13	3.90+/- 0.51
ME	12.64+/- 1.03	16.27	5.65+/- 0.22	33.24+/- 1.94	56.25+/- 7.14	0.06+/- 0.03	8.40+/- 0.93	4.51+/- 0.25	6.10+/- 1.49
KF	13.62+/- 1.20	20.15	6.16+/- 0.36	79.64+/- 7.96	37.48+/- 2.28	0.24+/- 0.03	19.40+/- 2.14	2.59+/- 0.17	4.90+/- 1.56
RT	12.32+/- 1.35	17.18	5.97+/- 0.21	40.25+/- 11.01	29.13+/- 3.57	0.23+/- 0.09	15.00+/- 1.00	6.65+/- 0.76	5.10+/- 1.49
GC	12.15+/- 1.00	17.14	5.03+/- 0.22	25.30+/- 1.29	37.63+/- 6.29	0.04+/- 0.01	4.00+/- 0.45	5.50+/- 0.51	6.60+/- 2.01
PS	12.21+/- 1.19	19.4	5.93+/- 0.26	52.80+/- 4.12	31.80+/- 3.50	0.13+/- 0.04	13.80+/- 1.93	7.45+/- 1.44	8.00+/- 3.91
PG	13.20+/- 1.63	25.26	6.16+/- 0.26	152.34+/- 22.65	25.25+/- 2.05	0.06+/- 0.02	44.80+/- 5.09	1.65+/- 0.33	7.60+/- 3.07
AS	14.50+/- 1.42	15.13	5.57+/- 0.24	26.66+/- 1.70	57.10+/- 7.25	0.20+/- 0.09	6.80+/- 0.73	7.23+/- 0.77	6.30+/- 2.70

Appendix 2 Invertebrate Data

Table 1 Invertebrate datasheet

[illegible]

Table 2. Invertebrate functional groups. FFG groups taken from available keys, (Centre for Freshwater Ecosystems, 2018). Stygofaunal groups taken from available literature.

Code	Sample Site	FFGs	Stygofaunal groups	Code	Sample Site	FFGs	Stygofaunal groups
Rotifera	Rotifera	gatherer	stygobiont	hydrhyRy	Hygrobatidae like Rynhaustrobates	predator	stygobiont
Aphanone	Aphanonerans	filterer	stygophile	hydrhync	Hygrobatidae? No claws heavily sclerotized	predator	stygobiont
Gastrotr	Gastrotricha ?	gatherer	stygophile	hydhyAu	Hygrobatidae cf. Austalorivarcus	predator	stygobiont
Tardigra	Tardigrada	gatherer	stygophile	hydrhyCo	Hygrobatidae c.f. Cortiacarus	predator	stygobiont
nematoda	Nematoda	predator	stygophile	hydrilmn	Limnesiidae	predator	stygobiont
nemerte	Nemertea	predator	stygophile	hydrmide	Mideopsidae	predator	stygobiont
Nematomo	Nematomorpha	parasite	stygophile	hydrunio	Unionicolidae	predator	stygobiont
dugesida	Dugesidae	scraper	stygophile	hydrspTG	Hydracarina / Hydrachnidae spTG190816	predator	stygobiont
dugesid2	Dugesidae sp. 2	scraper	stygophile	hydrspX	Hydracarina / Hydrachnidae sp. X sclerotised pedipalps long, scissors, no apparent eyes large genital flap	predator	stygobiont
tricladi	Tricladida unid.	scraper	stygophile	hydrsoft	Hydracarina/ front2coxa well sep from back 2, no eyes, soft bodied, pedipalps on thing trochanters, anal pores obvious, 2 claws on legs	predator	stygobiont
turbblobs	Turbellaria unid.1	scraper	stygophile	cypridid	Cyprididae	filterer	stygobiont
turbblo2	Turbellaria unid.2	scraper	stygophile	ostrimm	Ostracoda imm.	filterer	stygobiont
capillov	Capilloventridae (freshwater, marine)	gatherer	stygophile	ostrdarw	species 1 translucent thin shell	filterer	stygobiont
megadril	SO Megadrili	gatherer	stygophile	ostrcy2	Cyprididae sp 2. white shell, hairy, distinct furca, wider apical structure	filterer	stygobiont
tubific	Tubificidae	gatherer	stygophile	ostrcyca	Cyprididae white shell Cabanocypis like, white, long antennae narrow furca	filterer	stygobiont
lumbricu	Lumbriculidae (freshwater, introduced)	gatherer	stygophile	ostrblue	Cyprididae like round shelled tiny blue translucent	filterer	stygobiont
naididae	Naididae (freshwater, marine, stygal)	gatherer	stygophile	ostrcyp	Cypridae type no furca	filterer	stygobiont
phreodri	Phreodrilidae (almost entirely freshwater)	gatherer	stygophile	claddaph	Cladocera - Daphnidae	scraper	stygobiont
oligocha	Oligochaeta	gatherer	stygophile	copnaupl	Copepoda - Nauplii	filterer	stygobiont
acarimdo	Acarina.imm. long dorsal setae hairy	predator	stygophile	copcyclop	Copepoda - Cyclopoida	filterer	stygobiont
oribspa	Oribatida sp. A	scraper	stygoxen	copharpa	Copepoda - Harpacticoida	filterer	stygobiont
orib1	Oribatidae sp1	scraper	stygoxen	isophrea	Isopoda - Phreatoicidae	shredder	stygobiont
orib1KFR	Oribatidae sp1 KFR 091115	scraper	stygoxen	collemba	Collembola	gatherer	stygoxen
orib2	Oribatidae sp2	scraper	stygoxen	cerapogn	Diptera - Ceratopogonidae - Ceratopogoninae	predator	stygophile
orib3	Oribatidae sp3	scraper	stygoxen	ceraforc	Diptera - Ceratopogonidae- Forcipomyiinae	scraper	stygophile
oribatid	Oribatidae unid.	scraper	stygoxen	ephrydi	Diptera - Ephydriidae	shredder	stygophile
orib4	Oribatidae 4	scraper	stygoxen	psychod	Diptera - Psychodidae	gatherer	stygophile
orib5	Oribatidae 5	scraper	stygoxen	empidid	Diptera - Empididae	predator	stygophile
orib6	Oribatidae 6	scraper	stygoxen	culicida	Diptera - Culicidae	predator	stygoxen
orib7bea	Oribatidae 7 beak 1	scraper	stygoxen	muscid	Diptera - Muscidae	predator	stygophile
orib8	Oribatidae 8, soft,no labial palps, large (sim. <i>Achiptera coleopterata</i>)	scraper	stygoxen	aphroten	Chironomidae - <i>Aphroteniella</i> sp.	shredder	stygophile
orib9	Oribatidae sp 9. spider like, beaked	scraper	stygoxen	cricotop	Chironomidae - <i>Cricotopus</i> sp.	gatherer	stygophile
orib10	Oribatidae sp. 10 (feb17) very round legs from beneath flaps	scraper	stygoxen	rheocric	Chironomidae - syn. <i>Rheocricotopus</i> imm.	gatherer	stygophile
oribspUS	Oribatidae sp KFR wrinkly sim. US sp Aglipra 260	scraper	stygoxen	eukiefer	Chironomidae - <i>Eukieferella</i> sp.	gatherer	stygophile
orib17	Oribatidae sp 17 PP031216	scraper	stygoxen	theinem	Chironomidae - <i>Theinemaniella</i> imm	gatherer	stygophile
orib18	Oribatidae sp 18	scraper	stygoxen	ortho33	Chironomidae - Orthoclaadiinae - 33 couplet Don edwards V15	gatherer	stygophile
oribimm	Oribatidae imm.	scraper	stygoxen	orth33b	Chironomidae - Orthoclaadiinae - couplet 33 Madden sp. 1	gatherer	stygophile
acarimm	Acarina imm	scraper	stygoxen	chirorth	Chironomidae-Orthoclaadiinae imm	gatherer	stygophile
astigmat	S.O. Astigmata	predator	stygoxen	chirtany	Chironomidae - <i>Tanytarsus</i> sp.	gatherer	stygophile
halaimm	immature Halacarida imm. Long dorsal setae hairy	predator	stygoxen	chirpoly	Chironomidae - <i>Polypedium</i> sp.	gatherer	stygophile
halapez1	Halacaroida Pezidae sp 1 imm	predator	stygoxen	chirxylo	Chironomidae - <i>Xylochironomus</i> sp.	gatherer	stygophile
halapezi	Halacaroida Pezidae	predator	stygoxen	chirdicr	Chironomidae - <i>Dicrotendipes</i> sp.	gatherer	stygophile
halacar	Halacaroida Halacaridae sp1	predator	stygoxen	chirwood	Chironomidae - genus <i>woodminer</i>	gatherer	stygophile
halalong	Halacaridae super long	predator	stygoxen	chirpara	Chironomidae - <i>Paramerina</i> sp.	predator	stygobiont
halaX	Halacaridae square, hairy, spiny processes, single claw, thick setae on tarsus	predator	stygoxen	chirparo	Chironomidae - <i>Parochlus</i> sp.	predator	stygophile
mesostig	Mesostigmata sp.	predator	stygoxen	chirtelm	Chironomidae - <i>Telmatopelopia</i> sp.	predator	stygophile
mesoMH17	Mesostigmata same as MH250217	predator	stygoxen	tipulid	Diptera - Tipulidae	gatherer	stygophile
Meso14	Mesostigmata sp. 14 long large chelate chelicera	predator	stygoxen	stratod	Stratiomyidae - Odontomyia sp.	gatherer	stygoxen
mesoacWF	AcarinaMesostigmata sim WFL 02/17	predator	stygoxen	scirtida	Coleoptera - Scirtidae	filterer	stygophile
meso1psci	Mesostigmata sp1 sp scissors fluffly hairy peipalps	predator	stygoxen	circulio	Coleoptera - Curculionidae	shredder	stygophile
Meso16	Mesostigmata sp. 16	predator	stygoxen	carabad	Coleoptera - Carabidae adult	predator	stygophile
hydranis	Hydracarina / Hydrachnidae Anisitsiellidae	predator	stygobiont	colhydro	Coleoptera - Hydrophilidae	predator	stygoxen
hydrarre	Arrenuridae	predator	stygobiont	ephelept	Ephemeroptera - Leptophlebiidae	scraper	stygoxen
hydratur	Aturidae	predator	stygobiont	lepicram	Lepidoptera - Crambidae	shredder	stygoxen
hydrhydr	Hydrachnidae Hydrachna	predator	stygobiont	tricpoly	Trichoptera - Polycentropidae	predator	stygophile
hydrhygl	Hygrobatidae based on glandularia	predator	stygobiont	meconann	Mecoptera - Nannochorista	predator	stygophile
hydrhye	Hygrobatidae like with long spiky pedipalps	predator	stygobiont	odonargi	Odonata - Argiolestidae	predator	stygoxen

Chapter 4

Leaf litter decomposition processes in upland swamp streams; threatened ecological systems in the Blue Mountains of eastern New South Wales, Australia



Lorraine Hardwick

Department of Biological Sciences

Abstract

Peat swamps globally are under intense pressure from human activities. In the Blue Mountains, Australia, upland peat swamps are a small, but highly variable and endemic wetland type, which are under high levels of threat. Leaf litter bags were used to investigate functional processes in streams associated with peat swamps. Eucalypt leaf litter decay was measured to understand how swamp streams are affected by anthropogenic stress associated with a gradient of urbanization. Leaf litter decay was slow relative to comparative studies in other ecosystems but in line with understandings of peat swamp processes. Fresh leaves lost more than 70% mass over 265 days in swamps within catchments with the highest catchment development. Aged litter decayed poorly, with 21% mass loss, suggesting little use by microbes or invertebrates. Variable rates of decay were related to disturbance gradients of nutrients, pH, temperature, dissolved oxygen and intrinsic properties of the leaves. Both leaf litter decomposition and invertebrate metrics illustrated unimodal responses to increasing catchment imperviousness and disturbance. While there are few studies of peat swamps affected by urbanization, these results agree with other studies of streams suffering urban degradation. Therefore, upland swamps, once channelized, lose their buffering potential and ability to act as sinks for nutrients and other pollutants.

Introduction

Peat swamps are found throughout the world. They are most common in high latitudes such as Russia, Canada and South America, but are poorly represented in many areas, such as Australia (Pemberton, 2005). They account for between 50% and 70% of all wetlands and store twice the carbon of all forests combined but cover only 3% of the earth's surface (Parish, 2008). Peat swamps are under intense pressure globally, and greater knowledge is needed to manage and protect them into the future.

The accumulation and relatively slow breakdown of organic matter, typically leaf or other plant material, leading to peat formation is a defining attribute of these systems. Accumulation of decomposed plant material and formation of peat is a complex process dependent on waterlogged or alternately dry and saturated and often anoxic conditions (Pemberton, 2005). Rates of formation and loss are also highly variable, with anthropogenic loss extensive and rates of formation complicated and related to water availability and thickness of the aerobic surface layer (acrotelm) (Belyea & Clymo, 2001). Maintenance of peat swamps, with their huge carbon stores, relies on inundation and storage of water.

Leaf litter decomposition is an essential ecological process in river and wetland food webs generally (Graca, 2001). The process generally involves initial leaching, followed by microbial colonization and conditioning (Bergfur *et al.*, 2007; Imberger *et al.*, 2008; Kerr *et al.*, 2013) and then invertebrate shredding and decomposition (Graca, 2001). The rates of decomposition are variable and are dependent on temperature, leaf toughness, nutrient content and the presence of secondary metabolites (Graca, 2001), seasonality, stream flow variability (Dieter *et al.*, 2011), natural and human induced eutrophication (Ferreira *et al.*, 2015) and acidity (Dangles, 2004a; Holland *et al.*, 2012). While the role of these factors in influencing litter decomposition in streams and wetlands broadly is well known, little is known of these processes in peat swamps, particularly in Australia. In particular, information on the importance of invertebrate shredders and microbial decomposition, flow, pH and nutrient concentrations is sparse, even in Northern hemisphere boreal, temperate and tropical peat systems.

Peat swamps are common feature in the headwaters of low order streams, on low relief plateaus of eastern Australia, such as those in the Blue Mountains and Southern Highlands, near Sydney in New South Wales (Fryirs, 2014). They are typically small (0.04 to 42 ha) and support a range of endemic fauna and flora (Benson & Baird, 2012; Hollands *et al.*, 1991). They are extremely vulnerable to a variety of anthropogenic pressures including urban development, forestry, coal and gas mining, bushfires and associated climate change (Krogh, 2007; The Scientific Committee, 2007). Mechanisms such as drainage that renders swamps aerobic, leads to microbial decomposition of peat. Accordingly, they are listed as endangered ecological communities (Fryirs & Hose, 2012; Fryirs, 2016; Hose *et al.*, 2014) under Commonwealth (EPBC Act 1999) and NSW (TSCA 2005) legislation.

Catchment urbanization is a major threat to the upland peat swamps in the Sydney basin (Benson *et al.*, 2012). Impacts of catchment urbanization on streams are generally well known (Meyer *et al.*, 2005; Walsh *et al.*, 2005); impervious land surface cover and efficient drainage systems create fast, high volume discharge to streams following rainfall events, causing erosion and sedimentation (Yule *et al.*, 2015). Further predictable effects include the mobilization of nutrients and contaminants (Freidman, 2014; Fryirs, 2016; Kohlhagen *et al.*, 2013; St.Lawrence, 2014), weed infestation and increased litter decomposition rates, with changes to native vegetation and invertebrate communities (Besley & Chessman, 2008; Carey, 2007; St.Lawrence, 2014; Wright & Burgin, 2009) and a shift toward more tolerant invertebrate species (Meyer *et al.*, 2005). Associated loss of structural integrity and the presence of contemporary sands within urbanized Blue Mountains swamps, with lower water retention capacity (Cowley, 2017), indicate that impacted, channelized swamps function and respond differently to natural swamp systems. Evidence of flocs of iron bacteria in streams downstream of urbanized peat swamps continues to cause concern and indicates altered redox functions taking place within them (Bertrand *et al.*, 2011).

Aims

The aims of this study were to understand eucalypt leaf litter decomposition processes in streams associated with Blue Mountains peat swamps. It was hypothesized that streams associated with swamps in least urbanized catchments would have slower rates of litter decomposition than those

in more urbanized catchments because of lower temperature, low nutrients, less acidic conditions and lower stream flow than less urbanized swamps. It was further hypothesized that invertebrate assemblages in leaf packs in less urbanized catchments would differ from those in more urbanized catchments and contain fewer tolerant invertebrate taxa. To test these hypotheses, leaf litter filled mesh bags in three mesh sizes were deployed that would allow for total decomposition, small macroinvertebrate access and microbial access, filled with aged and fresh eucalypt leaves. These were then placed in streams running through channelized swamps or in small streams downstream of un-channelized swamps differing in their degree of catchment urbanization. The rates of litter decay and invertebrate communities inhabiting the leaf packs were quantified over time and compared.

Methods

Study Sites

The Blue Mountains region west of Sydney, Australia (Figure 1) is dominated by an incised sandstone plateau. Peat Swamps are common in the headwaters of low order streams (Fryirs, 2016) between 600 and 1100 m A.S.L and where mean annual rainfall exceeds 880 mm. Swamps form in valleys above bedrock steps of constriction, where eroded sediments deposit over time.

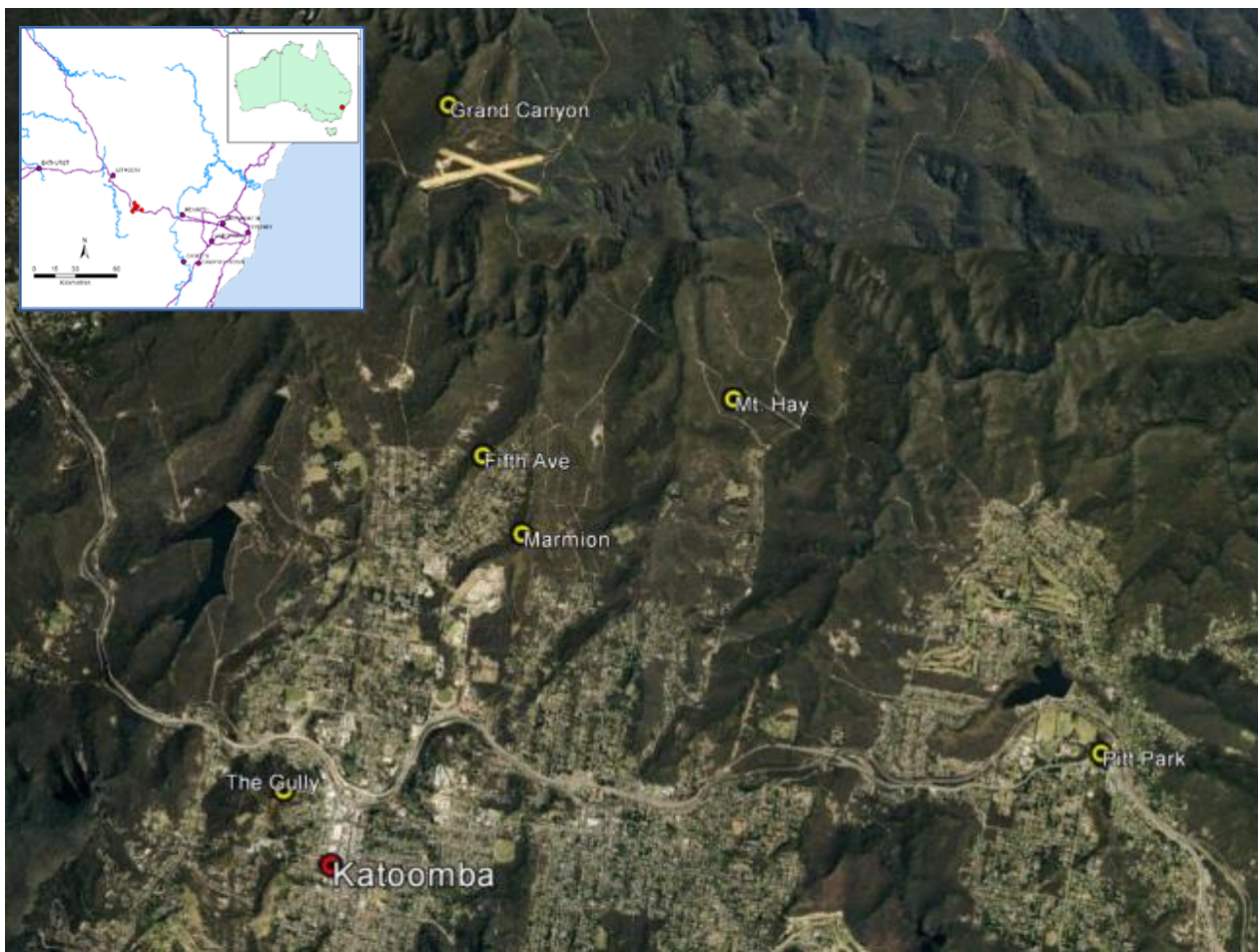


Figure 1. Sampling sites for Study (Source Google Earth Pro (©2017 CNES/Airbus, Aerometrix & Jacobs, Sinclair Knight Merz).

Six swamps were selected for this study (Figure 2). All were located near Katoomba (33° 40' S; 150° 20' E), approximately 120 km west of Sydney, NSW. Mean daily temperatures range between 3-10 °C to 13-23°C in winter and summer respectively, with mean annual rainfall of 1400 mm. Sites were chosen between 900-1000 m A.S.L. All swamps are termed THPSS swamps, which when undisturbed generally are entirely groundwater driven. Once urbanization occurs, increased impervious surfaces lead to higher runoff intensity and erosion may occur. This creates streams through the swamps, characteristically with bedrock bases.(Cowley *et al.*, 2018; Fryirs, 2016)

Mount Hay Swamp (MH) (-33.682646,150.34336, 926 m A.S.L) has a catchment area of 19.5 ha and a mean slope of 32.3%. The swamp is channelized in its lower reaches due to construction of a ford for vehicle access to powerlines. This has created a headcut upstream of the ford, with substantial tunneling and loss of groundwater depth. Downstream, the channel is narrow and alternately deep and shallow, with highly shaded pools, before reaching the 10 metre geomorphic control rock cliff.

Grand Canyon Swamp (GC) (-33.661944,150.319794, 944 m A.S.L) has a catchment area of 41.3 ha and a mean slope of 22.9%. GC is completely unchannelized above the 4 metre cliff, but with a small open and then highly shaded channel downstream. Both MH and GC are mostly undisturbed Blue Mountains upland shrub swamps, characterized in part by *Grevillea acanthifolia* subsp. *acanthifolia*, *Banksia spinulosa*, *Hakea teretifolia*, *Lepidosperma limicola* and *Epacris obtusifolia*.

Pitt Park Swamp (PP) (-33.707137,150.360739, 873 m A.S.L) has a catchment area of 39.5 ha and a mean slope of 7.9%). PP is surrounded by urban development and featuring an historic, failed dam, with a small continuous channel and is vegetated with exotic weeds, (*Rubus fruticosus* L. agg. , *Lonicera japonica* and *Nephrolepis cordifolia*) and *Carex appressa* and *Leptospermum juniperum* (Keith & Myerscough, 1993).

The Gully (Catalina Racetrack) (TG) (-33.712419,150.304785, 983 m A.S.L) has a catchment area of 50.6 ha and a mean slope of 4.6%). TG in Katoomba has similar vegetation to PP, dominated by *Carex appressa* and *Leptospermum juniperum* and has been similarly excavated,

initially as a lake and with adjacent mining (Blue Mountains City Council, 2004) with altered profile and geomorphology. It has a reduced profile and is subject to restoration as an important Indigenous cultural site.



Figure 2. The Blue Mountains sample sites, with distance and stream environment sites shown.

Fifth Avenue Swamp (5A) (-33.686452,150.325137, 916 m A.S.L) has a catchment area of 67.5 ha and a mean slope of 17.5%). The swamp, adjacent to Fifth Ave North Katoomba, has been encroached by housing to within the swamp boundary. The stream alternates between surface and groundwater flow, finally becoming a distinct channel before flowing into Yosemite Creek. While it retains original vegetation remnants of *Grevillea acanthifolia* subs. *acanthifolia* and *Gleichenia dicarpa*, it also supports *Leptospermum juniperum* and *Lepidosperma limicola*.

Marmion Swamp (MS) (-33.6693502,150.325513, 936 m A.S.L) has a catchment area of 1333 ha and a mean slope of 13.4%). MS is highly urbanized, with upstream hospital, cemetery and landfill site. It is fully channelized along the western edge, with a sandstone stream base eroded from the swamp. While further uphill, the swamp is vegetated with *Grevillea acanthifolia* subsp.

acanthifolia and *Gymnoschoenus sphaerocephalus*, further downstream riparian vegetation is dominated by *Nephrolepis*, *Blechnum* and *Carex* spp., with eucalypt overstorey.

Swamp catchments varied in their natural and anthropogenic characteristics (Fryirs, 2016), including catchment imperviousness which varied from 0 to 50% and swamps with and without in-swamp channelization (Figure 2). Catchment imperviousness is the percent of a catchment that is covered by impervious cover such as roads, roof surface or areas of concrete (Meyer *et al.*, 2005). Sites were chosen as channelized or semi channelized streams with alternating surface water/groundwater typology, or streams emanating from undisturbed un-channelized swamps (Cowley *et al.*, 2018). Naturally, swamps are entire, with complex groundwater hydrology, ending in a sandstone cascade (Figure 3).

Swamp water emanates from beneath the swamp as it is limited in its downward vertical movement by a sandstone aquitard. Downstream, a small stream with several ponds, is usually followed by spectacular waterfalls as the stream falls from the escarpment into deeply incised valleys. Eroded swamps feature well defined channels with permanent streams. Associated drying of swamps and loss of peat is common. A hydraulic conceptual model of the swamps has been developed by Cowley *et al.* (2018), reproduced in Appendix 1.

These carbon rich and acidic peat swamps are derived from terrestrial organic matter decomposition (Fryirs, 2014), are naturally shaded and groundwater linked (Cowley, 2017). They are the source of small streams, apparently heterotrophic in nature (Mulholland, 1981). A common aquatic feature of these peat streams are eucalypt leaf packs (accumulations of litter and detritus that form in pockets of low flow) in backwaters or small deep pools in either channelized or intact swamps. They are mostly very small habitat patches, below waterfalls, within alternating groundwater-surface water hydrology or as defined and eroded channels with a sandstone base.

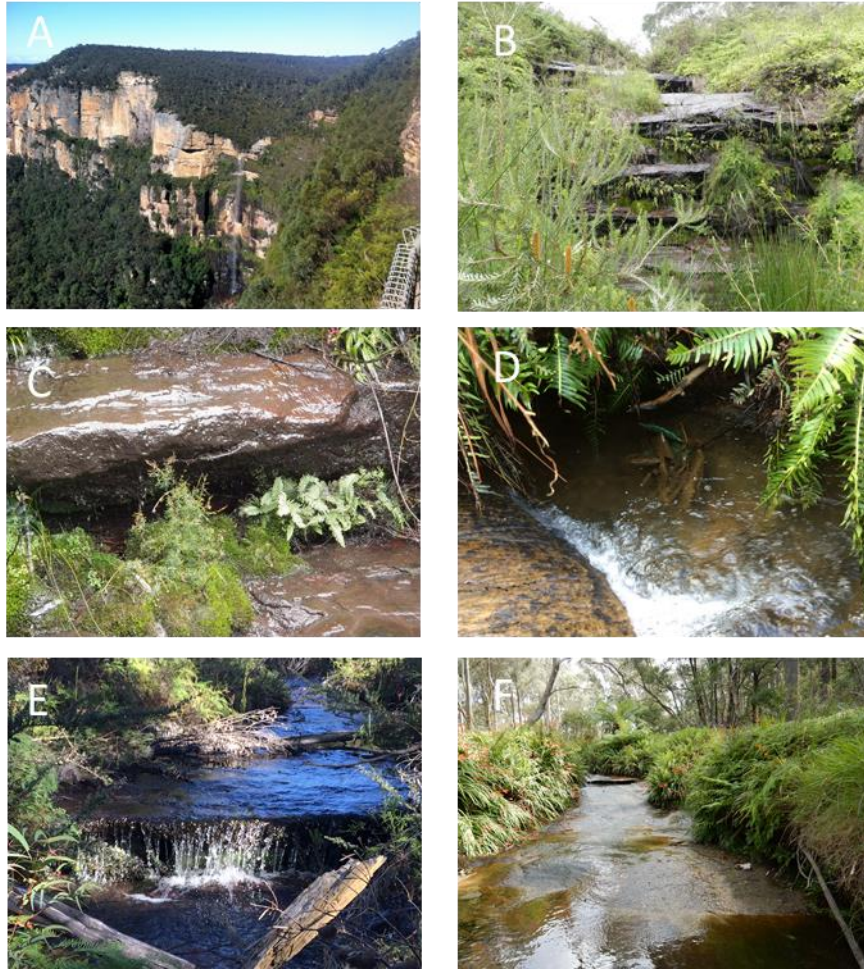


Figure 3. Blue Mountains peat swamp stream features. A, Govetts Leap waterfall, downstream of Perry St. peat swamp at Blackheath; B, Grand Canyon swamp cascade, downstream of swamp, Medlow Bath; C, Close up of Grand Canyon swamp seepage from base of swamp; D-F stepped sandstone aquitard of eroded swamp streams.

Assessment of Anthropogenic Disturbance

Swamps were initially chosen based on their catchment imperviousness as described above and based on that, were allocated a provisional disturbance group of undisturbed (GC and MH), disturbed (TG and PP) and most disturbed (5A and MS). Catchment areas were manually calculated using SIX (Spatial Information Exchange) maps including the NSW topographic basemap (Spatial Services, 2017) with a combination of Digital Topographic Database (DTDB), the Geocoded Urban and Rural Addressing System (GURAS) database and the Digital Cadastral

Database (DCDB) file identifier (018E63C0-56D2-4298-94D8-9BA17E26D347) remotely sensed data using contour based mapping, with swamp area and length also manually derived. Verification was performed with high resolution satellite imagery sourced from DSFI Spatial Services NSW. The layer depicts an imagery map of NSW showing a selection of SPOT5® satellite imagery, standard 50cm orthorectified imageries, High resolution 10cm Town Imageries, revised 17/7/2017. Quality control was performed by duplicate mapping to verify method, with <10% difference between derived area. Altitude and slope were derived from Google Earth Pro (Google Inc, 2015).

Pre-existing data for total percent impervious cover for each swamp were obtained (Fryirs, 2016) where catchment boundaries were derived from 2002 SPOT raster imagery imported to ARCGIS. Vegetated colourations were then removed and impervious area defined by two colour values then converted to vector polygons. These were then intersected with the catchment area layer and impervious area calculated followed by derived percentages. Verification was performed over several catchments by manually mapping impervious cover using SIX (Spatial Information Exchange) maps as above. Distance to stormwater and sewerage infrastructure was calculated using Google Earth Pro distance tool (Google Inc. 2015) overlain with Blue Mountains City Council (BMCC) infrastructure spatial layers, calculated manually.

Physical and Chemical Methods

Stream temperature was measured at 30-minute intervals using Onetemp Hobo ® 64 bit pendant light/temperature loggers, with mean daily temperature and variability calculated. Real time temperature was converted to degree days by summing mean daily temperatures of days since time 0 (Griffiths & Tiegs, 2016). On each sampling occasion, two replicates of water quality and habitat information were collected. Electrical conductivity ($\mu\text{S}/\text{cm}$), pH and dissolved oxygen (% saturation and mg/L) were collected using a pre-calibrated Hydrolab Surveyor 4 and Minisonde 5S multi probed water quality instrument. Water samples (125 mL) were filtered through 0.45 μm (Sartorius Minisart) sterile syringe filters into triple rinsed PET sample bottles and frozen until analysis by Sydney Analytical Laboratories (Seven Hills, NSW, Australia). Samples were analyzed for filtered Ammonia (NH_3 4500G), Nitrite/Nitrate (NO_2/NO_3 4500F), filterable reactive phosphorus (FRP 4500F) and dissolved organic carbon (DOC 5310C) (APHA, 2005).

Litter Bag Methods

Choice of plant litter

Blue Mountains swamps are nutrient poor and acidic, with sclerophyllous vegetation, comprised almost entirely of species exhibiting high leaf mass per area (LMA). Correlations between high LMA and leaf life span (LL) in such environments are well known (Wright *et al.*, 2002) and associated defense mechanisms including leaf toughness, presence of toxic secondary metabolites and high C:N ratios (Burghardt, 2015), mean that swamp vegetation isn't palatable or readily available. Most swamp species exhibit narrow or terete leaf structure, brittle phenology, with substantial secondary thickening and high SA/V ratios, so are inherently unsuitable for leaf litter decomposition studies. The nearest plants available with conventional leaf structure and comparable specific leaf area (SLA) as used in global comparisons (Boyero, 2016), were surrounding eucalypts, which while also exhibiting similar traits, enabled data collection such as leaf surface respiration, ease of access for invertebrates and with low fragmentation. They are known to be more readily available for decomposition in upland streams than others species in SE Australian upland streams (Stewart *et al.*, 2002). While they exhibit poor seasonality of leaf fall, typically lasting 2-3 years (CSIRO, 2019) and collecting recently abscised leaves is impossible, they were chosen as the best local option for standardized sampling. Furthermore, local tree species are commonly used in litter decomposition studies (Boulton, 1991; Boyero, 2016; Suter *et al.*, 2011). The use of globally standardized leaf species Alder (*Alnus glutinosa*) has been more commonly used in global studies, however it is reasonable to contend that by using the same species acquired locally is not scientifically valid for many reasons. Furthermore, recently abscised leaves are commonly used to standardize sampling protocols, However recent studies indicate that they may over-estimate decomposition rates (Abelho & Descals, 2019). Complicated decomposition processes related to age of litter and the time it would naturally take to access the aquatic system mean that standardized methods need to be stated and reported categorically and may not reflect natural rates. Therefore, in this study, freshly cut leaves of *E. mannifera* subsp. *gullickii* and *E. piperita* were accessed following pruning beneath power lines at Minihaha Reserve (adjacent to *Fifth Avenue Swamp* (5A) (-33.686452, 150.325137, 916 m A.S.L). Aged leaves were similarly accessed underneath trees at the same location. These formed

the bulk of leaf decomposition materials and reduced the need for destruction of further leaf canopy.

Litter bags of different mesh size were made using 9 mm PVC mesh gutterguard (www.whitesgroup.com.au), 1.0 mm nylon (Nytal 1000 μm - SEFAR 06-1000/44) and 150 μm (Nytex 150 μm SEFAR 03-150/38) (www.sefar.com.au). These mesh sizes were used to measure total leaf litter (9 mm mesh bags), smaller detritivore mediated (1 mm mesh bags) and microbial mediated decomposition (150 μm mesh bags) (Mora-Gomez *et al.*, 2015; Woodward *et al.*, 2012). Bags were all 100 mm x 150 mm. Leaf litter was air dried to constant mass before use (Suter *et al.*, 2011). Each bag was filled with a mixture of fresh recently pruned dried (3.0 \pm 0.1g), and aged *E. mannifera* subsp. *gullickii* and *E. piperita* leaves (2.5 \pm 0.1 g), similar to methods outlined by Boyero *et al.* (2011).

Six (6) replicated bags were placed in each of four (4) pools in close proximity in small streams within or immediately downstream of the swamps. Bags were deployed on 5 May 2016 and removed 30, 60, 90, 150 and 210 and 270 days after deployment, with one bag of each of the mesh size (150 μm , 1 mm and 9 mm) collected from each pool (i.e. four replicates) on each occasion. The 12 samples from each of the six (6) sites were placed in clean plastic bags on ice immediately and kept at 4°C until processing within 24 hours.

The litter bag contents were washed gently under tap water over a 150 μm sieve to separate litter from invertebrates and remove sediment. Samples were taken for measurement of respiration after 210 and 270 days as measured by colourmetric reduction of MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Gerlier & Thomasset, 1986; Stockert *et al.*, 2012), with two replicates from each 150 μm mesh fresh leaf sample. Microbial samples were taken with an ethanol sterilized 8 circle HDPE milled stencil, using a sterile cotton swab to randomly collect 4, 10 mm diameter areas into 1.0 mL sterile PBS buffer. Following processing, remnant cells were incubated in 160 μL Thiazolium/nutrient/buffer for 24 hours at 22° C. This was followed by addition and mixing of acidified isopropanol under dark conditions and measurement of formazan at between 570 and 690 nm (Pherastar microplate reader).

Washed leaves were then air dried to consistent dryness and weighed to ± 0.001 g for mass loss. Plant litter was subsequently separated into original components then oven dried at 60° C and further weighed ± 0.001 g. Remaining litter was then burnt off at 550°C for 4 hours and reweighed for Ash Free Dry Mass (AFDM) by loss on ignition (LOI) as before. Replicate samples of fresh and aged eucalypt leaves were analyzed for Carbon:Nitrogen ratios before inundation. Fern material was removed from analysis due to its brittleness and difficulties in accurately determining mass.

All remnant sieve contents, including invertebrates, sediment and detritus were collected into wide mouth samples jars and preserved in 100% ethanol. A subsample was sorted and identified, using available Australian aquatic invertebrate keys (Centre for Freshwater Ecosystems, 2018; Gooderham, 2002) to genus level where possible, except for Diptera (to family - except for Chironomidae and Ceratopogonidae which were identified to subfamily), microcrustaceans (super family), oligochaetes (class), nematodes and lower taxonomic groups (phylum). Functional feeding groups were elucidated from various local sources (Centre for Freshwater Ecosystems, 2018; Gooderham, 2002; Hawking, 2006).

Data Analysis

Catchment characteristics and mean water quality ($n=2$) for each sampling event were analyzed using principal components analysis (PCA) following normalization (Clarke, 2014). Correlations among environmental variables were tested using Pearsons correlations within Draftsman plots in PRIMER (Clarke, 2015), with those with greater than 0.95 (number of stormwater pits and industry in catchment) deleted from analysis. Cluster analysis and similarity of profiles (SIMPROF) were then used to identify site groupings.

Rates of total leaf litter decomposition (9 mm mesh bags), smaller detritivore mediated decomposition (1 mm mesh bags) and microbial mediated decomposition (150 μ m mesh bags) (Mora-Gomez *et al.*, 2015; Woodward *et al.*, 2012) of fresh and aged leaves were derived and transformed appropriately. Litter mass loss fitted a linear model better than exponential one, so no transformation was performed. Decomposition rates of aged and fresh leaves were also compared for total, detritivore and microbial decomposition. Percentage ash free dry mass

(AFDM) by loss on ignition (LOI) data were arcsine transformed before analysis. Both mass loss and AFDM were analyzed using a general linear model (GLM) with loss as the response, site and mesh size as factors and days/degree days as the covariate. Mass loss slopes were compared using Tukey's post hoc comparisons to identify significant differences at $P < 0.05$.

Invertebrate data were pooled from each mesh size within each site, with measures of richness and abundance using all data. For multivariate species analysis, taxa contributing less than 10% of total abundance were excluded followed by square root transformation in PRIMER (Clarke, 2014). Non-metric MDS using Bray Curtis similarity was used to identify visual differences among swamps over time, which were then tested using Analysis of Similarity (ANOSIM). Variability in community structure was analysed using multivariate index of dispersion (MVDISP). Abundance of Ephemeroptera, Plecoptera, and Trichoptera (EPT) (Resh, 1993) were used as an indicator of taxa sensitive to anthropogenic stress. SIGNAL2 (Chessman, 2003) was used as an indicator of pollution tolerance and percentage of chironomids and oligochaetes (% OC) in samples as a measure of low status invertebrates (Camargo *et al.*, 2011). Both of these groups have low SIGNAL values and are known to inhabit polluted aquatic habitats. Functional feeding groups (FFGs) were allocated using local invertebrate keys (Centre for Freshwater Ecosystems, 2018; Gooderham, 2002; Hawking, 2006), following removal of chironomid data due to insufficient taxonomy.

Litter decomposition rates (mass loss and AFDM) were compared between bag mesh sizes and swamp streams over time. Leaf litter decomposition rates were then related to catchment characteristics to identify how they affect ecological processes in swamps. These were further related to leaf litter invertebrate metrics. The mean of both leaf litter decomposition and functional feeding group data for each site and sampling event were both used to perform correlation analysis using Pearson's Rho. Multivariate analyses were performed in PRIMER (Clarke, 2015) and univariate analyses using Minitab 17.3.1, 2016, Minitab Inc. with a significance level of 0.05.

Results

Swamp Characteristics

The six catchments differed in terms of anthropogenic disturbance, with increasing catchment imperviousness and proximity to stormwater infrastructure (Table 1). Stream electrical conductivity and pH increased with catchment imperviousness. Mean daily water temperature ($F, 20.4, P<0.001$) was significantly higher for PP (12.4°C) and lower for TG (9.4°C) than the other sites. Mean daily temperature variability was highest in the heavily channelized MS, and lowest in less disturbed catchments, except for TG ($F, 174.5, P<0.001$).

There were few significant differences in mean dissolved oxygen, although TG had a mean of 43.9%, while the most channelized site, MS had 94.4% saturation. Ammonium concentrations were highest in PP and the most urbanized catchments had significantly higher NO_2/NO_3 (Table 1). PP released significantly higher DOC than MS and MH ($f, 3.84, P=0.008$). Iron reducing bacteria were evident at PP and dominant within the entire waterbody of TG. While PP and TG had lower total catchment imperviousness than 5A and MS, they were closer to sewer lines and possible sewage leakage, historic excavation and associated lack of slope (Table 1).

Principal components analysis of catchment properties separated the six swamps into four groups. The ordination explained 56% of the differences in the first two axes (Figure 4), with PC1 axis exhibiting disturbance trajectory. The least disturbed swamps, GC and MH, were characterized by high slope and long distance to stormwater and sewerage infrastructure. PP and to some extent TG were characterized by previous excavation, proximity to stormwater lines, DOC and $\text{NH}_4\text{-N}$ concentrations. Catchment imperviousness increased with catchment size, possibly due to available inhabitable land.

The catchments with highest % impervious catchment - 5A and MS, were also characterized by higher electrical conductivity, pH, DO% and $\text{NO}_x\text{-N}$. Cluster analysis (CLUSTER) and similarity of profiles (SIMPROF) analysis identified four groups: MH/GC, 5A/MS, and with PP and TG separating into two groupings at $\pi = 0.527$ $p<0.1\%$. These results indicated strong dissimilarity between sites. Greatest similarities were between MH/GC and MS/5A (Figure 4).

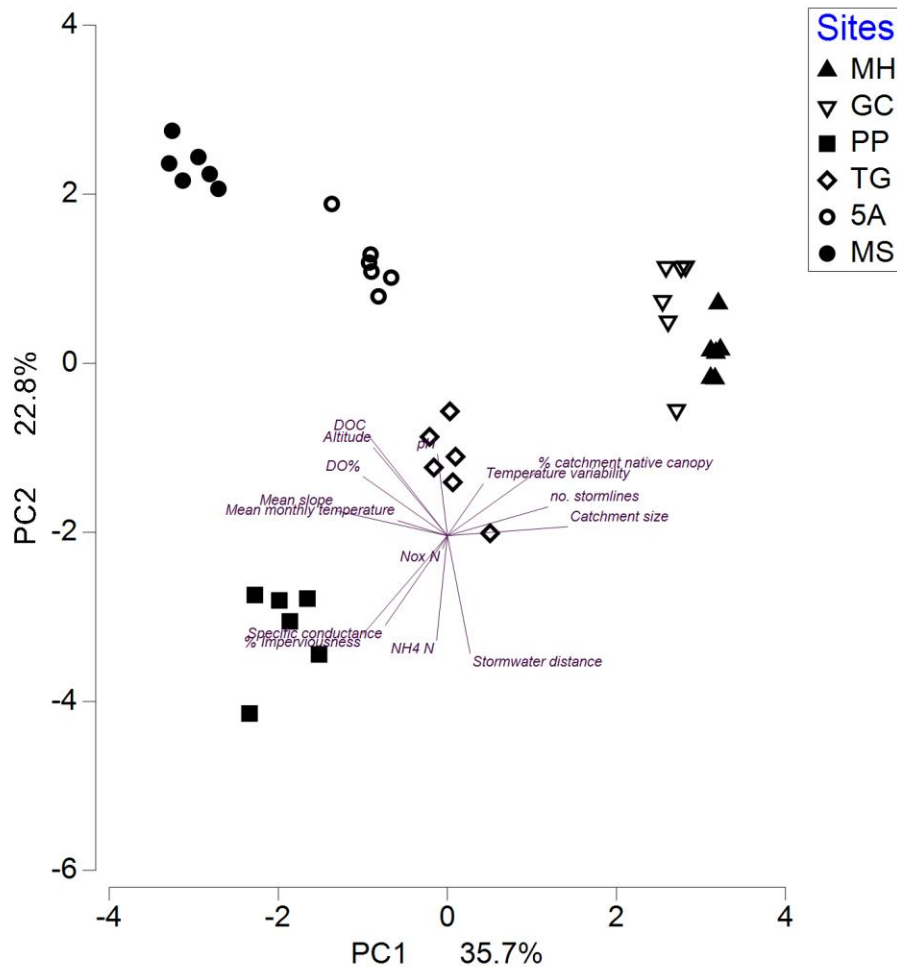


Figure 4. Principal Components Analysis (PCA) of catchment features of the Blue Mountains upland swamp streams (Normalised, correlated data removed, Euclidean distance). Parameters used were percent native vegetation (%vegN), distance to sewer(distsewar), Slope, altitude(Alt), excavation(Excav), distance to stormwater lines(DistormL), dissolved organic carbon(DOC), Ammoniacal nitrogen(NH4N), mean daily temperature (Temp),mean temperature variability(TempV), percent dissolved oxygen*DO%), percent impervious catchment(%ImpV), pH, electrical conductivity (Cond), catchment area (Area), nitrogen oxides(NoxN).

Table 1. Swamp catchment properties, increasing in percent impervious cover. Mean temperature data was derived from 30 minute measurements at duplicate points in each stream, n=265 days, May 2015-February 2016. Mean pH, electrical conductivity, dissolved oxygen, mean NH₄, NO₂/NO₃, FRP, DOC, n=6, nutrient methods according to APHA 2012. All parameters including range and +/- S.D.

Site	Mt. Hay MH	Grand Canyon GC	Pitt Park PP	The Gully TG	Fifth Ave 5A	Marmion MS
Mean Temperature, °C (Temp)	10.9±1.0 (6.3-17.0)	11.5 ±0.9 (7.7-15.3)	10.8±1.6 (4.4-19.4)	9.3±1.5 (4.2-18.3)	10.6±0.9 (7.2-15.2)	12.7±1.4 (6.7-19.7)
Mean Daily Temperature Variability, °C (tempV)	0.8±0.4	2.2±1.4	2.3±1.4	0.8±0.7	1.3±0.8	2.8±1.7
Mean pH	5.6±0.5 (4.8-7.0)	5.5±0.8 (4.0-6.9)	6.2±0.48 (5.4-6.7)	5.8±0.6 (4.6-6.6)	6.4 ±0.56 (5.07-7.25)	6.9±0.7 (5.1-7.8)
Mean Conductivity, µS/cm (Cond)	22.5±2.3 (18.2-27.2)	30.4±10.2 (11.2-51.0)	50.8±24.2 (5.37-77.1)	56.8±24.1 (1.9-85.1)	62.9±15.3 (45.5-96.3)	93.0±21.8 (58.1-138.3)
Mean Dissolved Oxygen, % Saturation (DO%)	86.0±8.3 (18.2-27.2)	81.1±25.8 (22.4-107.2)	71.7±17.7 (50.5-98.8)	43.9±21.2 (15.2-83.6)	88.5±2.61 (8.28-92.1)	95.0±5.4 (82.1-101.4)
Mean NH ₄ ⁺ , mg/L (NH ₄ N)	0-0.02	0.02 ±0.02 (0.1-0.7)	0.1±0.03 (0.05-0.14)	0.01-0.02	0.01 (0.01-0.02)	0.04±0.02 (0.01-0.06)
Mean NO ₂ /NO ₃ , mg/L (NO _x N)	0-0.01	0	0.05±0.04 (0-0.1)	0	0.68±0.16 (0.49-0.97)	0.8±0.2 (0.5-1.2)
Mean Filterable Reactive Phosphorus, mg/L (FRP)	0	0	0	0	0	0
Mean Dissolved Organic Carbon, mg/L (DOC)	1.92±0.47 (1.0-2.5)	2.3±06 (1.5-3.5)	2.8±0.65 (2.0-4.0)	2.63±0.68 (2.0-4.5)	2.14±0.43 (1.5-3.0)	1.8±0.25 (1.5-2.0)
% Catchment Native Canopy (%vegN)	87.2	82.3	11.1	40.3	23.4	3.75
Impervious catchment % (%Impv)	1.3	4.6	32	39.6	44.3	54.6
Stormwater pits in catchment (#stormp)	0	0	41	36	66	81
Stormwater lines distance, m (DistormL)	0	0	41	11	8	15
Distance to stormwater, m (Distorm)	1667	1290	30	175	73	247
Distance to sewer lines, m (Distsewar)	1946	3511	2	0	12	60
Upstream industry	no	no	yes	yes	yes	yes
Earthworks in swamp	YES (road)	NO	YES (excavation)	YES (excavation)	NO	NO

Characteristics of Leaf Litter Decay

Mean Carbon:Nitrogen ratios for fresh and aged air-dried leaves were (41:1) and (112:1), respectively and commensurate with known values for eucalypt litter in general (Snowdon, 2005). Mean carbon content of fresh and aged leaves was 49.5 and 49.7%, respectively; fresh leaves contained 1.2% and aged leaves 0.44% nitrogen.

Leaf litter decay measured by ANOVA of carbon loss (AFDM) of pooled data (leaf type, site and mesh size) over each time (days) were significant ($R^2=4.91\%$, $F_{46.6}$, $P<0.001$) but was poorly indicative of decomposition rates. Arcsine transformation was not effective at normalizing the data. An average of 95% of leaf carbon remained after nine months. Fresh leaves lost significantly more carbon (95.8%) on ignition than aged leaves (91.2%), ($R^2=15.94\%$, $F_{164.7}$, $P<0.001$), implying that fresh leaves and their microbial communities retained more secondary non-combustible components, for example silicates and nutrients such as Ca, Mg, K, and P (Berg, 2014). Analysis using time as degree days was less significant ($R^2=3.71$, $F_{29.71}$, $P<0.001$).

Analysis of mass loss of air-dried leaves using a general linear model with fixed factors exhibited greater differences between sites, mesh size and over time (Figure 4). Mass loss of fresh/aged combined leaves over time (days) were significant ($R^2=82.4\%$, $F_{1423.9}$, $P<0.001$). Fresh leaf mass decay rates at all sites were significant over time ($R^2=80.7\%$, $F_{1330.5}$, $P<0.001$) with an overall mean of 65.2% mass loss over 265 days. There were significant interactions between factors: days and site ($F_{20.02}$, $P<0.001$) and days and mesh size ($F_{20.55}$, $P<0.001$), but not site and mesh size (Table 2). Decomposition rates differed significantly between streams, ($F_{12.88}$, $P<0.001$).

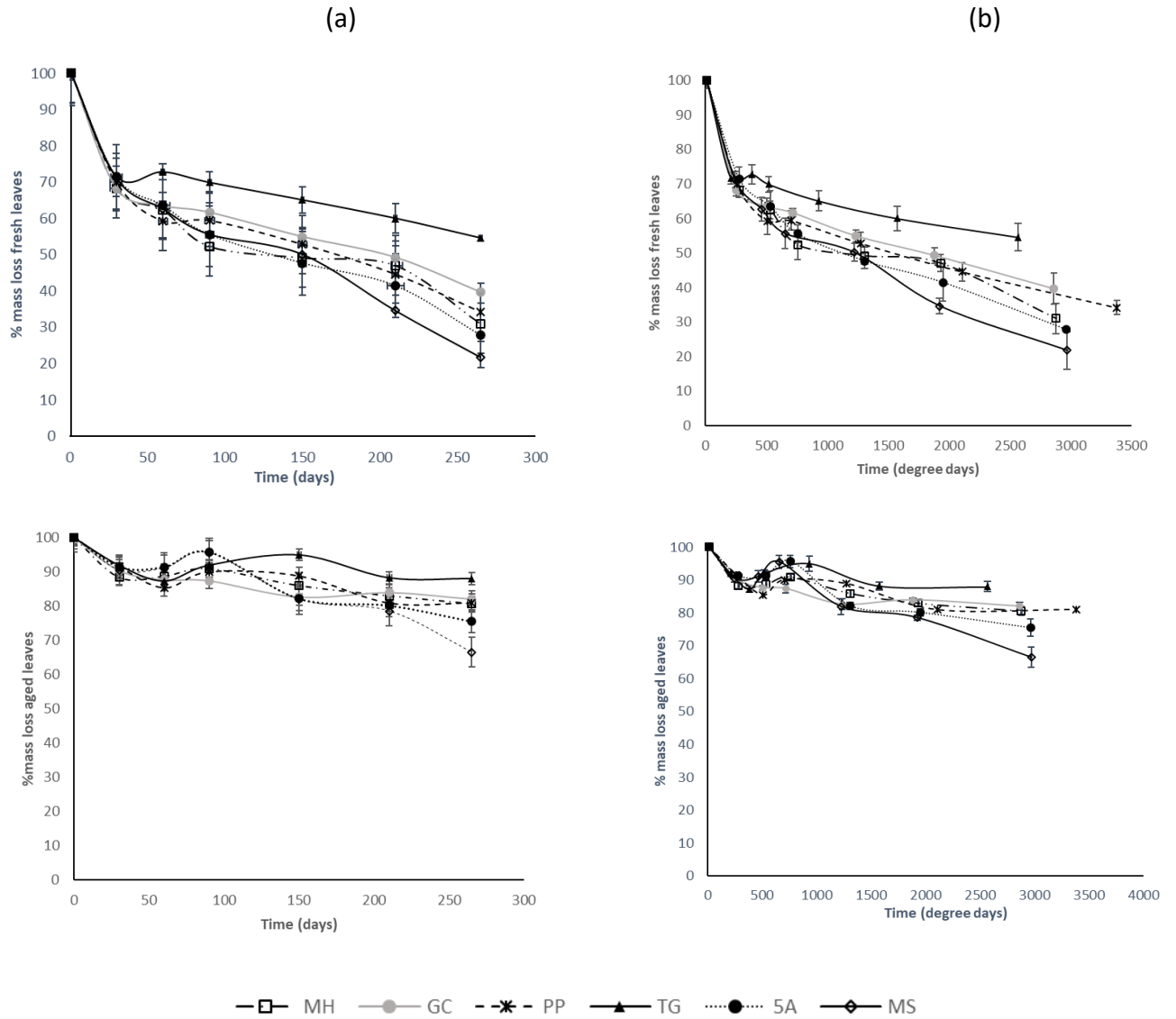


Figure 5. Percentage of air-dried mass loss of fresh and aged leaves over inundation period of (a) days and (b) degree days ($n=4 \pm S.E.$).

Aged leaf mass decay rates at all sites (21.1% mean mass loss) were slower but still significant over time ($R^2=41.8\%$, $F, 177.0$, $P<0.001$). There were significant interactions between factors: days and site ($F, 12.15$, $P<0.001.00$) and days and mesh size ($F, 8.14$, $P<0.001$), but not site and mesh size.

Analysis of mass loss using degree days marginally increased discrimination between sites. Mass loss of fresh/aged combined leaves over time (degree days) were significant ($R^2=82.9\%$, $F,968.6$, $P<0.001$). Fresh leaf mass decay rates at all sites were significant ($R^2=80.2\%$, $F,1243.2$, $P<0.001$) over 2567 degree days. There were significant interactions between factors: degree days and site ($F,15.6$, $P<0.001$) and days and mesh size ($F,20.8$, $P<0.001$), but not site and mesh size (Table 2). Aged leaf mass decay rates at all sites were significant ($R^2=43.6\%$, $F,186.1$, $P<0.001$) over 2567 degree days. There were significant interactions between factors: degree days and site ($F, 12.0$, $P<0.001$) and degree days and mesh size ($F,8.5$, $P<0.001$), but not site and mesh size.

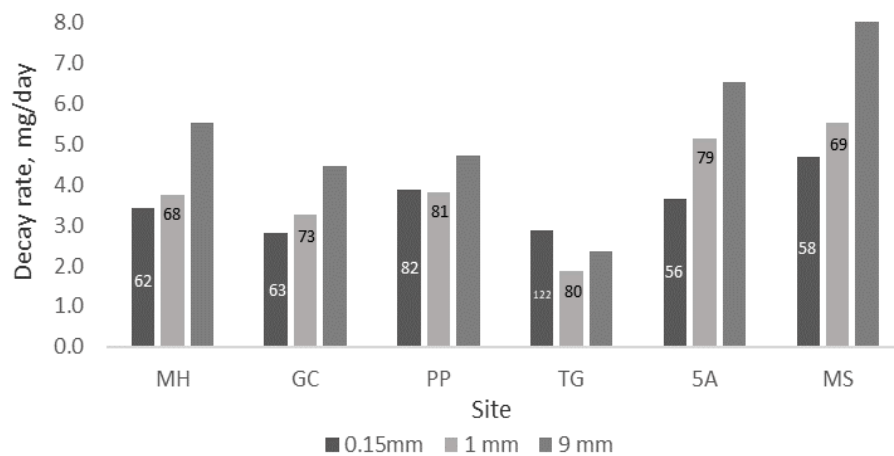


Figure 6. Fresh mass loss mg/day, microbial, small detritivores, versus total, with percentage of total decay rate. Values are regression slopes of GLM regression model of decay rates. Sites are ordered related to catchment imperviousness as given in Table 1.

Fresh leaf litter decay rates of all mesh sizes generally increased with total catchment imperviousness, with TG being an exception (Figure 6). Decay of fresh leaves in 150 μ m mesh bags that enabled microbial utilization contributed around 63% of total litter decay (9 mm mesh) for both undisturbed (MH and GC), 56% for the most heavily urbanized (5A and MS) sites, but between 85% and 122% of total decay rates for PP and TG.

Table 2. General Linear Model (with fixed factors) ANOVA results for decomposition rates, days and degree days, combined, fresh and aged leaves, non-linearized data.

	days			degree days		
	Mean mass loss	fresh	aged	Mean mass loss	fresh	aged
R ²	81.1%	80.7%	41.8%	82.9%	80.2%	33.6%
time*mesh*site D.F.=10	F,1.68, P=0.084	F,2.20, P=0.017	F,1.11, P=0.36	F,2.48, P=0.01	F, 1.85, P=0.05	F, 1.32, P=0.22
mesh*site D.F.=10	F,0.18, P=0.10	F,0.18, P=0.998	F,0.30, P=0.99	F,0.15, P=0.999	F,0.18, P=0.99	F,0.34, P=0.97
time*site D.F.=5	F,34.09, P<0.001	F,20.02, P<0.001	F,12.15, P<0.001	F,31.19, P<0.001	F,30.21, P<0.001	F,12.01, P<0.001
Time*mesh D.F.=2	F,22.91, P<0.001	F,20.55, P<0.001	F,8.14, P<0.001	F,32.30, P<0.001	F,24.45, P<0.001	F,8.53, P<0.001
Time	F,1338.6, P<0.001	F,1331.51, P<0.001	F,176.99, P<0.001	F,1353.84, P<0.001	F,1243.21, P<0.001	F,186.13, P<0.001
Mesh	F,7.43, P=0.001	F,9.94, P<0.001	F,0.7, P=0.93	F,6.25, P=0.002	F,9.15, P<0.001	F,0.08, P=0.92
Site	F,6.47, P<0.001	F,2.82, P=0.016	F,4.41, P=0.001	F,5.43, P<0.001	F,3.08, P=0.01	F,33.70, P=0.003

Decay in 1 mm mesh bags (that allowed ingress by microbes and small detritivores contributed an extra 8-10% rate of decay for undisturbed sites, 11-23% for the most urbanized sites but no contribution to total decay rates in PP and TG (-1 and -42%). Higher total decay rates (9 mm mesh) in most sites, other than TG, may be attributed to larger shredding invertebrates and fragmentation.

Aged leaf litter decay rates were more complicated than those for fresh leaves. Comparatively slow decay rates occurred at all sites, except for total decay at the heavily channelized Marmion swamp (Figure 6). Microbial versus total decay rates were around 65-75% (Figure 6). Microbial respiration on leaf surfaces (Figure 7), were not significantly different between sites (F, 2.01 P=0.085).

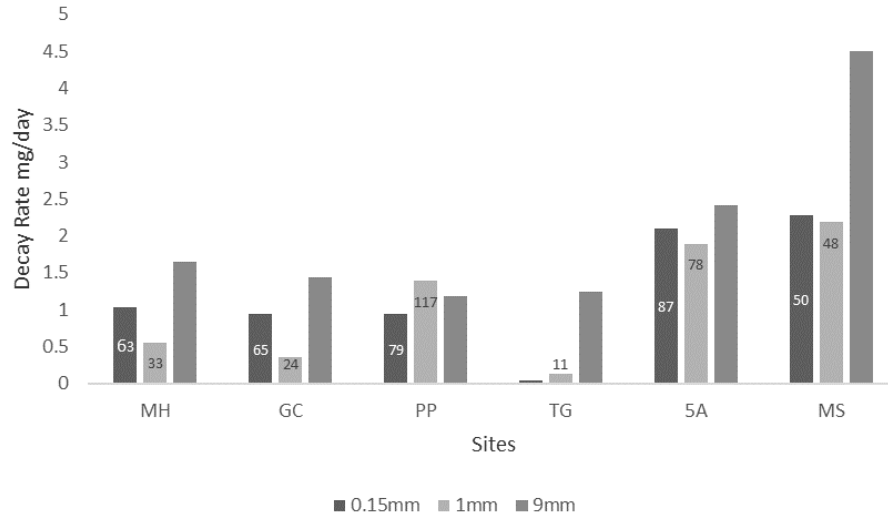


Figure 7. Aged mass loss mg/day, microbial, small detritivores versus total, with percentage of total decay rate (9 mm mesh). Values are regression slopes of GLM regression model of decay rate.s

Invertebrates in litter bags had between 9 and 16 taxa per sample. Invertebrate communities were dominated by Chironomidae, Oligochaeta and Nematoda. Less urbanized catchments (MH and GC) as depicted by percent catchment imperviousness (CI) and proximity to stormwater infrastructure supported fewer oligochaetes and more leptophlebiids, Aphroteninae and trichopteran families than other sites. More urbanized catchments, PP and MS, had large populations of Oligochaeta, Chironomidae and the grazing limpet *Ferrissia petterdi*. TG and 5A, also more urbanized, and with intermittent groundwater and surface water sections, both supported large populations of larval Scirtidae. TG supported comparatively few invertebrates, dominated by Oligochaeta and Chironomidae. Invertebrate abundance was significantly higher in PP and MS, the most channelized streams (F,7.4 P<0.001) with higher abundance of gatherers including Chironomidae and Oligochaeta (Table 3). These groups were numerous in biofilm collected on leaves but also on the mesh bags.

Characteristics of Microbial Respiration

Respiration rates on leaf surfaces in December followed similar patterns to invertebrate results, however the February results indicated higher rates of aerobic respiration.

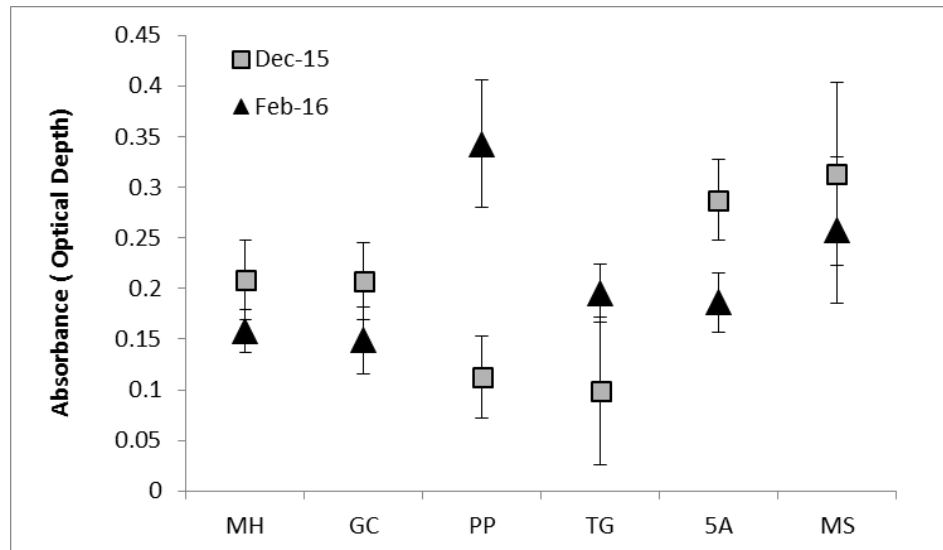


Figure 8. Respiration rates of leaf surfaces as measured by MTT incubation, December and February +/- S.E., $n=8$.

Aquatic Invertebrate Characteristics

Invertebrate taxa richness was not significantly different overall between sites with differences ($F_{3,89}$, $P<0.01$), driven by high taxa richness in MS and comparatively low richness in TG (Table 3). Invertebrate taxa richness was affected by multiple factors. Taxa richness was greatest at the most urbanized and channelized site (MS), but many of these were tolerant groups such as oligochaetes and chironomids and lower EPT taxa and densities. High abundance of tolerant invertebrates was also encountered at PP. *Ferrissia*, a grazing gastropod was only found at these two urbanized sites, whereas scraping, shredding and grazing leptophlebiids such as *Ulmerophlebia*, and *Neboissophlebia* and caddisflies, the shredder *Condocerus* (Leptoceridae) and the predaceous *Ecnomina* (Ecnomidae) were more common in less disturbed sites. Similar patterns of greater insect:gastropod dominance in less disturbed sites have been found (Yule *et al.*, 2015), suggesting some consistency in ecological processes.

EPT (Ephemeroptera:Plecoptera:Trichoptera) were most taxa rich at MH and significantly higher than PP and TG, the latter of which supported no EPT taxa. EPT taxa were also more numerous at MH and GC. Mean percentage of oligochaetes and chironomids (%OC) were higher at PP, TG and MS, with weighted SIGNAL2 metrics relatively low overall, but higher at MH and 5A and lower at TG.

Ordination of invertebrate community structure showed similarities between less developed catchment sites MH and GC, close similarity between PP and MS and individual communities at PP and TGs (Figure 9).

Table 3. Analysis of variance, invertebrate and EPT taxa and abundance, with Tukeys post hoc comparisons $P < 0.05$.

Site	Mt. Hay MH	Grand Canyon GC	Pitt Park PP	The Gully TG	Fifth Ave 5A	Marmion Swamp MS
N	6	6	6	6	6	6
Mean taxa per sample +/- S.E.	11+/-1.4	9.5+/-2.3	12.7+/-1.9	6.8+/-0.8	11.17+/-1.2	15.7+/-1.9
Diff. @ 0.05	AB	AB	AB	B	AB	A
Mean Individuals per sample +/- S.E	131.2+/-33.0	110.7+/-44.9	394.8+/-94.6	81.3+/-23.2	98.8+/-29.7	324.3+/-62.7
Diff.@ 0.05	BC	C	A	C	C	AB
Mean EPT Taxa +/- S.E.	4.5+/- 0.6	2.3+/-0.8	1.2+/-0.4	0	3.2+/-0.6	2.1+/-0.9
Diff. @ 0.05	A	ABC	BC	C	AB	AB
Mean EPT sum +/- S.E.	29.5+/-12.0	20.4+/-8.2	1.3+/-0.4	0	10.3+/-2.6	6.8+/-3.3
Diff. @ 0.05	A	AB	B	B	B	B
MV Disp	1.02	1.61	0.47	1.21	1.04	0.64
mean %OC	62.5	56.1	88.3	84.4	53.4	83.1
mean SIGNAL2	4.4	3.9	3.5	2.9	4.7	3.5

Analysis of similarity (ANOSIM – Primer 2015) returned an $R, 0.59$, with only two pairwise comparisons not significantly different (GC, MH - $R, 0.291$, sig. 2.2% and MS, PP - $R, 0.0048$, sig. 29.4%). Multivariate dispersion of invertebrate communities varied between 0.47 and 0.64 for PP and MS respectively to 1.61 for GC. GC was the most variable of invertebrate communities, commensurate with it being the smallest, least channelized and most variable habitat. PP and MS are the most channelized and supported the least variable invertebrate communities (Table 5). There were no consistent relationships between functional feeding groups and sites.

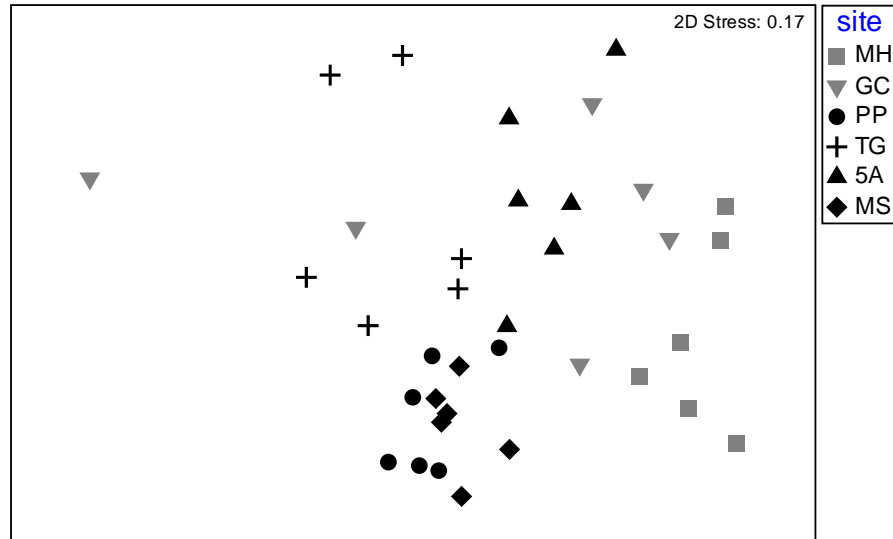


Figure 9. NMDS (Primer 2015) of invertebrates (>10% contribution, so rare taxa removed), data $\sqrt{}$ transformed prior to analysis.

Biodiversity-ecosystem function relationships between functional invertebrate groups and litter decomposition suggested a strong and significant relationship between scrapers and shredders for both fresh and aged leaves (Table 4).

Table 4. Correlation analysis of fresh leaf litter decomposition and functional feeding groups (chironomids removed from analysis), sum of all invertebrates per replicate litter bag and mesh sizes at each sampling event, for each stream

	Mean fresh leaves decomposition	Mean aged leaves decomposition
Filterers	R, 0.33, P=0.181	R, 0.124, P=0.623
Gatherers	R, - 0.028, P=0.911	R, -0.171, P= 0.496
Predators	R, 0.413, P=0.088	R, 0.309, P=0.212
Scrapers	R, 0.58, P<0.05 *	R, 0.709, P<0.005 *
Shredders	R, 0.758, P<0.001 *	R, 0.737, P<0.001 *

Table 5. Invertebrate taxa and functional feeding groups in litter bags, n=6, pooled mesh bags.

Taxa		MH	GC	PP	RT	SA	MS
Mollusca							
Bivalvia - Ancyliidae	<i>Ferrissia petterdi</i> - scraper	0	0	37	0	0	157
Gastropoda - Lymnaeidae	<i>Pseudosuccinea columella</i> - scraper	0	0	1	0	0	0
Oligochaeta	Oligochaeta - gatherer	16	32	978	265	87	761
Nematoda	Nematoda - predator	13	420	105	49	29	87
Nemertea	Nemertea - predator	0	0	13	0	0	1
Platyhelminthidae- Tricladida	Dugesidae - predator	0	0	6	4	0	0
	Dugesidae 2 -predator	0	2	126	12	11	6
Crustacea - Copepoda	Cyclopoida - filterer	45	9	16	9	5	27
	Harpacticoida - filterer	0	16	43	22	38	8
Crustacea - Cladocera	Chydoridae - scraper	3	0	5	0	0	5
	Cladocera - scraper	3	0	32	0	0	8
Crustacea - Isopoda	Phreatoicidae - scraper	0	2	0	0	0	0
Acarina	SO Oribatida - shredder	0	1	1	0	0	18
	SO Oribatida sp. 2 - shredder	0	2	0	0	0	0
	Halacaridae - predator	2	1	9	0	15	41
	Pezidae - predator	0	1	5	1	0	6
	SO Hydracarina - predator	0	1	8	1	0	2
	white oblong sp.1 - predator	0	0	1	1	0	0
Insecta							
Collembola	Isotomidae - gatherer	0	2	1	0	0	3
	Hypogasturidae - gatherer	1	0	0	0	0	0
	Collembola unid. - gatherer	0	0	0	0	1	1
Diptera	Chironomidae - various	428	167	1072	84	213	938
	Chiro - S.F. Aphroteninae - shredder	18	1	0	0	0	0
	Empididae - predator	1	1	0	0	0	0
	S.F.Ceratopogoninae - predator	32	11	12	9	21	19
	<i>Dasyhelea</i> spp.- scraper	0	1	0	0	2	2
	<i>Paradasyhelea</i> spp.- scraper	0	0	0	1	0	0
	Muscidae - predator	0	5	1	0	2	1
	Tipulidae - gatherer	2	1	1	0	2	3
	Dolichophidae - predator	0	0	0	0	0	1
	Ephydriidae - shredder	0	0	1	0	0	0
	Psychodidae - gatherer	0	0	0	0	0	4
	Athericidae - predator	0	1	0	0	0	0
Coleoptera							
Scirtidae	Scirtidae - filterer	0	0	2	29	96	0
Elmidae	<i>Notriolis</i> spp.- shredder	0	0	0	0	5	0
	<i>Simsonia</i> spp. - shredder	0	0	0	0	0	3
	Elmidae Adult - shredder	0	0	0	0	0	1
Dytiscidae	<i>Sternoprisus</i> spp.- predator	0	0	0	0	0	3
Psphenidae	<i>Sclerocyphus</i> spp.- scraper	0	0	0	0	0	6
Gyrinidae	<i>Macrogyrus</i> spp.- predator	1	0	0	0	0	0
Mecoptera							
Nannochoristidae	<i>Nannochorista</i> spp.- predator	0	0	0	1	4	0
Megaloptera							
Corydalidae	<i>Archichauliodes</i> spp.- predator	0	0	1	0	0	1
Ephemeroptera							
Leptophlebiidae	<i>Thraulophlebia</i> spp.- scraper	50	13	0	0	35	0
	<i>Nousia</i> spp.- shredder	1	3	0	0	3	0
	<i>Atalophlebia</i> spp.- shredder	0	0	1	0	4	6
	<i>Koornonga</i> type - shredder	0	0	0	0	4	0
	<i>Ulmerophlebia</i> spp.- gatherer	96	28	3	0	1	0
	<i>Neobissephlebia</i> spp.- shredder	18	0	2	0	0	3
	<i>Cloeon</i> spp.- shredder	0	0	0	0	0	1
Odonata							
Gomphidae	<i>Hemigomphus</i> spp.- predator	0	0	0	0	1	0
Argiolestidae	<i>Grisargiolestes</i> spp.- predator	0	0	1	0	0	0
	<i>Austroargiolestes</i> spp.- predator	6	0	0	0	0	0
Plecoptera							
Gripopterygidae	<i>Riekoperla</i> spp.- shredder	0	0	0	0	3	0
	<i>Trinotoperla</i> spp.- shredder	0	0	0	0	2	0
	<i>Leptoperla</i> spp.- shredder	0	0	0	0	0	3
Notonemouridae	<i>Austrocercella</i> spp.- shredder	0	0	0	0	0	7
	<i>Austrocercella</i> spp.- predator	0	0	0	0	1	3
Trichoptera							
Leptoceridae	<i>Condoceris</i> spp.- shredder	1	34	0	0	2	1
	<i>Lectrides</i> spp.- shredder	2	1	1	0	0	0
	<i>Tripletides</i> spp.- shredder	1	0	0	0	1	9
Ecnomidae	<i>Ecnomina</i> spp.- predator	51	5	0	0	0	0
	<i>Datonomina</i> spp.- predator	4	0	1	0	4	1
	<i>Ecnomus</i> spp.- predator	0	0	0	0	1	6
Hydroptilidae	<i>Hellyethira</i> spp.- scraper	1	0	0	0	0	0
Hydrobiosidae	<i>Psyllabetina</i> spp.- predator	0	0	0	0	0	1
Total		796	761	2486	488	593	2154

Discussion

Rate of Decomposition

This study illustrated the very slow aquatic decomposition rates of eucalypt leaves in poorly studied upland peat swamp streams. These peat swamps; nutrient poor, acidic and heavily vegetated with xeromorphic native species, generally provided conditions of carbon and nutrient retention. Human impact led to higher litter decay rates up to thresholds where decomposition was apparently impeded by toxicity (discussed below) (Figure 10). Several complex interactions were seemingly involved in litter decay, including litter species composition, stream and litter nutrients, stream pH, underlying geology and human impact.

Eucalypts comprise the canopy of Blue Mountains woodlands and are one of the few local tree species with an ovate to lanceolate leaf shape. The terrestrial derivation and age of the peat in the upland swamps (Fryirs, 2014), implies that eucalypts formed a significant component of peat layers in contemporary swamps. However, eucalypt leaf litter is generally refractile due to high levels of tannins and lignins (Boulton & Boon, 1991), which generally mitigate against rapid aquatic leaf decomposition.

Decay coefficients of fresh *Eucalyptus viminalis* in small Victorian streams have been reported as between $2.0\text{--}3.1 \times 10^{-2}$ g/day (Campbell, 1992). However, in this study, decay rates were $0.6\text{--}4.8 \times 10^{-3}$ g/day. This is therefore much slower than reported elsewhere for stream systems and reflects the role of peat swamps in conserving and storing carbon and nutrients (Pemberton, 2005). Litter mass loss rates in the wetlands varied between 12–78% over 265 days (Figures 6,7), while reported rates elsewhere are given as around 50% loss over periods of 84 days. Furthermore, AFDM rates ranged from 0.8 to 3.1×10^{-4} % /day. Other studies have reported % AFDM decomposition rates of 1.03 to 1.45% per day for softer, high quality alder leaves in Mediterranean streams (Pérez *et al.*, 2013) and globally 0.66–1.2%/day (0.02–0.04 g/day) (Boyero *et al.*, 2011).

Decay rates differed between fresh (65.2%) and aged leaves (21.1%) as encountered elsewhere for eucalypt decomposition in Australian streams (Boulton, 1991). Higher initial N and lower phenolic concentrations of fresh leaves (Boulton, 1991) make them more palatable. Greater

colonization of fresh leaves by microbes following inundation is known to increase accumulation of nutrients such as Ca, P, K, Mg and silicates Berg (2014), enhancing palatability, although these elements were not tested during this study.

In natural peat swamps generally, C:N ratios increase with depth, with surface litter approximating local leaf ratios, suggesting higher levels of N storage in surface peats (Wang *et al.*, 2015) even in xeromorphic shrub swamps studies depicted here. Comparatively high C:N ratios in eucalypt leaves relative to global leaf C:N ratios (greater than 40) however, mean that upland peat swamps in south eastern Australia may have comparatively lower nitrogen at the surface of peat swamps than peat swamps elsewhere becoming less with depth (Snowdon, 2005). Differential carbon loss from the peat surface as carbon dioxide and methane and atmospheric derived nitrogen becomes less of an influence at depth. Microbial decomposition is thought to lead to the peat incorporating microbial stoichiometry as a result (Wang, 2015).

Channelization reduces N storage by lowering groundwater tables, increasing depth of the vadose zone and stimulating aerobic peat decomposition (Cowley, 2016). Subsequent release of nitrogen and carbon has implications for loss of carbon sequestration. Even minor disruptions in longitudinal connectivity in upland streams have been shown to alter carbon storage, decomposition processes and invertebrate communities (Northington & Webster, 2017).

Role of Acidity and Nutrients

Peat swamp streams are naturally extreme environments; acidic (pH 4-5), low in nutrients, demonstrating slow rates of litter decay, low dissolved oxygen (Pemberton, 2005) and in common with other groundwater systems, low temperature variability (Boulton, 2014). Streams originating from Blue Mountains peat swamps reflect those conditions (Figure 10) where litter decay is impeded in acidic stream waters (Boyero *et al.*, 2016; Clivot *et al.*, 2013; Dangles, 2004a) and where microbial rather than invertebrate mechanisms are influential, particularly in affecting P metabolism in fungi and bacteria (Clivot *et al.*, 2013). In naturally occurring, boreal acid streams, there is evidence that acid tolerant invertebrate shredders dominate and compensate for altered microbial conditions, including decomposition processes (Dangles, 2004b), however, the results here suggest that invertebrate shredders were found across the pH gradient, instead

affected by other disturbance thresholds, such as nutrient enrichment. These results agree with those for tropical streams (Wantzen, 2008)

While there is continuing loss of natural acidic streams, there is a concurrent increase globally of anthropogenically derived acidic streams (Dangles, 2004a). Polluted acidic streams have relatively recent and adverse effects on stream ecology, whereas naturally acidic streams provide evolutionary unique chemical composition and adapted environments (Holland *et al.*, 2012). It was observed that streams in the most urbanized catchments had more neutral pH, as stormwater inputs contributed runoff of higher alkalinity. These swamps also exhibited higher rates of decomposition and larger proportions of tolerant invertebrates.

Australian soils are typically nutrient poor, particularly those on sedimentary bases, such as sandstone, which are naturally low in nutrients such as phosphorus (Thomson & Leishman, 2004). The xeromorphic nature of natural Blue Mountains swamp flora suggests poor levels of phosphorus and conversely, dominance of weeds in more urbanized swamps suggests a response to increased nutrients (Leishman & Thomson, 2005). Low nutrient soils and vegetation impedes microbial decomposition (Graça *et al.*, 2015) and hence rates of litter decay would also be expected to be comparatively slow under these low nutrient conditions (Wind-Mulder & Vitt, 2000). Generally, higher levels of nitrogen oxides and dissolved oxygen correlate with increased litter decomposition (Abelho, 2001) and widespread evidence indicates that nutrients are integral to litter decomposition (Biasi *et al.*, 2017), with both accelerated colonization by microbes and shredding invertebrates observed as a result of elevated nutrients (Cross *et al.*, 2007; Tant, 2015).

In this study, leaf decomposition rates were greater in streams with higher nutrient concentrations. It was observed that total catchment imperviousness up to greater than 50% of catchment area, led to higher nitrogen oxide concentrations, more neutral pH and higher conductivity. However, as with other studies, for example (Woodward *et al.*, 2012), responses to nutrient enrichment were not predictive or linear with either microbial or invertebrate responses. Other factors may override nutrient importance, such as extensive water logging, development of anoxia and alteration to decomposition pathways. Anoxic conditions and resulting microbial response to high water levels and disturbed substrata at TG may have resulted in nitrogen

reduction and production of NH_4 , with associated and obvious iron reduction and toxic conditions (Wind-Mulder & Vitt, 2000).

Microbial conditioning of leaf litter is integral to decomposition. Heterotrophs, including bacteria and fungal hyphomycetes, colonize leaf material rapidly, enhancing nutritional value and increasing detrital mass (Graça *et al.*, 2015; Krauss *et al.*, 2011) and palatability to detritivores. In this study, microbial decay rates of fresh leaves ranged from around 62-63% of total decomposition for the least disturbed sites, slightly less for the most urbanized sites (56-58%), to 82-100% for the most physically disturbed sites.

So, while decomposition rates were greater in more urbanized sites (5A and MS), the relative contribution of microbial decay was less. This could be due to channelization effects including abrasion and fragmentation or greater contribution by shredding invertebrates. On the other hand those swamps affected by loss of intact physical structure (PP and TG), had fewer invertebrates and higher levels of microbial decay as well as nitrogen and DOC release. These results are consistent with models of decomposition of organic matter and nutrient dynamics under conditions of physical disturbance of peat layer such as that exhibited by peat harvesting (Strack *et al.*, 2011). Physical disturbance of peat with associated altered water levels and loss of vegetation are known to create complex nutrient dynamics, including nitrification and denitrification, elevated pH and release of peat associated nutrients (Wind-Mulder *et al.*, 2000).

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Iron and Anaerobiosis

In increasingly anaerobic conditions, DO is replaced as the main electron acceptor for bacteria with accompanying selection for facultative anaerobes reducing ions of Fe^{3+} and NO_3^- (Sigee, 2005a). As hyphomycetes are largely aerobic they become less influential with increasing anoxia (Sigee, 2005b), with anaerobic bacteria dominating. Consequently, aerobic respiration measurements such as MTT (Gerlier *et al.*, 1986; Stockert *et al.*, 2012) used in this study, failed to distinguish between sites. Under anaerobic conditions, only taxa with ability to survive low oxygen conditions such as chironomids and oligochaetes, can survive underwater. Some taxa such as Coleoptera and Hemiptera may use oxygen at the surface. In swamps where anoxia was constant, conditions appear to impact stream invertebrate fauna. Geomorphic alterations of

excavation and disturbance of swamp, soil and iron rich sandstone strata were present at both PP and TG. These, combined with proximity to stormwater and sewerage infrastructure with upstream dam development at PP, appear to have altered their chemical and biotic character by altering invertebrate communities and biogeochemical dynamics as discussed above. Furthermore, obvious flocs of iron reducing bacteria draining the swamps indicated a further process occurring that suggests alternative conditions.

Iron is a common component in soils and swamps and is present in peat swamps globally. Fe(II) may be directly or indirectly toxic but in the presence of oxygen, is oxidized to iron oxides, which, at low temperatures and pH inhibits ecological processes (Vuori, 1995). This process is further inhibited by humic substances, so that toxic Fe(II) may persist downstream of disturbed peat swamps for extended periods of time. So, while Fe(II) may be a natural component of swamps and stream flow, disturbance such as mining and peat removal exacerbate levels, which may alternate between oxidation and reduction processes in complex ways. High oxygen levels, oxidation and formation of iron hydroxides, acidify stream water. Low oxygen, dissolved sulphides and reductive enzymatic reactions may all be involved (Vuori, 1995) in reduction to Fe(II). Both oxidative and reductive processes may be toxic (Vuori, 1995), with either acid stream conditions or physical and biological toxicity of Fe(II) bacterial deposits which clog gills and alimentary canals, and increase direct iron toxicity (Gerhardt, 1992, 1995; Vuori, 1995). Furthermore, high iron concentrations may limit plant growth, creating further complexity in swamps by altering vegetation communities (Alongi, 2010). Therefore, obvious iron reducing bacteria in streams downstream of swamps indicate poor ecological value and ecological conditions in The Gully and Pitt Park demonstrate what may be a common problem.

Relationships between Urbanization, Hydrology and Ecology

Globally, many swamps have been drained to permit agriculture while in the Blue Mountains, erosion and draining has been a secondary effect of urbanization and underground coal mining (Benson *et al.*, 2012). However, the effects are similar, independent of the cause. Peat drainage leads to dewatering of swamp strata, drying the soils and releasing carbon, nutrients and sediment downstream (Freidman, 2014). Consequently, swamp vegetation is dehydrated, but may be maintained by rainfall until a catastrophic event: bushfire or drought create threshold conditions

to permanently alter vegetation community to one of terrestrial woodland and shrub composition. Faunal communities are also impacted (Gorissen *et al.*, 2017). Therefore, channelization is a pervasive impact, along with groundwater extraction and underground mining. Impacts on terrestrial vegetation may not be evident for many years. It is clear though, that channelization has a current noticeable effect on invertebrate communities.

Longitudinal surface water connectivity in peat swamps is variable. Those with channels have distinct longitudinal surface aquatic connectivity, with colonization by stream dwelling invertebrates and dispersal by drift and active movement (Bilton *et al.*, 2001), whereas swamps where water movement is entirely through the hyporheic zone, means that dispersal processes are more restricted (Dole-Olivier, 2009). Some species, such as groundwater isopods and amphipods may employ active dispersal, however, obligate groundwater species exhibit low fecundity, lack of larval dispersal and long developmental stages, which limits their dispersal ability (Dole-Olivier, 2009).

Statistically, multivariate dispersion of invertebrate communities is used primarily to identify extent of degradation, with the argument being that more degraded environments lead to greater dispersion (Clarke, 2014). In this study, it was used to investigate longitudinal connectivity and channelization, on the premise that intact, natural peat swamp surface water habitat is poorly connected and small. On the other hand, channelized systems have continuous connectivity, and are less heterogeneous in habitat. In this study, both highly channelized systems, PP and MS, were most similar and least variable, and the least channelized and smallest habitat, GC, was the most variable.

The limited research on invertebrates in boreal peat swamps (Spitzer, 2005), suggests that communities are relatively rich overall because of the diversity of habitats within swamps. Evidence also points to a unique fauna within the confines of the groundwater, either obligate or facultative, with poor local diversity relative to regional diversity due to poor nutrient status, acidic and with limited dispersal (Gibert, 2009). Like hyporheic environments where surface water and groundwater meet (Tomlinson & Boulton, 2010), swamps may be viewed similarly as an ecotone that the range of invertebrates would, independently of acidity, be expected to be rich in taxa.

Further metrics of invertebrate ecological integrity suggest that multiple factors may be operating in these swamps. Firstly, the habitats are small, with few invertebrates per sample. Relatively high taxa richness per sample indicates that overall, Blue Mountains swamp streams may be diverse. However low SIGNAL2 values may be indicative of small habitats or of any chemical or physical pollutant (Chessman, 2003). Secondly discontinuous flow in more urban swamps such as 5A may filter sediment and nutrients and provide variable habitat for a larger range of invertebrates. Thirdly, high proportions of tolerant oligochaetes and chironomids in the more channelized and impacted swamp streams indicates that catchments upstream of these streams are not acting as sinks for pollutants, but rather as sources.

And finally, the combination of low SIGNAL scores and high % OC, as well as low EPT at TG, sends a strong signal of an ecosystem in a highly degraded state.

Therefore, combinations of invertebrate metrics may provide additional information and act to support others in typifying streams that may not be well studied or understood. While multiple metrics are not a new idea (Karr, 1999), and SIGNAL2 is a comprehensive metric (Gronwald *et al.*, 1997) incorporating others, there may be utility in investigating multiple metrics if they provide further information.

Basic correlation analysis between leaf litter decay rates as an ecological function and invertebrate traits of feeding groups suggested strong and significant relationships between abundance of scrapers and shredders and function. The role of shredders and scrapers in leaf decay would be expected to be an important 'effect' trait that adds to functional diversity (Schmera *et al.*, 2017; Truchy *et al.*, 2015) that may be further explored. This intuitive relationship requires a greater analysis to test the strength of the relationship.

The results suggested a unimodal model for leaf decomposition (Figure 10), with low nutrient and acidic undisturbed swamps exhibiting low decay rates, increased with increasing nutrients and channelization, but with a possible threshold response related to past excavation, anoxia with resultant iron reduction/oxidation processes dominating. Similar outcomes have been observed elsewhere, (Wenger *et al.*, 2009) with a range of causative factors, leaf palatability, including abrasion, iron oxidation and reduction (as identified here) or reduced abundance of shredders.

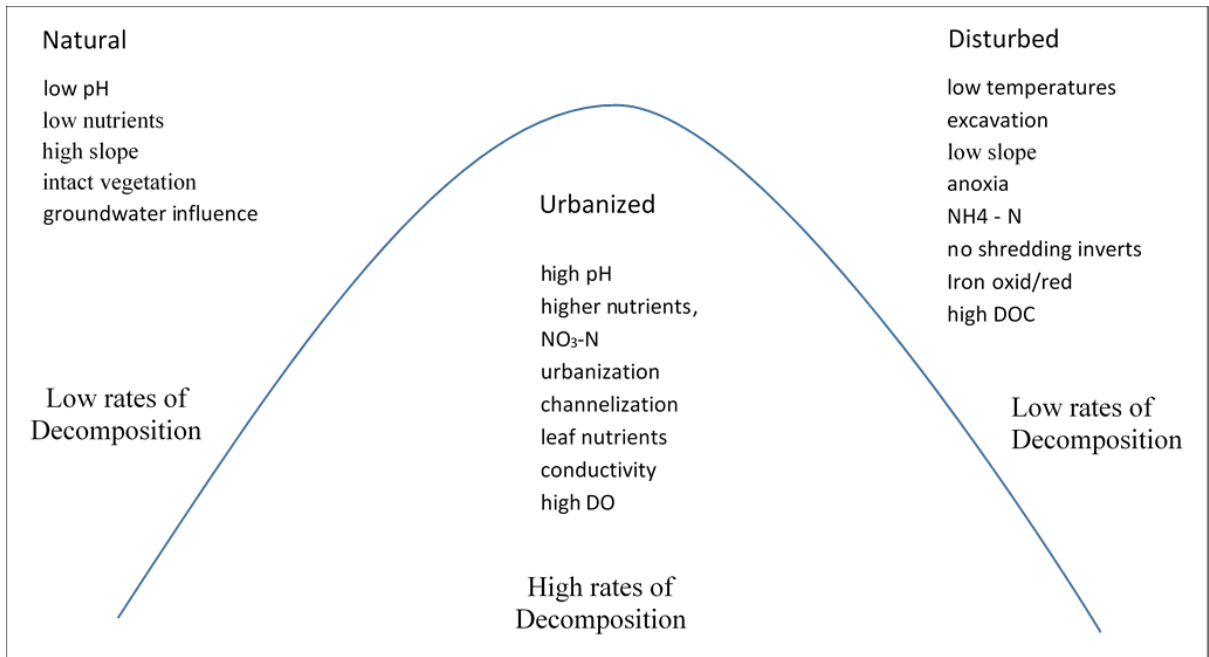


Figure 10. Conceptual model of leaf litter decomposition in upland peat swamp streams.

This unimodal relationship between litter decomposition and invertebrates in more urbanized and channelized catchments is well tested globally (Woodward *et al.*, 2012). Moderate enrichment is likely to stimulate microbial activity and consequent invertebrate consumption of leaf material. Further pollution leading to oxygen depletion and loss of sensitive shredding invertebrates may drive decomposition down.

While these results suggest a threshold response (Groffman *et al.*, 2006), it is not known at what point or what created the threshold. Conditions for peat development, including waterlogging, anoxia and heterotrophic plant decomposition, suggests that affected swamps may in fact be resetting into an early succession of peat swamp development. The trajectory of this succession is by no means clear, with long term outcomes unknown. Furthermore, the changes brought by channelization are likely to have occurred as a significant and rapid geomorphic event and could also be seen as a threshold event. In the absence of historic context, it is impossible to investigate these thresholds, but is important to manage and prevent further events. Current activities aimed at reducing stormwater impact into swamps by managing inflows are a start in reducing runoff intensity.

The extensive distribution and geographic location in the water supply catchment of Australia's largest city, give these swamps value in water treatment and storage (Fryirs, 2014) as well as their importance for local, regional and Australian ecosystem services. The future of these highly biodiverse swamps is uncertain, with not only increasing urbanisation creating three dimensional threats. Longitudinal effects from upstream impervious surfaces will increase runoff intensity creating erosion; lateral threats where alteration to groundwater levels in the peat swamps adjoining the streams lead to oxidation and drying of peat strata, loss of carbon and swamp functioning; and the additional vertical threat of alteration to rainfall patterns created by weather changes, that alter stream flow, are threats to their long-term protection. The future of the increasing urbanization, coal mining and climate change will determine the outcome for the health of the upland swamps on sandstone in eastern NSW.

Conclusion

Leaf litter decomposition processes in upland swamp streams in the Blue Mountains are naturally slow due to litter characteristics coupled with low pH, nutrients, temperature and dissolved oxygen. The six peat swamps investigated here represent a small percentage of the small valley fill swamps that dot the sandstone escarpment. These swamps are important to maintain; not only do they store water for periods of time before release to rivers downstream in Sydney's water catchment, but also store significant carbon in peat. Furthermore, they support highly endemic and threatened species. Knowledge of ecosystem processes such as decomposition, comprise an important part of understanding how to maintain these swamps. Decomposition rates of eucalypt leaf litter varied substantially under human influences with processes of increasing nutrients, channelization and flow leading to unimodal responses of higher decomposition rates of eucalypt leaf litter. Invertebrate communities have changed from insect shredder, scraper and predator-based communities to those dominated by the grazing gastropod *Ferrissia petterdi* and tolerant oligochaetes and chironomids. Historic excavation, altered geomorphology, hydrology and vegetation, have led to threshold conditions that inhibit litter decomposition while creating toxic conditions for all but the most tolerant invertebrates.

There are still many questions unanswered by this study, including native versus exotic leaf litter decay dynamics, microbial community structure involved in decay processes and vegetation

processes within urban and undisturbed swamps. The strong relationships between abundance of invertebrate scrapers and shredders and litter decomposition provide an interesting biodiversity-ecosystem function link that would be an avenue for future research. Several possibilities to be investigated include previous findings that in the absence of shredders, other invertebrates such as scrapers may become shredders, shredders also consume hyphomycete biofilm, which makes leaf surfaces more available for scrapers (Graca, 2001) and scrapers themselves may be opportunistic in consuming shredder leaf products.

Unimodal responses in leaf litter decomposition in the threatened Blue Mountains upland peat swamps suggest that some of these swamps may have already undergone irreversible ecological change. Management to alter excavated and channelized swamps is possibly pointless, trajectories are uncertain. However preventative management is required and possible.

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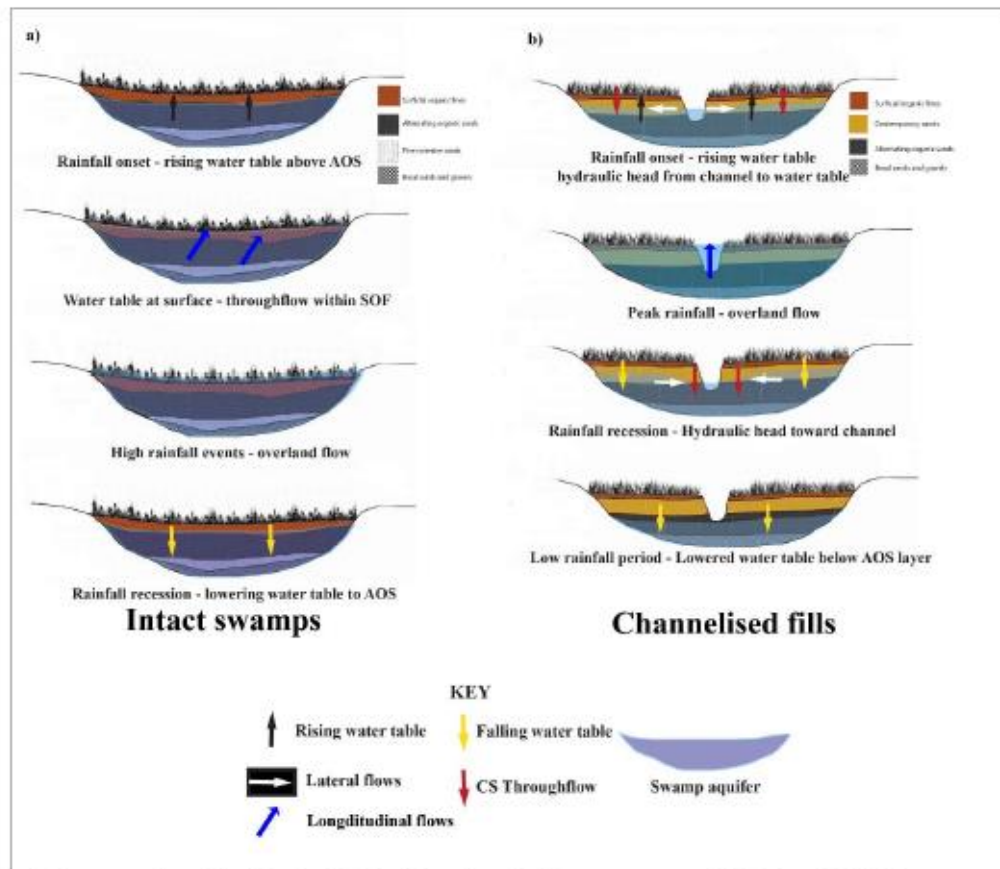
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Appendices

Appendix 1. Conceptual model of the hydrological function of a) intact swamps and b) channelized fill (copied from Cowley et al. (2018))



Chapter 5

Waterbody productivity in Mulwaree chain-of-ponds, a rare geomorphic aquatic system



Lorraine Hardwick

Department of Biological Sciences

Abstract

Freshwater ecosystems are increasingly at risk of anthropogenic impact, including agricultural runoff and water use and grazing, and this is particularly the case for chain-of-ponds systems which are uncommon and poorly understood. The Mulwaree chain-of-ponds system is such an aquatic feature. These ponds are unusual ecosystems, with uncharacteristically clear water and well vegetated and mostly rhizomatous marginal vegetation. They do not behave solely as a river, a shallow pond or a lake, but rather, functional aspects resemble all three in terms of their primary productivity.

When in flow, the river is productively a lotic system, with inflows, outflows, deposition and longitudinal connectivity of debris, nutrients and energy. At times of seasonal low flow, the ponds are disconnected from each other and become strongly temperature and oxygen stratified at around 2.5 metres to act as mesotrophic monomictic lakes.

This study examined ecological productivity in chain-of-pond systems using pelagic phytoplankton and microbial productivity of the Mulwaree Ponds in inland Australia as a model for deep pond productivity. Phytoplankton productivity and spatial patterns of microbial respiration increased during periods of disconnection, being associated with thermocline and oxycline development. Above the thermal mixing depth, there were few differences between primary production and microbial respiration in vegetated edges and mid pond, although $P:R < 1$ in summer and autumn amongst the marginal vegetation. Gross primary productivity, net ecosystem productivity and phytoplankton biomass followed predictable patterns of freshwater microbial loops related to other mesotrophic lake studies but were highly variable across the ponds and related to seasonal and connected conditions. GPP was mostly positive and decreased with depth (-0.2 to $+1.2$ g/m³/day), NEP became negative at depth (-0.6 to $+0.7$ g/m³/day), and PR was positive (-0.4 to $+1.4$ g/m³/day), except during winter. Variability between ponds was high, however. This study demonstrated that ecological processes in Mulwaree chain-of-ponds provide important carbon productivity benefits that are under risk under increasing temperature and more variable rainfall.

Introduction

Description of Chain-of-ponds

Globally, there is growing interest in the function of ponds in aquatic landscapes as their contribution to regional biodiversity (Williams, 2004), carbon sequestration, (Downing, 2008) and ecosystem services (Biggs *et al.*, 2017) are increasingly recognized (Céréghino, 2014). Ponds are defined as permanent lentic water bodies, between 1 and 5 ha (Meerhoff & Jeppesen, 2009; Oertli, 2010). Despite their small size, collectively they cover more land area than lakes (Downing, 2006) and unlike pools, which dry out, are permanent. Ponds outnumber lakes by 100:1 (Céréghino, 2014) and comprise 30% of global lentic surface (Downing & Melack, 2006). Despite being less studied than streams, ponds support a comparatively greater regional biodiversity (Williams, 2004).

Nutrient dynamics and productivity in ponds used for intensive human purposes, such as for fish farming and watering stock or irrigation are well known (Bichsel *et al.*, 2016; Oertli, 2010), but the need for further knowledge of natural pond functions, connectivity and management has been increasingly recognized (Biggs *et al.*, 2017; Oertli, 2010). Key concerns for global pond management include increasing eutrophication (Downing, 2010; Gao, 2016), loss of aquatic vegetation (Phillips, 2016), the impact of and response to climate change and the increasing threat of invasive species (Oertli *et al.*, 2009).

Surface water systems in Australia have been heavily altered since European colonization. One impact of vegetation clearing and the introduction of western farming has been widespread soil erosion and sedimentation in sloping catchments (Olley, 2003). This has been a frequent occurrence on the western slopes of the Great Dividing Range in eastern Australia. Commonly, what were significant chain of ponds systems, with vegetated and deep sided pools have almost disappeared (Eyles, 1977b). Remnant pools in south eastern Australia are now heavily sedimented, with high turbidity (Eyles, 1977b; Wasson *et al.*, 1998) and generally phytoplankton dominated, while deep vegetated ponds have become extinct (Eyles, 1977a; Olley, 2003). Hydrology of these streams have also changed as stream flows have become more perennial. Naturally occurring persistent thermal stratification that is a feature of chain of ponds is now rare, because flow is usually adequate to cause complete mixing (Turner & Erskine, 2005).

Generally, ponds across Australian landscapes, as elsewhere, exhibit substantial spatial heterogeneity, influenced strongly by hydrological connectivity and water quality (Boulton, 2014; Casanova *et al.*, 1997; Fairchild *et al.*, 2005; Meerhoff *et al.*, 2009). When disconnected from adjacent aquatic systems, they may represent distinct ecosystems, both geographically and functionally. Their role as perennial components in ephemeral systems is little known. Superimposed on these characteristics are the within-pool features imposed by bathymetry and seasonality; depth to width ratios, riparian vegetation, water quality and temperature and dissolved oxygen stratification. These aquatic systems therefore provide local and landscape-scale environmental benefit and associated complex ecological services for aquatic flora and fauna (Boulton, 2014; Casanova *et al.*, 1997; Fairchild *et al.*, 2005; Meerhoff *et al.*, 2009). In the global context the Mulwaree chain-of-ponds (“the ponds”) are unusual. The ponds are larger and deeper than kettleholes of Germany (Kazanjian *et al.*, 2018), and though similar in appearance at the surface to southern African dambos (von der Heyden, 2004), Mediterranean wadis and American arroyos (Mould & Fryirs, 2017), the Mulwaree ponds have deeper profiles. The Mulwaree ponds are defined by their high depth:surface area ratios, extensive marginal aquatic vegetation and hydrological permanence (Figure 1).

Little is known about how they function, how water quality and pond productivity change temporally and spatially and what affect the unusual morphology and water clarity have on pond ecosystem dynamics. The chain-of-ponds are a remnant of a more common pre-European stream type which is now rare. Elsewhere on the western slopes of the Great Dividing Range, the prevailing pattern for stream typology is one of eroded and infilled watercourses. The dominant stream types have, since European settlement, been sources of significant sediment downstream and are largely devoid of instream and riparian vegetation. Loss of catchment vegetation has led to higher groundwater levels, leading to more permanently flowing stream hydrology (Eberbach, 2003; Prosser, 1991; Zierholz, 2001) and consequently a mixed water source. The chain-of-ponds are unprotected by environmental legislation, despite their local and global rarity. They are not listed as wetlands of National Importance, or by the Ramsar Convention, so are therefore relatively unprotected (Hill *et al.*, 2018).



Figure 1. Mulwaree chain-of-ponds during flow, disconnection and drying. Presence of Gambusia holbrooki and grazing impact evident. (All photos: Lorraine Hardwick)

Productivity in Chain-of-ponds Systems

The Mulwaree chain-of-ponds provide an opportunity to study both productivity and seasonality related to flow dynamics in an unusual aquatic system. Generally, nutrient and carbon dynamics within chain-of-pond systems are not well known (Fairchild *et al.*, 2005). How the ponds respond to flow and loss of connection, how this affects carbon sequestration or release and whether macrophytes and phytoplankton interplay in these processes is interesting. In this system, connectivity varies as rainfall runoff maintains connection through the preferential flow paths until warmer temperature in spring reduces the stream into separate ponds. Water levels continue to fall until the ponds shrink beyond the marginal fringing vegetation. The individual ponds in this system are thought to be poorly connected to groundwater (Williams, 2017) so may represent a closed system when disconnected during summer dry periods

further hypothesized that phytoplanktonic respiration (PR) is consistent across depth; as a common feature of lakes elsewhere and that chlorophyll A concentration/activity/production is greater in the middle of the pond than in fringing macrophyte beds, and greater during conditions of disconnected flow. This latter hypothesis is based on the importance of phytoplankton biomass in locations of greater light intensity and lower competition for nutrient resources posed from aquatic macrophytes and their associated periphyton. While Mulwaree Ponds have been influenced by anthropogenic activities reflected in relatively poor water quality (GHD, 2013), their rare geomorphic nature provides an opportunity to study ecological concepts of seasonal disconnection, of autochthonous versus allochthonous productivity and vertical stratification dynamics in non-turbid ponds.

Aims

The aims of this study were to attempt to understand function of a rare geomorphic landscape feature by using productivity measures. The means used were to identify temporal changes; diurnal and seasonal, in thermal and oxygen stratification and water quality in the Mulwaree Ponds chain-of-ponds. It was hypothesized that gross primary productivity (GPP) and net ecosystem productivity (NEP; as measured by 24-hour light and dark bottle productivity/respiration) would vary with surface and air temperature, but decrease with depth. These are features commonly associated with deep lakes, rather than ponds. It was

Methods

Study Sites

Four seasonally connected and adjacent ponds were selected for study in the Mulwaree River at Kelburn (35° 52' 23.41''S, 149° 39' 02.31''E; Figure 2), near Goulburn, New South Wales, Australia. Streamflow metrics at Mulwaree River @ The Towers indicated three distinct flow periods since data collection commenced in November 1993. Mean daily flows for non-drought periods of 1993-2000 and 2010-2018 ranged from 29-44 ML/day, with a daily maximum of 16,737 ML/day on 9/12/2010. The 90th Percentile flow was 56 ML and Lanes Variability Index of 0.745 ML. These flow periods were interspersed by the Millenium drought between 2001-2009 (van Dijk *et al.*, 2013), when mean daily flow fell to 6.5 ML/day and the 90th percentile flow 2.88 ML/day and Lanes Variability Index of 0.3 ML.

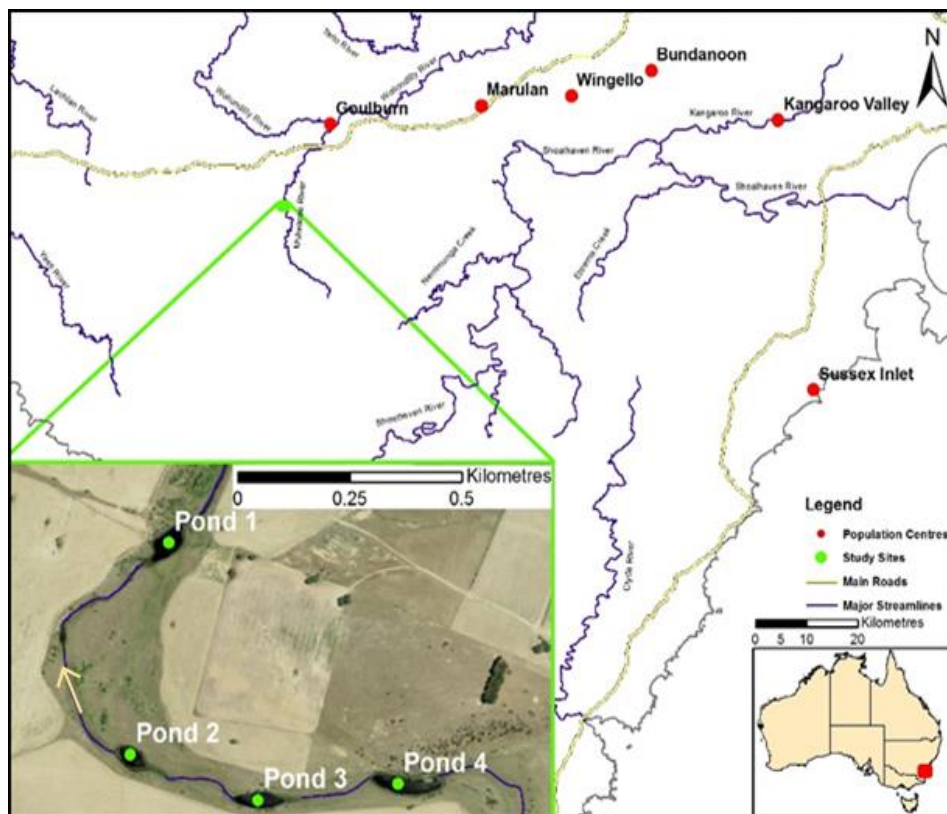


Figure 2. Location Map. Mulwaree chain-of-ponds, Kelburn NSW, ponds 1 to 4. Arrow in direction of flow (Source layer Credits © Land and Property Information 2015, map courtesy Will Farebrother 2018)

Long term (1988-2018) mean annual rainfall at Goulburn airport (070330) is 544 (mms and typically exhibits a bimodal maximum in June of 61mm and December of 58mm (Bureau of Meteorology, 2018). Months of low rainfall occurred in April with 26 mm and May with 33mm. Mulwaree chain-of-ponds represents a geomorphic anomaly; an apparently stable, vegetated chain-of-ponds system, with remnant large and deep ponds connected by swampy meadow preferential flow paths (Williams, 2017). The Mulwaree River, in which the ponds lie, is an intermittently flowing relictual river isolated in the late Tertiary Period from its source upstream (Abell, 1995). The river now receives water from the western edge of the catchment, running alongside Lake Bathurst, which is separated from the Mulwaree River by a deposited alluvial levee. The chain-of-ponds systems exist on low profiled floodplains, which are themselves little altered over time (Mould *et al.*, 2017; Williams, 2017). They are large and characterized by unusually deep bathymetry. Individual ponds may be up to 7.5 metres deep while disconnected, with width and length dimensions of 30 to 100 metres. The ponds exist in a highly agricultural landscape, with post European settlement and altered sediment movement dating from the 1830's (Portenga *et al.*, 2016). The ponds are under threat from ongoing land management practices such as continued grazing and cropping, extraction for stock and domestic use, upstream impact on water quality and altered weather conditions (Ji, 2016).

Catchment riparian conditions are highly altered (EnvironmentACT, 2004). The original vegetation in the area was open woodlands of *Eucalyptus melliodora*-*E. blakelyi*, with *Acacia mearnsii*, *Themeda australis* and *Danthonia pallida* understory, but this has been replaced by pasture species and exotic weeds (Dodson, 1986). The Mulwaree River at the site generally reflects its agricultural catchment, with locally poor water quality, typified by high electrical conductivity, total nitrogen and total phosphorus (EnvironmentACT, 2004; GHD, 2013).

Aquatic plants are seasonally abundant, with maximum coverage during summer. Most species were permanent rhizomatous perennial species (Roberts, 2011; Sainty, 2003), with littoral species variously present. Species diversity decreased with depth, with *Vallisneria gigantea* present to 3.5 metres, in the photic zone (Chapter 6). The ponds are within an agricultural landscape with variable to heavy cattle and sheep grazing.

Physical and Water Quality Methods

In order to measure water depth a piezometer (51 mm, diameter PVC pipe, with screen slots at 4 mm slot and width at 0.5 mm) was installed at 0.1 m in from the edge of pond 1. A Solinst Levelogger, with zero set at 0.2 m below cease-to-flow height, with a Barologger Edge (<https://www.hydroterra.com.au>) logged water depth and barometric pressure hourly from 16/4/15 to 28/9/17. Streamflow data was acquired for the nearest downstream site, Mulwaree River @ The Towers (2122725) (WaterNSW, 2018). Streamflow rate was calculated using River Analysis Package (EWater.com.au).

The temperature profile of the four ponds was recorded using thermistor chains comprised of a 30 cm polystyrene float, seven metres of 6 mm steel chain and a concrete brick installed in the deepest part of each pond in February 2016. Onetemp Hobo© 64 bit temperature and light pendent loggers (www.onetemp.com.au), logging temperature and relative light (lux) at 30 minute intervals were installed on the chain at +0.15, - 0.20, 0.60, 1.20, 2.00 metres and then at metre intervals to the depth of the pond. Data was collected from each pond for 18 months between February 2016 and August 2017. Euphotic depth ($Z_{eu} = 2 \times Z_{SD}$) (Boulton, 2014) was estimated on 11 occasions between February 2016 and September 2017 by a mean of duplicate secchi disk measurements (Kirk, 1994) deployed on the sunny side of the boat. This method gives a rough estimate of euphotic depth where more precise methods are not available.

Water quality profiles were taken on eight occasions, approximately every second month between February 2016 and September 2017 at 50 cm intervals to maximum depth in each pond, using a pre-calibrated Hydrolab Surveyor 4 and Minisonde 5S water quality probe (Aqualab.com.au). Nutrient samples were collected on six occasions between September 2016 and September 2017 at the centre of each pond at 25 cm depth using triple rinsed 250 mL PET sample jars which were filtered, chilled and frozen until analysis (APHA., 2005). Methods for analysis are summarized below (Table 1).

Table 1. Water quality parameter laboratory analysis methods. Results below detection limits were substituted to LOD/√(Croghan, 2003; Ogden, 2010)

Parameter	Determination	Method	Units of measurement
Ammonia	Colourimetrically	APHA 4500-NH ₃ F	mg/L
Total Nitrogen	Sum of total Kjeldahl Nitrogen plus NO _x	APHA 4500B + APHA 4500-NO ₂ -B, 4500 – NO ₃	mg/L
Oxidized N (NO ₂ , NO ₃)	Colourimetrically	APHA 4500-NO ₂ -B, 4500-NO ₃	mg/L
Alkalinity	Titrimetrically	APHA 2320-B	mg/L
Dissolved Organic Carbon	Filtered before analysis	APHA 5310B,C	mg/L
Total Phosphorus	Colourimetrically	APHA 4500BF	mg/L
Phosphate	Colourimetrically	EPA 365.S AND APHA 4500 PE.F	mg/L

Pond Productivity

Phytoplankton primary productivity and microbial respiration was measured on four occasions; twice when the ponds were connected (November 2016 and August 2017) and twice while disconnected (February and May 2017). Water was collected using a Van Dorn sampler (Wildco 2.2 litre Alpha sampler) mid-pond at one metre intervals between 0.5 to 4.5 metres depth; and at the pond margins within fringing aquatic vegetation at 3 random points at 0.5 metres depth. Samples were decanted to minimize addition of oxygen into acid washed (10% hydrochloric acid followed by deionized water) clear (light) and dark bottles (APHA., 2005) (300 mL BOD bottles (Wheaton No 1781N32 and Thomas Number 0914U63)). Dark bottles were coated with black epoxy paint). Dissolved oxygen was measured at the start of the experiment with bottles refilled to overflowing and after incubation using a YSI Ecosense self-stirring BOD probe (www.xylen-analytics.com.au) with a benchtop Eutech DO 700 Dissolved Oxygen meter (<https://www.thermofisher.com.au>). Each light and dark bottle was placed in pairs in transparent containers (Figure 3) attached at their original depth (as described above) on a 7 metre, 6 mm

steel chain, hanging from a 30 cm polystyrene float at each collection site. Each float was anchored to the adjoining thermistor chain or to a wooden stake amongst macrophytes to prevent movement and left in situ for 24 hours (Figure 3). One chain was installed in each pond, and three light and dark bottle pairs in fringing macrophyte beds. The caps of dark bottles were covered in double layers of aluminium foil to minimize light ingress. Following incubation, dissolved oxygen was measured in each bottle immediately.



Figure 3. Light and dark bottles, 0.5 m to 4.5 metre depth (left to right) before placement.

Chlorophyll A – Plankton biomass

For chlorophyll A, samples were collected from the same sample as for productivity incubations (500 mL – APHA 2005), filtered onto glass fibre filters (Whatman GF-C) and frozen immediately at -4.0°C. For analysis, the samples were thawed, later steeped in 90% acetone and chilled at 4 degrees for 24 hours, and then analysed using a spectrophotometer (Shimadzu 1600) methods according to Standard Methods 10200H. Chlorophyll (APHA., 2005). Samples were collected for phytoplankton community structure as a composite mid pool sample and three marginal vegetated sites as above. These were preserved in Lugols Iodine for later identification, but data is not included for this study.

Data Analysis

Depth data was compensated for barometric pressure before being offset to elevation at 640.95 m A.S.L., the height below which flow ceases between the ponds. Connected and disconnected periods were ascertained from data above and below that height A.S.L. Temperature change (ΔT) was calculated from mean daily temperatures using the difference between the surface and bottom temperatures (Whiterod, 2012). Calculation of the mixing depth (Z_{mix}), defined as the depth above the thermocline boundary, was imperative to understanding pond productivity (Coloso *et al.*, 2011) because the thermocline represents a barrier to metabolic processes in ponds. In each pond, Z_{mix} was calculated from 30-minute temperature data, as depth at which there was a persistent $-2^{\circ}\text{C}/\text{m}$ drop in temperature and the beginning of the thermocline (Losordo & Piedrahita, 1991). In lake studies the thermocline criterion varies between 0.01°C to 2°C per metre in depth, with very deep clear lakes using the former and more shallow lakes the latter (Nöges *et al.*, 2010; Zhang, 2015). More rigorous measures of the thermocline, Z_{mix} and metalimnion depth have been developed for deep lakes, (Obrador *et al.*, 2014; Staehr *et al.*, 2012), however were not addressed here due to the spatial scale of these small ponds. These data were manipulated to identify persistent stratification over the period of study and compared to photic depths (Z_{eu}) as calculated from secchi disk depths as described above (Boulton, 2014; Luhtala, 2013) and anoxic depth ($Z_{\text{anox}} < 2 \text{ mg/L O}_2$ - (Zhang, 2015). Water quality data were normalized and data with missing values removed before analysis by principal components (PCA), then two factor Permanova (Anderson, 2019) using event and ponds as factors. Water quality concentrations were related to Australian default trigger values (ANZECC, 2000).

Gross primary productivity (GPP), net ecosystem productivity (NEP) and planktonic respiration (PR) were calculated (APHA., 2005) from light and dark bottle dissolved oxygen results to grams $\text{C}/\text{m}^3/\text{day}$. There is strong evidence that stratified lakes support strongly productive layers in the meta- or hypolimnion, which are usually poorly sampled. So planktonic productivity is often underestimated in transparent lakes with sufficient light and nutrients to support primary productivity (Nöges *et al.*, 2010). Phytoplankton biomass as measured by Chlorophyll A (ChlA) was therefore calculated at each depth to mg/m^3 and analysed as mean and total ChlA for each sampling event, above and below the thermocline.

Differences in GPP, NEP, phytoplankton respiration and normalized (\log_{10}) ChlA data were analyzed using repeated measures ANOVA, with and between pond number, sample depth, sampling event and pond connectivity subjects and factors. Initial outlier analysis using Grubbs outlier test at $P < 0.05$ found one outlier, which was replaced by using the mid-point between the neighboring values. Tukeys HSD Post-Hoc tests were performed on all significant results.

P:R ratios were calculated for all samples, with mid pond and edge analyses performed by taking the mean of mid pond samples at 0.5 metres and the mean of the three edge samples. These were then analyzed using one way ANOVA for combined pond data for each sampling event.

Results

Hydrology

The Mulwaree River flowed for around 50% of the study period between April 2015 and September 2018. The piezometer provided flow data to 0.2 metres below the commence-to-fill (CTF) level of 640.95 m A.S.L in pond one, the most downstream site. The logger was set at 0.2 metres below CTF so was unable to measure pond height below that level. No flow-height rating table was developed.

During the study period, the ponds were connected between 16/4/2015-23/11/ 2015, 6/6/16-7/12/16 and 7/8/17-14/9/17. Rainfall events in 2016 represented some of the highest daily rainfall on record in New South Wales, being 2-3 times the long term mean monthly rainfall (44.9mm) at Goulburn in January (121.2 mm), June (166 mm) and September (95.8 mm) (Bureau of Meteorology, 2018). The ponds were subject to strong seasonal and thermal stratification once the ponds disconnected (Figure 4).

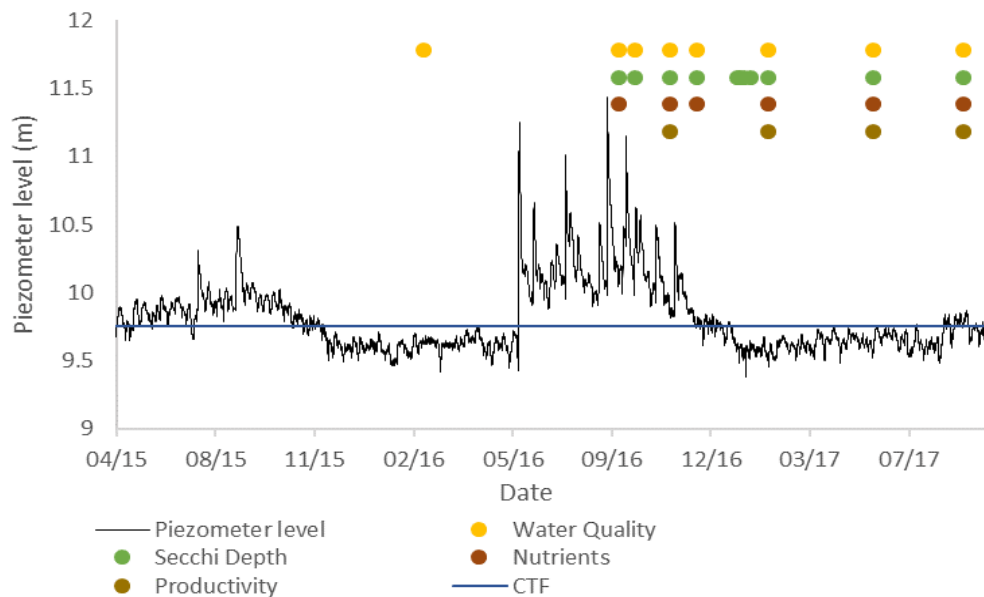


Figure 4. Water level in Mulwaree River as indicated by water level in piezometer in pond 1 with horizontal blue line denoting zero commence to flow. Dots denote dates of sampling events.

Thermal Behaviour

Maintenance of persistent stratification was less affected by flow than by air temperature and disrupted by cold overnight temperatures (Figure 5). ΔT exhibited a strong seasonal pattern related to connected/disconnected flow, but with short term hourly and daily variability during periods of diurnal stratification

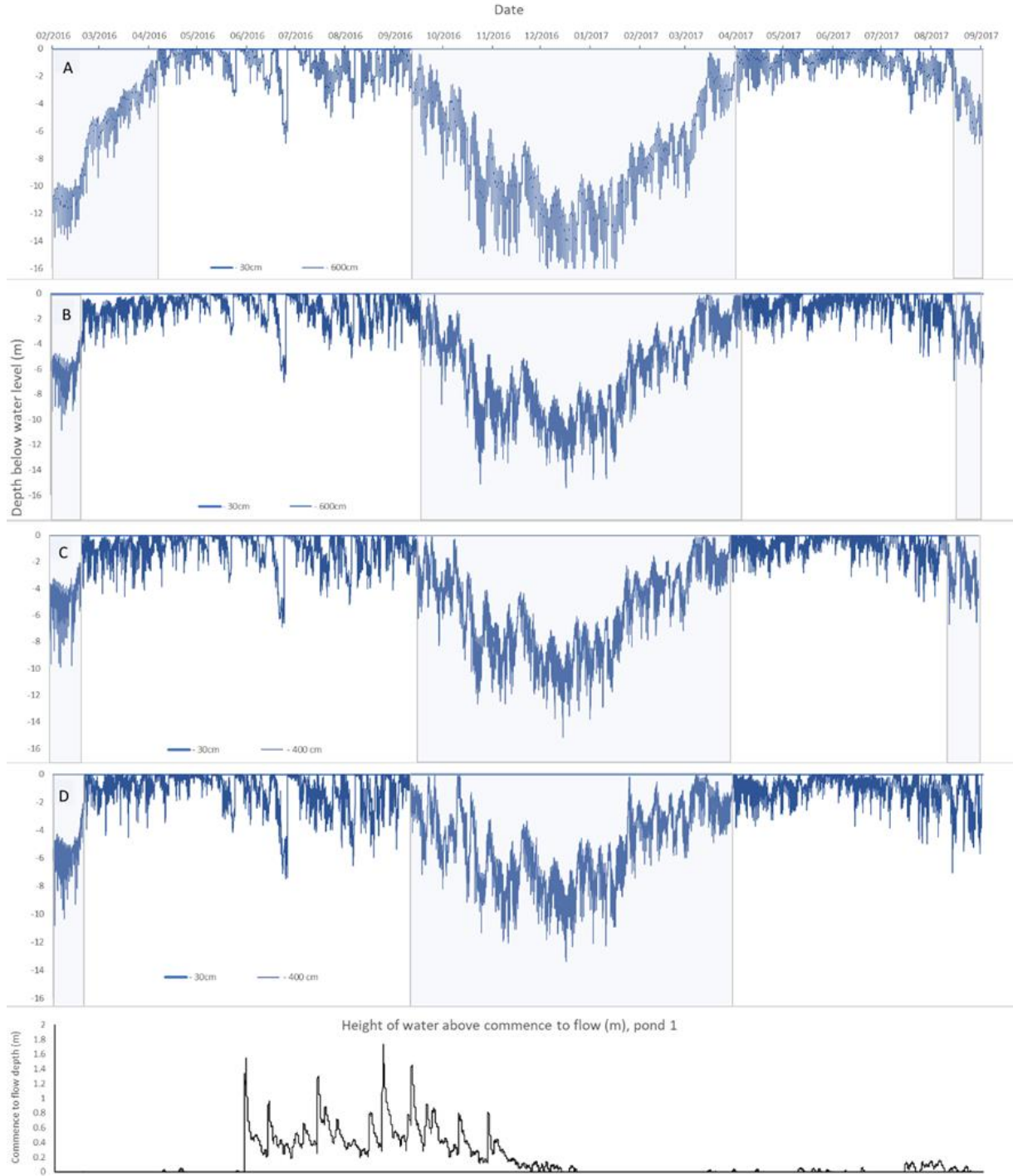


Figure 5 The maximum temperature difference between surface and bottom water (ΔT) for each of 4 ponds on Mulwaree River at Kelburn between February 2016 and September 2017. Periods highlighted in blue indicate periods of persistent stratification A=pond 1 (most downstream), B=pond 2, C=pond 3, D=pond 4 (most upstream). Hydrologic connection between the ponds is seen bottom of graphs, in metres above commence to flow.

Temperature, Light and Dissolved Oxygen

The thermocline, indicated by a temperature decrease of more than 2°C/m, became deeper related to seasons (Figure 6), starting around 1 metre and gradually deepening to 4 metres.

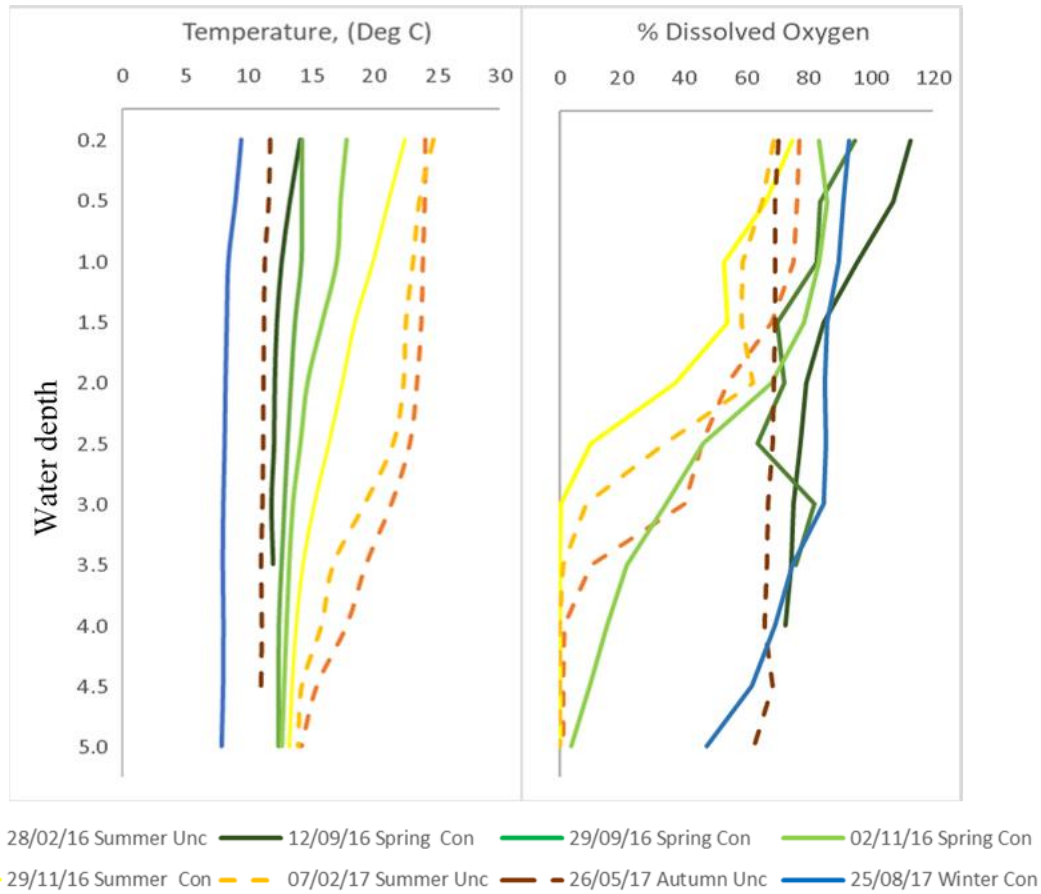


Figure 6. Mean Dissolved oxygen (% saturation) and temperature (°C) profiles for 8 sampling events from 28/2/2016 to 25/8/2017 averaged across all four ponds per time. Dotted lines represent events when there was no connection in the ponds, whereas solid lines represent events when the ponds were connected by flow. Winter (June-August) is represented by blue, Spring (September-November) by green, Summer (December-February) by yellow, Autumn (March-May) by brown.

During periods of connected flow and also between mid-April and mid-September, stratification was disrupted, and the ponds were thermally mixed. Secchi depth varied between 2-3 metres across ponds and seasons, with photic depth (Z_{eu}) varying between 4-6 metres (Figure 7). Generally, water transparency increased during periods of no or very low flow.

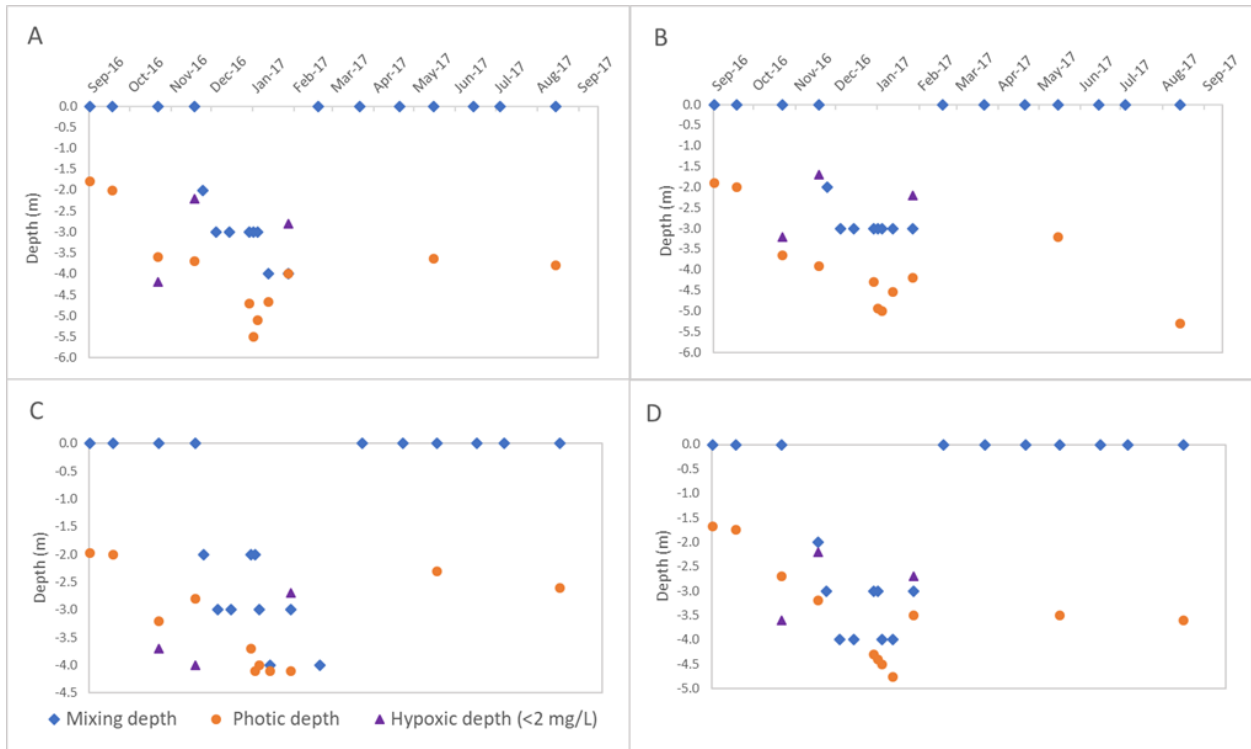


Figure 7. Mixing (Z_{mix}) - the depth above the thermocline, photic (Z_{eu}) - depth of effective light and hypoxic (Z_{anox}) - depth below which $DO < 2 \text{ mg/L}$, for each of 4 ponds on Mulwaree River at Kelburn between September 2016 and September 2017

During periods of persistent temperature stratification (where the ponds retained a thermocline consistently over 24 hours periods for days), photic depths (Z_{eu}) were consistently deeper than mixing depth (Z_{mix}) (Figure 7). The period when the ratio of photic depth to mixing depth was greater than 1 (Figure 7), coincided with thermal stratification and conditions where photosynthesis was possible below the thermocline. The oxycline ($Z_{anox} < 2 \text{ mg/L O}_2$ - (Bureau of Meteorology, 2017) (Figures 7, 8) when present, became shallower during disconnection, with hypoxia between 2.1 and 2.5 metres of the 4.5 to 6.5 metres depth during summer disconnected periods. Based on a roughly parabolic morphometry of the ponds (Appendix 1) (Williams,

2018), an estimated $\frac{1}{2}$ to $\frac{2}{3}$ of pond volumes were hypoxic during summer disconnected periods. The oxycline roughly coincided with the thermocline but was shallower than the photic zone during those times.

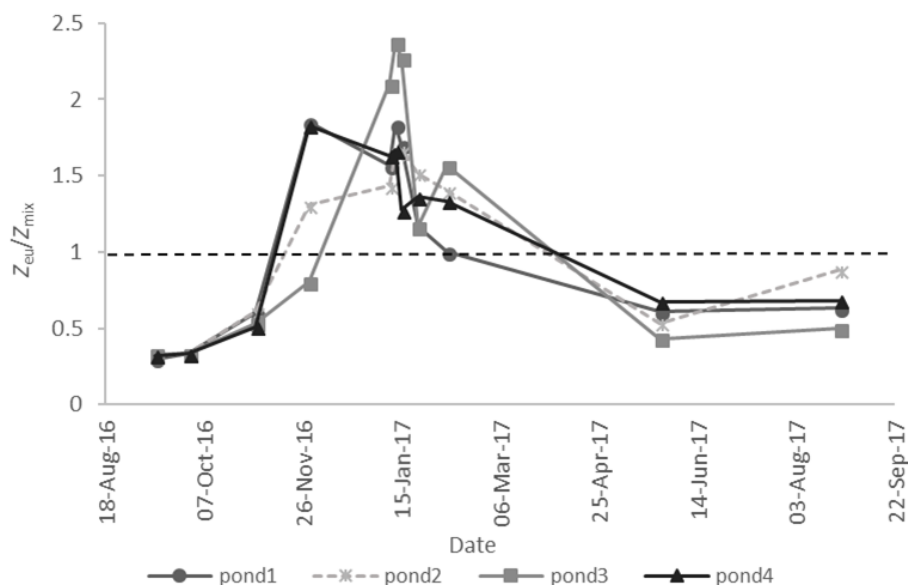


Figure 8. Ratio of Euphotic Depth (Z_{eu}) and Mixing Depth (Z_{mix}). Values above 1.0 indicate photosynthetic ability below the thermocline.

Water Quality and Nutrients

Water quality analysis revealed that the ponds were mesotrophic (Supplementary material, Appendix 2). It was assumed that organic matter was synonymous with DOC, as is usually the case (Boulton, 2014; Leenheer, 2003). With pH being approximately neutral, alkalinity was present almost entirely as bicarbonate ions (Supplementary material, Appendix 2, Table 2) a common occurrence (Boulton, 2014). Electrical conductivity (EC) increased over time as the ponds dried out and ions became more concentrated with the gradual evaporation of water (Supplementary information, Appendix 2, Table 2). Flows into the upper ponds in July 2017 were higher in EC than at any other sampling time, leading to a trickle-down inundation as the ponds filled from upstream. Variability in water quality was therefore based on seasonal and disconnected/connected status.

Principal Components Analysis (Figure 9) of environmental data was performed to identify the multivariate patterns separating seasonal changes in water quality. Seasonal differences explained 60.7% of the variability in the data in the first two axes of PCA. November, during strong flow connectivity, exhibited low nutrients and relatively good water quality, with February water quality related to high temperatures. By May, ammonia levels were high with August showing strong relationships to higher levels of nutrients, electrical conductivity, pH and alkalinity and low temperatures.

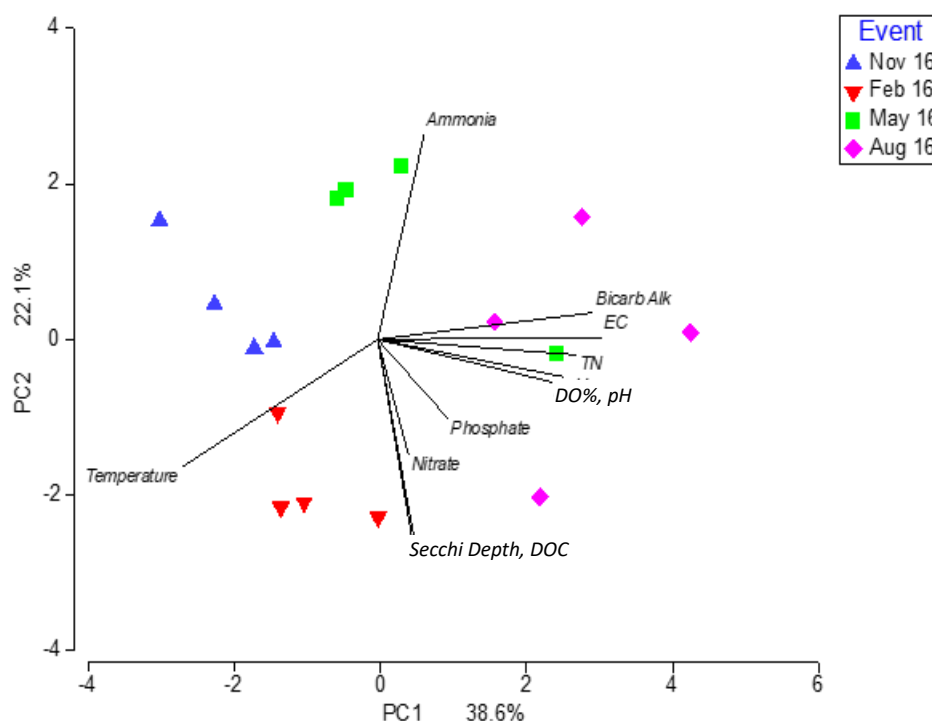


Figure 9. Water quality and nutrients, Principal Components Analysis (PCA), normalized data, (Primer Version 7.) for Mulwaree River @ Kelburn between November 2016 and September 2017. Four sampling events, mid pond for water quality and nutrients. Vectors clockwise from top – Ammonia, bicarbonate alkalinity, electrical conductivity, total nitrogen, pH, DO% saturation, phosphate, nitrate, secchi depth, dissolved organic carbon, temperature.

Multivariate significance testing of environmental data using Permanova (Anderson, 2008) indicated that overall, ponds were significantly different from each other ($p=0.001$, pseudo $F=17.8$), but the most significant differences were between events, ($p=0.001$, pseudo- $F=158.6$).

Community Production

Daily GPP was mostly positive for each sampling event during the study (Figure 10), increasing between November and February by at least a factor of four for all depths. Net ecosystem productivity was always positive to 1.5 metres and in autumn to 3.5 metres (Figure 10). During higher flows in spring, the ponds were well mixed, with low variability across all depths (Figure 10).

Flow connection and disconnection rather than seasonality had the most significant effect on GPP (connection*depth ($F_{(4,40)}$, 4.01, $P<0.05$), differences between pond 2 and 4 for NEP were small but significant ($F_{(3,40)}$, 2.91, $P<0.05$) and there were significant differences between events for PR ($F_{(3,40)}$, 2.95, $P<0.05$). GPP and NEP were mostly greater mid pond than that at the vegetated edge (Figure 11) but only significantly more so in summer for NEP ($F_{(1,6)}$, 12.15, $P<0.05$).

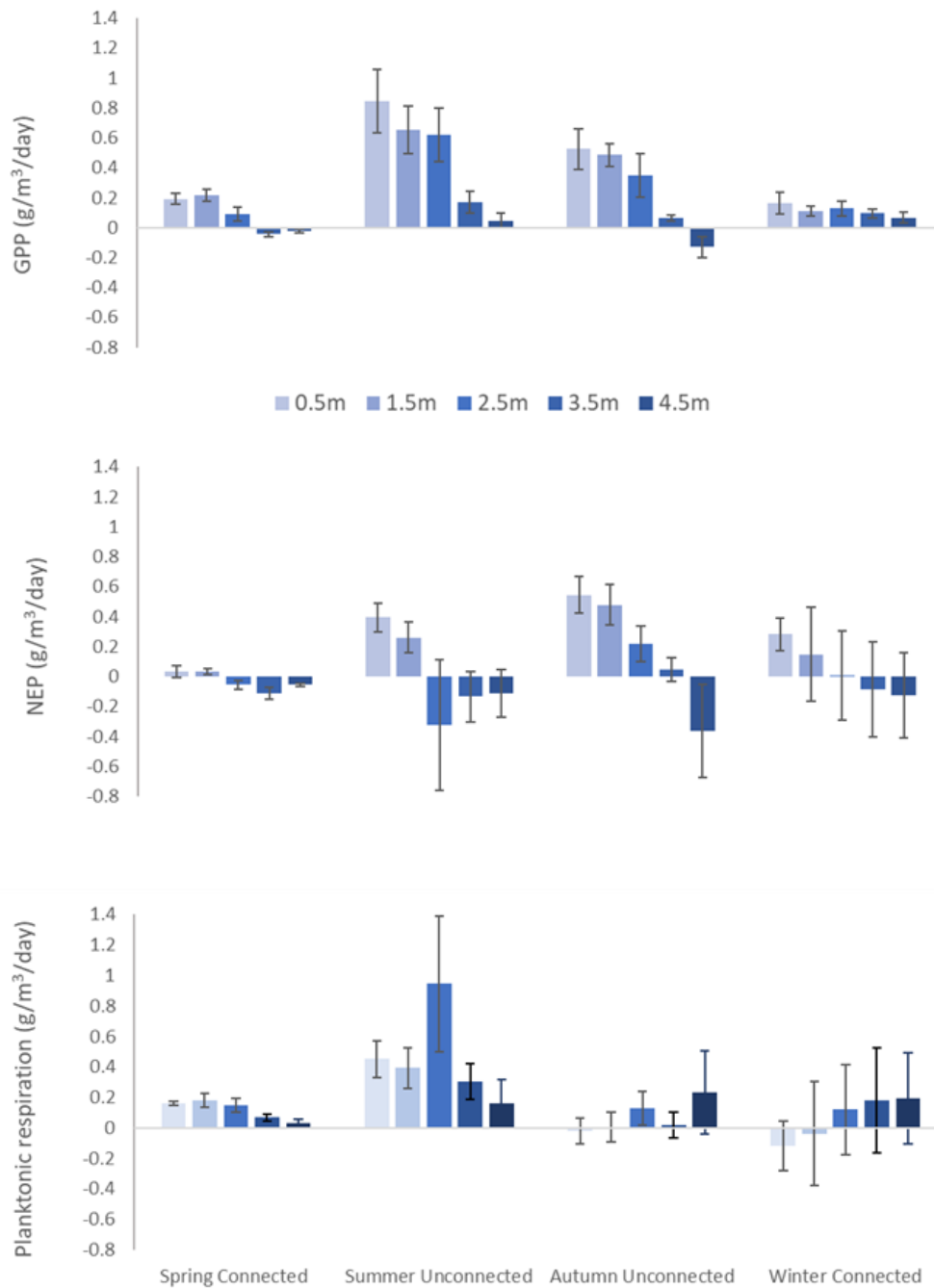


Figure 10. Gross Primary Productivity (GPP), Planktonic Respiration (PR) and Net Ecosystem Productivity (NEP) over four events, November 2016, February 2017, May 2017 and August 2017 at depths 0.5 to 4.5 metres, during seasonal flowing and disconnected flows in Mulwaree ponds (n=4, +/- S.E.).

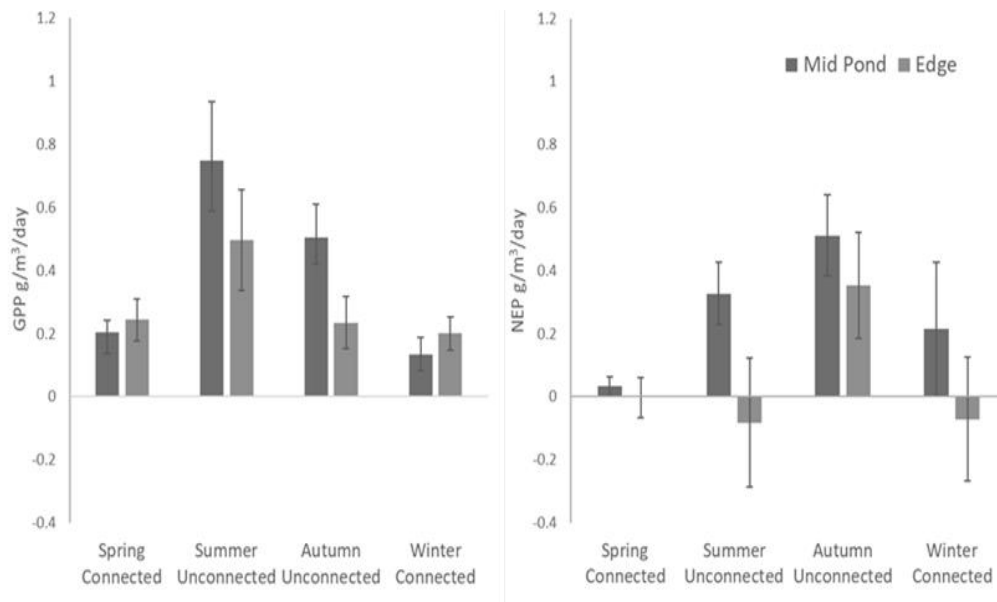


Figure 11. GPP and NEP of all samples from all ponds during connected and disconnected events, mean of mid pond (0.5 and 1.5 m) and 3 vegetated edges samples (0.5 m) \pm S.E., $n=4$.

GPP exhibited mostly positive relationships with planktonic respiration, strongest during spring and negative in autumn (Figure 12). Outlier analysis indicated that one sample from the autumn cohort be removed, altering the scatterplot to a negative relationship. The ratio was less variable in spring, most variable in summer as a result of one particular sample with extremely high planktonic respiration, and in winter exhibited very little difference in GPP with increasing planktonic respiration. Pearsons correlation for each of the events indicated significant correlation only in spring, when the ponds were highly connected. Overall there was a significant but small positive correlation for P:R.

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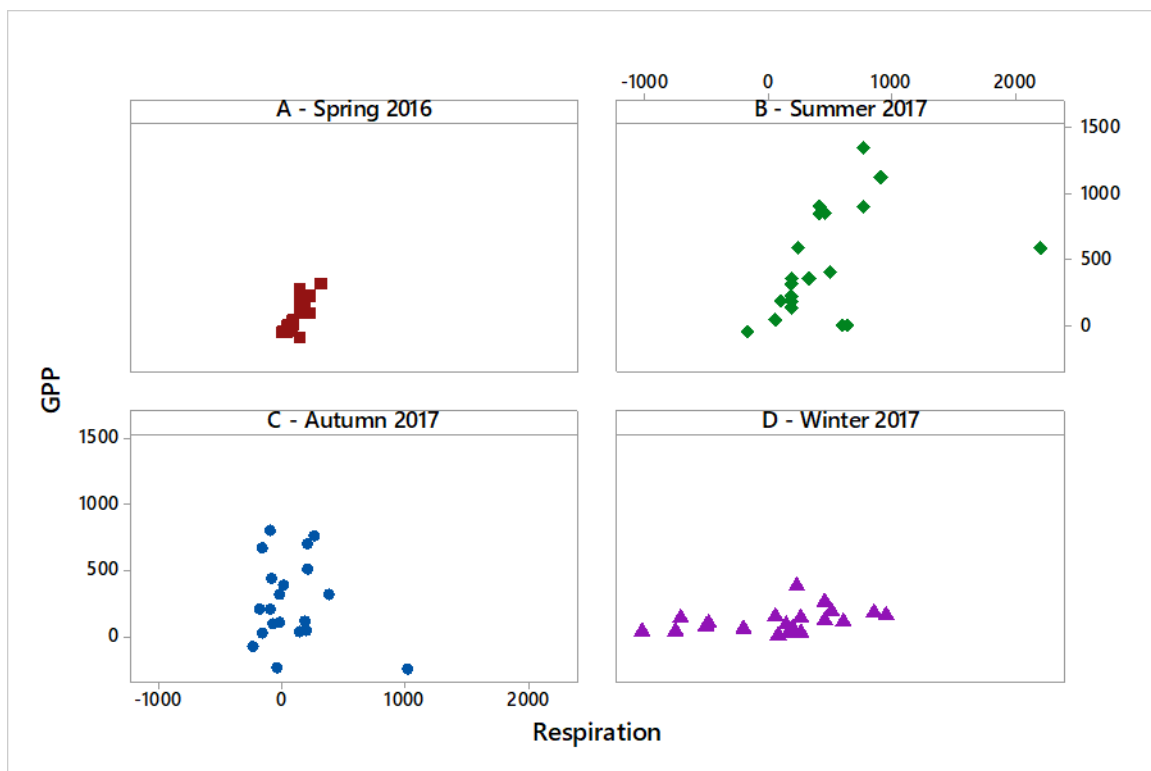


Figure 12. Scatterplot of GPP versus planktonic respiration – Pearsons correlation coefficient overall, 0.348, $P < 0.05$, for Spring – 0.786, $P < 0.001$, Summer – 0.420, $P = 0.065$, Autumn – 0.196, $P = 0.421$, Winter – 0.386, $P = 0.093$.

P:R ratios were greater than one over all events for all ponds and greatest in autumn. The main contributor to this pattern can be found mid pond in autumn, which had the greatest P:R ratios, but also high variability between the ponds. This suggests that the ponds were more similar during flow events and became more different following disconnection.

Table 2. Mean P:R ratios for all ponds and all depths over time and connection status. All pond samples include mid pond and mean of 3 vegetated edge samples at 0.5 m. (n=8). Mid ponds samples include 0.5m samples (n=4). Edge pond samples include mean of 3 random sampled points in the vegetated margins (n=4), all +/- S.E

P:R ratios +/- S.E.	November, Spring connected	February, Summer unconnected	May, Autumn unconnected	August, Winter connected
All ponds, 0.5 m, n=8	1.15 +/- 0.19	1.37 +/- 0.21	9.55 +/- 10.84	2.67 +/- 1.61
Mid pond, 0.5 m n=4	1.23 +/- 0.28	1.88 +/- 0.84	19.91 +/- 21.82	1.22 +/- 0.90
Edge pond, mean 3 points 0.5 n=4	1.08 +/- 0.28	0.86 +/- 0.18	-0.81 +/- 0.55	4.12 +/- 3.15

Vegetated edges of the ponds had generally lower P:R ratios, with respiration greater than production during summer and autumn. However, highly variable P:R values suggested dynamic and complicated productivity conditions at small scales within each pool.

Planktonic Biomass

Concentrations of $\log_{(10)}$ ChlA were seasonal and varied between ponds (event*pond $F_{(3,88)}$, 5.07, $P < 0.005$), during spring and winter when connected were significantly lower than during summer and autumn when disconnected ($F_{(1,126)}$, 40.61, $P < 0.001$). $\log_{(10)}$ ChlA exhibited low variability across all ponds during spring connected flows. Ponds were mixed, with ChlA at 4.5 metres depth similar to surface concentrations. Variability across the ponds and at depths increased once the ponds were disconnected (Appendix 2, Table 3). While concentrations of ChlA in summer to winter were often greater between 2.5 and 4.5 metres depth, in the mixing depth, high variability led to lack of statistical significance (Figure 13).

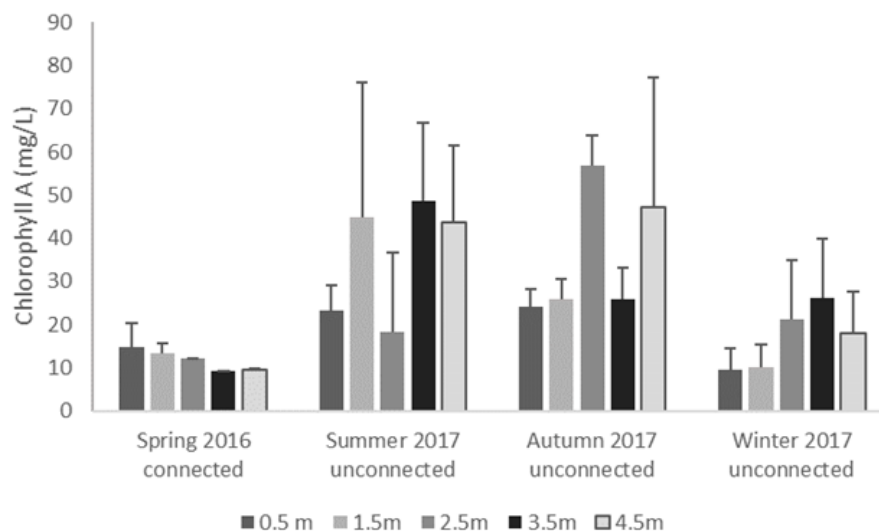


Figure 13. Chlorophyll A profiles, mid pond 0.5, to 4.5 metres, mean of all ponds, $n=4$

Mean pond log ChlA across all depths were less variable (Figure 14), with connection/disconnection more influential than seasonality (pond*connection $F_{(3,120)}$, 4.16, $P<0.01$). Mid pond concentrations of ChlA were greater, but not significantly different ($P=0.252$) than those for vegetated pond margins (Figure 15).

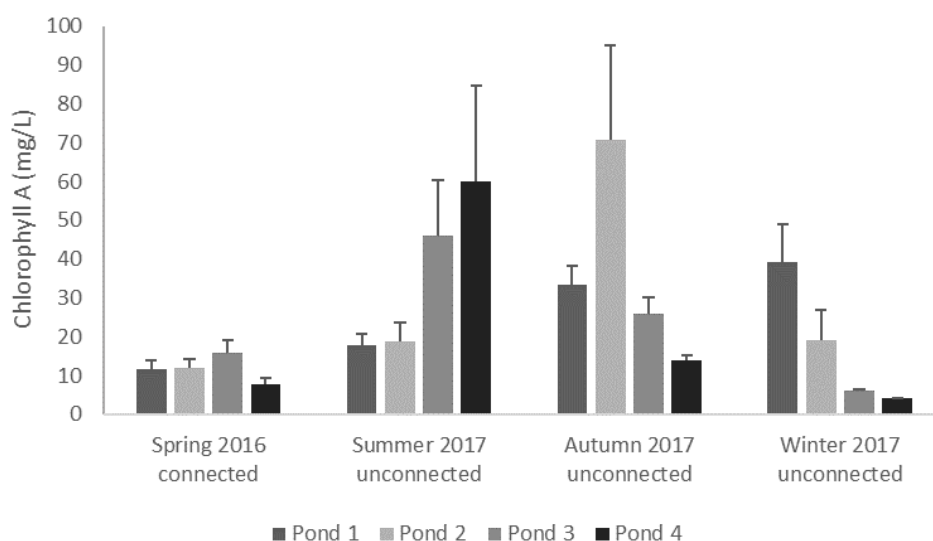


Figure 14. Mean Chlorophyll A concentrations for each pond, including depth profiles and vegetated edge samples, $n=8$ (\pm S.E.), for connected and disconnected flow/seasonal sampling.

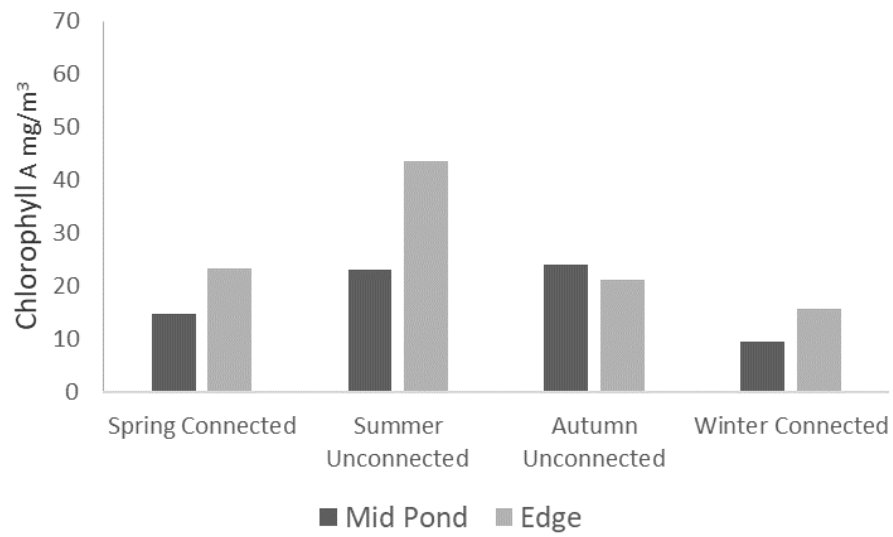


Figure 15. Mean Chlorophyll A concentrations across all ponds for depths of 0.5 metres, mid pond versus vegetated pond edges +/- S.E. n= 4.

Discussion

Characteristics of Flow and Non-flow

The Mulwaree river flowed and the chain-of-ponds were connected for around 50% of the study period, predominantly in the cooler months. The ponds are an intermittent stream system, a characteristic they share with up to 90% of other Australian dryland rivers (Boulton, 2014; Sheldon, 2010) and to a lesser extent, elsewhere (Larned, 2010). During large flow events, the ponds reconnected, exhibiting instream flood pulse behavior (Junk, 1989) and acted like a reconnected floodplain river (Baldwin *et al.*, 2016). Flows in the ponds were fully mixed, especially in spring, moving previously accumulated carbon and nutrients downstream, with lower P:R ratios. Inter-pond variability was low. For the rest of the time, the river formed a series of ponds in a strongly lentic system and P:R ratios increased over time. By the following winter, minor reconnection and lower primary productivity caused P:R ratios to decline. These conditions may occur in many other small rivers in Australia (Turner *et al.*, 2005), where natural deep scour pools or regulation structures exist. However, as unregulated streams (streams without major flow regulation) in southeastern Australia have become more permanent as a result of vegetation clearing and associated higher groundwater levels (Eberbach, 2003), intermittent stream drying into pools is now less common.

Several physical features of Mulwaree chain-of-ponds set them apart from other rivers, including spatial scale, deep photic zones, intermittent hydrology and extensive vegetation. Most ponds globally, including chain-of-ponds described elsewhere in Australia, have a maximum depth of 2 to 3 metres (Eyles, 1977a; Mould *et al.*, 2017; Pettit *et al.*, 2012) and widths to 15 metres (Williams, 2018) and between 25 m² and 2 ha (Davies, 2008). Mulwaree ponds are unusually large for Australian chain-of-ponds systems, with depths to 11 metres, (Eyles, 1977b) and exhibit bathymetry that is rare for Australian aquatic systems. This makes them act ecologically as lakes rather than ponds.

Mulwaree Ponds geomorphology is little changed, with distinct ponds and shallow preferential flow lines. Stratification of the water column occurs annually during disconnected flow and the ponds are permanent. Furthermore, the ponds and preferential flow paths are vegetated with perennial and rhizomatous semi-aquatic plants and finally, due to macrophyte dominance, water clarity within the ponds, as reported elsewhere, (Mulderij *et al.*, 2007) is unusually high. These factors all affect phytoplankton and microbial productivity within the ponds and with transfer of material downstream.

During flood events, rapid rewetting of pond sediments and intersecting preferential flow lines disrupted the thermocline (as shown in Figure 7), presumably exporting microbial, phytoplankton and aquatic macrophyte detritus downstream as experienced elsewhere (Larned, 2010; Puckridge, 2000). During smaller flows, depending on antecedent conditions, longitudinal connection was minimal, as occurred during spring 2016 and winter 2017. Under these conditions, within individual ponds, complex interactions with drying and rewetting sediments can liberate accumulated carbon as well as nitrogen and phosphorus, depending on seasonal conditions (Baldwin, 2000). These conditions then stimulate phytoplankton and macrophyte growth as shown here and in Chapter 6. In spring, with receding flows, damp marginal sediments dry and thermal stratification develops, trapping material below the thermocline.

In winter 2017, water trickled from upstream, slowly filling the ponds and eventually being released downstream, pond by pond. Initially, downstream flows were minimal (Figure 5) the concurrent release of carbon and nutrients would therefore be expected to be minimal (Weilhoefer, 2008). At these low flow rates, the dried sediments at the edge of the ponds were wet only slowly and to the limit of the commence-to-flow depth. Microbial and macrophyte involvement and primary productivity would be expected to be low due to seasonal low temperatures (Baldwin, 2000). Catchment-derived suspended sediments and higher conductivity water flowed firstly into pond 4. Water at low temperatures with a relatively high dissolved oxygen concentration, combined with aquatic plant detritus, phytoplankton carbon, microbial material, and sediment nutrients were released slowly downstream.

During summer, stratification was common but disrupted by occasional high flows. If flows were low, incoming warm water was unlikely to disrupt stratification, instead flowing over the top of the thermocline and releasing only surface water. If flows were high, and the thermocline disrupted, significant quantities of anoxic carbon and nutrient rich water were released downstream. The ponds were mesotrophic during the study, indicating that both nitrogen and phosphorus were abundant. Elevated phosphorus in streams is influential in increased plant growth (Boulton, 2014) and is generally the primary nutrient limiting productivity in freshwater systems globally (Vollenweider, 1976). Coupling of aquatic carbon and phosphorus can increase microbial activity, resulting in remineralization of phosphorus under higher concentrations of organic carbon (Anderson, 2018). However, there are many examples of nitrogen rather than phosphorus limitation and co-limitation with nitrate and other nutrients (Maberly *et al.*, 2002).

Varying nutrient limitation leads to alternative stable states for phytoplankton communities where different groups may exhibit competitive advantage over others (Sigg, 2005b). The results indicated that concentrations of total nitrogen increased over time compared to phosphorus, however low reported levels of phosphorus meant that it wasn't possible to identify nutrient limitation dynamics. Geochemical relationships between nitrogen and phosphorus in mesotrophic ponds such as Mulwaree ponds require more investigation, particularly in light of increasing weather variability, temperature and eutrophication of Australian aquatic systems (Davis & Koop, 2006).

In-pond Dynamics of Productivity

During disconnected conditions in summer and autumn, bathymetry influenced the development of pond productivity. Diurnal and later persistent thermal stratification and a well-developed oxycline, were able to develop in the presence of marginal vegetation and comparatively small surface area to depth. Once the ponds stratified, they behaved as monomictic lakes, developing a well-defined epilimnion, metalimnion and hypolimnion. These discrete layers were dynamic during periods of diurnal stratification but became permanent and slowly increased in depth during summer. Metabolic rates varied in each of the layers, underlying the importance of both monitoring productivity and algal biomass at depth, but also in estimating more accurate measures of total water column productivity (Obrador *et al.*, 2014).

Persistent thermal stratification prevented vertical mixing between the epilimnion, through the metalimnion to the hypolimnion, as reported elsewhere (Losordo *et al.*, 1991; Wetzel, 2001). Thermal stratification was accompanied by development of a mostly shallower oxycline. Oxycline development is well known to occur in mesotrophic waters where, as NEP decreases with depth, bottom layers become nutrient rich and anoxic (Bormans & Condie, 1997). In the Mulwaree ponds, disconnection and thermocline development was not complete before the oxycline developed, suggesting that there was considerable subsurface respiration occurring as disconnection progressed. Hypoxic conditions (< 2 mg/L) that are known to be toxic to fish and invertebrates (Boulton, 2014; Small *et al.*, 2014) occurred below 2.3 metres which accounts for up to $\frac{2}{3}$ of the depth and half of the volume of the ponds. When the photic zone extended to 6 metres depth in summer, there was a layer of up to 2.5 m of water capable of supporting photosynthetic activity, but without oxygen.

Gross primary productivity was strongly positive at depths to 2.5 metres, above the thermocline. However, as hypothesised, planktonic respiration was not strongly related to depth or euphotic conditions but to seasonality. Net ecosystem productivity and thus autotrophy was positive to 1.5 metres in summer around the oxycline, and in autumn to 3.5 metres, well below the thermocline. These well-developed barriers to phytoplankton and microbial movement may have been influential in the complexity and variability of autotrophy and heterotrophy both within and between ponds.

Mostly positive P:R ratios in the surface water suggests that the ponds may be acting as carbon sinks at small scales, but with production of reduced carbon at the hypoxic base of the ponds and no primary production below Z_{eu} , some release of carbon to the atmosphere. Variation in P:R ratios appears dynamic, perhaps related to mid pond autotrophy, competition for light and nutrients and possible allelopathy in the macrophyte edges and zooplankton grazing (Iacarella *et al.*, 2018). Definitely, variability in primary productivity and respiration was highest while the ponds were disconnected. This implies that ponds became more different from each other once disconnected and therefore were providing biological diversity or a 'pondscape' (Hill *et al.*, 2018). The development of variability in ponds across the landscape increases landscape diversity, thus supporting greater ecological functions at a landscape level.

Carbon Dynamics

Without knowing the benthic and macrophyte contribution to carbon dynamics it is not possible to know whether the ponds act as sinks or sources of carbon during disconnection and connection. There is strong evidence that benthic respiration in ponds contribute gaseous carbon as CO₂ and CH₄. For example, in temperate latitudes where this study was undertaken, CH₄ is usually more commonly released than CO₂ and more so in smaller than larger ponds (Holgerson & Raymond, 2016). Reasons for the greater proportional CH₄ fluxes from small ponds include shallower water, higher edge to water volume ratios, frequent mixing and high suspended sediment. These contribute to CO₂ and CH₄ supersaturation, limiting efficient CH₄ methane oxidation (Holgerson *et al.*, 2016).

Furthermore, smaller ponds are closer to terrestrial sources of carbon that may increase pond carbon stocks and marginal aquatic vegetation covering proportionally more surface area. Mulwaree ponds are heavily vegetated within their margins, enabling considerable carbon capture into plant biomass due to photosynthesis. Multiple processes may operate under these conditions to complicate carbon dynamics in the ponds. Release of gaseous carbon through sediment to air transpiration and annual decomposition and benthic oxidation of methane in macrophyte root zones related to high species richness (Bouchard *et al.*, 2007) may mean that carbon dynamics in Mulwaree ponds are complex and operate at fine spatial scales.

Ponds and lakes may move between alternative stable states, including between macrophyte and phytoplankton dominance. Macrophyte dominated states develop positive feedback loops based on provision of habitat for zooplankton, sequestration of nutrients, reduction of suspended particles and allelopathy (Scheffer, 1993). This increases water clarity and reduces nutrient availability. A strong imperative in maintaining macrophyte communities is the continued loss of macrophytes globally and across Australia (Phillips, 2016; Wassens *et al.*, 2017). The alternative phytoplankton state occurs when there are high concentrations of phytoplankton, high turbidity, nutrient enrichment (Phillips, 2016) and associated poor ecosystem function (Iacarella *et al.*, 2018). However, there is also evidence that phytoplankton dominance increases carbon burial in shallow lakes, which has an overall benefit in conditions of global warming (Mulderij *et al.*, 2007).

There is still not enough information in this study to answer the question of whether these ponds act as shallow lakes that might exhibit alternative stable states as is evident elsewhere (Brothers *et al.*, 2013), or whether they vary along a continuum undergoing hysteresis and more stochastically depending on current intrinsic and extrinsic drivers (Capon *et al.*, 2015; Scheffer & van Nes, 2007). Further investigation over longer temporal scales and multiple environmental conditions may provide more certainty.

Planktonic Biomass

Phytoplankton biomass was highly variable across ponds over all times and depths, but more so during disconnected periods in summer and autumn than in connected periods in winter and spring. Ponds developed individual phytoplankton biomass profiles in terms of chlorophyll A concentration, varying from each other and at depths. There was evidence of microbial photoinhibition, commonly encountered elsewhere (Staehr *et al.*, 2016), in the surface layers during summer and autumn and of substantial primary production below the thermocline during summer.

Phytoplankton variability in stratified waterbodies is well known, facilitated by active migration, passive movement and population change (Sigee, 2005a), including in temperature-stratified and turbid Australian rivers (Bormans *et al.*, 1997; Ganf & Oliver, 1982; Mitrovic *et al.*, 2010; Sherman & Webster, 1994; Thompson *et al.*, 2003), much of which is due to vertical mobility of cyanobacteria and its competitiveness compared to Chlorophyta and other algae. In Mulwaree ponds, light was unlikely to be limiting, with the photic zone extending to 6 metres and as evidenced by observations reported elsewhere. (Kromkamp, 2008). Temperature, oxygen stratification, macrophyte dominance and nutrient fluxes meant that phytoplankton may have exhibited dynamic community structure. There was no evidence of cyanobacterial blooms, but during stratification, green water was observed at 4.5 metres (pers.obs.), in anoxic conditions (Figure 7).

The presence of photosynthetic pigments below the oxycline suggested that either phytoplankton detritus was falling to below the thermocline, cyanobacteria were actively moving across thermal and oxygen boundaries or that anaerobic photosynthetic bacteria were utilizing light below the oxycline. It is possible that once disconnected, the complexity of stratification and water clarity in each pond led to intra-pond phytoplankton community variability at very small scale. Unfortunately, it was not possible to identify the complexities that were operating; peak absorption by bacterial chlorophyll (653nm) is too close to that of chlorophyll A (663 nm) (Caraco & Puccoon, 1986), making distinction impossible with the methods used. Repeated algal composition profiles and the use of alternative techniques such as flow cytometry, microbial and genomic methods (Karhunen *et al.*, 2013; Lucas *et al.*, 2010) could be useful in future studies.

During flow connection, phytoplankton biomass was mixed, particularly in spring high flows. Ponds responded differently over time. During winter 2017, slow trickling flows meant that dilution and flow downstream was slow and had not connected to pond 1. This meant that phytoplankton biomass remained higher than more upstream ponds. These temporal and spatial flow related differences would be expected to be a common occurrence in ponds with variable, intermittent flows.

Conclusion

Many permanently flowing Australian rivers have been altered from clear, macrophyte dominated systems to ones of high turbidity and phytoplankton dominance (Deegan & Ganf, 2008). The combination of Mulwaree chain-of-ponds deep bathymetry, high macrophyte species richness and extent, with high water clarity combined with mesotrophic water quality has created a unique system. Strong seasonal hydrology and flow disconnection, with thermal and oxygen stratification and with relatively low rates of planktonic productivity, coupled with significant respiration, has enabled the ponds to maintain macrophyte dominance.

This study provided an initial understanding of the functions of this unique system. It is now possible to understand that the combination of bathymetry and hydrology underlying the unusual geomorphology of Mulwaree Ponds has provided a template for a rare system of ponds that act as temperate monomictic lakes. The vegetation that extends around the margins and between the ponds maintain macrophyte dominance, that is at risk because of land management and possible changing climate. Increased variability in primary productivity and respiration during seasonal disconnection suggests that the ponds are acting individually and creating pondsapes that increase ecological function across the landscape.

More knowledge is needed to more fully enable learnings to be more transferable across pond functional ecology. Questions related to understanding diurnal dissolved oxygen patterns over time; macrophyte dynamics within the ponds and how they contribute to maintaining dominance; grazing and predator interactions that maintain or threaten macrophyte dominance; carbon dynamics and hysteresis and how the ponds move between macrophyte and phytoplankton dominance over time; the mechanisms for protection using futures thinking (Cork, 2010); and the effect of top down control of pond ecology are all important future studies.

The risks to Mulwaree ponds are many, but include:

- more nutrients, in particularly nitrogen, from upstream and from local grazing pressure
- global warming and variability, altering internal pond processes of temperature, microbial activity and flow variability
- macrophyte grazing, reducing the ability of macrophyte dominance.

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Appendices

Appendix 1.

Table. Morphology of Mulwaree chain-of-ponds

Taken from Williams, R.T., 2018, 'The Hydro-Geomorphic Structure, Function and Evolution of Chains-of-Ponds: Implications for Recognition of These Discontinuous Watercourses in River Management'. A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy, Macquarie University, Department of Environmental Sciences, December 2017.

Table 1. Morphometrics of the five ponds along the study reach. See Figure 1D for locations and Figure 3 for comparative cross-sectional and longitudinal profiles

Pond	Length (m)	Width (m)	Area (m ²)	Perimeter (m)	Volume (m ³)	Mean Depth (m)	Max Depth from water outflow level (m)
1	135.5	42.4	3962	305	10655	2.7	5.1
2	103.7	35.7	2474	228	7909	3.2	6.6
3	66.0	36.5	1818	167	4943	2.8	4.9
4	97.2	46.6	3001	237	10616	3.5	7.0
5	108.4	30.7	1738	244	1964	1.2	3.4

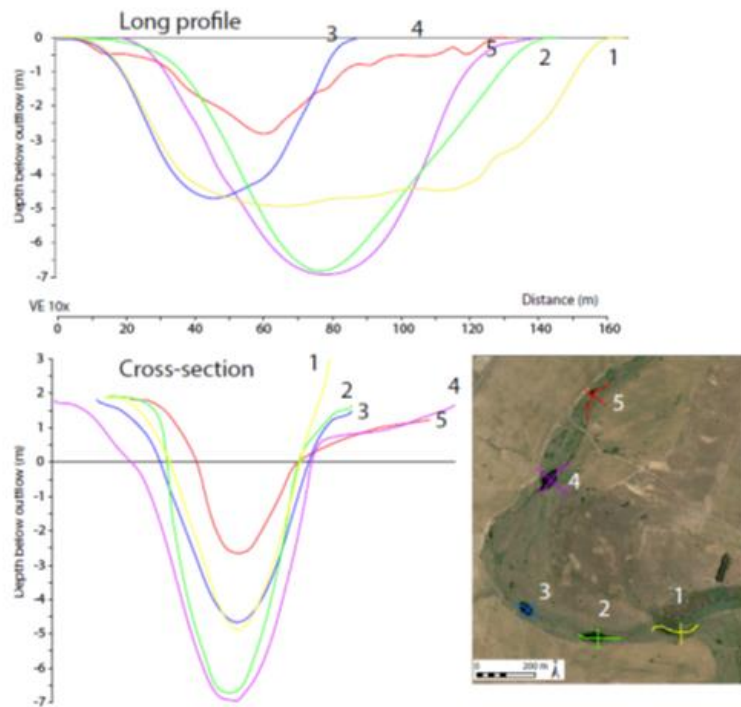


Figure 3. Longitudinal profile and cross-section of the five ponds. Numbers related to ponds on Figure 1D moving downstream in order.

Appendix 2.

Table 2. Water Quality (mean \pm S.E), $n=4$, over the course of the study, Mulwaree ponds

Mean \pm S.E.	12/09/2016	02/11/2016	29/11/2016	07/02/2017	26/05/2017	25/08/2017
Electrical conductivity(μ S/cm)	356.95 \pm 0.48	601.89 \pm 23.38	569.28 \pm 8.85	774.28 \pm 17.92	974.51 \pm 132.97	1294.74 \pm 199.64
pH	6.89 \pm 0.40	6.88 \pm 0.20	6.71 \pm 0.10	7.60 \pm 0.60	7.19 \pm 0.16	7.45 \pm 0.20
Total Nitrogen (mg/L)	0.55 \pm 0.60	0.83 \pm 0.11	0.95 \pm 0.30	1.18 \pm 0.60	1.18 \pm 0.30	1.95 \pm 0.10
Bicarbonate Alkalinity as CaCO ₃ (mg/L)			97.00 \pm 0.71		132.50 \pm 1.31	137.50 \pm 12.50
Total Alkalinity as CaCO ₃ (mg/L)	66.00 \pm 0.71	98.25 \pm 2.50	97.00 \pm 0.71		132.50 \pm 1.31	137.50 \pm 12.50
Ammonia (mg/L)	0.20 \pm 0.10		0.30 \pm 0.10	0.40	0.50	0.40 \pm 0.10
Nitrate (mg/L)	0.40 \pm 0.20		0.40	0.7 \pm 0.2	0.10	0.40 \pm 0.10
Nitrite (mg/L)	0.20 \pm 0.10		0.40	0.40	0.40	0.30 \pm 0.10
Phosphate (mg/L)	0.10		0.30 \pm 0.10	0.10	0.10	0.10
Dissolved Organic Carbon (mg/L)	18.00	13.75 \pm 1.65	12.75 \pm 0.25	13.25 \pm 0.25	12.25 \pm 0.25	13 \pm 0.41
Total Phosphorus (mg/L)	0.11 \pm 0.20		0.40	0.40	0.40	0.40

Table 3. Basic Statistics for Chlorophyll A

Variable	event2	N	Mean	SE Mean	Standard Deviation
Chlorophyll A	Spring 2016	32	13.69	1.29	7.29
	Summer 2017	32	40.26	5.91	33.41
	Autumn 2017	32	28.05	5.05	28.58
	Winter 2017	32	15.91	2.85	16.09

Chapter 6

Decomposition processes of *Cycnogeton procera*, a native rhizomatous aquatic macrophyte in Mulwaree Chain-of-Ponds



Lorraine Hardwick

Department of Biological Sciences

Abstract

Decomposition of leaf litter of the endemic macrophyte *Cychnogeton procerum* (R.Br.) Buchenau was investigated in Mulwaree chain-of-ponds, a morphologically rare intermittent river with deep ponds and vegetated preferential flow paths in eastern New South Wales, Australia. Due to anthropogenic pressures, it is an at-risk ecosystem, both structurally and functionally. The chain-of-ponds are unprotected by legislation, despite being the only remnant of large chain of ponds system in Australia and globally rare. The aims of the study were to try and understand how this rare system functions, by using decomposition processes; and to identify spatial and temporal patterns in macrophyte decomposition and the importance of microbial activity in that process. Leaf litter bags were incubated in situ over four events, with varying flow connection and seasonality. Decomposition rates and carbon loss over time were established. Decomposition rates were highest during spring and winter lotic conditions and slower during summer and autumn, when lentic conditions, persistent stratification, possible herbivory and net primary productivity were at their highest. Seasonal and complex interactions between macrophytes, periphyton and phytoplankton with nutrient availability, as well as palatability of *C. procerum* to grazers and top down pressure from predaceous fish *Gambusia holbrooki* are likely determinants. Decomposition was mostly performed by microbes, with herbivores possibly only a factor during spring lotic conditions. Temperature was a major factor, so expected climate change scenarios will put further pressure on this rare system supporting both macrophytes and phytoplankton.

Introduction

Globally, aquatic plants are under increasing stress from habitat loss. Since 1900, the global distribution of wetlands, a major habitat for aquatic plants (macrophytes), has declined by around two thirds following centuries of draining, infilling and conversion for human use. Up to 87% of wetlands are estimated to have been lost since 1700 (Davidson, 2014; Vorosmarty, 2000).

Wetlands in Southern Australia are particularly at risk due to relative aridity and competition for water (Specht, 1990). In the Murray-Darling Basin and associated slopes, vegetation clearing for agriculture and water regulation have severely altered stream hydrology. Timing of availability, quality and quantity of water available to support viable aquatic macrophyte communities have been affected across vast wetland areas (Brinson & Malvárez, 2002).

While macrophyte communities are common in some Australian rivers (Bunn, 2002; Paice *et al.*, 2017; Watson & Barmuta, 2011), high turbidity, altered hydrology and irrigation development have led to widespread reduction of macrophyte communities in others (Walker, 1992).

Eutrophication and changes to flow variability have both been influential in stimulating or impeding growth and resilience (Carr *et al.*, 1997; Mackay *et al.*, 2003; Walker, 1992) and climate change is expected to compound these effects for both riverine and wetland aquatic macrophytes (Balcombe *et al.*, 2011).

Australian macrophytes are adapted to unpredictable aquatic habitat in a continent that exhibits some of the most highly variable stream flows in the world (Poff *et al.*, 2006; Puckridge, 1998). Furthermore, as a likely result of continental isolation, the macrophyte community has a greater proportion of endemic species than elsewhere (Chambers *et al.*, 2008) so the relative risk of loss of biodiversity is high under climate change scenarios. In particular, macrophytes with limited dispersal strategies, perennial life cycles and local investment, such as rhizome production, are at relatively greater risk under conditions of increasing climate variability. Many native and widespread rhizomatous species such as bulrushes (*Typha domingensis*) and the common reed (*Phragmites australis*) disperse seed mainly by wind (anemochory), which facilitates effective gene flow. However, Water Ribbons (*Cynnogeton procerum*) is mostly dispersed by water (hydrochory) and occasionally by waterbirds (zoochory). It was selected for study because it disperses less readily and so has a heightened risk of local extinction.

Macrophytes are now recognized as an important component of the aquatic food web (Lodge, 1991). For more than half a century the paradigm of aquatic macrophytes as minor players in food web dynamics persisted (Shelford, 1918). Their use as a food source was exemplified by studies that indicated they were poorly grazed by invertebrates when fresh (Suren & Lake, 1989) and became more palatable following senescence. More recently, a growing body of work (Bakker *et al.*, 2016; Lacoul & Freedman, 2006; Lodge, 1991; Wood *et al.*, 2012) has emphasized the importance of herbivory of aquatic macrophytes in aquatic food webs. A recent meta-analysis of macrophyte biomass removal by herbivores reported losses of between 44-48%, significantly greater than for terrestrial systems (Wood *et al.*, 2017).

Functionally, macrophytes provide protection against erosion (Zierholz, 2001) and in the Mulwaree system are particularly important in maintaining geomorphic integrity of Mulwaree chain-of-ponds (Williams, 2018). Additionally, macrophytes are heavily used as a refuge habitat by invertebrates and fish, as a substrate for periphyton and are instrumental in sequestration and release of carbon to the surrounding environment (Bouchard *et al.*, 2007; Mitsch *et al.*, 2013). Their contribution to biodiversity in aquatic systems is integral to stream health, by increasing invertebrate abundance and diversity by orders of magnitudes greater than unvegetated habitats (Reid, 2008; Warfe & Barmuta, 2004). As well as its use in preventing erosion, *C. procerum* is utilized by waterfowl, and livestock who will walk into the water to graze on it. The loss of such a dominant macrophyte in this system would be likely to destroy the unique geomorphic structure of the ponds.

Mulwaree Ponds form a rare aquatic system due to their geomorphic nature (Mould & Fryirs, 2017). While more is known about their geological and geomorphic history (Williams, 2018), very little is known about their structural and functional ecology. By investigating functional processes of one of the key macrophytes in a macrophyte dominated system, the aim is to better understand the risks facing both. The ponds exist in a highly agricultural landscape, exhibiting stressors such as surface water extraction, riparian clearing and nutrient runoff. These negative effects are expected to increase in the future with predicted climate change. Modelled climate change scenarios including increased runoff variability and extreme events (CSIRO, 2016) are

expected to increase the risk of destruction of this rare ecosystem and its at-risk macrophyte communities.

Aims

The aims of this paper were firstly, to document aquatic macrophyte assemblages and their functional groupings in several ponds in Mulwaree chain-of-ponds and then to examine rates of decomposition of one at risk species. A particular objective was analysis of the seasonal rates of litter decay and the relative contribution of microbes versus herbivores. A further aim was to try to understand the risk of regime shift between phytoplankton and macrophyte dominance that appear to exist (Scheffer, 2003) in the ponds, related to abiotic drivers, such as temperature and flow. In this way, functional measures could be used to more fully understand processes within the ponds

It was hypothesized that decomposition would vary seasonally (sampled through four seasons) and spatially (between ponds) within the ponds. Further it is hypothesized that herbivory would dominate decomposition compared to microbial decay processes, emphasizing the importance of herbivores in Mulwaree chain-of-ponds vegetated margins.

Methods

Study Sites

The Mulwaree River is an intermittently flowing, relictual river that has been isolated historically from its source upstream (Abell, 1995). Intermittent flows on low profiled floodplains have led to the development of a chain-of-ponds system within the channel (Mould *et al.*, 2017; Williams, 2018). The ponds are large and characterized by unusually deep bathymetry. Individual ponds are up to 7.5 metres deep while disconnected, with width and length dimensions of 30 to 100 metres. The chain-of-ponds are unprotected by environmental legislation, despite their local and global rarity. They are not listed as wetlands of National Importance, or by the Ramsar Convention.

Four seasonally connected and adjacent ponds were chosen in the Mulwaree River at Kelburn (35° 52' 23.41''S, 149° 39' 02.31''E), near Goulburn, New South Wales, Australia (Figure 1). Streamflow metrics at Mulwaree River @ The Towers indicated three distinct flow periods since data collection commenced in November 1993. Mean daily flows for non-drought periods of 1993-2000 and 2010-2018 ranged from 29-44 ML/day, with a daily maximum of 16,737 ML/day on 9/12/2010.

The 90th Percentile flow was 56 ML and Lanes Variability Index of 0.745 ML. These flow periods were interspersed by the Millenium drought between 2001-2009 (van Dijk *et al.*, 2013), when mean daily flow fell to 6.5 ML/day and the 90th percentile 2.88 and Lanes Variability Index of 0.3 ML. Long term (1988-2018) mean annual rainfall at Goulburn airport (070330) is 544 mms and typically exhibits a bimodal maximum in June of 61mm and December of 58mm. Months of low rainfall occur in April with 26 mm and May with 33mm.

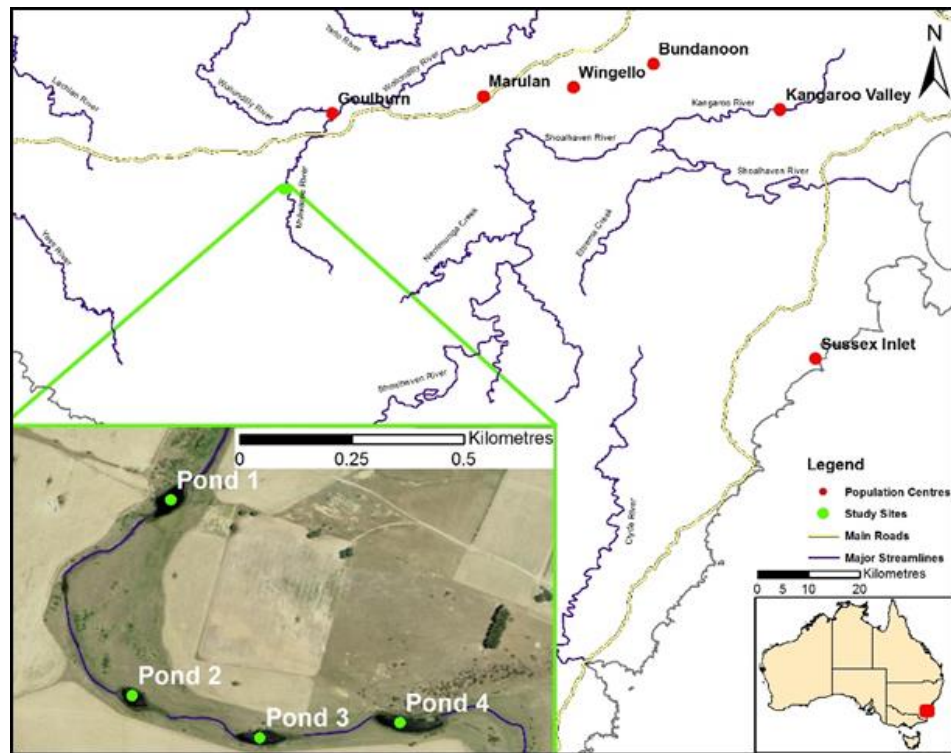


Figure 1. Location Map. Mulwaree chain-of-ponds, Kelburn NSW, ponds 1 to 4. Arrow in direction of flow (Source layer Credits © Land and Property Information 2015, map courtesy Will Farebrother 2018)

The ponds undergo temperature and dissolved oxygen stratification when disconnected and ambient temperatures exceed 18°C, acting as monomictic lakes (Chapter 5). During connected flows and occasional floodplain involvement, stratification is disrupted, with nutrients and material released downstream.

Catchment riparian conditions in the Mulwaree River are highly altered (EnvironmentACT, 2004). The once open woodlands of *Eucalyptus melliodora*-*E. blakelyi* on the low slopes and plains, with *Acacia mearnsii*, *Themeda australis* and *Danthonia pallida* understory have been replaced by pasture species and exotic weeds (Dodson, 1986). The Mulwaree River at the site generally reflects its agricultural catchment with locally poor water quality; high electrical conductivity, total nitrogen and total phosphorus (EnvironmentACT, 2004; GHD, 2013b). The ponds are within an agricultural landscape with cattle and sheep grazing. However, they support

a range of waterbirds including swans (*Cygnus atratus*), black ducks (*Anas superciliosa*) and dusky moorhens (*Gallinula tenebrosa*).

Choice of Study Plant

Cycnogeton procerum (R.Br.) Buchenau (von Mering, 2010) previously *Triglochin procerum* R.Br. is a fleshy leaved, rhizomatous and endemic semi-emergent perennial (Rea, 1992) which is morphologically variable and widespread across Australian and Malesia (Brummitt, 2001; Harden, 1993). It is a dominant macrophyte in Mulwaree Ponds, occupying a 1-3 metre margin of the ponds during maximum growth conditions (Figure 2). *C. procerum* exhibits a seasonal life cycle, but continually produces new growth, most heavily in winter (Rea, 1992) with seasonal decomposition. The leaves continually decompose rapidly and completely, so recently dead leaves cannot be used for decomposition studies. Its seasonal response to herbivores versus microbial decomposers has been poorly studied and there is a dearth of research into decomposition of native aquatic macrophytes generally.

The genus is Gondwanan in origin, but with current global distribution and ecological diversity (von Mering, 2015). The polyploid species ($2n=2x=16$ to $2n=8x=64$) (Aston, 1993, 1995) exhibits distinct morphological and geographic correlations to chromosome number, suggesting clumped distribution of polyploid groups (Robb & Ladiges, 1981). Based on morphological description and location, the Mulwaree population is likely to be $2n=64$ (Aston, 1995), although investigation of ploidy has not been performed. Variation in morphological traits related to polyploidy include flowering and fruit character differences and leaf width and thickness (Robb *et al.*, 1981). *C. procerum* is described functionally as a perennial emergent with water-dispersed seeds (Se) (Casanova, 2011). Flowering mostly between September and March (Aston, 1995), dispersal by water (hydrochory) is facilitated by buoyant seeds that are able to float for up to 5 weeks (James, 2013). While there is evidence that some gene transfer may be mediated by birds (zoochory) and to a lesser extent wind disposal of pollen (amenochory) (James, 2013), downstream seed dispersal would be the primarily viable means of gene and propagule transfer in the Mulwaree River. Recruitment in the catchment is confined to upstream ponds and farm dams, with Lake Bathurst and the Morass an unreliable source of aquatic plant seed (Abell,

1995). This has ramifications for long term viability of the species which is subject to an increasingly intermittent upstream seed source.

Vegetation Mapping

Macrophyte species distribution was mapped at each sampling event according to methods by (Gunn, 2010; Wetzel, 1991). Vegetation was surveyed by traversing the littoral zone of the four ponds on foot during each visit, noting plant species distribution and occurrence, followed by identification. This was followed by a series of random transects from the level of based flow perpendicular to extent of vegetation. Depth measurements were taken at species depth tolerance ranges by boat to measure horizontal and vertical species distribution at multiple random points in the ponds during event-based sampling (Figure 2).

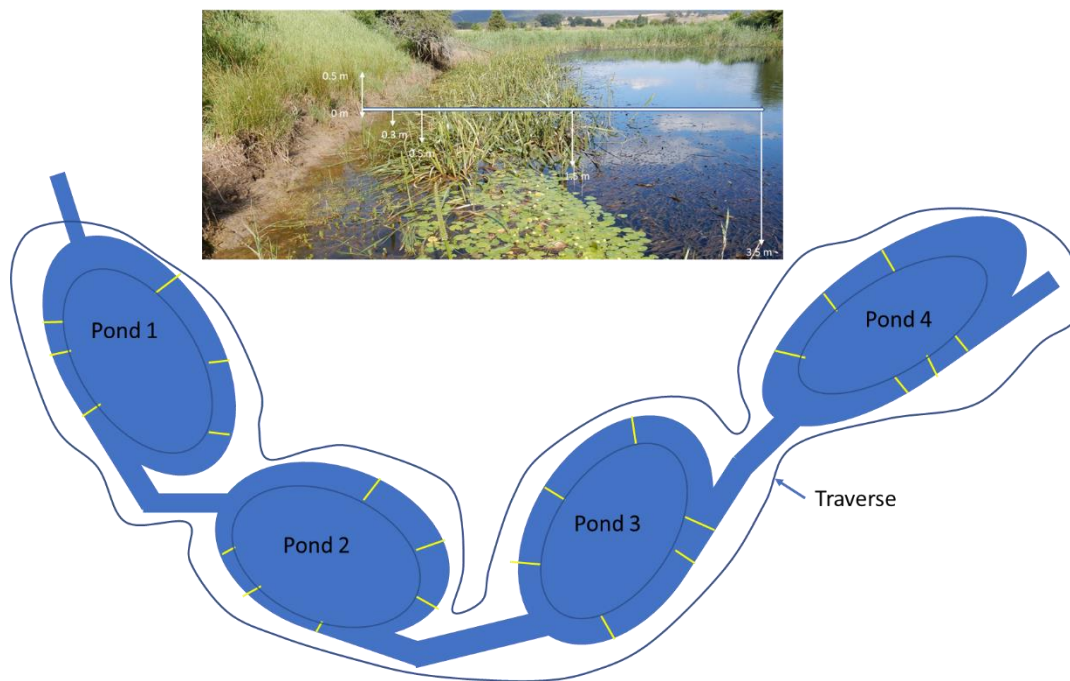


Figure 2. Schematic of vegetation mapping methods, including traverse around ponds and measurements taken during vegetation sampling.

Photographs were taken to verify plant distribution and identification. Identified plant taxa were allocated functional groups, based on growth form and dispersal traits (Brock, 1997; Casanova, 2011; Catford & Jansson, 2014).

Leaf Litter Decay

Cyanogeton procerum fresh leaves were collected from each of the ponds and washed in situ to remove periphyton, sediment and invertebrates. Leaf material was air dried for 7 days and finished in a drying oven at 30 °C for three days to constant mass. Five grams (+/- 0.1 g) of leaves were placed in 100 mm x 150 mm bags of different mesh size; 9 mm PVC mesh gutterguard (www.whitesgroup.com.au) and 150 µm (Nytex 150 µm SEFAR 03-150/38) (www.sefar.com.au) and sealed. These were used to measure total (9 mm mesh bags) and microbial mediated decomposition (150 µm mesh bags) by excluding invertebrates (Mora-Gomez *et al.*, 2015; Woodward *et al.*, 2012). Bags of each mesh size were placed in duplicate at 0.5 metres depth, attached to a buoyed chain in the centre of the pond. Further bags were deployed, attached on wooden stakes in three random locations in the vegetated margins of each of the ponds. Deployment was performed seasonally on 1 November 2016, 7 February 2017, 26 May 2017 and 25 August 2017.

Bags were harvested at 23-30 days depending on water and air temperature, in order to ensure remaining material on collection. During winter, bags were left for longer, while in summer they were removed after a shorter period of time. A separate mid-summer decay rate experiment was performed on 9 January 2017, with litter bags harvested at 3, 8, 15 and 32 days after deployment. To standardize decomposition rates to previous studies, a cotton strip decomposition assay was also performed during leaf litter decomposition, for the same period of time. Replicate bags were made containing sets of 5 strips of prewashed calico (35 mm x 15 mm) bound together to simulate leaves. Seventeen of such bags, with both mesh sizes as above, were deployed randomly with the leaf litter bags across all ponds and site types.

All litter bags were harvested, placed on ice and kept at 4°C overnight. Samples were rinsed into a 106 µm mesh sieve before being rinsed with absolute ethanol (AR grade) to prevent further decomposition, then filtered using a Whatmans Grade 1 cellulose filter paper (product 1001-150 www.austscientific.com.au) over a 100 mm glass funnel and flask to remove residual ethanol. Samples were then dried to constant weight at 37°C for three days. Mass loss was calculated before oven drying at 60°C and with carbon burnt off at 550°C (4 hours) to determine ash-free

dry mass remaining (AFDM) by Loss on Ignition (LOI) (A. Sluiter, 2008). Ash remaining was subtracted from initial dry weight to calculate organic carbon remaining following deployment. Cotton strips were washed, shaken in 100% ethanol to halt decomposition and dried to constant weight before storing at -4°C. Strips were reduced to 25 mm (60 threads) X 100 mm before measurement. Decomposition of cotton strips was measured by maximum load at break (N) using an Instron 5540 series benchtop electromechanical testing system with 25 mm grips and load frame and Bluehill Extended System and Software (M18-14443-EN, Revision A 2004). Carbon:Nitrogen:Hydrogen ratios from a sample of plant material collected in summer were determined by incineration (CHN900, LECO USA) of replicated micro-samples of air dried plant material in tin capsules (3.3 mm X 4 mm – LECO Part no. 502-227) weighed to 0.1 µg (Mettler UMX2 microscale).

Hydrology

A piezometer (uPVC – 51 mm with screen slots at 4 mm slot and width at 0.5 mm – Thermofisher.com.au/MGS05030ST18) was installed at 10 cm in from the edge of pond 1 at 0.2 m below cease to flow height. A Solinst Levellogger depth logger, and Barologger Edge (<https://www.hydroterra.com.au>) were installed in the piezometer to log hourly from 16/04/15 to 28/09/17 (Figure 3). Piezometer MK15_2_4 provided flow data to 0.2 metres below the commence to fill (CTF) of 640.95 m A.S.L in pond one, the most downstream site. The logger was set at 0.2 metres below CTF so was unable to measure pond height below that level. Streamflow data was acquired for the nearest downstream site, Mulwaree River @ The Towers (2122725 – 149° 41'53.2"E, 34° 46'54.7"S) (WaterNSW, 2018). Streamflow metrics were calculated using River Analysis Package (EWater.com.au).

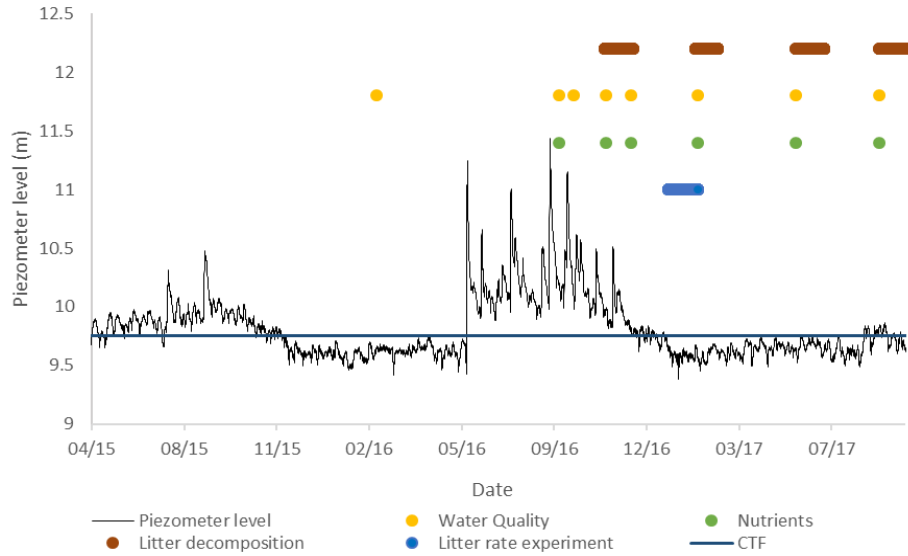


Figure 3. Piezometer level at pond 1, 2015-2017 with line denoting zero commence to flow (CTF). Dots denote sampling events and bars litter decomposition dates.

Water Quality

Temperature, pH, electrical conductivity and dissolved oxygen were measured in each pond during each sampling event between March 2015 and September 2017 (Figure 3), using a pre-calibrated Hydrolab minisonde (Aqualab.com.au). Nutrient samples were collected between September 2016 and September 2017 (Figure 3) at the centre of each pond at 25 cm depth into triple rinsed 250 mL PET sample jars. Water samples were filtered, chilled and frozen as appropriate before analysis (APHA., 2005). Methods for analysis are summarized in Table 1. Historical nutrient data was also acquired for the monitoring station Mulwaree River @ The Towers (2122725 – 149° 41' 53.2"E, 34° 46' 54.7"S) (WaterNSW, 2018) reported as part of the Audit of Sydney Drinking Water Catchment to identify deviation from the molar ratios of 16TN:1TP. This ratio, part of the Redfield ratio (Boulton, 2014) provides information on nutrient limitation that can potentially limit primary production in stream and river systems (Jarvie *et al.*, 2018).

Table 1. Water quality parameter laboratory analysis methods. Results below detection limits were substituted to limit of detection (LOD)/ $\sqrt{2}$ (Croghan, 2003; Ogden, 2010).

<i>Parameter</i>	<i>Determination</i>	<i>Method</i>	<i>Units of measurement</i>
Ammonia	Colorimetrically	APHA 4500-NH ₃ F	mg/L
Total Nitrogen	Sum of total Kjeldahl Nitrogen plus NO _x	APHA 4500B + APHA 4500-NO ₂ -B, 4500-NO ₃	mg/L
Oxidized N (NO ₂ , NO ₃)	Colorimetrically	APHA 4500-NO ₂ - B, 4500-NO ₃	mg/L
Alkalinity	Titrimetrically	APHA 2320-B	mg/L
Dissolved Organic Carbon (DOC)	Filtered before analysis	APHA 5310B,C	mg/L
Total Phosphorus	Colorimetrically	APHA 4500BF	mg/L
Phosphate (FRP)	Colorimetrically	EPA 365.s and APHA 4500 PE,F	mg/L

Data Analysis

All leaf decomposition data were initially analysed using general linear models (as described below) and one-way ANOVA (to identify cause of differences where significant GLM results) in Minitab ® 18.1 (Minitab Incorporated, 2017). Total leaf litter decomposition (9 mm mesh bags), and microbial mediated decomposition (150 µm mesh bags) (Mora-Gomez *et al.*, 2015; Woodward *et al.*, 2012) data were derived and transformed appropriately as follows. Samples were retrieved at 29 (29 November 2016), 23 (2 March 2017), and 30 days (5 July and 28 September 2017) respectively so data were standardized by number of days deployed, after transformation. A mid-summer decay rate experiment was performed to check if decomposition followed an exponential rate. Leaf decay data were consequently fitted to an exponential decay model, $M_t = M_i e^{-kt}$, where M_t is mass (µg) at time t , M_i is initial mass (µg), k is the first order rate constant (day^{-1}) and t is time in days. Proportional mass loss and ash free dry mass (AFDM) by loss on ignition (LOI) data ($(p_{\text{initial}} - p_{\text{final}})/p_{\text{initial}}$) of rate data were logit transformed ($\log(p/(1-p))$) prior to analysis. Both daily mass loss and LOI by AFDM were analyzed using a general linear model with loss of sample mass as the response, with the categorical variables of pond and event as fixed factors and mesh size as a covariate. Significant results were further tested with one-way ANOVA, which was also used to test differences between mean mid-pond ($n=2$) and mean ($n=6$)

vegetated edge data. Significant results were examined using Tukey's HSD post hoc test (Quinn, 2002) to identify which factors contributed to significance at $P < 0.05$.

Thirty-day logit transformed mass loss and % LOI for 9 mm and 150 μm data were analyzed against single value environmental data using Spearman Rho correlation analysis. Comparison with Pearson's correlation indicated that the former was the more robust method, also reported elsewhere (Quinn, 2002). To avoid collinearity, total phosphorus and nitrogen and alkalinity fractions were removed prior to analysis according to Quinn and Keough (2002). Productivity measures (outlined in Chapter 5) of Gross Primary Productivity, Net Ecosystem Productivity, planktonic respiration and Chlorophyll A were similarly analysed. Streamflow metrics were calculated using River Analysis Package (EWater.com.au) (results in Chapter 5) and N:P values in mg/L converted to moles and ratios calculated.

Results

Distribution of Aquatic Macrophytes

Aquatic macrophytes formed a seasonally dense margin of aquatic vegetation around the ponds, with *C. procerum* covering the largest surface area. Aquatic plant cover extended from above water's edge to around 3 metres depth and up to five metres horizontally at the shallower ends of the ponds (Figure 4). Gradation from terrestrial to submerged species was evident with *Carex* spp., *Cyperus* spp., *Eleocharis acuta* R.Br.APNI* and *Phragmites australis* TRIN APNI* (to 0 metres) grading to *Myriophyllum verrucosum* Lindl. APNI*, *Nymphoides geminate* R.Br. Buchenau, *Nitella* sp. (0 to 0.6 m) *C. procerum*, (0.6 to 1.5 m) to *Vallisneria australis* (S.W.L. Jacobs and Les (to 3 m depth). *Vallisneria* leaves extended on the surface to around 4 metres depth into the ponds. Other species were scattered along the littoral zone contributing to high biodiversity.

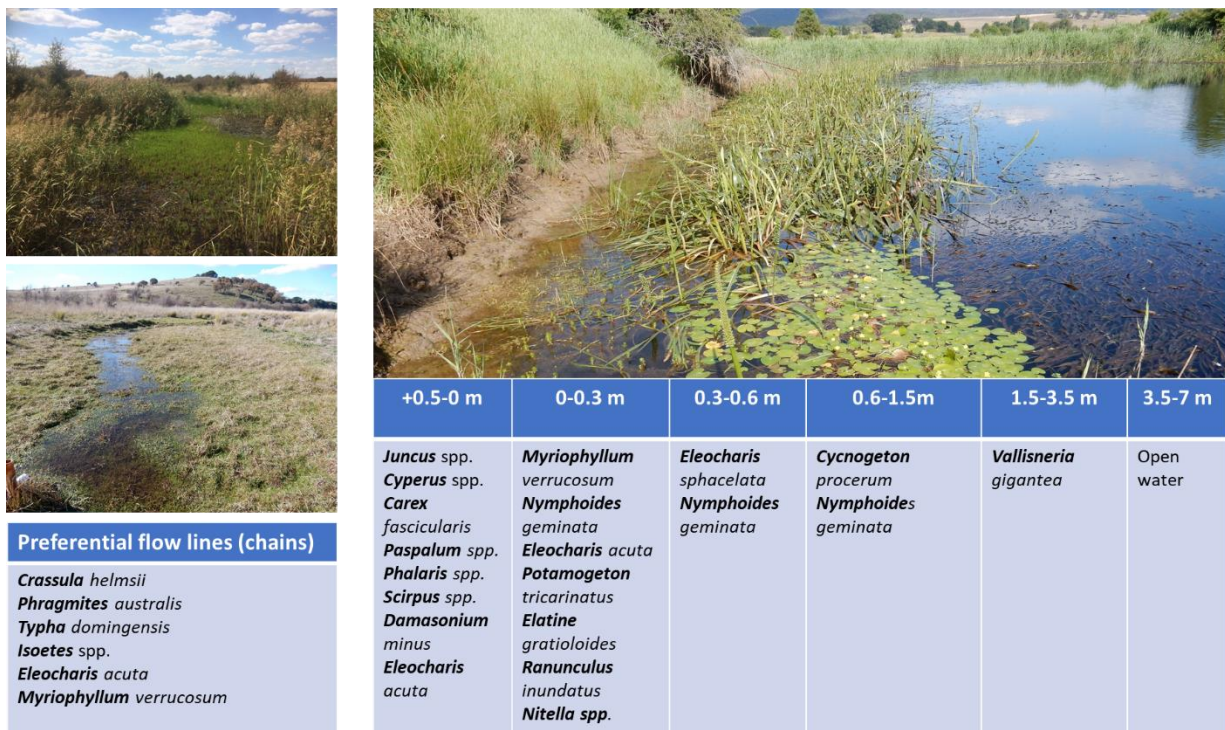


Figure 4. Aquatic macrophyte zonation related to water depth in Mulwaree Ponds and differential flow lines during connected flow, late spring, 2016.

The preferential flow lines between the ponds were populated variously by *Phragmites australis*, *Crassula helmsii*, *Typha domingensis*, *Isoetes* spp., *Eleocharis acuta* and *Myriophyllum verrucosum*. While plant cover was greater during summer, most species were perennial and mainly rhizomatous or stoloniferous. Other than localised vegetative reproduction, most species disperse primarily by water (hydrochory) (Table 2). Macrophyte beds were inhabited by the exotic snail *Physa acuta*, amphipods and leptophlebiid mayflies (pers.obs.) and by abundant waterfowl. The ponds were heavily populated by predators including the mosquito fish (*Gambusia holbrooki*), damselflies and dragonflies (Odonata).

Litter Decay of *Cycnogeton procerum*

Initial Carbon:Nitrogen ratios of leaf material were found to be 20.24 +/- 5.99 S.E. with nitrogen content of 4.1%. Decomposition rates for *C. procerum* during mid-summer followed an exponential decay curve, $y=2.74e^{-0.09x}$, $r^2=0.84$, with the exponential decay constant (kd^{-1}) = -0.092 (Figure 5). Decay rates in all ponds were similar and variability between samples low.

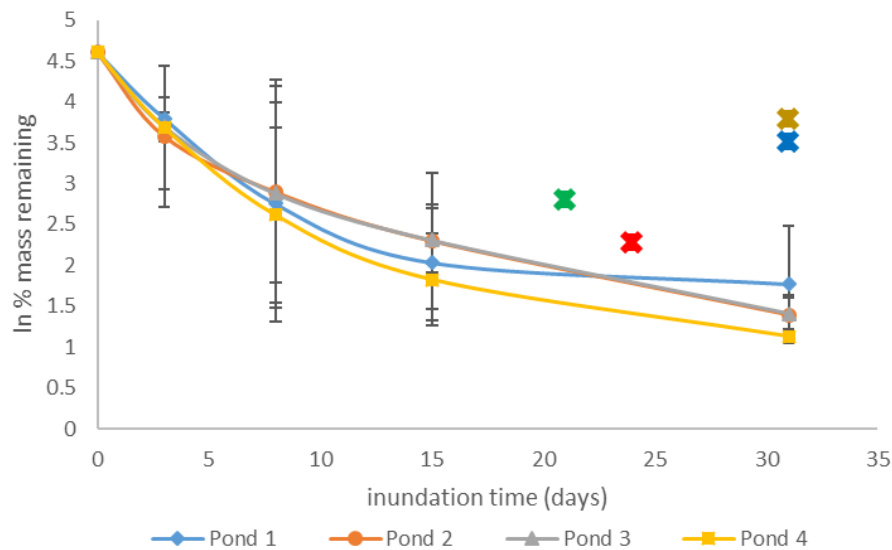


Figure 5. Mid-summer decomposition rates, *C. procerum* +/- S.E. (values for S.E., 0.04-0.09), January 09-February 09 2017. Mean exponential loss relationship, $y=2.742e^{-0.092x}$, $R^2=0.8425$. Coloured crosses highlight % mass loss end points for ✕ November 2016 (24 days), ✕ February 2017 (21 days), ✕ June 2017 (31 days), ✕ September 2017 (31 days).

Table 2. Functional groupings for common aquatic macrophytes encountered at Mulwaree Ponds. Tdr, terrestrial dry; Tda, terrestrial damp; ARf, amphibious fluctuation responder-floating; ARp, amphibious fluctuation responder-plastic; ATe amphibious fluctuation tolerator-emergent; Sk - submerged- k-selected, Sr – submerged-r-selected, Se Perennial – emergent (Casanova, 2011; Catford, 2017)

Taxa	Functional Group	Rhizomes/Stolons /Annuals	Dispersal	Reference
<i>Alisma plantage-aquatica</i> L. APNI*	Tda	R	hydrochory, zoochory	(Barrat-Segretain, 1996)
<i>Juncus spp.</i>	ATe	R	hydrochory, zoochory	http://www.cpbr.gov.au/cpbr/WfHC (Barrat-Segretain, 1996)
<i>Carex fascicularis</i> Sol. ex Boott APNI*	ATe	R	amenchory	http://plantnet.rbg Syd.nsw.gov.au
<i>Crassula helmsii</i> (Kirk) Cockayen	ARp	S	hydrochory, zoochory	(Denys <i>et al.</i> , 2014)
<i>Cyanogeton procerum</i> (R.Br.) Buchenau	Se	R	hydrochory	(Rea, 1992)
<i>Cyperus spp.</i>	ATe	R	amenchory	(Sainty, 2003)
<i>Damasonium minus</i> (R. Br.)	Tda	A	hydrochory	http://plantnet.rbg Syd.nsw.gov.au
<i>Elatine gratioloides</i> A.Cunn.	ARp	A	hydrochory	http://plantnet.rbg Syd.nsw.gov.au (Casanova, 2011)
<i>Eleocharis acuta</i> R. Br.	ATe	R	hydrochory	(Bell, 2004)
<i>Eleocharis sphacelata</i> R. Br.	ATe	R	hydrochory	(Sainty, 2003)
<i>Isoetes spp.</i>	Sk	R	amenchory	(Marsden, 1979)
<i>Myriophyllum propinquum</i> A. Cunn.	ARp	R	hydrochory, zoochory	(Catford <i>et al.</i> , 2017)
<i>Myriophyllum verrucosum</i> Lindl.	ARp	R	hydrochory, zoochory	(Casanova, 2011)
<i>Nitella sp.</i>	Sr	A	hydrochory, zoochory	(Barrat-Segretain, 1996; Casanova, 2009)
<i>Nymphoides geminata</i> (R.Br.) Kuntze APNI	Arf	R	hydrochory, zoochory	(Smits <i>et al.</i> , 1989)
<i>Paspalum spp.</i>	Tdr	R	amenchory	http://plantnet.rbg Syd.nsw.gov.au
<i>Phalaris spp.</i>	Tdr	R	amenchory, hydrochory	http://plantnet.rbg Syd.nsw.gov.au (Barrat-Segretain, 1996)
<i>Phragmites australis</i> (Cav.) Trin ex, Steud	Ate/Se	R	amenchory	(Barrat-Segretain, 1996; Casanova, 2011)
<i>Potamogeton tricarlinatus</i> A.Benn.	ARp	R	hydrochory, zoochory	(Barrat-Segretain, 1996; Smits <i>et al.</i> , 1989)
<i>Ranunculus inundatus</i> (R.Br.) Kuntze APNI	ATe	S	hydrochory, zoochory	www.herbiguide.com.au , (Goodrick, 1979)
<i>Scirpus spp.</i>	Tda	R	amenchory, hydrochory	(Barrat-Segretain, 1996)
<i>Typha domingensis</i> Pers. APNI	ATe	R	amenchory	(Barrat-Segretain, 1996; Sainty, 2003)
<i>Vallisneria australis</i> S.W.L.Jacobs & Les APNI*	Sk	R	hydrochory	(Les <i>et al.</i> , 2008)

Linearized mass loss analysis for the January rate experiment indicated that, independent of days inundated, which were all significantly different ($F_{(3,58)}=48.98$, $P<0.001$), there were no significant differences ($P>0.05$) between ponds, mesh size or between mid-pond and edges (Figure 6).

There was a significant difference in mass loss between events overall ($F_{(3,60)}=434.49$, $P<0.001$, $R^2_{(adj)} = 0.954$), with mass loss rates highest in spring and winter and lower in summer and autumn. Linearized mass loss analysis for seasonal decay varied between the four ponds. November and February mass loss were not significantly different from each other, but were significantly different from June and September ($F_{(3,60)}=145.82$, $P<0.001$) (Figures 6,7).

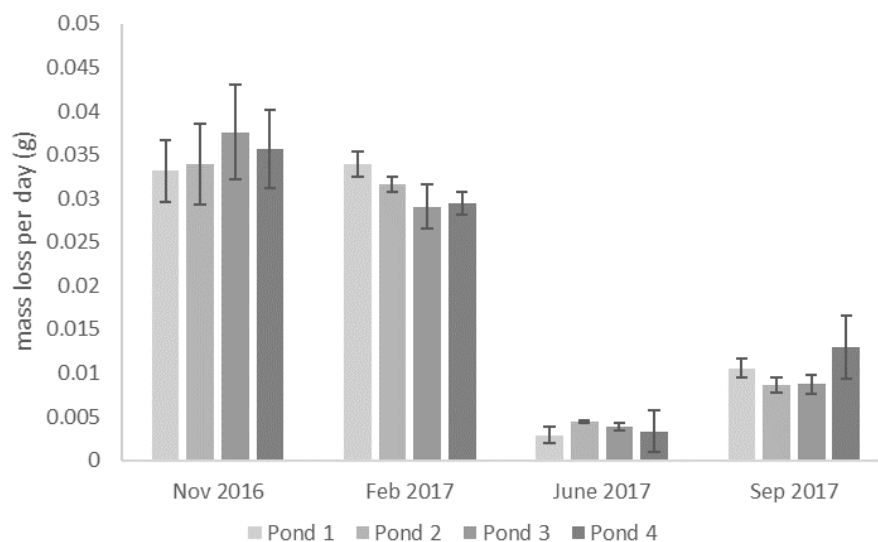


Figure 6. Mean daily mass loss (g) for all samples over four sampling events for all four ponds. \pm S.E., $n=4$.

Differences were significant for daily mass loss for mesh*event ($F_{(3,32)}=20.93$, $P<0.001$), with total decomposition greater than microbial decomposition for November 2016 ($F_{(1,14)}=79.06$, $P<0.001$), but not for February 2017, June 2017 and September 2017 (Figure 7A). There was no significant difference ($P>0.05$) between mid-pond and vegetated margins for any ponds at any time.

Organic matter remaining (ash free dry mass) by LOI was significantly different for mesh*event, ($F_{(3,32)}=5.19$, $P<0.01$) for mesh ($F_{(1,32)}=153.25$, $P<0.01$) and for event

($F_{(3,32)}=37.75$, $P<0.01$). There was more organic matter remaining in 150 micron bags compared to 9 mm bags, indicating that daily total organic matter use was greater than microbial use (Figure 7B). For events, summer and autumn samples decomposed more slowly than during winter and spring. There was no significant difference between ponds or between mid-pond and vegetated margins ($P>0.05$).

Cotton strip decomposition exhibited a slower daily decay response compared to leaves (Figure 8). Decay rates were different between sampling events ($F_{(3,47)}=261.35$, $P<0.001$), slower in summer and comparatively slower than plant material.

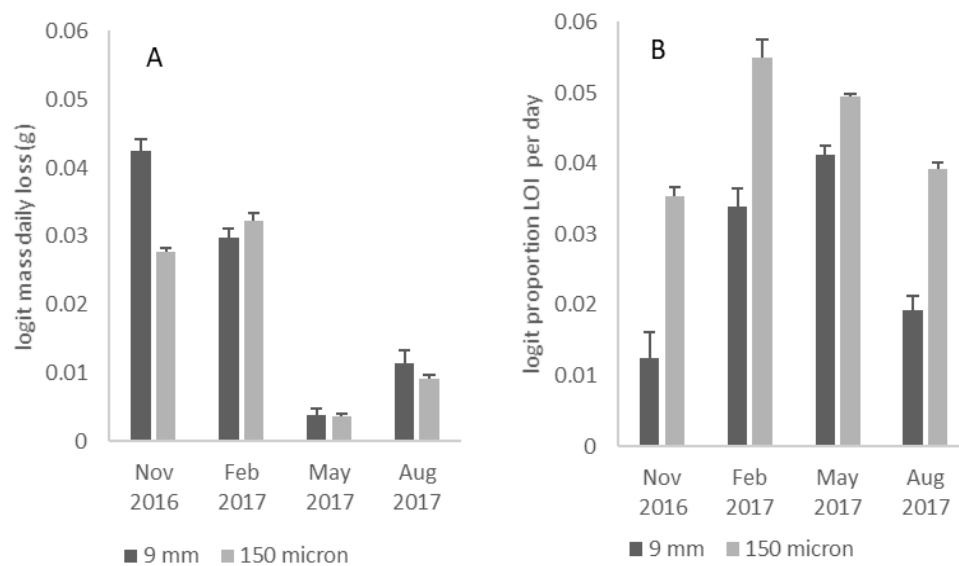


Figure 7. Proportional mean daily mass loss (A) and carbon content(g) remaining (LOI) (B) of *C. procerum* for 9 mm and 150 μ m mesh in four ponds in the Mulwaree River over time, \pm S.E., $n=8$.

There was no significant difference ($P>0.05$) between maximum load at break for controls and three of the four sampling times. There were no significant relationships ($P>0.05$) between measures of productivity (Chapter 5) and mass loss, organic matter use or cotton strip decay.

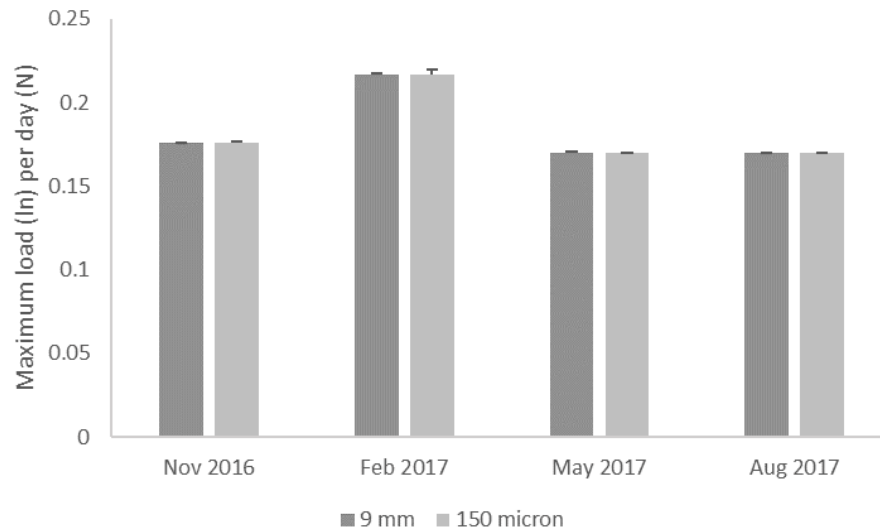


Figure 8. Mean (\pm S.E.) maximum load of break, (Newtons), for calico cotton strips (mean of 5 per bundle), from 9mm mesh and 150 μ m mesh bags, linearized (ln) per day, \pm S.E. N =various (7-10 bundles).

Hydrology, Water Quality and Nutrients

The Mulwaree River flowed for around 50% of the study period between April 2015 and September 2018. During the study period, Mulwaree River experienced record flows. Water quality analysis revealed that the ponds were mesotrophic (Supplementary material, Chapter 5, Appendix 1) during the period of this study. Mean \pm S.E. dissolved organic carbon (DOC) was 12.81 \pm 0.16 mg/L, remaining stable over time and across ponds. It was assumed that organic matter was synonymous to DOC, as is usually the case (Boulton, 2014; Leenheer, 2003).

Total nitrogen was 1.10 \pm 0.09 mg/L, increasing from 0.55 mg/L to 1.95 mg/L over the course of the study. Total phosphorus was consistently below the detection limits of 0.05 mg/L, so for most analysis purposes was converted to LOD/ $\sqrt{2}$. Molar TN:TP ratios using this data were therefore fraught, so ratios in this study were calculated at the maximum TP value of 0.05 mg/L. Use of additional historical data (GHD, 2013b) suggested that the TN:TP ratio for Mulwaree River and the ponds exceeded the ratio of 16:1 and were always more than 27 to >85:1 (Appendix 1, Table 2). ANZECC (2000) trigger values for TN (250-500 μ g/L) and for TP (20-50 μ g/L) for upland and lowland streams for slightly degraded ecosystems were exceeded substantially for all data measured above detection limits. pH was almost

neutral, meaning that alkalinity was present almost entirely as bicarbonate ions (Boulton, 2014) (average 116.3 +/- 6.1 mg/L), increasing during the period of the study.

The major differences in water quality were based on seasonal and unconnected/connected status. Electrical conductivity (EC) was highly related to flow events with high flows in Spring 2016 creating a dilution effect followed by increasing concentrations as the ponds dried out. Conductivity increased from 357 $\mu\text{S}/\text{cm}$ to 1295 $\mu\text{S}/\text{cm}$ over the period of the study as flow declined and the ponds dried out (Figure 9). Low passing flows in winter were a source of EC which were unable to dilute nutrients from the ponds.

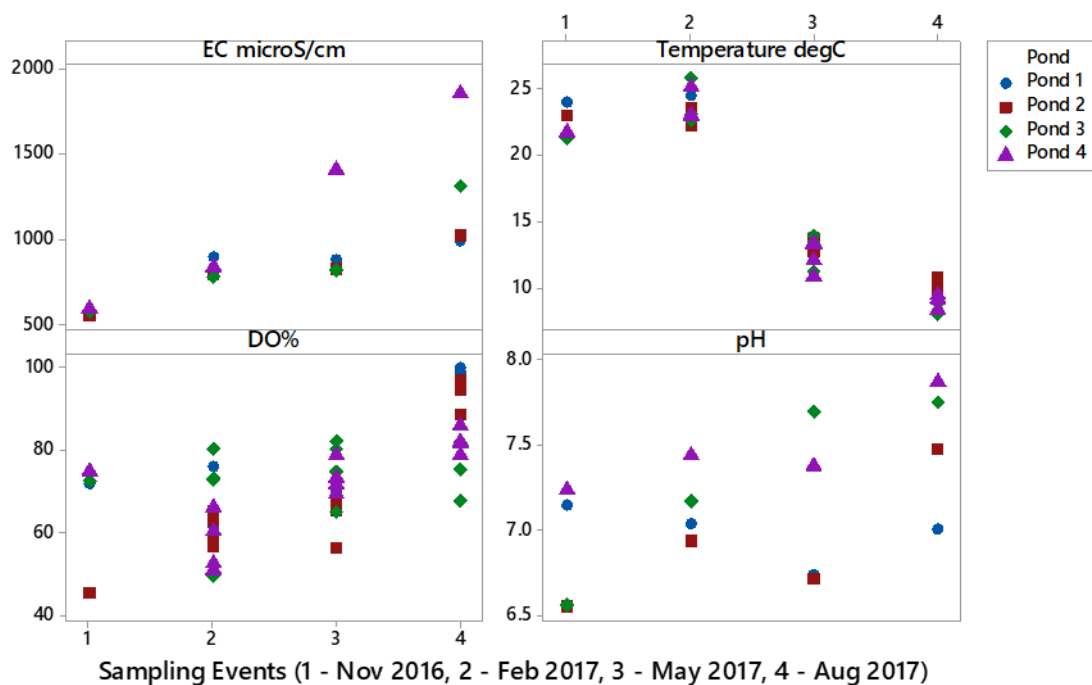


Figure 9. Water quality for four ponds in Mulwaree River, four sampling events, November - spring 2016, February - summer, 2017, May - autumn 2017, August - winter, 2017.

Principal Components Analysis (Clarke, 2015) of environmental data (first two axes explained 61% of the dissimilarity) suggested seasonal differences (Figure 10), where samples collected in November 2016 had lower nutrient concentrations than lower flow samples from summer through to winter. Disconnection of the ponds in summer led to elevated temperature and increasing DOC. Seasonal decomposition in autumn may have led

to higher ammonia and low flows were unable to dilute the high levels of nutrients accumulated in the ponds (Figure 10).

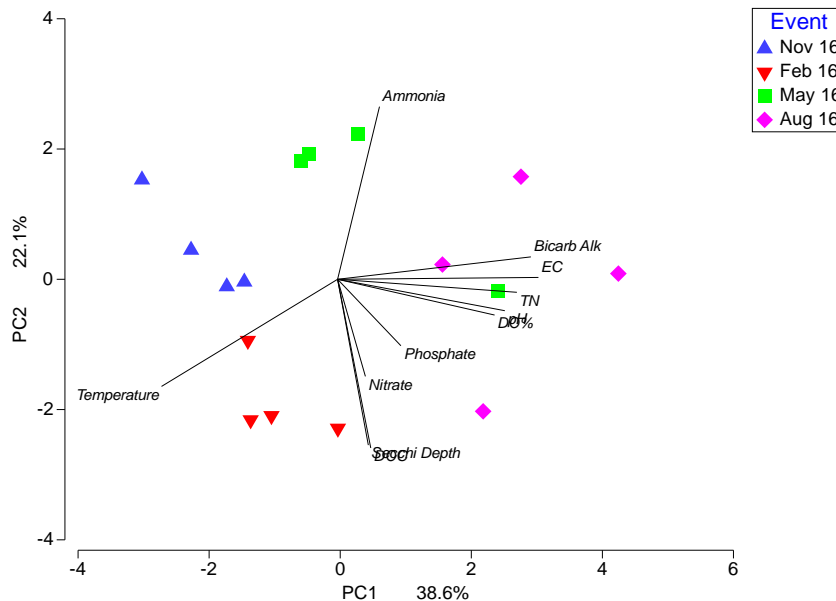


Figure 10. Water quality and nutrients, Principal Components Analysis (PCA), normalized data, (Primer Version 7.) for Mulwaree River @ Kelburn between November 2016 and September 2017. Four sampling events, mid pond for water quality and nutrients. Vectors clockwise from top – ammonia, bicarbonate alkalinity, electrical conductivity, total nitrogen, pH, DO% saturation, phosphate, nitrate, secchi depth, dissolved organic carbon, temperature.

Spearman's Rho Correlation analysis of abiotic drivers (Table 3) related to 30-day mass loss for total decomposition indicated that temperature was significantly and positively correlated (0.647, $P < 0.001$), with electrical conductivity (-0.649, $P < 0.001$) and bicarbonate alkalinity (-0.625, $P < 0.001$) negatively correlated.

Table 3. Relationship between *C. procerum* decomposition and organic matter content and abiotic drivers in four ponds of the Mulwaree River using Spearmans Rho Correlation. Data used were four sampling events, 30 day linearized mass loss and organic matter remaining (LOI) decomposition, for total (9 mm) and microbial (150 micron) mesh bags versus environmental data Values <0.001 ***, <0.005 **, <0.05*.

<i>Spearman's Rho Correlation</i>	<i>9 mm (total decomposition)</i>		<i>150 micron (microbial decomposition)</i>	
<i>Logit Mass Loss Proportion 30 day</i>				
<i>Variable</i>	<i>Statistic</i>	<i>P Value</i>	<i>Statistic</i>	<i>P Value</i>
<i>Temperature °C</i>	0.647	<0.001***	0.693	<0.001***
<i>pH</i>	-0.325	0.069	-0.191	0.294
<i>Dissolved Oxygen %</i>	-0.197	0.28	-0.268	0.138
<i>Electrical Conductivity µS/cm</i>	-0.649	<0.001***	-0.443	0.011*
<i>Total Nitrogen mg/L</i>	-0.537	0.002**	-0.287	0.111
<i>Alkalinity as Bicarbonate mg/L</i>	-0.625	<0.001***	-0.427	0.015*
<i>Ammonia mg/L</i>	-0.397	0.025*	-0.623	<0.001***
<i>Nitrate mg/L</i>	-0.36	0.043*	0.005	0.98
<i>Phosphate mg/L</i>	-0.495	0.004**	-0.154	0.4
<i>Dissolved Organic Carbon mg/L</i>	0.321	0.073*	0.433	0.013*
<i>Logit Organic Matter remaining (LOI) 30 day</i>				
<i>Temperature °C</i>	0.102	0.579	0.084	0.647
<i>pH</i>	0.016	0.931	0.093	0.611
<i>Dissolved Oxygen %</i>	-0.392	0.027*	-0.317	0.077
<i>Electrical Conductivity µS/cm</i>	0.134	0.466	0.256	0.158
<i>Total Nitrogen mg/L</i>	-0.012	0.95	0.109	0.553
<i>Alkalinity as Bicarbonate mg/L</i>	0.361	0.043*	0.434	0.013*
<i>Ammonia mg/L</i>	-0.067	0.717	-0.081	0.659
<i>Nitrate mg/L</i>	0.434	0.013*	0.428	0.015*
<i>Phosphate mg/L</i>	0.601	<0.001***	0.727	<0.001***
<i>Dissolved Organic Carbon mg/L</i>	-0.151	0.441	-0.148	0.419

All other significant correlations were less than 0.5. For microbial decomposition, temperature (0.693, P<0.001) was positively and significantly correlated, with Ammonia (-0.623, P<0.001) negatively correlated. On the other hand, water quality poorly explained organic matter decay rates in the study (Table 3). For organic matter, only phosphate was significantly and positively correlated with total (0.601, <0.001) and microbial (0.727, P<0.001) decomposition.

Discussion

Mulwaree Ponds, once connected, were a source of substantial aquatic macrophyte diversity in a mesotrophic and highly agricultural landscape. The most widespread macrophyte, *C. procerum*, formed an important component of productivity within the ponds. Continual microbial decomposition and grazer use of *C. procerum* leaves, in addition to its use as habitat for periphyton, made it an important species within the ponds.

Vegetation of Mulwaree Ponds

Aquatic vegetation in Mulwaree Ponds is a diverse ecological community. In comparison, local farm dams support an average of only two species (Casanova, 1997). The pond's vegetation communities are at risk in a south eastern Australian landscape that is increasingly devoid of wetland vegetation (Kingsford, 2000; Wassens *et al.*, 2017). Many of the common species such as *C. procerum*, *Vallisneria australis* and *Eleocharis* spp. are largely permanent populations in Mulwaree chain-of-ponds with predominant waterborne, and less commonly, waterbird dispersal (Table 2). As such, they rely heavily on regular water supply and lotic conditions. Intermittent to permanent hydrological conditions form the basis for their persistence. Distribution in the ponds in this study reflects their functional groupings based on plant traits (Brock, 1997; Casanova, 2011; Catford, 2017), suggesting a long history of suitable environmental conditions for growth, survival and dispersal.

Characteristics of Litter Decay

There was a significant difference in mass loss between events overall ($F_{(3,60)}=434.49$, $P<0.001$, $R^2_{(adj)} = 0.954$), with mass loss rates highest in spring and winter and lower in summer and autumn. Total mass loss (9 mm mesh bags) was only greater than microbial (150 μm mesh bags) decomposition in spring, suggesting that seasonal changes in palatability and microbial activity make decomposition a predominantly microbial process. Herbivory and influence of flow on decay rates was only evident during spring.

Cychnogeton procerum, characteristic of most plants, displays seasonal growth. In early spring, shoot development is typically at a maximum and species specific morphological features of *C. procerum*, including substantial lacunae, thin cuticles and rapid shoot growth

(Rea, 1992) make it an edible food source for herbivores, in accord with general knowledge that macrophyte palatability is higher in spring (Elger *et al.*, 2006). During the growth phase, aquatic plants integrate substantial nutrients, particularly nitrogen from the water column, they also use detrital carbon and nutrients deposited in sediments in complex processes related to oxygen availability (Bornette & Puijalon, 2011). This early macrophyte growth is readily available for herbivores such as invertebrates, which commonly graze on fresh and young leaves (Watson *et al.*, 2011). But generally, herbivory is not restricted to invertebrates, with fish and waterbirds both significant in reducing macrophyte abundance (Wood *et al.*, 2017). In this study, only small fish would have been able to access the 9 mm bags, so that total macrophyte grazing would have been expected to be greater than what was measured. Generally, herbivores of all sizes are influential in creating detritus by ingesting senescent plant material previously decomposed by heterotrophs, or on the periphyton that commonly colonizes *C. procerum* (Suren *et al.*, 1989).

Maturation of plant material may decrease palatability, increasing tissue toughness and anti-herbivore chemicals (Majak, 2001), combined with lower protein and nutrient concentrations in plant tissues (Elger *et al.*, 2006; Newman, 1991). This is partially supported by C:N ratios for *C. procerum* analysed in summer (20.24 and 4.1%N) that were higher than reported in other studies (11.7 and 4.3%N) (Watson *et al.*, 2011), suggesting that *C. procerum* may have been less palatable at that time. If that were the case, once *C. procerum* became less palatable, shredding and grazing invertebrates may then have used macrophyte periphyton as a food source rather than macrophyte material itself. Periphyton also increase shading, which may increase competition for light for macrophytes.

Dominance of microbial decomposition of *C. procerum* in the ponds is a common occurrence for macrophytes generally, with fungi comprising more than 90% of microbial biomass experienced elsewhere (Komínková, 2000; Kuehn *et al.*, 2000). The heterotrophic process of recycling plant material may follow various trajectories, including herbivory, but central is microbially mediated decomposition. The process usually involves initial leaching, followed by microbial colonization and conditioning (Bergfur *et al.*, 2007; Imberger *et al.*, 2008; Kerr *et al.*, 2013) and then invertebrate shredding and decomposition (Graca, 2001; Suren *et al.*, 1989). Fungal hyphomycetes are now known to be the dominant decomposers in both terrestrial and aquatic systems. Rates of decomposition are variable and dependent on leaf

toughness, nutrient content and the presence of secondary metabolites (Graca, 2001), seasonality, stream flow variability (Dieter *et al.*, 2011), natural and human induced eutrophication (Ferreira *et al.*, 2015) and acidity (Dangles, 2004; Holland *et al.*, 2012).

The lack of differences in mass and organic matter loss rates between mid-pond and vegetated edges and between ponds for any sampling event or mesh size, suggests that nutrient availability was homogeneous and the ponds well mixed. While the hypothesis assumed that the presence of macrophytes and associated periphyton would alter localised conditions, the results suggested otherwise. Generally, conditions within macrophyte beds are functionally different to open water habitats (Bodker *et al.*, 2015; Horppila & Nurminen, 2003; Wang Li., 2013), with differences in clarity, shade and nutrient availability related to flow, species attributes and richness as examples (Engelhardt & Ritchie, 2001; Takamura *et al.*, 2003). However, the hypothesis was not proven, with decomposition rates similar in both open water and vegetated sites. It can be assumed that complete lateral mixing across the ponds occurs, at least for leaf decomposition requirements. Lake cycling dynamics may support this assumption under some conditions. It was predicted that Mulwaree chain-of-ponds would act as mesotrophic monomictic lakes, due to their morphology; with high depth to width ratios (Chapter 5).

The mixing depth in lakes is controlled by inflow, outflow and wind, but in small lakes only wind and internal small waves are important in maintaining the mixing depth (Fee, 1996). In Mulwaree ponds, well developed thermoclines during summer (Chapter 5) gave a mixing depth of around 1.5 metres. Lake studies (Davies-Colley, 1988; Losordo & Piedrahita, 1991) indicate that effective diffusion coefficient (E_z) in the mixing zone is high at the water surface and decreases to zero at the thermocline. Incoming solar radiation acts to create vertical differences in temperature above the thermocline, but fetch, the area of the ponds affected by wave generating wind, works to achieve maximum diffusion. In areas where winds are strong, the effective mixing zone is to the thermocline. Long term average winds at Goulburn are comparatively high at 15 km/h compared to nearby locations including Canberra (13 km/h) and Bathurst (12 km/h) (www.timeanddate.com, 2018). This suggests that fetch, which is a crucial element in effective diffusion, may be substantive and influential in maximising homogeneous conditions above the thermocline in summer and to depth of the ponds at other times.

Generally, circulation within the epilimnion of lakes is facilitated easily by wind, due to fetch. So, while the premise that circulation within macrophyte beds would be minimized due to abundant macrophyte cover and associated micro habitat development, the effect of fetch was able to over-ride differences within the epilimnion that would encourage differences in leaf decomposition. Several factors may be involved in complicating resource availability between the edge and margins of the ponds. These include macrophyte use of nutrients from the water column in preference to sediment sources (Madsen & Cedergreen, 2002), which means that there may be dynamic nutrient fluxes within ponds. Also, herbivores able to access 9 mm mesh bags may have been able to reach the middle of the pond and vegetated margins and microbial processes may be similarly active across both.

Rates of Decay

Decomposition followed standard exponential decay metrics $M_t = M_i e^{-kt}$, similar to that exhibited by both terrestrial litter decomposition in aquatic systems (Kuehn *et al.*, 2000). The rate of decomposition varied seasonally, with a summer maximum of $kd^{-1} = -0.092$ (Figure 5). Over all seasons, daily mass loss of between 0.004 g (autumn) to 0.045 g (summer) were within global ranges for diverse terrestrial and aquatic leaf decomposition in lotic systems, but higher than those in lentic systems (Belova, 1993; Rezende *et al.*, 2018). This comparatively high rate of decomposition can be explained by several extrinsic and intrinsic factors. First, the ponds were mesotrophic, hence adequate nutrients were available to facilitate microbial decomposition and enhance trophic interactions (Bodker *et al.*, 2015; Rejmánková & Sirová, 2007). Secondly, the ponds were periodically flowing. Decomposition rates were higher during periods of flow, as reported elsewhere (Rezende *et al.*, 2018). Thirdly, temperatures at the surface were higher during warmer months, facilitating decompositional processes (Bornette *et al.*, 2011). Finally, species specific morphological features of *C. procerum* include rapid leaf turnover rate for *C. procerum* of between 10 and 30 days (Muller, 1994), possibly due to a smaller investment in secondary growth, (Rea, 1992), which suggests both high primary productivity and the possibility of equally rapid decomposition rate. Other factors, such as water quality, may have been influential as well.

In Mulwaree chain-of-ponds, DOC was greater than the 1-10mg/L usual in Australian natural waters (Boulton, 2014; Dobson, 1998), and above default trigger values for South Eastern Australian slightly disturbed ecosystems. Given that Mulwaree chain of ponds exists in a largely pastured catchment, with few trees, DOC would be expected to be dominated by algal and other readily available sources (Boulton, 2014; Hladysz, 2009). Electrical conductivity was higher during no flow periods and has been shown to slow decomposition, which may further explain the observed seasonality as documented elsewhere (Roache *et al.*, 2006). Mean total phosphorus at a mean of 0.035 mg/L and total nitrogen (1.11 mg/L) were both less than the National guidelines for (ANZECC, 2000) short term trigger values for irrigation water (TP<0.8-12mg/L, TN<25-125 mg/L) suggesting considerable stored carbon compared to both nitrogen and phosphorus. Pond nutrients would be expected to be dominated by autochthonous N and P, with comparatively low C:N ratios supplied by algae and macrophytes, especially during periods of disconnection. These results suggest conditions of rapid uptake of available nutrients, with abundant available carbon remaining (Reid, 2008).

Rates of both growth and decomposition also vary depending on seasonal factors – light, day length and temperature. Interactions with phytoplankton constrain both, as competition for nutrients, shading and life history are all influential in the decomposition process (Asaeda *et al.*, 2001). With increasing productivity, there are shifts in dominance of these groups which set up the template for macrophyte/algal dominance (Brönmark & Hansson, 2017) . At low productivity, periphyton may cover sediments, and at higher productivity, submerged macrophytes (Figure 11). As productivity increases, phytoplankton may dominate until eutrophic conditions only allow emergent macrophytes to survive and photosynthesize above the surface.

However, this conceptual model may be complicated by interactions between periphyton, phytoplankton and macrophytes. Allelopathic chemicals produced by some macrophytes may selectively act on phytoplankton, rather than periphyton (Eigemann, 2013; Mulderij, 2006). While it was impossible to say that this is occurring in Mulwaree Ponds, there is evidence elsewhere that dominance of macrophytes in shallow lakes is the result (Eigemann, 2013; Hilt & Gross, 2008).

Under these current mesotrophic conditions, macrophytes, periphyton and phytoplankton maintain some balance, with occasional floods, adequate but unbalanced nutrients, water clarity and depth controlling productivity. If the ponds move further into eutrophy, these conditions would be expected to change to more phytoplankton and emergent macrophyte dominance, but with substantial microbial impact.

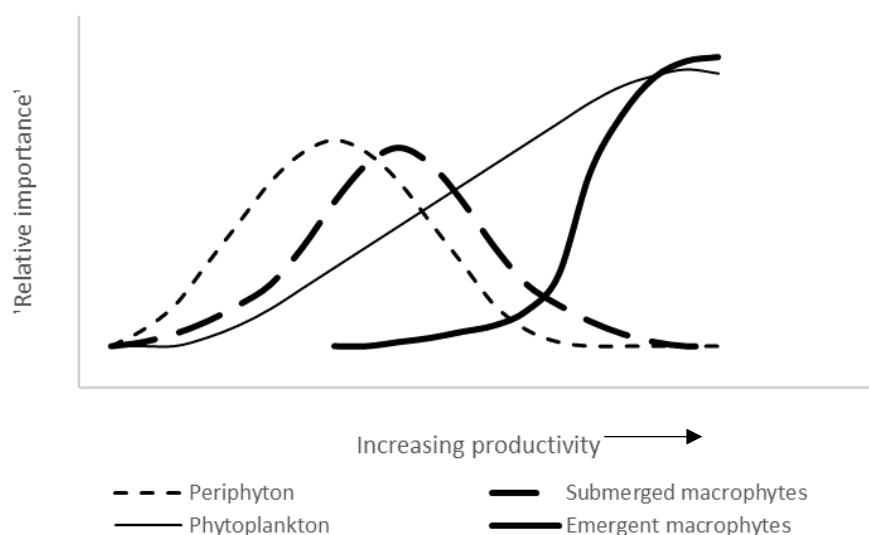


Figure 11. Conceptual 'relative importance' of primary producers related to increasing productivity within lake systems. Redrawn from (Brönmark & Hansson, 2005).

Phytoplankton productivity and planktonic respiration were at their highest during summer and autumn (Chapter 5) and may have increased competition for nutrients. This is a common phenomenon in planktonic systems, but it is not known how and if and how the pond planktonic and epiphytic algal/microbial loops are coupled (Figure 12).

Several factors specific to these ecological conditions may have influenced the results encountered. Recent flooding events during winter 2016 (Chapter 5), may have scoured sediments, detritus and fauna and reset succession in the system (Boulton, 2014).

Mesotrophic conditions may have stimulated both macrophyte and phytoplankton, then periphyton growth. Competition with phytoplankton was favoured in conditions of high light availability, competition for nutrients and slowing flow (Brönmark & Hansson, 2005).

Disconnection of flow and resulting lentic conditions in summer, with associated thermocline and oxycline development, space and nutrient resource constraints may have led to increasing top down controls. Invertebrate predators (odonates) and predaceous fish (*Gambusia holbrooki*) were ubiquitous during summer (L.Hardwick. *pers.comm.*) and may have been influential in increasing top down control in the ponds. The combination of periphyton growth supporting herbivores, including the exotic aquatic snail, *Physa acuta*, and increasing predator abundance may have further impacted on invertebrate led litter decomposition within the food web (Figure 12).

The interaction between algae and microbes in aquatic systems is a complex one. While the relationship between algae and bacteria in periphyton is often a positive, symbiotic one, for other aquatic relationships, they can be both negative and positive. Phytoplankton release photosynthetic carbon, which acts as a food source for bacteria (Danger *et al.*, 2013). At the same time, bacterial activity on dead algal cells release mineralized nutrients that are then accessible for algal growth (Sigee, 2005a). However, competition for nutrients between phytoplankton and bacteria can be strong (Sigee, 2005a) and while in oligotrophic conditions this would be more pronounced, any imbalance would alter trophic relationships. There is evidence that bacteria hold a competitive advantage over phytoplankton in conditions of phosphorus limitation, where nitrogen may be limiting, the reverse advantage to phytoplankton may prevail (Brussaard, 1998). Further, aquatic fungi also exhibit complex relationships with algae and other aquatic organisms (Sigee, 2005b). While important in litter decomposition generally (Gulis, 2006; Medeiros, 2009), fungi also exhibit parasitism on algae, rotifers, amoeba and invertebrates (Sigee, 2005b). The combination of fungi, algae and bacteria may have created complex interactions at very small scale, both in the macrophyte beds and open water in the ponds.

Furthermore, algal inhibitory processes include production of antibiotics (Jones, 1986) that are active against bacteria. Conversely, there is also evidence that algae may co-exist with decomposer heterotrophs, ‘priming’ leaf litter and increasing microbial decomposition, particularly in low nutrient systems (Danger *et al.*, 2013). However, this response may not be as effective in more eutrophic systems and Danger’s (2013) studies were conducted in mesocosms so represent simplified relationships to that experienced in real pond complex systems. The complexity of interactions between high levels of nutrients in such lotic/lentic

systems such as Mulwaree chain-of-ponds make conditions dynamic and strong predictions difficult. Simple conceptual models (Figure 12) have been constructed to illustrate how this seasonal trophic/geochemical system may operate.

Carbon remaining as measured by loss on ignition (AFDM) was unexpectedly higher in summer and autumn, for both total and microbial decomposition. The same response, while smaller, occurred to cotton strip decomposition despite higher water temperature and lentic conditions. These results suggest that carbon was abundant and decomposition processes driven by nutrients. Furthermore, limited decomposition of cotton strips indicated that more labile forms of carbon were likely available and that longer submersion times were required. Productive conditions within the ponds, including more than adequate carbon, suggests that the ponds may be a sink for carbon while disconnected, and a source while flowing for downstream ecosystems. However, this assumption would need to be tested.

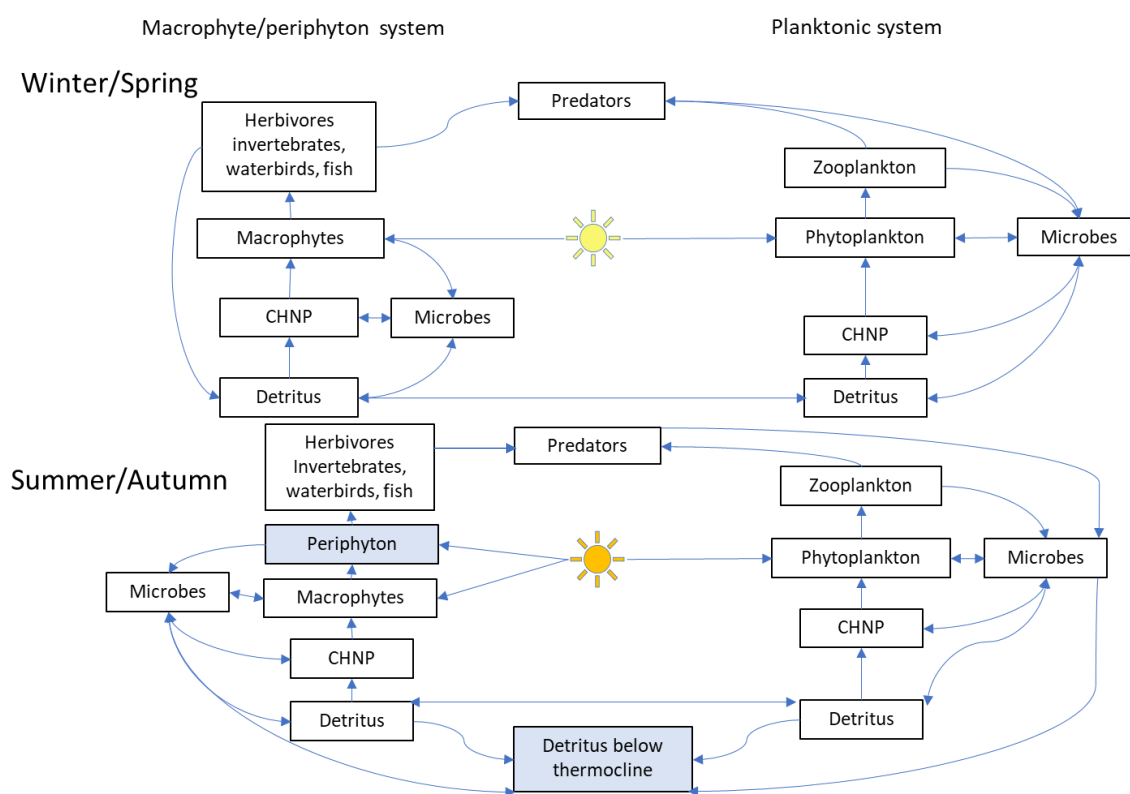


Figure 12. Simple seasonal conceptual models for trophic and energy relationships for macrophyte and phytoplankton mediated systems in Mulwaree Ponds.

Longer term, while total decomposition only dominated in spring while plants were likely to be more palatable, elevated temperatures may have altered this balance, increasing decomposition, herbivory and impacting biomass. This alteration to food web pressures may have pushed the ponds to phytoplankton dominance. Despite phosphorus levels being below measured detection limits, it was possible to augment the data from other studies (GHD, 2013a) which indicated high concentrations for both nutrients but with phosphorus limitation. The Redfield ratio of C,106: N, 16: P, 1 (Boulton, 2014) was exceeded substantially with N:P greater than 27:1, suggesting the possibility of long term bacterial dominance within the ponds.

A risk for Mulwaree ponds is that elevated concentrations of phosphorus under conditions of mesotrophy are known to increase decomposition rates and further risk of eutrophication if adequate nitrogen is available (Gulis & Suberkropp, 2003; Qualls & Richardson, 2000). If phosphorus levels were to increase in the ponds, this would presumably push the system into eutrophication. There is evidence that increasing periods of no flow in intermittent systems promotes heterotrophy (Acuña, 2015). This was not evident for Mulwaree Ponds (Chapter 5), during the study period, with net ecosystem productivity mostly positive despite high levels of planktonic respiration. However, the study period was uncharacteristically wet, so it may have been atypical that NEP was positive. Macrophyte dominance at the edge of the pool and phytoplankton availability mid pond, with loss of detritus to the sediments below the thermocline and oxycline during summer unconnected periods, represented a store of carbon and nutrients within the ponds. This was despite the known release of CO₂ and CH₄ derived from macrophyte and heterotrophic microbial activities (Dise, 2009).

Large accumulations of organic matter in deep and productive ponds like Mulwaree chain of pond can be problematic, as pond turnover in autumn brings nutrient rich sediments to the surface. Common in more eutrophic systems, this may cause cyanobacterial dominance, moving the ponds to a phytoplankton state, with heightened risk of increased heterotrophy (Sigee, 2005a). These processes are also complex. For example, microbes require critical C:N and C:P ratios that are close to that of their biomass in order to start mineralization. The critical ratio required for litter C:N is 15:1 and 60:1 for C:P, with Mulwaree C:N ratios (12.4:1) depauperate in nitrogen (Bridgham, 2009) and C:P (>664.6:1) even more limited. Given those conditions within the ponds, it is possible that microbial communities responded

to comparatively low nitrogen and phosphorus and were able to accommodate lower C:N and C:P critical ratios, as microbial decomposition was the dominant process for *C. procerum* mineralization. If nitrogen input were increased, it could alter dominance of microbial decomposition further.

Alternative Stable States

Mulwaree ponds are macrophyte-dominated around their margins, but due to their depth, mesotrophy and comparative clarity, exhibit substantial phytoplankton productivity (Chapter 5). Individual ponds may exhibit both macrophyte and phytoplankton activity and may exist in alternative stable states. Such conditions are common in shallow permanent lakes in Europe, but are relatively uncommon in Australia (Boulton, 2014). There is evidence for similar dynamics in nearby farm dams (Casanova, 1997) and recognized in eutrophic lakes elsewhere (Bakker *et al.*, 2016), but there has been considerable inconsistency in the understanding of other aquatic systems (Capon *et al.*, 2015; Scheffer & van Nes, 2007). However, where shifts between turbid phytoplankton and clear water macrophyte states occur, they have been attributed to increased nutrients, herbivory and temperature (Zhang *et al.*, 2018) all of which create a risk for Mulwaree ponds.

The intricate relationships that maintain macrophyte dominance as a stable state are complex, dynamic and subject to regime shifts (Scheffer, 2003). Macrophytes remove carbon and nutrients, stored in plant material, rhizosphere sediments and released into the atmosphere (Khan & Ansari, 2005), but in the absence of hydrological connection, much is retained within the nutrient pool (Dise, 2009) and the ponds a sink for nutrients. Mulwaree Ponds may lose much of incorporated biologically-derived material downstream once reconnected, so regular floods are important for maintenance of the current system. Flow connection may reset this system by flushing nutrients and organic matter downstream. Furthermore, there is evidence that higher temperatures increase rates of litter decomposition in streams (Ferreira, 2015), so human induced temperature alterations would be expected to further alter current macrophyte/phytoplankton states in the ponds.

One of the major changes under climate change scenarios in aquatic ecosystems is an increase of carbon, including DOC (Reitsema *et al.*, 2018). The exact reason for these increases and

differences in DOC quality and composition is unknown but thought to be related to climate change (Reitsema *et al.*, 2018). While climate change is not expected to have a huge effect on aquatic ecosystems in the short term, to 2030 (CSIRO, 2007), there will be long term increases in average temperature, more hot days, generally less rainfall during cool seasons, with increased intensity of extreme rainfall events (CSIRO, 2016). Natural variability at an annual and decadal scale is expected to enhance or mask the long term anthropogenic effect (CSIRO, 2016). Eventually, it is not increased temperature alone that will put pressure on Mulwaree Ponds macrophyte dominated status, but also variability in temperature and rainfall (Dise, 2009).

Conclusion

Decomposition dynamics of an endemic freshwater macrophyte in a rare geomorphic aquatic system underscored the importance and unpredictability of temperature and flow in pond processes. Summer and autumn decomposition rates were less than in winter and spring, potentially explained by the palatability of *C. procerum* and herbivory, possible nutrient imbalance and the presence of lotic and lentic conditions. Once flow connection ceased, thermoclines and oxyclines meant that inhabitable space for predators became limited.

Rather than maximum decomposition occurring because of higher temperatures, complex interactions between predators, vertebrate and invertebrate herbivores, periphyton, phytoplankton and macrophytes may have disrupted macrophyte decomposition rates. Mesotrophic conditions in the at-risk chain-of-ponds that are regulated by natural flow events appear to maintain a highly productive macrophyte-dominated aquatic system. Alteration posed by continued push disturbance of changing climate and anthropogenic land use place the ponds at high risk. As these ponds lose their clarity as phytoplankton dominate, macrophytes are likely to be restricted to a narrower margin of pond area, which is likely to exacerbate recruitment opportunities further. More than half of wetlands in Australia have already been lost (Bennett, 1997), with increasing risks posed by alteration to water supply and quality, exotic species and climate change (Catford, 2017).

All of these factors impact on Mulwaree chain-of-ponds. Continued geomorphic pressures that are leading to head cuts upstream of the ponds (Williams, 2016) add to trajectories that place the aquatic vegetation community at high risk. Once marginal vegetation and the macrophyte dominance is lost as an alternative stable state, if hysteresis prevails, it will be difficult to recover (Capon *et al.*, 2015). Given that *Cycnogeton procerum* and the other dominant macrophyte taxa have limited dispersal abilities, the community is at risk. Once macrophytes that stabilize the pond margins are lost, geomorphic thresholds are more likely to be breached, with the ponds' geomorphic structure at risk (Brierley *et al.*, 2010). Urgent action to minimize grazing pressure and manage upstream nutrients is important if the ponds are to be maintained, given the water quality and riparian status outlined by State of Environment reporting (EnvironmentACT, 2004; GHD, 2013). But ultimately, reducing the

impact of rising temperature and increased flow variability will be the main driver in protecting this rare and unprotected ecosystem.

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Appendices

Appendix 1. Table 1. Water Quality (mean \pm S.E), $n=4$, over the course of the study, Mulwaree ponds. Mean values are given for ponds over sampling events.

Mean \pm S.E.	12/09/2016	02/11/2016	29/11/2016	07/02/2017	26/05/2017	25/08/2017
Electrical conductivity(μ S/cm)	356.95 \pm 0.48	601.89 \pm 23.38	569.28 \pm 8.85	774.28 \pm 17.92	974.51 \pm 132.97	1294.74 \pm 199.64
pH	6.89 \pm 0.40	6.88 \pm 0.20	6.71 \pm 0.10	7.60 \pm 0.60	7.19 \pm 0.16	7.45 \pm 0.20
Total Nitrogen (mg/L)	0.55 \pm 0.60	0.83 \pm 0.11	0.95 \pm 0.30	1.18 \pm 0.60	1.18 \pm 0.30	1.95 \pm 0.10
Bicarbonate Alkalinity as CaCO ₃ (mg/L)			97.00 \pm 0.71		132.50 \pm 1.31	137.50 \pm 12.50
Total Alkalinity as CaCO ₃ (mg/L)	66.00 \pm 0.71	98.25 \pm 2.50	97.00 \pm 0.71		132.50 \pm 1.31	137.50 \pm 12.50
Ammonia (mg/L)	0.20 \pm 0.10		0.30 \pm 0.10	0.40	0.50	0.40 \pm 0.10
Nitrate (mg/L)	0.40 \pm 0.20		0.40	0.7 \pm 0.2	0.10	0.40 \pm 0.10
Nitrite (mg/L)	0.20 \pm 0.10		0.40	0.40	0.40	0.30 \pm 0.10
Phosphate (mg/L)	0.10		0.30 \pm 0.10	0.10	0.10	0.10
Dissolved Organic Carbon (mg/L)	18.00	13.75 \pm 1.65	12.75 \pm 0.25	13.25 \pm 0.25	12.25 \pm 0.25	13 \pm 0.41
Total Phosphorus (mg/L)	0.11 \pm 0.20		0.40	0.40	0.40	0.40

Appendix 1 Table 2. Mean (including number of samples taken) Molar TN:TP ratios with data for Mulwaree River @ The Towers (GHD, 2013b), including number of samples and study collected data for four sampling periods, #. Total Phosphorus was below detectable limits for the study, so ratios given are a minimum value.*

Year of data collection	N:P ratio	TN mg/L	TP mg/L
1998 (n=31)*	39.13	1.61	0.09
2001(n=23)*	34.01	1.71	0.11
2004(n=10)*	26.95	1.355	0.11
2007 (n=39)*	34.03	1.4	0.09
2010 (n=20)*	31.91	1.415	0.097
2013 (n=35)*	60.32	0.91	0.033
Nov 2016 (n=4)#	>41.5625	0.95	0.05
Feb 2017 (n=4)#	>51.40625	1.175	0.05
May 2017 (n=4)#	>51.40625	1.175	0.05
Aug 2017 (n=4)#	>85.3125	1.95	0.05

Chapter 7

Discussion and Conclusion



Lorraine Hardwick

Department of Biological Sciences

Introduction

The aim of this thesis was to investigate ecosystem function in two unusual, globally rare and poorly studied aquatic systems. The thesis objectives were then, to attempt to document ecological function, identify their relationship to abiotic drivers and biotic attributes to link structural biodiversity response and ecosystem processes. By investigating the structure and linkages between abiotic, biotic and functional characteristics, it was hypothesized that more knowledge of these rare systems could be found and important drivers could be identified. A final objective of the respective studies was to develop management tools so that conservation and restoration measures for these endangered ecosystems can be more effectively designed and implemented.

This discussion is divided into several components. First, there is a comment on the findings of each of the studies individually. The ecology of the study systems was poorly known and as such the scientific results provided new biological and ecological knowledge. The high variability within and between systems, their biological diversity, the impact of human influence on their ecology and their ecological importance is discussed. Second, the functional aspects of each of the studies and the value gained by adding functional aspects to biological research is discussed. Third, a discussion of the value of integrating biodiversity and ecosystem function for each of the studies and across the studies highlights the contribution of this work to the body of knowledge of the BEF hypotheses. There continues to be knowledge gaps which will be documented briefly. Finally, a short discussion on how knowledge gained by investigating both biodiversity and function and theories of restoration ecology may be used in future management.

Thesis Findings

Two poorly known but ecologically unusual aquatic systems were studied in order to describe their natural aquatic features. The wetland systems varied considerably in their rates of carbon and nutrient turnover. The upland peat swamps in the Blue Mountains, similar to peat swamps globally, have sequestered carbon since the last Glacial Maximum (around 15,000 years before present (Kybp)). Terrestrial vegetation is slow growing, sclerophyllous and the swamps thickly vegetated. Both groundwater and surface water exist in heavily shaded, low

light environments with low aquatic primary productivity. Carbon cycling is based on decomposition and is extremely slow, due to peat derived acidic conditions and the recalcitrant nature of contemporary terrestrial plant litter (Chapter 3, 4).

Table 1. Thesis aims, research approach and chapters.

Thesis aim: To identify the aquatic ecology and function of two rare and poorly known wetlands, relate that to abiotic drivers altered by human influences, then to investigate how this knowledge may be useful for aspects of Biodiversity-Ecosystem Function theory

Thesis Aim	Research approach	Associated chapters
Investigate the effect of urbanization on biodiversity and productivity of groundwater invertebrate communities in THPSS swamps	Use multivariate analysis to typify 12 THPSS swamps based on abiotic drivers Investigate stygofauna and groundwater productivity related to abiotic drivers related to urbanization	Chapter 3
Investigate eucalypt litter decomposition and associated stream invertebrate communities from THPSS streams related to urban impact	Measure microbial, detritivore and total litter decay rates related to leaf age and identify the aquatic invertebrates inhabiting leaf packs in THPSS associated streams Use abiotic drivers related to urban impact to investigate linkages to decomposition processes	Chapter 4
Investigate the effect of abiotic drivers on phytoplankton biomass dynamics and functional relationships to pelagic primary productivity in Mulwaree chain-of-ponds	Using connected and unconnected flow to investigate pelagic primary productivity at depth, mid pond and vegetated margins. Relate primary productivity and phytoplankton biomass to biotic drivers such as temperature	Chapter 5
Investigate the relative importance of seasonality of macrophyte/phytoplankton dominance by using decomposition processes of <i>Cyanogeton procerum</i> , a common but water dispersed and thus at-risk aquatic macrophyte in Mulwaree chain-of-ponds	Map aquatic macrophyte distribution related to depth across ponds. Measure seasonal decomposition of a common but at risk macrophyte, <i>Cyanogeton procerum</i> , and investigate processes of microbial compared to total litter decay Develop a conceptual model of macrophyte versus phytoplankton dominance	Chapter 6

Erosion and dewatering processes in disturbed and channelized swamps have been shown to be major source of both CO₂ and CH₄ in associated studies (Cowley, 2017), with significant

carbon storage and cycling occurs within the groundwater of undisturbed swamps (Cowley, 2017).

Mulwaree chain-of-ponds, on the other hand, is a system where organic matter is rapidly cycled. Due to high availability of light, nutrients and readily available organic matter, lotic and lentic conditions, carbon cycling is seasonal, with macrophyte dominance and phytoplankton primary productivity interacting with microbial decomposition (Chapter 5, 6).

The Upland Peat Swamps

The upland peat swamps in the Blue Mountains are naturally acidic and low in nutrients, reflected by the sclerophyllous vegetation (Benson & Baird, 2012; Keith & Myerscough, 1993) in undisturbed swamps. Groundwater fauna were naturally low in abundance, generally high in diversity and low in evenness, but not always so; inter-site variability was generally high. The variability among upland swamps, including topography, hydrology (Cowley, 2017) sedimentation (Cowley, 2016), vegetation (Keith *et al.*, 1993) and fauna (Baird, 2012; Gorissen, 2016), (Chapter 3), has created a diverse series of wetlands with many different natural values. Catchment urbanization has altered groundwater hydrology and quality in these swamps, as shown in Chapter 3. Response in groundwater to rainfall runoff is rapid, between 1 to 3 days, with erosion by stormwater a major problem in the swamps (Cowley, 2017). Channelization itself has affected the ecology of some swamps, with those, like Mt. Hay, exhibiting different groundwater faunal characteristics. Water quality changes little as it travels through the swamps. Increased water temperature variability, electrical conductivity, alkalinity and nutrients, pH at more neutral levels and lower dissolved oxygen are all features of swamps in urbanized catchments compared to undisturbed swamps (Chapter 3). These changes may have impacted on groundwater fauna within the swamps. It was not possible to identify the stygofaunal taxa to species level, suggesting that they are as yet unknown. They therefore represent a fauna that needs further attention before they are lost to urbanization. More study of high biodiversity groups such as the habitat specific Oribatidae (Déchéne & Buddle, 2009) and the locally common but taxonomically poorly known Copepod groups: Harpaticoida and Cyclopoida (Galassi, 2009) would be particularly rewarding. Moderate levels of catchment urbanization were related to higher diversity,

creating a unimodal pattern of taxa richness, with high variability. Carbon decomposition within the groundwater was higher in more urban swamps, perhaps as a result of several factors. These may include more available nutrients, but also higher microbial activity under conditions of phenolic inhibition (Chapter 4) associated with rates of drying and rewetting (Fenner & Freeman, 2011) as a result of groundwater variability and channelization (Cowley, 2017), which, combined with higher productivity, is likely to affect urbanized swamps substantially. Poor quality water entering swamps from urban catchments continues through the swamps and downstream, also impacting on rates of leaf litter decomposition, one of the major processes in swamp function (Chapter 4). Increased aerobic processing rates of leaf litter in these streams, with the release of CO₂, rather than slow and anaerobic decomposition in more waterlogged swamps, suggests that peat formation processes are also being altered as a result of human impact. These results suggest that the swamps require protection from pollutants in the stormwater itself, not just the hydrological, erosive impacts of urbanized runoff, in order to maintain natural character (Chapter 3).

Other anthropogenic impacts may be more difficult to correct. Peat swamps form under conditions of wet and anaerobic decomposition of plant material and have developed over the Holocene period to sequester 21% of the global store of soil organic carbon and covering around 3% of the world's surface (Leifeld & Menichetti, 2018) (Chapter 2). On the Blue Mountains sandstone and ironstone escarpment, past excavation in swamps have created ecological conditions under which litter decomposition has been impeded, probably as a result of iron redox processes and the formation of flocs of iron bacteria. These conditions, combined with low slope and proximity to stormwater may be suitable for peat development, however the trajectory for such conditions is unknown (Chapter 4). Restoration of such swamps may be difficult (Moreno-Mateos *et al.*, 2012; Zedler, 2000).

The mechanisms and importance of invertebrate shredders and scrapers in leaf litter decomposition rates was underscored by strong and significant biodiversity-ecosystem function correlations between invertebrate and litter decomposition metrics. These were not visible in other analyses, pointing to the value of linking biodiversity and function in studying ecosystems (Chapter 4).

The Mulwaree Chain-of-Ponds

Ponds are increasingly under focus for their regional heterogeneity and biodiversity (Oertli, 2010; Williams, 2004) and there is recognition that their importance in maintaining landscape aquatic biodiversity and function cannot be underestimated (Hill *et al.*, 2018). For these reasons alone, Mulwaree chain-of-ponds are important to understand and protect. Together with the other aquatic pond environments across the local landscape; farm dams (Casanova, 1997), which support variable biodiversity, the Mulwaree chain-of-ponds are integral to holistic aquatic ecology of the region.

Mulwaree chain-of-ponds exist in a highly agricultural landscape. The land surrounding the ponds has been cleared of native vegetation and current land used is dominated by annual cropping, cattle and sheep grazing. The hydrology varies seasonally, with periods of connected flow and disconnection between the ponds. The ponds are large and separated by vegetated preferential flow lines. They have unusual bathymetry, with high depth to width ratios. Water quality is relatively poor, mesotrophic and at times has relatively high electrical conductivity.

The bathymetry of the ponds provided the structural template for studies that increased knowledge related both to pelagic primary production (Chapter 5) and litter decomposition dynamics (Chapter 6). Rather than concentrating on biodiversity, these studies focused on ecological function, to attempt to understand how abiotic factors directly affect processes within the ponds. Chlorophyll A was used as a surrogate of phytoplankton biomass and plant functional traits to describe the aquatic macrophyte community.

The studies found that the ponds alternate between mixed, lotic conditions when available carbon and nutrients are released downstream and lentic conditions when the ponds, due to their bathymetry, act as small mesotrophic, monomictic lakes (Chapter 5). Strong thermal and oxygen stratification develops and unusually, due to high water clarity, significant photosynthesis is possible below the thermocline and oxycline. This unusual finding of the study suggests a need for greater investigation. During lotic conditions, the ponds act as a

river, the water body is mixed, phytoplankton biomass and net ecosystem productivity are relatively low and scouring of the pond sediment may occur (Williams, 2018).

Vegetation biodiversity is high with an extensive fringing macrophyte community in the ponds and preferential flow paths between the ponds (Chapter 6). This community maintains macrophyte dominance in the ponds, with high clarity of water (Brothers *et al.*, 2013; Scheffer & van Nes, 2007). One of the most abundant of these, *Cycnogeton procerum*, a rhizomatous emergent native species, is at risk as a result of primarily exhibiting hydrochory as a dispersal mechanism. Altered hydrology that would reduce seasonal inundation means that dispersal of the species may be interrupted. The dominance of rhizomatous plant species that stabilize the sediment and banks of the ponds, however may provide a buffer against grazing pressure during short term drought events.

Seasonal macrophyte decomposition is mostly affected by microbial activity, with invasive fish and invertebrates (*Gambusia holbrookii* and *Physa acuta*) presumably affecting trophic interactions within the ponds. However, decomposition rates were lower during periods of disconnection, suggesting that factors such as high carbon concentrations and limited nitrogen and phosphorus may drive an altered system state where decomposition is impeded. Macrophyte dominance may be under some threat under changing temperature and associated hydrological regimes.

Function: Influences, Values and Theories

Abiotic factors

Of all the abiotic factors that impacted on natural functioning of these two ecosystems, geomorphology was the most influential. At Mulwaree, geomorphic conditions of a relatively stable chain-of-pond river system have set the structural template outlined by Williams (2018) for macrophyte dominance that maintain the pond stability, at least in the short term. In the Blue Mountains, Holocene development of THPSS (Temperate Highland Peat Swamps on Sandstone) have left terrestrial/aquatic ecotones of considerable ecological endemism and variability. The effects of urban development on the physical structure of

these geomorphically fragile swamps are clearly obvious with impacts on carbon storage, pollutant filtering and ecological processes (Cowley, 2016).

Temperature is the most important water quality abiotic factor. Variation in stygofaunal assemblages was best explained by temperature (Chapter 3), and increased decomposition in upland peat swamps streams was also related to temperature (Chapter 4). Both primary productivity in Mulwaree chain of pond and decomposition of *C. procerum* were influenced heavily by thermal stratification (Chapter 5) and water temperature respectively (Chapter 6). All of these outcomes are temperature dependent and there is an abundance of evidence that temperature increases microbial respiration additively where nutrients are not limiting (Manning *et al.*, 2018), increases litter decomposition in upland streams (Ferreira, 2015) and in peat (Hilasvuori *et al.*, 2013); and increases macrophyte growth (Carr *et al.*, 1997) and phytoplankton productivity (Stomp *et al.*, 2011) in freshwater.

In all four experimental chapters of this thesis, temperature was a prominent feature influencing both biodiversity and functional measures. This has implications for continuing ecological function under modelled scenarios of changing climate (Bureau of Meteorology, 2017). In the short term, these scenarios predict greater variability in both temperature and rainfall, but decadal scenarios predict both higher temperatures (+1°C) and highly altered and reduced (-4%) seasonal rainfall patterns (www.climatechangeinaustralia.gov.au). The impact of changing climate would be expected to place greater pressure on ecology and function of both THPSS and Mulwaree chain-of-ponds, with alteration to intensity and variability of flow under conditions of higher temperatures

Hydrology as a feature of geomorphological condition, was an important abiotic factor influencing ecosystem processes. Water depth and variability, stream flow; erosive potential and carrier of pollutants were major abiotic factors in both wetlands. Introduction of nutrients that were related to increased leaf litter decay (Chapter 4) and carbon decomposition (Chapter 3) has altered swamp productivity. In Mulwaree chain-of-ponds, disconnection in summer and autumn increased the pressure on macrophyte dominance (Chapter 6) and created thermo- and oxyclines that alter phytoplankton productivity (Chapter 5). Both hydrology and resultant

temperature regimes created dynamic templates in both wetlands that have been critical for ecological productivity.

The value of including functional attributes in biodiversity studies

These four studies provided examples of the value of including functional attributes in studying both pristine and more impacted aquatic systems. The integration of abiotic drivers, taxa richness and functional responses provided an understanding of upland swamp streams, what drives them and how better to manage them. In the chain-of-ponds, using mainly functional attributes, it became possible to understand the workings of a long-impacted system and to highlight the imminent risk of collapse.

In the upland swamp streams (Chapter 4), by including functional measures, it was possible to more fully understand impacts of urbanization. Aquatic invertebrate richness was greater in more urbanized compared to less disturbed streams until thresholds above which taxa richness was impeded. Functional attributes of litter decomposition mirrored these patterns, thereby providing further evidence of urbanization impact. Furthermore, α diversity in stygofaunal communities also exhibited similar patterns. These results suggested a unimodal pattern of invertebrate taxa richness (Chapter 4) and stygofaunal α diversity (Chapter 3) and function across swamp streams, which at the outset could be seen as fitting theories of the Intermediate Disturbance Hypothesis (Connell, 1978). Connell's IDH has been highly cited as a defining theory of biodiversity, including for aquatic systems (Townsend *et al.*, 1997) but is at odds with recent research by Fox (2013) and ongoing discussion (Willig, 2018). How these disturbance hypotheses fit with biodiversity and ecosystem functions is grounds for further research.

In Mulwaree chain-of-ponds, studying undisturbed conditions of biodiversity traits that may have historically provided niches or contributed to resilience prior to European colonization is no longer possible. There are no reference conditions. However, chlorophyll A as a surrogate for phytoplankton biomass did not relate strongly to pond productivity over depths (Chapter 5). Invasive species such as *Gambusia holbrooki* have established in the ponds, preying on smaller invertebrates, such as zooplankton and chironomids (Pyke, 2008). Presence of larger

invertebrate grazers such as the exotic snail *Physa acuta*, isopods and large numbers of odonate nymphs observed, suggests that *Gambusia* may be creating top-down effects on the invertebrate community. The likely effect of this would be a reduction in invertebrate taxa richness and an increase in phytoplankton, with less periphyton and macrophytes as a result of dominance of *Physa acuta*. The question is then, how is extant function likely to be affected and how has it helped an understanding of the ponds? Macrophyte decomposition is mainly mediated by microbial means (Chapter 6), indicating a possible altered role for grazers and detritivores consuming both microbial and decomposed biomass within the system.

It is difficult to categorically state the effect of invasive aquatic faunal species are having on trophic relationships within ponds. Under current mesotrophic conditions, and ongoing pressure of further increases in nutrient conditions, it is likely that macrophyte dominance is under threat and further pressure from land use and hydrological change may push the system over the threshold and into an alternative state. This would alter the rare clear water macrophyte dominated ponds that act as lakes into phytoplankton dominated, turbid ponds. Only by studying function have these conclusions been possible.

In the upland swamp studies, there were strong links between invertebrate functional feeding groups (FFG) traits and litter decomposition and weaker ones between stygofaunal traits and cellulose decomposition. But in the chain-of-ponds studies, where there is no reference condition (Palmer *et al.*, 2016) lack of taxa richness information meant that these links could not be made. However, the use of functional attributes in this altered ecosystem delivered knowledge useful for management and restoration.

Features of upland swamps and ponds that make it difficult to identify relationships are:

1. Upland swamp stygofauna and surface water invertebrates in their streams may be naturally low in taxa richness at local scales, but those spatial scales are very small, admittedly at the scale encompassed by BEF. Only by sampling a greater number of swamps could one begin to understand α and then β diversity in a realistic way. However, there needs to be much greater emphasis on scale dependence to test the BEF relationship and an understanding that in naturally low diversity ecosystems,

disturbance may increase biodiversity and productivity, but the effect on stability requires much greater understanding.

2. Because upland swamps are an ecotone between terrestrial and aquatic ecosystem, they are highly varied, so definition of the ecosystem and the importance of scale again is important. Thompson *et al.* (2018) reported that there are three ways for the BEF relationship to be scale-dependent: variation among patches in local α diversity (within swamps and within groundwater or surface water); spatial variation in the local BEF relationships; and incomplete compositional turnover in species composition among patches. All of these may apply to upland swamps.
3. Knowledge of biodiversity in ground water is currently hampered by lack of taxonomic knowledge of species and many of the taxa collected may have been stygoxenes, accidental stygofauna. This means that they may have been peripheral to groundwater ecosystems, inhabiting terrestrial environments at small scale.
4. In Mulwaree chain-of-ponds, using chlorophyll A as a surrogate for phytoplankton biomass was not an effective biodiversity measure, but the ponds provide a useful ecosystem space for testing theories using limited experiments.
5. Macrophyte taxa richness was not measured at a scale that could be easily used to measure ecological function; obvious patch size would be at pond level, with results suggesting mixing of functions within the ponds and considerable compositional turnover. An alternative patch size would be much smaller at sub-macrophyte scale and a comparison between periphyton and phytoplankton functions.

Knowledge Gaps

There are knowledge gaps that are now more obvious in these rare and poorly known wetlands that are required for best management practice for protection and maintenance. These initial biodiversity and function studies have provided much useful information, but much more is needed.

This thesis provided more questions than answers, possibly as a result of studying two diverse ecosystems, rather than building knowledge within one. These systems, very different, meant that attempting to understand their function required a broad approach. Generally, PhD theses start with a hypothesis to be tested and the four data chapters fulfil that hypothesis. The hypothesis for this study revolved around the importance of adding functional knowledge in

studies of poorly studied and rare aquatic ecological systems. However, because two disparate ecosystems were studied and the methods varied, it was difficult to bring a single hypothesis together. One of the causes of choosing these two very different ecosystems was funding, which meant that funds were available for studies in both. A different approach would have been to focus the thesis on only one ecosystem, leaving knowledge of the other to another researcher. There is benefit, though, in using a similar idea in different ecosystems to try to find if the hypothesis holds. In this case, the overarching importance of temperature in the ecological processes within each of these systems tells us about the importance of climate change generally. The disadvantage of such an approach is the absence of greater depth of knowledge overall, within each ecosystem. Some of these possible avenues that were not explored include:

- Better stygofaunal identification by conventional and molecular (DNA)
- Microbial diversity using genetic methods would have enabled better measures of productivity in groundwater
- The influence of scale on ecosystem structure and processes would have been a useful addition to these studies to assist in the better estimation of α and β diversity that would better test BEF theories or alternative theories (Naeem, 2016)
- Better identification and development of stygofaunal and surface water invertebrate traits would assist in further development of links to function
- The abundance of toxins, from natural iron levels to more toxic heavy metals such as copper, lead and zinc, and field and experimental responses by peat swamp stream invertebrates and stygofauna would have been useful to further develop urbanization stormwater models in Blue Mountains swamps
- In Mulwaree chain-of-ponds, a further spread of pond investigation would have tested similarities and differences of the river/wetland system as a whole
- Identifying phytoplankton taxa richness at small spatial and temporal scales would have provided more biodiversity information to link to ecosystem processes and undertaking benthic respiration would have provided the information for whole pond energy budgets
- Integrating faunal diversity at temporal scales, including trophic interactions and performing enclosure experiments to identify the importance of top down predation on

macrophyte decomposition rates would have provided data to answer the importance of invasive species.

These possible areas of study could have provided more knowledge of each of the ecosystems studied. However, the central tenet is the importance of understanding ecological function of varied ecosystems, and the outcome that temperature is an overriding controller of that. This means that experimentation of the mechanisms of how temperature affects ecological processes and quantified knowledge of thresholds could have been a useful addition to this research.

The links to Restoration Ecology and Future Management

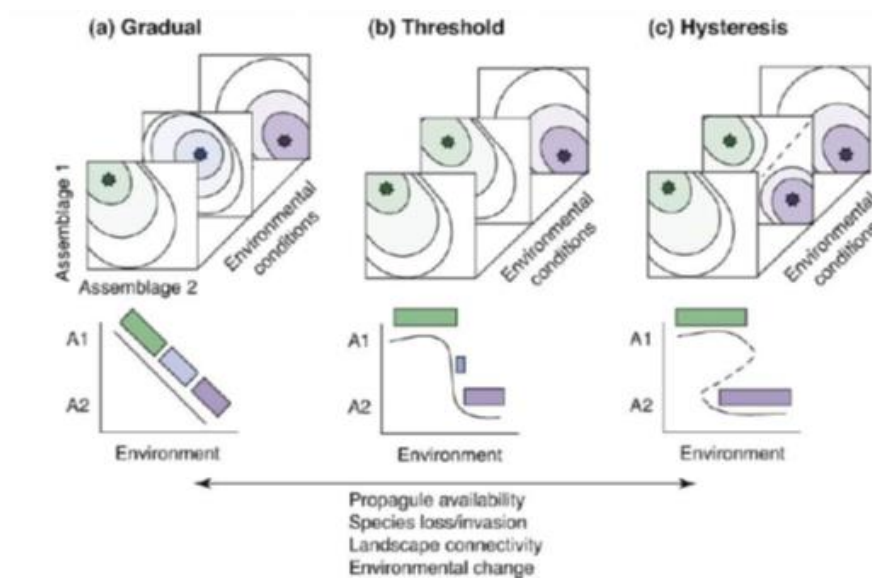
The future of biodiversity and function of the THPSS swamps and Mulwaree chain-of-ponds will depend on careful restoration and management of these degraded and threatened systems. In order to set some theory around how that might be done, it is necessary to frame the studies in a restoration context. This short section outlines how ecological restoration theory and practice can be used to restore and manage the two wetland systems.

Restoration ecology can be described as ‘the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed’ (McDonald *et al.*, 2016). Under conditions of the Anthropocene and growing uncertainty, restoration ecology faces many challenges. One of the most important of these is rate of change, making the need for restoration goals to be dynamic. Under changed environments, restoration to past condition may not be possible and goals must represent some prediction of future conditions (Harris *et al.*, 2006). That means that restoration ecologists must get better at understanding and predicting change in biodiversity, function and resilience (Scheffer, 2003) to better manage ecosystems for the future.

Restoration steps in ecological restoration are informed both by theory and methods of restoration science and follow a well-documented path (Palmer *et al.*, 2016). Firstly, restoration goals are set, based on analysis of contemporary or historic reference condition. Current condition is assessed scientifically using relevant indicators that are related to known drivers of ecological change. Then the restoration process is designed that can restore

functions and structure for maximum ecological benefits and long-term maintenance. Finally, restoration is undertaken (Palmer *et al.*, 2016).

Analysis of current condition can assist in setting objectives and paths for best restoration. Conceptual models of ecological dynamics such as those outlined by Scheffer *et al.* (2001) may be used to analyze current condition and therefore guide restoration trajectories. One of these, threshold based ecological dynamics (Suding & Hobbs, 2009), is commonly linked to restoration and management objectives and becoming mainstream in restoration ecology (Suding *et al.*, 2016) (Figure 1).



*Figure 1. Alternative models of ecosystem dynamics. Gradual change (a) and two threshold models, non-hysteresis (b) and hysteresis (c). Squares designate possible relative abundances of two state characters assemblage 1,2 (different species, functional groups or ecosystem processes). Stars represent mid points and ovals represent isoclines of standard units of perturbation strength (resilience). Changes in isoclines across the environmental gradient represent change in stability. Diagrams below the isoclines indicate the two-dimensional relationships between the biotic community and environment. Human activities can change the frequency and nature of threshold events influencing resilience, which can affect the arrangement of isoclines as well as shift the system from one to another type of dynamics. Sourced from Suding *et al.* (2009).*

This thesis, by investigating both biodiversity and functional aspects of two disparate aquatic systems, was able to begin to understand the ecological processes that are operating to drive them and use the Suding and Hobbs models (Figure 1).

Both of the wetland ecosystems studied are impacted by human activities; the THPSS swamps by many threats, including urbanization; and Mulwaree chain-of-ponds by long term agricultural practices. The threat of changing climate affects both. This thesis has revealed two important wetlands systems, are under differential threat (Figure 2). The THPSS swamps exhibited functional change, even at low levels of anthropogenic stress. Invertebrate communities varied, decomposition rates increased, and swamp structural integrity was altered. At higher levels of disturbance, decomposition rates decrease. Individual swamps exist at different levels of threat, so require individual management. Mulwaree chain-of-ponds maintains macrophyte dominance at present but is at risk of moving into an alternative stable state of phytoplankton dominance that may result in hysteresis conditions. Under these conditions, a return to previous macrophyte dominance may be difficult and the trajectory to another stable state uncertain. However, in the sense of describing changes in ecosystem states and setting goals, it is important to understand and analyze all of the drivers (Bugnot *et al.*, 2019) and then project how the system can be moved back to a less disturbed state.

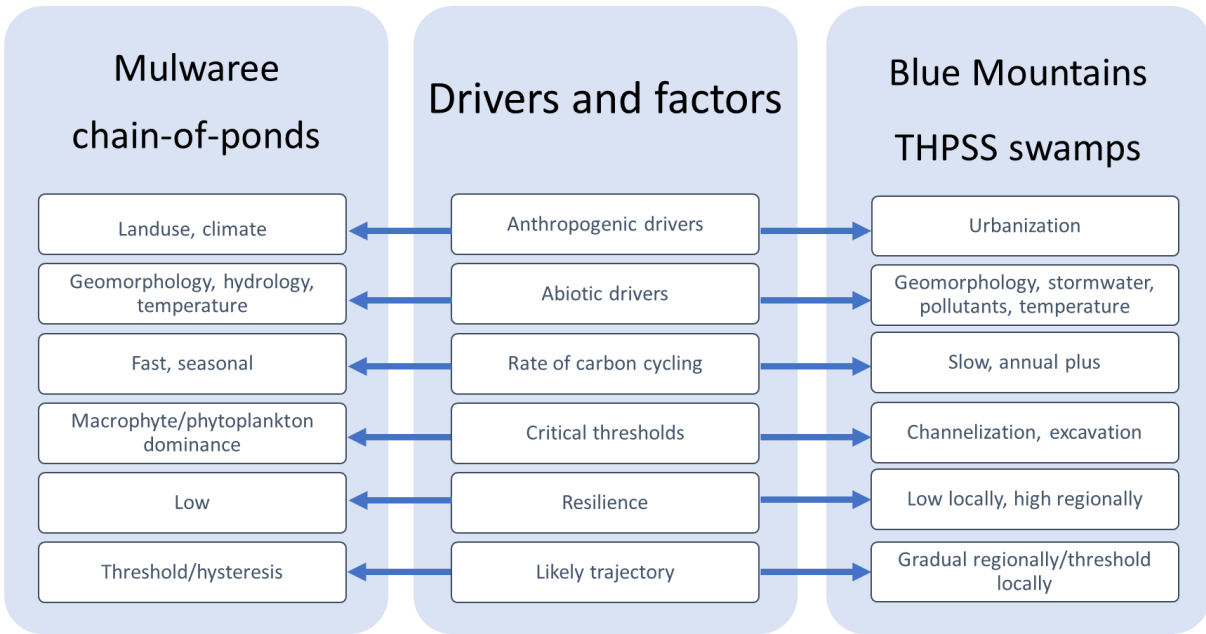


Figure 2. Translating drivers and important restoration factors for study sites.

Once current status and risk are known, design and planning for restoration is required. This is a challenging component of ecological management as identifying and predicting ecological responses is difficult (Liu *et al.*, 2015). For Mulwaree ponds, there is a wealth of information to inform the conditions under which lakes and ponds can move from macrophyte to phytoplankton dominance (Walker & Meyers, 2004), and indicators for upland swamp thresholds may be developed, however active monitoring is required to predict imminent change.

While many of the upland swamps are undisturbed and only require management, other wetlands studied in this thesis could be described as requiring (at least), biological modification (Figure 3). Several of the THPSS are so badly degraded that they require physical-chemical modification, and Mulwaree chain-of-ponds may require improved and urgent management at present or be at risk of significant change that may be irreversible. If thresholds in the ponds are exceeded, it is quite possible that hysteresis will deliver a new state that may not recover macrophyte dominance.

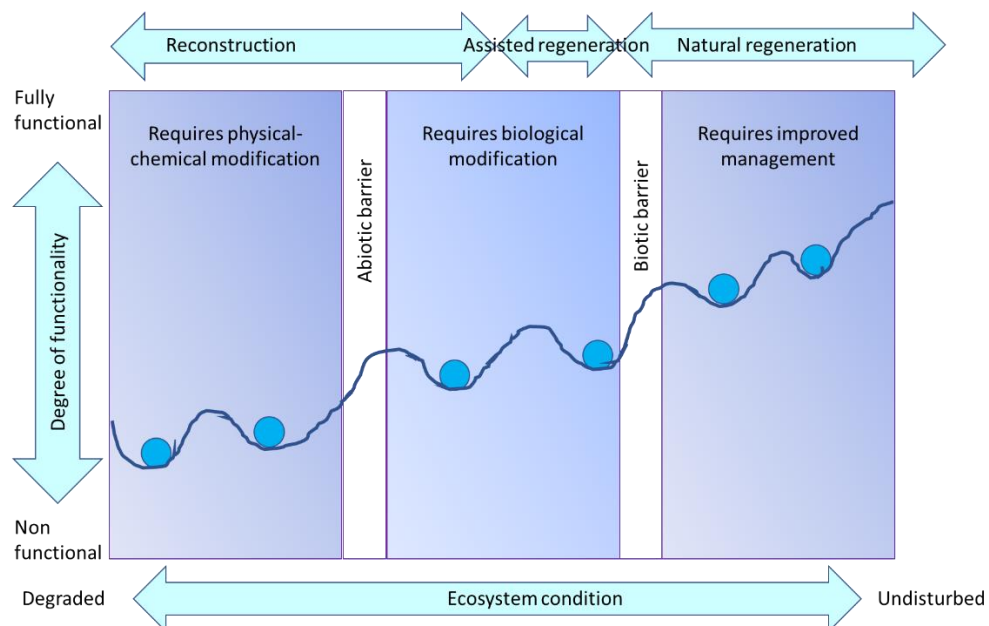


Figure 3. Conceptual model of ecosystem degradation and restoration (modified from McDonald (2016) and adapted from various authors). The troughs in the diagram represents basins of stability in which an ecosystem can remain in a steady state prior to being shifted by a restoration or a degradation event past a threshold (represented by peaks in a diagram) towards a higher functioning state or a lower functioning state.

Conclusion

This thesis was able to assess functional ecology of two important and rare wetland systems, and further, to use abiotic and biotic metrics to more fully understand how they are responding to current anthropogenic pressures. Both systems provide ecotonal habitat, comprising both terrestrial and aquatic biological and ecological traits and functions. As such they are biologically interesting and diverse and provide lessons in ecological theory.

The studies forming the scientific basis of this thesis tested many hypotheses, most of which could be accepted (Table 2).

Table 2. Hypotheses testing and outcomes.

Chapter	Hypothesis	Accepted/Not Accepted
3	There is a relationship between urban impact and the stygofaunal community structure and ecological function of the swamps	Accepted
3	Site and catchment attributes are strongly correlated with differences in the stygofaunal community structure and ecological function of the swamps	Accepted
4	Streams associated with swamps in least urbanized catchments exhibit slower rates of litter decomposition than those in more urbanized catchments due to low nutrients, less acidic conditions and lower stream flow	Accepted
4	Site and catchment attributes are strongly correlated with differences in the stygofaunal community structure and ecological function of the swamps	Partly accepted
5	Gross primary productivity (GPP) and net ecosystem productivity (NEP; as measured by 24-hour light and dark bottle productivity/respiration) varies with surface and air temperature, but decreases with depth	Accepted
5	Planktonic respiration (PR) is consistent across depth	Accepted
5	Chlorophyll A concentration/activity/production is greater in the middle of the ponds than in fringing macrophyte beds, and greater during conditions of disconnected flow	Not accepted Accepted
6	Decomposition varies seasonally and spatially within the ponds	Accepted
6	Herbivory (by shredders), rather than microbial action dominates <i>C.procerum</i> leaf litter decomposition, emphasizing the importance of herbivores in Mulwaree chain-of-ponds vegetation margins	Not accepted

There were some limitations in the ecosystems for the four studies:

- in Mulwaree chain-of-ponds, it was not possible to complete a carbon budget due to a lack of measuring benthic productivity, phytoplankton and microbial taxonomy.
- the studies in the Blue Mountains upland swamps did not enable full understanding of ecological function, due to lack of taxonomic knowledge and biodiversity of not only stygofaunal invertebrates, but also terrestrial invertebrates in swamps as well.

But the combination of the range of differences between these two important aquatic ecosystems has provided a contribution to both local ecological knowledge and an understanding of threats, with a means by which management for protection and sustainability may proceed.

These studies are the first to investigate ecological functions in two rare and endangered aquatic systems. Generally classed as wetlands, which are particularly endangered ecosystems globally, these two systems are now seen to be at risk of anthropogenic change. Human impact, while from varying processes exhibits central drivers, including temperature, nutrients and hydrological alteration. Temperature alteration has been found to be a central theme driving ecosystem change in both of these systems and in their functional processes. That being so, there is heightened risk of climate change that may exacerbate natural ecological functions in both Mulwaree chain-of-ponds and the THPSS. Eutrophication is a growing problem across ecosystems worldwide and is seen here as altering phytoplankton and macrophyte domination in chain-of-ponds, but also altering productivity in upland swamps and their associated streams. This has ramifications for long term maintenance of both nutrient poor swamp ecology, but also the unique nature of the chain-of-ponds. And finally, a further predicted impact of climate change that is already obvious, will be hydrological changes, which have been illustrated in this study and outlined more fully by Cowley *et al.* (2018) and Williams (2018) in their respective theses. Increased variability that includes more frequent storms and droughts mean that fragile geomorphic systems such as these may well be lost. These two rare ecosystems are symbolic of many around the world that may similarly be lost under the threat of climate change. By studying how they function, it is possible to understand the processes that may lead to loss as a more general threat facing ecosystems elsewhere. This is the use of a thesis that describes how several ecosystems function, that may be extrapolated to other wetland systems elsewhere and provides some management options for addressing the substantial risk facing them.

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