Response of Periphyton to Current Velocities in the Nepean River, NSW.

by

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All other research described in this thesis is my own original work. This work has not been submitted for a higher degree at any other university or institution.

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Abstract

Warragamba Dam is the largest water supply dam on the Hawkesbury-Nepean River system. It greatly reduces flow variability and volume downstream, impacting water quality, periphyton, macrophyte growth and ecological processes dependent on these. The benefits of variable environmental flows from Warragamba Dam have been investigated by the NSW Government. Environmental flows can scour nuisance algae and reset periphyton communities; however the flow regime required to achieve this in the Nepean River is unknown. The study was conducted in a cobble-dominated riffle in the Nepean River at Penrith, NSW. Eighty-four algal genera were identified. *Leptolyngbya* dominated all samples during the study. A flow of 5440 ML/day reduced biomass, overall abundance, particularly filamentous green algae and stimulated algal succession, resulting in increased community richness. Periphyton communities are influenced by current velocity; however, the similarity in flow velocities between scouring and non-scouring events suggests that thresholds may exist. This study investigated responses of periphyton to current velocity using Threshold Indicator Taxa Analysis (TITAN). *Scenedesmus, Navicula* and *Cymbella* had loss thresholds, whilst others exhibited positive associations with increasing current velocity. This study will help finalise an environmental flow regime for Warragamba Dam and supports the existence current velocity scouring thresholds for some periphyton taxa.

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1. Introduction / background

Natural flow regimes are the fundamental drivers of the structural and functional attributes of surface water ecosystems (Poff *et al.,* 1997; Rolls *et al.,* 2012). The abundance and diversity of aquatic organisms in rivers are influenced by the spatial and temporal effects of the flow regime on habitat structure, therefore, deviations from natural flow regimes, such as extraction and dam impoundments, threaten the diversity of aquatic organisms (Bunn and Arthington, 2002).

1.1 Periphyton

Periphyton, which includes benthic algae and is the focus of this study, can be used as an indicator of stream health (Biggs and Kilroy, 2000). Because periphyton responds rapidly (within days to weeks) to changes in environmental conditions, it can be used as an indicator of recent environmental change (Gaiser, 2009). Periphyton appears as a slimy coating on submerged rocks or other stable substrates such as logs, sediment and submerged plants. It can be a thin brown or greenish film, thick, dark-coloured mats, and long filaments of brown or green algae (Biggs and Kilroy, 2000). These mats are a highly diverse part of the riverine ecosystem, composed of bacteria, cyanobacteria, algae, protozoa, fungi and detritus (Biggs and Close, 1989). Periphyton is vital for ecosystem functioning as it takes up large amounts of nitrogen and phosphorus from the water column, is an important primary producer and provides a complex food source for grazing macroinvertebrates (Guariento *et al.,* 2009).

The presence of dams that alter and regulate flow can result in changes to the periphyton communities (Chester and Norris, 2006). Changes in flow regime, coupled with eutrophication, can result in periphyton proliferation (Hart *et al.*, 2013) causing water management issues (Biggs, 1996; Biggs, 2000). Instream values may be compromised by the proliferation of periphyton. These include reduced aesthetic values, odours, reduced benthic biodiversity due to loss of sensitive macroinvertebrate taxa, impacts on recreational use such as impairment to swimmers and slip hazards for waders, clogging of irrigation intake pumps and the fouling of sensory and flow monitoring equipment (Death *et al.*, 2007; New Zealand Department of Conservation, 2013). Proliferations can lead to reduced dissolved oxygen due to periphytic decomposition (Biggs, 2000) and the dominance of unpalatable algal communities leading to changes in invertebrate community composition (Welch *et al.*, 1988).

Models that can predict nuisance levels of periphyton growth and the flow velocities required to reduce or stimulate biomass are useful tools for water managers. The chlorophyll-a threshold developed to indicate nuisance growth has been estimated to be 150 – 200 mg/m^2 (Horner *et al.*, 1983; Welch *et al.*, 1988; Welch *et al.*, 1989; Luce *et al.*, 2010).

1.1.1 Nutrients and periphyton growth

Algae species vary in their nutrient requirements for growth (Biggs *et al.*, 1998). Both nitrogen and phosphorus have been reported to limit overall growth, and differ between genera within the periphyton community (Fairchild *et al.*., 1985). Both nitrogen and silica are vital for diatom growth, as silica is required for the development of the diatom cell walls, or frustules (Dodds, 2002), and any growth is impossible without nitrogen (Townsend *et al.*, 2012; Gilpin *et al.*, 2004). Increased nitrogen in rivers leads to increased silica uptake by diatoms (Muylaert *et al.*, 2009). Fairchild *et al.* (1985) showed that combinations of both nitrogen and phosphorus resulted in heavy growths of the filamentous green algae, *Stigeoclonium*, and the diatom, *Navicula* was found to be more abundant. Rosemond *et al.* (1993) found that both nitrogen and phosphorus were the limiting growth factors, which is consistent with Dodds and Welch (2000), who also reported maximum periphyton growth response in an experimental treatment when nitrogen and phosphorus were added simultaneously. Death *et al.* (2007) found that nitrogen was the limiting nutrient for periphyton growth in a New Zealand study. Experimental manipulation of nutrients has shown that periphyton growth occurs with only nitrogen added; however no stimulatory effects were seen when only phosphorus was added (Smith and Lee, 2006). This is contrary to Guariento *et al.* (2009) who reported that phosphorus was the key variable regulating periphyton biomass.

1.1.2 Flow and growth

There have been numerous studies of the relationship between benthic algal biomass and stream current (Opsahl *et al.*, 2003; Biggs, 1996). Current velocity has been shown to enhance the colonisation of periphytic algae (Reisen and Spencer, 1970), although high velocities tend to limit periphyton growth (Antoine and Benson-Evans, 1982). Many diatoms show a preference for slow current velocity areas such as pools over high current velocity areas in streams (Ghosh and Gaur, 1998).

Current velocity may affect the response of periphyton due to changes to nutrient availability. Townsend *et al.* (2012) found that increasing velocities reduced the thickness of the diffusive boundary layer, thus enhancing the rate of nutrient transfer by diffusion across the boundary layer

to the algal cell wall. Periphytic architecture determines the effects of current velocity, as adherent assemblages are more resistant to sloughing, whilst filamentous assemblages are susceptible to shear stress (Opsahl *et al.*, 2003). The resistant properties of the periphyton are divided into two types, inherent and conditional. Inherent relates to their shape, size, texture, tensile and attachment strength, whilst conditional relates to the community and its environment, which includes its age and acclimation (Biggs and Thomsen, 1995). Dense and coherent growth forms, such as mucilaginous diatoms and cyanobacterial mats, may be resistant to diffusion and dislodgment by shear stress. High velocities may, therefore, enhance biomass accrual by increasing rates of mass transfer, but without greatly increasing losses through sloughing. Long filaments of green algae with an open matrix and high rates of diffusion into the mats are often present at low current velocity (Biggs *et al.*, 1998). Increased current velocity can result in an increase in diatoms, supporting the periphytic growth form and architecture theory of velocity attenuation across different assemblages (Dodds and Biggs, 2002).

Arnon *et al.* (2007) reported that nutrients and flow play a key role in structuring benthic communities during manipulated experiments. They found different algal communities developed under three flow conditions, with low velocities (<0.2 m/s) dominated by filamentous green algae, which decreased at velocities greater than 0.5 m/s.

Moderate current velocity increases may stimulate periphyton growth in nutrient-rich streams by stimulating algal metabolism (Humphrey and Stevenson, 1992). Davie and Mitrovic (2014) reported sub-scouring flows of <0.9 m/s of nutrient-enriched water increased nutrient diffusion and uptake, leading to increased algal growth. Horner and Welch (1981) reported increased velocities of up to 0.5 m/s enhanced periphyton growth, but velocities exceeding this reduced accrual rate due to scouring from increased friction, overcoming the positive nutrient transfer.

Horner *et al.* (1983) reported that a change in current velocity at a location is more likely to impact on the periphyton assemblage than constant current velocity. The intensity and frequency of disturbance, influences succession and community composition (Larnad, 2010). Biomass loss occurs when the current velocity increases beyond that under which the periphyton community developed (Horner *et al.*, 1990). Spates of high flow reduce algal biomass and alter periphyton community composition (Biggs *et al.*, 1999; Davie *et al.*, 2012). Velocities of 1.2 m/s were reported to reduce filamentous algal biomass and velocities <0.9 m/s were found to have constant abundance or increased filamentous algae (Davie and Mitrovic, 2014). Observations of scouring and non-scouring events have suggested that loss / removal thresholds exist for certain taxa (Davie

& Mitrovic, 2014). Studies on current velocity resistance reported loss thresholds for *Fragilaria, Cymbella* and weakly attached algae such as *Gomphonema* and *Ulothrix,* but noted the degree of loss was determined by the concentration of suspended sediment (Luce *et al.*, 2010; Biggs and Thomsen, 1995).

Periphyton biomass is determined by the balance between the accrual and loss of periphyton (Biggs, 1996). Silt densities during flow events have a significant relationship with periphyton biomass (Jowett and Biggs, 1997). Biggs *et al.* (1999) reported that it was important to consider not only the frequency of high-current velocity events, but the degree of bed movement to reflect the change in periphyton biomass losses, as silt can act as a scouring agent.

A manipulated experiment conducted in a stream in Colorado, showed the different communities at varying velocities. It was found that the green algae *Ulothix* was dominant in slow velocities, at a medium current velocity, *Ulothix* was found to support the epiphytic diatoms *Achnanthes* and *Cocconeis* and at a high current velocity, the periphyton was dominated by diatoms, particularly *Fragilaria* and *Navicula*, and the filamentous cyanobacterium, *Lyngbya* (Wellnitz and Leroy Poff, 2006).

Current velocity can influence the interactions between algae and grazing macroinvertebrates (Opsahl *et al.*, 2003). Macroinvertebrate diversity decreases as the amount of filamentous algae increases (Tonkin *et al.*, 2014). Diatoms and green algae are more palatable and nutritious to invertebrates than are cyanobacteria (Hart, 1985; Opsahl *et al.*, 2003).

After high-flow scouring events, benthic algae must recolonize the surface of stream substrates. The interaction between scour events and available nutrients determines biomass accrual and structuring of algae species assemblages (Riseng *et al.*, 2004; Stevenson *et al.*, 1996). Thresholds for algae associated with low phosphorus possibly represent levels that exceed the maximum growth rate potential and define where along phosphorus-enrichment gradients species are no longer competitive (Stevenson *et al.*, 2006). Manoylov and Stevenson (2006) reported that *Achnanthidium minutissimum* has high growth rates in low-phosphorus conditions, but are not competitive with other taxa in high-phosphorus environments. As nutrient concentrations increase, new algae assemblages develop based on interactions between differences in optimal growth rates, competition for substrate during recolonization, and structural requirements for establishment of particular growth forms (Taylor *et al.*, 2014).

Peterson and Porter (2002) reported periphyton biomass and community structure are influenced by the availability of nutrients and turbidity of the water. They found that the abundance of cyanobacteria is often an indicator of limited available nitrogen due to competition and that algal biomass was low at sites where the abundance of the nitrogen fixing cyanobacteria was 50% or greater. Davie *et al.* (2012) found that chlorophyll-a was low on scoured cobbles with succession up to day 16, and then increased to a level comparable with reference cobbles. They found that early succession was dominated by diatoms, *Cocconesis, Synedra* and *Fragilaria* and later succession by filamentous green algae.

1.2 The Hawkesbury-Nepean River

The Hawkesbury-Nepean River is one of the most important river systems in NSW (DECC, 2009). It is the largest river/estuary system in the Sydney Metropolitan area (Figure 1), and its complex ecosystems provide habitat for a large diversity of native plant and animal species (Roberts *et al.*, 1999). Since European settlement, the river has been increasingly relied upon to meet the requirements of a growing city, and it now provides 97% of water for more than 4.8 million people living in and around Sydney (DECC, 2009). The Hawkesbury-Nepean River system also supports a $259 million agriculture industry, as well as tourism, fishing and oyster industries. It is also used for recreation, including swimming, boating and water skiing (NOW, 2015).

Figure 1 Hawkesbury-Nepean catchment, NSW

Source: http://www.water.nsw.gov.au/_images/deprecated/water/35/act_hawkesbury-nepean-wma.gif

The Hawkesbury-Nepean River catchment covers approximately 22,000 km^2, from Lithgow in the west, south to Goulburn and east as far as the Illawarra escarpment (AECOM, 2014). There are a total of 38 large dams and weirs on the Hawkesbury-Nepean River system, with 20 water supply dams and 13 weirs on the Nepean River above Penrith. These weirs maintain water levels to facilitate recreation, industry and irrigation. There are also 17 wastewater treatment plants (WWTPs) and one major recycled water plant in the catchment, with many discharging treated water into the Hawkesbury-Nepean River (Sydney Water, 2012).

The Hawkesbury-Nepean River has undergone profound morphological changes, transitioning from a gravel bed meandering system to a low sinuosity sand-bed river that is effectively inset within a gravel braid plain (Brierley & Fryirs, 2013). The lowland catchment of the Nepean River has a long history of land clearing since European settlement. The original form of the river at Penrith has been permanently lost since the construction of Penrith Weir in 1909 (Eco Logical Australia, 2014).

The site for Warragamba Dam was proposed in 1897, but it wasn't until the drought of 1934 to 1942, that saw Sydney with only three months' water supply, that the decision to build the dam was taken (Beasley, 1988). Work commenced in 1946 and was completed in 1960 (Beasley, 1988). In the interim, Warragamba Weir was constructed as an emergency supply.

The location of the dam offered advantages as it has a large catchment area, and is in the deep, narrow gorge of the Warragamba River, making the dam capable of holding a vast amount of water. Warragamba Dam is located approximately 65 km west of Sydney, and has a storage capacity of 2,027,000 ML. It is one of the largest domestic water supply dams in the world, with a storage lake that is four times the size of Sydney Harbour. It provides more than 80% of Sydney's water (WaterNSW 2015). The construction of dams and weirs on The Hawkesbury-Nepean River have resulted in a change to the natural flow of river (DECC, 2009). The alteration of natural flow regimes is recognised as a major factor contributing to the loss of biodiversity (NPWS, 2001).

River regulation and elevated nutrient levels from discharges from Wastewater Treatment Plants (WWTPs), agricultural runoff and urban stormwater together have led to a decline in the health of the Hawkesbury-Nepean River (DECC, 2009). Releases of water from dams to meet environmental outcomes, known as environmental flows, are essential for downstream river health (DECC, 2009). Prior to 2001, there was no water released from any of the dams or weirs for environmental purposes (Growns, 2016).

1.3 Environmental flows

In July 2001, the independent Hawkesbury-Nepean River Management Forum (The Forum) was commissioned by the NSW State government to consider ways to improve the health of the Hawkesbury-Nepean River (Albanese, 2006). Increasing severity and persistence of toxic cyanobacterial blooms and aquatic weed outbreaks were symptoms of declining waterway health. The release of environmental flows from the water supply dams was one of the major recommendations from the Forum (HNRMF, 2004; Growns and Reinfelds, 2014). In 2008, the Sydney Catchment Authority (SCA) began releasing variable environmental flows from Avon Dam, on a tributary of the upper Nepean River, with the volume released based on inflows to the storage. Variable environmental flows from the other Upper Nepean dams (Cataract, Cordeaux and Nepean) commenced on 1 July 2010 (Metropolitan Water Directorate 2010a; Growns, 2016). Environmental flows are planned for release from the Warragamba Dam in 2024 (Metropolitan Water2017; Growns, 2016). Figure 2 shows the location of the upper dams in reference to the study site.

Transparent / translucent environmental flows mimic the natural flow pattern of the river (Arthington, *et al.,* 2006; HNRMF, 2004)and are designed to minimise the ecological effects due to the changes in the flow regime (Rolls *et al.,* 2012). All inflows to a dam are measured or estimated and a portion of the inflow released daily. Low flows are protected by the "transparency rule" where all inflows up to a chosen percentile (for the Upper Nepean dams, all flows up to the 80th percentile flow) are released. For Avon Dam, this is 13.2 ML/d. In addition to the low flow, when inflows exceed the transparency percentile, an additional volume is released. For the Upper Nepean storages, this is an additional 20% of inflows, up to the required daily flow or the maximum capacity of the release infrastructure. This reintroduces flow variability to the river downstream of the dam. The variable environmental flows will restore or improve the frequency and duration of freshes (up to 5,000 ML/d), and reduce the persistence and frequency of periods of low flows.

It is expected that the variable environmental flows from Warragamba Dam will help benefit the Warragamba, Nepean and Hawkesbury rivers by:

- Improving water quality;
- reducing the number, severity and persistence of aquatic weed outbreaks and cyanobacterial blooms;

- improving conditions for native fish, frogs, water birds and river-dependent plants and animals that rely on different flows to trigger migration and breeding; and
- protecting or enhancing river conditions that are suitable for recreation such as boating and swimming (SCA 2010; WaterNSW, 2016; Metropolitan Water, 2017).

Environmental flows are the main restoration method used in NSW to ameliorate the ecological effects of river regulation (Arthington, 2012; Frazier, *et al.,* 2012; Growns, 2016). Although the natural flow of many rivers and streams in the Hawkesbury-Nepean River system has been significantly altered by dams, the river system is classed as unregulated under the Water Management Act 2000 as the water storages do not capture and then release water into the river downstream, for extraction by users (NOW, 2014b).

To sustain surface water and groundwater resources, it is essential to balance the competing needs of the environment and water users. Water sharing plans establish rules for sharing water between the environmental needs of the river or aquifer, and water users, and also between different types of water use such as town supply, rural domestic supply, stock watering, industry and irrigation (NOW, 2015). Water extraction in the Hawkesbury-Nepean River is regulated under the *Water Sharing Plan for the Greater Metropolitan Region Unregulated River Water Sources* (NOW, 2011).

The Nepean River downstream of Wallacia Weir receives water from a variety of sources, including environmental flow releases from Nepean Dam, WWTP discharges and inflows from tributaries. The Warragamba River, downstream from the dam, receives little water unless the dam is spilling and has had periods of poor water quality and experienced weed infestations (NOW, 2014a). Groundwater, dam leakage, and the runoff from the small local catchment are not sufficient to maintain flows in the section immediately downstream of the dam. Currently, water releases from Warragamba Dam are made from the pipeline (that supplies water filtration plants) into Megarrity's Creek, approximately 2.9 km downstream of the dam wall. This water discharges into the top of the 22 km long pool formed by Penrith Weir. The 22 – 30 ML/d release is made to dilute effluent and provide drinking water for extraction at North Richmond (NOW, 2011). Figure 2 shows the operations of the Hawkesbury-Nepean River with regard to dams and sewage treatment inputs into the river system.

1.4 Water quality in the Hawkesbury-Nepean River

Good water quality is fundamental for good river health. It sustains ecological processes that support aquatic ecosystems. The various uses of the river require water quality that is suitable for irrigation, watering stock, drinking, fishing and recreation, to meet cultural and spiritual needs, and to protect aquatic ecosystems (OEH, 2014). Trigger values provided in the ANZECC/ARMCANZ water quality guidelines (2000) provide thresholds against which water quality parameters can be compared.

Natural inputs of salt, metals and nutrients from the surrounding landscape influence the natural water quality. Degrading water quality can be attributed to point source activities, such as discharges from WWTPs, mining and industry. Diffuse source activities, including land clearing, agricultural activities and urban development also contribute to the degradation of water quality (DECC, 2009).

Inflows can also influence water quality, and point and diffuse discharges are often high in nutrients. This is the case in the Hawkesbury-Nepean, where urban, agricultural runoff and WWTPs contribute to increased levels of nitrogen and phosphorus, resulting in cyanobacterial blooms and macrophyte and periphyton proliferations. Historically, blooms of the potentially toxic cyanobacteria *Anabaena* (now *Dolichospermum*, Wacklin *et al.*, 2009) and *Microsystis* have been a major concern in the river during prolonged low flows and high summer temperatures (DECC, 2009). Saunders & White (1993) reported a bloom of *Microcystis aeruginosa* between Windsor and Wisemans Ferry in December 1991, coincided with warm water, low flows and total phosphorus (TP) concentrations of 33- 52 µg/L (slightly to moderately elevated, when compared with the ANZECC (2000) trigger value of 25 µg/L).

In 1996, studies by Sydney Water confirmed that phosphorus from WWTPs were a major contributor to eutrophication in the river, which in turn, was linked to cyanobacterial blooms. In response, Sydney Water embarked on a program of WWTP upgrades to reduce nutrient inputs (Sydney Water, 2012).

DECC (2009) described the water quality in the Hawkesbury-Nepean River as poor and eutrophic. Examination of historic water quality data showed poor water quality (as exceedances of the ANZECC guidelines) at many sites for TP, TN chlorophyll-a and dissolved oxygen. Table 1 shows the long-term water quality results for the Nepean River at Penrith and Yarramundi, compared to the Grose River. The Grose River, a large, unregulated tributary of the Hawkesbury River, has over 80%

of its catchment in the Blue Mountains National Park (DECCW 2008) had lower levels of all parameters when compared to the nearby Nepean River sites. At the Yarramundi, concentrations of TN are significantly higher than the other two sites and exceed the ANZECC/ARMCANZ (2000) Guidelines trigger value of 0.5 mg/L. Water quality results from 2011 – 2015 also show higher nutrients at Yarramundi (Table 2).

Table 1. Long-term median water quality monitoring of the Hawkesbury-Nepean River (1985 – 2007).

Site	Total Phosphorus (mg/L)	Total Nitrogen (mg/L)	Turbidity (NTU)	Chlorophyll-a (µg/L)
Nepean River at Penrith Weir	0.011	0.48	2.37	3.1
Nepean River at Yarramundi Bridge	0.028	0.97	3.4	8.3
Grose River at Yarramundi	0.0165	0.42	1.85	1.75
ANZECC guidelines	0.025	0.5	10	5

Source: DECC 2009, Table A1.2; ANZECC 2000, table 3.3.2.

Table 2. Average Water quality results from 14/3/2011 – 24/6/2014 and 16/7/2014 – 28/5/2015

Site	Years	Total Phosphorus (mg/L)	Total Nitrogen (mg/L)	Turbidity (NTU)	Chlorophyll-a (µg/L)
Nepean River at Penrith Weir	2011-2014	0.018	0.486	5.28	8.29
	2014-2015**	0.018	0.481	9.16	6.17
Nepean River at Yarramundi Bridge	2011-2014	0.031	0.624	5.97	12.34
	2014-2015**	0.032	0.603	7.26	12.02
Grose River at Yarramundi	2011-2014*	0.012	0.139	4.77	1.18

(Data obtained from WaterNSW and Sydney Water 2016).
*Water quality data was not collected after June 2014 at the Grose River at Yarramundi; 2015 data includes two high flows resulting in an increase in nutrients following flow events; 2011-2014 includes high flows in 2012 & 2013.

The Winmalee WWTP discharges up to 17 ML/d of treated wastewater into the Nepean River above Yarramundi. Before 2009, Penrith WWTP also discharged up to 24 ML/d of treated effluent below Penrith Weir. The Penrith area is highly urbanized, and the lower Blue Mountains villages contribute nutrients to the waterways on the western side of the river. North of Penrith there is some agricultural land, including turf farms.

In comparison, the Grose River, declared a "Wild River" (DECC, 2008), now has no WWTP discharges into it. There is a small amount of urban run-off from the villages in the mid and upper Blue Mountains, and only limited agriculture in the catchment. There is a small amount of extraction for agriculture near the confluence with the Nepean River, and high in the catchment around Katoomba / Medlow Bath there are five small water supply dams (extracting up to 5,000 ML/yr) (NOW, 2014b). Long-term median water quality results are unlikely to reflect the current water quality in the Nepean River.

Figure 2. The Hawkesbury-Nepean River system
Source: Water Recycling in Australia, 2007

1.5 Periphyton in the Hawkesbury-Nepean River

There is little information on the periphyton community structure of the Hawkesbury-Nepean River. Previous studies by Growns and Growns (2001) identified periphytic diatoms in the upper Nepean River, above and below the upper Nepean dams. They found differing assemblages between the regulated and unregulated sites for macroinvertebrates, although diatom assemblages did not differ significantly. Although Growns (2016) reported improvements in the macroinvertebrate assemblages downstream of the Nepean dams following the commencement of environmental flows in the upper Nepean, diatoms were not included in this recent study and so no information is available regarding changes in diatom community following environmental flows.

Prior to 2015, very little information was available on periphyton community structure or the effects of flow on periphyton in the Nepean River below Warragamba Dam. Prolific periphyton was observed coating macrophytes in the Warragamba River in 2009 (Figure 3)and a sample showed diatoms dominant (50%), cyanobacteria (41%) and green algae (9%)(NOW, 2014c). *Leptolyngbya* and *Achnanthididum* were the most abundant genera at 20% and 19% of the total abundance respectively (Keenan, unpublished data).

Figure 3. Periphyton coating *Egeria densa* in the Warragamba River (Photo: H. Keenan (NOW) 2009).

Lynch and Keenan (unpublished data) examined the effects of two flow events on periphyton in the Nepean River. They reported a moderate flow (1540 ML/d at Penrith (~40th percentile natural flow) and 2530 ML/d at Yarramundi (~30th percentile natural flow) reduced filamentous green algae and increased cyanobacteria, particularly *Leptolyngbya*. A high flow (24,900 ML/d in the Nepean River at Penrith and 46,482 ML/d in the Nepean River at Yarramundi) further scoured the cobbles of filamentous green algae but increased the total abundance of other algae. At Penrith,

this resulted in a change of community structure from an assemblage dominated by diatoms (45%) and green algae (38%) to cyanobacteria-dominant (>95%). Classification of flow classes for the Hawkesbury-Nepean River are included in Appendix 1.

Lynch and Keenan (unpublished data) also found a difference in the periphyton communities in the Nepean River below Warragamba Dam compared to the Grose River. A total of 74 genera of periphyton were identified in this study, including 16 cyanobacteria, 29 green algae and 22 diatoms. The major differences between the Nepean and Grose rivers were the contributions of *Leptolyngbya, Homoeothrix, Navicula, Heteroleibleinia, Coleochaete, Synechococcus* and *Cyclotella.* The study concluded that the two Nepean River sites were more similar to each other, (dissimilarity 43.94 - SIMPER) compared to the Grose River. The Yarramundi site was found to have higher overall abundance, greater biomass and more green algae genera, including *Palmellopsis, Scenedesmus* and *Coleochaete*, most likely due to higher nutrient levels.

1.6 Estimating periphyton biomass

Periphyton abundance can be estimated by taxonomic counts (converted to cells per cm^2) and / or biomass. Biomass can be estimated by chlorophyll-a, carbon biomass as ash-free dry mass (AFDM), or particulate organic carbon (POC). The chlorophyll-a method measures the photosynthetic pigment common to all types of algae, while AFDM and POC procedures measure the carbon in a filtered water sample (Hambrook *et al.*, 2007). Chlorophyll-a is the most common method for estimating periphyton biomass (e.g. Biggs and Kilroy, 2000; Dodds *et al.*, 2002; Kilroy *et al.*, 2013; Snelder *et al.*, 2013). Because all types of live algae contain chlorophyll-a, this metric indicates the total amount of live algae in a sample. The other two techniques do not distinguish between algae which is live or dead at the time of collection so a build-up of dead algae could give an unrepresentatively high biomass result. In addition, cell densities and biovolumes are also used to determine the proportions of biomass in different taxonomic groups of algae (Stevenson, 2014).

1.7 Chlorophyll-a analysis

Chlorophyll-a is often used as an indicator due to a direct relationship between the pigment content and the amount of live algal biomass (Henriques *et al.*, 2007). Extraction and measurement of chlorophyll-a is faster and more convenient than cell counting (Tett *et al.*, 1978), and requires less operator skill and training. The content of chlorophyll-a in a periphyton sample

may vary due to a number of factors including algal community composition, algal cell size, light and temperature (Baulch *et al.*, 2009).

There are three main methods for the determination of chlorophyll-a from algae: spectrophotometry, fluorometry, and high performance liquid chromotography (HPLC) (Biggs, 2000). Spectrophotometry methods have been most frequently used for chlorophyll measurement because they provide a quick, accurate and inexpensive estimation of chlorophyll concentration (Hu *et al.*, 2013).

1.7.1 Solvents for extraction

The degree to which a solvent can extract is dependent on the hydration and permeability of the algae cell wall (Henriques *et al.*, 2007). Acetone is widely used for extracting photosynthetic pigments from fresh plant tissues (Marker, 1972). As a solvent, acetone is miscible and provides sharp absorbance peaks resulting in its popular use (Porra *et al.*, 1989; Ritchie, 2008). Aqueous acetone, containing 10 – 20% water is used in preference to anhydrous solvent as it has been found to be more efficient in extracting pigments and eliminates the need to dry the plant tissue before extraction (Marker, 1972). However, acetone has been reported to be a poor extractor with some algae (Scheer, 1991; Scheer, 2006), particularly for members of Chlorophyceae and Cyanophyceae (Marker, 1972).

Alcohol-based solvents such as methanol and ethanol have also been used to extract chlorophyll. Both ethanol and methanol were found to be more efficient than acetone for extracting chlorophyll from the marine alga *Nannochloropsis gaditana*, with methanol the most suitable (Henriques *et al.*, 2007). Sumanta (2014) found methanol to be a very good extractant for chlorophyll, particularly from recalcitrant vascular plant and algae.

Freezing and thawing samples before processing was a more effective method than fresh samples for extracting chlorophyll (Henriques *et al.*, 2007). Eighty-six percent more chlorophyll was extracted when the sample was freeze-dried relative to fresh/frozen samples extracted with 90% acetone (Hagerthey *et al.*, 2006). Tett *et al.* (1978) found that boiling methanol was more efficient compared to acetone for the distinction of chlorophyll-a and pheophytin-a when samples were frozen (TETT *et al.*, 1978).

Extraction of the green algae, *Scenedesmus quadricauda* and *Selenastrum capricornutum* using methanol and ethanol was reported to be more efficient when compared to extraction with 90%

acetone (Sartory and Grobbelaar, 1984). *Scenedesmus* has been described as a difficult alga to extract pigments from (Wiltshire *et al.*, 2000). Nush (1980) also reported low extraction efficiencies from *Scenedesmus* using 90% acetone when compared to alcohol extractions. Dere *et al.* (1998) found methanol to be the best solvent for extraction of chlorophyll in *Cladophora glomerata* and *Ulva rigita* because of the diversity of the cell wall structures.

Although less volatile and flammable than acetone, methanol is notoriously toxic as it is readily absorbed by inhalation and through the skin (Porra *et al.*, 1989; Ritchie, 2008). The use of methanol is not generally encouraged (Ramluckan *et al.*, 2014).

When algal chlorophyll degrades, it forms a series of products from the loss of magnesium, resulting in phaeophytin and chlorophyllide (Carlson and Simpson, 1996). Lorenzen (1967) introduced an acidification step in the spectrophotometry method. When chlorophylls are acidified, the magnesium ion is lost resulting in the production of phaeophytin. Equations to correct the calculation of chlorophyll containing phaeophytin were developed by Lorenzen (1967).

The type of algal cells present in samples influences extraction efficiency (Jeffrey *et al.*, 1997). Buffan-Dubau and Carman (2000) found that some genera of benthic diatoms were difficult to extract chorophyll from. Methanol is an effective extractant for green algae, but was found to be not as effective as acetone for freshwater cyanobacteria or heavily silicified diatoms (Wright *et al.*, 1997).

Lynch and Keenan (2015, unpublished data) reported reduced chlorophyll-a concentrations at all sites after moderate and high flow events in the Nepean River. They found that chlorophyll-a did not provide an accurate indicator of periphyton biomass, due to the high abundance of small-celled cyanobacteria, low photosynthesis during early succession and the low extraction concentrations by acetone when cyanobacteria were dominant. They recommended trialling alternative solvents for extraction of photosynthetic pigments in the cyanobacteria dominated river.

1.8 Objectives

The aims of this study are to:

- establish the most efficient solvent for extracting photosynthetic pigments from periphyton present in the Nepean River, NSW;

- investigate if there is a relationship between current velocity and periphyton community structure in the Nepean River;
- assist in understanding the response of periphyton community structure to changes in current velocity and river flows events;
- provide information to enable prediction of how environmental flows from Warragamba Dam may improve periphyton community structure by assessing responses of periphyton to flow events during the study;
- determine whether periphyton taxa have current velocity thresholds for community change;
- report periphyton community structure present in the Nepean River, noting any longitudinal changes during the study and from the previous 2015 study.

2. Materials and methods

2.1 Study sites

A pilot study was conducted at two riffle sites in the Nepean River. Site R1, the Nepean River at Penrith and R2, the Nepean River at Yarramundi. Figures 4a – 4g show the location of the sites in the catchment along with specific features and locations of each of the sites. To reduce the confounding factor of increased nutrients from the catchment, R1was upstream of both Peachtree and Boundary creeks, which are contributors of urban stormwater. R2 is also impacted by discharges from Winmalee WWTP and agricultural runoff downstream of Penrith. This site was only sampled during the pilot study in November 2015. Both sites are surrounded by a combination of different landuses including urban, farming and industry extraction activities. Geographic locations of the study sites are listed in Table 3.

Following the pilot study, R1 was chosen as the preferred study site. It is similar to many other the cobble dominated riffle sites in the Nepean River downstream of Penrith Weir and has consistent temperature, turbidity and nutrient inputs across the whole riffle, as the water comes from Penrith Weir immediately upstream, either passing through the fishway or over the weir. Figure 4e). The riffle at R1 consisted of zones varying in current velocities, eliminating the need to construct artificial streams or flumes in a manipulated experiment. Figure 5 illustrates the zonation of the riffle into different velocities.

Table 3. Study site locations in the Nepean River, NSW.

Site Name	Longitude/latitude	Site description
R1	-33.740280, 150.685481	Nepean River@ Penrith
R2	-33.612254, 150.700223	Nepean River@Yarramundi

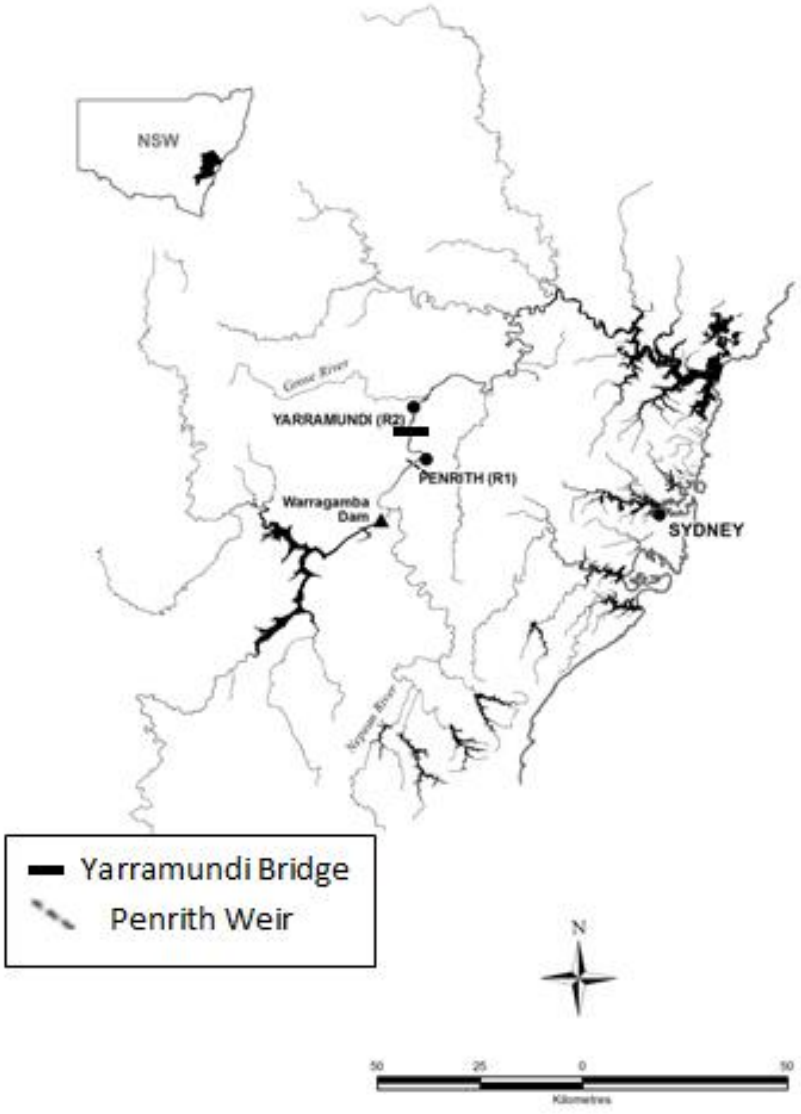

Figure 4. Periphyton sampling sites in the Nepean river. R1 (Nepean River at Penrith) and R2 (Nepean River at Yarramundi from November 2015 – May 2016.

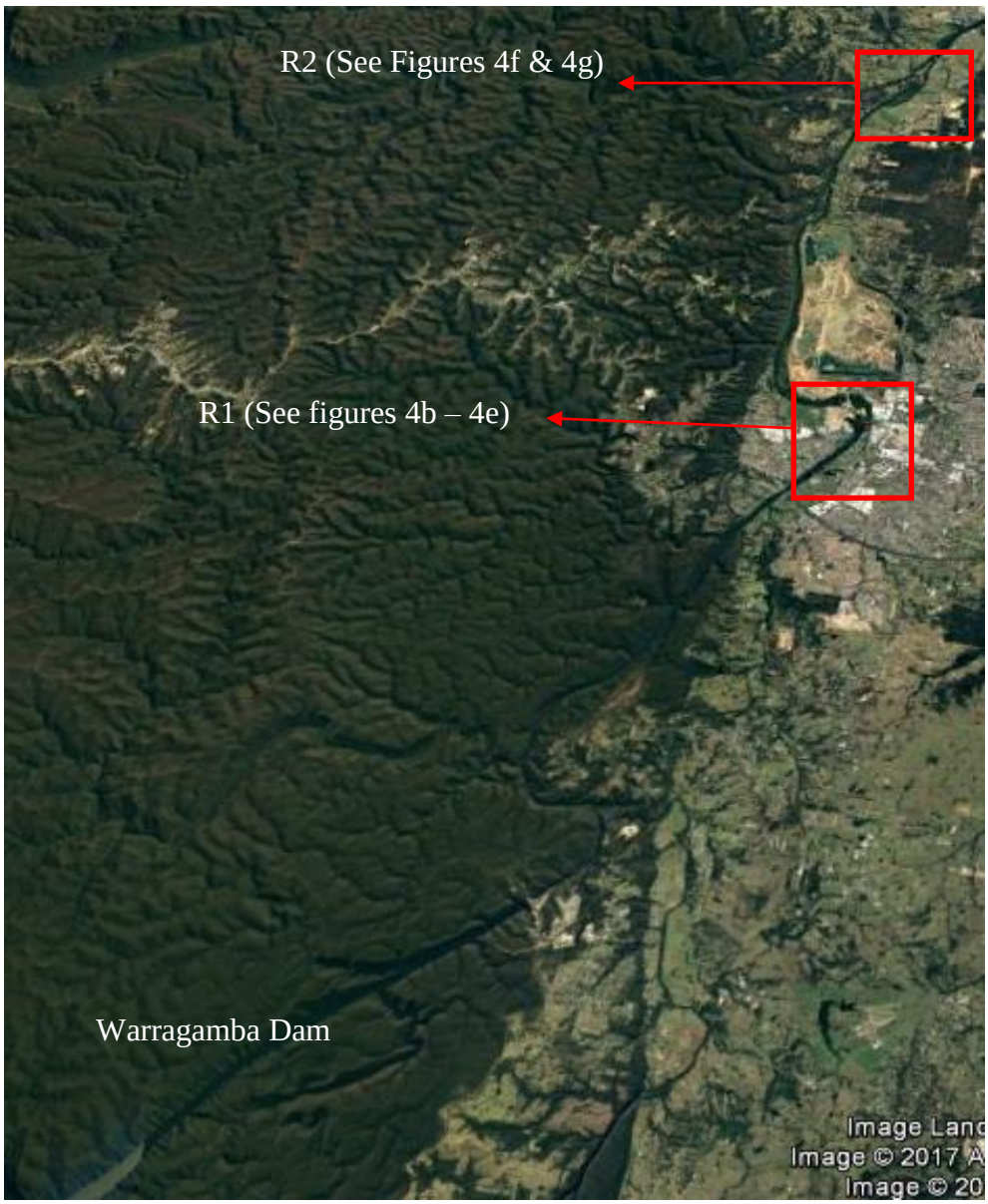

Figure 4b. Warragamba Dam shown with proximity to sampling sites in the Nepean River. Insets shown below

Figure 4c. Inset:Nepean River at Penrith sampling site (R1)

Figure 4d. Inset: Nepean River at Penrith sampling site (R1) showing distance of riffle from Penrith Weir (78 metres)

Figure 4e. Nepean River at Penrith sampling site (R1), showing Penrith Weir upstream (Photo: K Lynch 1/5/16), at a flow of 136 ML/d.

Figure 4f. Inset: Nepean River at Yarramundi sampling site (R2) and locationto the unregulated Grose River. This site was sampled during the pilot study in November 2015.

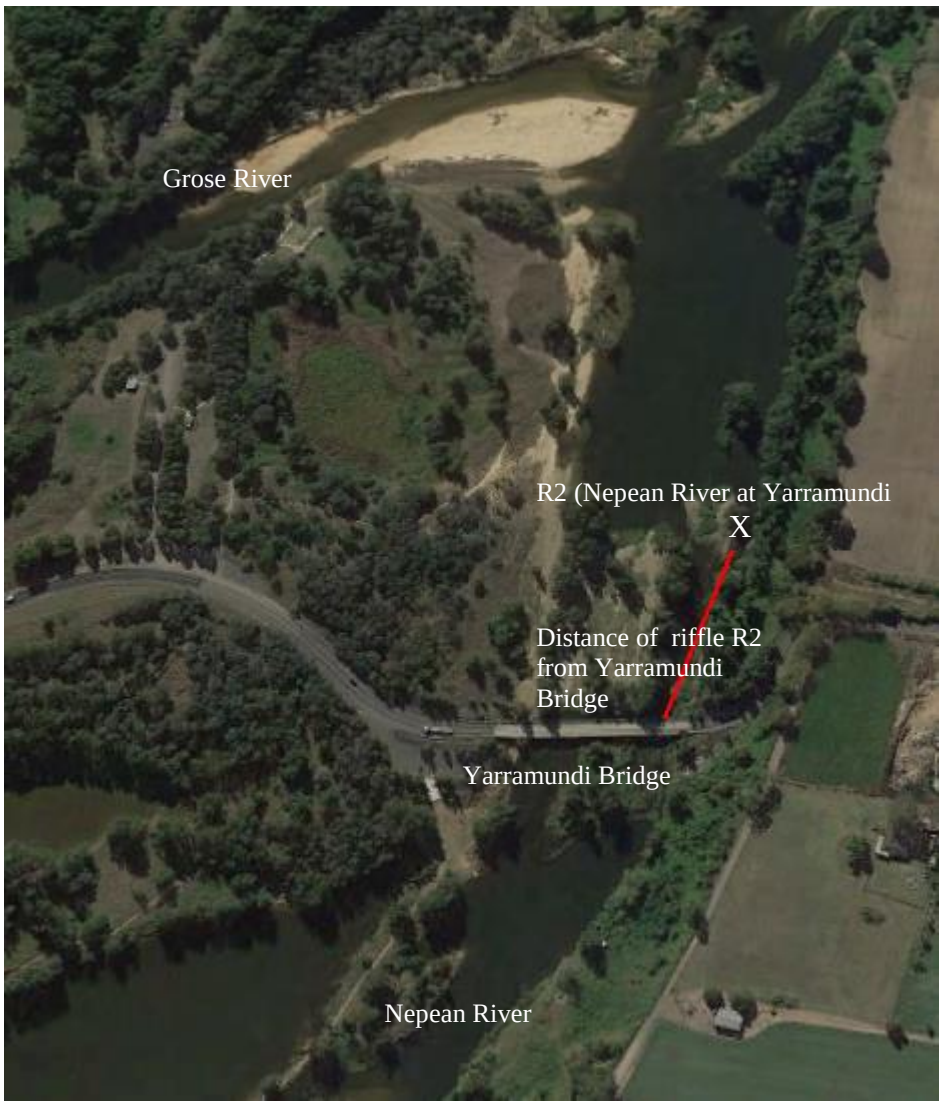

Figure 4g. Inset: Nepean River at Yarramundi sampling site (R2). This site was sampled during the pilot study in November 2015.

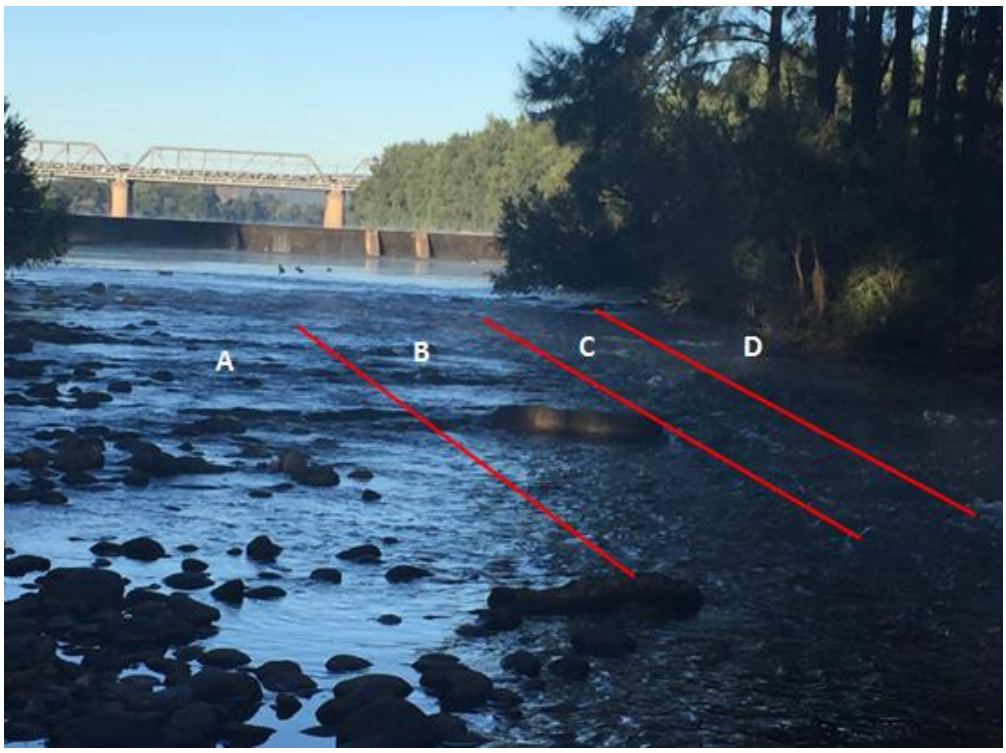

Figure 5. Riffle site R1 (Nepean River at Penrith) showing varying current velocity zones Current velocity ranges measured within the riffle during sampling were: (A)<0.4m/s;(B) 0.4-0.8m/s;(C)0.8-1.2m/s; (D)>1.2m/s

2.2 Current velocity

For each cobble sampled, the current velocity (m/s) immediately above the cobble was recorded using a Pygmy flow meter (CMC200, Hydrological Services, Australia). The depth (m) was also recorded for each sample.

2.3 Stream-flow gauging

Stream flow was measured at the two sites by WaterNSW. The gauges record water level at 15 minute intervals and then convert level to flow in Hydstra using ratings tables and cross sections. Site R1, Nepean River at Penrith is approximately 700 m downstream of the gauge at Penrith Weir (212201) and Site R2, Nepean River at Yarramundi, is around 100 m downstream of the gauge at Yarramundi Bridge (2122001). The daily river flow data are an indicator of any flow events that may affect the periphyton abundance or community structure. Flow measurements do not directly relate to current speed as they are a function of the stream cross-sectional area (Townsend *et al.*, 2012). Flow measurements were not converted to current velocity (m/s); current velocity is highly

variable based on local stream structure such as substrate type, bed configuration, cross-sectional shape and macrophyte abundance. current velocity

2.4 Water quality sampling

Water quality results were obtained from water quality sampling conducted by WaterNSW (monthly) and Sydney Water (3 weekly), these are shown in Table 4. On the days of periphyton sampling, in situ physico-chemical readings with a calibrated TPS 90FL-T multi-function water quality meter and logger (Thermo Fisher)were taken, and water samples collected for TP, TN and chlorophyll-a. The samples for TN and TP analysis were frozen and phytoplankton chlororophyll-a samples were chilled to 4^{o}C and transported to SGS Environmental (Alexandria) for analysis. Water quality parameter averages were taken over the sampling period of this study from the three analysis sources. The monthly water quality and grab samples provided on the day of sampling were considered a reasonable representation of the water quality in the river over the study period, particularly given the available data and budget constraints.. The effect of daily water quality changes on periphyton would not be seen in a sample taken on that day: periphyton growth is a result of longer-term water quality and will not respond instantly in response to a water quality event, but in days to weeks to changes in environmental conditions (Gaiser, *et al.*, 2005).

2.5 Periphyton sampling and analysis methods

Periphyton was sampled at R1 in November 2015, February and May 2016 for this study. On each occasion, cobbles were randomly selected across a 15 m by 40 m area of the riffle. Sampling was conducted from downstream to upstream to minimise disturbance (Lower *et al.*, 1996). The cobbles were numbered and placed in individual plastic containers with current velocity and depths recorded. Cobble size was deemed appropriate if it measured between 60 mm and 150 mm across the longest axis. Cobble surface area was quantified by using the aluminum foil wrapping technique described in Lowe *et al.* (1996) and applying the regression formula outlined in Hauer and Lamberti (2006), by measuring a known area of foil (i.e. 10 x 10cm). This was weighed, and an average of three foil weights was used in the formula (known weight). Colonised cobble surface area was normalised assuming only half of the total cobble surface area was exposed to light and therefore able to be colonised (Biggs and Hickey, 1994).

Over the study period, 93 cobbles were collected (including the 12 cobbles sampled from R2 during the pilot study). Appendix 4 shows the dates, deths and current velocities for the sampling times. Each cobble was scrubbed vigorously using a stiff nylon brush to remove periphyton. The material scrubbed from the rock was retained in a bucket. This technique of manually scrubbing the cobbles removes more than 95% of algae present (Hill *et al.*, 2008; Davie *et al.*, 2012). Both the scrubbing brush and the bucket were thoroughly rinsed with clean water in between samples, so as to avoid cross-contamination. The slurry from each sample that was retained in the bucket after scrubbing was transferred to a labelled bottle and made up to 500 mL. The slurry was then homogenised in a blender for approximately 50 seconds. From this volume, a subsample of 5 mL was removed for chlorophyll-a analysis. Three mL of the preservative Lugols' Iodine was added to the remaining sample, to preserve the algae for identification and enumeration. Samples were stored in a dark cool place until analysis.

Algal samples were analysed by the NSW DPI Water's Algal Laboratory at Menangle (NATA Accredited) using the following method: a 0.5 mL aliquot of the preserved sample in a Lund cell. Cells were counted under an inverted microscope with bright field illumination at 200x magnification. A minimum of 50 units (colonies, filaments or single cells) of the dominant cyanobacteria taxon, or 30 units of the dominant eukaryotic taxon, were counted, and all other algae seen identified and counted, to provide a precision of 28 and 36% respectively (Lund *et al.* 1958). Benthic algae were identified to genus level by using the keys of Gell *et al.* (1999) and Sonneman *et al.* (2000).

Results were reported in cells/ml. Total cell counts were then calculated using the original sample volume (500 ml) and divided by the normalised cobble surface area, providing cell counts per cm^2.

2.6 Chlorophyll-a method and analysis

The 5 mL subsample from the homogenised 500 mL sample was filtered onto Whatman GF/C glass fibre filters using a Buchner funnel with a handheld vacuum pump. The filters were removed with tweezers, folded in half, wrapped in aluminium foil, labelled and stored in a snap lock bag in the freezer until analysed.

2.7 Preparation of the extraction solvents

2.7.1 Acetone

A saturated $MgCO_3$ solution (1.0 g $MgCO_3$ in 100 ml deionised water) was added to the 90% acetone (90 parts acetone solution to 10 parts $MgCO_3$, resulting in a 90% buffered aqueous acetone solvent (900 mL acetone to 100 mL deionised water).

2.7.2 Ethanol

Magnesium carbonate (0.3 g) was added directly to 100% ethanol (as above) and mixed well, resulting in a buffered ethanol solution. Ethanol was pre-heated in a water bath to 78^{o}C (Sartory and Grobbelaar, 1984) before adding to the centrifuge tubes.

The folded filter pads (previously frozen) were placed in centrifuge tubes, and 10 mL of the buffered acetone solution or buffered Ethanol solution was added. A glass rod was used to grind and rupture algal cells, enabling greater penetration of the solvent to the photosynthetic material on the filter. The centrifuge tubes were then covered with aluminium foil excluding light and left at 4^{o}C in a refrigerator.

After 24 hours, the centrifuge tubes (still containing filters) were placed in the Eppendorf 5810R Centrifuge for 15 minutes at 4000 rpm.

2.7.3 Comparison of acetone and ethanol as extractants for chlorophyll-a analysis

Duplicate sub-samples were taken from the 500 mL periphyton slurry from each sample and filtered for chlorophyll-a analysis. Filters were wrapped in foil and frozen for four months before analysis, which was conducted using both acetone and ethanol to compare the effectiveness of the two solvents as an extractant for benthic algal pigments. Ethanol was chosen as the alcohol solvent for comparison over methanol, as similar results had been reported for the two solvents and ethanol was considered to be a less toxic alternative (Sartory and Grobbelaar, 1984; Porra et al., 1989; Ritchie, 2008). Calculations for the comparison were made for each 5 mL subsample and normalised to surface area of the cobble (mg/m^2). The UNESCO coefficient for the use of acetone and the Kaczmar coefficient for the use of ethanol were selected to compare the efficiency of the two solvents (Kaczmar, 2004; Henriques *et al.*, 2007).

A sufficient volume of the extract to read (>4 mL) was pipetted into a 1cm spectrophotometer cuvette. Solvent blanks were used to zero at 750 nm (Ritchie, 2006) and the absorption readings of chlorophyll-a in each sample was recorded using a Shimadzu UV-mini 1240 Spectrophotometer.

To correct for pheophytin, 0.1 mL 1 M HCl solution (0.83 mL HCl to 100 mL deionised water) was added to cuvettes and left for 60 seconds. The absorbance of the acidified extracts was read at 750 nm, 665 nm and 664 nm, in accordance with standard American Public Health Association methods (APHA, 1995).

2.8 Statistical Analysis

Univariate and multivariate analyses based on square root transformed data were used in combination to detect differences in abundance and community composition of periphyton over the three sampling occasions. Due to the high abundance of taxa present in samples, data were transformed using a square root transformation before analysis.

A stepwise, distance-based linear model (DistLM, McArdle and Anderson, 2001) was performed to identify which environmental variables contributed most to the variation in periphyton community structure. DistLM was conducted using PRIMER 7. Variables used in this analysis included time, current velocity, chlorophyll-a, depth and cobble size.

Differences in the periphyton taxonomic composition over the sampling period were examined using analysis of similarity (ANOSIM) and a similarity percentage (SIMPER) analysis. ANOSIM was used to examine statistical significance between samples and SIMPER analysis was used to identify algal taxa contributing most to the similarity within groups of samples. SIMPER analysis was used to identify taxa that contribute the most to the similarity in community composition between samples of the same group or taxa that contribute the most to dissimilarity in community composition across groups of samples. SIMPER, ANOSIM analyses and 2-dimensional nonmetric multidimensional scaling (nMDS) ordination plots were done using Primer 7 (Clarke & Gorley, 2015).

All data were square root transformed as it down-weighted the effect of abundant algal taxa sufficiently for the rarer taxa to be observed, whilst still enabling the relative differences in abundance of taxa to influence the patterns in assemblage structure. Pearson's correlation (r) and linear regression analyses (R^2) to relationships between current velocity measurements, depths, abundance and chlorophyll-a using EXCEL were performed.

Threshold Indicator Taxa Analysis (TITAN) (King and Baker, 2010; Baker et al., 2015) was used to identify changes in periphyton taxon abundance and frequency along the current velocity gradient. This assesses the synchrony among taxa change points as evidence for community thresholds (Spietz *et al.*, 2015). In order to accurately calculate an indicator value (IndVal) for each taxon, the taxon must be present in three or more samples. Therefore, taxa present in fewer than three samples were eliminated from each subsampled dataset. TITAN was run using R (R Development Core Team, 2015).

TITAN uses indicator species scores to integrate occurrence, abundance and directionality of taxa responses. It identifies the optimum value of a continuous variable, x, that partitions sample units while maximizing taxon-specific scores. Indicator z scores standardize original scores relative to the mean and SD of permuted samples along x, thereby emphasizing the relative magnitude of change and increasing the contributions of taxa with low occurrence frequencies but high sensitivity to the gradient. TITAN distinguishes negative (z−) and positive (z+) taxa responses and tracks cumulative responses of declining [sum(z−)] and increasing [sum(z+)] taxa in the community. Bootstrapping is used to estimate indicator reliability and purity as well as uncertainty around the location of individual taxa and community change points. Z-scores are further analysed across taxa filtered by purity and reliability associated with each indicator direction (- or +) at each level of the environmental gradient. Filtered taxa with a value of either 1 or 2 are pure and reliable decreasers or increasers, respectively, and are selected for summation. Filtered sums are more precise and are used to create estimates of robust community change (Baker *et al.*, 2015).

Chlorophyll-a was compared over the three sampling dates using a 1-way ANOVA. Comparisons were made between biomass over time and differences between acetone and ethanol as extractants (2-way ANOVA). The significance level (α) for all inferential analyses was 0.05.

3. Results

3.1 Pilot study results

On 12 November 2015, a pilot study was conducted where 13 individual cobbles were sampled at R1 (Nepean River at Penrith) and 12 cobbles from R2 (Nepean River at Yarramundi). The depth and current velocities were recorded for each cobble using a pygmy flow meter (CMC200, Hydrological Services, Australia). Depths and velocities for each cobble sampled are listed in Appendix 4.

3.1.2 Periphyton results in the pilot study

Comparisons between the two Nepean River sites showed that R2 was found to higher overall cell abundance compared to R1 (Figure 6). R2 was also found to have greater abundance of filamentous green algae and the charophyte, *Coleochaete* (Figure 7) and greater contributions from diatoms compared to R1 (Table 5). Both sites were dominated by cyanobacteria.

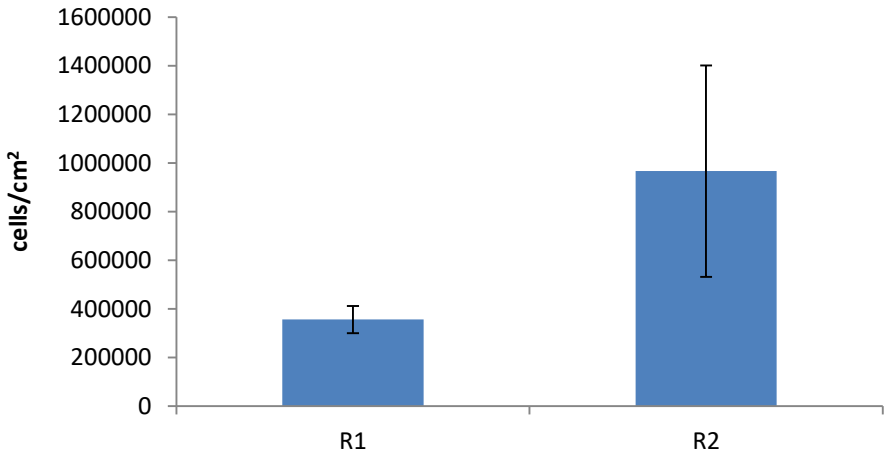

Figure 6. Average total cell abundance for periphyton sampling at R1 and R2 on 12/11/16. Error bars represent the standard error of the mean (SEM).

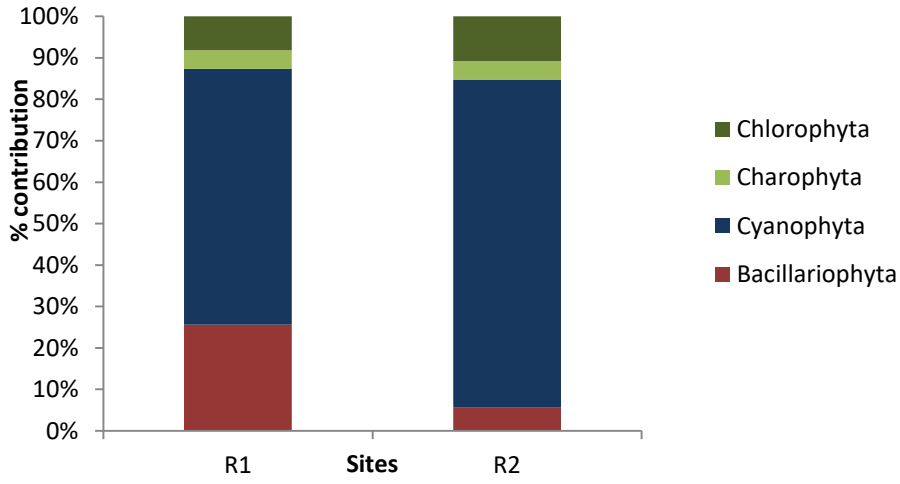

Figure 7. Percentage contribution of dominant algal groups present at R1 (Nepean River at Penrith) and R2 (Nepean River at Yarramundi) on 12/11/16.

Water quality measurements taken at R2 reported higher concentrations of total nitrogen (TN), total phosphorus (TP) and phytoplankton chlorophyll-a (Table 4) compared to R1. Site R2 had higher biomass as measured by chlorophyll-a concentrations (Figure 8). This was consistent with Lynch and Keenan (2015, unpublished data) which showed higher algal abundance and greater contributions from filamentous green taxa at R2 compared to R1 (Table 5). Forty-one genera were identified at R1 compared to 46 genera at R2. R2 was found to have more green algal genera compared to R1 (18 chlorophyta at R2 compared to 14 at R1. Water quality measurements taken at R2 reported higher concentrations of TN, TP and phytoplankton chlorophyll-a (Table 4) compared to R1.

Table 4. Average water quality results in the Nepean River between August – November 2015 (Sydney Water 2016). Standard error of the mean (SEM) is also shown.

Site		Total nitrogen	Total phosphorus	Temperature	Chlorophyll - a
		mg/L	mg/L	^{o}C	µg/L
R1 (Nepean River at Penrith)		0.566	0.019	17.5	5.93
	SEM	0.064	0.006	1.58	0.46
R2 (Nepean River at Yarramundi)		0.79	0.028	18.26	8.68
	SEM	0.09	0.004	2.01	0.74

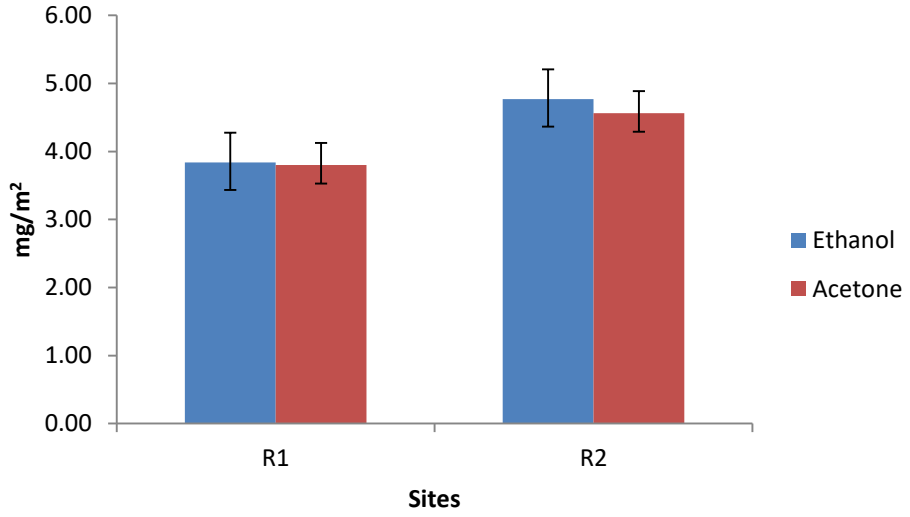

Figure 8. Average chlorophyll-a concentrations (mg/m^2) at R1(Nepean River at Penrith) and R2(Nepean River at Yarramundi) on 12 November 2015. Error bars show the standard error of the mean (SEM).

SIMPER analysis reported the main contributors of similarity for R1 (average similarity: 64.37) included *Leptolyngbya, Navicula, Achnanthes* and *Monoraphidium*. The major contributors to the differences in periphyton assemblages between R1 and R2 (dissimilarity = 42.38) were *Stigeoclonium, Cocconeis, Oscillatoria* and *Oedogonium*.

Table 5. Abundance and percentage contributions from dominant algal groups for samples taken 12 November 2015 at R1(Nepean River at Penrith) and R2(Nepean River at Yarramundi).

	R1 (Nepean River at Penrith)		R2 (Nepean River at Yarramundi).	
	Average cell abundance (cells/cm^2)	Percentage contribution (%)	Average cell abundance (cells/cm^2)	Percentage contribution (%)
Bacillariophyta	90,918	26	54,652	6
Cyanophyta	219,307	62	762,661	79
Charophyta	16,089	5	44,356	5
Chlorophyta	28,836	8	103,673	11

Non-metric multidimensional scaling (nMDS) separated R1 and R2 from each other based on community assemblage Figure 10). Current velocities of cobbles sampled ranged from 0.3 – 1.57

m/s at R1 and 0.58 – 1.31 m/s at R2. The nMDS plot (Figure 9) shows the clustering of similarities of community assemblages. The current velocity range is represented by the overlying bubbles. The clustering of assemblages was found in the current velocity ranges of 0.8 – 1.4 m/s for site R1 (blue).

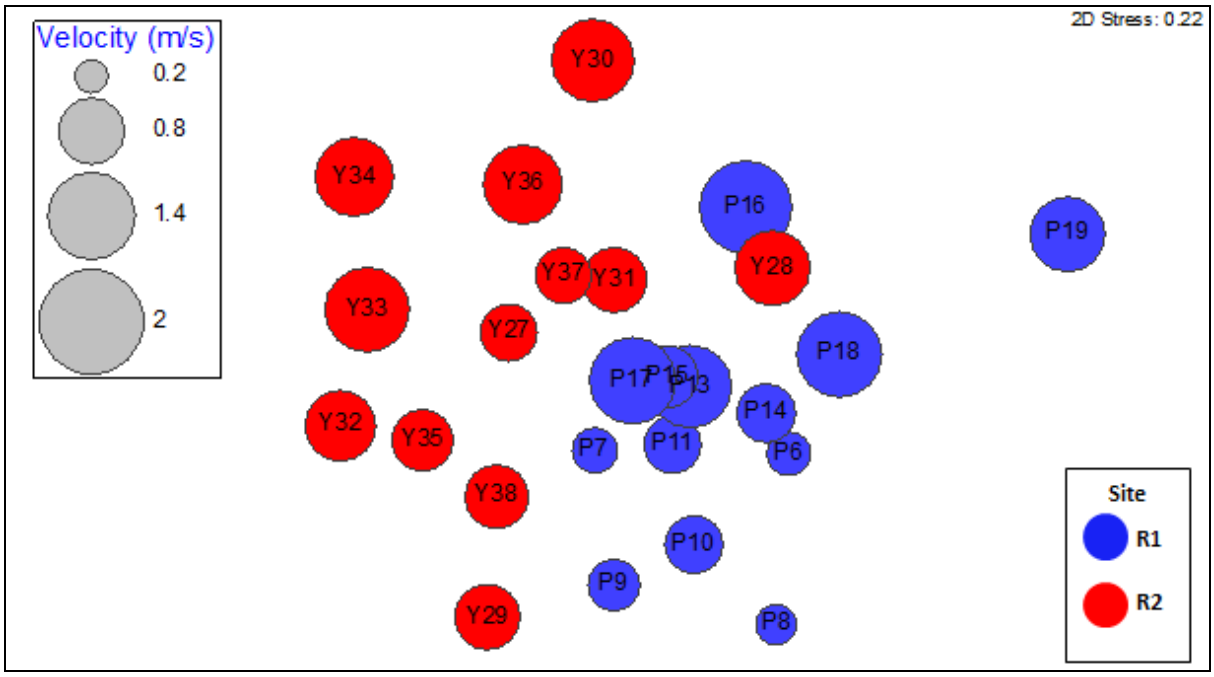

Figure 9. Non-metric multidimensional scaling (nMDS) plot of periphyton assemblages on individual cobbles at R1, Nepean River at Penrith (Blue) and R2, Nepean River at Yarramundi (Red) during sampling 12/11/2015. Circle size indicates current velocity (m/s) recorded immediately above the sampled cobble; the larger the circle, the greater the current velocity.

3.1.3 Site selection and solvent chosen for the study

R1 (Nepean River at Penrith) was selected for further study, as it was closest to Warragamba Dam and upstream of WWTPs, reducing the confounding factor of nutrient inputs. It was also a large, stable cobble-dominated rifle with multiple current velocity zones.

ANOVA analysis showed no significant difference in chlorophyll-a between the solvents, acetone and ethanol ($P>0.05$) at R1, however, ethanol extracted greater concentrations of chlorophyll-a from R2 samples compared to acetone and a significant difference between the solvents was reported ($P<0.01$) between samples at this site (Table 6).

Table 6. Analysis of variance (ANOVA) comparisons of acetone and ethanol, as solvents for extracting periphyton at R1 (Nepean River at Penrith) and R2 (Nepean River at Yarramundi) sample on 12 November 2015.

Source of Variation	*F*	*P-value*	*F crit*
R1 acetone and ethanol	0.929892	0.345372	4.30095
R2 acetone and ethanol	8.774672	0.007197	4.30095

As no significant difference was found between the solvents used for extracting chlorophyll-a from samples from R1, acetone was selected as the solvent to analyse chlorophyll-a to calculate biomass for the remainder of the study.

3.2 Results of the main study

3.2.1 Hydrology of the Nepean River during the study

Unregulated flows in the Nepean River were measured by WaterNSW at Penrith gauging station number 212201). Average daily flow data was supplied by WaterNSW for the 100 days prior to the study (1/8/2015) until after the completion of the study (10/7/2016). Average daily flows and peak flows preceding sampling are shown in Table 7. Figure 10 illustrates the flows in the Nepean River at Penrith. The gauge is approximately 800m upstream of the sampling site R1.

Table 7. Average daily river flow and peak flow magnitudes for the Nepean River at Penrith during periphyton sampling from 1/8/2015 – 10/7/2016.

Nepean River at Penrith flow (ML/day)	**T1**	**T2**	**T3**
	12/11/2015	**25/2/2016**	**30/5/2016**
Average daily flow	400	675	185
Peak flow	1046	5440	338

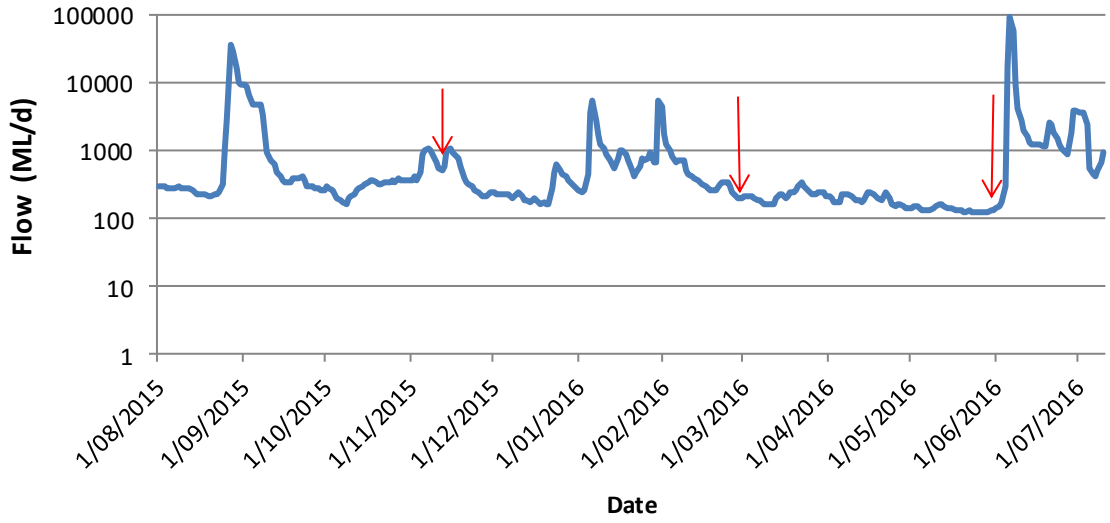

Figure 10. Flow at Nepean River at Penrith Weir daily average flow from 1/8/2015 – 10/7/2016 (212201). The red arrows represent sampling dates.

Between August 24th and 26th 2015, over 300 mm rain fell over the Sydney catchment area. This caused Warragamba Dam to spill on 26 August, leading to minor flooding in the Hawkesbury-Nepean River (BOM, 2016). A flow of over 36,000 ML/d was recorded in the Nepean River at Penrith Weir on 27/8/15 (Figure 10). Following this, flows averaged 400 ML/d until a peak flow of 1046 ML/d on 7/11/15. Sampling was conducted on the 12/11/15 (T1). Flows averaged 675 ML/d following this sampling with two peak flows of 5440 ML/d on 6/1/16 and 30/1/16. Sampling (T2) was conducted on 25/2/16, 26 days after the peak moderate flow. Flows remained very low after this sampling, averaging 185 ML/d up to T3 sampling on 30/5/16, with the highest daily flow recorded as 338 ML on 22/3/16 (Figure 10).

Near bed current velocities during sampling ranged from 0.3-1.57 m/s on T1, 0.14-1.79 m/s on T2 and 0.2-1.64 m/s on T3 Figure 11 & Appendix 4). The depth of sampled cobbles ranged from 0.05 – 0.45 m.

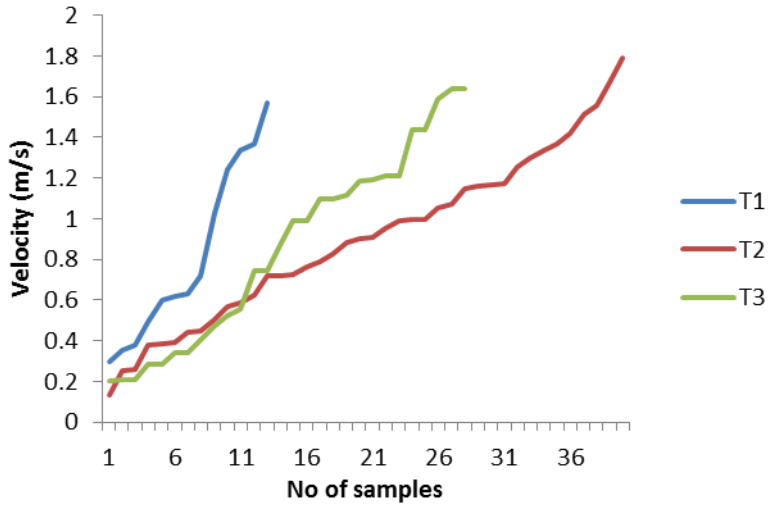

Figure 11. Current velocity ranges for periphyton sampling on T1 (12/11/2015),T2(25/2/2016 and T3(30/5/2016) at Nepean River at Penrith

There was a reduction in cell abundance between T1 and T2 which coincided with the river flow event of over 5440 ML/d on 30 January. This reduced the periphyton, but did not totally remove it. Average abundance increased during sampling at T3 from T2 (Figure 12).

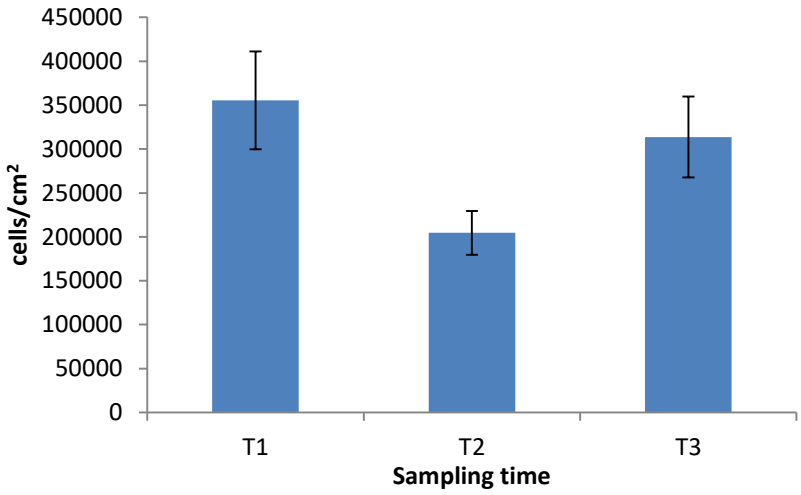

Figure 12. Average periphyton cell abundance from samples taken in the Nepean River at Penrith. T1 (12 November 2015) ,T2 (25 February 2016) and T3 (30 May 2016).

Percentage contribution for the total of all of the samples on each sampling date was calculated, Cyanobacteria dominated the samples over the three sampling occasions, at between 62% and 65% of the total abundance (Figure 13), with *Leptolyngbya* the most abundant genus. Changes in the contributions of diatoms (Bacillariophyta), filamentous green algae (Chlorophyta) and the charophyte, *Coleochaete* (Charophyta) were observed during the study. Diatoms were reduced at T2 but had increased by T3, contributing 30% of the total abundance. Charophytes were most abundant at T2, and green algae least abundant at T3.

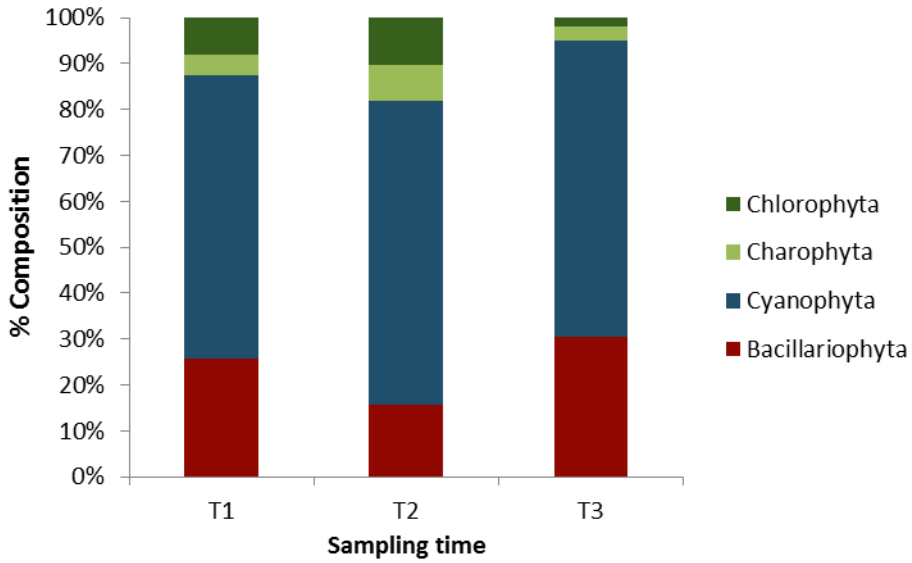

Figure 13. Changes in percentage abundance of the dominant algal groups for the three sampling occasions, November 2015 (T1), February 2016 (T2) and May 2016 (T3) in the Nepean River at Penrith.

3.2.2 Water quality in the Nepean River in relation to flow events

Figure 14 shows the response of water quality parameters to river flow. All parameters increased from T1 to T2, then decreased again at T3, consistent with the changes in flow over the study period. Temperature was consistent with seasonal temperatures, increasing after October 2015 and remaining above 19^{o}C until April 2016, where late autumn led to drops in temperature to 15^{o}C and 14.8^{o}C at T3 sampling.

TN increased from 0.57 mg/L to 0.6 mg/L at T2, and decreased to 0.42 mg/L at T3. Similarly, TP increased from 0.019 mg/L to 0.049 mg/L then decreased to 0.016 mg/L at T3 (Figure 14).

Phytoplankton chlorophyll-a was higher during the warmer seasons (December – April) but declined after each flow, as resident algal populations were likely washed out of the weir pool upstream by the higher flows. A large reduction in chlorophyll-a, to less than 1 µg/L, was reported by Sydney Water and WaterNSW after high flows in June 2016.

Table 8. Average water quality in the Nepean River at Penrith 1/8/15 – 30/5/16.

	T1	T2	T3
Water quality parameter	12/11/2015	25/2/2016	30/5/2016
Total nitrogen (mg/L)	0.566	0.601	0.419
Total phosphorus (mg/L)	0.019	0.049	0.016
Temperature (°C)	17.5	24.1	21.4
Chlorophyll-a (µg/L) (phytoplankton)	5.9	13.8	9.29

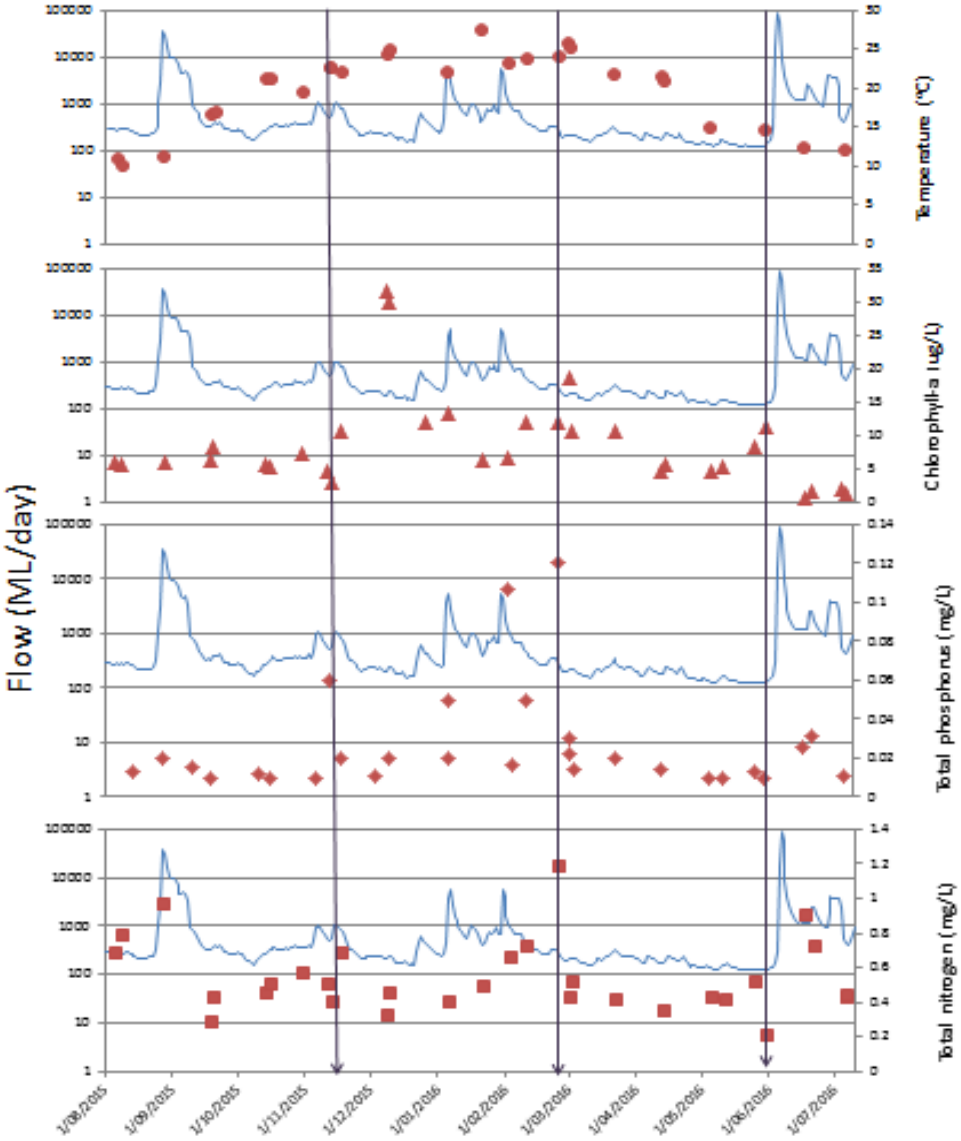

Figure 14. Nepean River flow and water quality parameters taken in the Nepean River at Penrith from 1/8/2015 – 10/7/2016. Red symbols show the results for each sampling occasion and the arrows indicate periphyton sampling dates (T1 – T3). Data provided by SydneyWater and WaterNSW, in addition to data collected during the study.

3.2.3 Changes in periphyton chlorophyll-a during the study

Concentrations of periphyton chlorophyll-a decreased slightly, but not significantly (P>0.05) between T1 and T2, and increased significantly (P<0.01) from T2 to T3. Figure 15 shows the decline from sampling T1 and then increase in chlorophyll-a concentrations during the study, at T3. The moderate flow of 5440 ML/d on 30/1/16 coincides with reduced overall abundance of periphyton and, in particular, filamentous green algae.

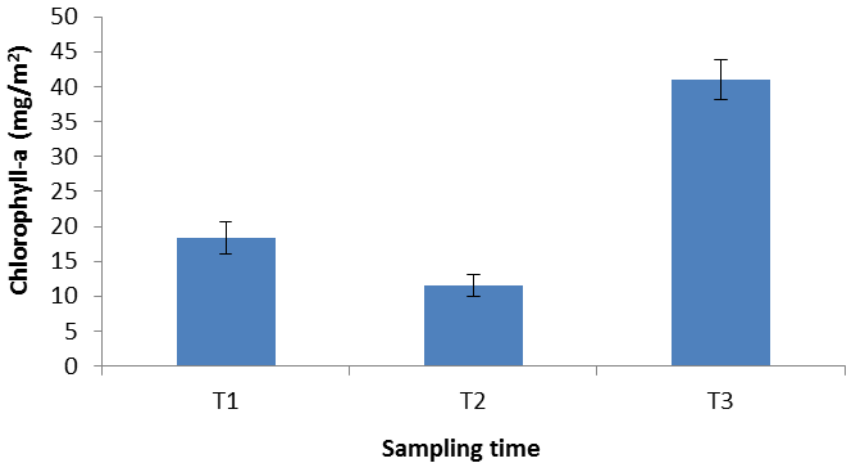

Figure 15. Changes in periphyton chlorophyll-a concentrations from T1 (12 November 2015) ,T2 (25 February 2016) and T3 (30 May 2016). in the Nepean River at Penrith.

For all sampling occasions, weak linear relationships between chlorophyll-a and current velocity were found (R^2=0.1) during the study. Correlations were found between current velocity and periphyton abundance (r=-0.45), however, abundance and chlorophyll-a during the study was not found to be correlated (r=-0.06).

3.2.4 Changes in cell abundance and periphyton assemblage during the study

There was a significant difference in community composition over the three sampling times (ANOSIM, Global R= 0.635; p = 0.01). An nMDS plot (Figure 16) highlights changes in assemblage over sampling times, with current velocity illustrated as bubble plots overlaid on the nMDS plot. T1 and T2 were clustered closer together than T3, suggesting that the variation in community composition was greater at T3 than on the preceding sampling periods. The overlain current velocity bubbles indicate clustering of comparable velocities in the 0.8 – 1.4 m/s range in all sampling times.

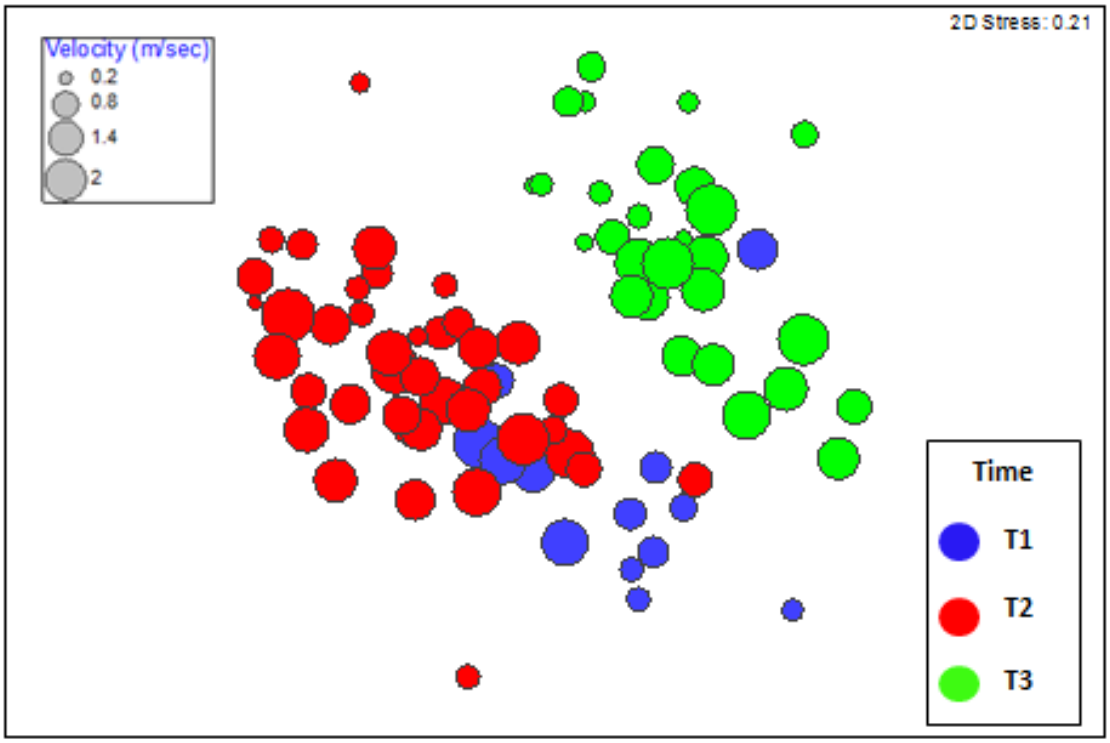

Figure 16. Non-metric multidimensional scaling (nMDS) plot of periphyton community changes on individual cobbles over sampling times.

The blue circles represent T1, red represents T2 and the green circles represent T3. The size of the circle represents the current velocity, with the greater the circle size, the greater the near bed current velocity above the sampled cobbles.

Distance based linear models (DistLM) reported time as the most significant variable (P=0.001) accounting for 11.6% of the differences. Current velocity and depth were also reported as significant variables. Cobble size was not considered significant (P=0.06,Table 9).

Table 9. Distance based linear models (DistLM) results of variables using transformed: square root data for periphyton sampling across the study, 11 November 2015 – 30 May 2016 in the Nepean River at Penrith.

Variable	Pseudo-F	P value	Proportion of variability (%)
Time	10.12	0.001	11.6
Velocity	3.54	0.001	4.4
Depth	2.3	0.012	2.9
Cobble size	1.79	0.06	2.2

When sampling period (time) was removed as a variable and individual sampling days were analysed, current velocity and depth were significant at T1, accounting for 19% and 16%

respectively. Current velocity was a significant variable (6% and 14% respectively) at T2 and T3, whereas depth and cobble size were not significant (Table 10).

Table 10. Distance based linear models (DistLM) results for variance, with sample time removed, using transformed: square root data for each sampling date T1 (12 November 2015) ,T2 (25 February 2016) and T3 (30 May 2016).

Time	**Variable**	**Pseudo-F**	**P value**	**Proportion of variability (%)**
T1	Velocity	2.63	0.006	19
	Depth	0.82	0.004	16
	Cobble size	2.57	0.6	5
T2	Velocity	2.35	0.012	6
	Depth	1.63	0.08	4
	Cobble size	0.84	0.6	2
T3	Velocity	4.36	0.001	14
	Depth	1.04	0.45	3
	Cobble size	0.82	0.6	3

Eighty-four genera were identified during the study. Diatoms, cyanobacteria and green algae were the dominant algal groups in the periphyton on each sampling occasion (Table 11). SIMPER analysis reported that six genera contributed to over 70% of the similarity of assemblages at T1 (*Leptolynygbya, Navicula, Heteroleibeinia, Achnanthes, Coleochaete* and *Synedra*). T2 had eight taxa that contributed to over 70% of the assemblage: *Leptolynygbya, Coleochaete, Fragilaria, Navicula, Monoraphidium, Heteroleibeinia, Cymbella* and *Oedogonium* (Appendix). T3 had five genera that together contributed over 70% similarity in assemblages (*Leptolynygbya, Navicula, Achnanthes, Fragilaria* and *Gomphonema*). There were a total of 63 genera at T1, 66 genera at T2 and 52 genera at T3 (Appendix 2).

Table 11. Dominant algal groups showing number of genera for periphyton sampled in the Nepean River at Penrith from T1 (12 November 2015) ,T2 (25 February 2016) and T3 (30 May 2016)..

	T1	T2	T3	
	12/11/2015	25/2/2016	30/5/2016	Total
Bacillariophyta	20	22	17	27
Cyanophyta	17	14	13	19
Charophyta	1	1	1	1
Chlorophyta	20	23	20	31
Other	5	6	1	7
Total genera	**63**	**66**	**52**	**84**

SIMPER analysis indicated that nine periphyton genera were associated with over 50% of the dissimilarity between T1 and T2 (average dissimilarity = 51.64). Differences between T1 and T2 were due to reductions in *Leptolyngbya, Heteroleibleinia, Navicula, Synedra, Achnanthes, Geitlerinema, Coleochaete* and *Palmellopsis,* while *Fragilaria* increased Table 12). Periphyton assemblages were most similar between T1 and T2. This was also indicated in the nMDS plot shown in Figure 16.

Ten genera were associated with over 50% of the dissimilarity between T2 and T3 (average dissimilarity = 55.00). Increases were observed in *Leptolyngbya, Achnanthes, Heteroleibleinia, Navicula, Coleochaete, Rhoicosphenia, Fragilaria, Geitlerinema* and *Achnanthidium.* Reductions in the abundance of *Monoraphidium* occurred from T2 to T3(Table 12). Eleven genera were associated with >50% of the difference between T1 and T3 (average dissimilarity = 52.11). A reduction in the abundances of *Leptolyngbya, Heteroleibleinia, Geitlerinema, Coleochaete, Synedra, Navicula, Palmellopsis* and *Phormidium* were observed between T1 and T3, while the abundances of *Fragilaria, Achnanthes* and *Rhoicosphenia* increased.

Table 12. Dominant algal groups showing number of genera for periphyton sampled in the Nepean River at Penrith from November 2015 – May 2016. SIMPER analysis of periphyton genera that contributed to differences (Bray-Curtis distance) in assemblages between sampling T1 (12/11/2015), T2 (25/2/2016) and T3 (30/5/2016) in the Nepean River at Penrith.

Genus	Mean abundance (Transformed)			Consistency ratio	Contribution (%)
	T1 12/11/2015	T2 25/2/2016	T3 30/5/2016		
Comparisons between sampling T1 and T2					
Leptolyngbya	351.61	283.16		1.17	9.21
Heteroleibleinia	148.97	82.04		1.40	7.09
Navicula	196.20	87.65		1.70	6.88
Synedra	109.55	17.01		1.51	5.82
Fragilaria	8.29	90.45		1.95	5.07
Achnanthes	110.49	32.57		1.28	4.84
Geitlerinema	86.42	23.45		1.12	4.32
Coleochaete	109.31	104.62		1.12	4.32
Palmellopsis	63.90	10.51		0.70	3.72
Comparisons between sampling T1 and T3					
Leptolyngbya	351.61		346.65	1.14	8.74
Heteroleibleinia	148.97		84.39	1.38	7.43
Fragilaria	8.29		93.74	1.77	4.90
Geitlerinema	86.42		45.86	1.34	4.90
Achnanthes	110.49		163.90	1.40	4.79
Coleochaete	109.31		67.48	1.24	4.78
Synedra	109.55		41.50	1.39	4.50
Navicula	196.20		155.52	1.30	3.59
Rhoicosphenia	7.96		67.70	1.65	3.57
Palmellopsis	63.90		12.13	0.69	3.54
Phormidium	60.21		17.23	0.70	3.47
Comparisons between sampling T2 and T3					
Leptolyngbya		283.16	346.65	1.11	9.07
Achnanthes		32.57	163.90	2.17	8.15
Heteroleibleinia		82.04	84.39	1.15	5.60
Navicula		87.65	155.52	1.60	5.22
Coleochaete		104.62	67.48	1.26	5.11
Rhoicosphenia		5.59	67.70	1.68	4.00
Monoraphidium		63.96	7.54	1.50	3.60
Fragilaria		90.45	93.74	1.42	3.48
Geitlerinema		23.45	45.86	0.82	3.43
Achnanthidium		4.34	57.04	1.80	3.39

Table 13 shows seven indicator taxa that reported high purity and reliability to velocity thresholds indicating consistently strong changes in response (>0.95) using Threshold Indicator Taxa ANalysis (TITAN) (King and Baker2010). Both negative and positive thresholds for periphyton scour and growth stimulation were identified.

Table 13. Threshold Indicator Taxa ANalysis (TITAN) results showing taxa with identified current velocity thresholds for periphyton sampled in the Nepean River at Penrith from November 2015 – May 2016.

Genus	Z score	5%ile (m/s)	95%ile (m/s)	Purity	Reliability (%)	Filter
Scenedesmus	4.76	0.27	1.23	0.95	96.2	1
Navicula	3.25	0.56	1.21	0.98	96.8	1
Cymbella	3.91	0.61	1.56	1.00	99.8	1
Aulacoseira	3.75	0.36	1.27	0.99	97.8	2
Homeothrix	3.96	0.38	1.26	0.99	98.0	2
Fragilaria	4.96	0.49	0.76	0.99	98.4	2
Leptolyngbya	3.69	0.29	1.43	0.97	97.0	2

Scenedesmus, Navicula and *Cymbella* all reported negative relationships, i.e. decreases in cell abundance, with increasing flow current velocity. *Aulacoseira, Homeothrix, Fragilaria* and *Leptolyngbya* had positive gain thresholds to increasing current velocity Figure 17).

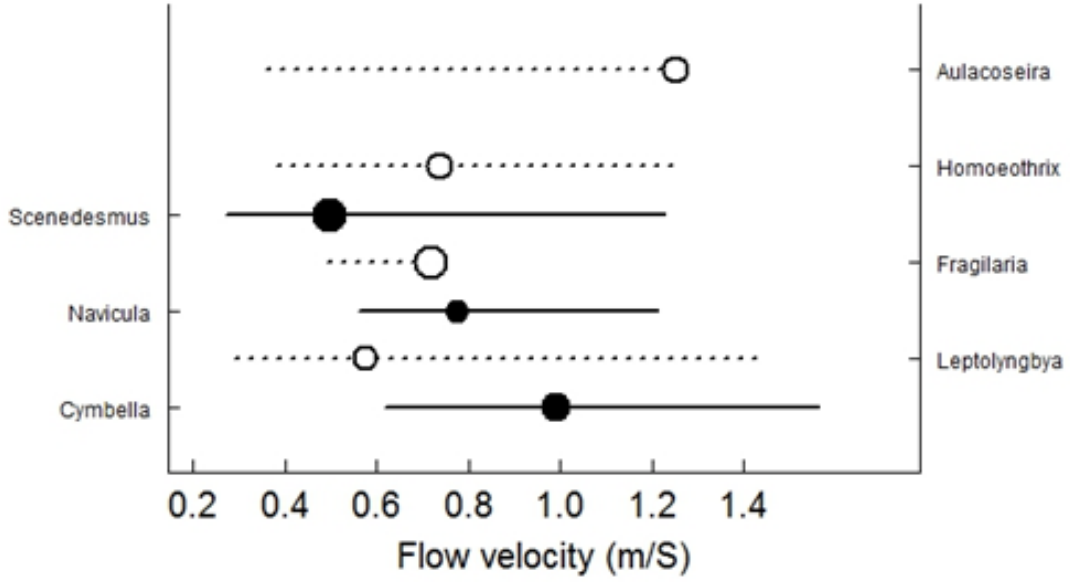

Figure 17. The results of Threshold Indicator Taxa ANalysis (TITAN) showing taxa with both loss and gain thresholds to increasing current velocity in the Nepean River at Penrith during sampling from November 2015 – May 2016.The left axis indicates algal genera with loss thresholds and the right axis shows genera that increased in abundance and frequency with increasing current velocity.

The black (filled) symbols correspond to negative (z−) indicator taxa (shown on the left axis), whereas unfilled circles correspond to positive (z+) indicator taxa (right axis). Symbols are sized in proportion to z scores. Horizontal lines overlapping each symbol represent 5th and 95th percentiles among 500 bootstrap replicates. The symbols in Figure 18 are sized in proportion to magnitude of the response of community change (z scores) across the environmental gradient.

4. Discussion

In this study, periphyton (benthic algae) was identified to genus level. Growns (1999) found that periphytic diatoms identified at either species or genus level were equally efficient in providing results for the impacts of river regulation in the Hawkesbury-Nepean River system. Very limited research has been conducted in the Hawkesbury-Nepean River on periphyton. Growns (1999) and Lynch & Keenan (2015 npublished data) are the only studies in this catchment reporting periphyton community structure and its response to flow events.

4.1 Pilot study

During a pilot study (November 2015), comparisons were made between acetone and hot ethanol extractants on duplicate samples from R1 (Nepean River at Penrith) and R2 (Nepean River at

Yarramundi) to test the most effective solvent for extraction in the Nepean River. No significant difference between the solvents was found at R1, however, R2 reported significant differences ($P<0.01$) in the chlorophyll-a concentration extracted by ethanol compared to acetone. R2 also had higher algal abundance, compared to concentrations at R1 and larger contributions from green algae. It is assumed that the extraction by solvents are not influencing the low chlorophyll-a results at the site, but rather the community structure containing low amounts of chlorophyll-a. Peterson and Porter (2002) reported biomass to be relatively low at sites where nitrogen-fixing blue-green algae were predominant. It is suggested that taxonomic identification and abundance may provide a more accurate assessment of river condition when using periphyton as an assessment tool in this river compared to chlorophyll-a density calculations as a method to calculate biomass. Samples with the highest abundance, were not found to contain the highest concentration of chlorophyll-a.

The site R2 was observed to have a higher cell abundance, greater contribution from filamentous green algal taxa and greater concentration of chlorophyll-a. The use of ethanol as the solvent for extracting chlorophyll-a was found to be more effective than the use of the more commonly used, acetone at this site during sampling in November 2015. Knowledge of the taxonomic contribution prior to extracting the pigments may prove more efficient in the selection of solvent to be used, as the use of ethanol on the cyanobacteria dominated samples, observed at R1, showed no statistical difference ($P>0.05$) between the two solvents. Acetone has previously been reported as a poor extractant for some algae (Sartory and Grobbelaar 1984; Porra *et al.* 1989; Jeffrey *et al.* 1997; Wright *et al.* 1997; Ritchie, 2006; Scheer, 2006; Sumanta *et al.*, 2014).

Results from the pilot study comparing the clustering of algal taxa composition showed a closer relationship between current velocity and algal assemblages for site R1. Figure 9 illustrated the clustering using multivariate analysis in a non-metric multidimensional scaling (nMDS) plot. The two sites (R1 and R2)were found to be separated from each other based on community assemblage. The current velocities of cobbles sampled ranged from 0.3 – 1.57 m/s at R1 and 0.58 – 1.31 m/s at R2. The clustering of assemblages was found in the current velocity ranges of 0.8 – 1.4 m/s for site R1. It is not known whether the small sample size contributed to the lack of clustering or the lack of differing velocity ranges within the riffle, as no clustering was observed for the R2 site on this sampling occasion. The sites were different from each other in dominant algal group composition. Both sites samples were dominated by cyanobacteria (particularly

Leptolyngbya), R1 had a higher contribution of diatoms and R2 was found to have high contributions from the green periphyton generas (Figure 7). Cell abundance and biomass were found to be higher at R2 compared to the R1 site and water quality parameters were higher at R2.

R1 was selected as the site for the main study following the results of the pilot. It showed a greater relationship between current velocity and community structure, less confounding issues of water quality as it was upstream of the majority of STP inlets and was closer to Warragamba Dam, therefore likely to reap the results of environmental flow releases in the future and can be used as a reference site in the future following releases

4.2 Relationship between current velocity and periphyton community structure

It is well accepted that current velocity influences periphyton community structure (Biggs and Thomsen, 1995; Jowett and Biggs, 1997; Beisel et al., 1998; Wetzel, 2012; Larson and Passy, 2013). Periphytic architecture determines the effects of current velocity as adherent assemblages are more resistant to sloughing, whilst filamentous assemblages are susceptible to shear stress (Opsahl *et al.*, 2003). Mucilaginous diatoms have been reported to have the greatest resistance to current velocity, whilst stalked or short filamentous diatoms have been shown to have low resistance >0.2 m/s (Jacoby and Welch, 2004).

Cardinale (2011) observed niche differences among genera to current velocity and nutrients during succession stages. He reported that filamentous algae, *Melosira* and *Stigeoclonium* were susceptible to shear and were abundant in low-current velocity habitats; single-celled, prostrate diatoms, *Achnanthidium* and *Synedra* achieved the highest densities in high-velocity habitats; early successional habitats were dominated by small diatoms, *Achnanthidium* and *Nitzschia* and late successional habitats were dominated by slow-growing cells, colonies or filaments (e.g. *Stigeoclonium, Spirogyra* and *Synedra*). During this study, *Synedra* was highly abundant during T1 sampling compared to the other two sampling occasions and was present in 92% of samples, compared to being present in only 50% of the samples at T2. *Stigeoclonium* was only found at R2 during the pilot study. Insufficient samples were available to draw any conclusions of its existence in velocities, however its presence only at the R2 site downstream of STP inputs and higher nutrient concentrations is interesting.

With the use of multivariate analysis in this study, by comparing periphyton assemblages and the current velocity of the cobble sampled with other samples of the same, clustering of algal assemblages within the same current velocity ranges were observed. These were more apparent for T1 and T2 sampling times within the ranges of 0.3 – 1.4 m/s and both sampling occasions were found to be similar in taxa. Figure 18 shows the clustering of algal communities with the current velocity overlain as a bubble. Sampling at T3 also showed the clustering of algal assemblages, however due to what is thought to be late succession communities, samples in the plot are separated from the first two sampling occasions based on taxa's presence and density. T3 also had a higher contribution from diatoms compared to the other two sampling dates.

Samples taken on all three sampling dates were dominated by the cyanobacteria, *Leptolyngbya*. It was present in 100% of the samples and accounted for 30% of the sample total on each sampling time. Results indicate that it is in higher abundance at current velocities above 0.45m/s. Average taxa composition on each sampling occasion reported similarities in the samples of 57%.

4.3 Response of periphyton to current velocity

Some periphyton genera were observed to either increase or decrease abundance with a change in current velocity. *Scenedesmus* showed losses above 0.5 m/s, *Navicula* had a loss threshold of 0.8 m/s and *Cymbella* showed losses at 1.0 m/s. Reductions of mucilaginous diatoms such as *Navicula* and *Synedra* and the stalked diatoms, *Gomphonema* and *Achnanthes* were observed in T2 after the moderate flow, compared to T1. Positive thresholds were also observed for some taxa, such as *Aulacoseira*, which increased at velocities above 1.4 m/s, *Homoeothrix* increased at 0.8 m/s and the major contributing cyanobacterium, *Leptolynbya* increased in abundance at flows above 0.6 m/s. Increases in *Leptolyngbya* were previously observed in the Nepean River after two high-flow events (Lynch and Keenan, 2015 unpublished data). Events that produce both scouring and non-scouring suggest that current velocity thresholds exist for some taxa (Davie and Mitrovic, 2014). Biggs and Thomsen (1995) also reported loss thresholds for *Cymbella* during resistance studies, whilst *Fragilaria* was found to maintain or increase abundance when shear stress flows were increased. *Fragilaria* in this study was found to increase in abundance at 0.7 m/s.

Greater losses in periphyton biomass occur when the current velocity increases beyond that velocity under which the periphyton developed, therefore current velocity change is an important consideration in estimating velocities required to reduce periphyton (Horner *et al.*, 1983; Horner *et al.*, 1990). During the study, cobbles were sampled from varying velocities ranging from 0.2 – 1.79 m/s. Previous studies vary in their estimations of velocities required to scour and reduce periphyton. Previous studies have reported optimum velocities for mature periphyton growth to be in the vicinity of 0.5 – 0.7 m/s, before scouring by shear stress is observed (Horner and Welch, 1981; Biggs and Stokseth, 1996). Davie and Mitrovic (2014) reported that flows of <0.9 m/s may actually increase filamentous algae biomass. Velocities less than 0.3 m/s have been reported to result in an increase in stalked and filamentous diatoms and filamentous green algae (Biggs and Stokesh, 1996; Jowett and Biggs, 1997; Biggs *et al.*, 1998; Horner *et al.*., 1990). A decrease in filamentous green algae, stalked and adnate diatoms was reported with velocities greater than 0.2m/s (Biggs and Gerbeaux, 1993; Flinders and Hart, 2009; Ryder *et al.*., 2006). Horner *et al.* (1990) reported velocities above 0.6 m/s decreased *Phormidium*, the pennate diatoms *Fragilaria* and *Synedra,* and the green filamentous *Mougeotia. Fragilaria* was found in higher abundance in higher velocities in this study. Davie and Mitrovic (2014) observed losses of filamentous green algae and biomass when current velocity was greater than 1.2 m/s. *Phormidium* was found predominantly at velocities less than <0.7 m/s during the study in the Nepean River.

4.4 Effect of suspended sediment on periphyton

Bed disturbance due to substrate stability and suspended sediment is a determining factor in the rate of scouring and colonisation of periphyton (Biggs and Thomsen, 1995; Biggs and Stokseth, 1996; Jowett and Biggs, 1997; Biggs, 1999; Luce *et al.*, 2010). Francoeur and Biggs (2006) reported increases in current velocity will remove benthic algal biomass; however, algal removal was greater with the addition of high suspended sediment. This study consisted of a cobble-dominated riffle with multiple current velocity zones and a sand bed upstream. As previously identified, the Hawkesbury-Nepean River has been transitioned from gravel beds to sand beds (Brierley and Fryirs, 2013). The availability of suspended sediments in the Nepean River may contribute to the effectiveness of flow to scour periphyton. The weir pool immediately upstream of the sampling site is a likely source of sediment, which would drop out of suspension in lower flows as the weir pool slows the flow to velocities that can't keep sediment suspended. Higher flows may

resuspend this sediment. Macrophyte beds in the weir pool will help to slow flows and encourage sediment deposition; the loss of macrophytes during floods may allow more sediment to be resuspended in subsequent higher flows. Yamada & Nakuma (2002) found a direct relationship between the decreased chlorophyll-a and the increase of fine sediment deposition in the Makomanai River due to channel works. Average turbidity of 3.9 NTU was recorded prior to T1 sampling. After the high flow on 30 January 2016, turbidity was recorded 46.5 NTU (WaterNSW 2/2/16), which dropped to 15 NTU (SGS Environmental) on the day of T2 sampling and remained <5 NTU (data supplied by WaterNSW) until T3.

Francoeur and Biggs (2006) found that biomass removal by current velocity and suspended sediments was community-specific and assemblages with a tightly adherent, cohesive mat were resistant to removal, even though they were taxonomically similar to easily disturbed assemblages. They found that some taxa were more susceptible to removal by disturbance than others. Heinlein (2000) found that high biomass (75 mg/m^2 chlorophyll-a) communities were more resistant to suspended sediment scour than low biomass (22 mg/m^2 chlorophyll-a) communities of similar taxonomic composition. Studies have shown that biomass removal is not consistent during high flows as most biomass losses (83-100%) occur within the first 5 – 10 minutes of the disturbance flow (Biggs and Thomsen, 1995; Francoeur and Biggs, 2006). Variable environmental flows in the Nepean River, of adequate volume that mimic natural spates, may prove more effective than continuous flows, resulting in biomass losses. This was observed during sampling at T2 where biomass was reduced from T1. The increased turbidity following the high flow event, prior to sampling suggests that there was a stirring of sediments, which would further contribute to the loss of periphyton biomass by current velocity alone.

4.5 Succession stages of periphyton during the study

Sampling in November 2015 (T1) was considered to be representative of a late succession algal community, as it had been 67 days since a flow of 4780 ML/d (7/9/2015). Average daily flow between September and sampling on T1 was 515 ML/d. T2 sampling was regarded as an early succession stage, although a number of days passed before sampling was undertaken. A flow of 5440 ML/d occurred on 30/1/2016. Sampling was undertaken 26 days later, with average flows of708 ML/d occurring between the flow event and sampling. T3 sampling was regarded as late succession algal stage, as no further moderate or large flow events occurred from the event of

5440 ML/d in late January until sampling at T3. Average flows from the peak (30/1/2016) until sampling were 290 ML/d and 121 days.

The flow of 5440 ML/d just prior to sampling on T2 coincided with a decrease in the overall abundance of periphyton and a decrease in the concentration of chlorophyll-a. The abundance of *Leptolyngbya*, *Heteroleibleinia, Navicula, Synedra, Achnanthes, Geitlerinema, Coleochaete, Palmellopsis, Phormidium, Homoeothrix, Pseudanabaena, Scenedesmus* and *Aulacoseira* were reduced compared to samples collected at T1, prior to the flow event. *Synedra* has been reported as an indicator of regulated rivers (Growns and Growns, 2001; Davie and Mitrovic, 2014). A reduction in abundance of this genus was observed after a moderate flow, and following this there was an increase in abundance when average flows were reduced. Increases in the abundance of *Desmodesmus, Fragilaria, Cocconesis* and the presence of the diatom *Nitzschia* at T2 also suggest early succession after disturbance. Davie *et al.* (2012) reported diatoms including *Cocconeis, Synedra, Navicula* and *Fragilaria* dominated the early succession assemblages. These taxa were succeeded in time by filamentous green alga (Davie *et al.*, 2012). *Achnanthidium* has been reported as an early colonizer, following instream disturbance (Cardinale, 2011); however, this taxon was present in greater abundance and present in 93% of all samples in T3 compared to T2 where it was in low abundance and only present in 20% of the samples. It is not known why this taxa had a significant presence in the late successional growth stage of the T3 sampling period. Further studies may answer the differing pattern to previous studies.

Flow for the three months prior to T3 sampling averaged 185 ML/d, with a maximum flow of 338 ML/d recorded on 22/3/2016. There were 121 days from the last flow disturbance until sampling on T3. It is therefore assumed that the periphyton community was late succession at T3. Presence of this *Achnanthidium* during late succession was also found by Borduqui and Ferragut (2012). Manoylov and Stevenson (2006) reported that *A. minutissimum* has high growth rates in low-P conditions, but are not competitive with other taxa in high-P environments. Low TP was observed before and during sampling at T3 which may have assisted competitiveness of *Achnanthidium,* allowing it to become abundant. Thresholds for algae associated with low TP possibly represent levels that exceed the maximum growth rate potential and define where along TP enrichment gradients species are no longer competitive (Stevenson *et al.*, 2006).

The number of periphyton genera numbers was found to be the highest in T2 compared to T1 and T3 (Appendix 3). Both T1 and T3 were considered late succession. Arnon *et al.* (2006) found that algal species richness in 120-day communities was low in all flow regimes. Periphyton richness was lowest in the late succession T3 (121 days since last disturbance flow), where 52 genera were identified compared to the early succession sampling in T2, which identified 66 genera of periphyton.

Cyanobacteria was the dominant contributor across all sampling occasions, contributing to 62 – 65% of the total abundance. *Leptolyngbya* was the most abundant taxa contributing 26 – 30% of all samples. Although there were reductions in both diatoms and green algal abundance in T2, sampling on this occasion was shown to have the greatest richness of periphyton genera present. There was a community change at this sampling time and taxa that had been removed or reduced (particularly filamentous green algae) were replaced with taxa that had either been reported in low abundance or were not present in T1. The green algae, *Desmodesmus*, was present in 78% of samples in T2 (31 samples) compared to being present in only 1 – 4 samples in T1 and T3 respectively. Increases, although low in abundance, of other green algae, including *Ankistrodesmus, Cosmarium, Euastrum, Monoraphidium* and *Rhizoclonium*, were also observed. The cyanbacterium, *Synechococcus* was also only present in T2. This genera has been reported as an early succession algae (Ferragut and de Campos Bicudo, 2012). The flow in January may have scoured late succession taxa, present in T1 and contributed to the resetting of early succession algae, encouraging the greater taxa richness.

4.6 Responses of periphyton to water quality

Both TP and TN average concentration increased after the moderate flow just prior to sampling T2.). Lynch and Keenan (unpublished data) reported increases in nutrients, in particular TP, in the Nepean River following two high flow events in 2015 which also resulted in an increase in algae abundance. Davie *et al.* (2012) also reported increased nutrient concentrations and increased benthic algae abundance following increased flow from dam releases. Nutrient enrichment can significantly alter algal community composition, biomass, and productivity (Stockner and Shortreed, 1978; Grimm and Fisher, 1986; Peterson and Grimm, 1992). Although abundance and chlorophyll-a were both reduced after the flow of 5440ML/d, the full effects of the magnitude of the flow may not be accurately assessed as recolonization of early succession taxa would have

taken place within the 26 days from the flow to sampling, due to increased nutrient availability, therefore minimising the impact of the flow event (Horner *et al.*,1990). Low flow and low nutrient levels were observed at T3. *Navicula* was found in lower abundance at T3 compared to T1. *Navicula* has been reported to be more abundant when combinations of both TP and TN are present (Fairchild *et al.*, 1985). The pilot study site R2 showed periphyton taxa presenence of a higher contribution of green algae. This site was shown to have higher nutrient levels compared to R1. The presence of *Stigeoclonium* a filamentous green periphyton was only found at the R2 site during the pilot study. Rosemarin (1980) reported that *Stigeoclonium* sp was commonly found downstream of sewage effluents in high nutrient waters. The R2 site was found to have higher nutrients, both TN and TP, higher biomass and more than double the cell abundance of the R1 site. Increased periphytic algal growth in the higher nutrient waters downstream of STP inlets is presented in this study.

4.7 Changes to periphyton biomass over sampling times

Chlorophyll-a analysis showed a reduction in biomass from T1 to T2, from 17.3 to 10.4 mg/m^2. Concentration of chlorophyll-a was reduced possibly as a result of the 5440 ML/day flow on 30 January, which also reduced overall algal abundance. Chlorophyll-a concentrations may be low during early succession by algal assemblages (Davie *et al.*, 2012). Early succession taxa were observed during sampling at T2, which may have further contributed to low biomass which was observed on this sampling day.

There was a significant ($P<0.01$) increase in algal biomass (measured as chlorophyll-a) from T2 (average 10.4 mg/m2) to T3 (average of 41 mg/m^2). Ferragut *et al.* (2010) reported the highest biomass values were recorded in winter and autumn, also consistent with Davie *et al.* (2012) found winter chlorophyll-a concentrations five times higher than the summer concentrations. The mean chlorophyll-a at T3 was 41 mg/m^2, with the highest concentration in a sample found to be 73 mg/m^2, which is below the nuisance proliferation threshold of 100 – 150 mg/m^2 (Horner *et al.*.1983; Welch *et al.*, 1988; Jacoby and Welch 2004; Luce *et al.*, 2010).

Chlorophyll-a was not linearly related with current velocity, consistent with Biggs and Close (1989). There was also no correlation found between chlorophyll-a and algal abundance. During the study, the samples with the highest abundance, did not have the highest chlorophyll-a

concentrations, consistent with the previous study of the same site (Lynch and Keenan, 2015). Baulch *et al.* (2009) reported that chlorophyll-a content may vary due to algal community composition and cautioned against using chlorophyll-a as an indicator of biovolume. Cell densities and biovolumes are also used to determine the biomass in different taxonomic groups (Stevenson, 2014). Cell biovolume has traditionally been calculated microscopically by calculating cell volume based on linear dimensions (Steinman *et al.*, 1991; Hillebrand and Sommer, 1997).

All samples from T1, T2 and T3, were dominated by the cyanobacteria, *Leptolyngbya* (contributing to approximately one third of the cell counts in all samples). *Leptolyngbya* has been reported to contain low concentrations of chlorophyll-a in its filamentous conical mat structure, including the cones and the filaments that make up its structure (Reyes *et al.*, 2013). A previous study at this site by Lynch and Keenan (unpublished data) also reported low chlorophyll-a concentrations in samples that were dominated by *Leptolyngbya*. Following the study, Lynch and Keenen (2015 unpublished data) recommended trialling the use of an alternative solvent to extract the pigment.

This study identified periphyton taxa contributions in the Nepean River at Penrith under varying current velocity conditions over three seasons from November 2015 – May 2016. It further builds on the previous 2015 study by identifying the response of periphyton to moderate flows through scouring and resetting of early succession assemblages, observing changes in community structure as a result of flow magnitudes. Statistical analysis of the response of the algae to increasing velocities supports previous studies that thresholds exist for specific taxa, as some flows are both scouring and non-scouring, and may actually increase abundance through the utilisation of nutrients. Ecological thresholds hold promise as a management tool because their identification might provide pre-emptive actions to prevent a system from moving to an alternate state (Hildebrand *et al.*, 2010). Taxa were identified in this study showing both positive and negative thresholds to increases in velocities.

Knowledge gained from this study will build on the previous 2015 study and provide the baseline data for periphyton assemblages prior to the release of environmental flows in 2024. It will also provide initial information to water managers regarding the estimation of releases to maximise the benefits of environmental flows. The flow of 5440 ML/d may have resulted in a reduction of filamentous green algae and stimulated the resetting of early successional algae. Modelling incorporating this volume can be compared to assess the benefits from this magnitude of flow.

Understanding the effects of various flows in the Hawkesbury-Nepean river system will help utilise the variable flow regime predicted for the e-flow releases. Objectives of environmental flows include the reducing of nuisance filamentous algal growth, encouraging the re-setting of periphyton at early succession stages, thus improving diversity. The results of the flow of 5440 ML/d support this volume in achieving the e-flows objectives. Further research into variable flows will add to this knowledge and support the most efficient volumes required to achieve the most favourable outcomes. During this study it was observed that a resetting of algal assemblages following a disturbance, increases the number of taxa genera (as seen at T2) and may provide an opportunity for a more palatable algae for grazers, thus increasing diversity (Brooks *et al*., 2005; Dyer and Thoms, 2006; Robson *et al*., 2009).

Spates of higher velocities will provide greater benefits to the riverine system as physical disturbance by spates are important mechanisms in structuring algal community composition (Biggs and Thomsen, 1995). The magnitude, frequency, duration, timing, and rate of change of hydrologic conditions are considered the critical components of the flow regimes that regulate ecological processes in river ecosystems (Poff and Ward, 1989; Poff *et al.*, 1997). Further research will assist water managers with the frequency and timing of the delivery of environmental flows to achieve an optimum outcome for the Nepean River.

4.8 Thresholds of periphyton taxa to current velocities

Many studies have suggested the existence of current velocity thresholds for periphyton taxa based on shape, size, architecture and adhesion to substrate. This study, with the use of a fairly new (2010) statistical package, TITAN (Baker and King, 2010), supports the theory of thresholds. Both loss and positive associations to current velocity were observed for periphyton genera along the current velocity gradient. Davie and Mitrovic (2014) suggested thresholds after they observed both scouring and stimulation of abundance and biomass at similar velocities. The results of this study support the theory of thresholds, as losses of *Scenedesmus* and *Navicula* were observed at the current velocity range of 0.5 – 0.8m/s whilst increases of *Leptolyngbya, Homeothrix* and *Fragilaria* were also observed in the same range. The results may have greater benefits for river managers of understanding periphyton thresholds in other cobble-dominated riffles within and outside of the Nepean River, Australia.

4.9 Limitations of the study

The major limitation to this study was the constrained time period in which to sample. Only three sampling opportunities were provided in the 10 month study, therefore seasonal influences were not taken into consideration. The relationship of periphyton to current velocity was only tested in one ripple in one river system (with the exception to a small sample taken during the pilot at R2). Further research may provide greater knowledge of the current velocity influencing periphyton community structure. Assumptions based on other studies were made regarding the succession stages of taxa. An additional study looking at succession may have provided further insight into the question of why T3 was different to the other two sampling days, although clustering of community structure on that day was evident, shown on the nMDS plot. This study also did not consider losses of periphyton biomass caused by macroinvertebrate grazing or autogenic sloughing once carrying capacity has been reached (Biggs, 1996; Biggs and Close, 1998). A concurrent macroinvertebrate study may have provided additional information to reductions in biomas to more than current velocity and provided additional information to the community structure and invertebrate presence.

Duplicate chlorophyll-a samples were analysed to compare acetone and ethanol as the preferred solvent for the Nepean River samples. However, only a small number of samples (13 from R1 and 12 from R2) were tested. Greater sampling size and replicates of the samples would have been more effective in establishing the most efficient solvent to be used as an extractant.

Conclusion

Results of this study support the objectives outlined in section 1. That is, similarly to a number of other studies, current velocity in the Nepean River was found to influence periphyton community structure as similarities of community assemblages were found in similar current velocity ranges. A flow figure of approximately 5000 ML/d can be investigated further as an effective volume to be released as an environmental/contingency flow, as this was found to have beneficial outcomes to periphyton, by sub-scouring, reducing filamentous green algae and stimulating early succession taxa. This was also supported by the Lynch & Keenan study in 2015 (unpublished data). Specific taxa were identified as having loss thresholds supporting other suggestions of the existence of community specific thresholds to current velocity. Results of this study identifying specific taxa thresholds may prove beneficial to periphyton studies and river managers to assess current

velocities required to decrease periphyton abundance. The study also builds on the previous study of the Nepean River (Lynch and Keenan 2015), identifying community structure which will provide a baseline prior to the release of environmental flows, to inevitably measure the benefits of environmental flows. The study also attempted to establish the most efficient solvent for the extraction of chlorophyll-a from benthic algae in the Nepean River, showing that with cyanobacteria dominant samples, there was no significant difference between acetone and ethanol in samples from the Nepean River at Penrith during sampling in November 2015.

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6. Appendices

Appendix 1a Flow classes for sites in the Hawkesbury Nepean River modelled on pre-development flows (i.e. "natural" – no dams, no extraction, no inputs)

Percentiles	Warragamba River (ML/d)	Nepean River at Penrith (ML/d)	Nepean River at Yarramundi (ML/d)
1	40,234	73,258	75,711
5	8,375	16,845	17,471
10	4,015	7,402	7,665
20	1,870	3,439	3,561
30	1,129	2,137	2,221
40	744	1,447	1,509
50	519	1,042	1,096
60	368	757	793
70	263	547	576
80	182	391	412
90	108	272	285
95	69	216	227
100	10	75	76

Source: NSW DPI 2014b

Appendix 1b. Flow classes and relevant River Flow Objectives

Flow class (Percentile)	Classification	Ecological relevance
10%	Flood	Restore natural flood variability; remove instream macrophytes; support healthy riparian zones & add floodplain inputs to regulate water quality
30 – 10%	Freshes	Protect / restore freshes; mimic natural flow variability; minimise impact of instream structures. Freshes transport sediment, nutrients, carbon, increase dissolved oxygen, minimise pool stratification; barrier inundation, riffle scour, habitat resetting Reduce risk of cyanobacterial blooms Maintain / rehabilitate estuarine processes
80 – 30%	Moderate flows	Mimic natural flows & maintain rates of rise and fall within natural bounds; protect and restore a number of moderate flows; barrier inundation, riffle scour, habitat resetting; minimise pool stratification; reduce risk of cyanobacterial blooms & transport carbon into the estuary
95 – 80%	Low flows	Protect low flows; provision of variable flows throughout the year & protection of low flow habitat
<95%	Very low flows	Protect water levels in rivers during dry periods; pool and riffle connectivity; protection of low flow habitat during summer

Source: NSW Office of Water (2001);Office of Environment and Heritage (2006).

Appendix 2. Algal genera present (periphyton and planktonic) during the pilot study sampling on 12 November 2015 at R1 (Nepean River at Penrith) and R2 (Nepean River at Yarramundi).

Genera	R1	R2
Achnanthes	x	x
Achnanthidium	x	
Anomoeoneis		x
Asterionella	x	
Aulacoseira	x	x
Cocconeis	x	x
Cyclotella	x	x
Cymbella	x	x
Fragilaria	x	x
Frustulia	x	
Gomphonema	x	x
Mastogloia		x
Melosira		x
Navicula	x	x
Nitzschia	x	x
Rhoicosphenia	x	x
Synedra	x	x
Tabellaria	x	
Anabaena (other species)	x	
Aphanocapsa	x	x
Aphanothece	x	
Chroococcus		x
Geitlerinema	x	x
Heteroleibleinia	x	x
Homoeothrix	x	x
Komvophoron		x
Leptolyngbya	x	x
Lyngbya		x
Merismopedia (small)		x
Oscillatoria	x	x
Phormidium	x	x
Pseudanabaena	x	x
Spirulina		x
Stigonema	x	
Coleochaete	x	x
Chlamydomonas		x
Closterium	x	
Coelastrum	x	
Cosmarium	x	x
Crucigenia	x	x
Desmodesmus	x	
Monoraphidium	x	x
Mougeotia	x	x
Oedogonium	x	x

Genera	R1	R2
Oocystis		x
Palmellopsis	x	x
Pediastrum		x
Scenedesmus	x	x
Staurastrum		x
Stauridium	x	
Staurodesmus		x
Stigeoclonium		x
Tetraedron	x	
Tetrastrum	x	
Peridinium		x
Euglena		x
Lepocinclis		x
Trachelomonas	x	x
Ophiocytium		x

Appendix 3. Algal genera present during sampling T1 (12 November 2015), T2 (25 February 2016) and T3 (30 May 2016) in the Nepean River at Penrith.

Genera	T1	T2	T3
Acanthoceras		x	
Achnanthes	x	x	x
Achnanthidium	x	x	x
Anomoeoneis	x	x	x
Asterionella	x		
Aulacoseira	x	x	x
Bacillaria		x	
Centritractus		x	
Cocconeis	x	x	
Cyclotella	x	x	x
Cymbella	x	x	x
Diploneis		x	
Diatoma			x
Epithemia		x	x
Eunotia	x	x	x
Fragilaria	x	x	x
Frustulia	x		
Gomphonema	x	x	x
Gyrosigma		x	x
Mastogloia	x		
Melosira	x	x	x
Navicula	x	x	x
Nitzschia	x	x	x
Rhoicosphenia	x	x	x
Surirella	x	x	
Synedra	x	x	x
Tabellaria	x		
Anabaena (other species)	x	x	x
Aphanocapsa	x	x	x
Aphanothece	x		
Blennothrix	x		
Chroococcus	x	x	x
Geitlerinema	x	x	x
Heteroleibleinia	x	x	x
Homoeothrix	x	x	x
Komvophoron	x	x	x
Leptolyngbya	x	x	x
Merismopedia (small)	x	x	
Lyngbya	x		x
Oscillatoria	x	x	x
Phormidium	x	x	x
Pseudanabaena	x	x	x
Spirulina	x		x
Stigonema	x		
Symplocastrum		x	
Synechococcus		x	
Coleochaete	x	x	x

Genera	T1	T2	T3
Acutodesmus	x		x
Ankistrodesmus		x	
Characium		x	x
Chlamydomonas	x		
Closterium	x	x	
Coelastrum	x	x	x
Cosmarium	x	x	x
Crucigenia	x	x	x
Desmodesmus	x	x	x
Euastrum		x	
Kirchneriella		x	x
Microspora		x	
Monoraphidium	x	x	x
Mougeotia	x	x	x
Oedogonium	x	x	x
Oocystis	x	x	
Palmellopsis	x	x	x
Pediastrum	x	x	x
Pseudopediastrum		x	x
Rhizoclonium		x	
Scenedesmus	x	x	x
Schroederia		x	
Sphaerocystis			x
Spirogyra			x
Staurastrum	x		x
Staurodesmus	x		
Stauridium	x	x	
Stigeoclonium	x	x	x
Tetraedron	x	x	x
Tetrastrum	x		
Ulothrix			x
Peridinium	x	x	
Euglena	x		
Lepocinclis	x	x	
Phacus		x	
Strombomonas		x	
Trachelomonas	x	x	x
Ophiocytium	x	x	

Appendix 4. Sampling times, site, depth and current velocity for each cobble sampled

Date sampled	Site	Sample I/D	Depth (m)	Current velocity (m/sec)
12/11/2015	R1	P6	0.1	0.354
12/11/2015	R1	P7	0.1	0.377
12/11/2015	R1	P8	0.2	0.300
12/11/2015	R1	P9	0.3	0.492
12/11/2015	R1	P10	0.3	0.617
12/11/2015	R1	P11	0.3	0.600
12/11/2015	R1	P13	0.05	1.243
12/11/2015	R1	P14	0.2	0.634
12/11/2015	R1	P15	0.4	0.718
12/11/2015	R1	P16	0.35	1.569
12/11/2015	R1	P17	0.2	1.369
12/11/2015	R1	P18	0.25	1.334
12/11/2015	R1	P19	0.25	1.028
12/11/2015	R2	Y27	0.15	0.598
12/11/2015	R2	Y28	0.15	1.036
12/11/2015	R2	Y29	0.2	0.786
12/11/2015	R2	Y30	0.1	1.256
12/11/2015	R2	Y31	0.3	0.779
12/11/2015	R2	Y32	0.3	0.923
12/11/2015	R2	Y33	0.2	1.311
12/11/2015	R2	Y34	0.3	1.134
12/11/2015	R2	Y35	0.3	0.697
12/11/2015	R2	Y36	0.12	1.148
12/11/2015	R2	Y37	0.12	0.579
12/11/2015	R2	Y38	0.12	0.746
25/02/2016	R1	P1	0.21	1.515
25/02/2016	R1	P2	0.23	0.718
25/02/2016	R1	P3	0.15	1.367
25/02/2016	R1	P4	0.16	0.725
25/02/2016	R1	P5	0.17	0.906
25/02/2016	R1	P6	0.13	0.721
25/02/2016	R1	P7	0.14	0.504
25/02/2016	R1	P8	0.15	0.443
25/02/2016	R1	P9	0.18	0.624
25/02/2016	R1	P10	0.15	1.169
25/02/2016	R1	P11	0.13	0.881
25/02/2016	R1	P12	0.18	0.786
25/02/2016	R1	P13	0.22	1.075
25/02/2016	R1	P14	0.15	1.560
25/02/2016	R1	P15	0.31	0.587
25/02/2016	R1	P16	0.31	0.993

Date sampled	Site	Sample I/D	Depth (m)	Current velocity (m/sec)
25/02/2016	R1	P17	0.31	1.149
25/02/2016	R1	P18	0.38	0.829
25/02/2016	R1	P19	0.36	0.951
25/02/2016	R1	P20	0.27	1.162
25/02/2016	R1	P21	0.34	1.421
25/02/2016	R1	P22	0.33	0.384
25/02/2016	R1	P23	0.27	1.055
25/02/2016	R1	P24	0.2	0.394
25/02/2016	R1	P25	0.13	0.258
25/02/2016	R1	P26	0.15	0.450
25/02/2016	R1	P27	0.23	0.136
25/02/2016	R1	P28	0.35	0.568
25/02/2016	R1	P29	0.42	1.257
25/02/2016	R1	P30	0.45	0.996
25/02/2016	R1	P31	0.32	1.170
25/02/2016	R1	P32	0.24	1.297
25/02/2016	R1	P33	0.15	0.996
25/02/2016	R1	P34	0.23	0.902
25/02/2016	R1	P35	0.21	1.668
25/02/2016	R1	P36	0.17	1.790
25/02/2016	R1	P37	0.33	1.337
25/02/2016	R1	P38	0.31	0.256
25/02/2016	R1	P39	0.26	0.382
25/02/2016	R1	P40	0.17	0.763
30/05/2016	R1	P1	0.12	0.285
30/05/2016	R1	P2	0.1	0.285
30/05/2016	R1	P3	0.12	0.206
30/05/2016	R1	P4	0.1	0.206
30/05/2016	R1	P5	0.1	0.344
30/05/2016	R1	P6	0.1	0.344
30/05/2016	R1	P7	0.1	0.205
30/05/2016	R1	P8	0.12	0.747
30/05/2016	R1	P9	0.12	0.746
30/05/2016	R1	P10	0.15	0.554
30/05/2016	R1	P11	0.15	0.471
30/05/2016	R1	P12	0.2	0.526
30/05/2016	R1	P13	0.2	0.405
30/05/2016	R1	P14	0.18	0.871
30/05/2016	R1	P15	0.3	1.209
30/05/2016	R1	P16	0.32	1.210
30/05/2016	R1	P17	0.35	0.988
30/05/2016	R1	P18	0.35	0.991
30/05/2016	R1	P19	0.15	1.186
30/05/2016	R1	P20	0.15	1.191

Date sampled	Site	Sample I/D	Depth (m)	Current velocity (m/sec)
30/05/2016	R1	P21	0.35	1.591
30/05/2016	R1	P22	0.3	1.435
30/05/2016	R1	P23	0.32	1.438
30/05/2016	R1	P24	0.25	1.097
30/05/2016	R1	P25	0.25	1.116
30/05/2016	R1	P26	0.2	1.097
30/05/2016	R1	P27	0.15	1.637
30/05/2016	R1	P28	0.2	1.642

*Pilot study - Sampling on 12/11/2015 at site R1 (Nepean River at Penrith)and R2 (Nepean River at Yarramundi