Food web dynamics in the benthic habitat of a groundwater-fed, freshwater pond

by

Yang Liu

A thesis submitted in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

School of Biological Sciences

THE UNIVERSITY OF ADELAIDE

SEPTEMBER 2017

Abstract

Human activities have significantly altered biogeochemical cycles of major elemental nutrients and hydrological cycles of water all over the world, causing degradation of many freshwater ecosystems. Enrichment in essential nutrients, especially nitrogen (N) and phosphorus (P), has been accused to progressively reduce the resilience of systems and pave the way to regime shifts in freshwater environments. Regime shifts mark profound changes in ecosystem structure and function, many of which have been carefully evaluated in certain types of habitat, for example, pelagic zones of lakes. However, the consequences of potential regime shifts are understudied in some unique inland waterbodies, such as groundwater-fed freshwater ponds.

In the southeast of South Australia, vast areas of peat swamps were drained and transferred into managed pastures, and remanent wetlands are highly valuable because of their importance in delivering critical ecological and socio-economic services. Even so, many of the remaining wetlands are under the threat of increasing nutrient concentrations due to pollution of groundwater by applied fertilizers and decreasing incoming flows owing to extraction of groundwater for agricultural use. Ewens Ponds, is an example of such wetland systems with deteriorated condition, as evidenced by gradual replacement of submerged vascular plants by benthic filamentous algae and episodic occurrence of algal blooms.

Traces of upcoming regime shifts may hide in gradual changes of key ecological processes, and many of these processes are connected through the energy and material flowing within the ecosystem. Determining trophic links and transfer efficiencies between food-web components, thus is critical to the understanding of how ecosystems are influenced by changes in environmental factors. Resources supporting the major benthic macroinvertebrate and fish consumers and their respective contribution to specific consumers were investigated in Ewens Ponds using a combination of gut content, stable isotope and fatty acid composition analysis. The results demonstrated that epiphytes and filamentous cyanobacteria were the most important sources of organic carbon for benthic macroinvertebrates and fish, with substantial supplement of macrophytes to the diet of these consumers. Contributions of different basal resources to consumer biomass were affected by their availability and quality. Disparity of stoichiometric ratios between primary producers and primary consumers were greater than that between fish and their prey, and population size of

several consumers may be constrained by the low availability of P in Ewens Ponds. Periphyton production was unlikely to be limited by light intensity, but was certainly limited by P concentration. It is likely that there is competition between benthic macroinvertebrates for epiphytes as a high-quality food resource. Accumulation of massive biomass of benthic filamentous algae was associated with the low transfer rate of this form of organic carbon production to animals. Complex trophic interactions related to feeding preferences and habitat selection between components of the food web in the benthic habitat of Ewens Ponds regulated the production of organisms at different trophic levels.

Ecosystem dynamics in freshwater environments may be greatly reshaped after the occurrence of regime shifts. This thesis emphasized the importance of considering trophic interactions among multiple groups of the members of the food web when assessing ecosystem responses to environmental factors and also generated information that are useful in an applied sense.

Table of Contents

List of Tables

List of Figures

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

Yang Liu

Signed on, 29 September 2017

Acknowledgements

I would like to express my deep appreciation and sincere gratitude to my supervisor, Dr. Justin D. Brookes. His wise knowledge, invaluable guidance and great patience has lead me into a wonderful world of freshwater ecology during the last four years. Without his support, my fantastic journey in this PhD programme would not have been possible, since I studied nutrient cycling in terrestrial ecosystems for my master subject. I enjoyed the study under his supervision, all I learn from him will be beneficial for my future career. I would also like to thank Dr. Anna Rigosi, whose constructive criticism and valuable advice considerably helped me.

This research was supported by the China Scholarship Council (CSC) and the University of Adelaide. Goyder Institute for Water Research provided funding for conducting field surveys, and Dr Philip Orr and his colleagues generously provided funding for attending the 10th International Conference on Toxic Cyanobacteria.

I thank Sanjina Upadhyay, Virginie Gaget, Deborah Furst, Adam Tomlinson, Chaturangi Wickramaratne, Kane Aldridge and Todd Wallace for their kind help in study and for bring me colourful life in Adelaide. I thank Professor Lian Pin Koh for his kind help in the estimation of aquatic plantation in the field. I also wish to thank other postgraduate student and staff of Department of Ecology & Environmental Science for their support and friendship. I would like to extend my gratitude to Leon van der Linden from SA water, Mark Rollog from School of Physical Sciences, John Carragher from School of Agriculture, Food and Wine for their help on sample analysis.

I sincerely thank my parents, parents in law and other family members for their encouragement and support over the past four years. Finally, I am indebted to my wife, Yanchen Zhang, in so many ways. Her unwavering love was the source of strength whenever I have a difficult time in this PhD programme.

Chapter 1 General introduction

1.1 Research background

1.1.1 Food-web structure and trophic link in aquatic ecosystems

Food webs describe the trophic interactions between consumers and resources, i.e. who eats who in ecosystems. Basal resources supporting the food webs of aquatic ecosystems can originate from three distinct habitats: as autochthonous primary production in pelagic and benthic habitats, and as allochthonous primary production in adjacent terrestrial habitats (Solomon et al. 2011). Fundamental differences between the relative availability of these sources of primary production to consumers and between the relative quality of these sources as food existed, affecting their contributions to consumers (Brett et al. 2009; Zanden et al. 2006).

The accessibility of terrestrial resource subsidies of carbon sources, including flows of dissolved and particulate organic carbon (DOC and POC) and terrestrial prey items, are often related to the land use of surrounding environments adjacent to aquatic ecosystems (Cole et al. 2006; Gratton et al. 2008; Pace et al. 2004). For example, consumer production tends to be supported by basal resources dominated by allochthonous carbon sources such as leaf litter in forested streams, whereas consumer production tends to be supported by basal resources dominated by autochthonous carbon sources such as benthic algae in grassland streams (Zanden et al. 2011).

Different forms of terrestrial organic carbon enter aquatic food webs through different pathways. Aquatic consumers such as fish and predatory macroinvertebrates can prey on terrestrial animals, representing direct carbon flux of allochthonous source into aquatic ecosystems (Mehner et al. 2005). Terrestrial inputs of DOC primarily support the catabolic

and anabolic processes of heterotrophic bacteria, which in turn fuel biomass production of primary consumers feeding in the pelagic habitat and consumers at higher trophic levels (Carpenter et al. 2005; Jansson et al. 2008), while terrestrial inputs of POC is processed by microbial communities, zooplankton and macroinvertebrates (Karlsson and Jonsson 2003; Pace et al. 2007).

The study of food webs in lakes have historically focused on pelagic processes, and neglected the contribution of benthic production to whole-lake production (Vadeboncoeur et al. 2002). However, recent work challenged this traditional tenet of pelagic domination of primary production in limnology by indicating a benthic domination of primary production in oligotrophic and shallow lakes (Ask et al. 2009a; Ask et al. 2009b; Vadeboncoeur et al. 2003), and benthic resources can play a large role in supporting fish (Hecky and Hesslein 1995; Karlsson and Byström 2005; Vander Zanden and Vadeboncoeur 2002). Lake size and morphology, as well as water chemistry in lakes, are considered as important determinants of the relative contributions of pelagic and benthic forms of primary production to whole-lake primary production (Vadeboncoeur et al. 2008). Habitat coupling regarding resource use may be more common than generally realized, for instance, benthic macroinvertebrates may utilize settled pelagic production, benthic algae may support zooplankton growth, and fish may consume benthic or pelagic prey (Karlsson and Säwström 2009; Solomon et al. 2011; Vander Zanden and Vadeboncoeur 2002). Within benthic habitats in lakes, consumers can be supported by autochthonous primary production in different forms including benthic algae, macrophytes and associated periphyton (Vadeboncoeur et al. 2003; Vadeboncoeur et al. 2002; Zanden et al. 2011).

1.1.2 Food quality and transfer efficiency in aquatic ecosystems

The amount of energy flowing from one trophic level to the next depends on the production at the lower trophic level and the efficiency at which it is converted to the production at the higher trophic level (Rowland et al. 2015). Insufficient transfer of primary production can result in low production of animals at higher trophic levels together with accumulation of nuisance blooms of primary producers (Persson et al. 2007). Food quality often constraints trophic transfer efficiency, and it can be expressed or determined as nutritional quality such as nutrient content, lipid content, caloric content, and edibility quality such as structure of carbon molecules of basal resources in aquatic ecosystems (Brett et al. 2009; Dickman et al. 2008; Lau et al. 2008).

Living organisms must regulate their elemental composition to keep it within a limited range for normal cell functioning, however, elemental ratios of food supplies generally deviate from elemental ratios of cells. Disparities in the elemental composition of consumers and food sources are considered elemental imbalances and significant elemental imbalances limit consumer growth and reproduction (Sterner and Elser 2002). Ecological stoichiometry studies the relative imbalance of key elements between the supply of resources and the need of consumers, and proposes that the elemental imbalance between consumers and food sources can be used to indicate food quality, because it reflects the degree of nutrient constraint (Sterner and Elser 2002).

Stoichiometric homeostasis describes the ability of organisms to keep a constant elemental composition regardless of the changes of elemental composition in the ambient environments, particularly that in their food for consumers (Hessen et al. 2013). An organism's degree of stoichiometric homeostasis can be characterized by the homeostasis coefficient, and strict stoichiometric homeostasis indicates that organismal elemental composition is completely independent of elemental composition of resources in ecological stoichiometry (Elser et al. 2000; Elser and Urabe 1999; Sterner and Elser 2002).

Ecological stoichiometry primarily assumes strict regulation of elemental ratios in animals, while autotrophs may exhibit relatively flexible stoichiometry (Persson et al. 2010; Sterner et al. 1998; Sterner and Elser 2002). The generality of stoichiometric homeostasis of consumers, however, was challenged as many studies revealed the plasticity of consumer stoichiometry (Cross et al. 2003; Fink and Elert 2006; Liess and Hillebrand 2005; Persson et al. 2010; Small and Pringle 2010). Intraspecific variation in elemental composition of consumers represents deviation from strict homeostasis, and may be associated with changes in local conditions such as food quality, reflecting nutritional limitation in organisms (Feijoó et al. 2014; Hillebrand et al. 2008; Small et al. 2011). Interspecific variation in nutrient stoichiometry can be attributed to long-term mechanisms such as phylogenetically conserved differences during evolutionary time (Lauridsen et al. 2012).

Certain nutrients become limiting when large elemental imbalances exist between heterotrophic consumers and their food sources, and these stoichiometric constraints on consumer growth and reproduction can occur at any levels of consumer elemental regulation (Anderson et al. 2004; Frost et al. 2005b). The framework of ecological stoichiometry began from illustrating the relationships between the primary production of phytoplankton and herbivore production of zooplankton in pelagic habitats, and has widened to include many other organism groups and ecosystem types ever since (Hessen et al. 2013; Sterner and Elser 2002).

Laboratory experiments indicating that consumer species vary in their responses to changes in food quality regarding elemental compositions reveal that food quality also depends on properties related to physiological and behavioural traits in animals, including assimilation efficiency, and feeding habits. The development of threshold elemental ratio theory addresses the effects of those factors, and threshold elemental ratios for C:N or C:P, above which N or P becomes limiting for consumer growth, were calculated using models incorporating data on

animal bioenergetics, body elemental composition and resource availability (Frost et al. 2006; Sterner 1997; Urabe and Watanabe 1992). Threshold elemental ratio theory has provided insights into detecting N or P limitation of secondary production, as well as indicating competition advantages between species and predicting responses of community composition to changes in food quality (Frost et al. 2006; Urabe and Watanabe 1992).

Ecological stoichiometry has proven to be useful in exploring causes of variable elemental nutrient ratios within and among organisms and predicting consequences of mismatches between elemental nutrient ratios of consumers and their food sources, it has also been applied to advance understanding of key biogeochemical cycling processes such as decomposition of organic matter and consumer-driven nutrient recycling (Cross et al. 2005; Frost et al. 2005a). The relative availability of C, N and P is of particular importance in determining population and community dynamics, and has implications for whole system productivity in aquatic environments.

Fatty acid composition of basal resources is also an important indicator of nutritional quality, which has critical impacts on consumer growth and reproduction as well as trophic transfer efficiency just as elemental nutrient composition has (Brett and Müller-Navarra 1997; Müller-Navarra et al. 2000; Persson et al. 2007). Most of the food quality research has focused on either elemental stoichiometry or polyunsaturated fatty acid composition in pelagic food webs of lacustrine systems where crustacean zooplankton feeds on phytoplankton and acts as major channel of carbon transfer through food webs (Guo et al. 2016; Persson et al. 2007).

1.1.3 Effects of light and nutrients on food quantity and quality in aquatic ecosystems

Light and nutrients often limit primary production in aquatic ecosystems, and the relative availability of these two resources also influence the food quality of primary producers for

consumers (Rowland et al. 2015). The light : nutrient hypothesis states that changes in food quantity and quality as regulated by the relative availability of light and nutrient limit the production of primary consumers and addresses the balance of supply of light and nutrients in supporting the production of primary consumers (Sterner et al. 1997). However, the effectiveness of using light : nutrient hypothesis to explain herbivore growth and production may be dampened in ecosystems where the overall importance of food quantity or quality control of the growth and production of herbivores is suppressed by other factors such as predator avoidance, resource depression and exploitation of mixotrophic food resources (Hill et al. 2010; Katechakis et al. 2005; Liess and Lange 2011).

1.1.4 Regime shifts and filamentous algae proliferation in aquatic ecosystems

The ecological theory that ecosystems can exist in more than one stable state and have more than one equilibrium has long been recognized (May 1977; Noy-Meir 1975). Observations in shallow lakes have led freshwater ecologists to speculate that these ecosystems may indeed possess two alternative stable states, a clear water state dominated by macrophytes and a turbid water state dominated by phytoplankton (Blindow et al. 1993; Scheffer 1989; Scheffer 1990; Scheffer et al. 1993).

Slow changes in environmental factors, such as gradual increase in nutrient concentrations, can undermine the resilience of a clear water state, and small stochastic perturbations or disturbances, such as heavy storm, can cause a regime shift from clear water state to turbid water state once the ecosystem stability is largely damaged (Scheffer 2001; Scheffer et al. 2001; Scheffer and Carpenter 2003; Scheffer et al. 1993; Scheffer and Jeppesen 2007). A large amount of empirical evidence supporting the theoretically predicted regime shifts have been published since the theory was first investigated in the 1980s (Bachmann et al. 1999; Bayley and Prather 2003; Ibelings et al. 2007; Jackson 2003; Lowe et al. 2001; Nes et al.

2002). The evidence of regime shifts in terrestrial and aquatic environments in relation to resilience of complex adaptive ecosystems and the functional roles of biological diversity in this context has also been reviewed in Folke et al. (2004).

Recent work has further expanded the concept of stable states to include regimes dominated by floating-leaf macrophytes and specific types of phytoplankton (Scheffer and Nes 2007; Scheffer et al. 2003), although most work still focused on the more commonly observed shifts between submerged macrophytes and phytoplankton (Zimmer et al. 2009). Studies attempting to provide early-warning indicators for ecosystem regime shifts have also emerged in recent years, with many of them focusing on lake ecosystems (Carpenter et al. 2008; Carpenter et al. 2011; Contamin and Ellison 2009; Guttal and Jayaprakash 2008; Nes and Scheffer 2007; Scheffer et al. 2009; Wang et al. 2012). Ecosystem management aims to prevent perturbations for long periods of time, however, new insights highlight the importance of managing gradual changes, which may be predicted, monitored and modified in ecosystems (Scheffer et al. 2001).

Although the process of eutrophication induced regime shifts is reasonably well understood in lentic systems such as lakes, conceptual understandings of how eutrophication triggers regime shifts in lentic systems are relatively scarce. A conceptual model describing changes of domination in autotrophic structure with eutrophication conditions in rivers has been developed by Hilton et al. (2006), based on mechanisms known to be important in lakes but modified by additional processes known to be important in rivers, and predictions of the model were supported by a number of filed observations in eutrophic rivers. Capon et al. (2015) pointed out that although the concepts of regime shifts and alternative stable states have become prominent in the scientific and management literature, their empirical underpinning is weak outside of a specific environmental setting.

Proliferation of benthic filamentous algae has been reported in many parts of the world, and blooms of those benthic filamentous algae dominated by green algae or cyanobacteria can be a result of inefficient trophic transfer of primary prodcution (Hart et al. 2013; Hudon et al. 2014; Power et al. 2009). Accumulation of the biomass of benthic filamentous algae depends on the relative strength of accrual process and loss process, and is determined by a range of environmental factors including light regime, hydraulic forces, algal grazers and, in particular, nutrient concentrations, which have been increasing worldwide owing to intensified agricultural activities and urban development (Carpenter et al. 1998; Hansen et al. 2014; Sturt et al. 2011). Field surveys, laboratory experiments, and simulating models generally confirmed that nutrient stimulate benthic filamentous algae growth, thus nutrient enrichments were generally considered as primary cause of benthic filamentous algae proliferation, largely ignoring other potential contributing factors (Biggs 2000; Dodds et al. 1997; Stevenson et al. 2006).

1.2 Study site

Ewens Ponds are a series of three small freshwater ponds located in the southeast of South Australia (38°01′36″S, 140°47′26″E), which flow into Eight Miles Creek, in turn, discharge to the sea ca. 2.5 km downstream (Wood 2011). The general characteriscs of the three ponds are given in Table 1.

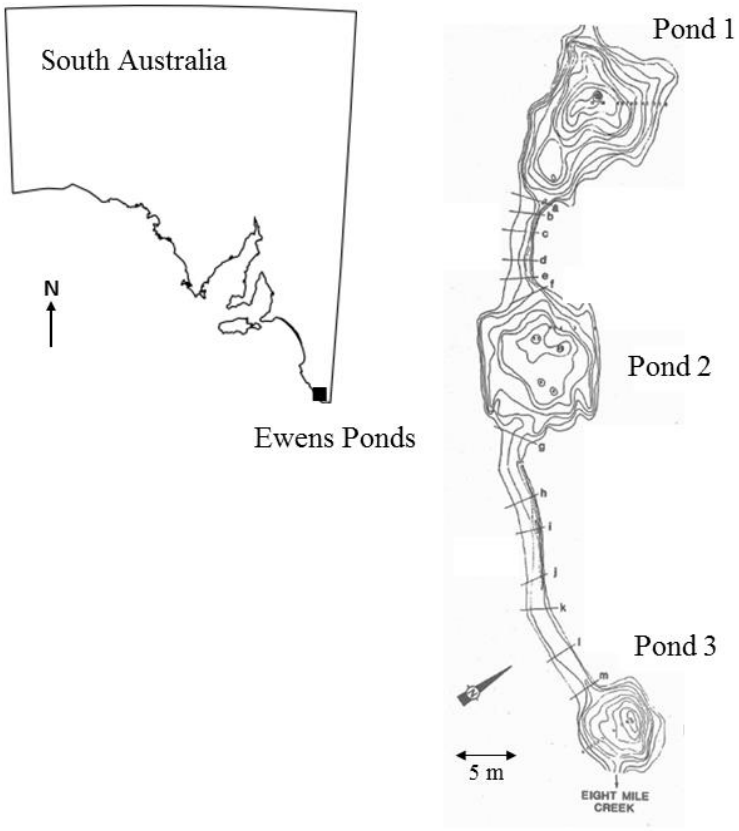


Figure 1-1 Location and map of Ewens Ponds, modified from Grandfield and Ashman (1984).

Table 1-1 General characteristics of the three ponds in Ewens Ponds Conservation Park in the southeast of South Australia

	Pond 1	Pond 2	Pond 3
Shape	Pear (75 m × 50 m)	Bowl (diameter 50m)	Bowl (diameter 35m)
Surface area (m^2)	3133	2423	977
Maximum depth (m)	11	6	9
Volume (m^3)	28000	11000	4400

Ewens Ponds is highly dependent upon underground water sourced from two distinct aquafers: the upper unconfined Tertiary Limestone Aquifer and the lower confined Tertiary Sand Aquifer, and estimated water source from surface runoff contributes less than 5% of the total water budget (Grandfield and Ashman 1984). The groundwater inflow entered the system mostly from Pond 1 (70% of the flow), and the rest came from Pond 3, with no groundwater input was observed in Pond 2 (Rigosi et al. 2015). Water can be clearly observed bubbling into the ponds through soft sediments at the bottom of Pond 1. The annual mean total discharge from Ponds is about 104, 845 m^3 day^{-1} in 2014 – 2015 and the retention time for water is around half a day for the whole system (Rigosi et al. 2015). Total dissolved solids (TDS) of all the three ponds are lower than 800 mg L^{-1} (Grandfield and Ashman 1984; Wood 2011). The water is extremely clear, with an extinction coefficient, K_d, of 0.2 m^{-1} measured on sunny days in austral summer. Some other abiotic variables measured during the sampling period of 2014-2015 were: water temperature 15.7-16.1 °C, conductivity 0.60-0.70 mS cm^{-1}, dissolved oxygen (DO) 6.7-7.3 mg L^{-1}, pH 6.7-7.3. Annula mean of total nitrogen (TN) and phosphorus (TP) concentrations of pond water measured during the sampling period of 2014-2015 were 5.774 and 0.020 mg L^{-1}, respectively. TN comprised mainly of nitrate (5.157 mg L^{-1}) and TP comprised mainly of orthophosphate (0.008 mg L^{-1}). There were only little variability in water physio-chemical properties between water of different depth and different sites within the system and temporal variations of those parameter were also minimal.

The dominant vegetation type immediately around Ewens Ponds and Eight Miles Creek is a closed-scrub formation of woolly tea tree (*Leptospermum lanigerum*) and scented paperbark (*Melaleuca squarrosa*) mixed with closed-grassland formation (Grandfield and Ashman 1984). In areas of more wet soil and swamp, the dominant species are common reed (*Phragmites australis*) and lesser bulrush (*Typha augustifolia*). In the channels between the

ponds, the dominant species are watercress (*Nasturtium officinale*) and common spike rush (*Eleocharis acuta*). The aquatic vegetation communities are abundant, and plants are variably distributed with depth and flow (Grandfield and Ashman 1984). A dense mat of semi-emergent water ribbon (*Triglochin procera*) rim the ponds, and below the water surface shield pennywort (*Hydrocotyle verticillata*) and river buttercup (*Ranunculus inundatus*) form on most littoral sediment where the slope is not too steep. The bottom zone where macrophytes can't establish is occupied by filamentous algae with two distinct pigmentation, with shallower part covered by light green algae (dominated by chlorophyte, *Rhizoclonium* sp.) and deeper part covered by dark green algae (dominated by cyanobacteria, *Lyngbya* sp.). Underwater macrophytes and filamentous algae are usually covered with a layer of biofilm (mainly diatoms as well as detritus and other microorganisms). Phytoplankton and seston are rare in the water column. Floating algal blooms have been increasingly reported in recent years, however this algae generally developed from benthic filamentous algae mats that became buoyant with trapped gas bubbles formed during photosynthesis.

The fluffy benthic algae mats and stretching macrophytes provide large surface areas with small standing biomass density, supporting large abundance of major macroinvertebrate species in the ponds. The macroinvertebrate assemblages include several taxonomic groups, such as insects (caddisfly larva, *Triplectides* sp. and diving beetles, *Antiporus* sp.), gastropods (Planorbidae snails, *Glyptophysa gibbosa* and Hydrobiidae snails, *Potamopyrgus antipodarum*) and small crustaceans (decapod, *Paratya australiensis* and *Amarinus lacustris*, amphipod, *Austrochiltonia* sp.). The mean mass of algae and macrophyte standing crop collected in December, 2014 at Ewens Ponds was approximately 54 g m^{-2} (AFDM) and 103 g m^{-2} (AFDM) respectively, and density of *Triplectides* sp., *G. gibbosa*, and *P. antipodarum* reached 4205 individuals m^{-2}, 1090 individuals m^{-2} and 8950 individuals m^{-2} respectively. Dense aquatic vegetation and associated macroinvertebrate community in the ponds support

several native fish species including southern shortfin eel (*Anguilla australis*), southern black bream (*Acanthopagrus butcheri*), river blackfish (*Gadopsis marmoratus*), congolli (*Pseudaphritis urvillii*), common galaxias (*Galaxias maculatus*), and southern pygmy perch (*Nannoperca australis*). The ponds also host the endangered large crustacean species, Glenelg spiny crayfish (*Euastacus bispinosus*).

Ewens Ponds are a popular recreation site, particularly for scuba diving and snorkelling and support several vulnerable and endangered species of aquatic fauna and flora, such as Ewens pygmy perch (*Nannoperca variegata*) and Glenelg spiny crayfish (*Euastacus bispinosus*) (Grandfield and Ashman 1984; National Parks and Wildlife SA 1999). Thus, Ewens Ponds are valued for the delivery of both ecological and recreational services, and are listed on the Register of the National Estate and under special protection by the National Parks and Wildlife Act 1972 and the National Parks and Wildlife Regulations 2001. Alteration of the regional hydrology due to increased water extraction and change of land use from native vegetation to managed pastures, decreased the quantity and quality of groundwater flowing into Ewens Ponds. Expansion of benthic filamentous algae mats and shrink of rooted vascular plant beds under water, together with episodic occurrence of cyanobacterial blooms have been observed in the ponds, and they are initial warning signals of an ongoing regime shift from domination by submerged macrophytes to domination by filamentous algae (Carmody 2006).

1.3 Aims, approaches and thesis structure

The overall objective of this thesis was to investigate food web structure and nutrition in the benthic habitats of a groundwater-fed freshwater wetland and assess potential consequences of altered nutrient and light regimes on growth and production of key components of that food web.

In order to achieve the goals, I combined field surveys and laboratory experiments to address four main questions:

(1) What are the basal resources supporting the food web of groundwater-fed ponds?

(2) How is the production of the system constrained by food quality regarding the stoichiometric ratios and fatty acid composition in basal resources and consumers?

(3) How do periphyton and grazing snail growth and stoichiometry response to changes in light levels and nutrient conditions?

(4) What are the influences of varying nutrient concentrations on benthic filamentous algae growth and stoichiometry?

I conducted field surveys of basic hydrology, biogeochemistry and biology conditions in Ewens Ponds, I collected samples of water from different sites and depth as well as samples of major components of food web. Food web structure and nutrition in the benthic habitats of Ewens Ponds were established. I conducted laboratory experiments to examine the dynamics of a few key components of the benthic food web including periphyton, snails and benthic filamentous algae as affected by condition of environmental factors such as light and nutrient.

Chapter 2 of this thesis investigated the major basal resources for the main aquatic animals in Ewens Ponds, including benthic macroinvertebrates and fish. The different contributions of basal resource groups to consumers were estimated using isotopic mixing models, and specific feeding patterns of different consumer species were recognized using results of mixing models combined with results of gut content analysis and fatty acid composition analysis.

In Chapter 3, the elemental nutrient stoichiometry and fatty acid composition of major basal resources and consumers in Ewens Ponds were determined, and the elemental imbalance

between consumers and the food assimilated was estimated. Furthermore, the standing stock of N and P in macroinvertebrates and water column and the relative availability of N and P for macroinvertebrates were evaluated.

In Chapter 4, I investigated the importance of light and nutrient conditions in determining periphyton quantity and quality and in turn affecting the growth and production of a grazing snail species.

Chapter 5 dealt with the effects of availability of N and P on algal growth and stoichiometry of the two dominant benthic filamentous algae species.

Chapter 6 integrated the findings from Chapter 2-5 and assesses the whole system production at different trophic levels. In particular, the limitation of efficient trophic transfer by insufficient nutrients or energy or palatable food and its ecological implications were investigated. The results of this thesis will improve our understanding of key ecological processes such as trophic transfer of energy and nutrients through food webs and provide useful information on the management and conservation of valuable freshwater habitats.

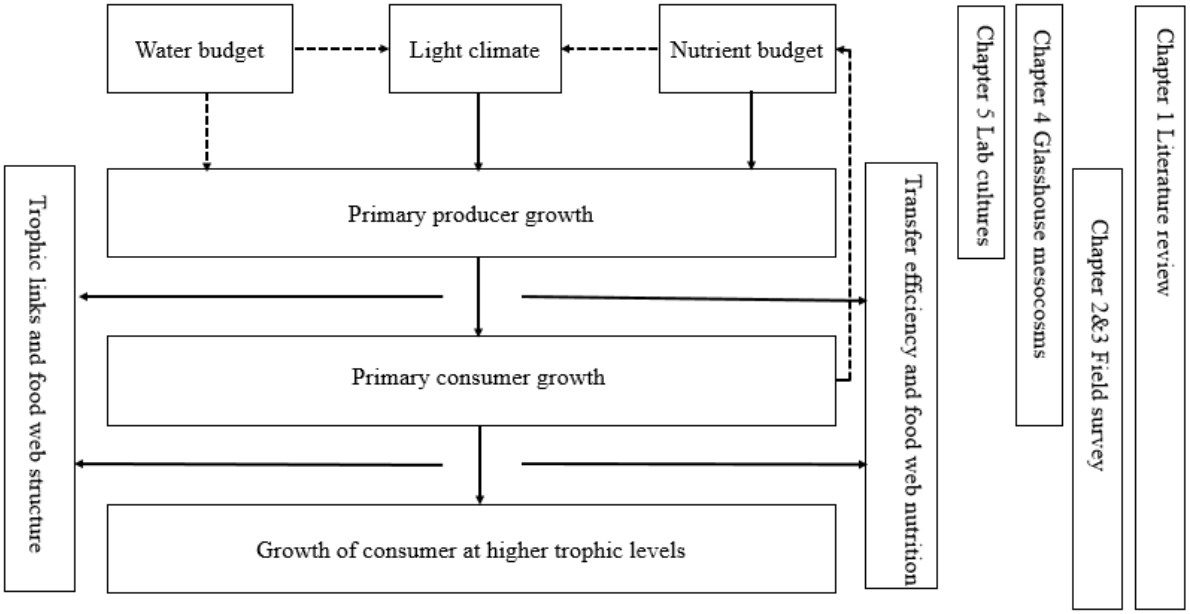


Figure 1-2 The conceptual model for this study. Solid lines represent what this study addresses, and dashed lines stand for future directions.

References

Anderson, T. R., M. Boersma & D. Raubenheimer, 2004. Stoichiometry: linking elements to biochemicals. Ecology 85:1193-1202.

Ask, J., J. Karlsson, L. Persson, P. Ask, P. Byström & M. Jansson, 2009a. Terrestrial organic matter and light penetration: Effects on bacterial and primary production in lakes. Limnology and Oceanography 54:2034-2040.

Ask, J., J. Karlsson, L. Persson, P. Ask, P. Byström & M. Jansson, 2009b. Whole-lake estimates of carbon flux through algae and bacteria in benthic and pelagic habitats of clear-water lakes. Ecology 90:1923-1932.

Bachmann, R. W., M. V. Hoyer & Daniel E. Canfield Jr., 1999. The restoration of Lake Apopka in relation to alternative stable states. Hydrobiologia 394:219-232.

Bayley, S. E. & C. M. Prather, 2003. Do wetland lakes exhibit alternative stable states? Submersed aquatic vegetation and chlorophyll in western boreal shallow lakes. Limnology and Oceanography 48:2335-2345.

Biggs, B. J. F., 2000. Eutrophication of streams and rivers: dissolved nutrient-chlorophyll relationships for benthic algae. Journal of North American Benthological Society 19:17-31.

Blindow, I., G. Andersson, A. Hargeby & S. Johansson, 1993. Long-term pattern of alternative stable states in two shallow eutrophic lakes. Freshwater Biology 30:159-167.

Brett, M. T., M. J. Kainz, S. J. Taipale & H. Seshan, 2009. Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. Proceedings of National Academy of Sciences 106:21197-21201.

Brett, M. T. & D. C. Müller-Navarra, 1997. The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshwater Biology 38:483-499.

Capon, S. J., A. J. J. Lynch, N. Bond, B. C. Chessman, J. Davis, N. Davidson, M. Finlayson, P. A. Gell, D. Hohnberg, C. Humphrey, R. T. Kingsford, D. Nielsen, J. R. Thomson, K. Ward & R. M. Nally, 2015. Regime shifts, thresholds and multiple stable states in freshwater ecosystems; a critical appraisal of the evidence. Science of the Total Environment 534:122-130.

Carmody, G., 2006. Save Ewens Ponds! Marine Life Society of South Australia Journal:55-65.

Carpenter, S. R., W. A. Brock, J. J. Cole, J. F. Kitchell & M. L. Pace, 2008. Leading indicators of trophic cascades. Ecology Letters 11:128-138.

Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley & V. H. Smith, 1998. Nonpoint pollution of surface waters with phosphorous and nitrogen. Ecological Applications 8:559-568.

Carpenter, S. R., J. J. Cole, M. L. Pace, R. Batt, W. A. Brock, T. Cline, J. Coloso, J. R. Hodgson, J. F. Kitchell, D. A. Seekell, L. Smith & B. Weidel, 2011. Early warning of regime shifts: a whole-ecosystem experiment. Science 332:1079-1082.

Carpenter, S. R., J. J. Cole, M. L. Pace, M. V. d. Bogert, D. L. Bade, D. Bastviken, C. M. Gille, J. R. Hodgson, J. F. Kitchell & E. S. Kritzberg, 2005. Ecosystem subsidies: terrestrial support of aquatic food webs from ^{13}C additon to contrasting lakes. Ecology 86:2737-2750.

Cole, J. J., S. R. Carpenter, M. L. Pace, M. C. V. d. Bogert, J. L. Kitchell & J. R. Hodgson, 2006. Differential support of lake food webs by three types of terrestrial organic carbon. Ecology Letters 9:558-568.

Contamin, R. & A. M. Ellison, 2009. Indicators of regime shifts in ecological systems: what do we need to know and when do we need to know it? Ecological Applictions 19:799-816.

Cross, W. F., J. P. Benstead, P. C. Frost & S. A. Thomas, 2005. Ecological stoichiometry in freshwater benthic systems: recent progress and perspectives. Frehswater Biology 50:1895-1912.

Cross, W. F., J. P. Benstead, A. D. Rosemond & J. B. Wallace, 2003. Consumer-resource stoichiometry in detritus-based streams. Ecology Letters 6:721-732.

Dickman, E. M., J. M. Newell, M. J. González & M. J. Vanni, 2008. Light, nutrients, and food-chain length constrain planktonic energy transfer efficiency across multiple trophic levels. Proceedings of National Academy of Sciences 105:18408-18412.

Dodds, W. K., V. H. Smith & B. Zander, 1997. Developing nutrient tragets to control benthic chlorophyll levels in streams: a case study of the Clark Fork River. Water Research 31:1738-1750.

Elser, J. J., W. F. Fagan, R. F. Denno, D. R. Dobberfuhl, A. Folarin, A. Huberty, S. Interlandi, S. S. Kilham, E. McCauley, K. L. Schulz, E. H. Siemann & R. W. Sterner, 2000. Nutritional constraints in terrestrial and freshwater food webs. Nature 408:578-580.

Elser, J. J. & J. Urabe, 1999. The stoichiometry of consumer-driven nutrient recycling: theory, observations, and consequences. Ecology 80:735-751.

Feijoó, C., L. Leggieri, C. Ocón, I. Muñoz, A. R. Capítulo, A. Giorgi, D. Colautti, N. Ferreiro, M. Licursi, N. Gómez & S. Sabater, 2014. Stoichiometric homeostasis in the food web of a chronically nutrient-rich stream. Freshwater Science 33:820-831.

Fink, P. & E. V. Elert, 2006. Physiological responses to stoichiometric constraints: nutrient limitation and compensatory feeding in a freshwater snail. Oikos 115:484-494.

Folke, C., S. Carpenter, B. Walker, M. Scheffer, T. Elmqvist, L. Gunderson & C. S. Holling, 2004. Regime shifts, resilience, and biodiversity in ecosystem management. Annual Review of Ecology, Evolution and Systematics 35:557-581.

Frost, P. C., J. P. Benstead, W. F. Cross, H. Hillebrand, J. H. Larson, M. A. Xenopoulos & T. Yoshida, 2006. Threshold elemental ratios of carbon and phosphorus in aquatic consumers. Ecology Letters 9:774-779.
Frost, P. C., W. F. Cross & J. P. Benstead, 2005a. Ecological stoichiometry in freshwater benthic ecosystems: an introduction. Frehswater Biology 50:1781-1785.
Frost, P. C., M. A. Evans-White, Z. V. Finkel, T. C. Jensen & V. Matzek, 2005b. Are you what you eat? Physiological constraints on organismal stoichiometry in an elementally imbalanced world. Oikos 109:18-28.
Grandfield, L. & G. Ashman, 1984. Ewens Ponds Management Plan Draft. Department of Lands, South East Region.
Gratton, C., J. Donaldson & M. J. V. Zanden, 2008. Ecosystem linkages between lakes and the surrounding terrestrial landscape in Northeast Iceland. Ecosystems 11:764-774.
Guo, F., M. J. Kainz, F. sheldon & S. E. Bunn, 2016. The importance of high-quality algal food sources in stream food webs - current status and future perspectives. Frehswater Biology 61:815-831.
Guttal, V. & C. Jayaprakash, 2008. Changing skewness: an early warning signal of regime shifts in ecosystems. Ecology Letters 11:450-460.
Hansen, A. T., M. Hondzo, J. Sheng & M. J. Sadowsky, 2014. Microscale measurements reveal contrasting effects of photosynthesis and epiphytes on fractional drag on the surfaces of filamentous algae. Freshwater Biology 59:312-324.
Hart, D. D., B. J. F. Biggs, V. I. Nikora & C. A. Flinders, 2013. Flow effects on periphyton patches and their ecological consequences in a New Zealand river. Freshwater Biology 58:1588-1602.
Hecky, R. E. & R. H. Hesslein, 1995. Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. Journal of the North American Benthological Society 14:631-653.
Hessen, D. O., J. J. Elser, R. W. Sterner & J. Urabe, 2013. Ecological stoichiometry: An elementary approach using basic principles. Limnology and Oceanography 2013:2219-2236.
Hill, W. R., J. G. Smith & A. J. Stewart, 2010. Light, nutrients, and herbivore growth in oligotrophic streams. Ecology 91:518-527.
Hillebrand, H., P. Frost & A. Liess, 2008. Ecological stoichiometry of indirect grazer effects on periphyton nutrient content. Oecologia 155:619-630.
Hilton, J., M. O'Hare, M. J. Bowes & J. I. Jones, 2006. How green is my river? A new paradigm of eutrophication in rivers. Science of the Total Environment 365:66-83.
Hudon, C., M. D. Sève & A. Cattaneo, 2014. Increasing occurrence of the benthic filamentous cyanobacterium *Lyngbya wollei*: a symptom of freshwater ecosystem degradation. Freshwater Science 33:606-618.
Ibelings, B. W., R. Portielje, E. H. R. R. Lammens, R. Noordhuis, M. S. v. d. Berg, W. Joosse & M. L. Meijer, 2007. Resilience of alternative stable states during the recovery of shallow lakes from eutrophication: Lake Veluwe as a case study. Ecosystems 10:4-16.
Jackson, L. J., 2003. Macrophyte-dominated and turbid states of shallow lakes: Evidence from Alberta lakes. Ecosystems 6:213-223.
Jansson, M., T. Hickler, A. Jonsson & J. Karlsson, 2008. Links between terrestrial primary production and bacterial production and respiration in lakes in a climate gradient in subarctic Sweden. Ecosystems 11:367-376.
Karlsson, J. & P. Byström, 2005. Littoral energy mobilization dominates energy supply for top consumers in subarctic lakes. Limnology and Oceanography 50:538-543.

Karlsson, J. & A. Jonsson, 2003. Control of zooplankton dependence on allochthonous organic carbon in humic and clear-water lakes in northern Sweden. Limnology and Oceanography 48:269-276.
Karlsson, J. & C. Säwström, 2009. Benthic algae support zooplankton growth during winter in a clear-water lake. Oikos 118:539-544.
Katechakis, A., T. Haseneder, R. Kling & H. Stibor, 2005. Mixotrophic versus photoautotrophic specialist algae as food for zooplankton: The light : nutrient hypothesis might not hold for mixotrophs. Limnology and Oceanography 50:1290-1299.
Lau, D. C. P., K. M. Y. Leung & D. Dudgeon, 2008. Experimental dietary manipulations for determining the relative importance of allochthonous and autochthonous food resources in tropical streams. Freshwater Biology 53:139-147.
Lauridsen, R. B., F. K. Edwards, M. J. Bowes, G. Woodward, A. G. Hildrew, A. T. Ibbotson & J. I. Jones, 2012. Consumer-resource elemental imbalances in a nutrient-rich stream. Freshwater Science 31:408-422.
Liess, A. & H. Hillebrand, 2005. Stoichiometric variation in C:N, C:P, and N:P ratios of littoral benthic invertebrates. Journal of the North American Benthological Society 24:256-269.
Liess, A. & K. Lange, 2011. The snail *Potamopyrgus antipodarum* grows faster and is more active in the shade, independent of food quality. Oecologia 167:85-96.
Lowe, E. F., L. E. Battoe, M. F. Coveney, C. L. Schelske, K. E. Havens, E. R. Marzolf & K. R. Reddy, 2001. The restoration of Lake Apopka in relation to alternative stable states: an alternative view to that of Bachmann et al. (1999). Hydrobiologia 448:11-18.
May, R. M., 1977. Thresholds and breakpoints of ecosystems with a multiplicity of stable states. Nature 269:471-477.
Mehner, T., J. Ihlau, H. Dörner & F. Hölker, 2005. Can feeding of fish on terrestrial insects subsidize the nutrient pool of lakes. Limnology and Oceanography 50:2022-2031.
Müller-Navarra, D. C., M. T. Brett, A. M. Liston & C. R. Goldman, 2000. A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. Nature 403:74-77.
National Parks and Wildlife SA, S. E. R., Heritage and Biodiversity division of the Department for Environment, Heritage and Aboriginal Affairs, 1999. Ewens Ponds Conservation Park Management Plan: Amendment to Plan of Management. Department for Environment, Heritage and Aboriginal Affairs, Adelaide, SA, Australia.
Nes, E. H. v. & M. Scheffer, 2007. Slow recovery from perturbations as a generic indicator of a nearby catastrophic shift. The American Naturalist 169:738-747.
Nes, E. H. v., M. Scheffer, M. S. v. d. Berg & H. Coops, 2002. Dominance of charophytes in eutrophic shallow lakes —when should we expect it to be an alternative stable state? Aquatic Botany 72:275-296.
Noy-Meir, I., 1975. Stability of grazing systems: an application of predator-prey graphs. Journal of Ecology 63:459-481.
Pace, M. L., S. R. Carpenter, J. J. Cole, J. J. Coloso, J. F. Kitchell, J. R. Hodgson, J. J. Middelburg, N. D. Preston, C. T. Solomon & B. C. Weidel, 2007. Does terrestrial organic carbon subsidize the planktonic food web in a clear-water lake? Limnology and Oceanography 52:2177-2189.
Pace, M. L., J. J. Cole, S. R. Carpenter, J. F. Kitchell, J. R. Hodgson, M. C. V. d. Bogert, D. L. Bade, E. S. Kritzberg & D. Bastviken, 2004. Whole-lake carbon-13 addtions reveal terreatrial support of aquatic food webs. Nature 427:240-243.

Persson, J., M. T. Brett, T. Vrede & J. L. Ravet, 2007. Food quantity and quality regulation of trophic transfer between primary producers and a keystone grazer (Daphnia) in pelagic freshwater food webs. Oikos 116:1152-1163.
Persson, J., P. Fink, A. Goto, J. M. Hood, J. Jonas & S. Kato, 2010. To be or not to be what you eat: regulation of stoichiometric homeostasis among autotrophs and heterotrophs. Oikos 119:741-751.
Power, M., R. Lowe, P. Furey, J. Welter, M. Limm, J. Finlay, C. Bode, S. Chang, M. Goodrich & J. Sculley, 2009. Algal mats and insect emergence in rivers under Mediterranean climates: towards photogrammetric surveillance. Frehswater Biology 54:2101-2115.
Rigosi, A., Y. Liu, M. Shanafield & J. D. Brookes, 2015. Determining environmental risks to Ewens Ponds in the South East. Goyder Institute for Water Research Technical Report Series No. 15/19, Adelaide, South Australia.
Rowland, F. E., K. J. Bricker, M. J. Vanni & M. J. González, 2015. Light and nutrients regulate energy transfer through benthic and pelagic food chains. Oikos 124:1648-1663.
Scheffer, M., 1989. Alternative stable states in eutrophic, shallow freshwater systems: a minimal model. Hydrobiology Bulletin 23:73-83.
Scheffer, M., 1990. Multiplicity of stable states in freshwater systems. Hydrobiologia 200/201:475-486.
Scheffer, M., 2001. Alternative attractors of shallow lakes. The Scientific World 1:254-263.
Scheffer, M., J. Bascompte, W. A. Brock, V. Brovkin, S. R. Carpenter, V. Dakos, H. Held, E. H. v. Nes, M. Rietkerk & G. Sugihara, 2009. Early-warning signals for critical transitions. Nature 461:53-59.
Scheffer, M., S. Carpenter, J. A. Foley, C. Folke & B. Walker, 2001. Catastrophic shifts in ecosystems. Nature 413:591-596.
Scheffer, M. & S. R. Carpenter, 2003. Catastrophic regime shifts in ecosystems: linking theory to observation. Trends in Ecology and Evolution 18:648-656.
Scheffer, M., S. H. Hosper, M.-L. Meijer, B. Moss & E. Jeppesen, 1993. Alternative equilibria in shallow lakes. Trends in Ecology and Evolution 8:275-279.
Scheffer, M. & E. Jeppesen, 2007. Regime shifts in shallow lakes. Ecosystems 10:1-3.
Scheffer, M. & E. H. v. Nes, 2007. Shallow lakes theory revisted: various alternative regimes driven by climate, nutrients, depth and lake size. Hydrobiologia 584:455-466.
Scheffer, M., S. Szabó, A. Gragnani, E. H. v. Nes, S. Rinaldi, N. Kautsky, J. Norberg, R. M. M. Roijackers & R. J. M. Franken, 2003. Floating plant dominance as a stable state. Proceedings of the National Academy of Sciences 100:4040-4045.
Small, G. E. & C. M. Pringle, 2010. Deviation from strict homeostasis across multiple trophic levels in an invertebrate consumer assemblage exposed to high chronic phosphorus enrichment in a Neotropical stream. Oecologia 162:581-590.
Small, G. E., J. P. Wares & C. M. Pringle, 2011. Differences in phosphorus demand among detritivorous chironomid larvae reflect intraspecific adaptations to differences in food resource stoichiometry across lowland tropical streams. Limnology and Oceanography 56:268-278.
Solomon, C. T., S. R. Carpenter, M. K. Clayton, J. J. Cole, J. J. Coloso, M. L. Pace, M. J. V. Zanden & B. C. Weidel, 2011. Terrestrial, benthic, and pelagic resource use in lakes: results from a three-isotope Bayesian mixing model. Ecology 92:1115-1125.
Sterner, R. W., 1997. Modelling interactions of food quality and quantity in homeostatic consumers. Frehswater Biology 38:473-481.
Sterner, R. W., J. Clasen, W. Lampert & T. Weisse, 1998. Carbon:phosphorus stoichiometry and food chain production. Ecology Letters 1:146-150.

Sterner, R. W. & J. J. Elser, 2002. Ecological stoiochiometry: The biology of elements from molecules to the biosphere. Princeton University Press.
Sterner, R. W., J. J. Elser, E. J. Fee, S. J. Guildford & T. H. Chrzanowski, 1997. The light : nutrient ratio in lakes: the balance of energy and materials affects ecosystem structure and process. The American Naturalist 150:663-684.
Stevenson, R. J., S. T. Rier, C. M. Riseng, R. E. Schultz & M. J. Wiley, 2006. Comparing effects of nutrients on algal biomass in streams in two regions with different disturbance regimes and with applications for developing nutrient criteria. Hydrobiologia 561:140-165.
Sturt, M. M., M. A. K. Jansen & S. S. C. Harrison, 2011. Invertebrate grazing and riparian shade as controllers of nuisance algae in a eutrophic river. Freshwater Biology 56:2580-2593.
Urabe, J. & Y. Watanabe, 1992. Possibility of N or P limitation for planktonic cladocerans: An experimental test. Limnology and Oceanography 37:244-251.
Vadeboncoeur, Y., E. Jeppesen, M. J. Vander Zanden, H.-H. Schierup, K. Christoffersen & D. M. Lodge, 2003. From Greenland to green lakes: Cultural eutrophication and the loss of benthic pathways in lakes. Limnology and Oceanography 48:1408-1418.
Vadeboncoeur, Y., G. Peterson, M. J. Vander Zanden & J. Kalff, 2008. Benthic algal production across lake size gradients: interactions among morphometry, nutrients, and light. Ecology 89:2542-2552.
Vadeboncoeur, Y., M. J. Vander Zanden & D. M. Lodge, 2002. Putting the lake back together: Reintegrating benthic pathways into lake food web models. BioScience 52:44-54.
Vander Zanden, M. J. & Y. Vadeboncoeur, 2002. Fishes as integrators of benthic and pelagic food webs in lakes. Ecology 83:2152-2161.
Wang, R., J. A. Dearing, P. G. Langdon, E. Zhang, X. Yang, V. Dakos & M. Scheffer, 2012. Flickering gives early warning signals of a critical transition to a eutrophic lake state. Nature 492:419-422.
Wood, C., 2011. Measurement and evalution of key groundwater discharge sites in the Lower South East of South Australia, DFW Technical Report 2011/14. Government of South Australia, through Department for Water, Adelaide.
Zanden, M. J. V., S. Chandra, S.-K. Park, Y. Vadeboncoeur & C. R. Goldman, 2006. Efficiencies of benthic and pelagic trophic pathways in a subalpine lake. Canadian Journal of Fisheries and Aquatic Sciences 63:2608-2620.
Zanden, M. J. V., Y. Vadeboncoeur & S. Chandra, 2011. Fish reliance on littoral-benthic resources and the distribution of primary production in lakes. Ecosystems 14:894-903.
Zimmer, K. D., M. A. Hanson, B. R. Herwig & M. L. Konsti, 2009. Thresholds and stability of alternative regimes in shallow Prairie-Parkland lakes of Central North America. Ecosystems 12:843-852.

Statement of Authorship

Title of Paper	Resources supporting the food web of groundwater-fed ponds
Publication Status	☐ Published ☐ Accepted for Publication ■ Submitted for Publication ☐ Unpublished and Unsubmitted work written in publication style
Publication Details	Submitted to Freshwater Biology

Principal Author

Name of Principal Author (Candidate)	Yang Liu		
Contribution to the paper	Designed the study, performed field experiments, conducted laboratory experiments, analysed the data, interpreted the results, wrote the manuscript		
Overall percentage (%)	80 %		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	22/09/2017

Co-author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution

Name of Co-author	Anna Rigosi		
Contribution to the paper	Provided input in conceiving the study and design of experiments, supervised data analysis and interpretation, reviewed the manuscript		
Signature		Date	22/09/2017

Name of Co-author	Justin Brookes		
Contribution to the paper	Provided input in conceiving the study and design of experiments, supervised data analysis and interpretation, reviewed the manuscript		
Signature		Date	4 Oct 2017

Chapter 2 Resources supporting the food web of a groundwater-fed freshwater pond

Abstract

Ponds, defined as inland waterbodies with small area < 50,000 m^2, are very numerous, and have important values for human being. However, many of the ponds around the world were threatened by a series of anthropogenic alterations of the surrounding environment, including land-use change and catchment development. Integrated understanding of ecosystem processes is required for better conservation of ponds. One critical ecosystem process is how organic carbon flows through components in the food web. Organic carbon transfer in ponds may differ from lakes or streams. Pelagic pathways of organic carbon transfer are thought to predominate in lakes, and allochthonous carbon is thought to provide the majority of organic carbon for consumers in food webs in streams. Epiphytes and filamentous algae, although often considered as symptoms for degradation of aquatic ecosystems, could be important basal resources for organisms in clear water ponds. We combined gut content analysis (GCA), stable isotope analysis (SIA) and fatty acid composition analysis to study carbon sources for animals in a groundwater-fed pond with very clear water and abundant macrophytes in the southeast of South Australia. MixSIAR model was used to interpret SIA results. Results confirmed that most macroinvertebrates and fish relied heavily on organic matter derived from epiphytes and filamentous cyanobacteria. One caddisfly larvae (*Triplectidae* sp.) and diving beetle (*Antiporus* sp.) derived their organic carbon from sources other than those sampled in this study, possibly methane-oxidizing bacteria (MOB) and terrestrial materials. Our study demonstrated that the combined use of several tracing techniques (GCA, SIA, and fatty acid composition analysis) was powerful in determining the trophic base of a complex food web. In addition, this tool kit provided new insights into

trophic interactions between organisms as influenced by consumer feeding capability and habitat use. While epiphytes and filamentous cyanobacteria are associated with degradation of aquatic ecosystem quality, the importance of their function in providing organic carbon for consumers at various trophic levels in food webs should not be overlooked, especially in clear water ponds where pelagic pathway of carbon transfer and subsidy of carbon source from materials beyond the boundary of the ecosystems are negligible.

2.1 Introduction

The world's inland lentic ecosystems are dominated by small waterbodies with an area smaller than 1 km^2 (Downing et al. 2006). Ponds are small waterbodies with an area between 1 m^2 and 50,000 m^2, and their number can be 100 times of that of larger lakes (Meester et al. 2005; Oertli et al. 2005). Ponds provide important functions and ecosystem services with social and economic benefits (Oertli et al. 2005; Oertli et al. 2009). Despite their significance, ponds are threatened by human activities, such as land use change and catchment development (Biggs et al. 2005; Oertli et al. 2005). In the southeast of South Australia, the loss of ponds has been attributed to infilling or drainage for agricultural land developments. Although these activities may have commenced in the 19^{th} century, the greatest loss occurred post Second World War (Grandfield and Ashman 1984). Land development has also promoted a decrease in the connectivity of remaining ponds, nutrient enrichment, contaminant inflow, hydrology modification and introduction of invasive species (Biggs et al. 2005; Oertli et al. 2005).

Management and conservation of ponds requires an integrated understanding of pond ecosystems. Research focusing on ponds and other small water bodies has increased drastically over the past few years, however ponds still receive less scientific attention than lakes, rivers and streams (Oertli et al. 2009). Determining patterns of carbon flow through

food-web components across time and space and how these are influenced by environmental changes is critical to understanding pond ecosystems (Pingram et al. 2014).

Pelagic primary production by phytoplankton has long been viewed as the basis for limnetic food webs, leading to the far less research of benthic food webs compared to pelagic food webs (Vadeboncoeur et al. 2001). Benthic production, however, could make appreciable contributions to whole-system primary production, in shallow, clear (Ask et al. 2009; Vadeboncoeur et al. 2001) or turbid systems (Bunn et al. 2003; Pingram et al. 2014). Major consumer groups such as fish and macroinvertebrate can rely largely upon benthic carbon sources in lakes (Hecky and Hesslein 1995; Karlsson and Byström 2005; Vander Zanden and Vadeboncoeur 2002).

Several potential sources of carbon are present in benthic habitats, including detritus, epiphyte, macrophyte, and mat-forming benthic algae (Bunn and Boon 1993; Hecky and Hesslein 1995; Jaschinski et al. 2011; Kelly and Hawes 2005). The relative abundance of these carbon sources and their relative contributions to whole system production may vary depending on several environmental factors (e.g. nutrient, light) (Bunn et al. 2003; Vadeboncoeur et al. 2008). Utilization of allochthonous carbon was found to be consistently low in all consumers with varied habitat use and feeding ability in a small, and shallow but naturally productive lake (Batt et al. 2012). Terrestrial organic carbon is believed to be important for aquatic food webs only in small and shallow systems with poor nutrient condition or low incident light (Carpenter et al. 2005; Holgerson et al. 2016; Karlsson et al. 2012). Organic carbon of autochthonous origin was found to fuel the majority of lake organism production in benthic habitats in clear-water lakes (Ask et al. 2009). The importance of macrophytes to ecosystem structure and function has focused on providing substrates for epiphytic food and refuges from predation (Newman 1991). Herbivory on living macrophytes is conventionally considered minimal in aquatic food webs, and the

pathway of organic matter produced by macrophytes entering aquatic food webs is mainly as detritus (Lodge 1991; Mann 1988; Newman 1991; Webster and Benfield 1986). However, more recent evidences indicated that direct consumption of macrophytes could form a major contribution to the diet of consumers (Batt et al. 2012; Deegan and Ganf 2008; Watson and Barmuta 2011). Epiphytes are believed to provide more nutritious and less repellent food than most macrophytes, thus are consistently ingested as major carbon source through direct grazing or detritus pathway in various aquatic environment (Bunn and Boon 1993; Hecky and Hesslein 1995; James et al. 2000; Jaschinski et al. 2011; Jones and Waldron 2003; Kelly and Hawes 2005). Benthic filamentous algae (including cyanobacteria and chlorophyte species) can sometimes form thick mats covering large areas of bottom in rivers and lakes (Hart et al. 2013; Hudon et al. 2014; Vis et al. 2008). These mat-forming alga, especially filamentous cyanobacteria, are often perceived as unsuitable food for aquatic consumers because of toxic metabolites and morphological defences, however, they don't deter some consumers who use them as a main food source (Camacho and Thacker 2006; Lévesque et al. 2015). Benthic filamentous algae have been demonstrated to support consumers in riverine food webs in various climatic zones in Australia (Bunn et al. 2003; Deegan and Ganf 2008; Hunt et al. 2012).

Gut content analysis (GCA) have traditionally been used to identify carbon sources or construct food web structure, but a few inherent limitations such as lack of indication of the degree of assimilation of dietary items means that only information of recently ingested items can be determined (Hyslop 1980; Parkyn et al. 2001). Stable isotope analysis (SIA), however, reflects food composition that are actually assimilated by the consumer, and this tool has gained increasing support to assess food web structure and function (Davis et al. 2012; Post 2002). Fatty acid profiles, usually using the relative content of different fatty acids, can also be used to trace trophic links between consumers and sources with particular phylogenetic

lineages (e.g. bacteria, diatoms, and green plants) in aquatic environments (Cashman et al. 2016; Gladyshev et al. 2009). The combined use of multiple analytical tools, such as gut content analysis, stable isotope analysis and fatty acid composition can overcome limitations of each individual method, so provide a useful toolkit to examine diet composition and trophic interaction.

Coastal spring-fed ponds in the Lower southeast of South Australia have been identified as crucial refuges for several vulnerable and endangered species (Harding 2012). The most striking features of these ponds are extremely high clarity of water and abundant submerged macrophytes. Benthic primary production is assumed to dominate these ecosystems, since phytoplankton biomass and productivity are extremely low as a result of continuous flushing of outflowing groundwater and nutrient limitation (Rigosi et al. 2015). The ponds are well protected by a belt of *Phragmites sp.*, and negligible surface inflow to the ponds occurs (Grandfield and Ashman 1984). Thus, food subsidy from surrounding terrestrial ecosystems is probably very low. Aquatic organisms are mostly sustained by a limited number of basal sources (macrophytes, epiphytes and benthic algae), but detailed knowledge of the benthic food web is still lacking.

In this study, we use multiple analyses to identify the major sources of organic matter supporting aquatic food webs in coastal, spring-fed clear-water ponds in the southeast of South Australia. Even if the ponds still have high water clarity and abundant submerged macrophytes, dieback and shrinking of macrophyte beds together with expansion of benthic filamentous algae mats have been reported (Carmody 2006). Furthermore, the outbreak of benthic filamentous algae is dense enough that buoyant mats reach the surface and form surface blooms. There are concerns that these phenomena are indicative of progressive eutrophication, and one final stage of filamentous or benthic algae domination was predicted in systems with low water retention time and high nutrient concentrations (Hilton et al. 2006).

We hypothesize that macrophytes provide the majority of organic carbon flows to consumers in Ewens Ponds although benthic and filamentous algae tend to gradually replace their place in food webs as benthic and filamentous algae may eventually prevail in such ecosystems while macrophytes are eradicated. In this work we assess the extent to which benthic macroinvertebrates and fish are supported by macrophytes, epiphytes and benthic filamentous algae.

2.2 Materials and methods

2.2.1 Study site

Ewens Ponds are a series of three small groundwater-fed freshwater ponds located in the limestone coast of lower southeast of South Australia, near the South Australia-Victoria border (38°01′36″S, 140°47′26″E) (Rigosi et al. 2015). The ponds are all small (977-3133 m^2), shallow (6-11 m) and unproductive. The annual mean of water total nitrogen and phosphorus concentrations were 5774 and 20 µg L^{-1}, respectively. The ponds had relatively low light absorbance across the spectral range of solar radiation from 400 to 700 nm with a mean extinction coefficient of 0.2 m^{-1} and were almost neutral with a mean pH of 7.02. The ponds had very low chlorophyll-a concentrations, usually lower than 0.1 µg L^{-1}, because of constant flush by discharged groundwater.

Ewens Ponds and adjacent areas included in the conservation park are a remnant of the peat bog ecosystem of the Eight Mile Creek swamp, which was drained and developed for pastures during the 1940s (Grandfield and Ashman 1984). Woolly tea tree (*Leptospermum lanigerum*) and scented paperbark (*Melaleuca squarrosa*) dominated the immediate surrounds of the ponds, and a belt of common reed (*Phragmites australis*) and common spike rush (*Eleocharis acuta*) occurs between them and open water surface of the ponds. Patchy distribution of several emergent macrophyte species including lesser bulrush (*Typha*

augustifolia) and watercress (*Nasturtium officinale*) scattered around the edge of the ponds and along bank of the channels. Semi-emergent and submerged macrophytes are abundant, with pond bottom around the rim and channel bed being covered by a few dominant species, including water ribbon (*Triglochin procera*), shield pennywort (*Hydrocotyle verticillata*) and river buttercup (*Ranunculus inundatus*). Sediments in the rest part of the ponds were colonized by benthic filamentous algae. The grazing snails *Glyptophysa gibbosa* and *Potamopyrgus antipodarum* are abundant, as is the caddisfly larvae *Triplectides* spp. The fish community is dominated by a number of small species, including the common jollytail (*Galaxias maculatus*) and the southern pergy perch (*Nannoperca australis*). The top predator in Ewens Ponds is the southern short-finned eel (*Anguilla australis*).

2.2.2 Sample collection

Aquatic macroinvertebrates, fish and their major potential food sources were collected from Pond 1 in December, 2014. Leaves of the common aquatic plants were collected by hand from a boat, or by divers. Samples of filamentous algae were sampled by divers. Samples of epiphytes were collected by scraping several plant stalks or leaves carefully with a scalpel. Plant material was washed clean with pond water and frozen in a potable freezer in the field. Epiphyte samples were divided into two parts, one was frozen for further analyses and the other was saved in ethanol for identification under a microscope. Macroinvertebrates were sampled from vegetated sites with scoop nets from a boat or by divers. Macroinvertebrate samples were kept alive in containers filled with pond water for one night, allowing them to clear their guts, and then frozen and transported to the laboratory. Fish were sampled using fyke nets and opera house traps deployed in Pond 1. Fish were euthanized using clove oil solution, frozen and transported to the lab. Ethical approval regarding the use of fish was acquired from the University of Adelaide Animal Ethics Committee (approval number: S-2014-204).

After returning to the lab, fish were dissected, foregut content taken for gut content analysis (GCA) and ventral muscle tissue of big fish (longer than 40 mm standard length) taken for stable isotope and fatty acid analyses. For fish < 40 mm standard length, whole fish excluding head, tail and viscera were used for stable isotope and fatty acid analyses. Physical separation of snail shells, small crustaceans' exoskeleton and caddisfly larva case from the bodies was conducted when possible and only flesh was used for analysis. Macrophyte and algae samples were thoroughly rinsed with distilled water and all small animals and particles attached were removed. Despite all efforts, there were still diatoms firmly attached to algae filaments when checked under microscope, but these diatoms only accounted for a negligible portion of the overall biomass of filamentous algae samples.

2.2.3 Gut content analysis

Gut content analyses were performed on fish samples that had more than 20% of their stomachs filled with food items. Viscera were excised from the abdominal cavity and stomachs were separated from other organs. Stomachs were then opened and all contained items were removed and transferred to glass dishes. The contribution of each food item to diet was determined by the volumetric method of Hyslop (1980) using microscope. Dietary items were identified to the lowest practical taxonomic level and grouped into the following categories: (a) Crustaceans, including small shrimps and crabs; (b) Gastropods, various species of snails; (c) Insects, including adults, larva and nymphs; (d) Detritus, mainly decomposed plant materials, and unidentified particulate organic matter (POM).

2.2.4 Stable isotope analysis

Samples for stable isotope analysis were oven-dried at 60 °C for 48 h to constant weight. The samples were ground with a grinder or mortar and pestle as fine as possible, and stored in airtight scintillation vials. Whole animals were analysed as individuals or, for small

invertebrates, individuals were pooled to obtain adequate sample mass. Samples of high carbonate content went through acid treatment before they were transferred into capsules for their carbon stable isotope composition analyses. Samples were analysed on a Nu Horizon IRMS (Nu Instruments Ltd, Wrexham, UK) at the Sprigg Geobiology Centre in the University of Adelaide. Ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ were expressed as the relative per mil (‰) difference between the sample and conventional standards. They were expressed as: $\delta X(‰) = [(R_{sample}/R_{standard})\text{-}1] \times 1000$, where $X = {}^{15}N$ or ^{13}C, $R = {}^{15}N/{}^{14}N$ or $^{13}C/^{12}C$.

2.2.5 Fatty acid analysis

Total lipids were extracted from weighed dried samples using a chloroform/methanol (2:1, v/v) solvent system. The extracted total lipids were dried under nitrogen gas and weighed to calculate crude fat %. The fatty acids were then methylated by heating at 70°C for 3 h in a solution of 1% H_2SO_4 in methanol. Fatty acid methyl esters were separated and quantified using a Hewlett Packard 6890 gas chromatography (GC) (Hewlett Packard, Palo Alto, CA, USA) equipped with a 50 m capillary column (0.32 mm ID) coated with BPX-70 (0.25 µm film thickness, SGE Pty Ltd, Ringwood, Victoria, Australia). The temperature of injector and flame ionization detector was set at 250 °C and 300 °C respectively. Helium was used as the carrier gas at the velocity of 35 cm s^{-1}. Individual fatty acids were identified by comparing their retention time with those of authentic lipid standard mixtures (GLC 463, Nu-Chek Prep, Inc., Elysian, MN, USA). Data were recorded and processed using Chemstation software and the results reported as % of total fatty acids. Fatty acid composition in basal resources and consumers was compared, and some polyunsaturated fatty acids (PUFAs) were used as biomarkers of certain groups of basal resources to trace diets of consumers.

2.2.6 Data analysis

The MixSIAR GUI programme was used to assess relative contributions of potential food sources to representative consumer species diet (Stock and Semmens 2016). MixSIAR is based on a Bayesian approach, which can give estimations of probability distributions of resource contributions to consumer's diets by accounting for all uncertainties of the input data (Parnell et al. 2010). We accounted for trophic fractionation using fractionation factors suggested in previous study (Post 2002). Samples of two dominant submerged macrophytes of different species, shield pennywort (*Hydrocotyle verticillata*) and river buttercup (*Ranunculus inundates*), were grouped to one aggregate denoted as "submerged plants" according to their proximity in isotopic signatures, reducing the number of sources and simplifying the analysing procedures in MixSIAR. Water ribbon (*Triglochin procera*), however, had distinct isotopic signatures from *H. verticillata* and *R. inundates*, and represent a distinct group of plant basal resource. The appropriate number of iterations was chosen according to the convergence diagnostics and the mean and variance were calculated from the resulting dietary proportions.

The MixSIAR was also used to estimate the contribution of resources to the biomass of fish. The contribution of potential macroinvertebrate prey groups to the biomass of fish was estimated using MixSIAR with the adjustment of trophic fractionation factors for them. Trophic fractionation factor of certain consumer was determined by its trophic position, and the consumer trophic position was evaluated by relating their $\delta^{13}C$ and $\delta^{15}N$ values to a site-specific trophic baseline derived from stable isotope values of non-predatory macroinvertebrate consumers (Zanden and Rasmussen 1999). Based on estimated contributions of basal sources to prey biomass, we used ratio calculation to estimate the resource contribution to predatory fish biomass (Brauns et al. 2011).

2.3 Results

2.3.1 Gut content analysis

Crustaceans (shrimps and crabs) dominated the diets of *A. australis* and *G. maculatus*, and gastropods (mainly New Zealand mud snail, *P. antipodarum*) dominated the diets of congolli *Pseudaphritis urvillii* and *N. australis* (Figure 2-1). The only fish species with gut content dominated by insects (mainly *Triplectides* larvae and chironomids larvae) was river blackfish *Gadopsis marmoratus* (Figure 2-1). Insects were also abundant in diets of *A. australis*, *P. urvillii* and *N. australis*. while crustaceans and gastropods were the second most abundant items in the diets of *G. marmoratus* and *G. maculatus* (Figure 2-1). Detritus was the least abundant item in gut content of fish species except *N. australis* (Figure 2-1). Crustaceans were the least abundant food items in the diets of *N. australis* (Figure 2-1).

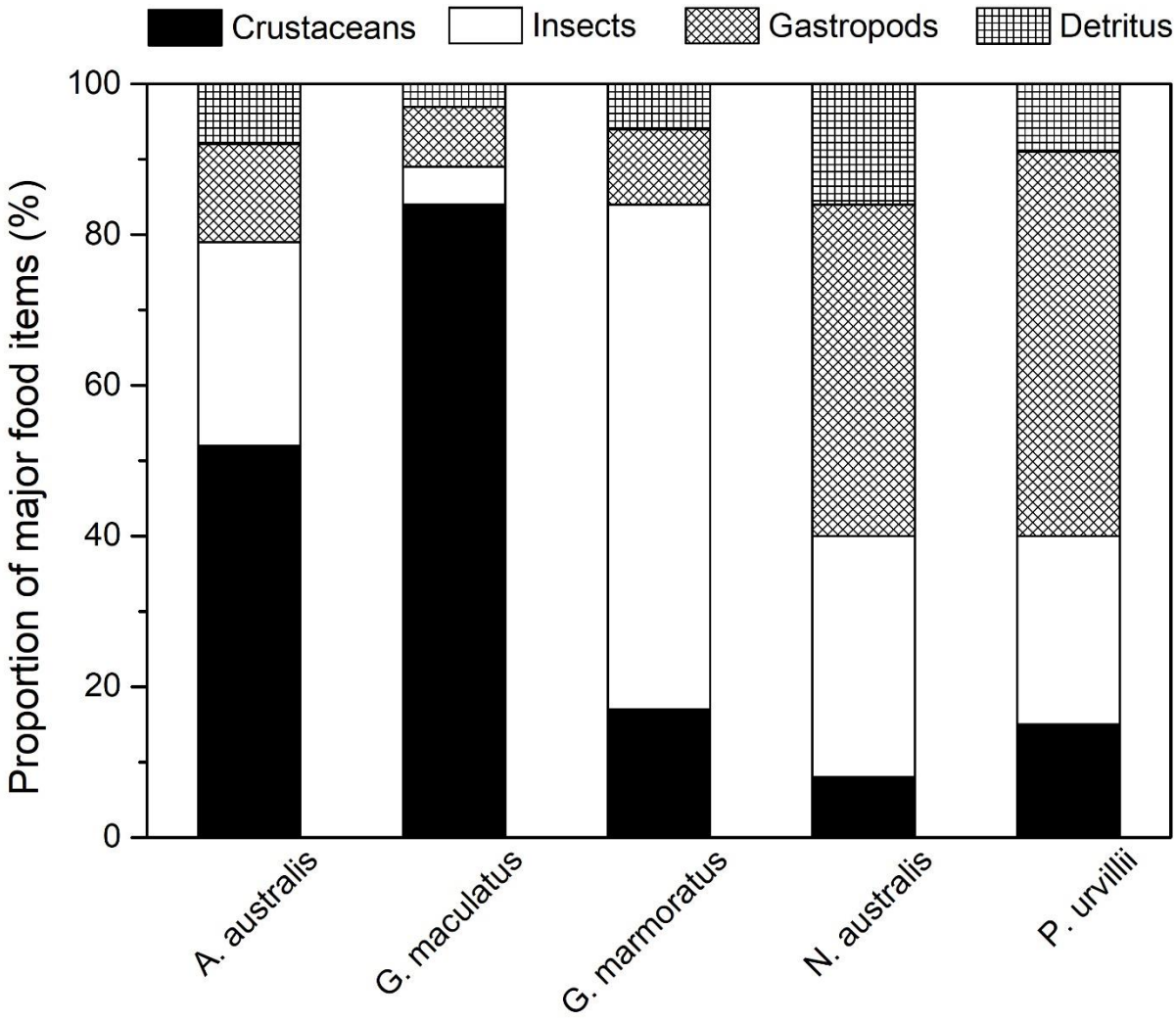


Figure 2-1 Volumetric proportions (%) of major prey items in diets of Ewens Ponds' fish species, *A. australis*, *G. maculatus*, *G. marmoratus*, *N. australis* and *P. urvillii* as determined by gut content analysis.

2.3.2 Stable isotope composition of food sources and consumers

Benthic filamentous cyanobacteria mats were dominated by *Lyngbya* sp. occupied the sediment surface in the pond. The algal mats were frequently covered with a layer of diatoms including *Fragilaria* sp., *Synedra* sp., *Encyonema* sp., *Rhoicosphenia* sp., and *Cocconeis* sp. The algal mats were more ^{13}C- and ^{15}N-depleted ($\delta^{13}C$ values lower than -39.2‰, $\delta^{15}N$ values

lower than -3.7‰) compared to all other primary producers (Figure 2-2). Epiphytic communities, which colonized on macrophyte leaves, were a mixture of small algae, other microorganisms, and deposited particulate organic matter. The Epiphytic communities had much higher $\delta^{13}C$ value than benthic filamentous cyanobacteria, but still much lower than macrophytes, while the $\delta^{15}N$ value of epiphyte was similar with some macrophyte species (Figure 2-2). The stable isotope signatures of typical macrophytes in the ponds scattered in a distinct region from other sources ($\delta^{13}C$ values -29.2‰ to -27.2‰, $\delta^{15}N$ values 2.7‰ to 6.2‰) (Figure 2-2).

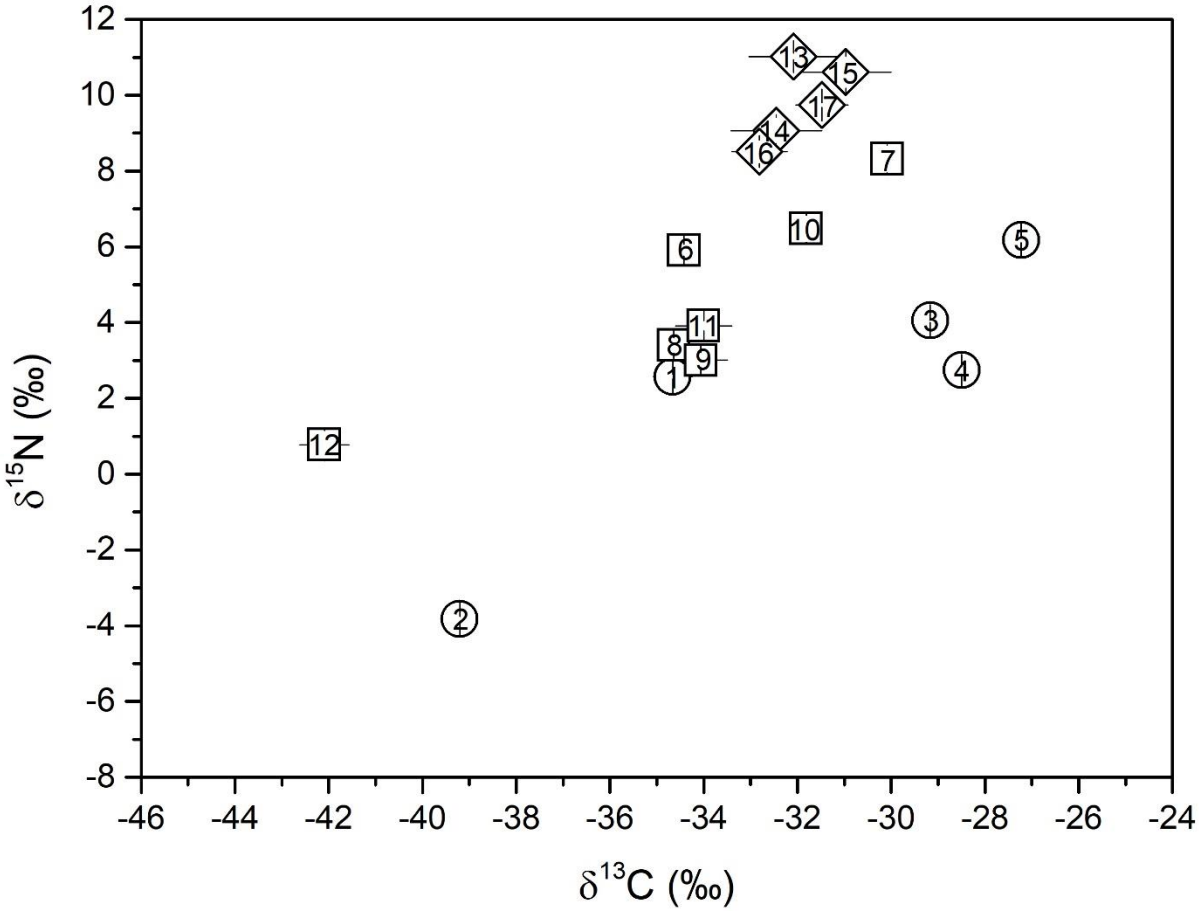


Figure 2-2 Stable isotope signatures of basal sources (circles), macroinvertebrate consumers (except crayfish) (white squares), and fish and crayfish (diamonds) in Ewens Ponds. Numbers designate particular basal resources and consumers: 1 – epiphyte; 2 – filamentous cyanobacteria; 3 – shield pennywort (*Hydrocotyle verticillata*); 4 – river buttercup (*Ranunculus inundates*); 5 – water ribbon (*Triglochin procera*); 6 – freshwater crab (*Amarinus lacustris*); 7 – diving beetle (*Antiporus* sp.); 8 – amphipod (*Austrochiltonia* sp.); 9 – pulmonate snail (*Glyptophysa gibbosa*); 10 – glass shrimp (*Paratya australiensis*); 11 – New Zealand mud snail (*Potamopyrgus antipodarum*); 12 – caddisfly (*Triplectides* sp.) larvae; 13 – southern shortfin eel (*Anguilla australis*); 14 – common galaxias (*Galaxias maculatus*); 15 – river blackfish (*Gadopsis marmoratus*); 16 – southern pygmy perch (*Nannoperca australis*); 17 – congolli (*Pseudaphritis urvillii*). Error bars represent ± 1 standard deviation; error bars not extending beyond the symbol perimeter are not shown.

The $\delta^{13}C$ value (-42.5‰ to -41.3‰) of *Triplectides* sp. was much lower than any other consumers and all basal sources sampled (Figure 2-2). Predatory diving beetles *Antiporus* sp. and glass shrimps *Paratya australiensis* had higher $\delta^{13}C$ values than the rest of macroinvertebrate species (Figure 2-2). Crab *Amarinus lacustris*, Amphipod *Austrochiltonia* sp., *G. gibbosa* and *P. antipodarum* had similar $\delta^{13}C$ values, close to that of epiphytic communities (Figure 2-2). *Antiporus* sp. had higher $\delta^{15}N$ value (7.7‰ to 8.6‰) than any other macroinvertebrates sampled except *E. bispinosus*, while *Triplectides* sp. had the lowest $\delta^{15}N$ value (0.5‰ to 1.4‰) of all consumers, and these $\delta^{15}N$ values were only higher than $\delta^{15}N$ values of benthic filamentous algae sampled (Figure 2-2). *P. australiensis* had higher $\delta^{15}N$ values than *A. lacustris*, *Austrochiltonia* sp., *G. gibbosa* and *P. antipodarum* (Figure 2-2). The $\delta^{15}N$ values of *Austrochiltonia* sp., *G. gibbosa* and *P. antipodarum*, like $\delta^{13}C$ values, were in the same range with epiphytes (Figure 2-2).

The $\delta^{13}C$ values (between -33.6‰ and -29.7‰) of fish were between the $\delta^{13}C$ values of epiphytes and macrophytes, had overlap with some macroinvertebrate species (Figure 2-2). The fish species generally had higher $\delta^{15}N$ values (between 8.1‰ and 11.1‰) than other consumers, except the predatory insect *Antiporus* sp. (Figure 2-2).

2.3.3 Mixing model analysis

Different macroinvertebrates obtained their carbon from different major food sources in Ewens Ponds. Contribution of different basal sources to *Triplectides* sp. and *Antiporus* sp. was not calculated using MixSIAR, because of lower $\delta^{13}C$ and $\delta^{15}N$ values of *Triplectides* sp. samples than those of any sampled basal resources and higher $\delta^{13}C$ and $\delta^{15}N$ values of *Antiporus* sp. samples than those of any sampled basal resources even after calibrating trophic fractionations. Epiphytes were largely consumed by all sampled benthic macroinvertebrates except *G. gibbosa* (Table 2-1). Filamentous algae acted as another major

food source for snail grazers and small amphipods (Table 2-1). Dependence on submerged plants was only obvious for *G. gibbosa* (Table 2-1). Contributions of *Triglochin* to body mass of the five benthic macroinvertebrates were always lower than 25% (Table 2-1). Epiphytes contributed a major part to the biomass of all fish species. Fish were also assimilating filamentous cyanobacteria, submerged plants and *Triglochin*, but the contribution of those sources to fish biomass was smaller than that of epiphytes (Table 2-2). Despite the use of isotopic mixing models to convert the isotopic data into estimates of food source contributions from the various components of an animal's diet has become an common practice in the study of food webs, we should always be aware of the numerous concerns raised (Bastos et al. 2017; Fry 2013; Phillips et al. 2014). Following best practices can help avoid the stable isotope mixing models being misused and misinterpreted (Phillips et al. 2014).

Table 2-1 Percentage contribution of food resources to benthic macroinvertebrate biomass in Ewens Ponds. Contributions were calculated excluding *Triplectides* sp. and *Antiporus* sp. The major primary energy sources (≥25% of assimilated material) for each consumer are shown in bold.

	Epiphytes	Filamentous cyanobacteria	Submerged plants	*Triglochin*
A. lacustris	**71.2 ± 6.8**	10.6 ± 2.9	10.0 ± 4.8	8.2 ± 4.9
Austrochiltonia sp.	**25.0 ± 7.3**	**47.8 ± 4.0**	16.5 ± 8.2	10.7 ± 7.5
G. gibbosa	10.1 ± 4.7	**52.8 ± 3.4**	**29.3 ± 9.6**	7.8 ± 7.2
P. antipodarum	**26.2 ± 8.1**	**41.3 ± 4.8**	20.1 ± 10.0	12.4 ± 9.5
P. australiensis	**49.1 ± 8.9**	7.4 ± 5.2	22.7 ± 14.6	20.9 ± 12.8

See text for detailed taxa names.

Table 2-2 Trophic position of fish and crayfish, and percentage contribution of food resources to fish and crayfish biomass in Ewens Ponds. The major primary energy sources (≥25% of assimilated material) for each consumer are shown in bold.

Consumers	Epiphytes	Filamentous cyanobacteria	Submerged plants	*Triglochin*
A. australis (3.7)[1]	**40.5**	20.0	20.2	15.2
G. maculatus (3.1)	**39.3**	22.5	19.4	13.9
G. marmoratus (3.6)	**38.1**	23.6	19.3	13.6
N. australis (2.9)	**40.0**	22.1	19.5	14.2
P. urvillii (3.3)	**42.5**	17.1	20.8	16.6

See text for detailed taxa names.

1 Trophic position (and therefore trophic fractionation of isotopes) of the consumer species was calculated following Zanden and Rasmussen (1999).

2.3.4 Biomarker fatty acids of food sources and consumers

The fatty acid 18:3(n-3) was used as biomarker for macrophyte food sources as the content of this fatty acid was much higher in macrophytes than other food sources (Figure 2-3a). The highest level of this fatty acid was found in caddisfly larvae, *Triplectides* sp. (13.7%) (Figure 2-3a). Other consumer species which had level of 18:3(n-3) higher than 5% were *A. australis*, *G. maculatus*, and *N. australis* (Figure 2-3a). The concentration of fatty acid 20:5(n-3) was much higher in epiphytes than other food sources, thus fatty acid 20:5(n-3) was used as biomarker for epiphyte (Figure 2-4b). Consumer species having significant amounts (≥10%) of this fatty acid were *P. australiensis*, and *A. lacustris* (Figure 2-3b). A specific fatty acid for filamentous cyanobacteria was not found.

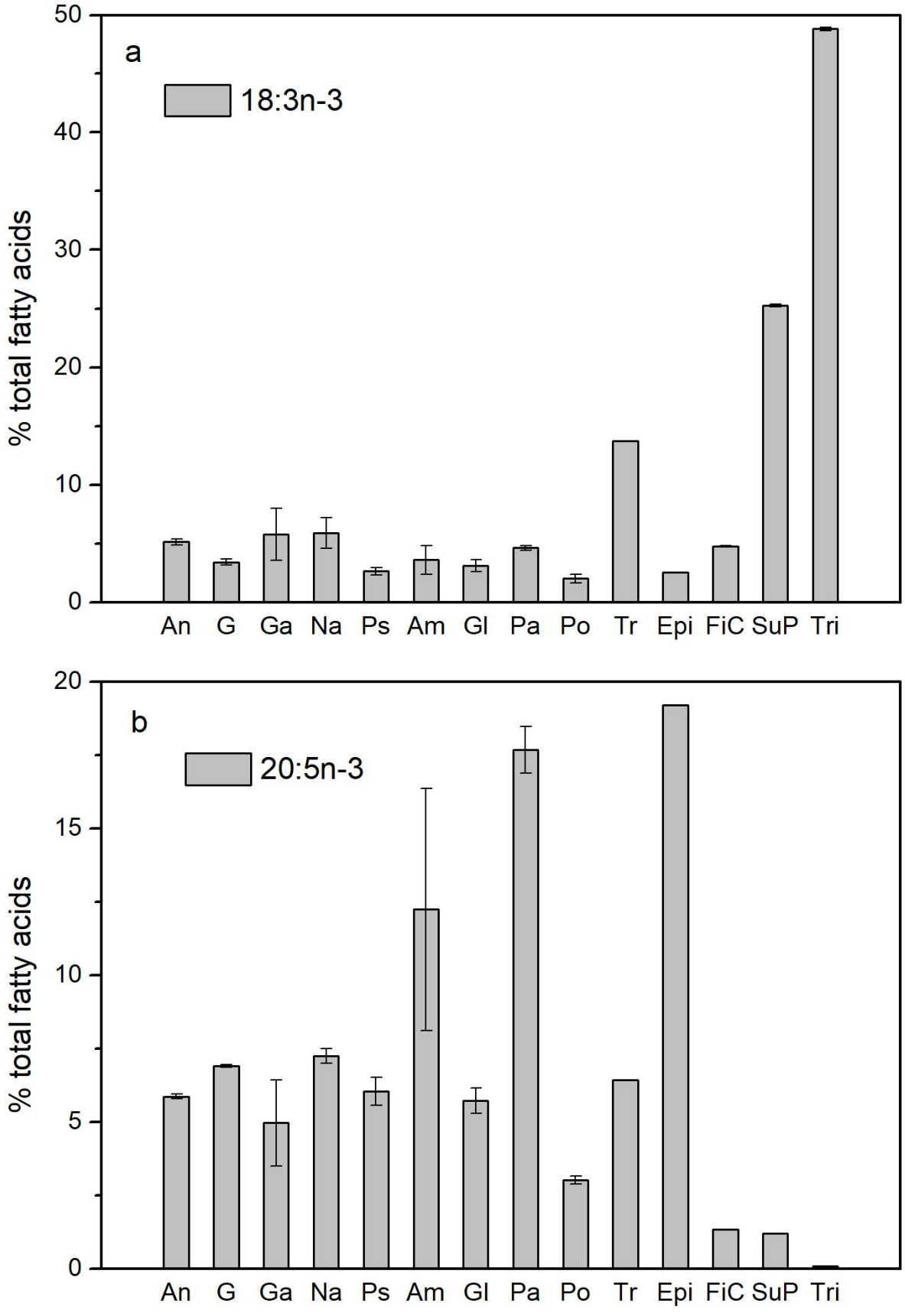

a
18:3n-3
% total fatty acids
50
40
30
20
10
0
An G Ga Na Ps Am Gl Pa Po Tr Epi FiC SuP Tri
b
20:5n-3
% total fatty acids
20
15
10
5
0
An G Ga Na Ps Am Gl Pa Po Tr Epi FiC SuP Tri

Figure 2-3 Biomarker fatty acids content in dominant consumers and primary food sources: (a) for macrophyte, and (b) for epiphyte. Symbol meaning are as follows: Am – *Amarinus lacustris*; An – *Anguilla australis*; Epi – epiphyte; FiC – filamentous cyanobacteria; G – *Gadopsis marmoratus*; Ga – *Galaxias maculatus*; Gl – *Glyptophysa gibbosa*; Na – *Nannoperca australis*; Pa – *Paratya australiensis*; Po – *Potamopyrgus antipodarum*; Ps – *Pseudaphritis urvillii*; SuP – submerged plants; Tr – *Triplectides* sp; Tri – *Triglochin procera*. Error bars represent ± 1 standard deviation.

2.4 Discussion

One of the essential requirements using mixing models to reliably interpret the results of stable isotope analyses is that the potential food sources have distinct isotopic signatures. The isotopic signatures of primary producers were determined by the signatures of elements in substances they rely on for energy and nutrition as well as the fractionation process when those substances are assimilated. We didn't sample the air nor charging groundwater for their stable isotopes, but samples of air and water should be measured and incorporated in future work to better understand the nutrient dynamics in pond food webs. Filamentous cyanobacteria, which grows on the bottom of the ponds, had the most depleted $\delta^{13}C$ (-39.3‰ to -39.1‰) and $\delta^{15}N$ (-3.9‰ to -3.8‰) values of all basal sources. The ^{13}C depletion of filamentous cyanobacteria is probably due to fixing of CO_2 that was derived from respiration and microbial decomposition of organic matter deposited on sediment surface, which was suggested to have extraordinary low $\delta^{13}C$ values (Hecky and Hesslein 1995; Rau 1978). It was also possible that the filamentous cyanobacteria used dissolved inorganic carbon (DIC) in the ponds by diffusion from atmosphere and groundwater. The normal range for $\delta^{13}C$ values of DIC of groundwater fed ponds is -17 to -7 ‰ (Hecky and Hesslein 1995; Marfia et

al. 2004; Vreca and Muri 2006). Well-mixed, lotic system such as Ewens Ponds, is less likely to develop carbon limitation for benthic algae photosynthesis, thus discrimination of ^{13}C between filamentous cyanobacteria and dissolved CO_2 from atmosphere and groundwater could potentially achieve maximum values of 29-30 ‰ (Hecky and Hesslein 1995; Hicks 1997; Osmond et al. 1981; Trudeau and Rasmussen 2003). Thus, the lower limit of $\delta^{13}C$ values should be between -47‰ and -36‰ for filamentous cyanobacteria using only DIC from atmosphere and groundwater, filamentous cyanobacteria had $\delta^{13}C$ values higher than the calculated lower limit in this study.

The more depleted $\delta^{15}N$ values of filamentous cyanobacteria relative to epiphytes and macrophytes could indicate a different source of nitrogen other than nitrogen in water column or sediment being incorporated, possibly N_2 from denitrification processes (Olsen et al. 2010). Ewens Ponds receives high loadings of nitrate from recharging groundwater, and the main land use within the watershed is grazed pastures. The NO_3^-- $\delta^{15}N$ values of two major agricultural sources are 10 to 20‰ for animal waste and -3 to 3‰ for synthetic fertilizer (Cole et al. 2004; McClelland et al. 1997). Processes such as volatilization and denitrification during transport of nitrogen in groundwater would make remaining nitrogen more ^{15}N enriched compared to original sources (Atkinson et al. 2014). High concentration of nitrogen in water lead to larger isotopic fractionation and lighter isotopic values in primary producers when DIN is assimilated (Cole et al. 2004). Different values of $\delta^{15}N$ of epiphytes and macrophytes in Ewens Ponds can be attributed to differences in taxonomy and local environment (Cole et al. 2004; Swart et al. 2014).

The decapods *P. australiensis* and *A. lacustris*, which are generally considered to be collectors, consumed mostly epiphytes and macrophytes in Ewens Ponds (Table 2-1). Previous studies of *P. australiensis* or other Atyidae food in creeks and rivers in Australia indicated that this species used attached algae, benthic algae or seston as significant food

source, and the main component of the attached algae was filamentous or colonial cyanobacteria, or filamentous chlorophytes (Burns and Walker 2000; Hunt et al. 2012; Reid et al. 2008). Allochthonous sources such as littoral plants or particulate organic matter derived from the plants were often included as other important food sources for *P. australiensis* where availability of these materials was obvious (Burns and Walker 2000; Reid et al. 2008). Bunn and Boon (1993) suggested that atyid shrimps could potentially assimilate littoral and fringing vegetation or epiphytes together with an additional highly ^{13}C-depleted source in billabongs, while Reid et al. (2008) found that the contribution of either epiphytes or macrophytes was small to the biomass of *P. australiensis* in streams. Omnivorous diet was likely to be popular in *P. australiensis* so that this opportunistic decapod could thrive in frequently disturbed systems where the availability of potential food sources differentiated both temporally and spatially (Burns and Walker 2000; Piola et al. 2008; Reid et al. 2008). *Austrochiltonia*, an endemic amphipod species, was abundant in filamentous cyanobacteria mats, and it used filamentous cyanobacteria as major food source (Table 2-1). Amphipod species, *Hyalella Azteca* and *Gammarus fasciatus* have been shown to consume filamentous cyanobacteria and chlorophyte, such as *Lyngbya* sp. and *Rhizoclonium* sp. (Camacho and Thacker 2006; Lévesque et al. 2015). *G. gibbosa* also derived most of their organic carbon from filamentous cyanobacteria (Table 2-1). *G. gibbosa* should be a flexible species regarding their diets, because they were able to consume allochthonous materials, epiphytes and macrophytes as major carbon sources (Reid et al. 2008). The invasive New Zealand mud snail, *P. antipodarum*, like native snail *G. gibbosa*, also assimilated organic carbon from different sources and *P. antipodarum* was believed to be able to graze on a wide range of materials, including filamentous green algae, macrophytes and benthic carbon (Hicks 1997; Kelly and Jellyman 2007; Pingram et al. 2014). Rounick et al. (1982) found that $\delta^{13}C$ signatures of *P. antipodarum* (-34.5‰) was closest to $\delta^{13}C$ signatures of *Cladophora* (-35‰)

or epilithic organic layer (-33.2‰), and they concluded that *P. antipodarum* had a high level of dependence on algae as a carbon source. Other studies also found $\delta^{13}C$ signatures of *P. antipodarum* were slightly higher than $\delta^{13}C$ signatures of epiphyton, and they suggested *P. antipodarum* diet consisted mainly of epiphyton (James et al. 2000; Kelly and Hawes 2005).

A. australis is an opportunistic generalist and considered a top predator in some aquatic ecosystems (Kelly and Jellyman 2007; Pingram et al. 2014). We found a high proportion of crustaceans (mainly *P. australiensis*) in the stomach of *A. australis*, together with some unidentified species of insects (Figure 2-2). Mixing model results showed organic carbon of filamentous cyanobacteria origin contributed most to the biomass of *A. australis*, which supported the gut content analysis result, because *P. australiensis* largely relied on filamentous cyanobacteria for carbon source. The ingestion of some insects, probably of terrestrial origin, was likely to cause the observed higher $\delta^{13}C$ value of *A. australis* than that of *P. australiensis*, and these food items were found to constitute the diet of *A. australis* (Hicks 1997; Jellyman 1989; Pingram et al. 2014). Terrestrial insects in the surrounding of Ewens Ponds should have higher $\delta^{13}C$ value than that of *A. australis* as their carbon source was mostly likely terrestrial plants, and C_3 plants have a $\delta^{13}C$ value of approximately -28 to -26‰ while C_4 plants are -14 to -12‰ on average (Hecky and Hesslein 1995). Gastropods such as the abundant *P. antipodarum* were less important for the energy source of *A. australis* in Ewens Ponds. Ontogenetic shift of *A. australis* diets was reported as they fed primarily on invertebrates (amphipods, insect larva and snails) when they were small, but snails became more important for medium sized individuals (Jellyman 1989; Ryan 1986). Fish including *G. maculatus* was the most important food as *A. australis* grew further, but the piscivorous feeding was shown to dominate within different size classes of *A. australis*, perhaps due to temporal and spatial variation and resulting differences in source abundance (Jellyman 1989; Kelly and Jellyman 2007; Ryan 1986).

We found large amounts of *P. australiensis* in the stomach of *G. maculatus*, although snails and insect larva were also very abundant (Figure 2-2). Mixing model results showed *G. maculatus* was mainly supported by epiphytes. A previous study of stream food webs in central Victoria indicated that *G. maculatus* was largely dependent on allochthonous carbon sources (Reid et al. 2008). Pollard (1973) reported that diet composition of *G. maculatus* from lotic environment such as streams contained more insects than crustaceans and molluscs, while *G. maculatus* from lentic environment such as lakes contained more crustaceans than other food items. Thus, our gut content analysis would put *G. maculatus* from Ewens Ponds as lake-dwelling populations because they had similar diet composition with those from lentic environment in the general observation. Fish are highly mobile and Eight Miles Creek connects Ewens Ponds to the sea. *G. maculatus* probably frequently moves between creeks and ponds and exploits every possible prey, including crustaceans, insects and molluscs. Gut content analysis of *G. maculatus* in this study only provided a snapshot of the feeding pattern of this species. Gastropods (*P. antipodarum*) and insects (*Triplectides* sp.), two of the most abundant benthic invertebrate fauna groups in Ewens Ponds, comprised the majority of *P. urvillii* gut content (Figure 2-2). *P. urvillii* was suggested to be opportunistic carnivores exploiting a wide range of benthic invertebrates, and their food composition might be closely related to the relative abundance of food sources, which could be affected by environmental factors (Giatas and Ye 2015; Hortle and White 1980).

Most consumers in this study typically had $\delta^{13}C$ values within the range of potential basal sources sampled, with the exception of larvae of one caddisfly species, *Triplectides* sp. and one beetle species, *Antiporus* sp. The extraordinary low $\delta^{13}C$ values of *Triplectides* sp. larva indicated that these case-dwelling insects must have consumed other highly ^{13}C-depleted sources (Bunn and Boon 1993). Methane-oxidizing bacteria (MOB) can be such a ^{13}C-depeleted source for providing food for *Triplectides* sp. larva. Trimmer et al. (2009) reported

the isotopic signature of two species of cased caddisfly (*Agapetus fuscipes* and *Silo nigrocornis*) larvae was consistently ^{13}C-depeleted compared to other taxa and basal sources. They also detected the greatest ratio of methane oxidation to chlorophyll for biofilms on the cases of caddisfly larva (Trimmer et al. 2009). They estimated up to 30% of the carbon of those caddisfly larva was provided by MOB in biofilms growing on gravel or cases (Trimmer et al. 2009). One condition for the major contribution of methane carbon to aquatic food webs in their site is that their samples were collected in a chalk stream where the water was supersaturated with methane from both groundwater aquifers and production in sediments (Trimmer et al. 2009). Ewens Ponds water was mixed because of a large recharge of groundwater and we didn't measure the methane content in water. Sediment surface beneath filamentous algae mats could develop suitable conditions for methane production and methane can then be used by MOB in biofilms on cases of caddisfly larva living in filamentous algae mats in Ewens Ponds. Studies have proven that carbon from methane can be transferred further up to consumers in higher trophic levels (Agasild et al. 2014; Jones and Grey 2011; Ravinet et al. 2010; Sanseverino et al. 2012). *Antiporus* sp. had assimilated ^{13}C-enriched sources, probably insects of terrestrial origin.

It is apparent that the food web of this oligotrophic to mesotrophic pond is complex, and generalism and omnivory may be prevalent. Organic matter is channelled through epiphytic/benthic compartments only because Ewens Ponds lacks the pelagic pathway of organic matter flux due to short water retention time and low water phosphorus concentration (Rigosi et al. 2015). The relative importance of available sources to different consumers in Ewens Ponds varied and may be strongly influenced by consumer feeding capability and habitat use. Epiphytes and benthic filamentous cyanobacteria, sometimes referred as or included in periphyton or attached biofilm, could be substantial sources of carbon to consumers, including snails, crustaceans and various species of fish (Bunn et al. 2003;

Deegan and Ganf 2008; Hunt et al. 2012; Reid et al. 2008; Sierszen et al. 2003). Although epiphytes and benthic filamentous cyanobacteria are considered problematic and symptoms of degradation of the aquatic ecosystems, in the absence of pelagic primary productivity they play a critical role in supporting consumers.

This study identified major organic matter sources for common aquatic organisms in Ewens Ponds freshwater wetland ecosystem, and determined the structure of food web. Overall, the combinations of GCA, SIA and fatty acid biomarker profiles provided complementary data about contributions of different basal sources to the diet of benthic macroinvertebrates and fish. Feeding patterns and trophic links of aquatic organisms in the ponds were also established, highlighting the contribution of benthic productivity by epiphytes and benthic filamentous cyanobacteria to the whole productivity in small waterbodies. Our findings also emphasize the importance of combining different tools when assessing freshwater food webs for better understanding of the variability in resource use among consumers in these ecosystems. This study suggests considering the integrity of components in ecosystems and focusing on the functions provided from few aspects only but overlooking others needs to be avoided in ecological studies.

References

Agasild, H., P. Zingel, L. Tuvikene, A. Tuvikene, H. Timm, T. Feldmann, J. Salujõe, K. Toming, R. I. Jones & T. Nõges, 2014. Biogenic methane contributes to the food web of a large, shallow lake. Freshwater Biology 59:272-285.

Ask, J., J. Karlsson, L. Persson, P. Ask, P. Byström & M. Jansson, 2009. Whole-lake estimates of carbon flux through algae and bacteria in benthic and pelagic habitats of clear-water lakes. Ecology 90:1923-1932.

Atkinson, C. L., A. D. Christian, D. E. Spooner & C. C. Vaughn, 2014. Long-lived organisms provide an integrative footprint of agricultural land use. Ecological Applictions 24:375-384.

Bastos, R. F., F. Corrêa, K. O. Winemiller & A. M. Garcia, 2017. Are you what you eat? Effects of trophic discrimination factors on estimates of food assimilation and trophic position with a new estimation method. Ecological Indicators 75:234-241.

Batt, R. D., S. R. Carpenter, J. J. Cole, M. L. Pace, T. J. Cline, R. A. Johnson & D. A. Seekell, 2012. Resources supporting the food web of a naturally productive lake. Limnology and Oceanography 57:1443-1452.

Biggs, J., P. Williams, M. Whitfield, P. Nicolet & A. Weatherby, 2005. 15 years of pond assessment in Britain: results and lessons learned from the work of Pond Conservation. Aquatic Conservation: Marine and Freshwater Ecosystems 15:693-714.

Brauns, M., B. Gücker, C. Wagner, X.-F. Garcia, N. Walz & M. T. Pusch, 2011. Human lakeshore development alters the structure and trophic basis of littoral food webs. Journal of Applied Ecology 48:916-925.

Bunn, S. E. & P. I. Boon, 1993. What sources of organic carbon drive food webs in billabongs? A study based on stable isotope analysis. Oecologia 96:85-94.

Bunn, S. E., P. M. Davies & M. Winning, 2003. Sources of organic carbon supporting the food web of an arid zone floodplain river. Freshwater Biology 48:619-635.

Burns, A. & K. F. Walker, 2000. Biofilms as food for decapods (Atyidae, Palaemonidae) in the River Murray, South Australia. Hydrobiologia 437:83-90.

Camacho, F. A. & R. W. Thacker, 2006. Amphipod herbivory on the freshwater cyanobacterium Lyngbya wollei: Chemical stimulants and morphological defenses. Limnology and Oceanography 51:1870-1875.

Carmody, G., 2006. Save Ewens Ponds! Marine Life Society of South Australia Journal:55-65.

Carpenter, S. R., J. J. Cole, M. L. Pace, M. V. d. Bogert, D. L. Bade, D. Bastviken, C. M. Gille, J. R. Hodgson, J. F. Kitchell & E. S. Kritzberg, 2005. Ecosystem subsidies: terrestrial support of aquatic food webs from ^{13}C additon to contrasting lakes. Ecology 86:2737-2750.

Cashman, M. J., F. Pilotto, G. L. Harvey, G. Wharton & M. T. Pusch, 2016. Combined stable-isotope and fatty acid analyses demonstrate that large wood increases the autochthonous trophic base of a macroinvertebrate assemblage. Freshwater Biology 61:549-564.

Cole, M. L., I. Valiela, K. D. Kroeger, G. L. Tomasky, J. Cebrain, C. Wigand, R. A. McKinney, S. P. Grady & M. H. C. d. Silva, 2004. Assessment of a $\delta^{15}N$ isotopic method to indicate anthropogenic eutrophication in aquatic ecosystems. Journal of Environmental Quality 33:124-132.

Davis, A. M., M. L. Blanchette, B. J. Pusey, T. D. Jardine & R. G. Pearson, 2012. Gut content and stable isotope analyses provide complementary understanding of ontogenetic dietary shifts and trophic relationships among fishes in a tropical river. Freshwater Biology 57:2156-2172.

Deegan, B. M. & G. G. Ganf, 2008. The loss of aquatic and riparian plant communities: implications for their consumers in a riverine food web. Austral Ecology 33:672-683.

Downing, J. A., Y. T. Prairie, J. J. Cole, C. M. Duarte, L. J. Tranvik, R. G. Striegl, W. H. McDowell, P. Kortelainen, N. F. Caraco, J. M. Melack & J. J. Middelburg, 2006. The global abundance and size distribution of lakes, ponds, and impoundments. Limnology and Oceanography 51:2388-2397.

Fry, B., 2013. Alternative approaches for solving underdetermined isotope mixing problems. Marine Ecology Progress Series 472:1-13.

Giatas, G. & Q. Ye, 2015. Diet and trophic characteristics of mulloway (*Argyrosomus japonicus*), congolli (*Pseudaphritis urvillii*) and Australian salmon (*Arripis truttaceus* and *A. trutta*) in the Coorong. SARDI Research Report Series No. 858. SARDI Aquatics Sciences.

Gladyshev, M., M. Arts & N. I. Sushchik, 2009. Preliminary estimates of the export of omega-3 highly unsaturated fatty acids (EPA + DHA) from aquatic to terrestrial

ecosystems. In Arts, M. T., M. T. Brett & M. Kainz (eds) Lipids in Aquatic Ecosystems. Springer, New York, 179-210.
Grandfield, L. & G. Ashman, 1984. Ewens Ponds Management Plan Draft. Department of Lands, South East Region.
Harding, C., 2012. Extension of the water-dependent ecosystem risk assessment framework to the south east NRM region, DFW Technical Report 2012/10. Government of South Australia, through Department for Water, Adelaide.
Hart, D. D., B. J. F. Biggs, V. I. Nikora & C. A. Flinders, 2013. Flow effects on periphyton patches and their ecological consequences in a New Zealand river. Freshwater Biology 58:1588-1602.
Hecky, R. E. & R. H. Hesslein, 1995. Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. Journal of the North American Benthological Society 14:631-653.
Hicks, B. J., 1997. Food webs in forest and pasture streams in the Waikato region, New Zealand: a study based on analyses of stable isotopes of carbon and nitrogen, and fish gut contents. New Zealand Journal of Marine and Freshwater Research 31:651-664.
Hilton, J., M. O'Hare, M. J. Bowes & J. I. Jones, 2006. How green is my river? A new paradigm of eutrophication in rivers. Science of the Total Environment 365:66-83.
Holgerson, M. A., D. M. Post & D. K. Skelly, 2016. Reconciling the role of terrestrial leaves in pond food webs: a whole-ecosystem experiment. Ecology 97:1771-1782.
Hortle, M. E. & R. W. G. White, 1980. Diet of Pseudaphritis urvillii (Cuvier & Valenciennes) (Pisces: Bovichthyidae) from South-eastern Tasmania. Australian Journal of Marine and Freshwater Research 31:533-539.
Hudon, C., M. D. Sève & A. Cattaneo, 2014. Increasing occurrence of the benthic filamentous cyanobacterium *Lyngbya wollei*: a symptom of freshwater ecosystem degradation. Freshwater Science 33:606-618.
Hunt, R. J., T. D. Jardine, S. K. Hamilton & S. E. Bunn, 2012. Temporal and spatial variation in ecosystem metabolism and food web carbon transfer in a wet-dry tropical river. Frehswater Biology 57:435-450.
Hyslop, E. J., 1980. Stomach contents analysis - a review of methods and their application. Journal of Fish Biology 17:411-429.
James, M. R., I. Hawes, M. Weatherhead, C. Stanger & M. Gibbs, 2000. Carbon flow in the littoral food web of an oligotrophic lake. Hydrobiologia 441:93-106.
Jaschinski, S., D. C. Brepohl & U. Sommer, 2011. The trophic importance of epiphytic algae in a freshwater macrophyte system (*Potamogeton perfoliatus* L.): stable isotope and fatty acid analyses. Aquatic Sciences 73:91-101.
Jellyman, D. J., 1989. Diet of two species of freshwater eel (*Anguilla* spp.) in Lake Pounui, New Zealand. New Zealand Journal of Marine and Freshwater Research 23:1-10.
Jones, J. I. & S. Waldron, 2003. Combined stable isotope and gut contents analysis of food webs in plant-dominated, shallow lakes. Frehswater Biology 48:1396-1407.
Jones, R. I. & J. Grey, 2011. Biogenic methane in freshwater food webs. Frehswater Biology 56:213-229.
Karlsson, J., M. Berggren, J. Ask, P. Byström, A. Jonsson, H. Laudon & M. Jansson, 2012. Terrestrial organic matter support of lake food webs: Evidence from lake metabolism and stable hydrogen isotopes of consumers. Limnology and Oceanography 57:1042-1048.
Karlsson, J. & P. Byström, 2005. Littoral energy mobilization dominates energy supply for top consumers in subarctic lakes. Limnology and Oceanography 50:538-543.

Kelly, D. J. & I. Hawes, 2005. Effects of invasive macrophytes on littoral-zone productivity and foodweb dynamics in a New Zealand high-country lake. Journal of North American Benthological Society 24:300-320.
Kelly, D. J. & D. J. Jellyman, 2007. Changes in trophic linkages to shortfin eels (*Anguilla australis*) since the collapse of submerged macrophytes in Lake Ellesmere, New Zealand. Hydrobiologia 579:161-173.
Lévesque, D., A. Cattaneo & C. Hudon, 2015. Benthic cyanobacterial mats serve as a refuge and food for the amphipod *Gammarus fasciatus*. Hydrobiologia 758:171-181.
Lodge, D. M., 1991. Herbivory on freshwater macrophytes. Aquatic Botany 41:195-224.
Mann, K. H., 1988. Production and use of detritus in various freshwater, estuarine, and coastal marine ecosystems. Limnology and Oceanography 33:910-930.
Marfia, A. M., R. V. Krishnamurthy, E. A. Atekwana & W. F. Panton, 2004. Isotopic and geochemical evolution of ground and surface waters in a karst dominated geological setting: a case study from Belize, Central America. Applied Geochemistry 19:937-946.
McClelland, J. W., I. Valiela & R. H. Michener, 1997. Nitrogen-stable isotope signatures in estuarine food webs: A record of increasing urbanization in coastal watersheds. Limnology and Oceanography 42:930-937.
Meester, L. D., S. Declerck, R. Stoks, G. Louette, F. v. d. Meutter, T. d. Bie, E. Michels & L. Brendonck, 2005. Ponds and pools as model systems in conservation biology, ecology and evolutionary biology. Aquatic Conservation: Marine and Freshwater Ecosystems 15:715-725.
Newman, R. M., 1991. Herbivory and detritivory on freshwater macrophytes by invertebrates: a review. Journal of North American Benthological Society 10:89-114.
Oertli, B., J. Biggs, R. Céréghino, P. Grillas, P. Joly & J.-B. Lachavanne, 2005. Conservation and monitoring of pond biodiversity: introduction. Aquatic Conservation: Marine and Freshwater Ecosystems 15:535-540.
Oertli, B., R. Céréghino, A. Hull & R. Miracle, 2009. Pond conservation: from science to practice. Hydrobiologia 634(1-9).
Olsen, Y. S., S. E. Fox, E. L. Kinney, M. Teichberg & I. Valiela, 2010. Differences in urbanization and degree of marine influence are reflected in $\delta^{13}C$ and $\delta^{15}N$ of producers and consumers in seagrass habitats of Puerto Rico. Marine Environmental Research 69:198-206.
Osmond, C. B., N. Valaane, S. M. Haslam, P. Uotila & Z. Roksandic, 1981. Comparisons of $\delta^{13}C$ values in leaves of aquatic macrophytes from different habitats in Britain and Finland: some implications for photosynthetic processes in aquatic plants. Oecologia 50:117-124.
Parkyn, S. M., K. J. Collier & B. J. Hicks, 2001. New Zealand stream crayfish: functional omnivores but trophic predators? Freshwater Biology 46:641-652.
Parnell, A. C., R. Inger, S. Bearhop & A. L. Jackson, 2010. Source partitioning using stable isotopes: Coping with too much variation. Plos ONE 5:e9672.
Phillips, D. L., R. Inger, S. Bearhop, A. L. Jackson, J. W. Moore, A. C. Parnell, B. X. Semmens & E. J. Ward, 2014. Best practices for use of stable isotope mixing models in food-web studies. Canadian Journal of Zoology 92:823-835.
Pingram, M. A., K. J. Collier, D. P. Hamilton, B. J. Hicks & B. O. David, 2014. Spatial and temporal patterns of carbon flow in a temperate, large river food web. Hydrobiologia 729:107-131.
Piola, R. F., I. M. Suthers & D. Rissik, 2008. Carbon and nitrogen stable isotope analysis indicates freshwater shrimp *Paratya australiensis* Kemp, 1917 (Atyidae) assimilate cyanobacterial accumulations. Hydrobiologia 608:121-132.

Pollard, D. A., 1973. The biology of a land-locked form of the normally catadromous salmoniform fish *Galaxias maculatus* (Jenyns) V. Composition of the diet. Australian Journal of Marine and Freshwater Research 24:281-295.
Post, D. M., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703-718.
Rau, G., 1978. Carbon-13 depletion in a subalpine lake: Carbon flow implications. Science 201:901-902.
Ravinet, M., J. Syväranta, R. I. Jones & J. Grey, 2010. A trophic pathway from biogenic methane supports fish biomass in a temperate lake ecosystem. Oikos 119:409-416.
Reid, D. J., G. P. Quinn, P. S. Lake & P. Reich, 2008. Terrestrial detrius supports the food webs in lowland intermittent streams of south-eastern Australia: a stable isotope study. Frehswater Biology 53:2036-2050.
Rigosi, A., Y. Liu, M. Shanafield & J. D. Brookes, 2015. Determining environmental risks to Ewens Ponds in the South East. Goyder Institute for Water Research Technical Report Series No. 15/19, Adelaide, South Australia.
Rounick, J. S., M. J. Winterbourn & G. L. Lyon, 1982. Differential utilization of allochthonous and autochthonous inputs by aquatic invertebrates in some New Zealand streams: a stable carbon isotope study. Oikos 39:191-198.
Ryan, P. A., 1986. Seasonal and size-related changes in the food of the short-finned eel, *Anguilla australis* in Lake Ellesmere, Canterbury, New Zealand. Environmental Biology of Fishes 15:47-58.
Sanseverino, A. M., D. Bastviken, I. Sundh, J. Pickova & A. Enrich-Prast, 2012. Methane carbon supports aquatic food webs to the fish level. Plos ONE 7:e42723.
Sierszen, M. E., M. E. McDonald & D. A. Jensen, 2003. Benthos as the basis for arctic lake food webs. Aquatic Ecology 37:437-445.
MixSIAR GUI user manual. Version 3.1 2016. https://github.com/brianstock/MixSIAR.
Swart, P. K., S. Evans, T. Capo & M. A. Altabet, 2014. The fractionation of nitrogen and oxygen isotopes in macroalgae during the assimilation of nitrate. Biogeosciences 11:6147-6157.
Trimmer, M., A. G. Hildrew, M. C. Jackson, J. L. Pretty & J. Grey, 2009. Evidence for the the role of methane-derived carbon in a free-flowing, lowland river food web. Limnology and Oceanography 54:1541-1547.
Trudeau, V. & J. B. Rasmussen, 2003. The effect of water velocity on stable carbon and nitrogen isotope signatures of periphyton. Limnology and Oceanography 48:2194-2199.
Vadeboncoeur, Y., D. M. Lodge & S. R. Carpenter, 2001. Whole-lake fertilization effects on distribution of primary production between benthic and pelagic habitats. Ecology 82:1065-1077.
Vadeboncoeur, Y., G. Peterson, M. J. Vander Zanden & J. Kalff, 2008. Benthic algal production across lake size gradients: interactions among morphometry, nutrients, and light. Ecology 89:2542-2552.
Vander Zanden, M. J. & Y. Vadeboncoeur, 2002. Fishes as integrators of benthic and pelagic food webs in lakes. Ecology 83:2152-2161.
Vis, C., A. Cattaneo & C. Hudon, 2008. Shift from chlorophytes to cyanobacteria in benthic macroalgae along a gradient of nitrate depletion. Journal of Phycology 44:38-44.
Vreca, P. & G. Muri, 2006. Changes in accumulation of organic matter and stable carbon and nitrogen isotopes in sediments of two Slovenian mountain lakes (Lake Ledvica and Lake Planina), induced by eutrophication changes. Limnology and Oceanography 51:781-790.

Watson, A. & L. A. Barmuta, 2011. Feeding-preference trials confirm unexpected stable isotope analysis results: freshwater macroinvertebrates do consume macrophytes. Marine and Freshwater Research 62:1248-1257.
Webster, J. R. & E. F. Benfield, 1986. Vascular plant breakdown in freshwater ecosystems. Annual Review of Ecology and Systematics 17:567-594.
Zanden, M. J. V. & J. B. Rasmussen, 1999. Primary consumer $\delta^{13}C$ and $\delta^{15}N$ and the trophic position of aquatic consumers. Ecology 80:1395-1404.

Statement of Authorship

Title of Paper	Elemental nutrient and fatty acid composition of organisms in benthic food web of a groundwater-fed, freshwater ecosystem
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ■ Unpublished and Unsubmitted work written in publication style
Publication Details	Prepared for submission to a journal

Principal Author

Name of Principal Author (Candidate)	Yang Liu
Contribution to the paper	Designed the study, performed field experiments, conducted laboratory experiments, analysed the data, interpreted the results, wrote the manuscript
Overall percentage (%)	80 %
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date: 04/10/2017

Co-author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution

Name of Co-author	Justin Brookes
Contribution to the paper	Provided input in conceiving the study and design of experiments, supervised data analysis and interpretation, reviewed the manuscript
Signature	Date: 4 Oct 2017

Chapter 3 Elemental nutrient and fatty acid composition of organisms in benthic food web of a groundwater-fed, freshwater pond

Abstract

There is an increasing focus on how food quality influences consumer growth and production in freshwater ecosystems, with elemental nutrient stoichiometry and fatty acid composition receiving more attention than other indices. We determined the elemental nutrient and fatty acid composition of major basal resources and most common consumers in a groundwater-fed freshwater wetland in the southeast of South Australia, and related variations in consumer stoichiometry to their feeding mode and taxonomic identity. Patterns of organismal elemental contents and ratios observed in this study were similar to previous studies which suggests broad generality of mechanisms determining animal stoichiometry. Compared with aquatic consumers, basal resources had higher C:N and C:P ratios, but N:P ratios of some consumers were higher than N:P ratios of epiphytes and filamentous algae. Detailed examination on factors affecting consumer stoichiometry revealed most of the variation in elemental C:N, C:P, and N:P ratios among benthic macroinvertebrates could be attributed to taxonomic identity. Fatty acid composition in consumers potentially reflected specific trophic links between benthic fauna with food sources. Elemental imbalances in C:N, C:P and N:P ratios between major consumer species and their resources were calculated, and larger imbalances between benthic macroinvertebrates and primary producers than that between fish and their food items were confirmed. Production of primary consumers was largely constrained by relatively low availability of P in food resources compared to N, although relative availability of N and P varied among benthic macroinvertebrates.

3.1 Introduction

Organismal growth occurs when energy and material transferred from autotrophic primary producers or heterotrophic bacteria to primary consumers or from primary consumers to consumers at higher trophic levels in aquatic ecosystems. The transfer of energy and material across multiple trophic levels in food webs, thus, is a key process in aquatic ecosystems (Brett and Müller-Navarra 1997; Persson et al. 2007). Inefficient transfer of energy and material from primary producers to consumers often leads to accumulation of primary producer biomass to nuisance levels in lakes (Brett and Müller-Navarra 1997; Persson et al. 2007). This accumulation of biomass is often associated with a series of adverse conditions, degrading water quality. The amount of energy and material that organisms at top trophic levels receive is considered to be strongly influenced by food quantity as well as food quality (Brett and Müller-Navarra 1997; Müller-Navarra and Lampert 1996; Sterner and Hessen 1994). Food quantity measurements such as biomass and chlorophyll-a content of primary producers have been widely used when assessing aquatic ecosystem resource use and consumer production. The importance of food quality control on consumer growth and production, however, only received attention in the past few decades (Arts et al. 2009; Sterner and Elser 2002). Food quality can be defined in many ways, including the proportion of digestible, toxic and nutritional materials in the food and caloric density (Karasov and Rio 2007). Most of the food quality research in freshwater food webs has focused on nutrient composition in the food, while elemental nutrient and fatty acid (FA) received more attention than many other inorganic nutrients and organic compounds (Persson et al. 2007; Volk and Kiffney 2012).

All organisms are composed of the same major elements (C, N and P) and a suite of trace elements (Elser et al. 2000a). In particular, nitrogen (N) and phosphorus (P) were regarded as essential nutrients and carbon (C) as an energy source (Persson et al. 2010). Autotrophic

producers typically have relatively high and variable elemental ratios (C:N:P), while heterotrophic consumers usually possess low and constrained elemental ratios (Cross et al. 2005; Cross et al. 2003; Elser et al. 2000a; Persson et al. 2010). The imbalances among key element ratios between consumers and their diets have been shown to strongly limit the growth and reproduction of consumers (Anderson et al. 2004; Cross et al. 2007; Frost et al. 2005; Frost et al. 2002; Sterner and Elser 2002).

Animals also require organic compounds, such as sterols, vitamins, amino acids and fatty acids in their diets to support physiological demands, because they are not able to use inorganic nutrients directly like autotrophic producers (Müller-Navarra 2008). Fatty acids play an important role in a wide range of critical physiological processes, including membrane fluidity regulation and bioactive molecule synthesis (Brett and Müller-Navarra 1997; Martin-Creuzburg et al. 2009; Sargent et al. 1999). Saturated fatty acids (SAFAs) and monounsaturated fatty acids (MUFAs), can be synthesized by most organisms and serve as energy storage molecules (Napolitano 1994). However, few animals are known to be able to synthesize highly unsaturated fatty acids (HUFAs), a subclass of polyunsaturated fatty acids (PUFAs) (Brett and Müller-Navarra 1997). Thus, most consumers must obtain HUFAs from their diet (Cashman et al. 2013). Several specific HUFAs, including linoleic acid (LIN, 18:2ω6), α-linolenic acid (ALA, 18:3ω3), arachidonic acid (ARA, 20:4ω6), eicosapentaenoic acid (EPA, 20:5ω3), and docosahexaenoic acid (DHA, 22:6ω3), are commonly considered essential for the survival of aquatic animals (Hill et al. 2011).

A number of field research or controlled laboratory studies have investigated the relationships between consumers and their resources in regard with either elemental nutrients composition or fatty acid composition. Most studies of freshwater food web stoichiometry have been conducted in pelagic systems, where consumers are suggested to be constrained to the elemental composition of primary producers that conforms to the Redfield ratio (Lauridsen et

al. 2012; Sterner and Elser 2002). Studies of HUFAs nutrition in animals also focused on zooplankton or domesticated taxa under laboratory conditions (Twining et al. 2016). Benthic habitats, an integral component of freshwater ecosystems, have different fauna communities and basal resources compared to pelagic systems. Consequently, significant differences in basic ecology concerning elemental nutrient and fatty acid limitation of consumer growth and community production would be expected between benthic and pelagic systems.

To date, few studies have combined elemental nutrients and fatty acid composition to assess the nutritional quality of food sources and requirement of consumers in benthic food webs (but see Volk & Kiffney, 2012). Accumulation of large amounts of benthic filamentous algae, have been observed in Ewens Ponds, a series of groundwater fed ponds in the southeast of South Australia where N concentrations are generally very enriched and P concentrations are limited (Grandfield and Ashman 1984; Rigosi et al. 2015; Wood 2011). Most of the primary consumers feed on macrophytes, epiphytic or filamentous algae in the benthic zone, because pelagic pathways of energy and nutrient transfer are negligible due to the high flow and so washout of pelagic phytoplankton. The nutritional quality of available food resources for consumers may vary considerably in the ponds, but no direct evidence was ever acquired. Negligible grazer control on the biomass of benthic filamentous algae was postulated in the ponds, and it may be caused by poor nutritional quality of these algae as resources. In contrast, grazers may prefer to consume epiphytes because stoichiometry of epiphytes matches better with stoichiometry of grazers, potentially constraining the biomass of epiphytes on macrophytes when exploitative competition between individuals are fierce. Thus, macrophyte loss in the ponds can be induced by shading of floating and deposited filamentous algae mats rather than the generally considered epiphytes. Examining benthic food web nutrition in Ewens Ponds provides valuable context for checking the validity of

ecological theories regarding stoichiometry and fatty acid composition and their applications to explain ecosystem structure and function.

In the present study, we quantified the elemental nutrient and fatty acid composition of basal resources and consumers in the food web of Ewens Ponds in the southeast of South Australia. We expected to find significant differences of body C, N and P contents among taxonomic groups of benthic macroinvertebrates based on previous studies (Bowman et al. 2005; Cross et al. 2003; Evans-White et al. 2005; Frost et al. 2003; Lauridsen et al. 2012; Liess and Hillebrand 2005; Small and Pringle 2010; Tsoi et al. 2011). The imbalances of stoichiometric ratios between consumers and their food resources at different trophic levels were inferred combining information on trophic dynamics. We were also interested in whether there were potential limitations of community production by stoichiometry of resources. We calculated the size of elemental pools contained in the standing stock of different consumer or resource groups and in water column, and used these data to forecast which benthic macroinvertebrates would most likely experience growth limitation with respect to their food.

3.2 Materials and methods

3.2.1 Study site

Ewens Ponds are a series of three small freshwater ponds located in the southeast of South Australia (38°01′36″S, 140°47′26″E), which flow into Eight Miles Creek, in turn, discharge to the sea ca. 2.5 km downstream (Wood 2011). Groundwater is the main source of water for Ewens Ponds, with estimated water source from surface runoff being less than 5% (Grandfield and Ashman 1984).The pond water is constantly flushed as the retention time for water is only 6 h (Grandfield and Ashman 1984; Wood 2011). The water is extremely clear, with an extinction coefficient, K_d, of 0.2 m^{-1}. The ponds are located in predominantly agricultural catchments and have semi-eutrophic conditions. Plantations in the ponds and

connecting channels are dominated by riparian, emergent and submerged macrophytes (e.g. common reed, *Phragmites australis*, water ribbon, *Triglochin procera*, and shield pennywort, *Hydrocotyle verticillata*), while filamentous algae mats (e.g. chlorophyte, *Rhizoclonium* sp., and cyanobacteria, *Lyngbya* sp.) cover most of the substrata which is either too steep or too deep for macrophytes.

3.2.2 Sample collection

We estimated the standing stock of basal resources and consumers from samples collected in December, 2015. We quantified macroinvertebrate density in samples collected with either Surber sampler or D-net sampler from 5 sites within pond basin or channel habitats. We also removed all plant or benthic filamentous algae material within the sampling area covered by samplers, and all attached macroinvertebrates were collected and combined with samples from the sampler. We sorted the macroinvertebrate samples, which were identified to the lowest possible taxonomic level, in the field. We collected additional samples of macroinvertebrate consumers to analyse the C, N and P content and fatty acid composition of the various components of the food web in Ewens Ponds. Macroinvertebrates were sampled from a boat or by divers. Other consumers including fish and crayfish were collected in December, 2015 with fyke nets. Information on the nutrient content of water and other physio-chemical properties in Ewens Ponds was obtained from another project estimating the environmental risks in Ewens Ponds (Rigosi et al. 2015).

3.2.3 Laboratory procedures

The numbers of major macroinvertebrate species were counted in the laboratory. Samples of macroinvertebrates species such as shrimp and crab were oven dried at 60 °C and weighed as whole. Samples of macroinvertebrates species such as snail and caddisfly larvae were first removed of the shell or case and only body flesh were oven dried at 60 °C and weighed. We

estimated dry body mass of macroinvertebrate species (e.g. snails) which had very high abundance and relatively narrow body size distribution from averaged measurements of dry body mass of randomly picked individuals. We estimated dry body mass of macroinvertebrate species (e.g. shrimps) which had low abundance and wide body size distribution from averaged measurements of dry body mass of all individuals. Fish were euthanized using clove oil solution. Fish and crayfish were dissected, ventral muscle tissue of big fish (longer than 40 mm standard length) and caudal muscle tissue of crayfish taken for elemental nutrient and fatty acid analysis. For fish < 40 mm standard length, whole fish excluding head, tail and viscera were used for analysis. Oven dried material were ground with mortar and pestle to fine powder and stored in air-tight vials for the analysis of C, N and P content and while fresh frozen samples were freeze-dried for fatty acid composition. Depending on individual body mass, the number of individuals required for elemental and fatty acid analysis varied, from 1 to several dozen as one pooled sample.

After rinsing the plant and filamentous algae, we vigorously shake the plant and filamentous algae in a bottle with RO water. Epiphytic algae were removed from macrophytes and algae mats, and collected by filtering the suspension. Macrophytes, filamentous algae and filters were oven dried at 60 °C and weighed to determine the biomass. Samples of macrophytes, filamentous algae and epiphytes from filtered suspension were ground with mortar and pestle or ball grinder to fine powder and then used to analyse the C, N and P content and fatty acid composition.

We measured the C and N content with an elemental analyser (Perkin Elmer 2400 Series II, Perkin Elmer, Waltham, MA, USA) at Sprigg Geobiology Centre in the University of Adelaide. We determined P content using ascorbic acid method with a spectrophotometer after hot acid digestion with hydrogen peroxide. Lipids were extracted in chloroform/methanol (2:1, v/v), and fatty acid composition of basal resources and consumers

were determined with a Hewlett- Packard 6890 gas chromatography (Hewlett Packard, Alto Palo, CA, USA) at Waite Analytical Service in the University of Adelaide.

3.2.4 Data analysis

A 1-way analysis of variance (ANOVA) was used to test for the effects of taxonomic group on invertebrate body C, N and P content. Tukey's Honestly Significant Difference (HSD) was used as a post hoc test to discriminate body C, N and P content between different species. A 1-way ANOVA was used to test for differences of C:N, C:P and N:P ratios among consumers FFGs and basal resources, followed by Tukey's HSD post hoc comparisons. All statistical analyses were conducted with SPSS 19.0 (SPSS, Chicago, IL, USA).

Elemental imbalances were calculated as the arithmetic difference between elemental ratios of 9 consumer species (5 invertebrate species and 4 fish species) and the resources consumed. Elemental ratios of the resources consumed was inferred using ratios of basal resource for invertebrates or ratios of prey groups (usually taxonomic, e.g. insect, gastropod) for fish predators and contributions of different basal resources or prey groups to the consumer from a previous study on food web structure in Ewens Ponds. Elemental pools of N and P contained with 5 invertebrate species and 3 basal resources in Ewens Ponds ecosystem were calculated from elemental composition and standing biomass. Elemental pools of N and P in the water were calculated from measured nutrient concentrations in the water, estimated water volume, and estimated surface area of three ponds (Grandfield and Ashman 1984; Rigosi et al. 2015). Relative availability of N and P from resources were calculated as the sum of elemental pool of basal resources weighted by their specific contribution to certain consumer divided by elemental pool contained in that consumer species.

3.3 Results

3.3.1 Consumers and resources elemental nutrient content and stoichiometry

The mean C content of consumer FFGs varied between 38.0 ± 4.1% for collectors and 50.6 ± 0.4% for invertebrate predators (Table 3-1). The mean C content of basal resources ranged from 25.6 ± 0.5% in filamentous algae to 40.5 ± 2.3% in macrophytes (Table 3-1). The mean N content of consumer FFGs ranged from 6.6 ± 1.3% in shredders to 11.9 ± 1.6% in fish predators (Table 3-1). The mean N content of basal resources ranged from 2.0 ± 0.1% in benthic organic matter (BOM) to 3.2 ± 0.9% in macrophytes (Table 3-1). Consumers at higher trophic levels, including fish and invertebrate predators and invertebrate omnivores had significantly higher N content than consumers ingesting primarily autotrophs. The mean P content of consumer FFGs ranged from 0.63 ± 0.02% in invertebrate predators to 1.93 ± 0.19% in collectors (Table 3-1). The mean P content of basal resources varied between 0.14 ± 0.01% for BOM and 0.32 ± 0.01% for epiphyte (Table 3-1).

Table 3-1 Mean and standard error (SE) of C, N, and P contents of major consumer functional feeding groups (FFGs) and basal resources in Ewens Ponds.

FFGs or resources	C (%)		N (%)		P (%)	
species	Mean	SE	Mean	SE	Mean	SE
Collector	38.0	4.1	7.3	1.3	1.93	0.19
A. lacustris	35.8	4.7	6.4	1.4	1.79	0.17
P. australiensis	40.2	1.6	8.3	0.2	2.07	0.07
Fish predator	48.2	5.7	11.9	1.6	1.28	1.10
Invertebrate predator	50.6	0.4	9.8	0.2	0.63	0.02
Invertebrate omnivore	43.9	0.4	11.8	0.9	0.94	0.02
Scraper	41.6	4.4	8.8	0.9	0.82	0.06
G. gibbosa	44.7	3.3	9.4	0.5	0.81	0.05
P. antipodarum	38.4	2.7	8.3	0.8	0.82	0.08
Shredder	43.7	9.7	6.6	1.3	1.64	0.99
Austrochiltonia sp.	34.8	2.3	5.7	0.8	2.46	0.75
Triplectides sp.	52.6	4.0	7.6	0.8	0.81	0.06
BOM	32.6	0.1	2.0	0.1	0.14	0.01
Epiphyte	30.9	0.4	2.9	0.3	0.32	0.01
Filamentous algae	25.6	0.5	2.4	0.1	0.28	0.01
Macrophyte	40.5	2.3	3.2	0.9	0.18	0.06

BOM, benthic organic matter

The mean C:N ratios of consumer FFGs ranged from 4.4 ± 0.3 in invertebrate omnivores to 7.7 ± 1.0 in shredders (Figure 3-1a). The mean C:P ratios of consumer FFGs ranged from 50.1 ± 2.6 in collectors to 224.4 ± 26.0 in invertebrate predators (Figure 3-1b). The mean N:P ratios of consumer FFGs ranged from 8.9 ± 0.5 in collectors to 34.0 ± 3.8 in invertebrate predators (Figure 3-1c).

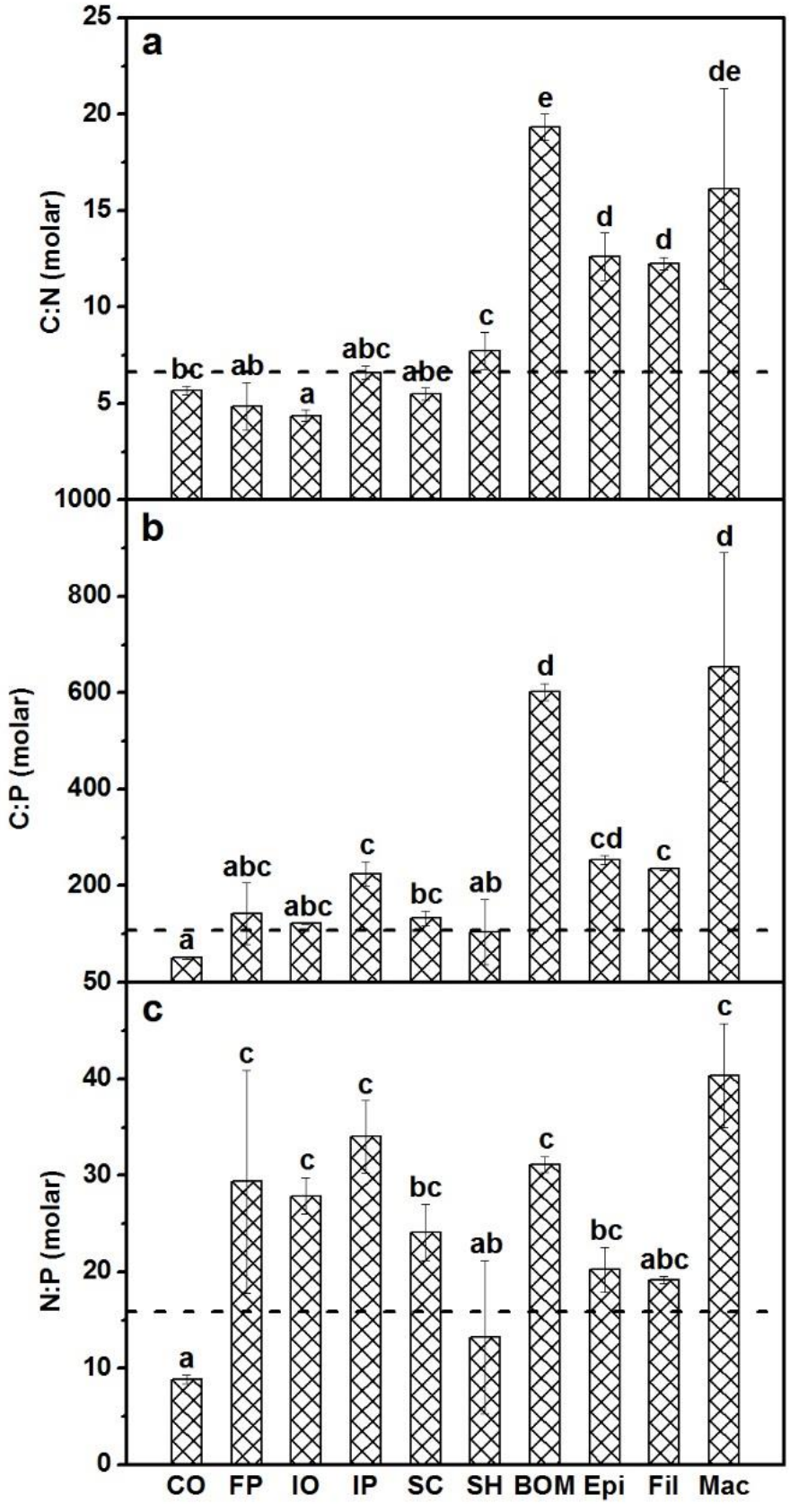

a
b
c
C:N (molar)
C:P (molar)
N:P (molar)
CO
FP
IO
IP
SC
SH
BOM
Epi
Fil
Mac

Figure 3-1 Mean C:N (a), C:P (b), and N:P (c) molar ratios of consumer functional feeding groups (FFGs) and basal resources in Ewens Ponds. Bars are standard errors. The dashed line indicates Redfield ratio. Groupings that share the same letter are not significantly different. CO – collectors, FP – fish predators, IO – invertebrate omnivores, IP – invertebrate predators, SC – scrapers, SH – shredders, BOM – benthic organic matter, Epi – epiphytes, Fil – filamentous algae, Mac – macrophytes.

3.3.2 Consumers and resources fatty acid composition

The contribution of 28 identified FAs to consumer total FA contents ranged from 90.9-97.8% (Table 3-2). FA compositions varied a lot among consumer FFGs and basal resources. SAFA accounted for 21.2-35.6 % of total FA in consumer FFGs, and 34.1-51.9 % in basal resources (Table 3-2). The percentage of SAFA in consumer FFGs except shredders were lower than the percentage of SAFA in basal resources. 16:0 was the most common SAFA in all consumer FFGs and basal resources, and the percentage of 18:0 was the second most in all consumer FFGs and basal resources except epiphytes. MUFA and PUFA accounted for 24.2-34.5 % and 27.5-40.3 % of total FA contents in consumer FFGs respectively (Table 3-2). The abundance of MUFA and PUFA in basal resources, however, had much greater ranges than consumer FFGs, with MUFA abundance ranging from 5.8 % in macrophytes to 46.0 % in BOM and PUFA abundance ranging from 12.4 % in BOM to 57.9 % in macrophytes (Table 3-2). 16:1ω7 and 18:1ω9 were the two most abundant MUFAs in consumer FFGs except scrapers, while the percentage of 18:1ω7 and 20:1ω11 higher than other MUFAs in scrapers. Higher percentages of 16:1ω7 and 18:1ω9 than other MUFAs were also observed in macrophytes and filamentous algae. Although the abundance of 18:1ω9 was highest among all MUFAs, the abundance of 16:1ω7 was lower than many other MUFAs in BOM and was

not detected in epiphytes. The percentage of DHA was highest among all PUFAs in fish predators, and the percentage of DHA in fish predator was much higher than the percentage of DHA in other consumer FFGs. The percentage of EPA was highest among all PUFAs in collectors, and the percentage of EPA in collectors was higher than the percentage of EPA in other consumer FFGs. The abundance of ARA was highest among all PUFAs in scrapers and invertebrate omnivores, and ARA abundance in other consumer FFGs were lower than the abundance in scrapers and invertebrate omnivores. ALA was the most abundant PUFA in shredders, and the abundance of ALA in shredders was higher than the abundance of ALA in consumer FFGs of other macroinvertebrates and fish. The percentage of ALA was the highest among PUFAs in macrophytes and filamentous algae, followed by LIN. The percentage of LIN was the highest among PUFAs in BOM, and the percentage of EPA was the highest among PUFAs in epiphytes. The abundance of ALA and LIN were much higher than the abundance of these two PUFAs in other basal resources. Total ω3 PUFA percentage ranged from 15.5-28.0 %, 2.5-40.6 % in consumer FFGs and basal resources respectively, and total ω6 PUFA percentage ranged from 6.9-23.1 %, 8.1-17.3 % in consumer FFGs and basal resources respectively (Table 3-2). The ratio between ω3 and ω6 varied between 0.3 and 3.0 in all consumer FFGs and basal resources (Table 3-2).

Table 3-2 Fatty acid compositions (percentages relative to total fatty acids, mean ± SE) of consumer functional feeding groups (FFGs) and basal resources in Ewens Ponds.

FFGs or resources	CO	FP	IO	SC	SH
14:0	1.9 ± 0.8	2.5 ± 1.7	0.3 ± <0.1	1.6 ± 0.4	4.7 ± -
15:0	0.6 ± 0.1	0.5 ± 0.1	0.2 ± <0.1	0.8 ± 0.1	0.3 ± -
16:0	17.1 ± 2.1	23.2 ± 3.7	13.0 ± 1.5	18.1 ± 5.6	22.4 ± -
17:0	1.2 ± 0.3	0.8 ± 0.2	0.8 ± 0.4	1.9 ± 0.9	2.0 ± -
18:0	5.7 ± 1.3	6.4 ± 1.2	6.4 ± 0.6	9.8 ± 0.9	5.9 ± -
20:0	0.6 ± 0.3	0.2 ± 0.1	0.3 ± <0.1	0.2 ± <0.1	0.2 ± -
22:0	0.6 ± 0.5	0.1 ± <0.1	0.1 ± <0.1	0.9 ± 0.7	0.1 ± -
24:0	0.2 ± 0.1	0.1 ± 0.1	<0.1 ± <0.1	0.2 ± <0.1	<0.1 ± -
SAFA	28.0 ± 2.9	33.7 ± 5.6	21.2 ± 2.5	33.4 ± 8.4	35.6 ± -
14:1	0.1 ± <0.1	0.1 ± 0.1	-	0.1 ± 0.1	0.2 ± -
16:1ω7	10.8 ± 2.9	9.0 ± 4.2	5.9 ± 2.8	4.3 ± 0.6	12.0 ± -
17:1	<0.1 ± <0.1	<0.1 ± <0.1	<0.1 ± <0.1	0.1 ± 0.1	<0.1 ± -
18:1ω9	10.9 ± 2.9	10.2 ± 4.4	25.3 ± 3.8	4.2 ± 0.8	18.3 ± -
18:1ω7	7.1 ± 0.3	4.9 ± 1.8	2.5 ± 0.2	8.5 ± 4.3	3.1 ± -
20:1ω11	0.6 ± 0.5	0.3 ± 0.4	0.1 ± <0.1	7.1 ± 0.9	<0.1 ± -
20:1ω9	0.7 ± 0.4	0.4 ± 0.2	0.6 ± 0.3	0.5 ± 0.1	0.2 ± -
22:1ω9	0.1 ± <0.1	0.1 ± <0.1	<0.1 ± <0.1	0.1 ± <0.1	<0.1 ± -
24:1	0.1 ± 0.1	0.3 ± 0.1	-	0.1 ± 0.1	<0.1 ± -
MUFA	30.5 ± 2.9	24.2 ± 7.0	34.5 ± 6.8	25.0 ± 4.3	33.8 ± -
18:2ω6	3.6 ± 0.4	3.8 ± 1.5	4.4 ± 1.8	3.6 ± 2.1	4.1 ± -
18:3ω6	0.3 ± <0.1	0.3 ± 0.1	0.1 ± <0.1	0.3 ± 0.1	0.4 ± -
18:3ω3	4.1 ± 0.9	4.0 ± 2.1	2.7 ± 0.8	2.6 ± 0.7	13.7 ± -
20:2ω6	0.6 ± 0.4	0.3 ± 0.2	0.8 ± 0.1	2.4 ± 0.1	0.1 ± -
20:3ω6	0.3 ± 0.1	0.4 ± 0.1	0.2 ± <0.1	0.8 ± 0.2	0.2 ± -
20:4ω6	5.4 ± 1.3	5.0 ± 2.7	17.4 ± 3.5	6.7 ± 4.7	2.1 ± -
20:3ω3	0.5 ± 0.1	0.5 ± 0.3	0.4 ± 0.1	0.8 ± 0.1	0.1 ± -
20:5ω3	15.0 ± 4.0	7.8 ± 3.9	11.7 ± 2.4	4.4 ± 1.5	6.4 ± -
22:4ω6	0.5 ± 0.5	1.1 ± 0.6	0.1 ± <0.1	3.3 ± 2.0	<0.1 ± -
22:5ω3	1.8 ± 1.0	4.7 ± 1.5	0.6 ± 0.2	4.3 ± 3.1	0.2 ± -
22:6ω3	3.9 ± 0.7	11.0 ± 4.5	1.9 ± 0.1	3.5 ± 3.6	0.1 ± -
PUFA	36.0 ± 5.5	39.0 ± 8.0	40.3 ± 4.6	32.7 ± 4.7	27.5 ± -
ω3	25.3 ± 4.7	28.0 ± 6.7	17.2 ± 3.1	15.5 ± 4.6	20.6 ± -
ω6	10.7 ± 2.1	11.0 ± 3.3	23.1 ± 1.6	17.2 ± 8.8	6.9 ± -
ω3/ω6	2.4 ± 0.6	2.8 ± 1.2	0.7 ± 0.1	1.3 ± 0.9	3.0 ± -
Sum	94.5 ± 0.4	97.0 ± 0.7	96.1 ± 0.4	91.2 ± 0.4	96.9 ± -

CO – collectors, SH – shredders, SC – scrapers, IP – invertebrate predators, FP – fish predators.

Table 3-2 (continued)

FFGs or resources	BOM	Epi	Fil	Mac
14:0	1.8 ± 0.1	6.0 ± -	4.1 ± 0.2	1.3 ± 0.6
15:0	0.5 ± 0.1	0.5 ± -	0.4 ± <0.1	0.3 ± 0.1
16:0	22.1 ± 0.5	38.6 ± -	41.2 ± 0.2	26.4 ± 5.2
17:0	0.7 ± 0.1	0.2 ± -	0.7 ± <0.1	0.8 ± 0.4
18:0	4.6 ± 0.1	1.5 ± -	4.7 ± 0.1	2.3 ± 0.6
20:0	2.8 ± <0.1	0.1 ± -	0.3 ± <0.1	0.6 ± 0.4
22:0	2.6 ± 0.1	0.1 ± -	0.2 ± <0.1	0.8 ± 0.5
24:0	3.9 ± 0.1	0.9 ± -	0.5 ± <0.1	1.6 ± 0.8
SAFA	39.0 ± 0.8	47.8 ± -	51.9 ± 0.2	34.1 ± 6.5
14:1	-	0.1 ± -	0.2 ± <0.1	0.1 ± <0.1
16:1ω7	2.8 ± 0.1	-	11.8 ± 0.1	2.1 ± 2.3
17:1	0.2 ± <0.1	0.2 ± -	0.3 ± <0.1	0.1 ± <0.1
18:1ω9	20.2 ± 0.3	9.9 ± -	7.3 ± 0.2	2.0 ± 0.6
18:1ω7	2.3 ± 0.1	2.3 ± -	3.0 ± <0.1	1.0 ± 0.7
20:1ω11	0.4 ± 0.1	-	0.4 ± <0.1	0.1 ± 0.1
20:1ω9	5.1 ± 0.3	0.1 ± -	0.3 ± <0.1	0.2 ± 0.1
22:1ω9	14.1 ± 0.8	<0.1 ± -	0.3 ± <0.1	0.2 ± 0.1
24:1	0.9 ± <0.1	<0.1 ± -	0.3 ± <0.1	0.1 ± 0.2
MUFA	46.0 ± 1.2	12.7 ± -	24.0 ± 0.1	5.8 ± 3.2
18:2ω6	9.0 ± 0.3	3.3 ± -	4.2 ± 0.1	16.7 ± 7.9
18:3ω6	0.4 ± <0.1	3.9 ± -	2.7 ± <0.1	0.2 ± 0.1
18:3ω3	1.4 ± <0.1	2.5 ± -	6.7 ± <0.1	39.3 ± 10.9
20:2ω6	0.4 ± <0.1	<0.1 ± -	0.2 ± <0.1	0.2 ± 0.1
20:3ω6	-	0.1 ± -	0.3 ± <0.1	0.1 ± 0.1
20:4ω6	0.2 ± <0.1	0.8 ± -	1.4 ± <0.1	0.1 ± 0.1
20:3ω3	-	-	0.2 ± <0.1	0.1 ± <0.1
20:5ω3	0.4 ± <0.1	19.2 ± -	3.8 ± <0.1	0.9 ± 0.9
22:4ω6	-	<0.1 ± -	0.1 ± <0.1	<0.1 ± <0.1
22:5ω3	-	0.5 ± -	0.7 ± <0.1	0.1 ± 0.1
22:6ω3	0.8 ± 0.1	0.1 ± -	0.5 ± <0.1	0.4 ± 0.3
PUFA	12.4 ± 0.4	30.4 ± -	20.7 ± <0.1	57.9 ± 8.7
ω3	2.5 ± 0.1	22.3 ± -	11.9 ± <0.1	40.6 ± 10.2
ω6	9.9 ± 0.3	8.1 ± -	8.8 ± 0.1	17.3 ± 8.0
ω3/ω6	0.3 ± <0.1	2.8 ± -	1.3 ± <0.1	2.8 ± 1.2
Sum	97.4 ± 0.1	90.9 ± -	96.6 ± 0.1	97.8 ± 0.6

IO – invertebrate omnivores, Mac – macrophytes, Fil – filamentous algae, Epi – epiphytes, BOM – benthic organic matter.

3.3.3 Elemental imbalances between consumers and resources and standing stock of N and P in consumers and resources

Imbalances in C:N between invertebrate primary consumers and their food were greater than imbalances in C:N between fish predators and their food (Figure 3-2a). Imbalances in C:P had similar pattern to imbalances in C:N when comparing invertebrate primary consumers with fish predators (Figure 3-2b). All taxa of fish had positive values of imbalances in C:P ratios (Figure 3-2b). Imbalances in N:P were much more variable, and all invertebrate primary consumers had negative values while all fish predators had positive values (Figure 3-2c).

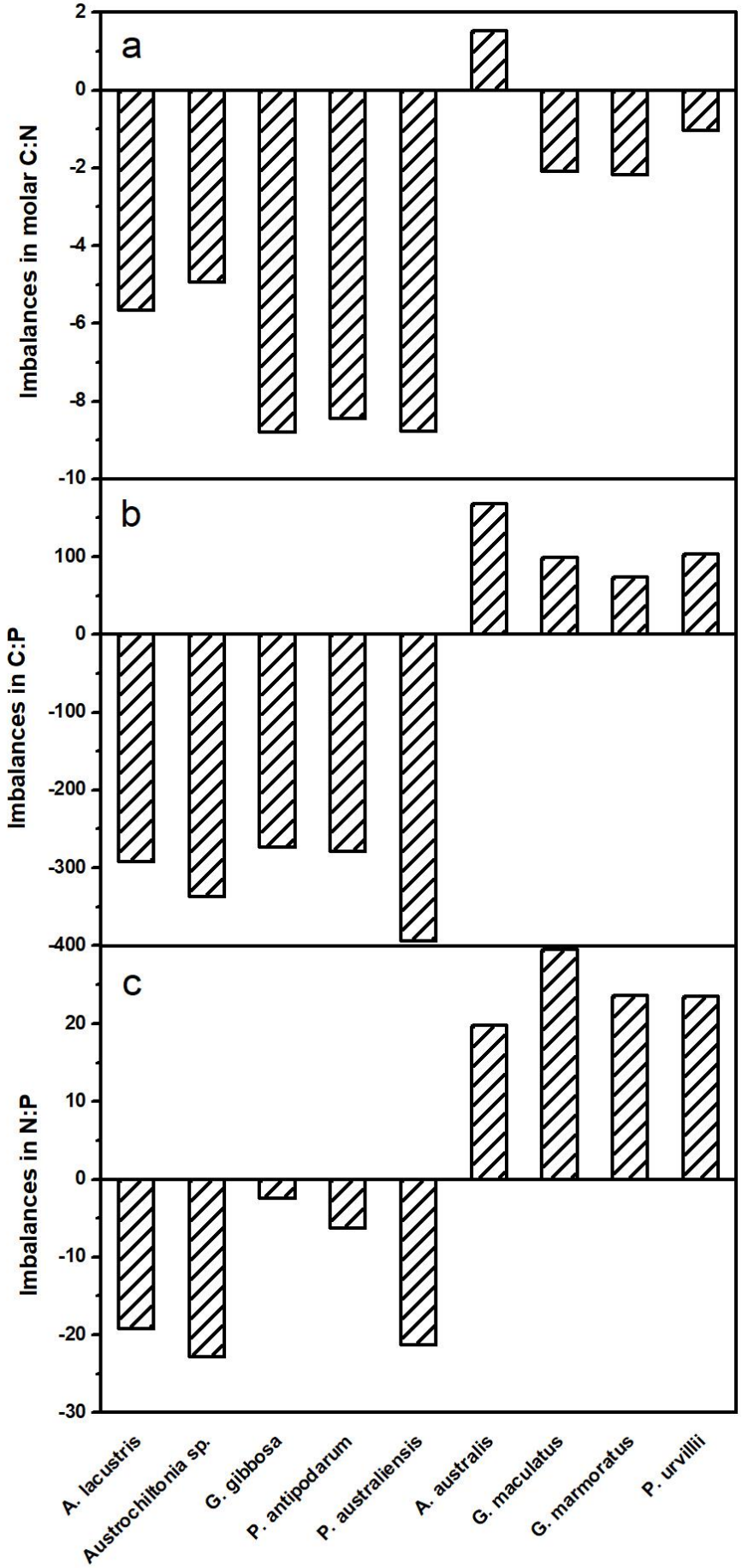

a
b
c
Imbalances in molar C:N
Imbalances in C:P
Imbalances in N:P
2
0
-2
-4
-6
-8
-10
100
-100
-200
-300
-400
20
10
-20
-30
A. lacustris
Austrochiltonia sp.
G. gibbosa
P. antipodarum
P. australiensis
A. australis
G. maculatus
G. marmoratus
P. urvillii

Figure 3-2 Elemental imbalances in C:N (a), C:P (b), and N:P (c) molar ratios between invertebrate and fish consumers and their food sources. Positive bar represents the ratios in consumers were higher than resources and vice versa.

The standing stock of five most common benthic macroinvertebrates and three major basal resources contained 5.96 g N m^{-2} and 0.62 g P m^{-2}. More than half (55.2 %) of the standing stock of N was contained in the form of macrophytes, and large percentage (22.2 %) was contained in the form of filamentous algae (Figure 3-3a). However, the N pool size in these organisms was much smaller than the N pool size in water column, with instantaneous standing stock of 32.0 g N m^{-2} dissolved inorganic nitrogen (DIN) (Figure 3-3a). Macrophytes and filamentous algae accounted for 29.5% and 24.6 % of the total P standing stock within the most common benthic organisms, and one macroinvertebrate primary consumer, freshwater crab (*A. lacustris*) accounted for 26.7% (Figure 3-3b). Unlike the N pool size in water column, the P pool size in water column was much smaller than the P pool size in benthic organisms, with instantaneous standing stock of 0.048 g P m^{-2} soluble reactive phosphorus (SRP) (Figure 3-3b). The relative availability of N was much greater for shredders, *Austrochiltonia* sp. than for other macroinvertebrates, and the relative availability of N was smaller for collectors than for scrapers (Figure 3-3c). The relative availability of P was much smaller for collectors, *A. lacustris* and *P. australiensis* than for other macroinvertebrates, while the relative availability of P was similar in shredders and scrapers. (Figure 3-3d).

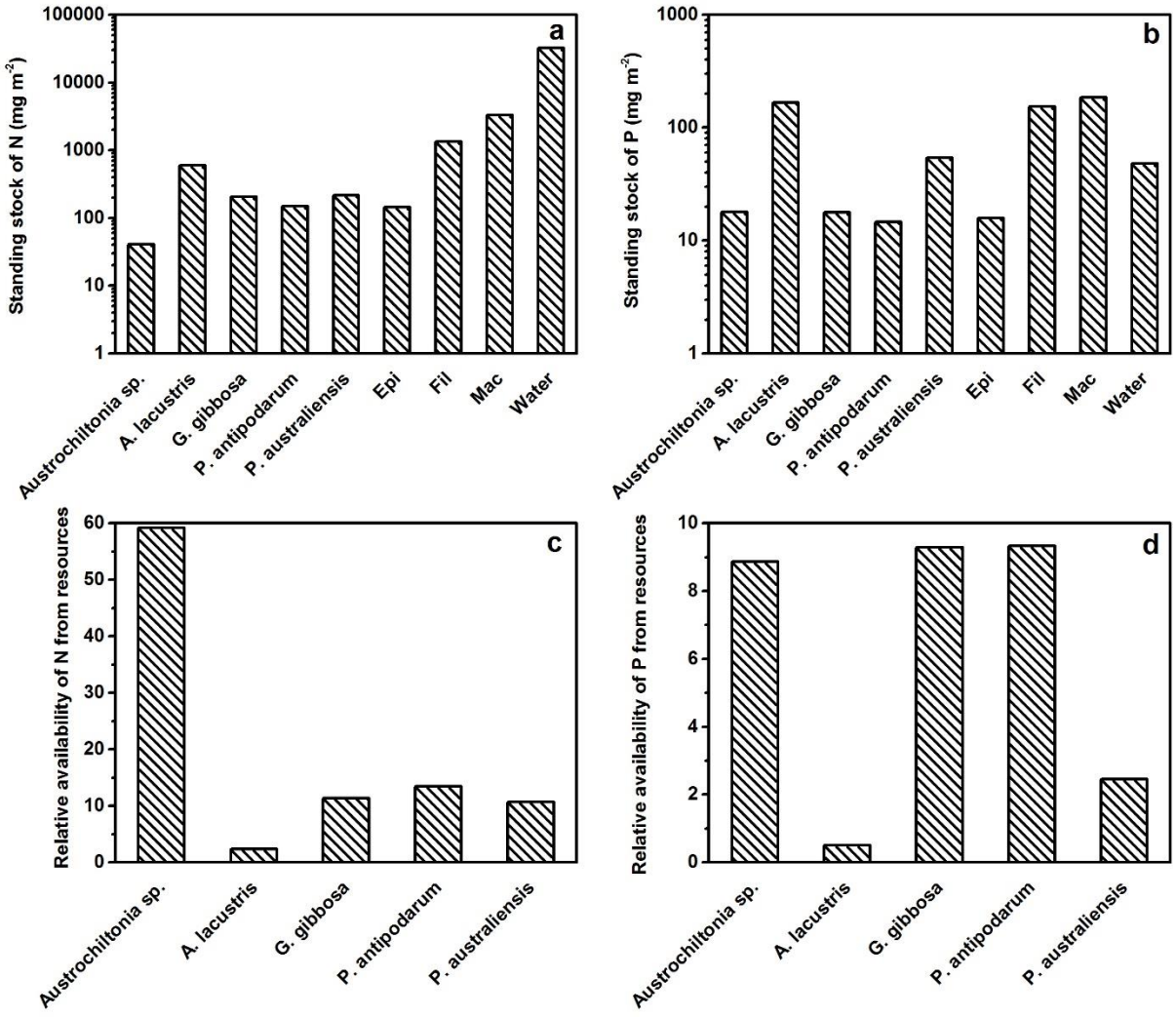


Figure 3-3 Elemental pool size within five invertebrate species and basal resources of N (a) and P (b) and instantaneous standing stock in water and relative availability of N (a) and P (b). Epi – epiphytes, Fil – filamentous algae, Mac – macrophytes.

3.4 Discussion

We investigated the elemental nutrient composition of basal resources and consumers in the food web of oligotrophic to mesotrophic ponds constantly flushed by large amount of recharging groundwater. The elemental nutrient in Ewens Ponds water are characterized by highly enriched N and relatively low P (DIN, 5.156 mg L^{-1}, SRP, 0.008 mg L^{-1}). Macrophyte

and BOM had higher C content but lower P content than filamentous algae and BOM in Ewens Ponds (Table 3-1). In contrast with the results shown in Evans-White et al. (2005), we found higher N and P content in epiphyte than filamentous algae in this study (Table 3-1). In consistent with by Evans-White et al. (2005), The N and P content of BOM was lower than either filamentous algae or epiphyte in this study (Table 3-1). We found the highest and lowest N content of basal resources was in macrophyte and BOM respectively while the highest and lowest P content of basal resources was in epiphyte and BOM respectively (Table 3-1), similar to findings in Lauridsen et al. (2012). C:N ratio of macrophyte was similar to that of BOM, but higher than that of epiphyte in Ewens Ponds (Figure 3-1). C:N ratio of macrophyte could be similar to or lower than C:N ratio of BOM or POM, depending on the origin of these organic matter (Feijoó et al. 2014; Lauridsen et al. 2012). Filamentous algae had similar C:N ratio with epiphyte, and the value was lower than that of macrophyte and BOM (Figure 3-1). Evans-White et al. (2005) reported similar C:N ratio in filamentous algae and epilithon, and higher C:N ratio in POM than filamentous algae and epilithon in streams. Other studies reported higher C:N ratio in POM than epilithon and higher C:N ratio in macrophyte than filamentous algae (Cross et al. 2003; Tsoi et al. 2011). Epiphyte had similar C:P and N:P ratios with filamentous algae, and the values were much lower than that of macrophyte and BOM (Figure 3-1). Lower C:P and N:P ratios in epiphyton or periphyton than macrophyte and BOM or POM were previously reported in streams having much higher water P concentration than Ewens Ponds (Feijoó et al. 2014; Lauridsen et al. 2012). Whereas the N:P ratios of epilithon, filamentous algae and POM were very close to each other, the C:P ratios of epilithon and filamentous algae were similar and higher than that of POM in Evans-White et al. (2005).

C:P and N:P ratios of basal resources, especially those with low level of stoichiometric homeostasis such as epiphyte, may be very sensitive to increase in water P concentrations in

systems with low P availability. Small and Pringle (2010) investigated epilithon stoichiometry in a series of streams that ranged widely in P levels, and found that P content, C:P and N:P ratios of epilithon were all negatively related with stream SRP. Bowman et al. (2005) compared epilithon stoichiometry between upstream and downstream sites of wastewater treatment plant discharges in Canadian Rocky mountains, and showed significantly lower epilithon C:P and N:P ratios with only slightly higher TP and DIN concentrations in downstream sites than upstream sites. Cross et al. (2003) found lower C:P and N:P ratios of basal resources (except N:P ratios of epilithon) in long-term treatment streams enriched with N and P compared to control streams, and the increased N:P ratios of epilithon in treatment streams were because higher times of N than P was taken up by epilithon in treatment streams than control streams.

We found significantly lower N and P contents in basal resources than consumer FFGs. Previous studies also observed the same pattern when comparing N contents of basal resources to N contents of consumer FFGs in lakes or streams having similar or higher available N concentrations (Evans-White et al. 2005; Lauridsen et al. 2012; Liess and Hillebrand 2005). Basal resources were reported to have lower P contents than that of consumers in oligotrophic to mesotrophic aquatic ecosystems where SRP concentrations were lower than 100 µg L^{-1} (Bowman et al. 2005; Evans-White et al. 2005; Liess and Hillebrand 2005). However, Lauridsen et al. (2012) reported periphyton P content as high as 1.31 ± 0.09%, this value was higher than the P contents of all basal resources and consumers in Ewens Ponds except small crustacean species. Such high P content in preiphyton may be due to luxury take-up of P in environments with very high SRP concentrations (123 µg L^{-1}).

We found higher C content in invertebrate predators and fish predators relative to other consumers (except one shredder species *Triplectides* sp.) (Table 3-1). The species of invertebrate predators in our study were diving beetles *Antiporus* sp., and relatively high C

content of beetles, Coleoptera, compared to other invertebrate orders was reported in lakes and streams in North America (Evans-White et al. 2005; Frost et al. 2003). Previous studies found that caddisflies, Trichoptera, had relatively high C content compared to other invertebrate orders (Bowman et al. 2005; Evans-White et al. 2005; Frost et al. 2003). *Austrochiltonia* sp., one Amphipod species, had the lowest C content among all consumers (Table 3-1). Frost et al. (2003) and Evans-White et al. (2005) also found much lower C content in Amphipoda than insects and molluscs. We found predators and invertebrate omnivores, which were at higher trophic levels in Ewens Ponds than other consumers indicated by their higher $\delta^{15}N$ values (Yang Liu, under review), had higher N content than invertebrate primary consumer FFGs, including collectors, shredders and scrapers (Table 3-1). Our findings coincided with the findings in previous studies where higher N content in predators relative to other FFGs were shown (Cross et al. 2003; Evans-White et al. 2005; Small and Pringle 2010). Higher nutrient content in the diets of predators or the crayfish with relatively big body size in our study might be responsible for this pattern (Fagan et al. 2002). N content of *Triplectides* sp. was much lower than other insects, but much higher than *A. lacustris* and *Austrochiltonia* sp. Only crustaceans, were found to have lower N content than Trichoptera when all consumers were compared in previous studies (Cross et al. 2003; Evans-White et al. 2005; Frost et al. 2003; Liess and Hillebrand 2005; Small and Pringle 2010). Scrapers in this study all belong to gastropods, and N content of gastropods was found to be slightly higher than that of Trichoptera, but lower than other insects in Evans-White et al. (2005). We found crustacean, *Austrochiltonia* sp. had the highest P content and Coleoptera, *Antiporus* sp. had the lowest P content among all consumers in this study (Table 3-1). Frost et al. (2003) and Evans-White et al. (2005) both observed similar patterns, and relatively rich P content in amphipods was attributed to less structural material and greater RNA content in their body. The low P content in Coleoptera may be related to the more structural material,

such as chitin, in their body (Liess and Hillebrand 2005). Our results had a lot in common with the results of Frost et al. (2003) in term with C, N, and P content in consumers, and much of this similarity can be explained by the close taxonomic relationships between consumer species in this study and theirs. Most of the Coleoptera consumers in their study belong to the family Dytiscidae, to which *Antiporus* sp. in this study also belongs. Both studies had Trichoptera consumers from the same family Leptoceridae, such as *Oecetis* sp. in theirs and *Triplectides* sp. in ours. Amphipod *Austrochiltonia* sp. in this study used to be in genus *Hyalella*, and most of the amphipods in their study belongs to this genus as well.

Fish predators and invertebrate omnivore contained high concentrations of nitrogen, leading to significantly lower C:N ratios in these two groups than in other FFGs (Table 3-1, Figure 3-1). Previous studies also found the lowest C:N ratios in fish among all consumers, and authors of those papers suggested that this pattern is because fish had the high proportion of muscle tissue in their bodies (Lauridsen et al. 2012; Tsoi et al. 2011). Shredders had the highest C:N among all FFGs in this study (Figure 3-1), which is consistent with the results from other studies comparing stoichiometry of invertebrate FFGs in streams (Cross et al. 2003; Evans-White et al. 2005; Lauridsen et al. 2012; Small and Pringle 2010). Shredders composed of two groups of organisms, *Triplectides* sp. and *Austrochiltonia* sp. in this study, and the relative high C:N in Trichoptera compared to most other insect orders was confirmed in previous studies (Cross et al. 2003; Evans-White et al. 2005; Frost et al. 2003; Liess and Hillebrand 2005; Small and Pringle 2010). Scrapers had lower C:N than invertebrate predators in this study, but other studies reported results contrary to ours (Bowman et al. 2005; Cross et al. 2003; Feijoó et al. 2014; Lauridsen et al. 2012; Small and Pringle 2010). Differences in taxonomic groups of the organisms in scraper and invertebrate predator FFGs in this study compared to that in others may be able to explain the contrasting results. For example, scrapers belong to Gastropoda and invertebrate predators belong to Coleoptera in

this study, but scraper consisted of Trichoptera and Ephemetoptera species and invertebrate predator consisted of Odonata species in Cross et al. (2003) and Small and Pringle (2010). Odonata predators were previously found to have lower C:N ratios than Trichoptera and Ephemetoptera scrapers (Cross et al. 2003; Evans-White et al. 2005; Frost et al. 2003; Small and Pringle 2010), however, the relationships between C:N ratios of Odonata and Gastropoda can be variable, with lower, equal or higher C:N ratios in Odonata than Gastropoda have been reported (Evans-White et al. 2005; Lauridsen et al. 2012; Tsoi et al. 2011). Lower C:N ratios in Gastropoda than Coleoptera was previously reported in several studies (Evans-White et al. 2005; Lauridsen et al. 2012; Liess and Hillebrand 2005), as well as lower C:N ratios in Odonata than Coleoptera (Evans-White et al. 2005; Frost et al. 2003; Lauridsen et al. 2012). Collectors consisted of crustacean species, Atyidae shrimp and Hymenosomatidae crab in this study, and they had similar C:N ratios with invertebrate predators (Figure 3-1). Lauridsen et al. (2012) found that Malacostraca collectors had the third highest C:N among all consumer orders, only after Ephemeroptera and Trichoptera scrapers, higher than Coleoptera and Odonata, but Tsoi et al. (2011) found Atyidae shrimp had the lowest C:N among all invertebrate consumers, although their C:N ratios was still higher than that of fish. Collectors and shredders had the lowest C:P and N:P ratios among all consumer FFGs, because of the high P content of some crustacean consumers (e.g. *A. lacustris* and *Austrochiltonia* sp.) within these two consumer FFGs (Table 3-1). Other ecologists also found that Malacostraca, or lower taxonomic groups within Malacostraca, Amphipoda, Decapoda or Isopoda had lower C:P and N:P than most other consumers (Evans-White et al. 2005; Frost et al. 2003; Lauridsen et al. 2012; Liess and Hillebrand 2005). An inherently higher ribosomal RNA (a P-rich cellular constituent) content of benthic crustaceans than other invertebrate consumers may account for the higher P content, and lower C:P and N:P in their body compared to other invertebrate consumers (Elser et al. 2000b; Evans-White et al. 2005). High content of P

associated with the calcium (Ca) in benthic crustacean carapaces or other calcified structures within their body may be another possible reason for the observed pattern (Evans-White et al. 2005; Vrede et al. 1999). Liess and Hillebrand (2005) found C:P and N:P of Trichoptera, the no crustacean shredder, in this study could be as low as that of Isopoda. Invertebrate predators had the highest C:P and N:P ratios among all consumer FFGs, a result that could potentially be explained by the high content of structural molecules in their hard exoskeleton, such as chitin, which was suggested to contain mostly C, little N, and no P (Elser et al. 1996; Elser et al. 2000b).

The taxonomic differences in C:N, C:P, and N:P ratios of invertebrates have been linked to differences in C, N, and P pool size associated with important biomolecules (Frost et al. 2003). Distinct biomolecular composition of various taxa can be a result of different allocations of cellular materials as animals select different life-history strategies in the process of growth (Frost et al. 2003). For example, high content of P-rich rRNA is associated with rapid growth, leading to low C:P and N:P in animals, while high content of protective tissue, which can be rich in C and/or N, is associated with slow growth, resulting in high C:P and N:P (Elser et al. 1996; Frost et al. 2003). P storage in animal body components (e.g. haemolymph in insects and carapace in crustaceans), other than in the form of rRNA (e.g. α-glycerophosphate or P-Ca complexes), have been mentioned for causing high P content in certain taxa too (Cross et al. 2003; Evans-White et al. 2005). The relationships between stoichiometric ratios of consumer FFGs can be largely influenced by the composition of species within each consumer FFG among different studies. Ontogenetic changes in body elemental composition, although largely unclear, was shown in some studies, thus body size and/or life stage should be taken into consideration when comparing C:N:P stoichiometry among invertebrate taxa (Cross et al. 2003; Frost and Elser 2002).

The most abundant SAFA in all basal resources was 16:0 (palmitic acid), and the percentage of 16:0 was the highest among all fatty acids in basal resources except macrophytes, in which the percentage of 18:3ω3 was higher than that of 16:0 (Table 3-2). Our finding was in consistent with Kelly and Scheibling (2012), as they also reported 16:0 was the most abundant FA in primary producers of various phyla or classes except Tracheophyta (vascular plant). MUFA in macrophytes consisted mainly of 16:1ω7 and 18:1ω9, but the percentages of these two MUFAs were low (<5%) (Table 3-2). PUFA in macrophytes consisted mainly of 18:3ω3 and 18:2ω6, and the percentage of 18:3ω3 and 18:2ω6 were much higher than that in other basal resources, typical for vascular plants (Kelly and Scheibling 2012). Fatty acid composition of filamentous algae was dominated by 16:0, while 16:1ω7 and 18:1ω9 were the most abundant MUFAs (Table 3-2). Vargas et al. (1998) investigated the fatty acid composition of 12 strains of filamentous, heterocystous, nitrogen-fixing cyanobacteria and found a similar pattern, thus the fatty acid profile in filamentous algae reflected the dominance of cyanobacteria. We found relatively high percentage of 20:5ω3, a commonly used fatty acid biomarker for diatoms, in epiphytes, but 16:1ω7, another fatty acid biomarker, was not detected (Kelly and Scheibling 2012; Taipale et al. 2013). BOM had much high percentage of 18:1ω9 and 22:1ω9 than other basal resources (Table 3-2). Lowe et al. (2014) conducted regression between biomarker fatty acids and particulate organic matter components and showed that 18:1ω9 was the best putative biomarker for detritus in particulate organic matter.

Functional feeding guild (FFG) groupings of macroinvertebrates were often used as a surrogate for diet in many studies on ecological stoichiometry (Lauridsen et al. 2014). However, FFG actually described feeding mode rather than real diet, and consumers in nature frequently exhibit variations in diet flexibility. Information on food composition of consumers in this study were from a parallel study using stable isotope analysis and gut

content analysis to determine source of organic carbon in Ewens Ponds. Improvement of the precision of elemental imbalances calculation was thus expected, although other obstacles such as bulk sampling of food sources still remained (Evans-White et al. 2005). Elemental imbalances between fish predators and their preys were generally lower than elemental imbalances between macroinvertebrate primary consumers and resources, presumably because of the much higher contents of N and P in preys consumed by fish predators than in resources consumed by macroinvertebrate primary consumers. C:N, C:P, and N:P imbalances between fish predators and their preys in Ewens Ponds were similar to or smaller than the imbalances between fish predators and their preys in a nutrient-enriched stream in England (Lauridsen et al. 2012). In fact, we even observed some positive values in C:P and N:P imbalances between fish predators and their preys, indicating C and N rather than P limitation for those fish species. C:N imbalances between collector and shredder FFGs and resources in Ewens Ponds were lower than that in Cross et al. (2003) and Lauridsen et al. (2012), but C:N imbalances between scraper FFG and resources was higher in Ewens Ponds. C:P imbalances between collector and scraper FFGs and resources in Ewens Ponds were greater than or similar to that in Lauridsen et al. (2012) where water SRP concentrations were much higher, but smaller than that in Cross et al. (2003) where water SRP concentrations were lower. C:P imbalances between collector and scraper FFGs and resources was reduced to the level comparable to that in Ewens Ponds after enriching the stream water artificially, and SRP concentrations reached 46 μg L^{-1} in Cross et al. (2003). In the same study, Cross et al. (2003) reported shredders, which relied on leaf-detritus for food, had much higher C:P imbalances than shredders in Ewens Ponds, which mostly relied on macrophytes. Cross et al. (2003) and Lauridsen et al. (2012) reported N:P imbalances between collector and shredder FFGs and resources in streams were negative, while N:P imbalances between collector and shredder FFGs and resources in Ewens Ponds were still positive, although some values were very

small. This differences in N:P imbalances between collector and shredder FFGs and resources among studies was related to relatively low available N:P ratios of ambient environment in the system. In a hypereutrophic stream, SRP concentrations were more than 10 times higher than that in Ewens Ponds while total oxidisable nitrogen concentrations were only 20% higher than that in Ewens Ponds, leading to the much lower total oxidisable nitrogen to SRP ratios in that stream than DIN:SRP ratios in Ewens Ponds (Lauridsen et al. 2012). In another study, the much lower DIN:SRP ratios in some oligotrophic streams than that in Ewens Ponds was caused by much lower DIN concentrations but similar or only a bit higher SRP concentrations in those streams (Cross et al. 2003). The much greater N:P imbalances between scraper FFG and resources in Cross et al. (2003) than ours were due to much higher N:P of epilithons from their sites than N:P of epiphytes from Ewens Ponds. Although elemental imbalances calculated as arithmetical differences were widely used in stoichiometry papers, we need to be cautious that these differences may be not entirely meaningful because these ratios themselves vary in magnitude.

The pools of N and P in macrophyte and filamentous algae were close to or larger than those of macroinvertebrate consumers (Figure 3-3a, b). N bound in benthic organisms was relatively scarce compared to N in the water column, while P abundance in benthic organisms and in water column differed not as much as N (Figure 3-3a, b). The calculated standing stocks of N and P in water column are instantaneous, and do not account for the constant replenishment by charging groundwater. From the discharge and concentration of nutrients, 541 kg DIN and 0.814 kg SRP was transported past the pond system daily. Despite relatively small instantaneous P pool size in water column, rapid replenishment might alleviate P shortage to some extent. Similar with Lauridsen et al. (2012), *Austrochiltonia* sp., a shredder, had the highest relative availability of N among all macroinvertebrates in this study, presumably because of the lowest body N content among all macroinvertebrates. Collectors

had much bigger body size and weight, and resultant bigger standing stock of N than scrapers, leading to the lower relative availability of N for collectors than scrapers. Much larger size of standing stock of P locked in *A. lacustris* biomass than other macroinvertebrates was due to the relative heavy body weight and high body P content among macroinvertebrates, resulting in the lowest relative availability of P among macroinvertebrates. The second highest body P content in the biomass of *P. australiensis* among all macroinvertebrates, combining with the much greater body mass than shredders and scrapers, makes standing stock of P in *P. australiensis* higher than that in shredders and scrapers. Higher dependence on epiphytes, which had much lower standing stock of P than other basal resources, of *P. australiensis* than shredder, *Austrochiltonia* sp. and two snail species in scraper FFG possibly accounted for the lower relative availability of P for *P. australiensis* than for shredders and scrapers. From the perspective of ecological stoichiometry, snails are the best choice of food for fish because of the smallest elemental imbalances between them and fish. However, the digestion and assimilation of some snails, such as P. antipodarum, was suggested to be difficult in some fish species (Vinson and Baker 2008). The diets of top predator species *A. australis* and the most abundant native species *G. maculatus* in Ewens Ponds was actually found to be dominated by crustaceans, especially *P. australiensis* (Yang Liu, under review). Gastropods dominated the diets of two other fish species, *A. butcheri* and *P. urvillii* in Ewens Ponds. Although the production of snails was less likely to be limited by N and P availability than *P. australiensis*, as they had higher relative availability of N and P than that of *P. australiensis*, population size of these two snails may be limited by a combination of food quality and quantity in Ewens Ponds. While there were plenty of benthic filamentous algae in Ewens Ponds, it is a poor food resource owing to factors such as sick sheath and toxins production in them, rather than nutritional quality. The supply of epiphytes, the other basal resources which had stoichiometric ratios

closer to snails than macrophytes and BOM, may be insufficient because snails raised in mesocosms at much lower density than in the field exhausted the whole periphyton community within days (Yang Liu, unpublished data). Severe competition for very limited epiphyte resources of high quality may have forced snails to ingest high portions of other food items in their diets in Ewens Ponds (Riley et al. 2008).

Stoichiometric imbalances did exist in Ewens Ponds, in particular between primary producers and primary consumers, i.e. at the plant-animal interface. Furthermore, consumer stoichiometry was mainly constrained by taxonomic identity. It appears that, the production of macroinvertebrates and fish was somewhat limited by the shortage of nutritious food supply, while large proportions of inorganic N and P supply were not available to most consumers in Ewens Ponds. Our results describe taxonomic and FFG-related differences in the C:N:P ratios and fatty acid contents of benthic macroinvertebrates and fish of a coastal groundwater-fed freshwater wetland. Although we used some of the documented patterns in this study to explain the relationships between organisms in the benthic habitats, the ecological consequences of differences in elemental nutrient and fatty acid composition between the food sources and consumers still need to further explored in future studies. In the meantime, proximate and ultimate causes leading to differences in the C:N:P and fatty acid contents of benthic invertebrate taxa still remain important gaps in our knowledge.

References

Anderson, T. R., M. Boersma & D. Raubenheimer, 2004. Stoichiometry: linking elements to biochemicals. Ecology 85:1193-1202.

Arts, M. T., M. T. Brett & M. J. Kainz, 2009. Lipids in aquatic ecosystems. Springer.

Bowman, M. F., P. A. Chambers & D. W. Schindler, 2005. Changes in stoichiometric constraints on epilithon and benthic macroinvertebrates in response to slight nutrient enrichment of mountain rivers. Freshwater Biology 50:1836-1852.

Brett, M. T. & D. C. Müller-Navarra, 1997. The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshwater Biology 38:483-499.

Cashman, M. J., J. D. Wehr & K. Truhn, 2013. Elevated light and nutrients alter the nutritional quality of stream periphyton. Freshwater Biology 58:1447-1457.

Cross, W. F., J. P. Benstead, P. C. Frost & S. A. Thomas, 2005. Ecological stoichiometry in freshwater benthic systems: recent progress and perspectives. Frehswater Biology 50:1895-1912.
Cross, W. F., J. P. Benstead, A. D. Rosemond & J. B. Wallace, 2003. Consumer-resource stoichiometry in detritus-based streams. Ecology Letters 6:721-732.
Cross, W. F., J. B. Wallace & A. D. Rosemond, 2007. Nutrient enrichment reduces constraints on material flows in a detritus-based food web. Ecology 88:2563-2575.
Elser, J. J., D. R. Dobberfuhl, M. Neil A & J. H. Schampel, 1996. Organism size, life history, and N:P stoichiometry: towards a unified view of cellular and ecosystem processes. BioScience 46:674-684.
Elser, J. J., W. F. Fagan, R. F. Denno, D. R. Dobberfuhl, A. Folarin, A. Huberty, S. Interlandi, S. S. Kilham, E. McCauley, K. L. Schulz, E. H. Siemann & R. W. Sterner, 2000a. Nutritional constraints in terrestrial and freshwater food webs. Nature 408:578-580.
Elser, J. J., R. W. Sterner, E. Gorokhova, W. F. Fagan, T. A. Markow, J. B. Cotner, J. F. Harrison, S. E. Hobbie, G. M. Odell & L. J. Weider, 2000b. Biological stoichiometry from genes to ecosystems. Ecology Letters 3:540-550.
Evans-White, M. A., R. S. Stelzer & G. A. Lamberti, 2005. Taxonomic and regional patterns in benthic macroinvertebrate elemental composition in streams. Freshwater Biology 50:1786-1799.
Fagan, W. F., E. Siemann, C. Mitter, R. F. Denno, A. F. Huberty, H. A. Woods & J. J. Elser, 2002. Nitrogen in insects: implications for trophic complexity and species diversification. The American Naturalist 160:784-802.
Feijoó, C., L. Leggieri, C. Ocón, I. Muñoz, A. R. Capítulo, A. Giorgi, D. Colautti, N. Ferreiro, M. Licursi, N. Gómez & S. Sabater, 2014. Stoichiometric homeostasis in the food web of a chronically nutrient-rich stream. Freshwater Science 33:820-831.
Frost, P. C. & J. J. Elser, 2002. Growth responses of littoral mayflies to the phosphorus content of their food. Ecology Letters 5:232-240.
Frost, P. C., M. A. Evans-White, Z. V. Finkel, T. C. Jensen & V. Matzek, 2005. Are you what you eat? Physiological constraints on organismal stoichiometry in an elementally imbalanced world. Oikos 109:18-28.
Frost, P. C., R. S. Stelzer, G. A. Lamberti & J. J. Elser, 2002. Ecological stoichiometry of trophic interactions in the benthos: understanding the role of C:N:P ratios in lentic and lotic habitats. Journal of North American Benthological Society 21:515-528.
Frost, P. C., S. E. Tank, M. A. Turner & J. J. Elser, 2003. Elemental composition of littoral invertebrates from oligotrophic and eutrophic Canadian lakes. Journal of North American Benthological Society 22:51-62.
Grandfield, L. & G. Ashman, 1984. Ewens Ponds Management Plan Draft. Department of Lands, South East Region.
Hill, W. R., J. Rinchard & S. Czesny, 2011. Light, nutrients and the fatty acid composition of stream periphyton. Freshwater Biology 56:1825-1836.
Karasov, W. H. & C. M. d. Rio, 2007. Physiological ecology: how animals process energy, nutrients, and toxins. Princeton University Press.
Kelly, J. R. & R. E. Scheibling, 2012. Fatty acids as dietary tracers in benthic food webs. Marine Ecology Progress Series 446:1-22.
Lauridsen, R. B., F. K. Edwards, M. J. Bowes, G. Woodward, A. G. Hildrew, A. T. Ibbotson & J. I. Jones, 2012. Consumer-resource elemental imbalances in a nutrient-rich stream. Freshwater Science 31:408-422.

Lauridsen, R. B., F. K. Edwards, W. F. Cross, G. Woodward, A. G. Hildrew & J. I. Jones, 2014. Consequences of inferring diet from feeding guilds when estimating and interpreting consumer-resource stoichiometry. Freshwater Biology 59:1497-1508.
Liess, A. & H. Hillebrand, 2005. Stoichiometric variation in C:N, C:P, and N:P ratios of littoral benthic invertebrates. Journal of the North American Benthological Society 24:256-269.
Lowe, A. T., A. W. E. Galloway, J. S. Yeung, M. N. Dethier & D. O. Duggins, 2014. Broad sampling and diverse biomarkers allow characterization of nearshore particulate organic matter. Oikos 123:1341-1354.
Martin-Creuzburg, D., E. Sperfeld & A. Wacker, 2009. Colimitation of a freshwater herbivore by sterols and polyunsaturated fatty acids. Proceedings of the Royal Society B 276:1805-1814.
Müller-Navarra, D. & W. Lampert, 1996. Seasonal patterns of food limitation in *Daphnia galeata*: separating food quantity and food quality effects. Journal of Plankton Research 18:1137-1157.
Müller-Navarra, D. C., 2008. Food web paradigms: the biochemical view on trophic interactions. International Review of Hydrobiology 93:489-505.
Napolitano, G. E., 1994. The relationship of lipids with light and chlorophyll measurements in freshwater algae and periphyton. Journal of Phycology 30:943-950.
Persson, J., M. T. Brett, T. Vrede & J. L. Ravet, 2007. Food quantity and quality regulation of trophic transfer between primary producers and a keystone grazer (Daphnia) in pelagic freshwater food webs. Oikos 116:1152-1163.
Persson, J., P. Fink, A. Goto, J. M. Hood, J. Jonas & S. Kato, 2010. To be or not to be what you eat: regulation of stoichiometric homeostasis among autotrophs and heterotrophs. Oikos 119:741-751.
Rigosi, A., Y. Liu, M. Shanafield & J. D. Brookes, 2015. Determining environmental risks to Ewens Ponds in the South East. Goyder Institute for Water Research Technical Report Series No. 15/19, Adelaide, South Australia.
Riley, L. A., M. F. Dybdahl & J. Robert O. Hall, 2008. Invasive species impact: asymmetric interactions between invasive and endemic freshwater snails. Journal of North American Benthological Society 27:509-520.
Sargent, J., L. McEvoy, A. Estevez, G. Bell, M. Bell, J. Henderson & D. Tocher, 1999. Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture 179:217-229.
Small, G. E. & C. M. Pringle, 2010. Deviation from strict homeostasis across multiple trophic levels in an invertebrate consumer assemblage exposed to high chronic phosphorus enrichment in a Neotropical stream. Oecologia 162:581-590.
Sterner, R. W. & J. J. Elser, 2002. Ecological stoiochiometry: The biology of elements from molecules to the biosphere. Princeton University Press.
Sterner, R. W. & D. O. Hessen, 1994. Algal nutrient limitation and the nutrition of aquatic herbivores. Annual Review of Ecology and Systematics 25:1-29.
Taipale, S., U. Strandberg, E. Peltomaa, A. W. E. Galloway, A. Ojala & M. T. Brett, 2013. Fatty acid composition as biomarkers of freshwater microalgae: analysis of 37 strains of microalgae in 22 genera and in seven classes. Aquatic Microbial Ecology 71:165-178.
Tsoi, W. Y., W. L. Hadwen & C. S. Fellows, 2011. Spatial and temporal variation in the ecological stoichiometry of aquatic organisms in an urban catchment. Journal of the North American Benthological Society 30:533-545.
Twining, C. W., J. T. Brenna, N. G. H. Jr. & A. S. Flecker, 2016. Highly unsaturated fatty acids in nature: what we know and what we need to learn. Oikos 125:749-760.

Vargas, M. A., H. Rodríguez, J. Moreno, H. Olivares, J. A. D. Campo, J. Rivas & M. G. Guerrero, 1998. Biochemical composition and fatty acid content of filamentous nitrogen-fixing cyanobacteria. Journal of Phycology 34:812-817.

Vinson, M. R. & M. A. Baker, 2008. Poor growth of rainbow trout fed New Zealand mud snails Potamopyrgus antipodarum. North American Journal of Fisheries Management 28:701-709.

Volk, C. & P. Kiffney, 2012. Comparison of fatty acids and elemental nutrients in periphyton, invertebrates, and cutthroat trout (*Oncorhynchus clarki*) in conifer and alder streams of western Washington state. Aquatic Ecology 46:85-99.

Vrede, T., T. Andersen & D. O. Hessen, 1999. Phosphorus distribution in three crustacean zooplankton species. Limnology and Oceanography 44:225-229.

Wood, C., 2011. Measurement and evalution of key groundwater discharge sites in the Lower South East of South Australia, DFW Technical Report 2011/14. Government of South Australia, through Department for Water, Adelaide.

Statement of Authorship

Title of Paper	Regulations of light and nutrients on growth and stoichiometry of periphyton and snail grazers
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ■ Unpublished and Unsubmitted work written in publication style
Publication Details	Prepared for submission to a journal

Principal Author

Name of Principal Author (Candidate)	Yang Liu		
Contribution to the paper	Designed the study, performed field experiments, conducted laboratory experiments, analysed the data, interpreted the results, wrote the manuscript		
Overall percentage (%)	80 %		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	04/10/2017

Co-author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution

Name of Co-author	Justin Brookes		
Contribution to the paper	Provided input in conceiving the study and design of experiments, supervised data analysis and interpretation, reviewed the manuscript		
Signature		Date	4 Oct 2017

Chapter 4 Effects of light and nutrients on growth and stoichiometry of periphyton and snail grazers

Abstract

Ecological stoichiometry posits that herbivore growth may be frequently limited by elemental nutrient contents rather than available energy due to commonly observed large stoichiometric dissimilarity between primary consumers and their food. The light : nutrient hypothesis describes how the relative availability of light and nutrients affects food quality and food quantity, thus constrains herbivore growth by low food quality or low food quantity in aquatic ecosystems. We tested predictions of this hypothesis by examining the effects of light and nutrients on periphyton and grazer growth and stoichiometry in mesocosm experiments. Two levels of light and two levels of nutrients were provided, forming total combination of four different conditions for periphyton and grazing snails (*Glyptophysa gibbosa*) in the laboratory. Periphyton had higher chlorophyll-a contents, ash-free dry mass and total C contents under high nutrient conditions, indicating relatively large quantities of periphyton available to snails driven by rich nutrient supplies. Periphyton elemental nutrient contents was significantly affected by nutrient conditions but not by light intensities. Measurements of stoichiometric ratios of periphyton dry mass can be used to describe quality of periphyton as food for snails, and periphyton C:P and N:P ratios differed between low and high nutrient conditions. The absence of any light effects on periphyton nutrient contents and elemental ratios may be due to insufficient manipulations of light to cause any substantial changes in algal nutrient contents in this study as the irradiances provided in treatments with low light were still higher than suggested saturation levels for periphyton photosynthesis. Snails had higher P contents and lower C:P ratios under low light conditions, reflecting a deviation from strict homeostasis. Snail growth rates were measured as changes in blotted wet weight and periphyton consumption rates were measured as changes in percentage cover of periphyton

on panels. Faster snail growth under high nutrient conditions may be due to reduced C:P imbalances between snails and periphyton, although higher N:P ratios in snails than periphyton indicated the growth of snails was more limited by N than P. Faster snail growth rates under low light conditions were related to higher consumptions rates of periphyton, and snails tend to graze more actively under dark environments. Snails did not exhibit active compensatory feeding when exposed to periphyton with lower quality under low nutrient conditions. Complex interactions between environmental factors and herbivores have the potential to interfere in the process of herbivores assimilating C and nutrients, leading to observations different from the predictions of ecological stoichiometry and light : nutrient hypothesis.

4.1 Introduction

It was traditionally assumed that the rate of supply of food determined the growth of herbivores (Hill et al. 2010; Liess and Lange 2011). Therefore, food quantity measurements such as biomass and chlorophyll-a content of primary producers have been widely used when assessing production of herbivores in aquatic ecosystems (Guo et al. 2016; Stelzer and Lamberti 2002). However, emerging research in the last few decades challenged the importance of food quantity to aquatic herbivore growth as much of the research focused on the role of food quality in mediating herbivore growth (Brett and Müller-Navarra 1997; Sterner and Hessen 1994).

The mineral limitation hypothesis suggested the production of herbivores with high nutrient demands was often limited by relatively scarce elemental nutrients rather than available energy in their food (Sterner and Hessen 1994). Elemental imbalances measure the stoichiometric dissimilarity of one or more elemental nutrients between the supply of resources and the need of consumers (Sterner and Elser 2002). Nutrient limitation of

herbivore growth was often predicted when elemental imbalances occurs in aquatic ecosystems (Evans-White et al. 2005; Frost and Elser 2002b; Stelzer and Lamberti 2002). Aquatic primary consumers frequently have lower carbon : nutrient ratios than their food, indicating a surplus of carbon (C) and inadequate supply of nutrients such as nitrogen (N) and phosphorus (P), although this C is needed as an energy source (Cross et al. 2003; Fink et al. 2006; Plath and Boersma 2001).

According to the light : nutrient hypothesis (LNH), not only nutrient availability but also light intensity determine algal nutrient content and elemental ratios, and a negative effect of light on algal C : nutrients ratios is hypothesized (Sterner et al. 1997). Algae receiving high light intensities relative to nutrients would have high C : nutrient ratios, while algae receiving low supplies of light relative to nutrients would have low C : nutrients ratios (Sterner and Elser 2002; Sterner et al. 1997). C fixation is predicted to proceed more rapidly than nutrient uptake when irradiance is elevated, leading to the relative depletion of nutrients by C-rich compounds in algae cells (Hill et al. 2010). In addition, the requirements for N (and possibly P) is reduced under higher irradiance, possibly because of the decreased production of light-harvesting compounds such as chlorophyll which have a high N content (Healey 1985).

The combined effects of food quantity and food quality make the relationship between herbivore growth and light : nutrient ratios to be unimodal (Hill et al. 2010). Herbivore growth is C-limited under very low light because of the very low food quantity (Urabe and Sterner 1996). Herbivore growth is then expected to respond positively to increasing light at first, because increasing light intensity would boost algae C fixation and enhance primary production, increasing the quantity of food supplied to herbivores (Hill et al. 2010). Herbivore growth shifts to be nutrient-limited when light intensity passes certain level and limitation of light is relieved (Hessen et al. 2002; Urabe and Sterner 1996). Herbivore growth is expected to respond negatively to increasing light, because increasing light intensity would

raise algal C : nutrients ratios, enlarging the elemental imbalance and decreasing the food quality.

The LNH has been proven to be able to predict patterns of primary production and herbivore growth change with different light levels and nutrient conditions in laboratory cultures (Hessen et al. 2002), lakes (Qin et al. 2007; Urabe et al. 2002), reservoirs (Dickman et al. 2006) and streams (Ohta et al. 2011). However, most of the empirical evidences supporting the prediction of the LNH is derived from experiments on species of freshwater crustacean zooplankton consuming abundant phytoplankton with highly flexible stoichiometric ratios in pelagic habitats. Freshwater benthic habitats have distinct primary producers and herbivores from those of the pelagic habitats as well as more extreme condition with regard to environmental factors, thus relationship between light and nutrients supply and macroinvertebrate growth in benthic habitats may significantly differ from the predictions of LNH.

Periphyton, the commonly considered main food source for benthic herbivores, contained not only attached algae but also detritus and associated microbes (Stelzer and Lamberti 2002). Katechakis et al. (2005) indicated that predictions of the LNH may not work for systems where herbivores have access to phototrophic and mixotrophic algae food resources. This is because the biomass and stoichiometry of mixotrophic algae would be affected by alterations in the supply of light and nutrients as mixotrophic algae have the ability to compensate for light or nutrient deficiency by heterotrophic nutrition (Katechakis et al. 2005). Shifts in the relative abundance between different periphyton components with changing light and nutrient conditions could cause changes in periphyton stoichiometry different from the expectation of LNH (Liess and Lange 2011). Rowland et al. (2015) reported that the response of phytoplankton to nutrient manipulations was more consistent with the LNH than the response of periphyton as periphyton C : nutrient ratios only decreased with nutrient additions at low

light. They suggested the no response of periphyton C:P to nutrient additions may be explained by compositional shift in the periphyton community, with a higher proportion of autotrophic species with higher C:P, fungi or bacterial biomass in biofilms (Rowland et al. 2015). Hall et al. (2007) revealed that the LNH pattern only emerged in the presence of crustacean grazers, and they addressed the importance of grazing for the LNH.

Application of the LNH may be only valid under a limited set of conditions, but environmental factors in benthic habitats can be much more variable and located within a much wider range than those in pelagic habitats. The negative effects of light on algal C:P ratios may only be evident in P-deprived environments, because benthic algae with high availability of nutrients are less likely to experience nutrient limitation and thus could be less subjected to the diluting effects of much more fixed C by photosynthesis under high light (Fanta et al. 2010; Liess et al. 2009; Sanches et al. 2011). Similarly, researchers failed to find significant effects of shaded light on benthic algae C:P ratios in shallow, well-illuminated littoral environments of lakes with very low P concentrations where the supply of light relative to P was very high (Frost and Elser 2002a). Furthermore, algal phosphorus content was previously reported to be not significantly affected by light even in streams where phosphorus concentrations were apparently growth-limiting in several studies (Hill and Fanta 2008; Hill et al. 2009). It was not quite clear why that was the case, but they postulated that amount of carbon available to dilute phosphorus within algal cells under high light was possibly limited as photosynthesis was limited by depleted inorganic carbon resulting from high production within the periphyton community matrix (Hill and Fanta 2008). Hill et al. (2010) found snail growth was not affected by light-induced variation in the stoichiometry of periphyton, but was mostly related to primary production in two oligotrophic streams. They attributed the nearly linear response of snail growth to increasing light : nutrient ratios to exploitative competition between individuals for a limited food resource (Hill et al. 2010).

Herbivores have developed special behavioural or evolutionary alternations to deal with low quality of the food resources (Elser et al. 2000). Strategies adopted by herbivores to mitigate adverse effects of low-quality food include grazing selectively on food with higher nutrient content and increasing consumption rates to compensate for shortage of nutrients (Fink and Elert 2006; Hill et al. 2010; Liess 2014; Stelzer and Lamberti 2002). Stelzer and Lamberti (2002) found the snail grazer, *Elimia*, had much higher growth feeding on periphyton of high quality than low quality when offered low quantity of food, but the growth of *Elimia* was not affected by food quality when offered high quantity of periphyton. *Elimia* was relieved from severe nutrient limitation by consuming more periphyton when offered a high quantity of food, although this reaction of *Elimia* was passive as they didn't increase removal rates to compensate for the low food quality when the same amount of each food type was offered (Stelzer and Lamberti 2002). Benthic herbivores can have lower body P content than crustacean zooplanktons, thus may exhibit higher tolerance of low food quality than crustacean zooplankton (Frost et al. 2003). Ecologists also proposed that herbivores have involved a lower dependence on N due to the chronic existence of low dietary N (Fagan et al. 2002).

In this study, we conducted serial mesocosm experiments to examine effects of light and nutrients on periphyton nutrient content and elemental ratios and their consequent effect on invertebrate grazer growth. Periphyton was cultured in water with different nutrient concentrations or under different light intensities, and this periphyton was grazed by herbivorous snails. We determined whether the biomass, nutrient content and stoichiometry was affected by the relative supply of light and nutrients. We also measured the consumption rate of periphyton by snails, the growth rate and stoichiometry of snail in different treatments to detect the influence of periphyton quantity and quality on snail performance. The usefulness of LNH in predicting the relationship between light and nutrient conditions and

algal and grazer growth was then explicitly tested in benthic habitats. We also examined if snails adopted any strategies to deal with possible food quality limitation.

4. 2 Methods

4.2.1 Field site

Ewens Ponds is located in the lower southeast of South Australia, approximately 30 km south of Mount Gambier (38°01′36″S, 140°47′26″E). It consists of three karst wetlands connected by channels, which in turn feed into Eight Miles Creek discharging to the sea. Ewens Ponds is a macrophyte-dominated, clear-water ecosystem of nationally recognized importance supporting endangered species such as Ewens pygmy perch (*Nannoperca variegata*) and Glenelg spiny crayfish (*Euastacus bispinosus*). Ewens Ponds is highly dependent on groundwater source with more than 95% of its water coming into the system through crevices in limestones making up the wall and bottom of pond basin. Changes in land use in the surrounding area from natural peatlands to managed pastures and croplands in the past decades have contributed to the very high nitrate concentrations in pond water. However, soluble reactive phosphorus (SRP) concentrations in pond water are relatively low, probably due to the unique geological property of the ponds as they are actually limestone sinkholes. Recent observations of algal blooms and continuing increases in N and P concentrations indicate that the ecosystem is highly vulnerable and at risk of undergoing a regime shift towards filamentous or benthic algae domination.

4.2.2 Determination of experimental conditions

Most of the groundwater-fed freshwater ecosystems in the southeast of South Australia are already highly enriched with nitrogen, and the risk of eutrophication is mainly related to gradual increase of phosphorus (Rigosi et al. 2015). Nitrogen and phosphorus concentrations in low nutrient concentration treatments were set at 0.5 mg L^{-1} and 0.02 mg L^{-1} respectively

in the main and parallel mesocosm experiments in this study, and these values were close to observed lowest total nitrogen and phosphorus concentrations in wetlands from the southeast of South Australia (Rigosi et al. 2015). We found the density of some of the grazer species, such as invasive New Zealand mud snail, *Potamopyrgus antipodarum*, can be as high as 8950 individuals m^{-2} (unpublished data) in Ewens Ponds. The density of *G. gibbosa* ranged from 540 to 1640 individuals m^{-2} during the survey of benthic macroinvertebrates in Ewens Ponds in December, 2015 (unpublished data). Considering much more complex structure and larger surface areas provided by macrophytes in the ponds, we set the target grazer density in the main and parallel mesocosm experiments at 200 individuals m^{-2}. The light intensity of full sunlight was around 600 µmol m^{-2} s^{-1} at water surface on sunny days in Ewens Ponds, and light intensity was around 100 µmol m^{-2} s^{-1} at the depth of 8 m, which is close to the maximum depth of possible periphyton distribution range. We determined the light intensity in shaded treatments was around 120 µmol m^{-2} s^{-1} because light intensity below this value would be very unlikely for periphyton in Ewens Ponds , but this value still exceeded the estimated saturation irradiance of 100 µmol m^{-2} s^{-1} for photosynthesis of most benthic algal assemblages (Hill 1996).

4.2.3 Mesocosm Experiments

Native snail, *Glyptophysa gibbosa*, commonly found on macrophytes and in filamentous algae mats in groundwater-fed freshwater ponds in the southeast of South Australia, were used as grazers in this study. Snails were collected from Ewens Ponds and were acclimated to ambient aquaria conditions for 3 weeks prior to exposing them to the experimental conditions. The grazer density in this study was set at 200 individuals m^{-2}. Two randomly picked snails within the length range from 8 to 12 mm were released into each experimental unit on the first day of all mesocosm experiments. The snails were restricted within cages (10 × 10 × 10 cm) in aquaria with the floor totally covered by blocks or panels, and cages were

cleaned every day to remove any periphyton grown on walls and roofs, ensuring that snails only had access to periphyton grown on blocks and panels.

A preliminary mesocosm experiment to evaluate the potential growth of periphyton under enriched nutrients and shaded light with or without grazing was conducted at the greenhouse in the University of Adelaide in December, 2014. Wood blocks (10 × 10 cm) were inoculated with periphyton by submerging them under water at 5 cm depth *in situ* in Ewens Ponds for two months between October and December, 2015. The blocks were found to be covered with a thin layer of periphyton after inoculation in the ponds and considered as initial state for the culture and returned to the laboratory for further experimentation. All the blocks in initial state were carefully checked and those having similar periphyton development were selected and used in the culture. The blocks used in the culture were then divided into nine groups, with one group immediately sampled for measurement of variables in initial state and the rest eight groups subjected to different treatments. The blocks in treatments were placed in 5L plastic buckets filled with water collected from Ewens Ponds and cultured for 21 days. The eight treatments were fractional combinations of two water nutrient conditions (with or without nutrient addition), two ambient light levels (with or without screen shade) and two grazer activity conditions (with or without snail grazing). Treatment without nutrient addition used water collected from Ewens Ponds, and treatment with nutrient addition used water enriched with Osmocote Plus controlled release fertiliser. Treatment without light shade received ambient sunlight irradiance, and treatment with light shade were covered by mirror window screen. Two *G. gibbosa* snails within the length range from 8 to 12 mm were released into buckets in treatments with invertebrate grazing. All the buckets went through natural light/dark cycles (14/10 hours), and glasshouse temperature was maintained at around 20 °C. Each of the eight treatments was replicated five times and all experimental units were randomly distributed on the bench.

During the preliminary mesocosm experiment it was found that in a treatment without nutrient addition all snails died after 7 days of the initiation of the experiment because periphyton biomass was too low to provide sufficient food for the survival of snails. So culture of periphyton in treatments without nutrient addition had to be terminated after 7 days, while culture of periphyton continued in treatments with nutrient addition. Results of the preliminary mesocosm experiment showed that the chlorophyll-a content was 7.22 mg m^{-2} on average in treatments without nutrient addition. From visual perspective, this much biomass was only a very thin layer of periphyton, with a light transmission rate higher than 90% through periphyton. In order to get enough periphyton cover with reasonable thickness on panels for snail grazing as well as sampling and measurements, we conducted pilot trails and determined the P concentration to be at least 0.02 mg L^{-1} for acceptable periphyton growth on panels, although this P concentration was considered as eutrophic in many natural aquatic ecosystems. The target N and P concentrations used in the main and parallel mesocosm experiments were then chosen by taking the results of pilot trials and values of N and P concentrations in many wetlands from the southeast of South Australia in a previous study into consideration (Rigosi et al. 2015).

To determine the effects of light and nutrients on periphyton quantity and quality with snail grazing, a main experiment was conducted at the greenhouse in the University of Adelaide in February, 2015. Periphyton was grown under different water nutrient concentrations and ambient light levels in 1L plastic containers with plastic panels (10 cm × 10 cm) used as substrate for periphyton growth. Panels were put in large incubation containers for periphyton development with combinations of water nutrient concentrations and ambient light levels as four treatments in a matrix design. Treatments comprised high nutrient high light (N, 2.5 mg L^{-1}; P, 0.1 mg L^{-1}; light, 100%), high nutrient low light (N, 2.5 mg L^{-1}; P, 0.1 mg L^{-1}; light, 20%), low nutrient high light (N, 0.5 mg L^{-1}; P, 0.02 mg L^{-1}; light, 100%), low nutrient low

light (N, 0.5 mg L^{-1}; P, 0.02 mg L^{-1}; light, 20%). N was added as $NaNO_3$ and P as KH_2PO_4. Light was a percent of the ambient incoming solar radiation which is in the Austral summer reaches a midday intensity of 2000 µmol m^{-2} s^{-1}. Nutrients other than N and P were provided by adding stock solutions following the formula of BG-11 algae culture media (Stanier et al. 1971). The incoming light intensity was varied by placing shade cloth over the aquaria. Periphyton inoculation was achieved by submersing wooden blocks in Ewens Ponds water for two months in the incubation containers. When periphyton developed on all panels and the percentages of periphyton cover of panels were similar and appropriate, the panels were transferred to 1 L aquaria with the same water nutrient concentrations and under the same light levels to initiate the main mesocosm experiment. Two *G. gibbosa* snails within the length range from 8 to 12 mm were released into aquaria in all treatments. Water in aquaria was replaced every day to minimize the fluctuation of water nutrient concentrations. The experiment lasted 21 days and all the incubation containers and experimental aquaria were placed in the greenhouse receiving natural light/dark cycles (14/10 hours). Each of the four treatments was replicated by five times and all experimental aquaria were randomly distributed on the bench.

To assess the impact of light and nutrients on periphyton removal by snail grazers, a parallel mesocosm experiment was conducted at the greenhouse in the University of Adelaide in February, 2015. Panels with appropriate percentage of periphyton cover from incubation containers were transferred to 1 L aquaria. Beside the four treatments having snails in cages, another four treatments without snails in cages were added. All the experimental units in eight treatments were kept for the same time period under the same conditions as used in the main mesocosm experiment.

4.2.4 Sample collection

Periphyton material on the up-facing side of treatment blocks was carefully scraped using a steel scraper at the end of the preliminary mesocosm experiment. The scrapped periphyton material was washed into 50 mL deionized water and formed periphyton slurry samples. Periphyton material on panels in all treatments was carefully scrapped using a surgical blade at the end of the main mesocosm experiment. The scrapped periphyton material was also washed into 50 mL deionized water and formed periphyton slurry samples. Snails in all treatments were caught and kept in small vessels for one night to allow for gut clearance at the end of the main mesocosm experiment. Percentage of periphyton cover on panels and snail blotted weight mass (BWM) were measured at the beginning and end of the parallel mesocosm experiment.

4.2.5 Laboratory analysis

Periphyton slurry sample from the preliminary mesocosm experiment was filtered on Whatman 47 mm GF/F filter and filter was frozen and used for chlorophyll-*a* determination. Each periphyton slurry sample from the main mesocosm experiment was divided into three subsamples. The first subsample was filtered on 47 mm Whatman GF/F filter and filter was frozen and used for chlorophyll-*a* determination. The second subsample was transferred into pre-weighed crucibles and used for dry weight and ash-free dry mass (AFDM) determination. The third subsample was transferred into plastic tubes, frozen and used for C, N and P content analysis. Snail samples were removed of shells, frozen and used for C, N and P content analysis.

Chlorophyll a concentrations were analysed using the hot ethanol extraction method adapted from Sartory and Grobbelaar (1984). The samples on filters were extracted in 90% ethanol at 78 °C for 5 minutes and at room temperature for 24 hours. Chlorophyll-a concentrations in solvent were measured using a spectrophotometer (Libra S22, Biochrom, Cambridge, UK).

Dry weights of periphyton subsamples were measured by firstly drying the samples in an oven at 60 °C in crucibles and weighing until constant weight was maintained. Oven-dried periphyton samples and crucibles were then combusted in a muffle furnace at 500 °C and reweighed to calculate AFDM. Periphyton subsamples and snail samples for C, N and P content analysis were dried in an oven at 55 °C and ground to fine powder with mortar and pestle or grinder. Periphyton and snail C and N content were analysed using an elemental analyser (Perkin Elmer 2400 Series II, Perkin Elmer, Waltham, MA, USA) at Sprigg Geobiology Centre in the University of Adelaide. Periphyton and snail P content were analysed using ascorbic acid method with a spectrophotometer (Libra S22, Biochrom, Cambridge, UK) after hot acid digestion with hydrogen peroxide (APHA 2005). Periphyton total C was calculated by multiplying periphyton dry weight by the percentage of C.

Periphyton growth rate in the preliminary mesocosm experiment was calculated based on chlorophyll-a concentration ($\mu_{chl\text{-}a}$) using the equation:

$$\mu_{chl\text{-}a} = \ln(X_t/X_0)/t$$

where X_t and X_0 were final chlorophyll-a concentrations in treatments and initial chlorophyll-a concentrations respectively, and t was duration time of the experiment. Periphyton consumption rates by grazers were determined as the percentage cover of consumed periphyton in the parallel mesocosm experiment divided by the number of grazers and days. Pilot trials were conducted for choosing panels with appropriate percentages of preiphyton cover, ensuring periphyton cover on panels with grazers was greater than 0% and periphyton cover on panels without grazers was smaller than 100% during the parallel mesocosm experiment. Percentage of periphyton cover on panels in treatments with grazers in cages was recorded at the beginning and end of the experiment to quantify periphyton removal rates by grazers. Percentage of periphyton cover on panels in treatments without grazers in cages was

also recorded at the beginning and end of the experiment to quantify periphyton accrual rates under different combinations of nutrient condition and light level. Percentage of periphyton cover was measured using a quadrat the same dimensions as the panel (10 cm × 10 cm), divided into 100 small grids. Periphyton consumption rates by grazers were then calculated by combining percentage of periphyton cover as removal from panels in treatments with grazers in cages and percentage of periphyton cover as accrual on panels in treatments without grazers in cages. Snail growth rate in the parallel mesocosm experiment was calculated as changes in snail BWM divided by the number of days. BWM of snails were measured by weighing them after blotting them with paper towels for 1 minute.

4.2.6 Data analysis

The effects of nutrient addition, light shade and grazer presence on periphyton chlorophyll-a contents in the preliminary mesocosm experiment were analysed using three-way analysis of variance (ANOVA). The effects of nutrient concentrations and light intensities on measures of periphyton quantity and quality, and snail stoichiometry were analysed using two-way ANOVA in the main mesocosm experiment. The effects of nutrient concentrations and light intensities on final percentage of periphyton cover in grazed and non-grazed treatments, periphyton consumption rates and snail growth rates were analysed using two-way ANOVA in the parallel mesocosm experiment. Additional one-way ANOVAs were performed on periphyton chlorophyll-a contents, periphyton and snail C, N and P contents and C:N, C:P and N:P ratios. Significance was set at $\alpha < 0.05$ and where significant effects of factors were detected, Tukey's post-hoc tests were used to determine the differences between treatments. Pearson's correlations were used to relate snail growth rate with periphyton food quantity and quality variables and periphyton consumption rate. Statistics were performed using SPSS 19.0 (IBM, Armonk, NY, USA). Linear fitting parameters were estimated using OriginPro 9.0 (OriginLab, Northampton, MA, USA).

4.3 Results

4.3.1 Periphyton performance in the preliminary mesocosm experiment

Periphyton chlorophyll-a contents were significantly higher in treatments with nutrient addition than treatments without nutrient addition, whereas light and grazing had no significant effect on periphyton chlorophyll-a contents (nutrient, $p < 0.001$). At the end of the experiment, periphyton chlorophyll-a contents in treatments with nutrient addition were nearly 100 mg m^{-2} and more than ten times higher than chlorophyll-a contents in treatments without nutrient addition (Table 4-1). The growth rates of periphyton were on average 0.017 and 0.112 d^{-1} in treatments without nutrient addition and treatments with nutrient addition (Table 4-1).

Table 4-1 Periphyton chlorophyll-a contents per unit block area and growth rates in different treatments in the preliminary mesocosm experiment.

Treatment	Periphyton chl-a content (mg m^{-2})	Periphyton growth rate (d^{-1})
Initial	4.43 ± 3.19	-
N-L-G-	8.27 ± 7.20	0.022
N-L-G+	4.71 ± 2.62	0.002
N-L+G-	8.29 ± 0.70	0.022
N-L+G+	7.59 ± 0.51	0.019
N+L-G+	106.47 ± 0.62	0.114
N+L-G-	101.53 ± 8.05	0.112
N+L+G-	98.91 ± 17.58	0.111
N+L+G+	96.89 ± 15.00	0.110

Periphyton chl-a contents are presented as means ± standard error. N-L-G-, no nutrient addition no light shade no invertebrate grazing; N-L-G+, no nutrient addition no light shade invertebrate grazing; N-L+G-, no nutrient addition light shade no invertebrate grazing; N-L+G+, no nutrient addition light shade invertebrate grazing; N+L-G-, nutrient addition no light shade no invertebrate grazing; N+L-G+, nutrient addition no light shade invertebrate grazing; N+L+G-, nutrient addition light shade no invertebrate grazing; N+L+G+, nutrient addition light shade invertebrate grazing.

4.3.2 Periphyton biomass and chl-a:C

Periphyton chlorophyll-a contents per unit panel area differed significantly between high and low nutrient treatments (Table 4-2; Figure 4-1a), but did not change significantly with light intensity. Periphyton AFDM and total C per unit panel area differed significantly between high and low nutrient treatments and between high and low light treatments (Table 4-2; Figure 4-1b, c). Periphyton chl-a:C ratios differed significantly between high and low nutrient treatments (Table 4-2; Figure 4-1d), but did not change significantly with light intensity.

Table 4-2 Results of ANOVAs for the effects of light and nutrient on periphyton biomass variables.

Periphyton biomass variables	Chl-a (mg m^{-2})		AFDM (mg m^{-2})		Periphyton total C (mg m^{-2})		Chl-a:C	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Nutrient	9.506	**0.007**	78.165	**<0.001**	90.329	**<0.001**	6.274	**0.023**
Light	3.365	0.085	21.876	**<0.001**	25.591	**<0.001**	2.093	0.167
Nutrient × Light	0.569	0.462	0.052	0.823	0.006	0.939	1.956	0.181

AFDM, ash-free dry mass. Figures in bold indicate significance at $p < 0.05$.

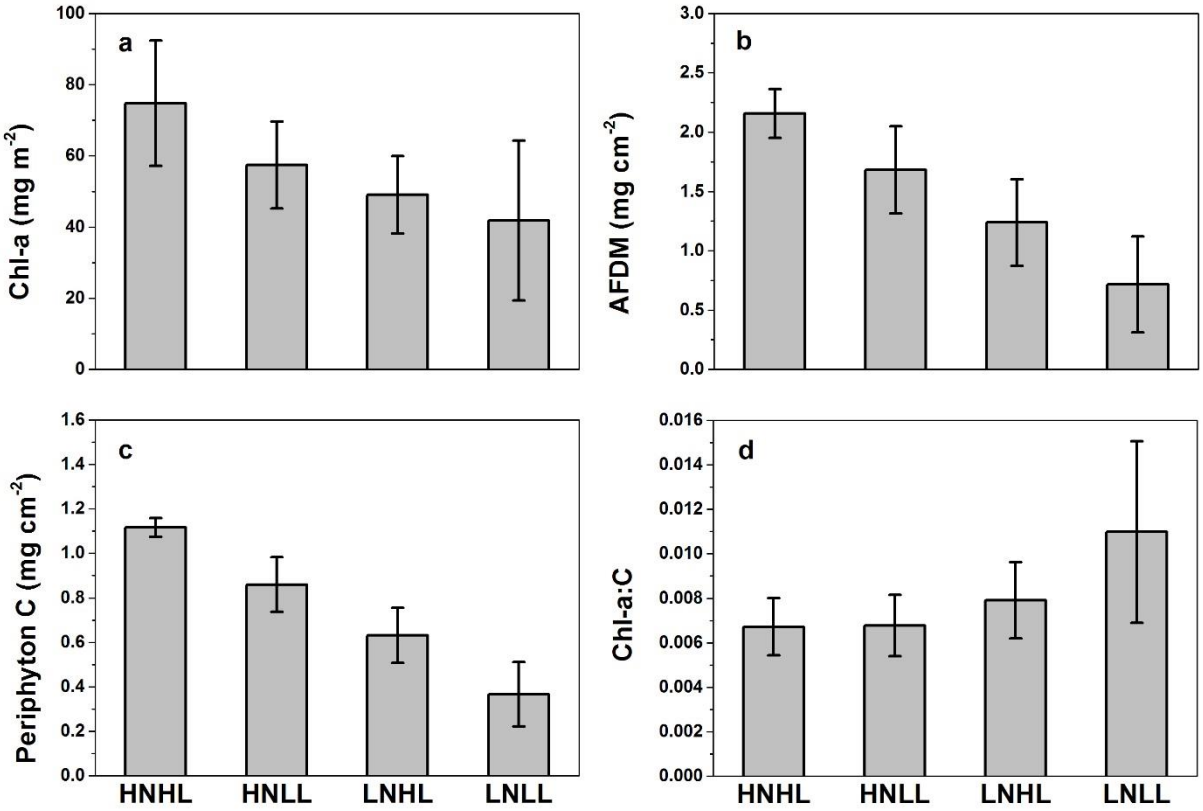


Figure 4-1 Periphyton chlorophyll-a contents per unit area (a), ash-free dry mass (AFDM) (b), total C (c), and chlorophyll-a:C (d) in four treatments (HNHL, high nutrient high light; HNLL, high nutrient low light; LNHL, low nutrient high light; LNLL, low nutrient low light) at the end of the main mesocosm experiment. Error bars represent ± 1 standard deviation.

4.3.3 Periphyton and snail C, N and P contents and stoichiometry

Periphyton C, N and P content differed significantly between high and low nutrient treatments (Table 4-3, Figure 4-2a-c). Snail P content differed significantly between high and low light treatments (Table 4-3, Figure 4-2c). Snail C, N and P contents were significantly higher than periphyton C, N and P content in all treatments except P content in high nutrient high light treatment (Figure 4-2a-c). C:P and N:P ratios of periphyton were significantly affected by nutrient conditions of treatments (Table 4-3, Figure 4-3b, c). Light levels had

significant effect on C:P ratios of snails (Table 4-3, Figure 4-3b). C:N ratios of snails were significantly lower than that of periphyton in all treatments (Figure 4-3a), while N:P ratios of snails were significantly higher than that of periphyton in all treatments (Figure 4-3c).

Table 4-3 Results of analysis of variances (ANOVAs) for the effects of light and nutrient on periphyton and snail C, N and P contents and C:N, C:P and N:P molar ratios and on periphyton consumption rates and snail growth rates.

Dependent variable		Source	*F*	*p*
Periphyton	C (%)	Nutrient	5.173	**0.037**
		Light	1.447	0.246
		Nutrient × Light	0.087	0.772
	N (%)	Nutrient	5.449	**0.033**
		Light	0.114	0.740
		Nutrient × Light	0.006	0.940
	P (%)	Nutrient	36.327	**<0.001**
		Light	2.230	0.155
		Nutrient × Light	0.306	0.588
	C:N	Nutrient	2.567	0.129
		Light	0.133	0.720
		Nutrient × Light	0.087	0.771
	C:P	Nutrient	14.938	**0.001**
		Light	1.082	0.314
		Nutrient × Light	0.165	0.690
	N:P	Nutrient	8.738	**0.009**
		Light	0.693	0.417
		Nutrient × Light	0.019	0.893
Snail	C (%)	Nutrient	0.030	0.865
		Light	0.847	0.371
		Nutrient × Light	0.637	0.436
	N (%)	Nutrient	0.033	0.859
		Light	0.042	0.840
		Nutrient × Light	0.398	0.537
	P (%)	Nutrient	1.819	0.196
		Light	9.249	**0.008**
		Nutrient × Light	1.087	0.313
	C:N	Nutrient	0.055	0.817
		Light	0.021	0.887
		Nutrient × Light	0.051	0.824
	C:P	Nutrient	1.469	0.243
		Light	7.894	**0.013**
		Nutrient × Light	1.577	0.227
	N:P	Nutrient	0.983	0.336
		Light	3.335	0.087
		Nutrient × Light	1.240	0.282
Periphyton consumption (% grazer^{-1} d^{-1})		Nutrient	2.527	0.131
		Light	158.639	**<0.001**
		Nutrient × Light	0.015	0.904
Snail growth (mg d^{-1})		Nutrient	17.653	**0.001**
		Light	4.552	**0.049**
		Nutrient × Light	1.984	0.178

Figures in bold indicate significance at $p < 0.05$.

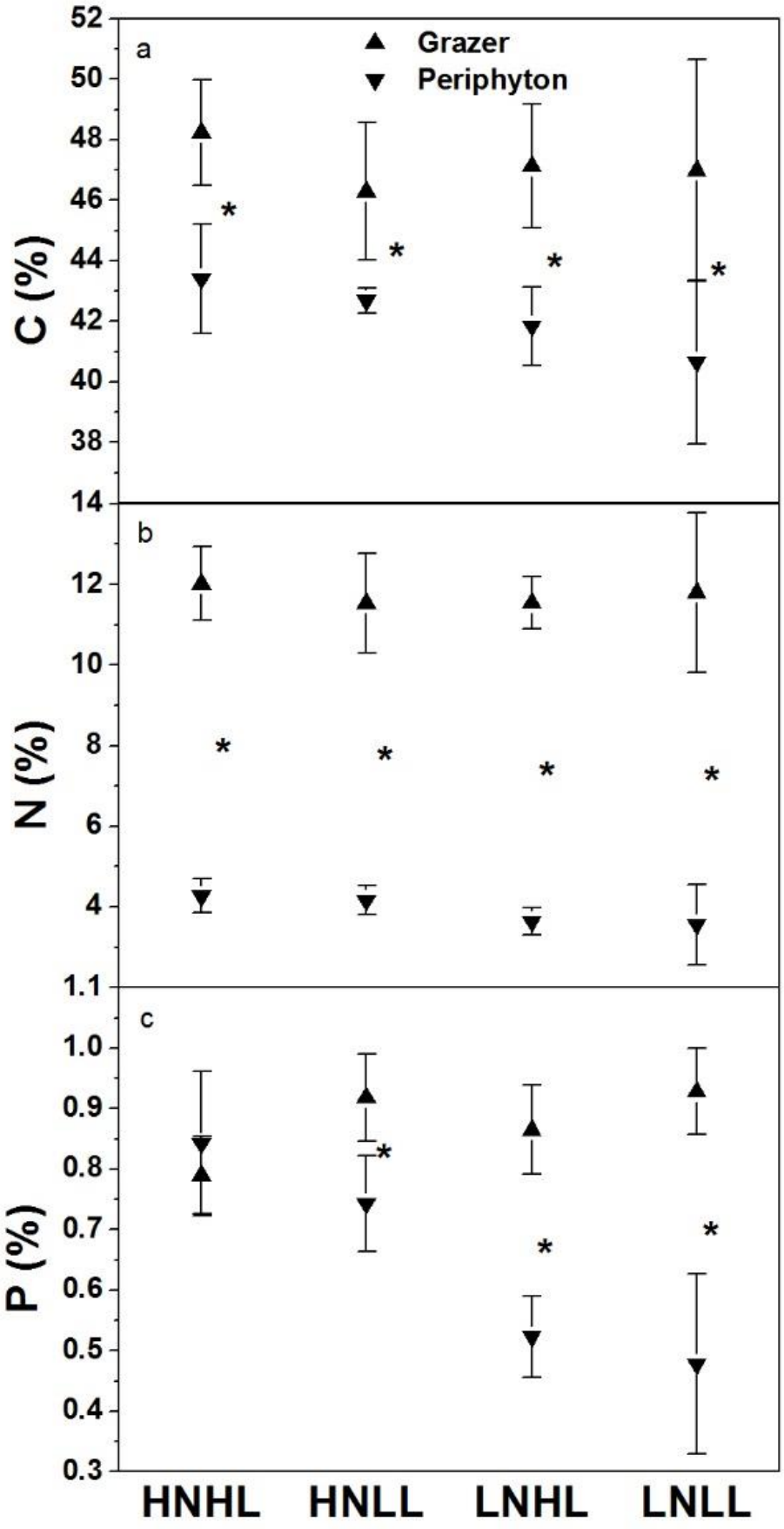

a
Grazer
Periphyton
C (%)
52
50
48
46
44
42
40
38
b
N (%)
14
12
10
8
6
4
c
P (%)
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
HNHL
HNLL
LNHL
LNLL

Figure 4-2 Elemental composition of C (a), N (b), and P (c) expressed as percentage dry weight of grazer and periphyton in four treatments (HNHL, high nutrient high light; HNLL, high nutrient low light; LNHL, low nutrient high light; LNLL, low nutrient low light) at the end of the main mesocosm experiment. Asterisks indicate significant differences between grazer and periphyton according to Tukey's post-hoc tests at $p < 0.05$. Error bars represent ± 1 standard deviation.

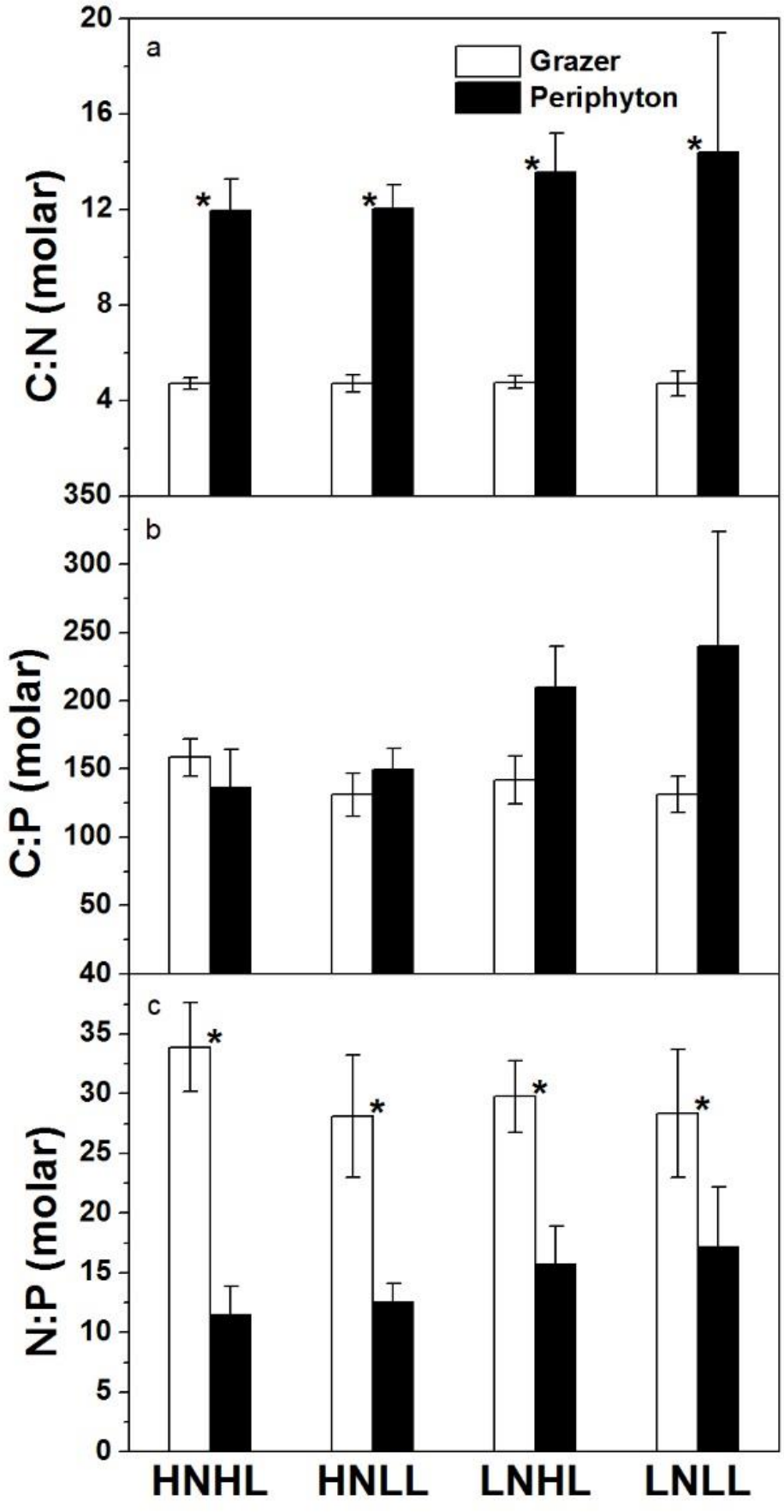

a
Grazer
Periphyton
C:N (molar)
20
16
12
8
4
350
b
C:P (molar)
300
250
200
150
100
50
40
c
N:P (molar)
35
30
25
20
15
10
5
0
HNHL
HNLL
LNHL
LNLL

Figure 4-3 C:N (a), C:P (b), and N:P (c) molar ratios of grazer and periphyton in four treatments (HNHL, high nutrient high light; HNLL, high nutrient low light; LNHL, low nutrient high light; LNLL, low nutrient low light) at the end of the main mesocosm experiment. Asterisks indicate significant differences between grazer and periphyton according to Tukey's post-hoc tests at $p < 0.05$. Error bars represent ± 1 standard deviation.

4.3.4 Periphyton consumption by snail and snail growth

In the presence of grazers, significantly higher final percentages of periphyton cover were found in treatments with high nutrient compared to treatments with low nutrient, the same pattern was also found when comparing final percentages of periphyton cover in treatments with high and low light (nutrient, $p < 0.001$; light, $p < 0.001$, Figure 4-4). In the absence of grazers, significantly higher final percentages of periphyton cover were found in treatments with high nutrient compared to treatments with low nutrient (nutrient, $p < 0.001$, Figure 4-4). Periphyton consumption rates by snails were significantly affected by light level (Table 4-3, Figure 4-5a). There were significant effects of nutrient conditions and light levels on snail BWM growth rates (Table 4-3, Figure 4-5b). Snail growth rates was negatively related to periphyton chl-a:C ratios ($p < 0.05$, $r^2 = 0.183$, Figure 4-6a) but positively related to periphyton consumption rates ($p < 0.05$, $r^2 = 0.191$, Figure 4-6b).

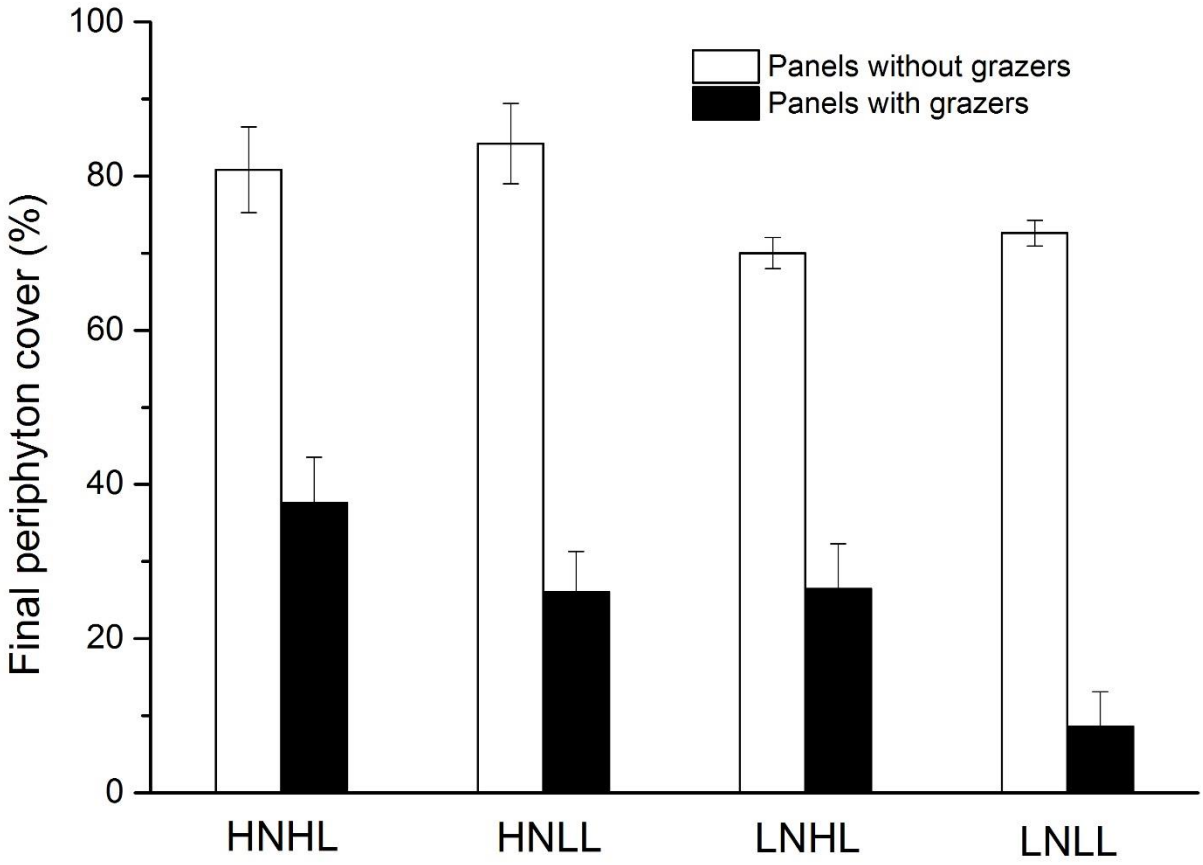


Figure 4-4 Final percentage of periphyton cover in four treatments (HNHL, high nutrient high light; HNLL, high nutrient low light; LNHL, low nutrient high light; LNLL, low nutrient low light) at the end of the parallel mesocosm experiment. Error bars represent ± 1 standard deviation.

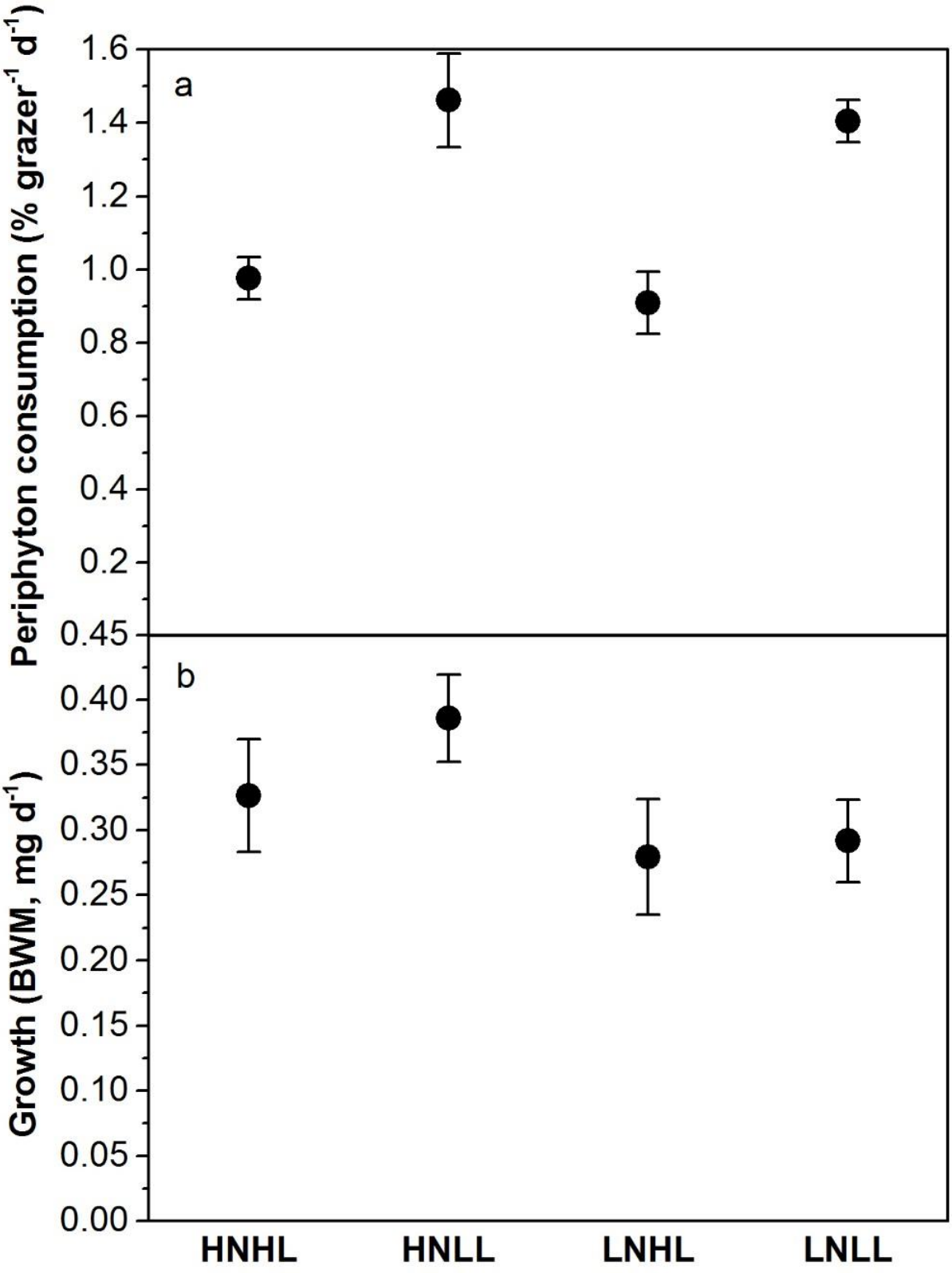
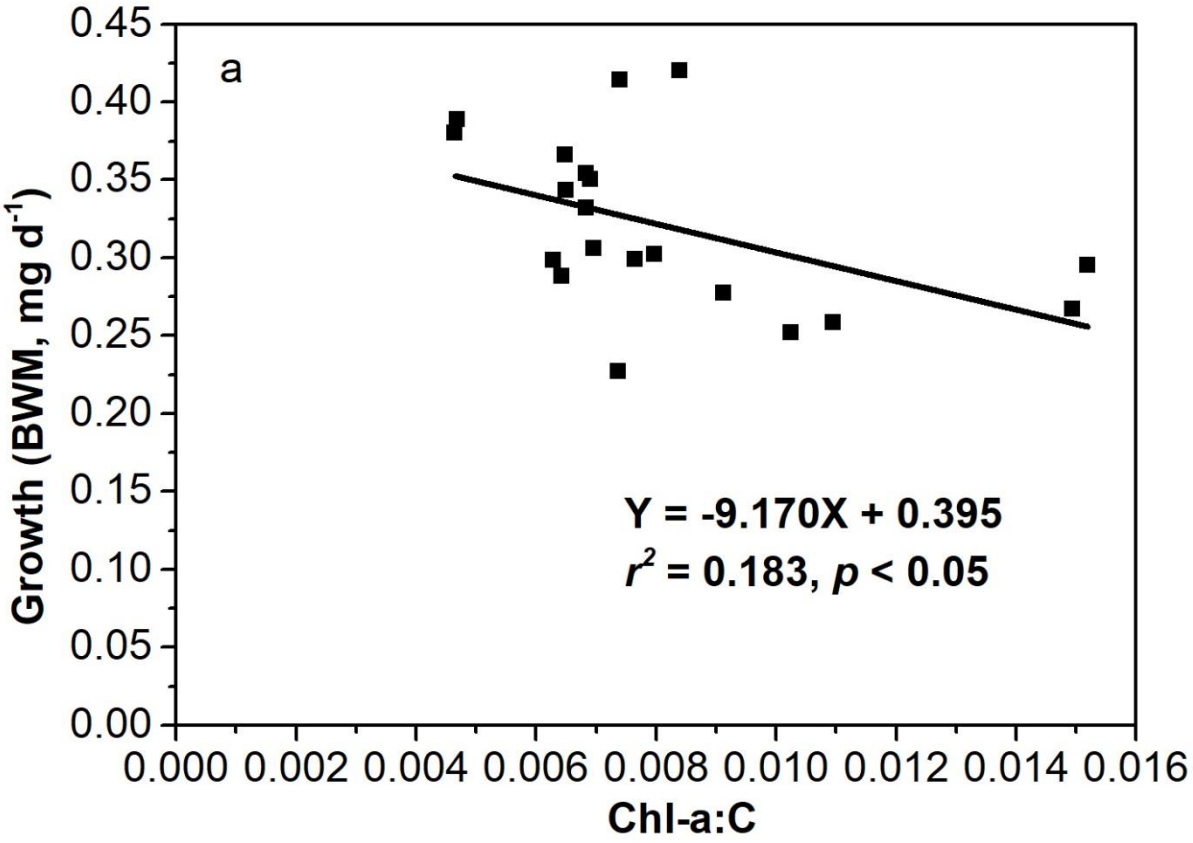

a
b
Periphyton consumption (% grazer^{-1} d^{-1})
Growth (BWM, mg d^{-1})
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
HNHL
HNLL
LNHL
LNLL

Figure 4-5 Periphyton consumption rate (% cover per grazer per day) (a) and snail growth rate (blotted weight mass change per day) (b) in four treatments (HNHL, high nutrient high light; HNLL, high nutrient low light; LNHL, low nutrient high light, LNLL, low nutrient low light) during the parallel mesocosm experiment. Error bars represent ± 1 standard deviation.


a
Growth (BWM, mg d-1)
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.000 0.002 0.004 0.006 0.008 0.010 0.012 0.014 0.016
Chl-a:C
Y = -9.170X + 0.395
r2 = 0.183, p < 0.05

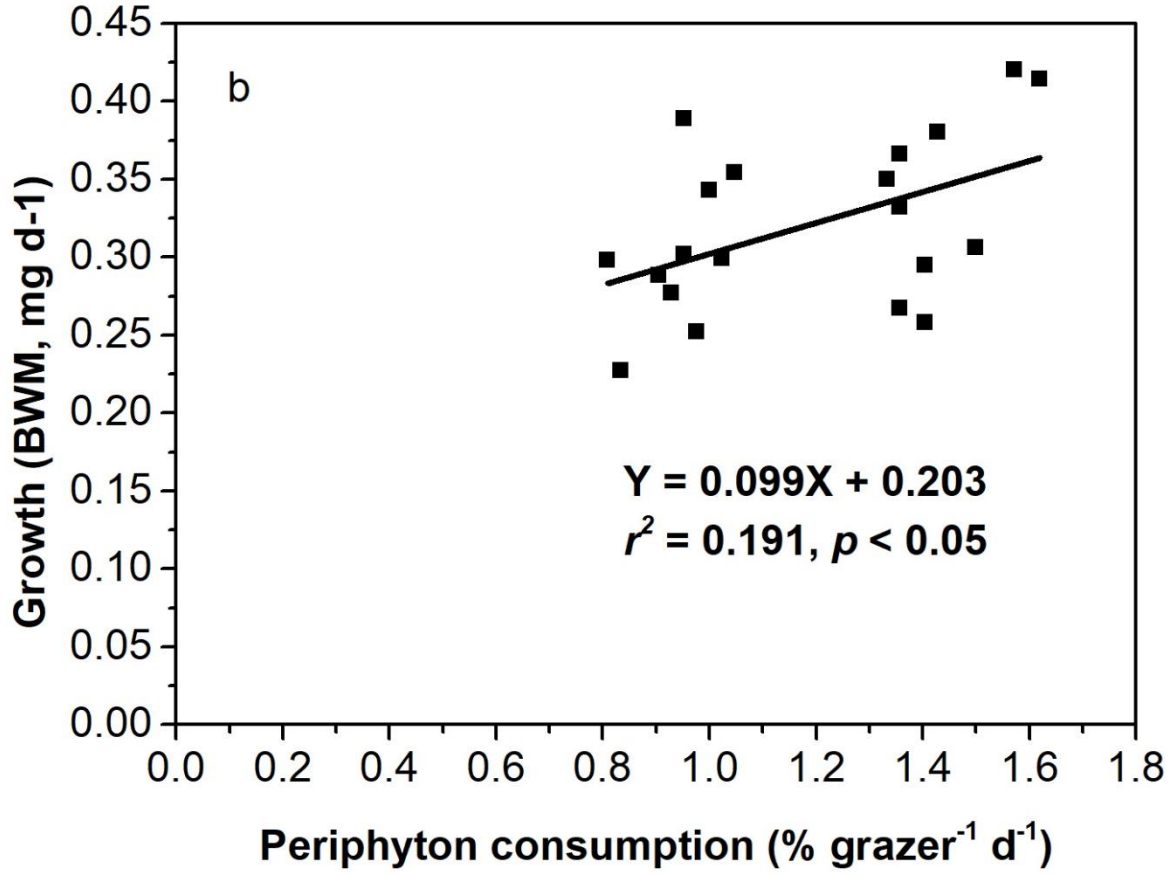

b
Growth (BWM, mg d-1)
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8
Periphyton consumption (% grazer-1 d-1)
Y = 0.099X + 0.203
r2 = 0.191, p < 0.05

Figure 4-6 Relationships between snail growth rate (blotted weight mass change per day) and chlorophyll-a:C (a) and periphyton consumption rate (% cover per grazer per day) (b).

4.4 Discussion

4.4.1 Periphyton growth

Nutrient addition significantly increased the growth of periphyton in the preliminary mesocosm experiment, suggesting the growth of periphyton in Ewens Ponds was limited by the supply of available nutrients (probably mainly P). There were no significant effects of light on periphyton growth, probably because light intensities in shaded treatments were still saturated for periphyton growth. Grazing effects on periphyton chlorophyll-a contents could not be detected in the preliminary mesocosm experiment due to extremely low periphyton biomass in treatments without nutrient addition and very high periphyton growth in treatments with nutrient addition (Table 4-1). Periphyton cover change can only be observed when removal of periphyton by grazers is faster than accrual of periphyton. It was necessary to have periphyton cover higher than certain minimum value at the beginning of the feeding trial so that grazers wouldn't exhaust the food supply during the course of the feeding trial. Chlorophyll-a contents of periphyton grown *in situ* for two months were very similar with chlorophyll-a contents of periphyton cultured in treatments with or without invertebrate grazing and without nutrient addition in the preliminary mesocosm experiment (Table 4-1). In treatments with invertebrate grazing and without nutrient addition, consumption by two *G. gibbosa* snails had led to almost total depletion of periphyton developed on blocks, suggesting periphyton in those treatments were under very high grazing pressure. The very low chlorophyll-a contents of periphyton grown on blocks *in situ* could be due to extremely low growth rate and insufficient time for development, but that doesn't expel the possibility

that periphyton in Ewens Ponds may also be under high grazing pressure just like periphyton cultured in treatments with invertebrate grazing and without nutrient addition (Table 4-1). In fact, we observed large amount of caddisfly larva, *Triplectides* sp., on the blocks when we retrieved them from Ewens Ponds. Due to limitation of available glasshouse space, we were not able to create more than two levels of each treatment, and that eliminated the possibility of establishing the relationship curves between light and nutrient conditions and algal and grazer growth. It was also unfortunate that the target values of light intensity and nutrient concentration can't be pushed to the very limits observed in field. Altogether, the power of explanation resulting from this study was somehow compromised, and it is necessary to replicate the experiments in the field as have been carried out in some other similar studies.

4.4.2 Periphyton biomass and chl-a:C

There were significant differences in periphyton chlorophyll-a contents, AFDM, total C and Chl-a:C between treatments receiving low and high nutrients, and two of the four parameters, AFDM and total C of periphyton were also affected by light intensities in the main mesocosm experiment. Hill et al. (2009) suggested that irradiance above 100 µmol m^{-2} s^{-1} would be able to saturate periphyton growth. The irradiances used in the main mesocosm experiment were around 120 µmol m^{-2} s^{-1}, and likely to have been saturating thus would not cause light limitation of periphton growth. Furthermore, light intensities effectively much lower than 100 µmol m^{-2} s^{-1} in Ewens Ponds are probably uncommon. The P concentrations used in treatments with low nutrients was 20 µg L^{-1} SRP, just below the relatively robust saturation threshold of P concentrations for stream algae at around 25 µg L^{-1} SRP (Hill et al. 2009). The lower chl-a:C ratios of periphyton in treatments with high nutrients than in treatments with low nutrients suggested a higher abundance of organic matter that were not autotrophic algae, possibly heterotrophic bacteria or detritus in those treatments (Liess and Hillebrand 2006). Alternatively, differences of periphyton chl-a:C ratios between treatments with high and low

nutrient could be caused by nutrient-induced changes in microbial community composition or chlorophyll a concentrations within algae cells (Stevenson 1996). The lower AFDM and total C in treatments with low light was related to the higher preiphyton removal rate by snails under low light intensities (See *snail growth and periphyton consumption*).

4.4.3 Periphyton and snail stoichiometry

Periphyton nutrient content was significantly increased by elevated nutrient concentrations in treatments in the main mesocosm experiment, but there were no effects of light levels on periphyton nutrient content (Table 4-3). Frost and Elser (2002a) found a similar pattern when they examined the response of periphyton nutrient content to manipulated nutrient concentrations as well as light in Canadian lakes. They suggested that manipulations of light were insufficient to cause any substantial changes in algal nutrient content in their study, it could also explain the results in this study because we provided irradiances higher than saturation as discussed above. N concentrations in the low and high nutrient treatments were 0.5 mg L^{-1} and 2.5 mg L^{-1} respectively, and dissolved inorganic nitrogen (DIN) concentrations in Ewens Ponds were higher than 5 mg L^{-1}. The values were higher than estimated saturating DIN concentration at 308 µg L^{-1} for stream periphytic algae (Rier and Stevenson 2006). The very high availability of N compared with P in the mesocosm experiments indicated that it was unlikely that the supply of N played a role in changing periphyton nutrient contents. Responses of stoichiometric ratios, C:N, C:P and N:P basically reflected responses of C, N and P contents to manipulated light and nutrient in different treatments. The P-limited nature of periphyton growth lead to greater increase of P percentages in the biomass of periphyton from treatments with low nutrient to treatments with high nutrient than C and N. Much lower C:N ratios and higher N:P ratios of *G. gibbosa* than that of periphyton in the corresponding treatments indicated strong N limitation for the growth of this snail in mesocosms, while P limitation was only evident in treatments with low

nutrient. However, *G. gibbosa* in the mesocosms can only feed on periphyton grown on panels, while snails in field could also consume other food sources, such as organic matter derived from macrophytes and benthic filamentous algae. The macrophyte *Triglochin procerum* contributed as much as 35-53% to the biomass of *G. gibbosa* in summer in Reid et al. (2008), while a range of 46-61% of the organic matter of *G. gibbosa* was determined to be derived from macrophytes in Ewens Ponds (Yang Liu, under review). Therefore, the observed elemental imbalances between snails and periphyton in the mesocosms may not reflect the real situations in field. *G. gibbosa* was found to suffer from severe P limitation in Ewens Ponds rather than little or no P limitation as shown here in the mesocosms, and N limitation of growth also existed for snails in field (unpublished data). N limitation of snail growth has also been reported in previous studies (Liess 2014; Liess and Hillebrand 2006; Liess and Lange 2011). It appears that we need to be very cautious when extrapolating the real situations in field from findings in laboratory controlled experiments.

4.4.4 Snail growth and periphyton consumption

We calculated the growth rate of grazers by diving the change of wet weight of snails by incubation time, but it should be noted that this assessment of animal growth using wet weight can be highly unreliable. Measuring final dry mass of the whole assemblage of snails in each experimental unit after incubation and estimating their initial dry by destructively sampling a control group, representative of the size of snails stocked in each experimental unit, at the beginning of the incubation would yield results with much better confidence. The reliability of data can be further strengthened by using larger experimental unit so that each one can contain more snails, and including more individuals in the measurement would reduce the stochastic errors. Also, longer experiments seem to be necessary to detect meaningful mass differences of these animals among treatment groups because they grow slow.

Snail growth rates were higher in treatments with low light under both low and high nutrient conditions, and snail growth rates were higher in treatments with high nutrient under both high and low light conditions (Figure 4-5b). The highest growth rate was in treatment with high nutrient low light, and the lowest growth was in treatment with low nutrient high light (Figure 4-5b). The LNH predicts that herbivore growth could be limited by food quantity under low light : nutrient ratios and by food quality under high light : nutrient ratios, while the highest growth is expected at intermediate light : nutrient ratios (Guo et al. 2016; Hill et al. 2010; Sterner et al. 1997). However, the negative effect of low light : nutrient ratios on periphyton food quantity can't be observed in this study because snail grazers were all provided with sufficient food in the parallel mesocosm experiment. We did that for precise measurement of periphyton removal by snails because entire removal of periphyton on panels would potentially cause an under-estimate of periphyton removal rates. Food quantity limitation of growth for snails in the field was highly probable, as snails used up all periphyton grown on blocks and couldn't survive within 7 days in treatments without nutrient addition in the preliminary mesocosm experiment. In one study, Hill et al. (2010) found the growth of snails peaked when light : nutrient ratios were highest in two oligotrophic streams where there was an inverse relationship between irradiances and dissolved nutrients. They suggested that exploitative competition between individuals for a limited food resource caused snail production was mainly determined by primary production, which in turn was driven by light intensity (Hill et al. 2010). The effectiveness of the LNH explaining herbivore growth is questionable in ecosystems where food supplies are far too plentiful or in big deficit.

Periphyton food quality could also potentially limit snail growth, and increasing light : nutrient ratios will create increasingly negative effects on algae stoichiometry and start to exert negative effects on herbivore growth when it passed the point where positive effects of

increasing light are counterbalanced (Hessen et al. 2002; Urabe and Sterner 1996). We didn't observe significant effects of light intensity on periphyton C:N or C:P ratios. Higher nutrient concentrations in water resulted in greater snail growth in this study, probably because reduced C:P ratios of periphyton in treatments with high nutrient diminished elemental imbalances between snails and periphyton (Figure 4-3, 5). We are not sure whether the different C:P ratios of periphyton in treatments with low or high nutrients were related to changes in taxonomic composition of the algal community or cellular stoichiometry of constituting algae species. However, N was relatively more limiting for snail growth than P based on stoichiometric imbalance between snails and periphyton (Figure 4-3). Although this study aims to evaluate the effects of periphyton stoichiometry on snail grazing, how grazing rate differences may, in turn, feed back to affect periphyton stoichiometry or comprising algae species represent an important link between producer and consumer in freshwater benthic habitats and should be examined in future studies.

No significant differences of periphyton consumption rates by *G. gibbosa* between treatments with low and high nutrients indicated that *G. gibbosa* didn't actively increase consumption rate to compensate for low quality of periphyton food. Stelzer and Lamberti (2002) offered high quantity of periphyton grown under different nutrient regimes to *Elimia livescens* snails in a 2-d feeding trial, and they also found periphyton removal rates by *E. livescens* were not affected by nutrient regimes. Failure of observing a response in snails to low quality of food when high quantity of food is supplied may be due to the inability of behavioural adjustment in *G. gibbosa* as in *E. livescens* proposed by Stelzer and Lamberti (2002).

Light intensities can sometimes influence snail growth, not by altering the quantity or quality of food for snails but by affecting snails' consumption rate or activity (Liess and Lange 2011). We found consumption rates of periphyton by *G. gibbosa* were significantly higher in treatments with low light than in treatments with high light, but periphyton food quality was

not significantly different from each other in treatments with low or high light (Table 4-3; Figure 4-3, 5). Our results were consistent with that of Liess and Lange (2011), as they found snail growth rates were higher in shaded than unshaded stream channels under high nutrient addition. It appears that the lower growth of snails under high light compared to low light could be caused by reduced active grazing as snails hide more frequently for avoidance of predators or UV radiation under high light (Liess and Lange 2011). Although we didn't measure the snail activity under different light intensities, the positive correlation between snail growth and periphyton consumption supported this postulation, and we noticed that snails preferred to stay in relatively dark environments in the mesocosms. Snail growth rates could also be mediated through changes in periphyton community composition when different conditions of light and nutrient were provided in treatments. Periphyton consisted mainly of diatoms, with little other microorganisms and detritus in the mesocosms in this study. Diatom community compositions have been shown to be strongly affected by changes in light intensities and nutrient levels in streams (Lange et al. 2011). Periphyton community composition under high light may be unfavourable to *G. gibbosa*, containing higher proportion of algae species that are unpalatable than under low light in this study.

Our conclusion is that complex interactions between environmental factors and herbivores have the potential to interfere in the process of herbivores assimilating C and nutrients, leading to observations different from the predictions of ecological stoichiometry and light : nutrient hypothesis.

References

APHA, 2005. Standard Methods for the Examination of Water and Wastewater, 21st ed. American Public Health Association, Washington DC, USA.

Brett, M. T. & D. C. Müller-Navarra, 1997. The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshwater Biology 38:483-499.

Cross, W. F., J. P. Benstead, A. D. Rosemond & J. B. Wallace, 2003. Consumer-resource stoichiometry in detritus-based streams. Ecology Letters 6:721-732.

Dickman, E. M., M. J. Vanni & M. J. Horgan, 2006. Interactive effects of light and nutrients on phytoplankton stoichiometry. Oecologia 149:676-689.
Elser, J. J., W. F. Fagan, R. F. Denno, D. R. Dobberfuhl, A. Folarin, A. Huberty, S. Interlandi, S. S. Kilham, E. McCauley, K. L. Schulz, E. H. Siemann & R. W. Sterner, 2000. Nutritional constraints in terrestrial and freshwater food webs. Nature 408:578-580.
Evans-White, M. A., R. S. Stelzer & G. A. Lamberti, 2005. Taxonomic and regional patterns in benthic macroinvertebrate elemental composition in streams. Freshwater Biology 50:1786-1799.
Fagan, W. F., E. Siemann, C. Mitter, R. F. Denno, A. F. Huberty, H. A. Woods & J. J. Elser, 2002. Nitrogen in insects: implications for trophic complexity and species diversification. The American Naturalist 160:784-802.
Fanta, S. E., W. R. Hill, T. B. Smith & B. J. Roberts, 2010. Applying the light : nutrient hypothesis to stream periphyton. Freshwater Biology 55:931-940.
Fink, P. & E. V. Elert, 2006. Physiological responses to stoichiometric constraints: nutrient limitation and compensatory feeding in a freshwater snail. Oikos 115:484-494.
Fink, P., L. Peters & E. V. Elert, 2006. Stoichiometric mismatch between littoral invertebrates and their periphyton food. Archiv für Hydrobiologie 165:145-165.
Frost, P. C. & J. J. Elser, 2002a. Effects of light and nutrients on the net accumulation and elemental composition of epilithon in boreal lakes. Freshwater Biology 47:173-183.
Frost, P. C. & J. J. Elser, 2002b. Growth responses of littoral mayflies to the phosphorus content of their food. Ecology Letters 5:232-240.
Frost, P. C., S. E. Tank, M. A. Turner & J. J. Elser, 2003. Elemental composition of littoral invertebrates from oligotrophic and eutrophic Canadian lakes. Journal of North American Benthological Society 22:51-62.
Guo, F., M. J. Kainz, D. Valdez, F. Sheldon & S. E. Bunn, 2016. The effect of light and nutrients on algal food quality and their consequent effect on grazer growth in subtropical streams. Freshwater Science 35:1202-1212.
Hall, S. R., M. A. Leibold, D. A. Lytle & V. H. Smith, 2007. Grazers, producer stoichiometry, and the light : nutrient hypothesis revisited. Ecology 88:1142-1152.
Healey, F. P., 1985. Interacting effects of light and nutrient limitation on the growth rate of *Synechococcus linearis* (Cyanophyceae). Journal of Phycology 21:134-146.
Hessen, D. O., P. J. Færøvig & T. Andersen, 2002. Light, nutrients, and P:C ratios in algae: grazer performance related to food quality and quantity. Ecology 83:1886-1898.
Hill, W. R., 1996. Effects of light. In Stevenson, R. J., M. L. Bothwell & R. L. Lowe (eds) Algal Ecology: Freshwater Benthic Ecosystems. Academic Press, New York, NY, USA, 121-148.
Hill, W. R. & S. E. Fanta, 2008. Phosphorus and light colimit periphyton growth at subsaturating irradiances. Freshwater Biology 53:215-225.
Hill, W. R., S. E. Fanta & B. J. Roberts, 2009. Quantifying phosphorus and light effects in stream algae. Limnology and Oceanography 54:368-380.
Hill, W. R., J. G. Smith & A. J. Stewart, 2010. Light, nutrients, and herbivore growth in oligotrophic streams. Ecology 91:518-527.
Katechakis, A., T. Haseneder, R. Kling & H. Stibor, 2005. Mixotrophic versus photoautotrophic specialist algae as food for zooplankton: The light : nutrient hypothesis might not hold for mixotrophs. Limnology and Oceanography 50:1290-1299.
Lange, K., A. Liess, J. J. Piggott, C. R. Townsend & C. D. Matthaei, 2011. Light, nutrients and grazing interact to determine stream diatom community composition and functional group structure. Freshwater Biology 56:264-278.

Liess, A., 2014. Compensatory feeding and low nutrient assimilation efficiencies lead to high nutrient turnover in nitrogen-limited snails. Freshwater Science 33:425-434.
Liess, A. & H. Hillebrand, 2006. Role of nutrient supply in grazer-periphyton interactions: reciprocal influences of periphyton and grazer nutrient stoichiometry. Journal of the North American Benthological Society 25:632-642.
Liess, A. & K. Lange, 2011. The snail *Potamopyrgus antipodarum* grows faster and is more active in the shade, independent of food quality. Oecologia 167:85-96.
Liess, A., K. Lange, F. Schulz, J. J. Piggott, C. D. Matthaei & C. R. Townsend, 2009. Light, nutrients and grazing interact to determine diatom species richness via changes to productivity, nutrient state and grazer activity. Journal of Ecology 97:326-336.
Ohta, T., Y. Miyake & T. Hiura, 2011. Light intensity regulates growth and reproduction of a snail grazer (Gyraulus chinensis) through changes in the quality and biomass of stream periphyton. Frehswater Biology 56:2260-2271.
Plath, K. & M. Boersma, 2001. Mineral limitation of zooplankton: stoichiometric constraints and optimal foraging. Ecology 82:1260-1269.
Qin, P., C. M. Mayer, K. L. Schulz, X. Ji & M. E. Ritchie, 2007. Ecological stoichiometry in benthic food webs: effect of light and nutrients on periphyton food quantity and quality in lakes. Limnology and Oceanography 52:1728-1734.
Reid, D. J., G. P. Quinn, P. S. Lake & P. Reich, 2008. Terrestrial detrius supports the food webs in lowland intermittent streams of south-eastern Australia: a stable isotope study. Frehswater Biology 53:2036-2050.
Rier, S. T. & R. J. Stevenson, 2006. Response of periphytic algae to gradients in nitrogen and phosphorus in streamside mesocosms. Hydrobiologia 561:131-147.
Rigosi, A., Y. Liu, M. Shanafield & J. D. Brookes, 2015. Determining environmental risks to Ewens Ponds in the South East. Goyder Institute for Water Research Technical Report Series No. 15/19, Adelaide, South Australia.
Rowland, F. E., K. J. Bricker, M. J. Vanni & M. J. González, 2015. Light and nutrients regulate energy transfer through benthic and pelagic food chains. Oikos 124:1648-1663.
Sanches, L. F., R. D. Guariento, A. Caliman, R. L. Bozelli & F. A. Esteves, 2011. Effects of nutrients and light on periphytic biomass and nutrient stoichiomerty in a trophic black-water aquatic ecosystem. Hydrobiologia 669:35-44.
Sartory, D. P. & J. U. Grobbelaar, 1984. Extraction of chlorophyll *a* from freshwater phytoplankton for spectrophotometric analysis. Hydrobiologia 114:177-187.
Stanier, R. Y., R. Kunisawa, M. Mandel & G. Cohen-Bazire, 1971. Purification and properties of unicellular blue-green algae (order *Chroococcales*). Bacteriological Reviews 35:171-205.
Stelzer, R. S. & G. A. Lamberti, 2002. Ecological stoichiometry in running waters: periphyton chemical composition and snail growth. Ecology 83:1039-1051.
Sterner, R. W. & J. J. Elser, 2002. Ecological stoiochiometry: The biology of elements from molecules to the biosphere. Princeton University Press.
Sterner, R. W., J. J. Elser, E. J. Fee, S. J. Guildford & T. H. Chrzanowski, 1997. The light : nutrient ratio in lakes: the balance of energy and materials affects ecosystem structure and process. The American Naturalist 150:663-684.
Sterner, R. W. & D. O. Hessen, 1994. Algal nutrient limitation and the nutrition of aquatic herbivores. Annual Review of Ecology and Systematics 25:1-29.
Stevenson, R. J., 1996. An introduction to algal ecology in freshwater benthic habitats. In Stevenson, R. J., M. L. Bothwell & R. L. Lowe (eds) Algal Ecology: Freshwater Benthic Ecosystems. Academic Press, New York, NY, USA, 3-30.

Urabe, J., M. Kyle, W. Makino, T. Yoshida, T. Andersen & J. J. Elser, 2002. Reduced light increases herbivore production due to stoichiometric effects of light/nutrient balance. Ecology 83:619-627.
Urabe, J. & R. W. Sterner, 1996. Regulation of herbivore growth by the balance of light and nutrients. Proceedings of National Academy of Sciences 93:8465-8469.

Statement of Authorship

Title of Paper	Growth and stoichiometry of two benthic filamentous algae under varying nutrient conditions
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ■ Unpublished and Unsubmitted work written in publication style
Publication Details	Prepared for submission to a journal

Principal Author

Name of Principal Author (Candidate)	Yang Liu
Contribution to the paper	Designed the study, performed field experiments, conducted laboratory experiments, analysed the data, interpreted the results, wrote the manuscript
Overall percentage (%)	80 %
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 04/10/2017

Co-author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution

Name of Co-author	Justin Brookes
Contribution to the paper	Provided input in conceiving the study and design of experiments, supervised data analysis and interpretation, reviewed the manuscript
Signature	Date 4 Oct 2017

Chapter 5 Growth and stoichiometry of benthic filamentous chlorophyte and cyanobacteria under varying nutrient conditions

Abstract

Proliferation of the benthic filamentous algae poses risks to ecosystem integrity and reduces aesthetic and recreational water uses in many freshwater environments all over the world. Recent observation of expansion of benthic filamentous algae mats and shrink of rooted vascular plant beds are symptoms of a shift from domination by submerged macrophytes to domination by benthic filamentous algae in Ewens Ponds, a groundwater-fed freshwater wetland system in the southeast of South Australia. This shift could significantly influence key ecological processes such as consumer production and nutrient recycling. It is generally postulated that this kind of shift may be associated with nutrient enrichment in the system, especially nitrogen (N) and phosphorus (P), and a gradual increase of N concentrations in Ewens Ponds water was monitored, but the exact responses of benthic filamentous algae growth and stoichiometry to nutrient concentrations were largely unknown. We cultured *Lyngbya* and *Rhizoclonium*, the two most common benthic filamentous algae in media with varying N or P concentrations as primary nutrient while the concentrations of the other nutrient in the media were either low or high following the experiment design of a previous study. Algal growth rates, chlorophyll-a contents (chl-a), carbon: chlorophyll-a (C:chl-a) ratios, C, N, P contents and molar C:N:P ratios were measured after 20 days of incubation. Different N and P concentrations in media induced large variations in *Lyngbya* and *Rhizoclonium* growth and stoichiometry. The growth rates of both *Lyngbya* and *Rhizoclonium* responded positively with increasing medium N or P concentrations as primary nutrient, and the responses were not different between algae cultured in media with low and high concentrations of the secondary nutrient. Algal chl-a contents increased with increasing

medium N or P concentrations, which was an effect that was altered by the concentrations of the secondary nutrient. Algal C:P and N:P ratios increased with greater medium N concentrations and decreased with greater medium P concentrations, with effect of the secondary nutrient concentrations only present in *Lyngbya* C:P and N:P ratios. Strong homeostasis regarding N:P ratios was found for both *Lyngbya* and *Rhizoclonium*. Relatively strong tendencies of homeostasis were found for N content in both *Lyngbya* and *Rhizoclonium*, whereas no tendencies of homeostasis were found for P content in those two algae. In accordance with previous studies, the relative invariant growth and stoichiometry appears to be common physiological traits of benthic filamentous algae species that prevail over others in freshwater environments, and these traits add to the difficulties managers meet when tackling proliferation of those algae.

5.1 Introduction

The increased occurrence of harmful algae blooms, mainly due to human activity, has considerable impacts on aquatic ecosystems in many parts of the world (O'Neil et al. 2012; Paerl et al. 2016a). While planktonic algal blooms have been intensively studied, comparatively little is known about the more recent problems caused by benthic filamentous algae in freshwater environments (Hudon et al. 2014). Large accumulations of benthic filamentous algae, such as chlorophytes *Cladophora*, *Ulva* and cyanophytes *Lyngbya*, *Phormidium* generally attached loosely with the bottom sediment, and occasionally float to the surface or drift around in water column because of trapped gas bubbles and dislodge caused by wind, wave or other disturbance in eutrophic freshwater habitats (Stevenson et al. 2007; Wetzel 1996). The proliferation of these benthic filamentous algae can yield greater biomass than the combination of primary production by all other algal groups and rooted vascular macrophytes, representing the occurrence of a regime shift from macrophyte domination to benthic filamentous algae domination in the autotrophic structure of aquatic

ecosystems (Hilton et al. 2006; Irfanullah and Moss 2005). After the completion of such shift, benthic filamentous algae become the dominant primary producers and constitute the main trophic pathway between dissolved nutrients and higher trophic levels, and the changes in autotrophic production can lead to changes in community diversity and composition of invertebrate assemblages and loss of habitats for organisms at higher trophic levels (Carpenter et al. 1998; Hudon et al. 2012). Besides their capability to simplify food webs, decrease ecosystem biodiversity and alter biogeochemical cycling, large floating masses of benthic filamentous algae can also obstruct waterways and release toxins, decreasing the recreational and aesthetic value of many freshwater environments (Gaget et al. 2017; Hudon et al. 2014; Middleton and Frost 2014; Stevenson et al. 2007; Wood et al. 2017).

Despite of the many possible detrimental effects caused by benthic filamentous algae, the basic physiology and ecology of many benthic filamentous algae are largely unknown. The accumulation and proliferation of benthic filamentous algae are subjected to relative strength of accrual and removal processes in them, and accrual may be affected by a range of environmental factors, including light levels and nutrient concentrations, while removal may be determined by factors, including hydraulic disturbances and grazing controls (Sturt et al. 2011). Most of the studies examining the links between chemical, physical and biological characteristics of freshwater ecosystems and the biomass of filamentous algae were conducted in field (Hart et al. 2013; Lévesque et al. 2015; Nifong 2017; Stevenson et al. 2012; Sturt et al. 2011; Wood et al. 2017) with a few exceptions (Middleton and Frost 2014; Stevenson et al. 2007). Among all the contributing factors, nutrient enrichment is generally recognized as a primary cause of the excessive growth of macroscopic algal species in aquatic ecosystems (Sturt et al. 2011). Although growth rates, nutrient contents and stoichiometric ratios of benthic filamentous algae grown in field can be used to assess proliferation risks and reflect nutrient availability in the immediate growing environments,

interpretation of the results are potentially complicated by interferences such as interactions among co-existing species (Middleton and Frost 2014). The exact responses of algal growth and elemental stoichiometry in specific species of benthic filamentous algae to changes in nutrient concentrations needs to be examined using unialgal cultures grown in isolation.

Ewens Ponds, a wetland consisting of three small groundwater-fed freshwater ponds in the southeast of South Australia, have shown the extension of benthic filamentous algae mats and shrink of rooted vascular macrophytes beds occurring concurrently with gradual increase of nitrate concentrations in the ponds (Carmody 2006). Such changes in the percentages of sediment covered by macrophytes or filamentous algae are considered as symptom of potential regime shifts from macrophyte domination to benthic filamentous algae domination. The benthic filamentous algae community contains many species of cyanobacteria as well as chlorophytes, with the two most common benthic filamentous algae species are *Lyngbya* sp. and *Rhizoclonium* sp. in Ewens Ponds. The most profound difference between these two taxa is that *Lyngbya* to cyanobacteria, which indicates that it could potentially fix atmospheric nitrogen gas and *Rhizoclonium*, as chlorophyte, has to obtain nitrogen nutrients from available sources such as dissolved nitrogen in water column. There appears to be a large surplus of soluble nitrogen (N) relative to phosphorus (P) in Ewens Ponds, however, previous report evaluating environmental risks in the ponds predicted potential increase in P loading into the ponds while N loading should have reached the peak (Rigosi et al. 2015). Management strategy involving practical measures may lead to reduction in N loading and prevention of abrupt or gradual increase in P loading.

The current work takes a critical look at a study conducted by Middleton and Frost (2014) documenting the relatively slow and steady growth of one filamentous green algae *Mougeotia* under relatively low N and P supplies. They tested stoichiometric and growth responses of a freshwater filamentous green alga to varying nutrient supplies. One aim of the current study

was to test the hypotheses while enhancing the scientific rigor of the work by Middleton and Frost (2014). The same methodology was employed except for modifications to the N and P concentrations and resultant N:P ratios used in culture media. We expect to find the relatively invariant growth of *Lyngbya* and *Rhizoclonium* despite the wide range of N and P concentrations in the medium. We also hypothesize both taxa would show homeostatic tendency regarding their N content and non-homeostatic tendency regarding their P content given the long-term extremely high N availability but relatively low P availability in Ewens Ponds. These two taxa were chosen as experimental species because of their dominance in filamentous algal communities in Ewens Ponds as well as their distinct physiological traits regarding nutrient uptake. We conducted field surveys, collecting samples of these two algae grown in field and comparing the nutrient contents and elemental ratios between natural samples and cultured samples. In addition to adding knowledge to the body of research on filamentous algae, this study also attempts to evaluate potential influences of possible reduction in N concentrations and elevation in P concentrations on benthic filamentous algae dynamics in Ewens Ponds.

5.2 Methods

5.2.1 Study site

Ewens Ponds is approximately 30 km south of Mount Gambier along the limestone coast in the lower southeast of South Australia (38°01′36″S, 140°47′26″E). It is a series of three basin-shaped small lakes connected by shallow, winding channels, which in turn feed into Eight Miles Creek discharging to the sea around 2.5 km downstream. Ewens Ponds is a groundwater-dependent system, as discharge from several aquifers makes up more than 95% of the water filling the ponds (Rigosi et al. 2015). The ponds are constantly flushed by high flows of groundwater, which prevents stable establishment of phytoplankton communities,

and water retention time in the ponds is as short as 6 hours (Grandfield and Ashman 1984). Vegetation in the ponds mainly consists of various types of aquatic vascular plants and benthic filamentous algae. Aquatic plants grow on sediments with flat underwater terrain along the edge of ponds and the channels. The rest of bottom zone where aquatic plants couldn't establish is usually with steep slopes and mostly occupied by filamentous algae mats with two distinct pigmentation, with shallower part (2-5 m in depth) covered by light green algae (dominated by Cladophoraceae, *Rhizoclonium* sp.) and deeper part covered by dark green algae (dominated by Oscillatoriaceae, *Lyngbya* sp.). The overall condition of Ewens Ponds ecosystem is a stable state characterized by clear water and abundant macrophytes (Rigosi et al. 2015). However, benthic biofilms dominated by filamentous algae can sometimes acquire extra buoyance from oxygen bubbles produced within the algal matrix and start to rise to the surface, forming floating algal blooms when photosynthesis activity is high during periods of high sunlight irradiance and temperature (Scheffer 1998; Wetzel 1996). Increasing frequencies of floating algal blooms have been reported in the ponds in recent years, together with extension of benthic filamentous algae mats and shrink of submerged macrophytes (Carmody 2006).

5.2.2 Field procedures

Benthic filamentous algae samples were collected from five different sites by divers from algal mats with contrasting colours and located in two distinct zones, with the dark green mats occurring on sediments in the depth from 5 to 8 m dominated by *Lyngbya* and light green mats occurring on sediments in the depth from 2 to 5 m dominated by *Rhizoclonium* respectively in Ewens ponds in December, 2015. Fresh algae samples were quickly put onto ice in the dark and transported to laboratory immediately.

5.2.3 Laboratory procedures

After returning to the laboratory, samples were washed with distilled water, cleared of any visible attached macroinvertebrates as well as other contaminant particles using forceps and checked under microscope to confirm that the mats were dominated by either *Lyngbya* or *Rhizoclonium* in the corresponding samples. Each sample was then divided into two portions. One portion was used as initials for creating stock cultures of *Lyngbya* and *Rhizoclonium*, while the other portion was used for determination of nutrient contents and stoichiometric ratios of algae grown in field.

Stock cultures of *Lyngbya* and *Rhizoclonium* were established by putting twenty cleaned filaments of these two alga in 500 mL glass jars filled with autoclaved BG11 media (Stanier et al. 1971). Germanium dioxide (GeO_2) was added to growth media at the concentration of 1 mg L^{-1} to inhibit diatom growth (Andersen 2005; Lewin 1966). The jars were covered with a piece of glass to prevent contamination from air, and gas exchange was permitted by putting a small pipette tip between the cover glass and the rim of the jar. Algal cultures were incubated at 20 °C in a constant temperature room, receiving an irradiance of 100 µmol m^{-2} s^{-1} at the surface of the growth medium with a light/dark cycle of 16/8 h provided by plant growth lamps. Newly grown trichomes out of the old filaments were cut and transferred to other clean glass jars filled with freshly autoclaved growth media fortnightly and this process was repeated several times until pure cultures of *Lyngbya* or *Rhizoclonium* was observed under microscope. Although we didn't add bacteria inhibitors to growth media, no obvious contamination of bacteria was observed in our cultures.

In order to evaluate responses of *Lyngbya* or *Rhizclonium* algal growth and stoichiometry to varying N and P concentrations in water, we conducted one N bioassay experiment and one P bioassay experiment following an close parallel to a previous study by Middleton and Frost (2014). A series of 500 mL glass jars were used as experimental units, and 500 mL autoclaved N- and P-free BG11 medium was poured into each experimental unit. Media was

added with six different N (as nitrate) concentrations (10, 25, 100, 500, 900 and 1500 µg L^{-1}) acting as primary factor, while P (as orthophosphate) was supplied at either low (10 µg L^{-1}) or high (100 µg L^{-1}) levels as secondary factor in N bioassay experiment. Media was also added with six different P (as orthophosphate) concentrations (5, 10, 25, 50, 100 and 150 µg L^{-1}) acting as primary factor, while N (as nitrate) was supplied at either low (25 µg L^{-1}) or high (900 µg L^{-1}) levels as secondary factor in P bioassay experiment. The combinations of N and P concentrations in N and P bioassay experiments created 20 different treatments and 17 different medium N:P ratios ranging from 0.22 to 398. We set the gradients of N and P levels as this to cover a wide range in the concentrations of both nutrients and in the ratios of N:P. Every treatment was replicated by four times, and prevention of airborne contamination and facilitation of gas exchange in all experimental units were supported by using the same method as described above in stock cultures. Filaments of either *Lyngbya* or *Rhizoclonium* were taken from homogenized mixture of stock cultures and blotted dry with paper tissue, around 10 mg (blotted dry weight) of thoroughly rinsed filaments were weighed first and then added into each experimental unit to start the experiments. All experimental units were under the same temperature and light conditions as provided for stock cultures and the experiments lasted for 20 days. Earlier trials had determined that the growth rates of these two algae were very slow, thus we chose to use relatively big initial biomass and long incubation time to ensure sufficient samples for measurements and analysis at the termination of the experiments.

On the last day of the experiments, we collected algal materials in each experimental unit by filtering the media after mixing. The collected algal materials were cut into very small filaments and added into deionized water, homogenized slurry samples were then made using stirrer. Each slurry sample of filamentous algae was divided into three subsamples. The first subsample was filtered on 47 mm Whatman GF/F filter with pore size of 0.45 µm and filter

was frozen and used for chl-a determination. The second subsample was transferred into pre-weighed aluminium pan and used for dry weight determination. The third subsample was transferred into plastic tube, kept frozen at -20 °C and used for C, N and P content analysis.

5.2.4 Chemical analysis

Chlorophyll a concentrations of cultured filamentous algae subsamples were quantified using a spectrophotometer (Libra S22, Biochrom, Cambridge, UK) after extracting the filters with the hot ethanol extraction method adapted from Sartory and Grobbelaar (1984). Dry weights of cultured filamentous algae subsamples were measured by drying the samples at 60 °C in an oven in aluminium pans and weighing the samples repeatedly until constant weight was maintained. Despite all efforts, there were still filaments of non-target algae species entangled in filaments of *Lyngbya* or *Rhizoclonium* in benthic filamentous algae samples used for determination of nutrient contents and stoichiometric ratios of algae grown in field, and firmly attached diatoms on algae filaments were still present. However, those contaminant filamentous algae and diatoms would only account for a negligible portion of the overall biomass of filamentous algae samples representing natural *Lyngbya* or *Rhizoclonium* because of their relatively small cell numbers and size compared to *Lyngbya* or *Rhizoclonium* filaments. Subsamples of both natural and cultured *Lyngbya* and *Rhizoclonium* for C, N and P content analysis were dried in an oven at 60 °C and ground to fine powder with grinder. Algal C and N contents were analysed using an elemental analyser (Perkin Elmer 2400 Series II, Perkin Elmer, Waltham, MA, USA) at Sprigg Geobiology Centre in the University of Adelaide. Algal P contents were analysed using ascorbic acid method with a spectrophotometer (Libra S22, Biochrom, Cambridge, UK) after hot acid digestion with hydrogen peroxide (APHA 2005). Total C of benthic filamentous algae samples in cultures was calculated by multiplying algal dry weight by the percentage of C. Data of Ewens Ponds water dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP)

concentrations were acquired from routine sampling and measurement of those parameters carried out in another project evaluating environmental risks to Ewens Ponds (Rigosi et al. 2015).

5.2.5 Data analysis

Growth rate (d^{-1}) in the N and P bioassay experiments was calculated using the following formula:

Growth rate = $\ln(m_2/m_1)/t$

where m_2 and m_1 were final and initial dry mass respectively, and t was duration time of the experiment. Initial dry mass of filamentous algae was calculated by multiplying the blotted dry weights of initial filaments by average percentage of oven dry weight in blotted dry weight. The average percentages of oven dry weight in blotted dry weight for *Lyngbya* and *Rhizoclonium* were estimated by weighing blotted dry weights of clusters of filaments of both algae genera from homogenized mixtures and reweighing oven dry weights of the same clusters after oven drying.

Similar to the experiment design, methods of analysing data in this study were also sourced from the paper by Middleton and Frost (2014). An organism's degree of stoichiometric homeostasis was characterized by the homeostasis coefficient H (Sterner and Elser 2002). The reciprocal of the regulation coefficient (1/H) can be estimated from the slopes of regression lines of logarithms of organism's nutrient stoichiometry versus logarithms of resource's nutrient stoichiometry based on transformed version of stoichiometric homeostasis equation (Halvorson and Small 2016; Persson et al. 2010; Sterner and Elser 2002). Organisms having strict homeostasis would be indicated by 1/H approaching 0, whereas organisms showing great plasticity would be represented by 1/H approaching 1 (Halvorson and Small 2016; Persson et al. 2010; Sterner and Elser 2002). We conducted linear regression

between logarithms of algal N:P ratios and logarithms of medium N:P ratios, and used the values of the slopes of the regression line to characterize the degree of N:P homeostasis in *Lyngbya* and *Rhizoclonium*.

Standard major axis (SMA) regression analysis was used to test whether there were significant differences in the slopes of regression lines of logarithms of response variables versus logarithms of the concentrations of the primary nutrient between the low and high concentrations of the secondary nutrient (Falster et al. 2006). Response and predictor variables went through necessary transformations to meet the requirements for parametric tests and to linearize respective relationships prior to analyses. Regression parameters from SMA were examined to assess growth and stoichiometric responses of *Lyngbya* and *Rhizoclonium* to varying medium primary nutrient concentrations with low and high medium secondary nutrient concentrations in both N and P bioassay experiments. Estimates of the parameters of respective correlation and linear regression between compiled algal N:P ratios, N content and P content from both N and P bioassay experiments and medium N:P ratios, N and P concentrations was determined using OriginPro 9.0 (OriginLab, Northampton, MA, USA).

5.3 Results

The results presentation of this study also followed the way used in Middleton and Frost (2014), and such arrangement offers an wonderful opportunity to better compare and contrast the results of this study and theirs.

5.3.1 Algal and water elemental nutrient composition in Ewens Ponds

Elemental nutrient compositions of benthic filamentous algae mats dominated by either *Lyngbya* or *Rhizoclonium* collected from Ewens Ponds varied a lot (Table1). For algal nutrient contents, the most variability was found in algal P contents, while the least variability

was found in algal C contents for both algae genera (Table 5-1). For algal stoichiometric ratios, the most variability was found in algal C:P ratios for both algae genera, while the least variability was found in algal C:N ratios for both algae genera (Table 5-1). Levels of variability in algal N and P contents as well as C:N, C:P and N:P stoichiometric ratios for *Rhizoclonium* was higher than for *Lyngbya*. *Rhizoclonium* had higher mean algal C and N contents but lower algal mean P contents than *Lyngbya*, thus mean C:P and N:P ratios of *Rhizoclonium* were higher than that of *Lyngbya* (Table 5-1). SRP ranged from 1-15 μg L^{-1}, and DIN ranged from 5000-5991 μg L^{-1} in Ewens Ponds water. There were great differences between variability in SRP (45.63%) and DIN (6.25%). DIN:SRP had an extremely wide range (824-11563), and a great deal of variability (98.55%) in DIN:SRP was found.

Table 5-1 Range, mean value, standard deviation (SD) and coefficient of variation (CV) of *Lyngbya* and *Rhizoclonium* C, N and P contents and C:N, C:P and N:P molar ratios in natural samples and of dissolved inorganic nitrogen (DIN) concentrations, soluble reactive phosphorus (SRP) concentrations and DIN:SRP molar ratios in Ewens Ponds water.

Response variables		Range	Mean	SD	CV (%)
Lyngbya	C (%)	27.520-37.341	30.542	3.854	12.62
	N (%)	2.009-3.487	2.826	0.568	20.08
	P (%)	0.140-0.409	0.242	0.107	44.43
	C:N	10.081-16.558	12.942	2.254	17.41
	C:P	230.534-541.893	369.768	118.849	32.14
	N:P	18.402-34.591	28.279	6.041	21.36
Rhizoclonium	C (%)	28.366-37.314	34.570	3.503	10.13
	N (%)	2.927-6.006	4.136	1.168	28.25
	P (%)	0.065-0.336	0.202	0.106	52.64
	C:N	5.722-14.602	10.671	3.571	33.46
	C:P	236.086-1447.157	659.440	481.338	72.99
	N:P	29.627-99.108	56.175	25.324	45.08
DIN (μg L^{-1})		5000-5991	5451	341	6.25
SRP (μg L^{-1})		1-15	8.17	3.73	45.63
DIN:SRP		824-11563	2139.89	2108.80	98.55

5.3.2 Algal elemental nutrient composition, growth, chl-a and C:chl-a in cultures

Similar to variabilities in elemental contents of *Lyngbya* and *Rhizoclonium* natural samples, the most variability was found in algal P contents, while the least variability was found in algal C contents of both *Lyngbya* and *Rhizoclonium* cultured samples in laboratory (Table 5-2). For algal stoichiometric ratios, the least variability was found in algal C:N ratios for both algae genera (Table 5-2). Variability in C:P ratios of cultured *Rhizoclonium* was more than variability in N:P ratios, but variability in N:P ratios of cultured *Lyngbya* was more than variability in C:P ratios (Table 5-2). Levels of variability in algal C and N contents as well as C:N ratios for *Rhizoclonium* was higher than for *Lyngbya* (Table 5-2). Mean C, N and P contents of *Lyngbya* cultured in laboratory were 39.5 %, 5.4 % and 0.32 % respectively, higher than the corresponding C, N and P contents of *Rhizoclonium* at 35.4 %, 2.4 % and 0.18% (Table 5-2). *Rhizoclonium* had higher mean C:N and C:P ratios but lower mean N:P ratios than *Lyngbya* in cultures (Table 5-2). The ranges of growth rates were 0.031-0.098 d^{-1} and 0.006-0.103 d^{-1} for *Lyngbya* and *Rhizoclonium* respectively in N and P bioassay experiments (Table 5-2). The minimum values of *Lyngbya* and *Rhizoclonium* chl-a was approximately 28 µg L^{-1} and 29 µg L^{-1} respectively, while the maximum values of *Lyngbya* and *Rhizoclonium* chl-a was approximately 891 µg L^{-1} and 334 µg L^{-1} respectively, exhibiting more than 30 folds of increase in *Lyngbya* and more than 10 folds of increase in *Rhizoclonium* (Table 5-2). C:chl-a ratios of both *Lyngbya* and *Rhizoclonium* in N and P bioassay experiments also covered wide ranges (Table 5-2).

Table 5-2 Range, mean value, standard deviation (SD) and coefficient of variation (CV) of of *Lyngbya* and *Rhizoclonium* C, N and P contents, C:N, C:P and N:P molar ratios, growth, chl-a and C:chl-a ratios in cultured samples in both N and P bioassay experiments.

Response variables	*Lyngbya*				*Rhizoclonium*			
	Range	Mean	SD	CV (%)	Range	Mean	SD	CV (%)
C (%)	35.517-42.010	39.506	1.441	3.65	30.708-39.575	35.429	1.883	5.31
N (%)	4.636-6.482	5.393	0.422	7.83	1.522-3.753	2.405	0.405	16.85
P (%)	0.161-0.637	0.320	0.143	44.69	0.103-0.280	0.183	0.051	27.87
C:N	7.392-10.133	8.594	0.685	7.98	11.552-28.688	17.703	3.360	18.98
C:P	162.833-640.985	381.811	150.929	39.53	322.684-920.947	542.346	159.889	29.48
N:P	16.563-81.762	45.337	19.508	43.03	14.482-52.186	30.928	7.926	25.63
Growth rate (d^{-1})	0.031-0.098	0.063	0.016	25.12	0.006-0.103	0.056	0.027	48.07
Chl-a ($\mu g\ L^{-1}$)	27.586-891.035	288.046	216.833	75.28	29.195-334.023	131.546	79.743	60.62
C:chl-a	11.315-165.890	59.285	41.215	69.52	38.474-660.929	176.797	100.838	57.04

5.3.3 Responses of algal growth, chl-a and C:chl-a to varying N and P concentrations

The growth rates of *Lyngbya* and *Rhizoclonium* both increased significantly with increasing medium N concentrations, with no differences found in slopes of regression lines of algal growth rates against log-transformed medium N concentrations between algae cultured under low and high P concentrations (Table 5-3; Fig. 5-1a, b). Chl-a of *Lyngbya* and *Rhizoclonium* also increased with greater medium N concentrations, but stronger responses of algal chl-a to increasing N concentrations was found in cultures with high P concentrations (Table 5-3, Fig. 5-1c, d). C:chl-a ratios of *Lyngbya* and *Rhizoclonium* were negatively related with medium N concentrations, and the responses of C:chl-a ratios to increasing medium N concentrations in cultures with low P concentrations were stronger than in cultures with high P concentrations (Table 5-3, Fig. 5-1e, f). Effects of medium P concentrations on growth rates and chl-a of *Lyngbya* and *Rhizoclonium* were similar with that of medium N concentrations (Table 5-3, Fig. 5-2a-d). C:chl-a ratios of *Lyngbya and Rhizoclonium* both decreased with increasing medium P concentrations, and there were no significant differences in regression slopes of C:chl-a ratios versus logarithms of medium P concentrations between algae in cultures with low and high N concentrations (Table 5-3, Fig. 5-2e, f). Increases in medium P concentrations boosted growth rates and chl-a of *Lyngbya and Rhizoclonium* more effectively than increases in N concentrations (Table 5-3). While growth rates of *Rhizoclonium* showed stronger responses to increases in medium N or P concentrations than that of *Lyngbya*, the responses of chl-a of *Lyngbya* was stronger than that of *Rhizoclonium* (Table 5-3). Increases in medium N or P concentrations influenced algal C:chl-a ratios at faster pace in *Rhizoclonium* than in *Lyngbya* (Table 5-3).

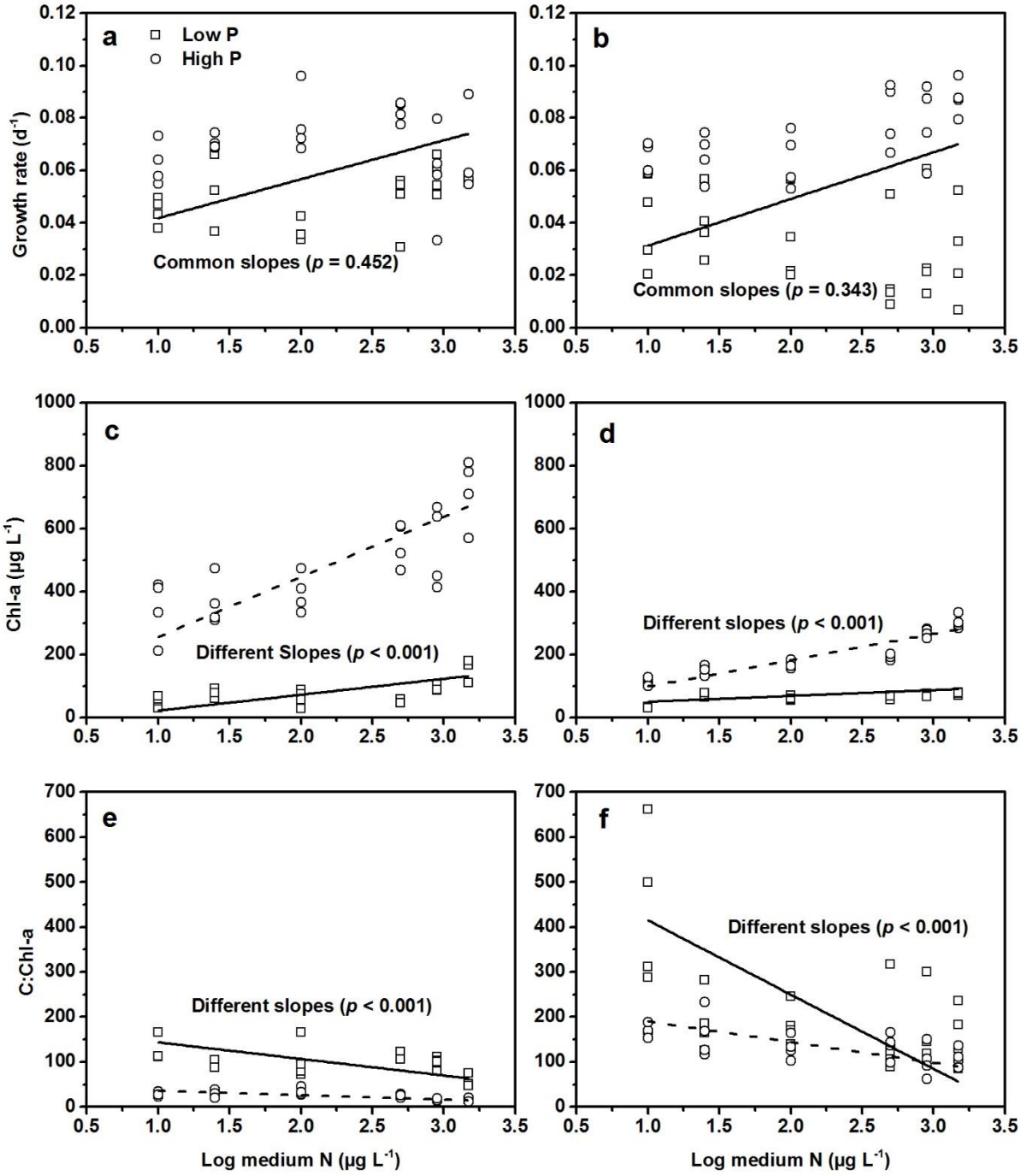

a
Low P
High P
Common slopes (p = 0.452)
b
Common slopes (p = 0.343)
c
Different Slopes (p < 0.001)
d
Different slopes (p < 0.001)
e
Different slopes (p < 0.001)
f
Different slopes (p < 0.001)
Growth rate (d-1)
Chl-a (µg L-1)
C:Chl-a
Log medium N (µg L-1)
Log medium N (µg L-1)

Figure 5-1 The effect of medium N concentration on *Lyngbya* (a) and *Rhizoclonium* (b) growth, *Lyngbya* (c) and *Rhizoclonium* (d) chl-a, *Lyngbya* (e) and *Rhizoclonium* (f) C:chl-a ratios in treatments with low and high P concentrations. In cases where the slopes were not significantly different between treatments with low and high P concentrations, a single regression line based on all data together is plotted. Otherwise, regression lines for treatments with low and high P concentrations are shown with solid and dashed lines respectively. Additional information on regression lines shown in each panel can be found in Table 5-2.

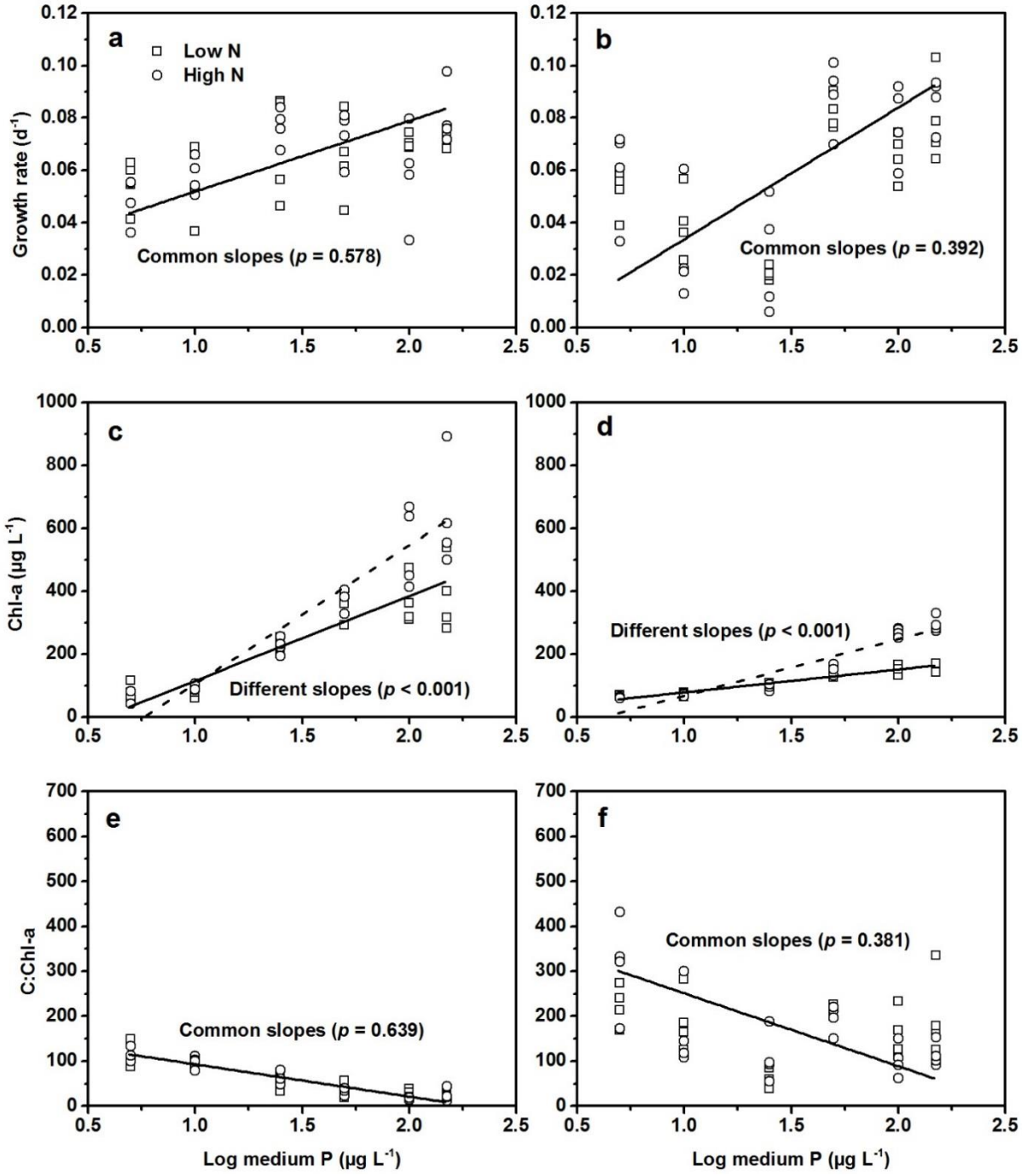

a
Low N
High N
Common slopes (p = 0.578)
b
Common slopes (p = 0.392)
c
Different slopes (p < 0.001)
d
Different slopes (p < 0.001)
e
Common slopes (p = 0.639)
f
Common slopes (p = 0.381)
Growth rate (d-1)
Chl-a (µg L-1)
C:Chl-a
Log medium P (µg L-1)
Log medium P (µg L-1)

Figure 5-2 The effect of medium P concentration on *Lyngbya* (a) and *Rhizoclonium* (b) growth, *Lyngbya* (c) and *Rhizoclonium* (d) chl-a, *Lyngbya* (e) and *Rhizoclonium* (f) C:chl-a ratios in treatments with low and high N concentrations. In cases where the slopes were not significantly different between treatments with low and high N concentrations, a single regression line based on all data together is plotted. Otherwise, regression lines for treatments with low and high N concentrations are shown with solid and dashed lines respectively. Additional information on regression lines shown in each panel can be found in Table 5-2.

Table 5-3 Standard major axis (SMA) regression statistics for growth rates, chl-a, C:chl-a, C:N, C:P and N:P molar ratios of *Lyngbya* and *Rhizoclonium* in N and P bioassay experiments. In cases where there were no detections of significantly different responses to concentrations of the secondary nutrient, common slopes are provided for response variables. Otherwise, slopes for each respective concentrations of secondary nutrient are provided for response variables.

Experiment	Response variables	First factor	Second factor	Slope	*p*
Lyngbya N	Growth rate	Log N		0.015	<0.001
	Chl-a	Log N	Low P	50.55	<0.01
			High P	191.0	<0.001
	C:chl-a	Log N	Low P	-36.85	<0.05
			High P	-10.14	<0.01
	C:N	Log N		-0.755	<0.001
	C:P	Log N	Low P	93.49	<0.01
			High P	43.37	<0.001
	N:P	Log N	Low P	13.69	<0.001
			High P	6.994	<0.001
Lyngbya P	Growth rate	Log P		0.027	<0.001
	Chl-a	Log P	Low N	269.3	<0.001
			High N	440.4	<0.001
	C:chl-a	Log P		-71.92	<0.001
	C:N	Log P		0.842	<0.001
	C:P	Log P		-297.3	<0.001
	N:P	Log P		-37.07	<0.001
Rhizoclonium N	Growth rate	Log N		0.018	<0.001
	Chl-a	Log N	Low P	18.46	<0.001
			High P	83.75	<0.001
	C:chl-a	Log N	Low P	-165.1	<0.01
			High P	-45.92	<0.001
	C:N	Log N		-3.545	<0.001
	C:P	Log N		126.3	0.617
	N:P	Log N		10.47	<0.001
Rhizoclonium P	Growth rate	Log P		0.050	<0.001
	Chl-a	Log P	Low N	72.83	<0.001
			High N	179.2	<0.001
	C:chl-a	Log P		-161.9	<0.05
	C:N	Log P		-5.311	<0.001
	C:P	Log P		-319.7	<0.001
	N:P	Log P		-11.45	<0.001

5.3.4 Responses of algal nutrient stoichiometry to varying N and P concentrations

C:N of *Lyngbya and Rhizoclonium* decreased with increasing medium N concentrations, but C:P and N:P of *Lyngbya and Rhizoclonium* increased with increasing medium N

concentrations (Table 5-3, Fig. 5-3a-f). Responses of C:P and N:P ratios of *Lyngbya* to medium N concentrations varied with P concentrations in media, as those ratios increased more faster with increasing medium N concentrations under low P concentrations than high P concentrations (Table 5-3, Fig. 5-3c, e). Increases in medium P concentrations led to significant decreases in C:P and N:P ratios of *Lyngbya* and C:N, C:P and N:P of *Rhizoclonium*, but resulted in slight increases in C:N ratios of *Lyngbya* (Table 5-3, Fig. 5-4a-f). None of the stoichiometric ratios exhibited different responses to increases in medium P concentrations between cultures under low and high medium N concentrations (Table 5-3, Fig. 5-4a-f). C:P ratios of *Rhizoclonium* had more stronger responses to changes in medium N or P concentrations than *Lyngbya* (Table 5-3).

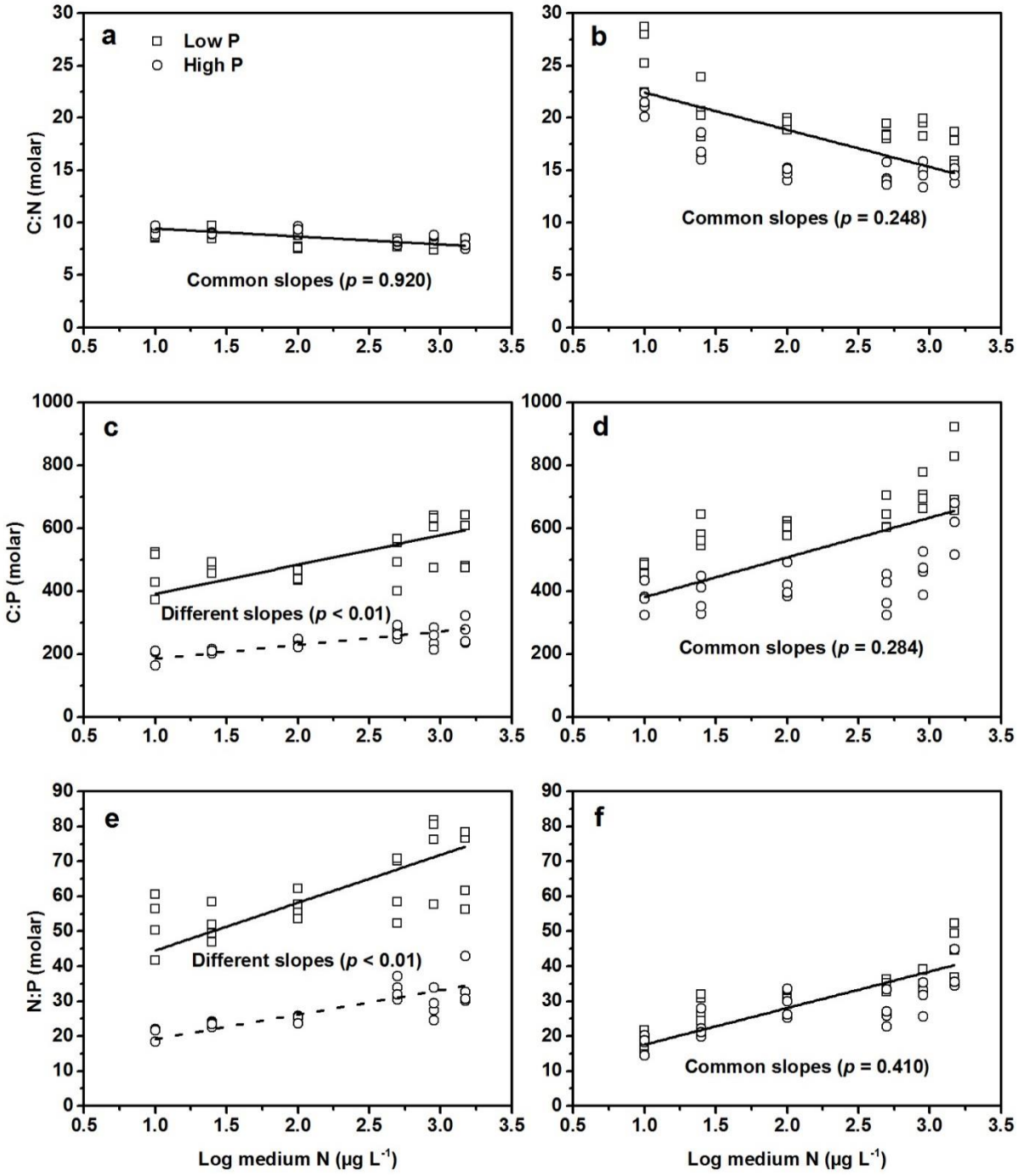

a
Low P
High P
C:N (molar)
Common slopes (p = 0.920)
b
Common slopes (p = 0.248)
c
C:P (molar)
Different slopes (p < 0.01)
d
Common slopes (p = 0.284)
e
N:P (molar)
Different slopes (p < 0.01)
f
Common slopes (p = 0.410)
Log medium N (µg L-1)
Log medium N (µg L-1)

Figure 5-3 The effect of medium N concentration on *Lyngbya* (a) and *Rhizoclonium* (b) molar C:N ratios, *Lyngbya* (c) and *Rhizoclonium* (d) molar C:P ratios, *Lyngbya* (e) and *Rhizoclonium* (f) molar N:P ratios in treatments with low and high P concentrations. In cases where the slopes were not significantly different between treatments with low and high P concentrations, a single regression line based on all data together is plotted. Otherwise, regression lines for treatments with low and high P concentrations are shown with solid and dashed lines respectively. Additional information on regression lines shown in each panel can be found in Table 5-2.

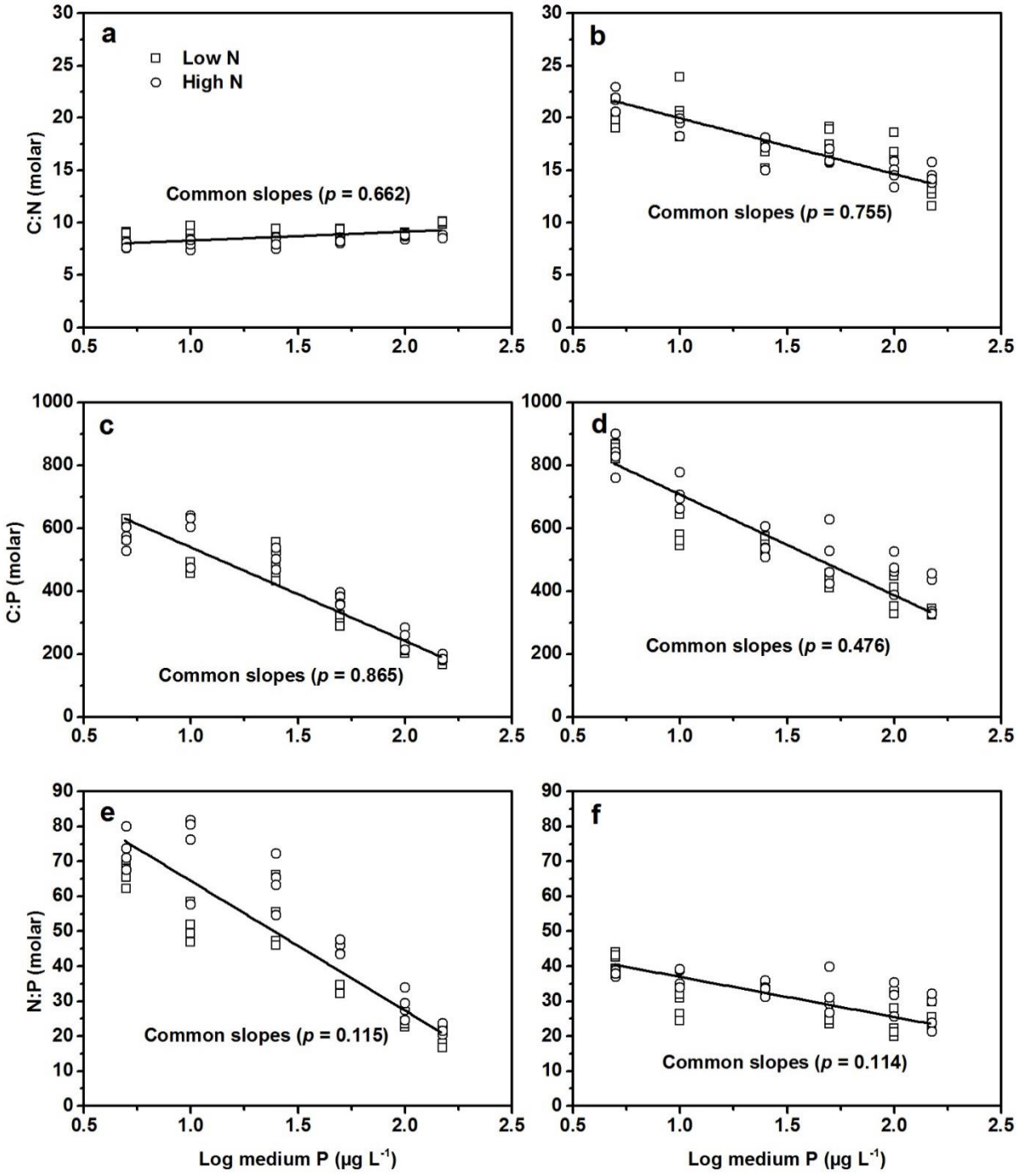

a
Low N
High N
Common slopes (p = 0.662)
b
Common slopes (p = 0.755)
c
Common slopes (p = 0.865)
d
Common slopes (p = 0.476)
e
Common slopes (p = 0.115)
f
Common slopes (p = 0.114)
C:N (molar)
C:P (molar)
N:P (molar)
Log medium P (µg L-1)
Log medium P (µg L-1)

Figure 5-4 The effect of medium P concentration on *Lyngbya* (a) and *Rhizoclonium* (b) molar C:N ratios, *Lyngbya* (c) and *Rhizoclonium* (d) molar C:P ratios, *Lyngbya* (e) and *Rhizoclonium* (f) molar N:P ratios in treatments with low and high N concentrations. In cases where the slopes were not significantly different between treatments with low and high N concentrations, a single regression line based on all data together is plotted. Otherwise, regression lines for treatments with low and high N concentrations are shown with solid and dashed lines respectively. Additional information on regression lines shown in each panel can be found in Table 5-2.

5.3.5 Homeostatic properties of algal elemental nutrient composition

Algal N:P ratios increased significantly with medium N:P ratios (Fig. 5-5a, b). The reciprocal of the regulation coefficient (1/H), represented by the slopes of regression lines between logarithms of algal N:P ratios and logarithms of medium N:P ratios were 0.154 and 0.089 for *Lyngbya* and *Rhizoclonium* respectively, and the regression lines met the 1:1 line passing through origin of coordinate system at around X = 1.716 and 1.518 for *Lyngbya* and *Rhizoclonium* respectively (Fig. 5-5a, b). Algal N or P contents were elevated in cultures with greater N or P concentrations (Fig. 5-5c-f). Increasing medium P concentrations exert stronger influences on algal P contents than the influences of increasing medium N concentrations on algal N contents (Fig. 5-5c-f). While responses of algal N contents to higher medium N concentrations in *Lyngbya* was similar with that in *Rhizoclonium*, much sharper increases of algal P contents with increasing medium P concentrations was found in *Lyngbya* than in *Rhizoclonium* (Fig. 5-5c-f).

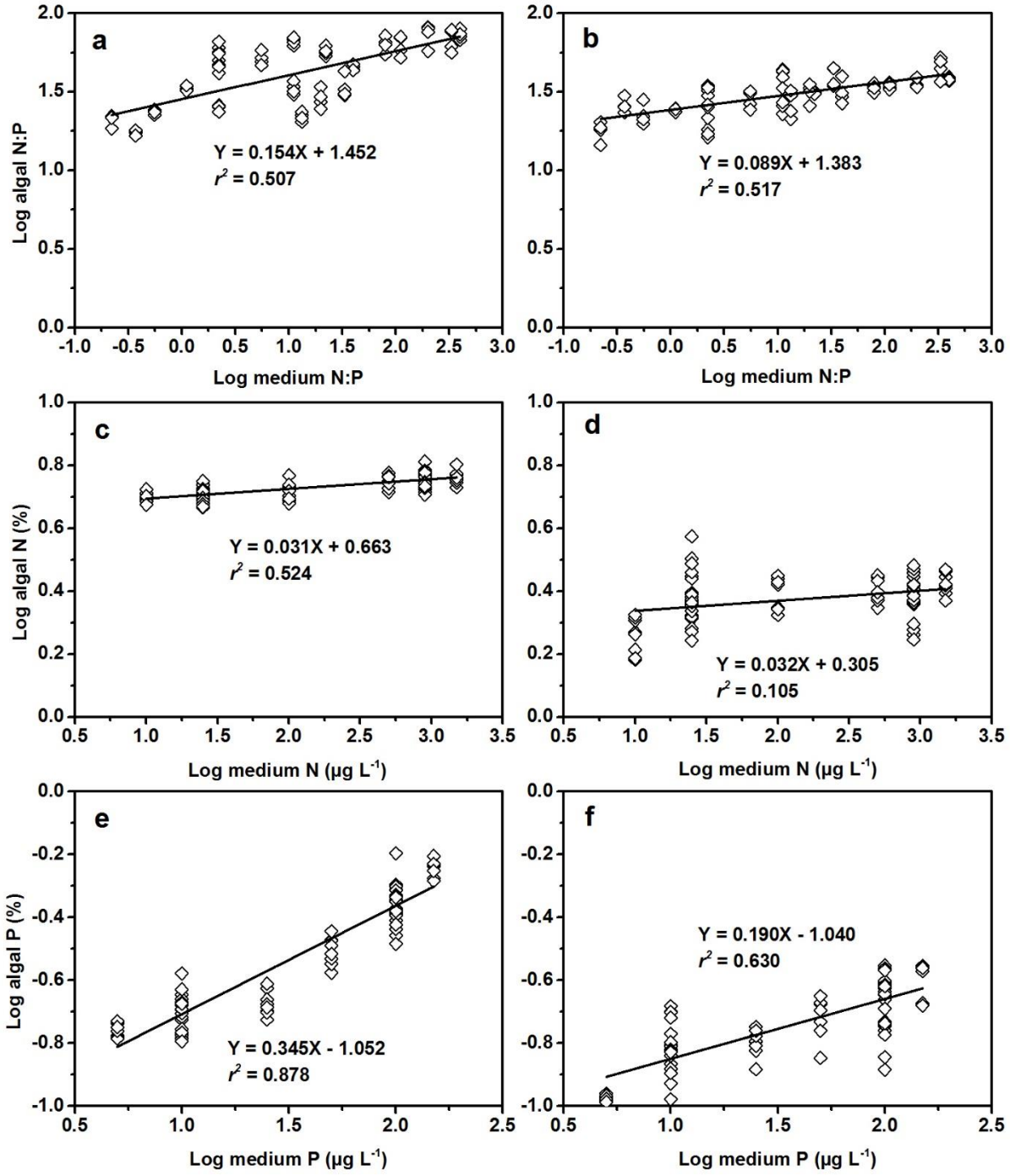


Figure 5-5 Relationship between the logarithms of medium N:P and *Lyngbya* N:P molar ratios (a), medium N:P and *Rhizoclonium* N:P molar ratios (b), medium N concentrations and *Lyngbya* N contents (c), medium N concentrations and *Rhizoclonium* N contents (d), medium P concentrations and *Lyngbya* P contents (e), medium P concentrations and *Rhizoclonium* N contents (f) in cultured samples.

5.4 Discussion

Increases in either N or P concentrations stimulated growth of *Lyngbya* and *Rhizoclonium*, and the extent to which the growth was elevated was irrespective of the levels of the secondary nutrient. A few previous studies have reported results contrary to that of ours, as they found *Lyngbya* cultured under high nutrients had similar or even slightly lower growth rates than *Lyngbya* cultured under low nutrients (Cowell and Botts 1994; Yin et al. 1997). The extremely low growth of *Lyngbya* under very high N and/or P concentrations may be due to direct inhibitory effect of nutrient toxicity or because of the limited availability of other minerals especially trace elements (Pinowska et al. 2007; Yin et al. 1997). However, the continuing accumulation of biomass of filamentous algae, despite severe limitation of N and/or P, has also been observed in other studies (Albertin et al. 2007; Middleton and Frost 2014). Albertin et al. (2007) found that *Lyngbya wollei* mats could still maintain growth rates at values ranging from 0.04 to 0.10 d^{-1} in recirculating stream channels after 28 days of experiment when nitrate and SRP concentrations in several treatments were as low as 0.005 and 0.001 mg L^{-1} respectively. Middleton and Frost (2014) reported the growth rates of *Mougeotia* between 0.25 and 0.32 d^{-1} in laboratory cultures, and the lowest concentrations of N and P were only 56 and 3 µg L^{-1}. The lack of nutrient limitation of growth may partially be explained by physiological acclimation of algae to low ambient nutrient concentrations, such as reduced quota in algal cells grown under low nutrient conditions compared to high nutrient conditions (Middleton and Frost 2014). If these filamentous algae need to be suppressed, control of both N and P inputs into the ponds should still be addressed when making management plans for the conservation of Ewens Ponds ecosystem (Paerl et al. 2016b).

The maximum growth rate of filamentous algae was approximately 0.1 d^{-1}, which was lower or similar to the growth rates of other species of filamentous algae (Borchardt 1996). The growth rates of *Lyngbya* cultured in jars in this study were lower than that cultured in

microcentrifuge tubes or circular raceways as microcosms in Stevenson et al. (2007), but similar to that cultured in flasks or dishes in Yin et al. (1997) and Cowell and Botts (1994). The growth rates of *Rhizoclonium* cultured in jars in this study were lower or similar to that cultured in petri dishes in (Chao et al. 2005). The relatively slow growth of *Lyngbya* and *Rhizoclonium* in this study could be an inherent nature of the strains of these two algal from Ewens Ponds, alternatively it could also be a result of biased representation of the filamentous algal growth under controlled laboratory conditions. Previous studies have shown that the growth rates of filamentous algae varied with the amount of sample biomass or length used in the measurement (Albertin et al. 2007; Chao et al. 2005; Stevenson et al. 2007). Stevenson et al. (2007) found *Lyngbya* cultured in mesocosms starting from mats with initial fresh weight at around 1 g had lower growth rates than *Lyngbya* cultured in microcosms starting from only one filament or mats with initial fresh weight at around 10 mg. Pinowska et al. (2007) found that the growth rates of *Lyngbya* in the microcosms with five filaments or high fresh weight of the mat fragments were lower than that in the microcosms with only one filament or low fresh weight of the mat fragments, while Chao et al. (2005) observed that *Rhizoclonium* filaments with short length (2 mm) had higher specific growth rates than filaments with long length (20 mm). It appears that the growth rate of filamentous algae is negatively related to the size of the community, and this could potentially be a result of either light or nutrient limitation of algal growth within the clump of filaments. Filaments on the outside of the clump would experience greater light intensities and shade filaments on the inside of the clump, similarly there may be a nutrient gradient through the cluster of filaments with preferential uptake by filaments on the outside of the cluster. We measured the growth rates of *Lyngbya* on algal mats with initial blotted weight at around 10 mg, but the minimum and maximum growth rates we measured was still lower than that measured on algal mats cultured in microcosms starting from an initial fresh weight

similar with ours. The range of growth rates of *Lyngbya* was between 0 and 0.1 d^{-1} in this study, overlapping with the range of the growth rates of *L. wollei* mats cultured under conditions of low P in mesocosms starting from much greater initial fresh weight at around 1 g (Albertin et al. 2007). In addition to differences in initial biomass or filaments' length of algae used in culture, different growth rates between studies could also arise from the differences in time length of the experiment. Albertin et al. (2007) measured the growth rates of *L. wollei* at different stages of the experiment, and found a gradual decrease in the growth rates of *L. wollei* with time. We only measured the dry weight of *Lyngbya* and *Rhzoclonium* at the end of the experiment and calculated growth rates averaged during the whole experiment period, thus potentially masking faster growth occurring at the beginning of the experiments compared to the currently measured growth. The relatively slow but steady growth of both *Lyngbya* and *Rhizoclonium*, as seen here, could have significant effects on community composition and food-web structure in Ewens Ponds by forming large masses of floating mats and subsequently smothering other primary producers, altering trophic pathways.

Chl-a in *Lyngbya* and *Rhizoclonium* responded positively to increases in medium N or P concentrations as primary nutrient, and the responses were stronger in treatments receiving high dose of the secondary nutrient. Lower chlorophyll contents of phytoplankton cultures have been reported during N and P deficiency (Riemann et al. 1989). The synthesis of cellular photosynthetic apparatus could be constrained by the availability of N, due to the high protein content of photosynthetic apparatus (Raven 1984). P limitation is also known to reduce chlorophyll production because insufficient P would constrain synthesis of phospholipid, an important component of the membrane-rich chloroplasts (Hessen et al. 2002). Decreasing C:chl-a ratios with increasing medium N/P concentrations was caused by disproportionate enhancement of chlorophyll-a production to carbon fixation under high

medium N or P concentrations. While there were differences in the responses of algal C:chl-a ratios to medium N concentrations between treatments with low and high P concentrations, the responses of algal C:chl-a ratios to medium P concentrations between treatments with low and high N concentrations were similar, indicating that C fixation was more sensitive to P deficiency. Change of C:chl-a ratios observed for *Lyngbya* and *Rhizoclonium* largely matched that expected for algae with different nutrient availability, and this parameter may be used to indicate nutrient condition for algae growth in the field.

We found both *Lyngbya* and *Rhizoclonium* exhibited flexible stoichiometry in response to varying N and P concentrations in laboratory cultures. Greater N availability decreased algal C:N ratios and increased C:P and N:P ratios, regardless of P concentrations in this study. This indicates greater P limitation associated with elevated N concentrations, as have been observed in Ewens Ponds and many other freshwater ecosystems with heavy N loadings (Elser et al. 2009; Liess et al. 2009; Rigosi et al. 2015). Similar results were found for *Lyngbya* mats cultured in mesocosms simulating recirculating stream channels where the P concentrations was low at 0.009 mg L^{-1} (Albertin et al. 2007). The increasing C:P ratios with elevated medium N concentrations may be due to more C uptake stimulated than P uptake, as suggested by Sistla et al. (2015). Middleton and Frost (2014) found the C:N ratios for benthic filamentous algae *Mougeotia* decreased with increasing N concentrations irrespective of the P level, but algal C:P and N:P ratios increased with increasing N concentrations only under low P level. The fact that C:P and N:P ratios of *Mougeotia* were largely unresponsive to changes in medium N concentrations under high P level may be a violation of the speculation that more uptake of C compared to P was stimulated by N addition. We observed stronger responses of C:P and N:P ratios to increases in medium N concentrations in treatments with low P than high P for *Lyngbya* in this study. Higher N concentrations have been suggested to be able to enhance the ability of organisms to take up P, and more uptake of P can counteract

some effects of the dilution of cellular P by increased C and N with increasing medium N when P supply was sufficient under high P level (Bracken et al. 2015). However, increasing P uptake efficiency may not be able to mitigate the dilution effects under low P level because of the existence of co-limitation of both N and P supply, leading to the faster increases in algal C:P and N:P ratios to increases in medium N concentrations in treatments with low P than high P. Increases in medium P concentrations increased C:N ratios of *Lyngbya* but not C:N ratios of *Rhizoclonium*, suggesting P addition boosted N uptake more than C fixation in *Rhizoclonium*. The synergistic effects of P availability on N use was not observed for both *Lyngbya* and *Rhizoclonium*. Saturation of N uptake in *Lyngbya* would have eliminated the synergistic effects of P availability on N use in *Lyngbya* in treatments with both low and high N level, considering that *Lyngbya* may be not limited by N at all in P bioassay experiments because of their potential ability of N fixation as cyanobacterium (Yin et al. 1997). Alternatively, the synergistic effects of P availability on N use in *Lyngbya* and Rhizoclonium may not exist due to the lack of P regulation of processes associated with N uptake such as protein synthesis (Hessen et al. 2017). The physiological acclimation as seen here would increase nutrient-use efficiency, delivering nutrients to the most essential processes in cells, and such regulation at least partly explained the continuing growth of both algae under severe nutrient limitation in this study.

Relatively high variability in algal P contents largely contributed to the relatively high variability in algal C:P and N:P ratios, and algal C and N contents and C:N ratios showed less variations. We found greater flexibility of C:N ratios in response to N enrichment and greater flexibility of C:P ratios in response to P enrichment in *Rhizclonium* than *Lyngbya*, which were consistent with the hypotheses by Sistla et al. (2015). They hypothesized the C:nutrient stoichiometric flexibility in response to fertilization with that nutrient would increase as environmental or biological fertility declined for that given nutrient, and we found higher

mean algal N and P content, as well as lower C:N and C:P ratios, in *Lyngbya* than *Rhizoclonium*, suggesting *Rhizoclonium* was more N- and P-limited than *Lyngbya*. Two distinct but potentially complementary mechanisms may be responsible for the reduced stoichiometric flexibility with greater nutrient availability, firstly organisms with relatively high content of one elemental nutrient are less likely to take up additional amount of that specific nutrient, and secondly organisms with relatively high content of one elemental nutrient might have greater ability to rapidly increase C fixation in response to the enrichment of that specific nutrient (Sistla et al. 2015). We observed stronger responses of N:P ratios to increasing medium P concentrations in *Lyngbya* than *Rhizoclonium*, while responses of N:P ratios to increasing medium N concentrations in *Rhizoclonium* were not always stronger than *Lyngbya*. Sistla et al. (2015) suggested relatively small stoichiometric plasticity in response to fertilization of certain nutrient when that nutrient is less limiting. Our results were in accordance with that hypothesis since *Lyngbya* experienced stronger P limitation than *Rhizoclonium* in P bioassay experiment. We observed that increases in medium N concentrations increased algal C:P ratios and the impacts on algal C:P ratios was stronger for *Rhizoclonium* than *Lyngbya*, more severe N limitation in *Rhizoclonium* than *Lyngbya* may have created greater potential of N addition to increase C acquisition in N bioassay experiment (Sistla et al. 2015). Future work should focus on determining molecular mechanisms controlling the stoichiometric plasticity of these algae.

We conducted linear regressions between logarithms of algal N:P ratios and logarithms of medium N:P ratios in Cartesian coordinate system and calculated parameters of the linear equations to assess degree of N:P homeostasis in *Lyngbya* and *Rhizoclonium*. A high level of N:P homeostasis was revealed in *Lyngbya* and *Rhizoclonium* as reciprocal of the homeostasis coefficient 1/H, represented by the slopes of regression lines, were 0.154 and 0.089 respectively, which were in the range between 0 and 0.25 and can be classified as

homeostatic according to Persson et al. (2010). *Lyngbya* has been shown to be highly homeostatic regarding tissue stoichiometry (Nifong et al. 2014). The X coordinate of the meeting point of the regression lines with 1:1 line was 1.716 and 1.518 for *Lyngbya* and *Rhizoclonium* respectively. Thus, *Lyngbya* and *Rhizoclonium* N:P ratios tend to be higher than medium N:P ratios when medium N:P ratios were below 52 and 33 respectively, otherwise algal N:P ratios tend to be lower than that in medium. The lower N:P ratio at the inflection point in *Rhizoclonium* than *Lyngbya*, suggesting *Rhizoclonium* might have lower tolerance to P limitation than *Lyngbya*. Although variability in medium N concentrations induced variability in algal N contents, the magnitude of this response was relatively small. The very little response of N contents in *Lyngbya* and *Rhizoclonium* to varying medium N concentrations reflected very limited species-specific capacity for N storage in these algae from this site. The highly stable cellular N contents in these two algae may be due to the extended persistence of very high N levels in Ewens Ponds from where the strains were collected. In contrast with N, variability in medium P concentrations induced much larger variability in algal P contents, indicating high level of physiological plasticity in processes related to P uptake and storage. Stronger acclimation of cellular P contents to changes in environmental P availability in *Lyngbya* than *Rhizoclonium* may reflect ecological or evolutional adaptions to high degree of mismatch between environmental resource availability and organismal demand (Sistla et al. 2015).

Middleton and Frost (2014) presented similar results with ours, but they used another test species, *Mougeotia*. Thus, replication studies should be conducted more often to examine and consolidate the previously recognized scientific facts. In that sense, by using the same experiment design and data analysis method, we were able to confirm that noxious benthic filamentous algae species may share common physiological traits which seem to contribute to their success of proliferation in many of the world's freshwater ecosystems.

References

Albertin, A., J. O. Sickman, M. Anderson, A. Pinowska & R. J. Stevenson, 2007. Macroalgal responses to nitrate additions under conditions of low phosphorus in recirculating stream channels. Florida Department of Environmental Protection, Tallahassee, FL, USA.

Andersen, R. A., 2005. Algal culturing techniques. Elsevier Academic Press, London, UK.

APHA, 2005. Standard Methods for the Examination of Water and Wastewater, 21st ed. American Public Health Association, Washington DC, USA.

Borchardt, M. A., 1996. Nutrients. In Stevenson, R. J., M. L. Bothwell & R. L. Lowe (eds) Algal Ecology: Freshwater Benthic Ecosystems. Academic Press, New York, NY, USA, 183-227.

Bracken, M. E. S., H. Hillebrand, E. T. Borer, E. W. Seabloom, J. Cebrian, E. E. Cleland, J. J. Elser, D. S. Gruner, W. S. Harpole, J. T. Ngai & J. E. Smith, 2015. Signatures of nutrient limitation and co-limitation: responses of autotroph internal nutrient concentrations to nitrogen and phosphorus addition. Oikos 124:113-121.

Carmody, G., 2006. Save Ewens Ponds! Marine Life Society of South Australia Journal:55-65.

Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley & V. H. Smith, 1998. Nonpoint pollution of surface waters with phosphorous and nitrogen. Ecological Applications 8:559-568.

Chao, K.-P., C.-S. Chen, E. I.-C. Wang & Y.-C. Su, 2005. Aquacultural characteristics of Rhizoclonium riparium and an evaluation of its biomass growth potential. Journal of Applied Phycology 17:67-73.

Cowell, B. C. & P. S. Botts, 1994. Factors influencing the distribution, abundance and growth of *Lyngbya wollei* in central Florida. Aquatic Botany 49:1-17.

Elser, J. J., M. Kyle, L. Steger, K. R. Nydick & J. S. Baron, 2009. Nutrient availability and phytoplankton nutrient limitation across a gradient of atmospheric nitrogen deposition. Ecology 90:3062-3073.

Falster, D. S., D. I. Warton & I. J. Wright, 2006. SMATR: Standardised major axis tests and routines, ver 2.0. (online) Available at: http://www.bio.mq.edu.au/ecology/SMATR/. (accessed Spetember, 2016). In. 2016.

Gaget, V., A. R. Humpage, Q. Huang, P. Monis & J. D. Brookes, 2017. Benthic cyanobacteria: A source of cylindrospermopsin and microcystin in Australian drinking water reservoirs. Water Research 124:454-464.

Grandfield, L. & G. Ashman, 1984. Ewens Ponds Management Plan Draft. Department of Lands, South East Region.

Halvorson, H. M. & G. E. Small, 2016. Observational field studies are not appropriate tests of consumer stoichiometric homeostasis. Freshwater Science 35:1103-1116.

Hart, D. D., B. J. F. Biggs, V. I. Nikora & C. A. Flinders, 2013. Flow effects on periphyton patches and their ecological consequences in a New Zealand river. Freshwater Biology 58:1588-1602.

Hessen, D. O., P. J. Færøvig & T. Andersen, 2002. Light, nutrients, and P:C ratios in algae: grazer performance related to food quality and quantity. Ecology 83:1886-1898.

Hessen, D. O., O. T. Hafslund, T. Andersen, C. Broch, N. K. Shala & M. W. Wojewodzic, 2017. Changes in stoichiometry, cellular RNA, and alkaline phosphatase activity of *Chlamydomonas* in response to temperature and nutrients. Frontiers in Microbiology 8:Article 18.

Hilton, J., M. O'Hare, M. J. Bowes & J. I. Jones, 2006. How green is my river? A new paradigm of eutrophication in rivers. Science of the Total Environment 365:66-83.
Hudon, C., A. Cattaneo, A.-M. T. Poirier, P. Brodeur, P. Dumont, Y. Mailhot, J.-P. Amyot, S.-P. Despatie & Y. d. Lafontaine, 2012. Oligotrophication from wetland epuration alters the riverine trophic network and carrying capacity for fish. Aquatic Sciences 74:495-511.
Hudon, C., M. D. Sève & A. Cattaneo, 2014. Increasing occurrence of the benthic filamentous cyanobacterium *Lyngbya wollei*: a symptom of freshwater ecosystem degradation. Freshwater Science 33:606-618.
Irfanullah, H. M. & B. Moss, 2005. A filamentous green algal-dominated temperate shallow lake: variations on the theme of clear-water stable states? Archiv für Hydrobiologie 163:25-47.
Lévesque, D., C. Hudon, J.-P. Amyot & A. Cattaneo, 2015. Wave exposure and current regulate biomass accumulation of the benthic cyanobacterium *Lyngbya wollei* in a large fluvial lake. Freshwater Science 34:867-880.
Lewin, J., 1966. Silicon metabolism in diatoms. V. Germanium dioxide, a specific inhibitor of diatom growth. Phycologia 6:1-12.
Liess, A., S. Drakare & M. Kahlert, 2009. Atmospheric nitrogen-deposition may intensify phosphorus limitation of shallow epilithic periphyton in unproductive lakes. Freshwater Biology 54:1759-1773.
Middleton, C. M. & P. C. Frost, 2014. Stoichiometric and growth responses of a freshwater filamentous green alga to varying nutrient supplies: slow and steady wins the race. Freshwater Biology 59:2225-2234.
Nifong, R. L., 2017. Experimental effects of grazers on autotrophic species assemblages across a nitrate gradient in Florida springs. Aquatic Botany 139:57-64.
Nifong, R. L., M. J. Cohen & J. Wendell P. Cropper, 2014. Homeostasis and nutrient limitation of benthic autotrophs in natural chemostats. Limnology and Oceanography 59:2101-2111.
O'Neil, J. M., T. W. Davis, M. A. Burford & C. J. Gobler, 2012. The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate change. Harmful Algae 14:313-334.
Paerl, H. W., W. S. Gardner, K. E. Havens, A. R. Joyner, M. J. McCarthy, S. E. Newell, B. Qin & J. T. Scott, 2016a. Mitigating cyanobacterial harmful algal blooms in aquatic ecosystems impacted by climate change and anthropogenic nutrients. Harmful Algae 54:213-222.
Paerl, H. W., J. T. Scott, M. J. McCarthy, S. E. Newell, W. S. Gardner, K. E. Havens, D. K. Hoffman, S. W. Wilhelm & W. A. Wurtsbaugh, 2016b. It takes two to tango: when and where dual nutrient (N & P) reductions are needed to protect lakes and downstream ecosystems. Environmental Science & Technology 50:10805-10813.
Persson, J., P. Fink, A. Goto, J. M. Hood, J. Jonas & S. Kato, 2010. To be or not to be what you eat: regulation of stoichiometric homeostasis among autotrophs and heterotrophs. Oikos 119:741-751.
Pinowska, A., R. J. Stevenson, J. O. Sickman, A. Albertin & M. Anderson, 2007. Integrated interpretation of survey and experimental approaches for determining nutrient thresholds for macroalgae in Florida springs. Florida Department of Environmental Protection, Tallahassee, FL, USA.
Raven, J. A., 1984. A cost-benefit analysis of photon absorption by photosynthetic unicells. New Phytologist 98:593-625.

Riemann, B., P. Simonsen & L. Stensgaard, 1989. The carbon and chlorophyll content of phytoplankton from various nutrient regimes. Journal of Plankton Research 11:1037-1045.

Rigosi, A., Y. Liu, M. Shanafield & J. D. Brookes, 2015. Determining environmental risks to Ewens Ponds in the South East. Goyder Institute for Water Research Technical Report Series No. 15/19, Adelaide, South Australia.

Sartory, D. P. & J. U. Grobbelaar, 1984. Extraction of chlorophyll *a* from freshwater phytoplankton for spectrophotometric analysis. Hydrobiologia 114:177-187.

Scheffer, M., 1998. Ecology of Shallow Lakes, 1st Edition. Chapman and Hall, London.

Sistla, S. A., A. P. Appling, A. M. Lewandowska, B. N. Taylor & A. A. Wolf, 2015. Stoichiometric flexibility in response to fertilization along gradients of environmental and organismal nutrient richness. Oikos 124:949-959.

Stanier, R. Y., R. Kunisawa, M. Mandel & G. Cohen-Bazire, 1971. Purification and properties of unicellular blue-green algae (order *Chroococcales*). Bacteriological Reviews 35:171-205.

Sterner, R. W. & J. J. Elser, 2002. Ecological stoiochiometry: The biology of elements from molecules to the biosphere. Princeton University Press.

Stevenson, R. J., B. J. Bennett, D. N. Jordan & R. D. French, 2012. Phosphorus regulates stream injury by filamentous green algae, DO, and pH with thresholds in reponses. Hydrobiologia 695:25-42.

Stevenson, R. J., A. Pinowska, A. Albertin & J. O. Sickman, 2007. Ecological condition of algae and nutrients in Florida springs: The synthesis report. Florida Department of Environmental Protection, Tallahassee, FL, USA.

Sturt, M. M., M. A. K. Jansen & S. S. C. Harrison, 2011. Invertebrate grazing and riparian shade as controllers of nuisance algae in a eutrophic river. Freshwater Biology 56:2580-2593.

Wetzel, R. G., 1996. Benthic algae and nutrient cycling in lentic freshwater ecosystems. In Stevenson, R. J., M. L. Bothwell & R. L. Lowe (eds) Algal Ecology: Freshwater Benthic Ecosystems. Academic Press, New York, NY, USA, 641-667.

Wood, S. A., J. Atalah, A. Wagenhoff, L. Brown, K. Doehring, R. G. Young & I. Hawes, 2017. Effect of river flow, temperature, and water chemistry on proliferations of the benthic anatoxin-producing cyanobacterium *Phormidium*. Freshwater Science 36:63-76.

Yin, Q., W. W. Carmichael & W. R. Evans, 1997. Factors influencing growth and toxin production by cultures of the freshwater cyanobacterium *Lyngbya wollei* Farlow ex Gomont. Journal of Applied Phycology 9:55-63.

Chapter 6 General Discussion

This thesis investigated the major basal sources supporting consumers in the benthic food web of a groundwater-fed freshwater wetland, as well as the elemental nutrient stoichiometry and fatty acid composition of basal resources and consumers in that food web. Based on the results of these two field surveys, we further conducted laboratory experiments to assess effects of light and nutrient conditions on periphyton and grazing snail growth and stoichiometry and responses of benthic filamentous algal growth and stoichiometry to varying N and P concentrations. This thesis improves our understanding of food web structure and nutrition in aquatic ecosystems, provides new insights into influences of environmental factors on producers and consumers in benthic habitats, and has application in the protection of valuable freshwater environments.

While stable phytoplankton communities are unable to establish in Ewens Ponds because of high flush rate, consumers have access to several basal food resources including epiphytes and benthic filamentous cyanobacteria in Ewens Ponds. These basal food resources varied with regard to their availability and quality. Early studies hypothesized that aquatic macrophytes may have low content of N, thus not preferred by grazers, but this study (Chapter 3) together with others refute that hypothesis as there were no significant differences in nitrogen content between algae and macrophytes (Lodge 1991). Macrophytes were postulated to be a poor quality food because of higher C:N and C:P ratios in the tissue than epiphytic algae and benthic algae (Chapter 3), and they were suggested to contain secondary compounds that may reduce grazing such as phenols (Lodge 1991). Macrophytes had low content of essential highly unsaturated fatty acids (HUFAs), however, the content of linoleic acid (LIN) and α-linolenic acid (ALA), which were the precursor of synthesis of HUFAs, were much higher in macrophytes than other basal resources in Ewens Ponds (Chapter 3). Dietary LIN and ALA can be further desaturated and elongated to form HUFAs,

including arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in many invertebrates and fish with varying efficiencies (Bell and Tocher 2009; Guo et al. 2016), but this conversion generally occurs at a level that is too low to support optimal growth (Brett and Müller-Navarra 1997). Therefore, the nutritional quality of macrophytes was also potentially constrained by the content of essential fatty acids, in particular as the food sources for macroinvertebrates.

Elemental stoichiometry may not constrain nutritional quality of benthic filamentous algae as food resources for consumers (Chapter 3), and the poor assimilation of benthic filamentous algae carbon in aquatic consumer may be related to low content of HUFAs, especially EPA, in those algae (Müller-Navarra et al. 2000). Alternatively, the low edibility of benthic filamentous algae to consumers may be attributed to the morphological properties, such as the formation of large filaments with thick sheaths, which may interfere with the ingestion process of consumers, and increase energetic costs by inducing higher rejection and respiration rates (Camacho and Thacker 2006; Martin-Creuzburg et al. 2008). Toxin production was also likely to hamper consumption of benthic filamentous algae and rendered benthic filamentous algae unpalatable for aquatic consumers (Martin-Creuzburg et al. 2008). Epiphytic algae are usually considered as food of high quality for aquatic consumers in Ewens Ponds, because of the similar stoichiometric ratios between this food source and major consumer species, as well as high contents of EPA in the epiphytic algae. In fact, this study demonstrated that epiphytes were the most important source of organic carbon for most benthic macroinvertebrates and fish consumers in a groundwater-fed freshwater wetland system with high macrophyte coverage (Chapter 2). It should be noted that elemental imbalances themselves may not be good indicators of the most likely limiting elements because they don't explicitly consider losses of carbon through respiration. The threshold elemental ratio (TER), critical C : nutrient ratio, above which limitation of growth shift from

C to nutrients, provided meaningful index of the elemental imbalance between a consumer and its food resource (Andersen et al. 2004; Frost et al. 2006; Liess and Lange 2011; Urabe and Watanabe 1992). TER reflects organism's adaption to energy and nutritional constraints with regard to the ambient environment, taking feeding mode, growth rate and body stoichiometry of organism into consideration (Doi et al. 2010; Frost et al. 2006; Liess and Lange 2011). A negative correlation between TER and maximum specific growth rate has been found for aquatic invertebrates, reflecting high demand of P-rich RNA for fast growth (Frost et al. 2006; Hessen et al. 2013).

Some forms of periphyton (e.g. epiphytic algae) were implicated in causing light limitation of macrophyte growth, leading to a regime shift from clear-water state with macrophyte domination to turbid-water state with phytoplankton, benthic or filamentous algae domination (Hilton et al. 2006; Sand-Jensen and Borum 1984; Sand-Jensen and Søndergaard 1981). The possible regime shift in Ewens Ponds, however may be not induced by overgrowth of epiphytic alga because they are postulated to be under heavy grazing pressure (Chapter 4). Periphyton receiving nutrients solely from water collected from Ewens Ponds was only a very thin layer of attached algae, detritus, and other associated organisms when continuously grazed by *G. gibbosa* snails at a density of 200 individuals m^{-2}, which was much lower than the density of grazers in field, in the preliminary mesocosm experiment. We calculated that more than 85% light could pass through periphyton of similar amount of biomass with periphyton in those treatments using established linear regression of periphyton biomass vs light transmission in another study (Figure 6-1). It is speculated that almost all of the periphyton biomass measured on blocks in treatments without nutrient addition was produced within few days of sampling in the preliminary mesocosm experiment. Periphyton quantity can only be lower in field than in mesocosms because of lower periphyton accrual rates due to lower P concentrations in Ewen ponds and higher periphyton removal rates due to higher

grazer density and possibly higher consumption rates per snail. *G. gibbosa* and *P. antipodarum* certainly have the potential to increase feeding rates when provided preferential habitat conditions having low light, as shown in this study and Liess and Lange (2011). Therefore, the adverse effect of periphyton shading on macrophytes should be negligible if the relatively low P concentrations and high snail densities can be maintained in Ewens Ponds.

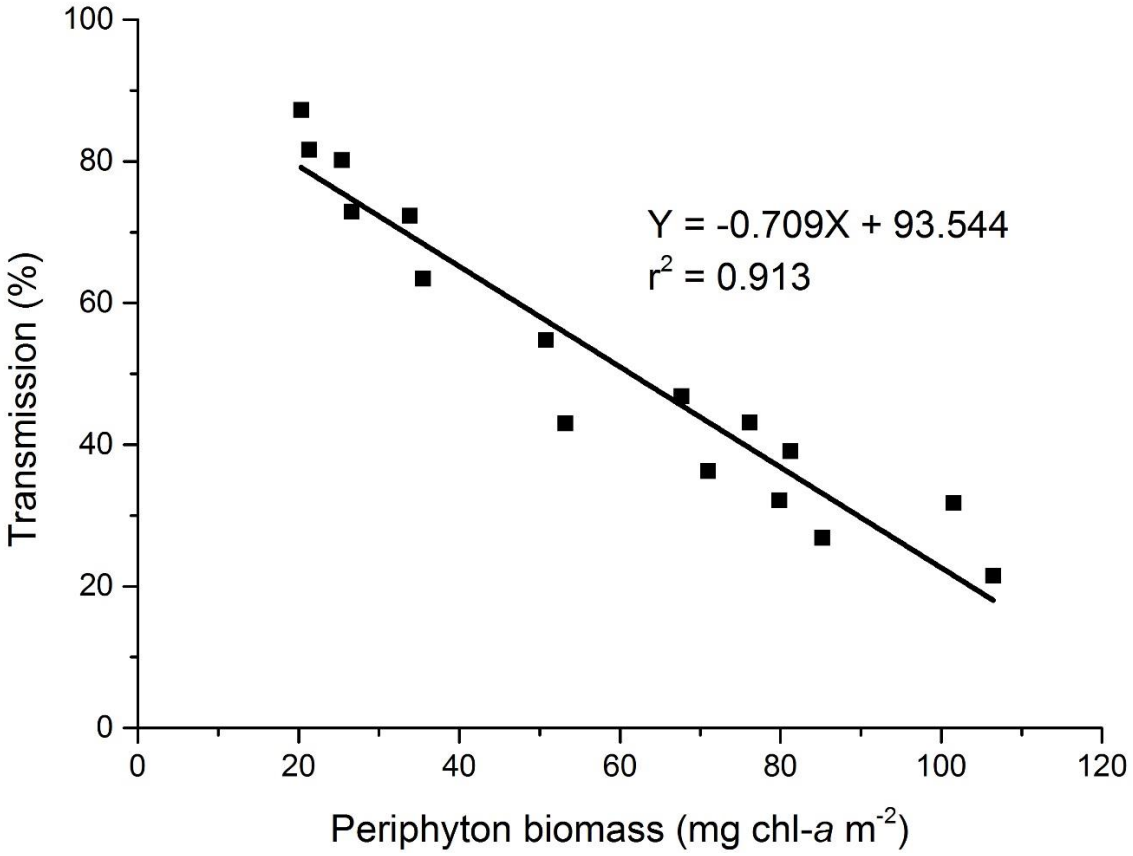


Figure 6-1 Linear regression for light transmission through periphyton vs periphyton biomass.

A considerable risk of deteriorated light climate for submerged macrophyte growth may come from benthic filamentous algae instead of epiphytes. Large floating patches of benthic algae can shade macrophytes for extended periods of time, as long as several months, when

surface bloom of benthic filamentous algae occurs, and deposited mats of those algae tangle around macrophytes after senescence starts, totally blocking any incoming sunlight. Historical trends in N and P concentrations show that N has increased much more than P in Ewens Ponds (Figure 6-2). Strong contrast between variability in DIN and SRP resulted from combinational effects of hydrogeology and land use in the surrounding areas. Changes in land use in the surrounding area from natural peatlands to managed pastures and croplands in the past decades have contributed to the very high nitrate concentrations in pond water. However, SRP concentrations in pond water are relatively low, probably due to the unique geological property of the ponds as they are actually limestone sinkholes. In comparison with medium N:P ratios at the inflection points in both algae genera, DIN:SRP was very high, suggesting strong limitation of algal growth by P in Ewens Ponds. Given historical records of total N (mostly in the form of nitrate) being already higher than 3 mg L^{-1} back to 1980s, which was far more than enough to saturate *Lyngbya* N requirements, recent observations of surface blooms of benthic filamentous algae dominated by *Lyngbya* may not necessarily caused by increases in N concentrations (Stevenson et al. 2007). Heffernan et al. (2010) showed that nitrate concentration didn't explain algal cover and biomass in Florida springs, which have very similar hydrological and geological settings to Ewens Ponds.

In addition to nutrient enrichment, two other factors have been invoked to be responsible for increasing frequency and magnitude of algal blooms recently, climate change acting through hydrological cycles and biological invasion acting through trophic interactions (Knoll et al. 2008; O'Neil et al. 2012). Flush rates of the whole system have increased from around 0.22 days in 1983 to 0.48 days in 2015, and a decrease in flow velocities near pond bottom would be expected (Grandfield and Ashman 1984; Rigosi et al. 2015). According to subsidy-stress mechanisms propose by Biggs et al. (1998), higher velocity can positively affect algal biomass accrual via greater mass transfer of limiting nutrients, but negatively affect algal

biomass accrual via greater sloughing risk due to increased drag and friction. The negative effects may suppress the positive effects of high velocity on benthic algal biomass, leading to an overall adverse effect induced by flush, because N was apparently not limiting and benthic algae could use P released from sediments and P recycled with mats in the field (Pinowska et al. 2007). In contrast, vascular macrophytes usually have firm roots into sediments, thus may be less vulnerable to sloughing under higher flow rates than benthic filamentous algae, thus reduced flow rate are more advantageous to benthic filamentous algae than macrophytes.

It is not clear when the New Zealand mud snail *P. antipodarum* invaded Ewens Ponds and if their invasion affected the abundance of native grazer species in the ponds. However, previous study have reported high densities of *P. antipodarum* were associated with low colonization of native macroinvertebrates (Kerans et al. 2005). The native snail species, *G. gibbosa* may be a more efficient grazer of benthic filamentous algae than *P. antipodarum* as we found greater proportion of filamentous algae in the diets of *G. gibbosa* than *P. antipodarum* (Yang Liu, under review), but the different contributions of filamentous algae to biomass between the two snail species could also be due to niche divergence for food resources caused by strong inter-species competition between the two snail species (Riley et al. 2008). Future studies need to investigate the biomass accumulation of benthic filamentous algae taking interactions of a suite of controlling factors of both accrual and loss processes into consideration.

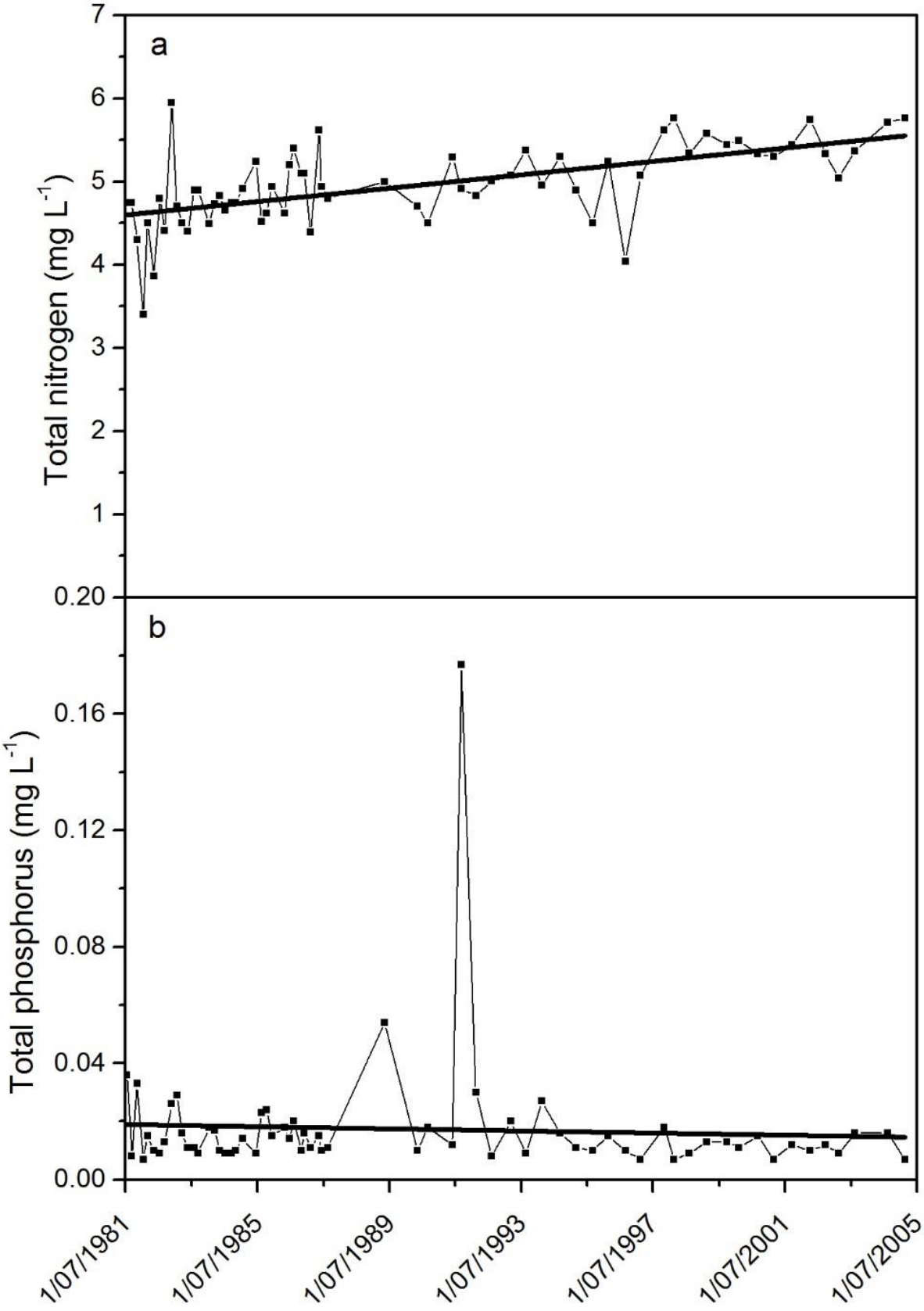

a
b
Total nitrogen (mg L-1)
Total phosphorus (mg L-1)
7
6
5
4
3
2
1
0.20
0.16
0.12
0.08
0.04
0.00
1/07/1981
1/07/1985
1/07/1989
1/07/1993
1/07/1997
1/07/2001
1/07/2005

Figure 6-2 Changes in total nitrogen (a) and total phosphorus (b) concentrations in Ewens Ponds water with time.

The importance of macrophytes in providing critical habitats has been recognized for a long time, they not only serve as substrates for periphyton and epiphytic food, but also act as refuges for avoidance of predators for benthic invertebrates (Newman 1991). Regime shift from macrophytes domination to benthic filamentous algae domination reduced the availability of ideal habitats for many benthic macroinvertebrates, and probably forced many of them to select habitats inferior to macrophytes in some aspects. Snails may prefer to stay in dark environments owing to their instinct to avoid predation or exposure to harmful UV radiations (Liess and Lange 2011). The native bladder snail, *G. gibbosa* was found to spend more time actively grazing under low light than high light, resulting in higher consumption rates of periphyton under low light (Chapter 4). A substitute of macrophyte habitats with low light conditions for snails in Ewens Ponds would be the benthic filamentous algae mats. The loss of macrophyte habitats may negatively affect the population production of these grazers, because of low trophic transfer efficiency from benthic filamentous algae to snails caused by the low food quality of benthic filamentous algae compared to epiphytes. *G. gibbosa* was found to lack the ability to increase their feeding rates to compensate for the low quality of food, and higher C:P ratios in periphyton food significantly decreased its growth (Chapter 4). Although snails may still be able to selectively graze on attached diatoms on algae filaments, the availability of this food source was very low. Benthic macroinvertebrates may also occupy the surface of hard substrate such as limestone where there are periphyton food resources with high quality, however, the risk of predation by fish or crayfish would significantly increase for them. A shift in the feeding preference of benthic macroinvertebrates may be induced by intra- and inter-species competition for the limited

amount of epiphytes, especially when several species with high degree of niche overlap coexist (Aberle et al. 2005). We don't know if this is the case for *G. gibbosa* and *P. antipodarum* in this study, as we sampled the periphyton community as whole. Advances in methods such as the development of compound-specific stable isotope analysis may provide ecologists a potential technical tool to solve this puzzle. Choice of food sources by many small benthic macroinvertebrates may primarily depend on food accessibility and palatability, habitat choice, and their interactions. The age and size of large predators, such as fish, may play a role in determining food resource use and organic carbon assimilation in those animals. There may be ontogenetic shifts in the diet composition of consumers, especially apex predators such as southern short-finned eel, and this topic should be examined in the future.

While many benthic macroinvertebrates species struggle to deal with degraded habitat conditions, some other species have the ability to transform the environment they live in and make it better for their survival. Some species of case-bearing caddisfly larvae and sediment-dwelling chironomids are able to be able to cultivate methane-oxidizing bacteria food on their case or in their tubes, and that they are important for survival during periods of food shortage (Jones and Grey 2011; Trimmer et al. 2009). One species of caddisfly larvae, *Triplectides* sp., was postulated to assimilate organic carbon from basal resources with much lower $\delta^{13}C$ and $\delta^{15}N$ values than macrophytes, epiphytes and filamentous algae, possibly methane-oxidizing bacteria with extremely negative isotopic signatures of both ^{13}C and ^{15}N. It is likely that the organic matter with extraordinary depleted ^{13}C signatures used by *Triplectides* sp. in Ewens Ponds derives from grazing of methanotrophic bacteria from their own cases or those of neighbouring larvae. The feeding strategy may be the key to facilitate their high abundance in Ewens Ponds, given that they are usually found in high density on macrophytes or in benthic

filamentous algae mats where the epiphyte resources should be readily depleted due to fierce intra- and inter-species competition (Chapter 4).

Primary production is probably mostly locked in the form of live macrophytes and benthic filamentous algae in Ewens Ponds, indicated by the distribution of standing stock of N within benthic organisms (Chapter 3). Great accumulation of benthic filamentous algae in Ewens Ponds reflected that the effective grazing control on the biomass of benthic filamentous algae by benthic macroinvertebrates was missing in Ewens Ponds, although benthic macroinvertebrates still frequently inhabit those mats and reached high densities. Production of benthic primary consumers are all severely P-limited (Chapter 3), however, freshwater crab *A. lacustris* represent an important proportion of standing stock of P (Chapter 3). Benthic macroinvertebrates may adopt a series of strategies to cope with this large elemental nutrient imbalances between supply of food resources and demand of their body, such as selective foraging, nutrient retention and case grazing in caddisfly larvae (Mooney et al. 2016). Periphyton on cases of caddisfly larvae may be a dietary resource of high quality, because P limitation of case periphyton may be alleviated by larval excretion, and grazing the P-rich periphyton from the case of conspecifics case potentially promotes P remineralization in P-limited aquatic ecosystems such as Ewens Ponds (Mooney et al. 2014). The production of fish appeared to be more limited by N than P based on the elemental imbalances calculated from actual gut contents (Chapter 3). Although the abundance of caddisfly larvae was high in Ewens Ponds, this prey item was not incorporated into the body tissue of major predator species (Chapter 2). Snails, especially *P. antipodarum*, are also abundant and the mismatch in elemental nutrient stoichiometry between them and fish is the smallest (Chapter 3). However, assimilation of *P. antipodarum* may be hindered by resistance of digestion owing to the hard shell and operculum (James et al. 2000). Consuming shrimp *P. australiensis* possibly require large energetic expenditure as they are agile and fast-moving prey, although the calorific

value of shrimps should be high. Putative consumption of *A. lacustris* and *Austrochiltonia* sp. was not important because they often hide deep within the benthic filamentous algae mats and may be beyond reach of fish predators. Predators may face several obstacles to acquire food of sufficient quantity and quality to meet their physiological demands, and the balance between benefits and costs of taking preys may explain the relatively small production of fish in Ewens Ponds.

Altogether, we demonstrated that epiphytes provided major organic carbon to consumers in Ewens Ponds, and population production of many consumer species was limited by the availability of nutritious food. Elevated food quality of periphyton can be achieved by adding plentiful supply of P. Filamentous cyanobacteria were largely unaffected by nutrient concentrations in water and were not much affected by grazing control, although ingested by some macroinvertebrates. Thus, filamentous cyanobacteria instead of epiphytes pose the greatest threat to the highly prosperous yet fragile ecosystems.

References

Aberle, N., H. Hillebrand, J. Grey & K. H. Wiltshire, 2005. Selectivity and competitive interactions between two benthic invertebrate grazers (*Asellus aquaticus* and *Potamopyrgus antipodarum*): an experimental study using ^{13}C- and ^{15}N-labelled diatoms. Frehswater Biology 50:369-379.

Andersen, T., J. J. Elser & D. O. Hessen, 2004. Stoichiometry and population dynamics. Ecology Letters 7:884-900.

Bell, M. V. & D. R. Tocher, 2009. Biosynthesis of polyunsaturated fatty acids in aquatic ecosystems: general pathways and new directions. In Arts, M. T., M. T. Brett & M. J. Kainz (eds) Lipids in Aquatic Ecosystems. Springer, New York, NY, USA, 211-236.

Biggs, B. J. F., D. G. Goring & V. I. Nikora, 1998. Subsidy and stress responses of stream periphyton to gradients in water velocity as a function of community growth form. Journal of Phycology 34:598-607.

Brett, M. T. & D. C. Müller-Navarra, 1997. The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshwater Biology 38:483-499.

Camacho, F. A. & R. W. Thacker, 2006. Amphipod herbivory on the freshwater cyanobacterium Lyngbya wollei: Chemical stimulants and morphological defenses. Limnology and Oceanography 51:1870-1875.

Doi, H., M. Cherif, T. Iwabuchi, I. Katano, J. C. Stegen & M. Striebel, 2010. Integrating elements and energy through the metabolic dependencies of gross growth efficiency and the threshold elemental ratio. Oikos 119:752-765.

Frost, P. C., J. P. Benstead, W. F. Cross, H. Hillebrand, J. H. Larson, M. A. Xenopoulos & T. Yoshida, 2006. Threshold elemental ratios of carbon and phosphorus in aquatic consumers. Ecology Letters 9:774-779.

Grandfield, L. & G. Ashman, 1984. Ewens Ponds Management Plan Draft. Department of Lands, South East Region.

Guo, F., M. J. Kainz, F. sheldon & S. E. Bunn, 2016. The importance of high-quality algal food sources in stream food webs - current status and future perspectives. Frehswater Biology 61:815-831.

Heffernan, J. B., D. M. Liebowitz, T. K. Frazer, J. M. Evans & M. J. Cohen, 2010. Algal blooms and the nitrogen-enrichment hypothesis in Florida springs: evidence, alternatives, and adaptive management. Ecological Applictions 20:816-829.

Hessen, D. O., J. J. Elser, R. W. Sterner & J. Urabe, 2013. Ecological stoichiometry: An elementary approach using basic principles. Limnology and Oceanography 2013:2219-2236.

Hilton, J., M. O'Hare, M. J. Bowes & J. I. Jones, 2006. How green is my river? A new paradigm of eutrophication in rivers. Science of the Total Environment 365:66-83.

James, M. R., I. Hawes, M. Weatherhead, C. Stanger & M. Gibbs, 2000. Carbon flow in the littoral food web of an oligotrophic lake. Hydrobiologia 441:93-106.

Jones, R. I. & J. Grey, 2011. Biogenic methane in freshwater food webs. Frehswater Biology 56:213-229.

Kerans, B. L., M. F. Dybdahl, M. M. Gangloff & J. E. Jannot, 2005. *Potamopyrgus antipodarum*: distribution, density, and effects on native macroinvertebrate assemblages in the Greater Yellowstone Ecosystem. Journal of the North American Benthological Society 24:123-138.

Knoll, L. B., O. Sarnelle, S. K. Hamilton, C. E. H. Kissman, A. E. Wilson, J. B. Rose & M. R. Morgan, 2008. Invasive zebra mussels (Dreissena polymorpha) increase cyanobacterial toxin concentrations in low-nutrient lakes. Canadian Journal of Fisheries and Aquatic Sciences 65:448-455.

Liess, A. & K. Lange, 2011. The snail *Potamopyrgus antipodarum* grows faster and is more active in the shade, independent of food quality. Oecologia 167:85-96.

Lodge, D. M., 1991. Herbivory on freshwater macrophytes. Aquatic Botany 41:195-224.

Martin-Creuzburg, D., E. v. Elert & K. H. Hoffmann, 2008. Nutritional constraints at the cyanobacteria-*Daphnia magna* interface: The role of sterols. Limnology and Oceanography 53:456-468.

Mooney, R. J., E. A. Strauss & R. J. Haro, 2014. Nutrient recycling by caddisflies alleviates phosphorus limitation in case periphyton. Freshwater Science 33:1086-1092.

Mooney, R. J., E. A. Strauss & R. J. Haro, 2016. Nutrient-specific foraging by *Glossosoma intermedium* larvae leads to conspecific grazing. Freshwater Science 35:873-881.

Müller-Navarra, D. C., M. T. Brett, A. M. Liston & C. R. Goldman, 2000. A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. Nature 403:74-77.

Newman, R. M., 1991. Herbivory and detritivory on freshwater macrophytes by invertebrates: a review. Journal of North American Benthological Society 10:89-114.

O'Neil, J. M., T. W. Davis, M. A. Burford & C. J. Gobler, 2012. The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate change. Harmful Algae 14:313-334.

Pinowska, A., R. J. Stevenson, J. O. Sickman, A. Albertin & M. Anderson, 2007. Integrated interpretation of survey and experimental approaches for determining nutrient thresholds for macroalgae in Florida springs. Florida Department of Environmental Protection, Tallahassee, FL, USA.

Rigosi, A., Y. Liu, M. Shanafield & J. D. Brookes, 2015. Determining environmental risks to Ewens Ponds in the South East. Goyder Institute for Water Research Technical Report Series No. 15/19, Adelaide, South Australia.

Riley, L. A., M. F. Dybdahl & J. Robert O. Hall, 2008. Invasive species impact: asymmetric interactions between invasive and endemic freshwater snails. Journal of North American Benthological Society 27:509-520.

Sand-Jensen, K. & J. Borum, 1984. Epiphyte shading and its effect on photosynthesis and diel matabolism of *Lobelia dortmanna* L. during the spring bloom in a Danish lake. Aquatic Botany 20:109-119.

Sand-Jensen, K. & M. Søndergaard, 1981. Phytoplankton and epiphyte development and their shading effect on submerged macrophytes in lakes of different nutrient status. International Review of Hydrobiology 66:529-552.

Stevenson, R. J., A. Pinowska, A. Albertin & J. O. Sickman, 2007. Ecological condition of algae and nutrients in Florida springs: The synthesis report. Florida Department of Environmental Protection, Tallahassee, FL, USA.

Trimmer, M., A. G. Hildrew, M. C. Jackson, J. L. Pretty & J. Grey, 2009. Evidence for the the role of methane-derived carbon in a free-flowing, lowland river food web. Limnology and Oceanography 54:1541-1547.

Urabe, J. & Y. Watanabe, 1992. Possibility of N or P limitation for planktonic cladocerans: An experimental test. Limnology and Oceanography 37:244-251.