

# **The role of submerged macrophytes in seasonally-flowing agricultural streams**



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## **Cover photo**

Clockwise from top left: *Cycnogeton huegelii* growing in the Caribunup River, enclosure for macrophyte transplants in the lower Vasse River, a tributary of Mary Brook flowing through farmland, a reach of the Buayanyup River with remnant riparian vegetation (R. Paice).

## **Dedication**

I dedicate this thesis to two unforgettable men who have inspired me in different ways on the same path. To my father, A. David Paice, and to Dr. Luke J. Pen.

## **Declaration**

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

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Robyn L. Paice

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Date

## **Statement of Contribution of Others**

Chapters 3, 4 and 5 of this thesis have been published in scientific journals, and the supervisors of Robyn L. Paice are included as co-authors for these papers. As first author, Robyn L. Paice has completed this research and prepared these manuscripts, and co-authors have had intellectual input through conceptual guidance, experimental design, data analysis and review.

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## **Permits**

Lower Vasse River sampling: Licence to take fauna for scientific purposes, licence number SF008312, Department of Environment and Conservation, Western Australia; Murdoch University Ethics Permit No RW247912.

Catchment sampling: Licence to take fauna for scientific purposes, licence number SF010065, Department of Environment and Conservation, Western Australia; Fish Resources Management Act 1994 exemption number 2121, Department of Fisheries Western Australia; Murdoch University Ethics Permit No. RW252912.



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## Thesis Abstract

Although submerged macrophytes are well known to support aquatic fauna and influence physical and chemical processes in many perennial freshwaters, they are rarely studied in seasonally-flowing streams. However, these streams often support seasonal plant assemblages that may support key ecological processes, particularly in agricultural landscapes with degraded riparian vegetation. This thesis investigated the distribution and contribution to ecosystem processes of submerged macrophytes in seasonally-flowing agricultural streams in a mediterranean climate, and their capacity to improve biodiversity and water quality in degraded reaches. It comprised: a survey of macrophyte distribution in relation to environmental factors; a food web study using stable  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopes; and a transplant experiment. Sufficient duration of flow and/or pools limited macrophyte establishment in these seasonal streams. Two plant assemblages were found with distinct distributions associated with riparian shading: strap-leaved *Cycnogeton* occurred as remnant populations in shaded habitats in both undisturbed and degraded reaches; whereas the more structurally-complex *Potamogeton/Ottelia* assemblage colonised degraded reaches with little riparian vegetation. Both assemblages supported greater macroinvertebrate abundance and richness than reaches without macrophytes. Stable isotopes indicated macrophyte and epiphyte material were a valuable food web resource in reaches with limited riparian vegetation, supporting macroinvertebrates and native fish, thereby potentially compensating for lost allochthonous resources. Low-light tolerance of *Vallisneria australis* (morphologically similar to *Cycnogeton*) and protection from waterbirds enabled successful transplantation and growth (85-100% cover after six months) in a phytoplankton-dominated lentic river reach. A regime of macrophyte/phytoplankton co-dominance achieved through transplantation delivered substantial biodiversity outcomes (macroinvertebrate abundance 18.5 times; diversity 3 times bare sediment controls) and in the longer term may contribute to improved water quality. Provision of higher faunal biodiversity and other ecosystem services suggests submerged macrophytes are worthy of conservation and inclusion in river restoration in agricultural catchments.





# Chapter 1. General Introduction

## Research aim

While it is widely accepted that submerged macrophytes<sup>1</sup> are important components in a diverse range of perennial aquatic ecosystems (Haslam 1978, Bornette and Puijalon 2011), their role in intermittent or seasonal streams has rarely been studied. This is despite the fact that their presence is often associated with high abundance and diversity of aquatic invertebrates compared to other habitats, (Heck and Crowder 1991, Phillips 2003, Warfe and Barmuta 2006, Shupryt and Stelzer 2009), and that their role in maintaining water clarity in shallow lakes is well-known and drives re-establishment as a goal of restoration (Van Donk and van de Bund, 2002, Sondergaard et al., 2007). In mediterranean-climate regions, many streams experience seasonal low flow velocities and form a 'chain-of-ponds' or resemble elongated shallow lakes for several months of the year, with the potential to show lentic rather than lotic dynamics. Indeed, other primary producers, such as benthic stream algae, show markedly different dynamics in seasonal or intermittent streams and rivers than they do in perennial systems (e.g. Bunn et al. 2003, Robson et al. 2008), so it might be expected that submerged macrophytes will also function differently in non-perennial streams. Lentic or slow-flowing conditions are likely to be conducive to the seasonal growth of submerged macrophytes (through sediment deposition and reduced damage by currents), yet there is little understanding of their distribution and ecological role in seasonally-flowing streams or how these may have changed in response to agricultural development of their catchments.

Anthropogenic changes, such as those caused by agriculture, alter the distribution of submerged macrophytes in streams through changes to physical factors (substrate and flow) and resource availability (light and nutrients) (Riis and Biggs 2001, Riis and Sand-Jensen 2001). In agricultural streams, extensive loss of riparian vegetation has consequences for the relative importance of submerged plants. While riparian-

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<sup>1</sup> The term submerged macrophyte is used throughout this thesis to refer to rooted aquatic angiosperms. It includes plants which are completely submerged (roots in sediment, all leaves submerged) and those with floating leaves (roots in sediment, some leaves submerged, some floating). Aquatic plants rooted in the sediment with both submerged and emergent leaves or stems are described as semi-emergent macrophytes. The term macrophytes in this study does not include emergent rushes or charophytes.

derived sources of habitat and food become limited, reduced shading may promote growth of aquatic plant growth, which potentially may compensate for the loss of riparian resources. So, provided that growth is not excessive, populations of submerged macrophytes may be ecologically important in seasonally-flowing agricultural streams. Furthermore, if submerged macrophytes do support aquatic fauna in degraded streams, this would indicate that there are potential benefits of their use in restoration (Table 1.1).

This thesis investigated the importance of submerged macrophytes in seasonally-flowing streams in a mediterranean agricultural landscape, through identifying:

- (i) the drivers of submerged macrophyte distribution and relationships between macrophytes and environmental factors;
- (ii) their contribution to ecosystem processes; and
- (iii) their capacity to improve biodiversity and water quality in degraded reaches.

It includes three related research projects which contribute jointly to this overall aim (Table 1.1): a landscape-scale survey of submerged macrophyte distribution in relation to environmental factors in agricultural streams (Chapter 3); a food web study of their comparative importance in reaches with good and poor condition riparian vegetation (Chapter 4); and a restoration trial using transplants in a eutrophic impounded river (Chapter 5).

## **Theoretical Context**

In seasonally-flowing streams, periods of low flow and pooling dictate that existing knowledge from both lentic and lotic systems is relevant. It is valuable to reflect how seasonal and perennial streams differ in their provision of key requirements for macrophytes (such as flow and light) and how human modification of this landscape may also affect these variables. It is also valuable to consider the ecological role of submerged macrophytes in the context of both streams and lakes, as seasonally-flowing streams are potentially a combination of these environments. The following literature review applies existing ecological research for submerged macrophytes in freshwater ecosystems to an understanding of plant communities in seasonally flowing streams.

## ***Consequences of anthropogenic change for the distribution of submerged macrophytes in seasonally-flowing streams***

Flood disturbance, flow velocity and substratum type are primary drivers of submerged macrophyte distribution in perennial streams (Sand-Jensen 1998, Gurnell et al. 2006, Franklin et al. 2008) and where these conditions are suitable, light climate and nutrient availability are important in providing resources for growth (Barrat-Segretain 1996, Bornette and Puijalon 2011). Macrophytes are more common in lowland rivers, which have moderate velocity, more diverse substratum for colonisation, and greater light availability owing to width (Madsen et al. 2001; Lacoul and Freedman, 2006) compared with upland streams. In agricultural catchments, hydrology, riparian vegetation cover and water quality have changed dramatically (Allan 2004), with likely consequences for macrophyte distribution. In cool temperate regions, anthropogenic change is generally associated with a change in macrophyte species composition, and increased abundance of species more tolerant to the altered conditions (Riis and Sand-Jensen, 2001; Dodkins et al. 2012). However, the response of native macrophyte populations to these changes has not been assessed for seasonally-flowing streams in drier climate regions, for which the seasonal water regime has likely implications for distribution in both undisturbed and degraded environments.

### **Flow and substratum**

Flow regime has consequences for submerged macrophyte distribution and species composition owing to differing tolerances of species to fluctuating water levels and seasonal drying (Riis and Hawes 2002). Velocity is also important in terms of its direct physical effect on plants, and variable vulnerability to breakage and uprooting (Riis and Biggs 2001; Bornette et al. 2008). Most macrophytes have a positive growth response to low velocity (Suren and Riis 2010), and seasonally-flowing streams can support conspicuous growth of macrophytes during periods of low flow and pooling (eg. Watson and Barmuta 2011). Artificial drainage, impoundment, water extraction and land clearing alter stream discharge volumes and flow regimes (Allan 2004, Bornette et al. 2008). Increased frequency of short, high intensity flow events may have negative physical impacts on macrophytes (Riis and Biggs 2001, Franklin et al. 2008). While reduced base flow velocity may favour plant growth in

perennial systems, in seasonal streams it may correlate with increased drying and thus a shortened growth season for macrophytes (Deegan et al. 2012).

Flow is also important in defining substratum characteristics (Sand-Jensen 2008), which affects potential for recruitment, anchorage (Franklin et al. 2008) and growth (Suren and Riis 2010). Erosion from land clearing and degradation of riparian vegetation increases stream sediment load (Allan 2004, Power et al. 2013).

Sedimentation has potentially negative effects on plant growth through burial and increased turbidity (Sculthorpe 1985), but may also benefit plant establishment by creating new areas of colonisable, fine substrate (Gurnell et al. 2006, Suren and Riis 2010). The presence of macrophytes also influences local flow conditions, and velocity reduction is associated with sediment deposition in the vicinity of plant beds (Sand-Jensen 1998, Cotton et al. 2006, Madsen et al. 2001), while diversion of flow may create channelization around stands (Gurnell et al. 2006). Accumulation of fine sediments within and around plant beds, which are enriched in nutrients and organic material, may be beneficial to plant growth (Sand-Jensen 1998; Cotton et al. 2006).

### **Light and nutrients**

In both lotic and lentic environments, available light is a key resource that affects the biomass and community composition of submerged macrophytes, mediated by plant growth forms which differ in their light requirements (Haslam 1978; Chambers and Kalff 1987). Most studies consider the effects of factors such as depth, colour and turbidity, due suspended sediments and phytoplankton, on light availability to submerged plants (Barrat-Segretain 1996, Bornette and Puijalon 2011). Depth is generally not limiting in seasonal streams as they are often shallow, and although highly turbid conditions may restrict growth in shallow waters, declining water levels during the spring growth period can potentially compensate for high turbidity (Bucak et al., 2012).

The degree of shading from riparian zones also affects light availability (Canfield and Hoyer 1988), and its effects on light climate are dependent on channel width, type and condition of riparian vegetation, and reach orientation (Ali et al. 2011). Loss of riparian vegetation in agricultural landscapes increases light availability to streams, which can promote macrophyte growth (Canfield and Hoyer 1988, Julian

et al. 2011). However, reduced shading may also increase evaporation and prolong desiccation in streams with seasonal flows, limiting macrophyte growth and reducing availability of moist refuges (Rea and Ganf 1994). Additionally, turbidity from suspended sediments (Allan 2004) may counteract positive effects of higher incident light.

Growth of macrophytes in lotic systems is often not nutrient limited (Sand-Jensen 1998), however species and growth forms vary in response to nutrient availability (Chambers and Kalff 1987), influencing both abundance and composition of submerged macrophyte communities (Haslam 1978, Sculthorpe 1985). Increased water-column nutrients arising from agricultural land use has been found to enhance macrophyte growth (Mebane et al. 2014), but often this is not the case (Madsen and Cedergreen 2002; Chessman and Royal 2010). In low flow environments, nutrient enrichment of the water column may reduce available light by supporting increased growth of epiphytes and phytoplankton (Jupp and Spence 1977, Phillips et al. 1978, Twilley et al. 1985, Hilton et al. 2006), or floating aquatic plant species (Haslam 1978). Additionally, although nutrient availability in sediments is generally correlated with increased macrophyte biomass (Chambers 1987), high algal productivity may lead to accumulation of poorly cohesive organic matter, which can restrict anchorage and recruitment of macrophytes (Barrat-Segretain 1996).

### **Potential for problematic growth**

Aquatic plants are often perceived as a symptom of degradation, with potential for excessive growth (Bunce et al. 2002) and subsequent impacts on channel capacity, recreational values and aquatic fauna (Shultz and Dibble 2012). This perception may explain why they are not commonly used in restoration of degraded streams, in which increased availability of light and nutrients may promote plant growth (Canfield and Hoyer 1988, King and Buckney 2000, Mebane et al. 2014). This response is more common for non-native species (Hastwell et al. 2008, Quinn et al. 2011), for which invasive species traits such as high growth rates, allelopathy and broad tolerance of habitat conditions, facilitate excessive<sup>2</sup> growth (Shultz and Dibble 2012) and a positive growth response to nutrient enrichment (Hastwell et al.

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<sup>2</sup> The term excessive growth is used to describe prolific, spreading, high density growth which may impact aesthetics and navigability, and have potential adverse impacts on aquatic organisms.

2008). As with native species, non-native plants can support aquatic fauna by providing structural habitat (Strayer et al. 2003, Theel et al. 2008), but faunal assemblage may be substantially altered and adversely affected in very dense plant stands by anoxia owing to increased decomposition of accumulated detritus (Bunn et al. 1998, Stiers et al. 2011). Although native macrophytes may also exhibit invasive traits, they often have a neutral or negative response to increased resources (Quinn et al. 2011) and may pose a lower risk of excessive growth. In streams with seasonal drying, limitations on growth imposed by water availability may mediate positive effects of increased resource availability.

### ***Ecological role of submerged macrophytes in seasonally-flowing streams***

Compared to other habitats, aquatic plant communities are often associated with high abundance of aquatic invertebrates (Heck and Crowder 1991, Phillips 2003, Warfe and Barmuta 2006, Shupryt and Stelzer 2009), which in turn support vertebrate fauna such as frogs, fish and birds (Dvorak and Best 1982). The three-dimensional structure of macrophytes provides greater surface area (Hutchens et al. 2004), increased availability and complexity of niches (Warfe et al. 2008), and protection from physical effects of flow (Garcia et al. 2012), providing more opportunities for colonisation and refuge from predation in comparison to bare substrate (Diehl and Kornijów 1998). While the potential habitat provided by submerged macrophytes in seasonally-flowing streams would be comparable to other systems, the hydrological regime restricts the time period for their presence. However, their annual growth in these systems parallels annual life-cycles of many aquatic fauna which are also adapted to periodic drying, and submerged macrophytes may therefore provide important seasonal habitat.

In addition to structural habitat, macrophytes provide substrate for growth of epiphytic algae (Warfe and Barmuta 2006, Ferreiro et al. 2011) and facilitate deposition of organic matter (Koetsier and McArthur 2000, Fritz et al. 2004), which also increase available food sources for aquatic fauna. However, the contribution of macrophytes themselves to food webs is uncertain. River food-web studies often focus on the relative importance of terrestrial versus algal carbon in driving food webs, based on classic river food-web theories (Vannote et al. 1980, Junk et al.

1989, Thorp and DeLong 1994). Consequently, submerged macrophytes have been excluded from many studies of food webs and ecological processes owing to a presumed lack of importance (e.g. Bunn et al. 1999, Hadwen et al. 2010). However, some recent studies indicate that macrophytes can be a nutritious basal resource streams (Deegan and Ganf 2008, Watson and Barmuta, 2011), at least seasonally (Reid et al. 2008). In seasonally-flowing streams, the annual growth of macrophytes during spring and summer corresponds to a period of recovery and growth of animal populations following high winter flows (Hershkovitz and Gasith 2013). The supply of additional resources at this time may be beneficial to support metamorphosis, the formation of desiccation-resistant life stages or reproduction. Furthermore, where a limited biomass of allochthonous organic matter is available owing to lost riparian vegetation, the relative contribution of macrophytes to food webs may be higher.

### ***Potential for restoration outcomes using submerged macrophytes***

Given their potential to support important ecosystem processes, it is reasonable to expect that restoration of aquatic plant communities would increase biodiversity, although direct transplantation of submerged macrophytes to achieve this outcome is rare in lotic systems (Riis et al. 2009, Larned et al 2006). Their establishment is not amongst traditional river restoration practices, with the dominant reach-scale approaches being physical stream modifications (eg. channel reshaping, introduction of rock and wood structure) and riparian zone rehabilitation (Roni et al. 2008, Feld et al. 2011). Modification of physical habitat to increase heterogeneity often has been shown to have limited outcomes for biodiversity (Palmer et al. 2010), although where restoration projects have resulted in recolonisation of macrophyte assemblages, greater macroinvertebrate abundance and diversity are reported (Lorenz et al. 2012). The role of riparian vegetation in stream ecosystem health, by providing habitat and shade, stabilising banks, intercepting nutrients from adjacent land use (Feld et al. 2011) and supporting food webs (Bunn et al. 1999, Reid et al. 2008, Power et al. 2013), makes their protection and rehabilitation a priority for river restoration (Davies 2010). However, revegetation of riparian zones requires a great deal of effort, expense and on-going maintenance, and

unfortunately such projects often incorporate an inadequate width of vegetation (Lake 2005), potentially limiting effects in-stream (e.g. Becker and Robson 2009).

Inclusion of submerged macrophytes in restoration has the potential to contribute to improved water quality, supporting longer-term management of diffuse nutrient sources. Effects of submerged macrophytes on nutrient cycling can be significant at the reach-scale (Clarke 2002), resulting from nutrient uptake by plants and epiphytes (Clarke 2002), locally reduced disturbance, increased sedimentation of particulate organic material (Svendsen and Kronvang 1993, Sand-Jensen 1998), and oxygenation of sediments by plant roots (Clarke 2002). Assimilation processes potentially reduce downstream transport of nutrients, especially phosphorus (Svendsen and Kronvang, 1993; Sand-Jensen, 1998). Submerged macrophytes have a competitive advantage over phytoplankton owing to their ability to obtain nutrients from sediments (Barko and James 1998), and can limit phytoplankton growth through uptake of water column nutrients by plants (Ozimek et al. 1990) and epiphytes (Blindow 1987). In seasonal systems, this may contribute to water clarity in pools and impoundments during the spring-summer period. This role is widely recognised in shallow lakes (Scheffer et al. 1993), where macrophytes limit phytoplankton growth by nutrient uptake, enhanced sedimentation, reduced resuspension (Van Donk and van de Bund 2002), allelopathy (Vanderstukken et al. 2011) and provision of habitat for herbivorous zooplankton (Timms and Moss 1984). It is the basis for restoration of submerged macrophytes in many northern temperate lakes (Ozimek et al. 1990, Meijer et al. 1994, Søndergaard et al. 2007) and provides relevant theory for seasonally-lentic stream reaches, but is rarely applied in their management.



## **Thesis structure**

Following the Introduction and Study Area chapters, three chapters present three related pieces of research undertaken in the Geographe Bay catchment, an agricultural catchment in the mediterranean-climate region of south-western Australia. A general description of each of these chapters is provided below, together with specific research questions and the contribution of each chapter to the overall thesis aims (Table 1.1). Chapter 6 provides a detailed synthesis of the findings of this research and their implications for our understanding and management of freshwater ecosystems. As the research chapters (3-5) have been published in different scientific journals, they differ in referencing style. They have joint authorship with my supervisors and therefore use collective terms such as “we”.

### ***Chapter 3. Native submerged macrophyte distribution in seasonally-flowing, south-western Australian streams in relation to stream condition.***

The distribution of submerged macrophytes in seasonally-flowing streams in agricultural catchments is likely to have been affected by changes to hydrology, substratum and riparian condition. Understanding factors driving macrophyte distribution in seasonally-flowing streams and their response to conditions in agricultural catchments is important for assessing their role in these ecosystems (Table 1.1). This study surveyed macrophytes, environmental variables and macroinvertebrates was conducted at fifty-three reaches across seven adjacent seasonally-flowing river systems in the Geographe Bay Catchment (Western Australia), to identify factors driving their distribution and relationships with environmental variables, and assess their importance in supporting macroinvertebrate assemblages. This chapter is published in *Aquatic Sciences: Research Across Boundaries* (in press).

### ***Chapter 4. Potential of aquatic macrophytes to support food webs in lowland agricultural streams.***

Regular periods of low rainfall in mediterranean-climate regions often create conditions of slow flow and pooling, which can support abundant seasonal growth

of submerged macrophytes. While the provision of structural habitat is an important function of these plants, their contribution to river food webs is uncertain. They have potential to provide a nutritious seasonal resource in seasonally-flowing streams, and furthermore may to some extent compensate for the food supply lost when riparian vegetation is cleared. Evidence for this role would suggest these plants are important components of agricultural stream ecosystems, and may stimulate consideration of their role in restoration (Table 1.1). In this study, stable isotope analysis was used to investigate the contribution of submerged macrophytes as a basal food source for food webs and to compare this contribution between reaches with good or poor riparian vegetation. This study is in review in *Marine and Freshwater Research*.

### ***Chapter 5. Outcomes of submerged macrophyte restoration in a shallow impounded, eutrophic river.***

Agricultural streams in mediterranean-climate regions commonly experience periods of little flow and extensive pooling during summer, and impoundments are common owing to high reliance on abstraction during seasonal periods of low rainfall. In these seasonally lentic environments, nutrient enrichment can cause excessive phytoplankton growth. Restoration of submerged macrophytes in these systems may contribute to improved water clarity, as has been demonstrated in cool-temperate shallow lakes, and provide biodiversity benefits (Table 1.1). However, phytoplankton dominance can prevent establishment of submerged macrophytes, and increasing water clarity by other means to enable their recolonisation is often unachievable. This study trialled direct establishment of submerged macrophytes in a eutrophic, phytoplankton-dominated impoundment (the lower Vasse River) without attempting to first increase water clarity, by planting a species tolerant of low light and high nutrient conditions. Protection from waterbird disturbance and a substratum for root anchorage in the flocculent sediment were provided. This study has been published in *Hydrobiologia* (online 2015).

**Table 1.1** Overview of specific research questions for each chapter in the thesis, and their contribution to the overall research aim.

Chapter title	Specific research questions	Links to overall thesis research aim: to investigate the importance of submerged macrophytes in seasonally flowing agricultural streams.
Chapter 3. Native submerged macrophyte distribution in seasonally -flowing, south -western Australian streams in relation to stream condition	3.1 What environmental variables are associated with macrophyte occurrence? 3.2 Is the presence of submerged macrophytes associated with stream degradation? 3.3 Do reaches with native macrophytes support a more abundant and diverse aquatic invertebrate assemblage than those without?	
Chapter 4. Potential of aquatic macrophytes to subsidise food webs in degraded lowland streams	4.1 Do macrophytes contribute to food webs in these streams? 4.2 Is the contribution of macrophytes to food webs is greater in degraded reaches than those in good condition?	
Chapter 5. Outcomes of submerged macrophyte restoration in a shallow impounded, eutrophic river	5.1 Can submerged macrophytes be established in turbid, nutrient rich waters when protected from herbivorous waterfowl? 5.2 What influence do submerged macrophyte transplants have on the aquatic invertebrate assemblage in this degraded system? 5.3 Does the presence of submerged macrophytes improve water quality in terms of reduced nutrient concentrations, turbidity and phytoplankton growth?	

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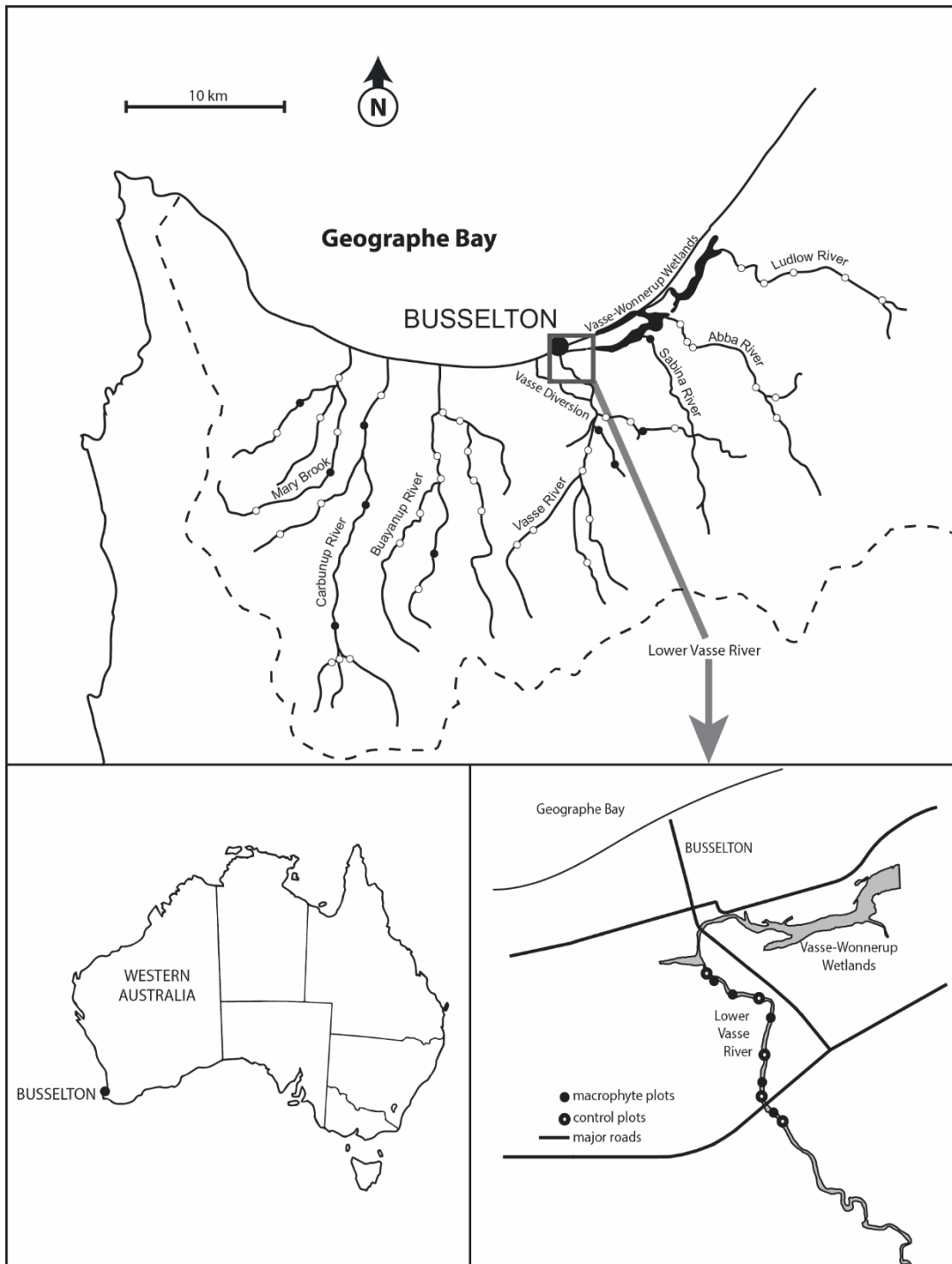
## Chapter 2. Study Area

### Location

This research was undertaken in seasonally-flowing streams within the Geographe Bay catchment, located in the mediterranean-climate south-west region of Australia, approximately 250 km south of Perth, Western Australia (Figure 2.1) (latitude: 33°24.41 – 33°54. 10 S; longitude 115°05.46 – 115°03.52 E). The catchment covers an area of approximately 2000 km<sup>2</sup>, encompassing several river systems with intermittent headwaters in the Darling Range, the Whicher Range and the Leeuwin-Naturaliste Ridge, and lowland reaches crossing the extensive Swan Coastal Plain. The ranges bounding the catchment are dominated by lateritic soil profiles, while deep sands and sandy loams characterise the coastal plain.

The river systems included in this research are relatively short, with main channel lengths of 20-45km, and are described as streams throughout the thesis owing to their relatively small size in comparison to typical rivers in a global sense. Seven adjacent river systems were included in the survey (Chapter 3): the Ludlow, Abba, Vasse, Sabina, Buayanyup and Carburnup Rivers and Mary Brook (Figure 2.1). Potential study reaches for the stable isotope study (Chapter 4) were identified during the survey, and did not include the Ludlow or Abba Rivers (Figure 2.1). The transplantation trial (Chapter 5) was located in the lower reach of the Vasse River (Figure 2.1, Figure 2.9).

Geographe Bay is of regional significance for tourism, owing to protected waters and stunning beaches, and contains extensive seagrass meadows of ecological importance (DEC 2006). The Ramsar-listed Vasse-Wonnerup wetland system is located within the catchment (Figure 2.1), a relatively large shallow coastal system of international significance as waterbird habitat. These wetlands regularly support more than 30,000 waterbirds annually, including many migratory species and more than 1% of the population of four species (Lane *et al.* 2007).



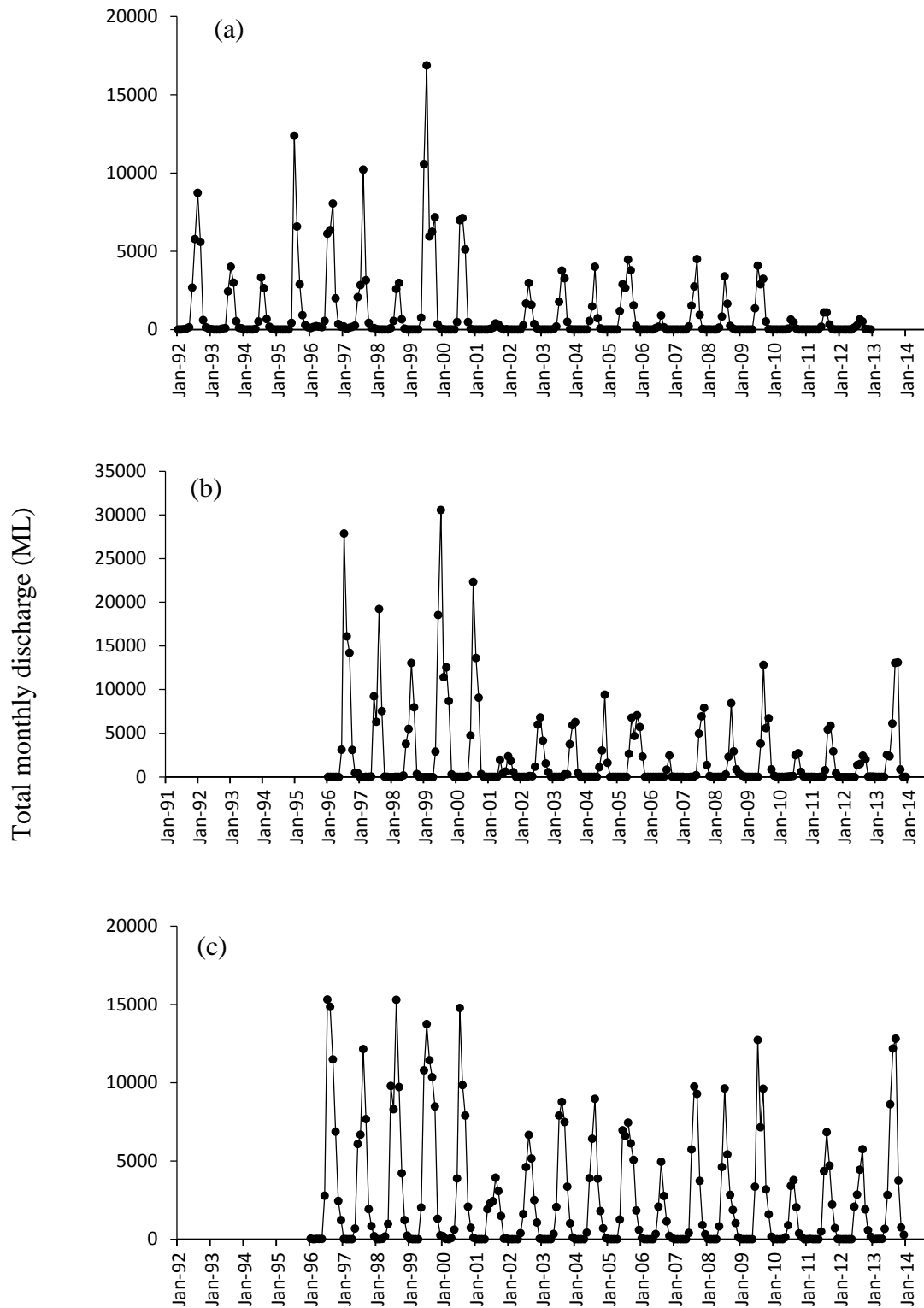
**Figure 2.1.** Location of study area in the Geographe Bay catchment (dashed line) relative to the town of Busselton in Western Australia. Survey reaches are indicated by all circles (black and white) on main map, and black circles are stable isotope sampling reaches. Enlargement shows the lower Vasse River with location of macrophyte and control sites included in the transplantation trial.

## **Climate and hydrology**

The region experiences a mediterranean climate of mild, wet winters and hot, dry summers. Average maximum temperature is 29 °C in summer and 17 °C in winter (BoM 2014). Average annual rainfall is 740 mm (1991-2010), but the 20-year average has declined by 15% over fifty years (1941-1960: 863 mm; BoM 2014). Seasonal rainfall results in typical hydrographs of peak winter-spring flows and summer-autumn drying (Figure 2.2). There has been a decline in stream flows since commencement of monitoring (Figure 2.2), most likely as the result of both reduced rainfall and increased water extraction and impoundment.

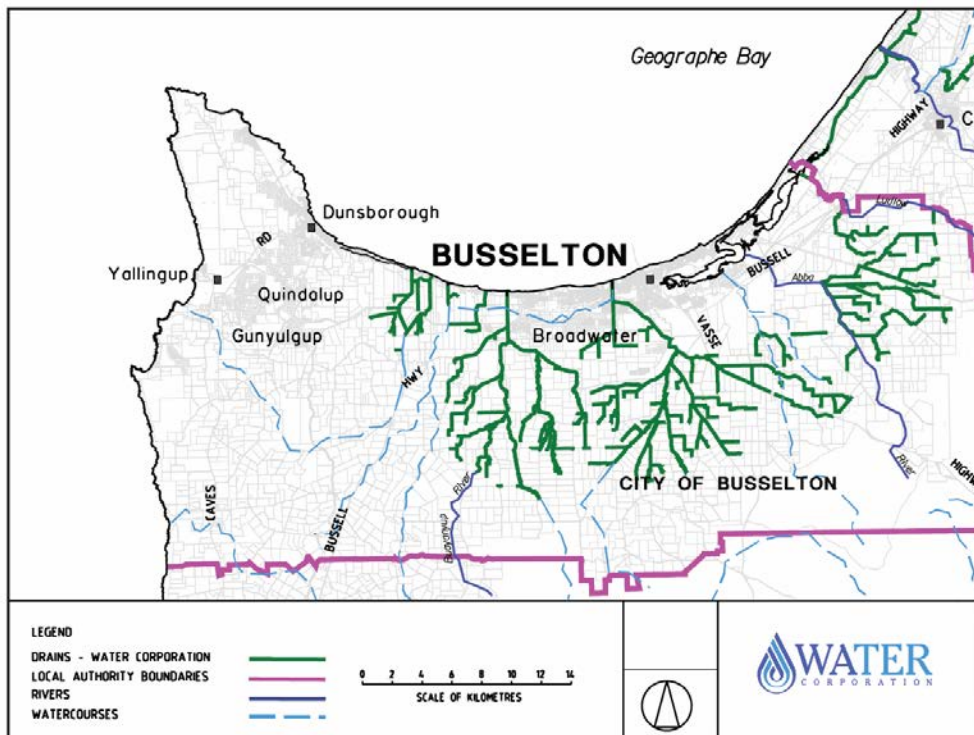
Natural hydrology on the Swan Coastal Plain subjects low-lying areas to seasonal flooding, and an extensive artificial drainage network was constructed in the 1920s to allow successful agricultural development (GeoCatch 2008), including the widening and straightening of existing channels and creation of new drains to improve conveyance (Figure 2.3). On-stream impoundments are common within the catchment, with surface water extracted for agricultural and domestic use.

The Buayanyup, Caribunup and Marybrook Rivers discharge directly into Geographe Bay, while the Ludlow, Abba, and the lower sections of the Sabina and Vasse Rivers, discharge to the Vasse-Wonnerup Wetlands and subsequently into Geographe Bay (Figure 2.1). Approximately 90% of the Vasse River catchment and 65% of the Sabina River catchment discharge directly to Geographe Bay via the Vasse Diversion drain, constructed in 1927 to protect the town of Busselton from flooding (GeoCatch 2008). All river systems in the study area are seasonally-flowing with the extent of summer-autumn drying and pooling dependent on inter-annual variation in winter-spring rainfall volumes. Permanent inundation occurs only in tidal reaches near the coast and in the lower Vasse River, where stop boards are used to maintain water levels for aesthetic purposes within the town of Busselton (Chapter 5).



**Figure 2.2.** Monthly flow discharge for rivers with continuous gauging station data: Ludlow River (a), Vasse Diversion drain (b) and Carburnup River (c) (DoW 2015, unpublished data).





**Figure 2.3.** Surface water drainage within the study area. Green lines are modified watercourses and artificial (provided by Water Corporation).



**Figure 2.4.** The Carburnup River with good condition riparian vegetation and submerged *Cycnogeton huegelii*.

## Natural environment

The south-west region of Western Australia is one of twenty-five recognised global biodiversity hotspots, owing to a high level of endemism and diversity of vascular plants combined with large areas of habitat loss (Myers *et al.* 2000, Davies and Stewart 2013). Terrestrial vegetation in the Geographe Bay Catchment include diverse complexes associated with variation in landform and soil profiles. Mixed forests and woodlands of *Corymbia calophylla* (Lindl.) and *Eucalyptus marginata* (Sm.) with diverse understorey are dominant in the ranges, with *Eucalyptus patens* common nearer streamlines. *C. calophylla* woodlands occur in drier parts of the coastal plain and extensive *Melaleuca* woodlands and heathlands are present in low-lying and seasonally-inundated flats. Low forests of *Agonis flexuosa* (DC.) are common near the coast. Riparian canopy vegetation includes these dominant tree species, and also the flooded gum, *Eucalyptus rudis* (Endl.), in lowland areas. ‘Teatree’ species such as *Agonis linearifolia* (DC.) and fringing and emergent rushes (Restionaceae) dominate the riparian understorey (Figure 2.4).

Aquatic fauna is also highly endemic to this region, including many species of macroinvertebrates, amphibians (Davies and Stewart 2013), freshwater fish and crayfish (Morgan *et al.* 2011). There are no published studies for macroinvertebrate assemblages in river systems in the study area, (most research has focussed on surveys of Swan Coastal Plain wetlands, Horwitz *et al.* 2009). More than 80% of native freshwater fish and all known native species of freshwater crayfish found in the region are endemic (Morgan *et al.* 2011). Recent surveys of streams in the study area have found five native fish species (Morgan *et al.* 2011), three of which are endemic to the south-west, including the Mud Minnow *Galaxiella munda* (McDowall 1978), which is listed as Vulnerable (Wildlife Conservation Act 1950 (Western Australia)). Additionally, one species of turtle, the snake-necked Turtle *Chelodina colliei* (Kuchling 1988) is known to occur in the study area (personal observation).

Although the aquatic plants in this region are not as species rich as the terrestrial flora (Davies and Stewart 2013), they form a distinct biogeographical assemblage (Jacobs and Wilson 1996). Current knowledge of aquatic plant distribution is

limited to sporadic records held by the Western Australian Herbarium, and there have been no previous surveys of submerged aquatic plants in the study area.

## **Land use change and impacts**

Aside from regional tourism, and commercial and residential development around Busselton and several other small urban centres, current land use in the catchment is predominantly livestock grazing for beef and dairy, viticulture and horticulture, with ongoing intensification of these industries (DoW 2010). Widespread clearing of native vegetation in the Geographe Bay catchment has occurred since the implementation of the Group Settlement Scheme in the 1920s (WAPC 1998). Less than 35% of native vegetation remains (GeoCatch 2008) and much of this is within State Forest areas in the Darling and Whicher Ranges; the lowland coastal plain areas were largely cleared for agriculture (Figure 2.5). This has resulted in extensive loss and degradation of riparian vegetation (Figure 2.6), both directly and as a result of channel instability and livestock damage, with implications for stream biota including loss of habitat and food resources, and increased exposure to drying and high temperatures (Davies 2010). In addition, many artificial drainage channels have no natural riparian vegetation (Figure 2.7a).

Clearing and altered hydrology have created channel instability and extensive sediment mobilisation and deposition is evident in lowland streams throughout the catchment (Figure 2.7b). Increased nutrient inputs from diffuse agricultural sources have led to elevated nutrient concentrations in many streams (DoW 2010; Figure 2.8) and increased nutrient loads to receiving environments. This has been exacerbated by loss of assimilative capacity within the catchment owing to loss of riparian vegetation and increased conveyance via drainage. The catchment is a recognised national nutrient hot spot, owing to extensive nutrient enrichment problems and the risk posed to high value natural assets of Geographe Bay and the Ramsar-listed Vasse-Wonnerup wetland system (DoW 2010). At the reach scale, during the warmer months in spring-summer, seasonal pools and impoundments are also prone to excessive phytoplankton growth. The lower Vasse River (Chapter 5) is the most prominent example, where seasonal phytoplankton dominance in waters within the town centre presents a public health risk (Figure 2.9) (Paice 2001).



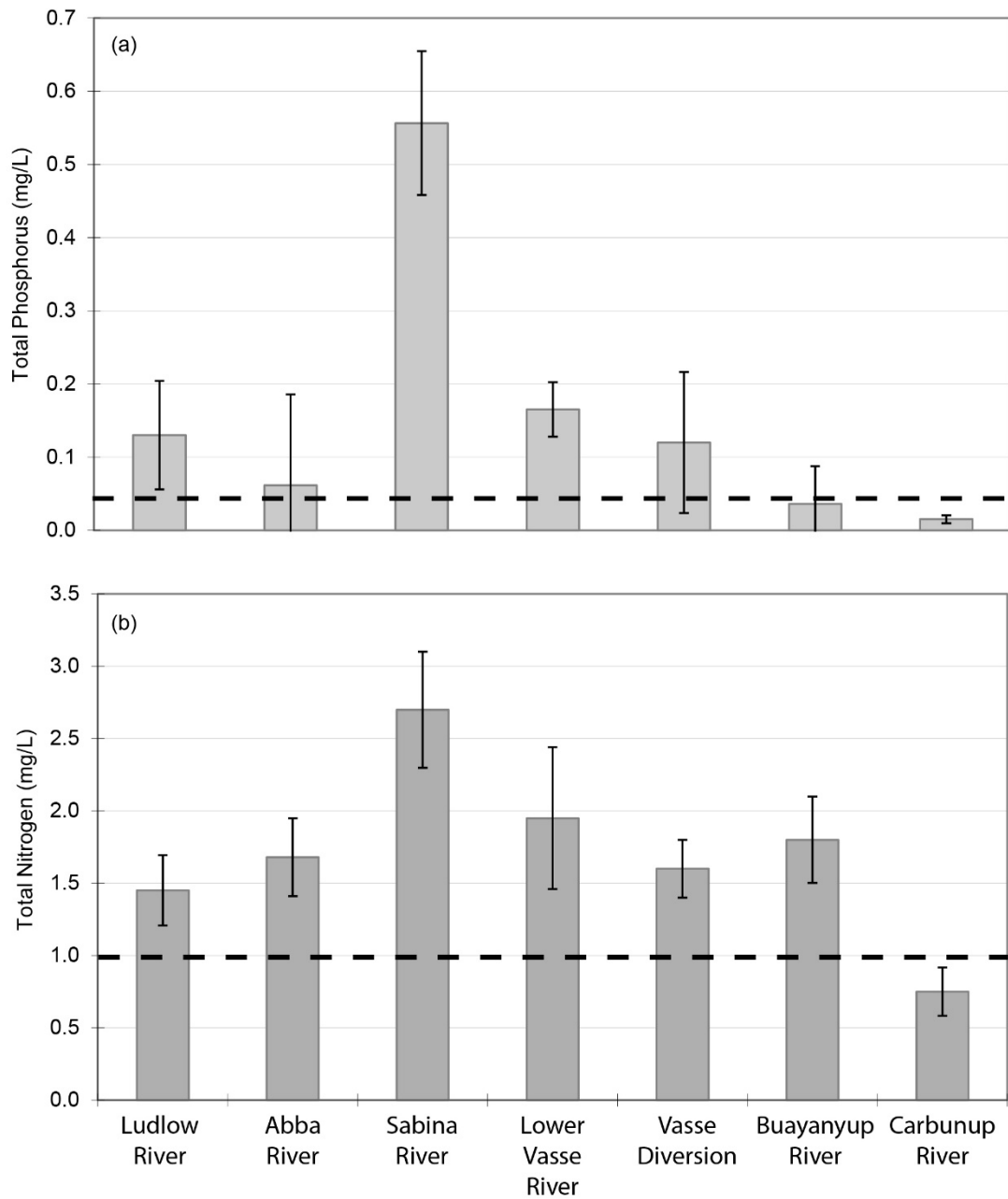
**Figure 2.5.** Aerial photograph image of the Vasse River catchment area showing widespread clearing, particularly in coastal plain areas, and remnant vegetation areas within State Forest to the south (Google Earth 2015).



**Figure 2.6.** Degraded riparian vegetation on natural streamlines in the study area. The reach of Mary Brook in (a) retains some riparian canopy cover and a bed of *Cycnogeton huegelii* is located here (inset). The tributary of the Buayanyup River in (b) has only isolated remnant trees remaining and experiences complete drying in the summer.



**Figure 2.7.** A tributary drain of the Buayanyup River (a) with no natural riparian vegetation, but which contains beds of *Potamogeton drummondii* and *Ottelia ovalifolia*, and another nearby tributary (b) showing bank slumping and severe sedimentation in the absence of riparian vegetation.



**Figure 2.8.** Median winter concentrations of total phosphorus (a) and total nitrogen (b) in monitored study reaches 1998-2007. Error bars are 95% confidence intervals. Reference lines are local water quality guidelines for ecosystem protection in lowland rivers (ANZECC and ARMCANZ 2000). Adapted from DoW (2010), original figure by Joel Hall.



**Figure 2.9.** As a combined result of increased nutrient inputs, impoundment and flow diversion, the lower reach of the Vasse River experiences serious algal blooms each year during summer and autumn, closing the waters to public use. The structure in the river is an enclosure used in the restoration study (Chapter 5).

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### **Chapter 3. Native submerged macrophyte distribution in seasonally-flowing, south-western Australian streams in relation to stream condition**

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## Abstract

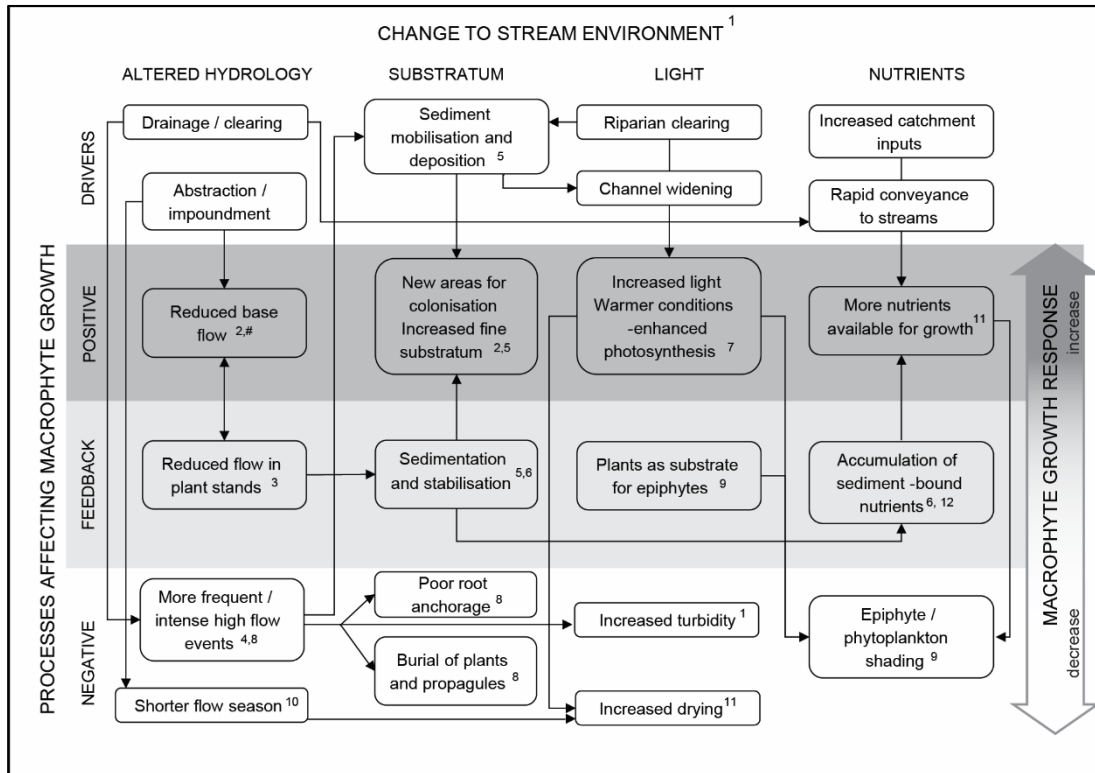
Submerged macrophytes are important structural and biological components of many lowland streams with potential to support ecosystem processes in degraded streams, provided that growth is not excessive. In a low-gradient agricultural landscape, a survey was used to explore associations between submerged macrophyte growth, biodiversity and variables assessing stream condition in seasonally-flowing streams. These variables were sampled across fifty-three reaches on seven adjacent streams in the mediterranean climate region of south-western Australia. Native submerged macrophytes were present in 43% of sampled reaches, forming two distinct macrophyte assemblages dominated either by *Potamogeton* spp. together with *Ottelia ovalifolia*, or by *Cycnogeton* spp. The *Potamogeton/Ottelia* assemblage was present in degraded reaches with higher light availability and deposition of fine sediments, but did not show excessive growth, even under nutrient-enriched conditions. Conversely, *Cycnogeton* spp. were associated with shaded conditions and greater flow. Reaches with macrophytes present had significantly higher macroinvertebrate abundance and family richness than those without, although rarefied family richness was similar among reaches with and without submerged macrophytes. The more structurally complex *Potamogeton/Ottelia* assemblage supported a greater abundance of grazers, shredders and predators than the simpler *Cycnogeton* spp. In degraded agricultural streams, remnant and colonising populations of submerged macrophytes may compensate for loss of riparian-derived habitat and resources for macroinvertebrates, and thus the food supply for predatory species.

## Introduction

Submerged macrophytes provide structural habitat and food sources for aquatic fauna, (Carpenter and Lodge 1986; Heck and Crowder 1991; Warfe and Barmuta 2006; Shupryt and Stelzer 2009), interact with flow and sediment dynamics (Sand-Jensen 1998), and influence nutrient cycling (Clarke 2002), but these plants are rarely studied in seasonally-flowing streams. However, many streams in mediterranean-climate regions have conditions of low flow velocity and pooling for several months of the year that are suitable for growth of submerged macrophytes, which potentially support important ecosystem processes. While processes relating to submerged macrophyte distribution have been widely studied in perennial temperate rivers, their growth and response to anthropogenic change in seasonally-flowing streams is not well-understood. Changes to hydrology, sediments and resource availability in agricultural landscapes has likely altered the distribution of submerged macrophytes, and their ecological role under these conditions warrants further investigation.

Submerged aquatic macrophyte distribution in perennial rivers is driven primarily by the interrelated factors of flow and substratum type (Sand-Jensen 1998; Gurnell et al. 2006; Franklin et al. 2008), and secondarily by light climate (determined by riparian vegetation cover, turbidity and colour) and nutrient availability (Barrat-Segretain 1996; Bornette and Puijalon 2011). Stream degradation affects each of these factors, with potential for both positive and negative impacts on macrophyte growth; and the presence of macrophytes within a stream also has feedback effects on the stream environment (Figure 3.1). Reduced base flow velocity resulting from drainage, abstraction and impoundment may improve physical conditions for growth, but also affect flow duration and shorten the growth season (Figure 3.1). Increased runoff from cleared lands and artificial drainage can intensify high-flow events and impact plant establishment and anchorage; but also create wider, well-lit environments, with more suitable substrata due to mobilisation and deposition of fine sediments (Figure 3.1). Increased availability of light (through riparian clearing) and nutrients in agricultural catchments can promote aquatic plant growth (Figure 3.1) (Canfield and Hoyer 1988; Mebane et al. 2014). In some cases this may cause excessive (prolific, spreading, high density) growth, with potential to impact

aquatic fauna through altered assemblage structure (Schultz and Dibble 2012) and increased risk of anoxia (Stiers et al. 2011). However, growth response is constrained by flow and substratum characteristics (Riis and Biggs 2001) (Figure 3.1) and will therefore vary between systems.



**Figure 3.1.** Conceptual model of native submerged macrophyte growth response to change in stream environment in an agricultural landscape. Interrelated changes to hydrology and resources (light and nutrients) create a suite of interacting processes with both positive and negative impacts of macrophyte growth, including feedback mechanisms as a result of the presence of plants (<sup>1</sup>Allan 2004; <sup>2</sup>Suren and Riis 2010; <sup>3</sup>Sand-Jensen 1998; <sup>4</sup>Riis and Biggs 2001; <sup>5</sup>Bornette et al. 2008; <sup>6</sup>Gurnell et al. 2006; <sup>7</sup>Bornett and Puijalon 2011; <sup>8</sup>Franklin et al, 2008; <sup>9</sup>Hilton et al 2006; <sup>10</sup>Deegan et al 2012; <sup>11</sup>Rea and Ganf 1994; <sup>12</sup>Mebane et al. 2014; <sup>13</sup>Clarke et al. 2002). #Flow reduction from abstraction/impoundment; excludes irrigation channels. The term ‘growth response’ is used in a general sense as it will vary with macrophyte community characteristics.

While flood disturbance is a fundamental driver of submerged macrophyte distribution in perennial temperate rivers (Riis and Biggs 2001), seasonal drying may be more important in mediterranean-climate streams and will influence the growth response to altered stream conditions. For example, macrophyte responses to nutrient enrichment may be limited either by drying of seasonal pools or by phytoplankton or epiphyte growth (under lentic conditions) if it reduces light availability (Hilton et al. 2006). Similarly, the reduced shading resulting from riparian clearing may exacerbate seasonal drying and create intolerably high temperatures for macrophyte persistence. So, although increased light availability, nutrients and fine sediment input to streams are a common consequence of catchment clearing and damaged riparian vegetation (Power et al. 2013, Figure 3.1), seasonal flow regimes may limit responses by macrophytes. Growth restrictions imposed by drying may prevent the excessive growth of macrophytes that is observed in perennial streams (Shultz and Dibble 2012).

Provided that growth is not excessive, submerged macrophytes may have an important ecological role in degraded stream reaches, particularly when habitat and food resources from riparian zones are limited. The heterogeneous habitat and refuge provided by macrophytes is well known to support aquatic fauna (Heck and Crowder 1991; Bell et al. 2013), and even small patches can contribute greatly to reach-scale populations of macroinvertebrates (Shupryt and Stelzer 2009).

Furthermore, while river food webs in mediterranean-climate streams are generally assumed to be based on allochthonous and algal sources rather than macrophyte carbon (Power et al. 2013), there is increasing recognition that macrophytes may provide food resources, both directly (e.g. Watson and Barmuta 2011) and through supporting algal epiphytes (Warfe and Barmuta 2006).

This study investigated the ecological role of submerged macrophytes in degraded streams in an agricultural landscape, to evaluate their potential in river restoration. A survey of macrophytes, environmental variables and macroinvertebrates was conducted across seven adjacent seasonally-flowing river systems in the Geographe Bay Catchment (Western Australia). Specifically, the study aimed to (i) identify environmental variables associated with macrophyte occurrence; (ii) determine whether the presence of submerged macrophytes was associated with stream

degradation; and (iii) assess whether reaches with native macrophytes support a more abundant and diverse aquatic invertebrate assemblage than those without.

## Methods

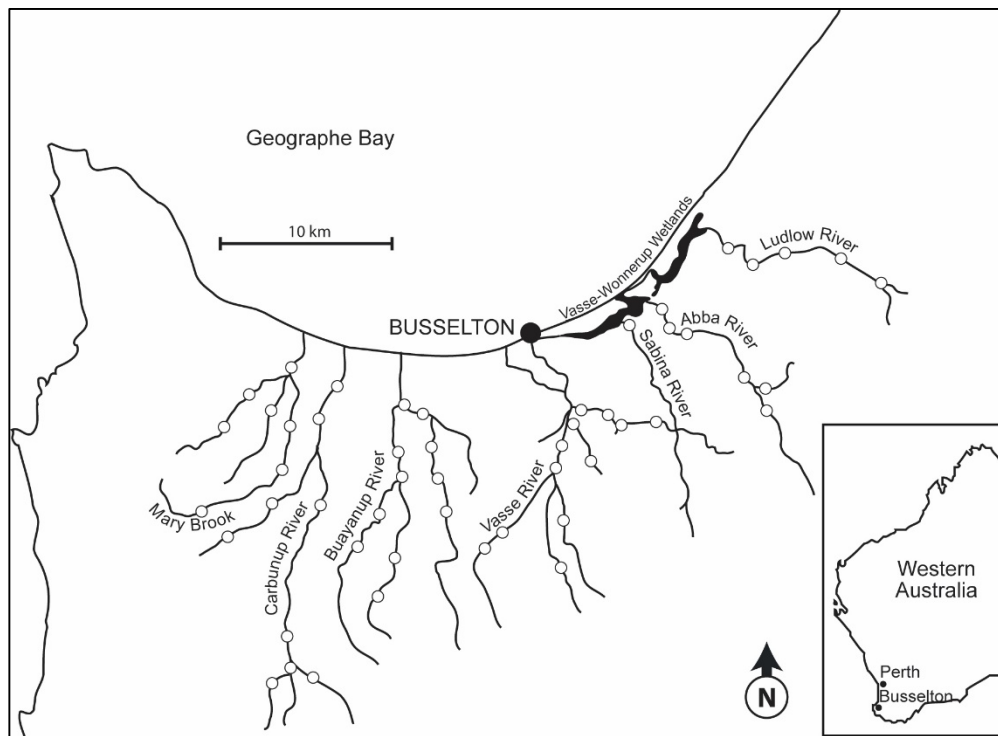
### *Study area*

The study area included seven adjacent river systems in the Geographe Bay catchment, located approximately 250 km south of Perth, Western Australia (Figure 3.2): the Ludlow, Abba, Vasse, Sabina, Buayanyup, Carbunup and Marybrook Rivers. The region experiences a mediterranean climate of mild, wet winters and hot, dry summers. The catchment covers an area of approximately 2000 km<sup>2</sup>, encompassing a series of short river systems (20 – 45 km in length) with headwaters in the lateritic Whicher Scarp, and traversing the Swan Coastal Plain to discharge into Geographe Bay. Swan Coastal Plain soils are characterised mainly by sandy-loam and deep sands with large areas of low-lying, seasonally inundated flats. Current land use is primarily dairy, livestock grazing, viticulture and horticulture. Stream modifications for drainage and impoundment are common, and surface water is extracted for agricultural and domestic water use. The catchment has been extensively cleared for agriculture, particularly coastal plain areas (37% of native vegetation remains, mainly in forested headwaters: Connell et al. 2000). Riparian vegetation has been impacted through direct clearing, livestock damage, and undermining of unstable banks. Natural riparian vegetation commonly includes an over-storey of *Eucalyptus* spp., *Corymbia calophylla*, *Agonis flexuosa* trees; mid storey of *Melaleuca* spp. and *Taxandria* spp.; and a mixed understorey of shrubs and rushes, with common emergent rushes including: *Baumea*, *Juncus* and *Leptocarpus* spp.

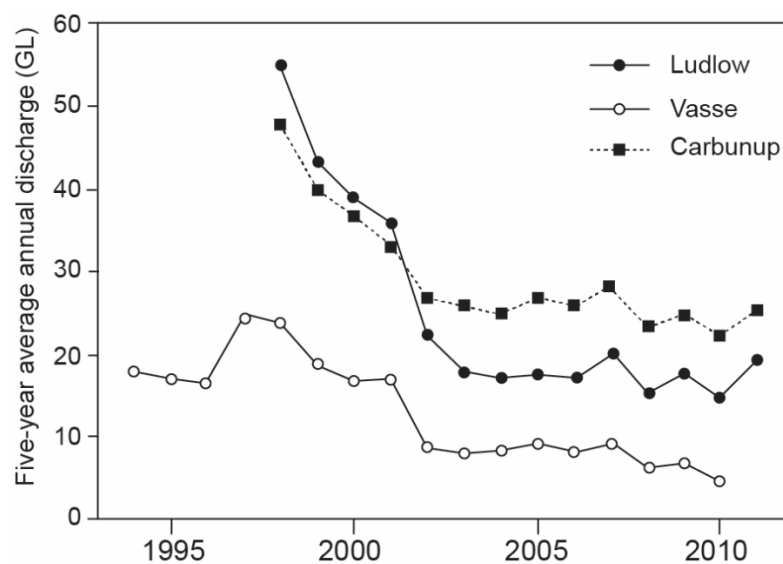
Average maximum temperature is 29 °C in summer and 17 °C in winter (BoM 2014). Average annual rainfall is 740 mm (1991-2010), but the 20-year average has declined by 15% over fifty years (1941-1960: 863 mm; BoM 2014). Seventy-five percent of rainfall occurs between May and September (DoW 2010) resulting in typical hydrographs of peak winter-spring flows and summer-autumn drying. Reduced rainfall and increased water extraction and impoundment have led to a



substantial decline in river flows (Figure 3.3). Also, gauging data show a reduced frequency of high flow events and base flow rate (DoW 2015).



**Figure 3.2.** Location of the fifty-three study reaches (open circles) included in the survey. The location of Busselton is also shown with reference to the capital city of Perth in Western Australia.



**Figure 3.3.** Mean annual discharge for the Ludlow, Vasse and Caribunup Rivers (only three rivers are gauged). Five-year average is calculated from the given year and the two years before and after (DOW 2015).

## ***Sampling design and field methods***

Fifty three reaches (20 m length) in seven seasonally-flowing river systems were surveyed in November 2013 (Figure 3.2) during spring base flow conditions. These included all accessible wadeable reaches with consistent flow (reaches on private land could not be accessed). Study reaches were upstream of road bridges where possible to exclude any influences, but in all cases, crossings were elevated bridges that did not impound or flow or influence substratum. When reaches were sampled downstream of bridges, they were located at a sufficient distance to reflect stream characteristics irrespective of the crossing. Most reaches were predominantly run (glide) habitat; riffles and pools were rare.

Native species of submerged (roots in sediment, all leaves submerged), floating-leaved (roots in sediment, some leaves submerged, some floating) and semi-emergent (roots in sediment but with some emergent leaves/stems) aquatic plants were recorded at each site. Identifications were confirmed by the Western Australian Herbarium. No native free-floating species were encountered and emergent rushes were not sampled. Cover for each native species, and for combined exotic species were assessed using the Braun-Blanquet scale: r = solitary plant, small cover; x = few plants, small cover; 2 = cover 5-25%; 3 = cover 25-50%; 4 = cover 50-75%; 5 = cover > 75%). Riparian condition assessment was completed using a method widely adopted in this region (WRC 1999) based on vegetation condition and stream stability, which grades each reach from pristine (A) to degraded (D), with three levels of condition within each grade (Table 3.1). Livestock access was noted as present or absent.

Physical stream characteristics (shading, periphyton growth, erosion, deposition, and substratum type) were scored using a scale of occurrence adapted from Chessman and Royal (2010): none (0) isolated (1), scattered (3), common (5) or abundant (7), with intermediate scores as appropriate. For substratum, scores were given for each of bedrock, boulders (> 256 mm diameter), cobbles (64-256 mm), pebbles (16-64 mm), granules (4-16 mm) and fines (< 4 mm) following Chessman and Royal (2010). Further classification of physical characteristic based on these scores for frequency analysis is described below.

**Table 3.1.** Riparian condition assessment category descriptions (adapted from WRC 1999).

Category	Description	Level
A	Stable, with pristine to slightly disturbed riparian vegetation.	A1: Pristine condition. A2 Occasional weeds. A3: Localised disturbance.
B	Stable, with all components of riparian vegetation, but significant weed invasion.	B1: Native vegetation dominant. B2: Native vegetation and weeds co-dominant. B3: Weeds dominant.
C	Native understorey degraded, but some remnant vegetation; variable stability.	C1: Erosion prone, weeds maintain stability. C2: Some erosion. C3: Actively eroding.
D	Streams devoid of native vegetation; artificial drains	D1: Some areas of stability. D2: Extensive erosion and deposition. D3: Highly eroded, but fenced and colonised with weeds.

Depth and velocity measurements taken at intervals along a cross section provided outputs of mean velocity, total discharge, depth, width and cross-sectional area for each reach (Flowtracker Handheld-ADV®). Cross sections were selected at a point which approximated average conditions for the reach, devoid of obstructions or pools, to maintain consistency across reaches. Reaches were visited monthly to determine the time of flow cessation because this is important in terms of length of growth season for macrophytes. Flow cessation was categorised as early-summer (December), mid-summer (January) or late summer (February). No reaches flowed after February. All reaches with late-summer flow retained pools which persisted beyond flow cessation, reflecting the local unconfined groundwater table, but these pools dried out during autumn.

Temperature, dissolved oxygen, conductivity, salinity and pH were measured *in situ* (YSI 556 MPS multiparameter probe). Turbidity was measured on site with a Hach 2100P turbidimeter. Samples were collected for laboratory analysis of total phosphorus (TP) and total nitrogen (TN) (Valderrama 1981); and colour (dissolved organic carbon) as gilvin (absorbance at 440 nm x 2.303 x 100, Kirk 1986).

Macroinvertebrate samples were initially taken from a random subset of twenty sites, including ten reaches with no macrophytes and ten with native macrophytes. When it became clear that two distinct macrophyte assemblages were present in these rivers, additional (randomly chosen) reaches were sampled. Aquatic

invertebrates were sampled using a single ten-metre sweep (D-frame net, mesh size 250  $\mu\text{m}$ ) at twenty-five reaches: ten without macrophytes, eight with the *Cycnogeton* plant assemblage and seven with the *Potamogeton/Ottelia* plant assemblage (Braun-Blanquet score  $\geq 2$ ). Whole samples were preserved in ethanol in the field and invertebrates identified to family level (except Copepoda, Cladocera, Collembola, Clitellata, Hydracarina and Hirudinea, which were not identified further) and counted in the laboratory.

### ***Data analysis***

Two macrophyte assemblage types were identified, named by genus: *Potamogeton* and *Ottelia*; and *Cycnogeton*. A series of two-way contingency tables were constructed to test associations between occurrence of native macrophytes, stock access and physical reach characteristics. Frequency analysis was performed twice: once for macrophyte presence and absence categories; and secondly comparing absence, *Potamogeton/Ottelia*, and *Cycnogeton* categories. Riparian condition was collapsed into three categories: A and B condition reaches were grouped as there were only 4 reaches in A condition, and both these grades contain intact remnant riparian vegetation. Reaches were classified into three categories for stream shading, periphyton growth, erosion and deposition, based on the 0-7 scoring scale: none or isolated (0-1), scattered (2-4) and abundant (5-7). Substratum scores were used to derive three categories: mostly sandy, mixed substrate and mostly rocky. Braun-Blanquet scores for aquatic weed cover were pooled as either none or few (0, r, x) or common (2-5). Flow cessation categories of early- mid- and late-summer were used. Stream width was classified into three groups: < 2.5m, 2.5-4.5m and > 4.5m.

Where there were observed frequencies less than 5 within these categories, Fisher's exact tests were used in addition to Pearson's Chi-square, with the Freeman-Halton extension for tables larger than two by two (Quinn and Keogh 2003). Odds and maximum likelihood (ML) odds ratios were calculated for tables where a significant association was found, with subdivision of larger tables into subsets of two by two tables. Where tables contained cells with zero observed frequencies, 0.5 was added to each cell for calculation of odds ratios (Quinn and Keogh 2003).

Multivariate analysis using all environmental variables was undertaken using canonical analysis of principal coordinates (CAP) to discriminate among reaches with *a priori* grouping based on macrophytes. Separate analyses were completed for presence and absence categories; and absence, *Potamogeton/Ottelia*, and *Cycnogeton* categories. A correlation matrix of environmental variables was constructed using Spearman's correlation coefficient.

Single factor ANOVA (SPSS version 21) was used to determine whether mean aquatic invertebrate abundance and mean family-level richness differed between macrophyte assemblage type (three levels, fixed: macrophytes absent, *Potamogeton/Ottelia*, *Cycnogeton*). All ANOVAs were checked for homoscedasticity (Levene's test) and normality (Shapiro-Wilk test) and abundance data was transformed ( $\log_{10}(x+1)$ ) to meet the normality assumption. Planned contrasts compared differences among means for these two dependent variables between the presence or absence of each macrophyte type (*Potamogeton/Ottelia* and *Cycnogeton*). Because taxa richness is positively correlated with abundance (the well-known species-abundance relationship, Gotelli and Colwell 2001), mean rarefied family richness was calculated using the EcoSim program (Gotelli and Ensminger 2000). The ANOVAs and contrasts described above were repeated using the rarefied data.

Differences in invertebrate assemblage composition between reaches with and without macrophytes, and between reaches with different macrophyte assemblages, were examined using single-factor ANOSIM (analysis of similarity) based on a Bray-Curtis similarity matrix, using untransformed abundance data. SIMPER (similarity percentages) was used to identify the families responsible for the differences between reach groups and were presented using a graphical method developed by Lind et al. (2006). All multivariate analyses were completed using PRIMER-E 6 (Plymouth Routines in Multivariate Ecological Research: Clarke and Warwick 2001).

## Results

### *Spatial variation in environmental characteristics*

Seventy-five percent of reaches had poor riparian condition (C and D). Stream size varied considerably in terms of width, discharge and velocity (Table 3.2). Although velocity varied, all reaches had a part of the channel with low velocity, most commonly bank edges, and many reaches had areas with zero or negative velocity due to backwater areas at the bank edges. Average depth was variable (Table 3.2), but similar depth ranges were observed across all catchments. Wider stream reaches were found in the Vasse, Buayanyup, Caribunup and Marybrook systems, which had more reaches with good riparian condition and lower salinity (conductivity < 500  $\mu\text{Scm}^{-1}$ ) (Table 3.2). The two most downstream reaches in the Sabina River were slightly brackish and likely influenced by the nearby Vasse Estuary. Nutrient concentrations, turbidity and colour were variable across the catchment (Table 3.2). Total phosphorus (TP) and total nitrogen (TN) in the Sabina and Vasse rivers were well above local guideline values for ecosystem protection, and TN was also high in the Abba and Buayanyup catchments. Both TN and TP were positively correlated with decreasing riparian condition (TP:  $r = 0.44$ ,  $P = 0.001$ ; TN:  $r = 0.49$ ,  $P < 0.001$ ).

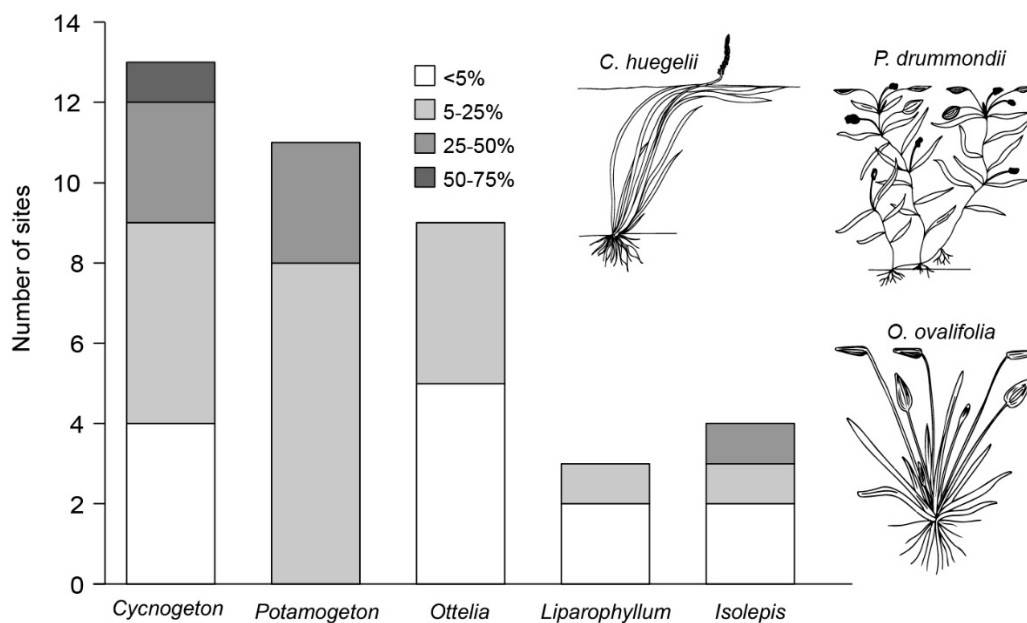
### *Native macrophyte assemblages*

Native submerged and semi-emergent macrophytes were observed at 23 of the 53 surveyed reaches (43%), with six species identified: *Cycnogeton huegelii* (Endl.), *Liparophyllum lasiospermum* (F. Muell.) Tippet and Les, *Potamogeton drummondii* Benth., *Potamogeton ochreatus* Raoul, *Ottelia ovalifolia* (R. Br.) Rich. and *Isolepis* sp. (absence of inflorescences prevented identification). *Cycnogeton* species in this region (*C. huegelii* and *C. lineare* (Endl.) Sond.) are very similar (previously both *Triglochin procerum*, DPaW 2014) and both may have occurred but were not distinguishable due to a lack of fruiting material. A maximum of only three species were found in any reach. Cover of native macrophytes did not suggest excessive growth, with cover at most reaches below 25% and only one reach had more than 50% cover (of *Cycnogeton* spp., Figure 3.4).

**Table 3.2.** Site characteristics for each river system in the survey. Values shown are number of sites for riparian condition and macrophyte community categories; and mean values with standard error in parentheses for physical and water quality variables. Nutrient values in italics indicate results in excess of local guidelines for ecosystem protection (ANZECC and ARMCNZ, 2000). Extent of clearing from Pen (1999) (dash indicates no data available). In Mary Brook, one reach contained only *Isolepis sp.* and so did not fall into the three macrophyte community groupings.

River	Ludlow	Abba	Sabina	Vasse	Buayanyup	Carbunup	Mary Brook
Number of sites	5	6	4	10	11	9	8
Macrophyte Community:							
Potamogeton /Ottelia	0	2	0	4	4	0	1
Cycnogeton	1	1	2	0	0	5	2
None	4	3	2	6	7	4	4
Riparian condition:							
A/ B	0	0	0	2	3	5	3
C	5	4	2	3	3	3	4
D	0	2	2	5	5	1	1
Catchment area (ha)	21081	13763	7643	30582	17400	16516	10913
Extent of clearing (%)	25	80	30	65	-	55	-
Width (m) (min-max)	3.9-6.4	1.6-5.6	2.15-3.3	1.55-8.05	2.15-9.2	2.9-9.9	1.9-5.5
Max depth (m)	0.50	0.35	0.66	0.31	0.34	0.44	0.25
Min velocity (ms <sup>-1</sup> )	-0.003	-0.008	0.00	-0.004	-0.011	-0.002	-0.038
Max velocity (ms <sup>-1</sup> )	0.126	0.112	0.130	0.160	0.220	0.314	0.381
Discharge (m <sup>3</sup> s <sup>-1</sup> )	0.041	0.026	0.045	0.072	0.102	0.300	0.129
Conductivity (µScm <sup>-1</sup> )	756 (49)	720 (66)	1227 (346)	445 (43)	418 (25)	389 (10)	479 (26)
pH	6.56 (0.3)	6.80 (0.2)	6.55 (0.2)	5.88 (0.5)	5.93 (0.2)	4.93 (0.3)	5.75 (0.4)
Colour (gilvin; g <sub>440</sub> m <sup>-1</sup> )	16.2 (2.5)	10.3 (1.9)	14.7 (6.0)	9.2 (2.5)	12.2 (2.1)	30.0 (18.5)	19.2 (5.7)
Turbidity (NTU)	6.1 (0.6)	5.6 (0.3)	11.0 (1.6)	14.8 (6.3)	3.2 (0.3)	11.2 (3.7)	5.1 (0.8)
TP (µgL <sup>-1</sup> )	38.0 (3.7)	27.8 (3.4)	179 (85.5)	110.4 (53.5)	32.27 (8.8)	17.33 (2.8)	21.71 (7.5)
TN (µgL <sup>-1</sup> )	874 (46)	1273 (546)	1925 (470)	1607 (350)	1635 (513)	610 (33)	804 (128)

*Cycnogeton* spp. occurred at thirteen sites, with *L. lasiospermum* also present at three of these. *Ottelia ovalifolia* and *Potamogeton* spp. often grew together, co-occurring at seven reaches and each growing alone at two reaches. Isolated *Cycnogeton* plants occurred in two reaches with *O. ovalifolia* and/or *Potamogeton* spp. *Isolepis* sp. occurred at four reaches, three of which had *O. ovalifolia* and/or *Potamogeton* spp. present. Thus two distinct macrophyte assemblages with differing distribution were identified, herein referred to as *Cycnogeton* and *Potamogeton/Ottelia*. These assemblages also differ in morphology (Figure 3.4): *Cycnogeton* is characterised by smooth strap-shaped leaves growing from the base of the plant; *Potamogeton/Ottelia* has more complex structure, because *O. ovalifolia* and *P. drummondii* have both submerged and floating leaves and *P. drummondii* and *P. ochreatus* form dissected submerged canopies.



**Figure 3.4.** Number of sites within each Bran-Blanquette category for each genus of aquatic macrophyte. Macrophyte drawings show differing structural complexity of the two plant assemblages.



Introduced aquatic plants were present in 31 reaches (58%) including *Callitriche stagnalis* Scop., *Mentha pulegium* L., *Juncus microcephalis* Kunth, *Isolepis nodosa* (Rottb.) R.Br. and *Elodea canadensis* Michx. Grasses such as *Pennisetum clandestinum* Chiov. and various species of *Paspalum* L. also grew in some stream channels. Exotic species occurred mainly in poor condition reaches (28 C and D condition reaches) at varying levels of colonisation, and were also present at three B condition reaches. There was no significant association between native macrophyte and exotic aquatic plant presence in either frequency analysis or CAP.

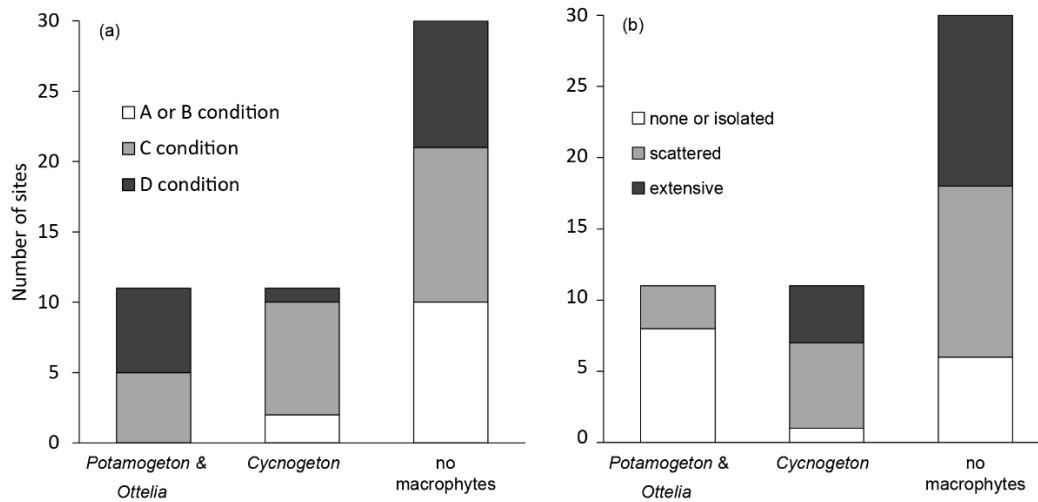
Although macrophytes occurred at only three good (A or B) condition reaches (two with *Cycnogeton* and one with *Isolepis* sp.), presence/absence data did not indicate an association between riparian condition and macrophyte occurrence. Absence of macrophytes was significantly associated with stock access ( $X^2_1 = 4.6$ ,  $P = 0.034$ ), because macrophytes were present at only one reach that had stock access.

Macrophytes were more likely to be present at reaches with no stock access compared to those with stock access (odds ratio = 8; CI<sub>95</sub>: 0.92-69.44), however most reaches (83%) did not have direct stock access, and odds for presence and absence of macrophytes were equal (0.5) among reaches without access.

Macrophyte presence/absence was not significantly associated with other categorical factors (riparian condition, shading, periphyton cover, aquatic weeds, erosion, deposition, substratum type, flow duration). Furthermore, sites without macrophytes were equally likely to be in D, C or A/B levels of riparian condition (Figure 3.5a).

Analysis of three groupings (absence, *Potamogeton/Ottelia*, and *Cycnogeton* categories) found a significant association between macrophyte assemblage type and both riparian condition ( $X^2_4 = 10.0$ ,  $P = 0.037$ ) and shading ( $X^2_4 = 15.0$ ,  $P = 0.005$ ). Results for these two variables were very similar (Figure 3.5) and highly correlated ( $r = -0.693$ ,  $P < 0.001$ ). Importantly, the two macrophyte assemblages responded differently, explaining the lack of association with macrophyte presence/absence categories. The odds of *Potamogeton/Ottelia* occurring were greater in C and D condition reaches with little or no shade, while odds of *Cycnogeton* occurring were greater in shaded C condition reaches (Table 3.3). Odds ratios also indicated that association with riparian condition was due mainly to the presence of *Cycnogeton* in C condition compared with D condition reaches; and the

association with shading was due to the presence of *Potamogeton/Ottelia* in reaches with no or isolated shade (Table 3.3).

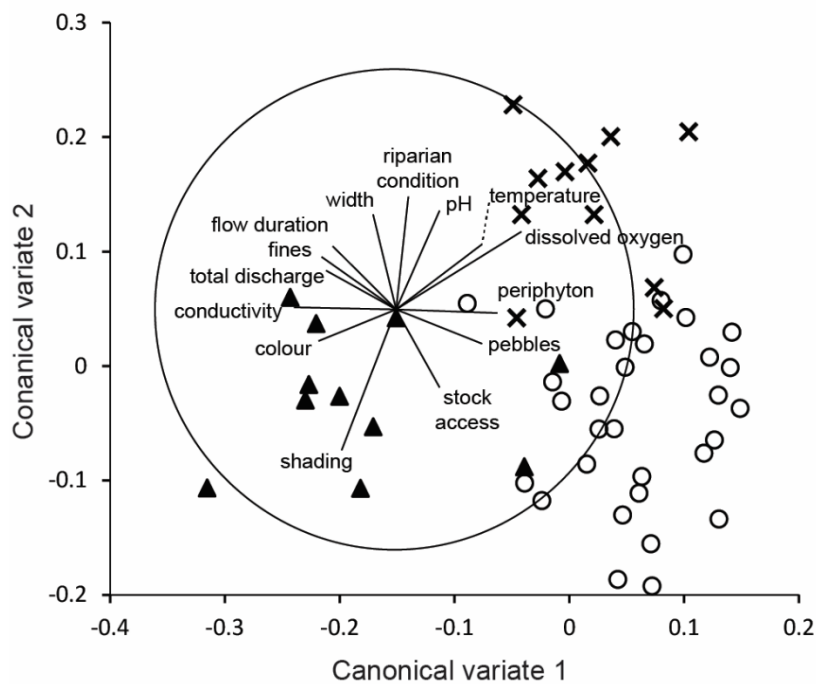


**Figure 3.5.** Distribution of each macrophyte assemblage group by riparian condition (a) and level of shading (b).

**Table 3.3.** Odds and odds ratios for riparian condition and shading.

Odds	Category	Potamogeton/ Ottelia	Cycnogeton
Foreshore condition	A or B	0.00	0.20
	C	0.45	0.73
	D	0.67	0.11
Shading	none or isolated (1)	1.33	0.17
	scattered (2)	0.25	0.50
	extensive (3)	0.00	0.33
<b>Odds ratios</b>	<b>Comparison</b>		
Foreshore condition	AB vs C	0.10 (0-2.03)	0.32 (0.06-1.66)
	AB vs D	0.07 (0-1.41)	1.51 (0.17-13.70)
	C vs D	0.70 (0.17-2.9)	4.68 (0.67-32.47)
Shading	2 vs 3	7.00 (0.33-150.06)	1.44 (0.34-6.07)
	1 vs 3	32.69 (1.62-660.28)	0.65 (0.08-5.11)
	1 vs 2	4.67 (0.98-22.33)	0.44 (0.06-3.32)

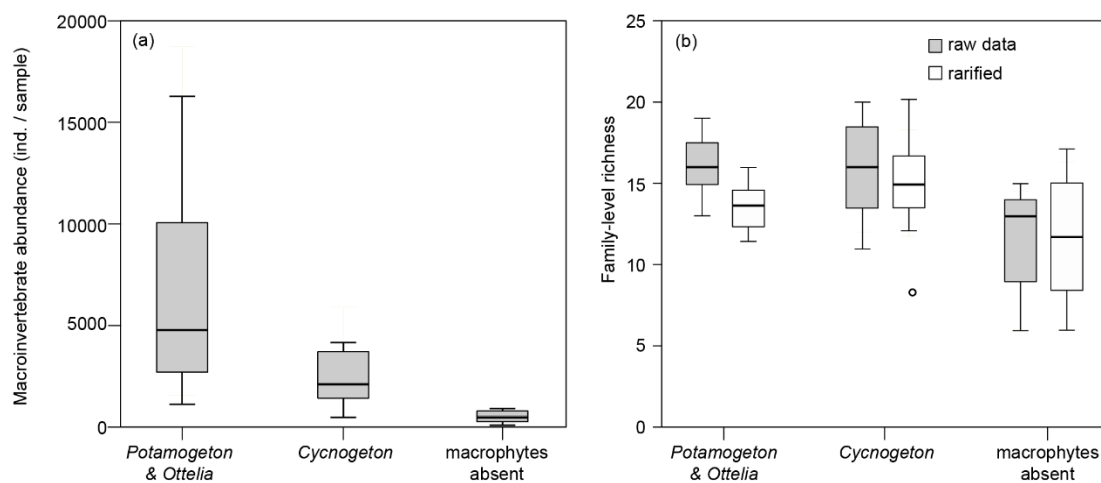
The three groupings of *Potamogeton/Ottelia*, *Cycnogeton* and macrophyte absence were clearly discriminated (axis correlations of 83% and 73%) on the basis of the measured environmental variables (Figure 3.6). Spearman rank correlation vectors indicated that presence of *Cycnogeton* was associated with increased shade and water colour; while *Potamogeton/Ottelia* was associated with higher temperatures and daytime dissolved oxygen (potentially resulting from plant photosynthesis), and inversely correlated with shade and colour (Figure 3.6). Plant absence was associated with substratum type and flow, having a positive correlation with pebbles and inverse correlation with fines, discharge velocity and flow season length (Figure 3.6). Turbidity and nutrients were not important determinants of macrophyte presence. Although the presence of neither plant assemblage was correlated with substratum variables, Braun-Blanquette scores did indicate a positive association with fine substrate. All reaches with macrophyte cover greater than 5% had at least scattered fines, and more than 85% of these reaches had common-abundant fine substratum particles.



**Figure 3.6.** Canonical analysis of principal components (CAP) for macrophyte assemblage groups: *Cycnogeton* (triangles), *Potamogeton/Ottelia* (crosses) and no macrophytes (open circles).

## *Invertebrates*

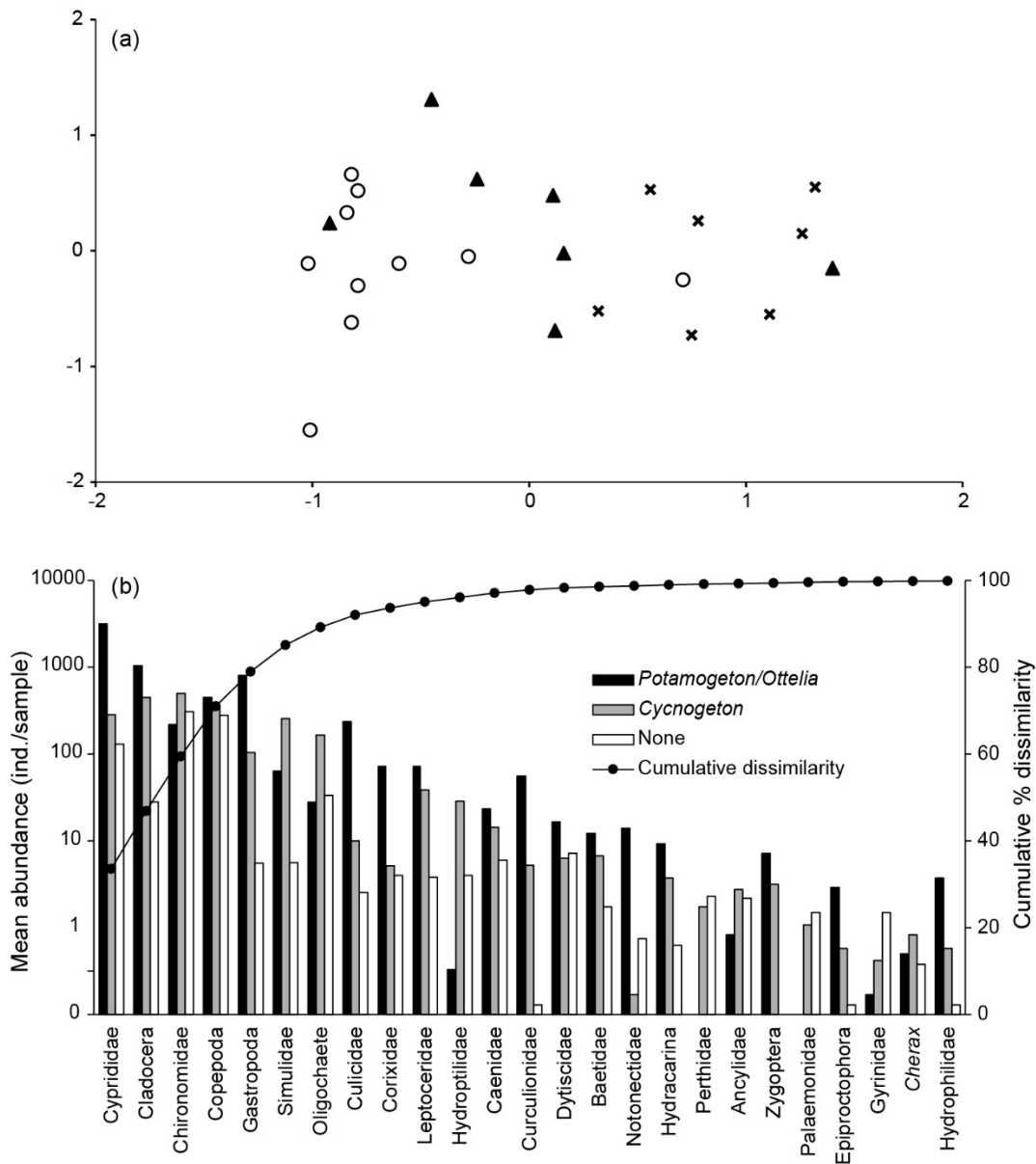
Mean aquatic invertebrate abundance ( $F_{2,21} = 12.04$ ,  $P < 0.001$ ) and mean family level richness ( $F_{2,21} = 7.13$ ,  $P = 0.004$ ) differed between macrophyte assemblage types. Macroinvertebrate abundance was greater in the presence of macrophytes overall ( $t_{21} = 4.55$ ,  $P < 0.001$ ); and in each macrophyte assemblage compared with macrophyte absence (*Potamogeton/Ottelia*:  $t_{21} = -4.82$ ,  $p < 0.001$ ; *Cycnogeton*: ( $t_{21} = 2.84$ ,  $P = 0.010$ ) (Fig. 7a). Mean abundance did not differ between *Potamogeton/Ottelia* and *Cycnogeton* due to high variation among reaches (Fig. 7a;  $t_{21} = -1.83$ ,  $P = 0.083$ ). Family-level richness was also higher in reaches with *Potamogeton/Ottelia* ( $t_{21} = -3.27$ ,  $P = 0.004$ ) and *Cycnogeton* ( $t_{21} = 3.07$ ,  $P = 0.006$ ) compared with reaches with no macrophytes (Fig. 7b), but did not differ between the two macrophyte assemblages ( $t_{21} = -0.18$ ,  $P = 0.858$ ). Mean rarefied taxa richness did not differ between reaches with or without macrophytes ( $F_{2,21} = 1.57$ ,  $P = 0.232$ ) (Fig. 7b), suggesting that increased family richness occurred as a consequence of higher invertebrate abundance amongst macrophytes. Reaches without macrophytes had seven fewer families than those with macrophytes, with Lestidae and Coenagrionidae (Zygoptera) notably absent. The only taxon exclusive to reaches without macrophytes was Ceratopogonidae (Diptera), but these were found in only one reach.



**Figure 3.7.** Macroinvertebrate abundance (a) and family-level richness (b) for each macrophyte assemblage. Boxplots show median (line), 25-75<sup>th</sup> percentile range (box), 5-25<sup>th</sup> and 75-95<sup>th</sup> percentile range (error bars) and outliers (o).

Invertebrate assemblage composition differed significantly between reaches with and without macrophytes ( $R = 0.29$ ,  $P = 0.008$ ) and between reaches with *Potamogeton/Ottelia* and those with *Cycnogeton* ( $R = 0.29$ ,  $P = 0.002$ , Figure 3.8a). Reaches with macrophytes had much higher abundance of grazing taxa, with Cyprididae, Cladocera, Copepoda and Gastropoda accounting for 67% of dissimilarity (Figure 3.8b). Damselfly nymphs were only recorded in the presence of macrophytes and many other taxa were more abundant in their presence (Figure 3.8b). A few families occurred with approximately equal abundance regardless of macrophyte presence or absence: the generalist feeders Chironomidae and Copepoda, predatory Dytiscidae and detritivorous caenid mayfly nymphs. Overall, some grazers (gastropods (excluding limpets), Curculionidae), suspension feeders (Simuliidae), shredders (Leptoceridae) and some predators (Odonata, Leptoceridae, aquatic mites) occurred more frequently in reaches with macrophytes than in those without (Figure 3.8b).

Reaches with *Potamogeton/Ottelia* had higher abundances of grazing Cyprididae, Cladocera and Gastropoda (except limpets) than those with *Cycnogeton*, and also of Culicidae, Corixidae, Curculionidae and Notonectidae. Hydroptilid caddisfly larvae, oligochaetes, Simuliidae larvae, freshwater limpets and crayfish were most abundant in reaches with *Cycnogeton* (Figure 3.8b). Amphipods (Perthidae) and Decapods (Palaemonidae, Parastacidae) were found only in reaches with *Cycnogeton* or with no macrophytes; although the two sites where Decapods occurred without submerged macrophytes did have good riparian condition together with emergent rushes.



**Figure 3.8.** Comparison of macroinvertebrate community composition for macrophyte assemblages using MDS ordination of invertebrate abundance data (a) (2D stress = 0.14); comparison of taxon abundance within each assemblage (b: bars); and cumulative contribution to dissimilarity between presence and absence of macrophytes (b: line). Data points in (a) indicate macrophyte assemblage groups: *Cycnogeton* (triangles), *Potamogeton/Ottelia* (crosses) and no macrophytes (open circles).

## Discussion

### *Native macrophyte occurrence*

Native submerged macrophytes were relatively common in these seasonally-flowing streams but species richness was low, a maximum of three per reach, consistent with reach-scale macrophyte richness in eastern Australian streams (Quinn et al. 2011). Two distinct macrophyte assemblages were observed: *Cycnogeton* spp.; and *O. ovalifolia* co-occurring with *Potamogeton* spp. (*P. ochreatus* and/or *P. drummondii*). While both assemblages were associated with poor riparian condition, they had contrasting associations with shade (discussed further below).

The association of macrophyte absence with low total discharge and short hydroperiod suggests larger streams with a longer growing season were more favourable for development of macrophyte beds. This influence of flow contrasts with larger perennial systems, where flood disturbance and velocity are primary limiting factors for macrophyte distribution (Riis and Biggs 2003; Franklin et al. 2008). Physical limitation of plant growth by current is unlikely in these streams; instead, lower flow rates and shorter hydroperiods are more likely to influence macrophyte distribution (Figure 3.1). Hydrology within the study area has changed dramatically over the last five decades, with reduced streamflow and hydroperiod as a consequence of both catchment-scale land use (drainage, impoundment and abstraction) and declining regional rainfall; and reach-scale base flow velocity was generally low. While low flows can enhance macrophyte growth due to physical stability (Suren and Riis 2010) and both assemblages can clearly persist under seasonally dry conditions, shorter periods of flow may be detrimental because plants must complete their life cycle within the flow period to grow successfully the following year (Warwick and Brock 2003).

Substrate type is important in terms of recruitment and anchorage of macrophytes (Bornette and Puijalon 2011), with loose stones providing poor substrate (Butcher 1933). Not surprisingly, macrophyte absence was associated with pebble substrate in these reaches, and plants were not found in reaches without fine substrate. Runoff from cleared lands and erosion of unstable banks has resulted in substantial

mobilisation of sediment in these river catchments. Depositional forces often dominate in streams with low gradient (Bornette et al. 2008), as found on the Swan Coastal Plain, and extensive sediment deposition along stream beds was common. This accumulation of finer-textured sediment provides new areas of suitable substratum for colonisation (Figure 3.1; Gurnell et al. 2006; Suren and Riis 2010). Larger macrophyte stands occurred in reaches with sediment deposition, indicating colonisation of deposited sediment (Lind et al. 2009) and sediment accumulation within plant stands is also likely (Figure 3.1; Gurnell et al. 2006).

Stock access appeared to preclude macrophyte growth, although presence and absence of macrophytes were equally likely in reaches with no stock access. Livestock impede macrophyte growth by grazing, and trampling during the dry season can prevent re-establishment during the following flow period (Pettit et al. 2012). Cattle access can increase nutrient and chlorophyll a concentrations and decrease light penetration (Pettit et al. 2012) which, together with indirect effects of reduced riparian cover and reduced bank stability, may have variable effects on macrophyte growth (Figure 3.1).

Elevated nutrient concentrations were correlated with poor riparian condition, as would be expected in this agricultural landscape. Although elevated stream nutrient levels in agricultural regions may enhance macrophyte growth (Chambers 1987; Mebane et al. 2014), there was no association between macrophytes and nutrients in this study. The few other Australian studies have also not found strong correlations between nutrient status and macrophyte distribution (Chessman and Royal 2010; Mackay et al. 2010), and increased water column nutrients may have little positive effect on macrophytes in rivers and streams (Madsen and Cedergreen 2002, Hilton et al. 2006).

Although clearly part of lotic ecosystems, with flow providing an important dispersal pathway (Nilsson et al. 2010), the growth of submerged macrophytes in these streams occurs during seasonal conditions of low- to no-flow, which at times more closely resemble lentic environments. However, the focus of macrophyte research in lentic and lotic systems has differed markedly. In rivers, much work examines macrophyte interactions with the abiotic conditions of flow, substrate, light and nutrients (e.g. Sand-Jensen 1998; Barrat-Segretain 1996; Clark 2002;



Gurnell et al. 2006; Franklin et al. 2008) and the variable response of different growth forms (e.g. Chambers and Klaff 1987; Rea and Ganf 1994; Blanch et al. 1998; Deegan et al. 2012). In shallow lentic systems, research has focussed on interactions of macrophytes with chemical processes (e.g. Wigand et al. 1997; Barko and James 1998) and trophic relationships (e.g. Timms and Moss 1984; Jeppesen et al. 1999; Norlin et al. 2005), and the implications for lake restoration (e.g. Moss 1990; van Donk and van de Bund 2002; Sondergaard et al. 2007). Historically, these streams would have experienced greater flow volumes and velocities, and in some cases perennial flows; have been less dominated by fine substratum; and been heavily shaded, even in the lower reaches. However, rainfall decline, altered hydrology and extensive clearing has changed the character of these streams, so they are less limited by flow disturbance, substratum characteristics and riparian shading. Rather, as seen in seasonal wetlands, macrophytes in these streams are limited to environments with sufficient hydroperiod to complete their life cycle and grow successfully the following year (Warwick and Brock 2003). Furthermore, as in shallow lakes, these data suggest that macrophytes influence trophic relationships by increasing grazer numbers and overall invertebrate richness and abundance. Under some circumstances then, as in shallow lakes, it may be appropriate to consider macrophytes in restoration of seasonally-flowing lowland streams (discussed further below).

### ***Macrophyte assemblage response to riparian condition***

While both macrophyte assemblages were associated with poor riparian condition, high light availability appeared to be important for the *Potamogeton/Ottelia* assemblage, while *Cycnogeton* spp. were associated with cooler, shaded reaches. The occurrence of distinct macrophyte assemblages commonly arises from differing autecology among species leading to different patterns of competitive advantage at particular locations (Barrat-Segretain 1996). Our observations suggest these species' differing responses to shade may explain their different distribution pattern in degraded streams. The *Potamogeton/Ottelia* assemblage, which was associated with poorly-shaded reaches, has potential for wider distribution in the Geographe Bay catchment where riparian vegetation is commonly degraded. This positive

response is less likely for *Cycnogeton* spp, which was associated with shadier reaches.

Growth of *Cycnogeton* spp. in shaded sites reflects their ability to reallocate resources from tubers to support rapid elongation of shoots to maximise exposure to light (Middelboe and Markager 1997). Their association with shade may also relate to a lower risk of seasonal desiccation in shaded reaches, where moist microclimates would extend the growing season of *Cycnogeton* spp., providing more favourable conditions for both vegetative growth and regrowth from perennial tubers, and increasing success of sexual reproduction (Rea and Ganf 1994). The presence of *Cycnogeton* spp. in shade in both good and poor condition reaches may indicate that these are remnant populations persisting despite changes to riparian condition and hydrology. The association with degraded riparian zones may simply reflect the predominance of reaches in this condition in the Geographe Bay catchment.

Restriction of the *Potamogeton/Ottelia* assemblage to degraded sites with little shade suggests dependence on high light availability. This is consistent with other Australian studies in which low riparian canopy cover has been associated with *P. ochreatus*, the ecologically similar *Potamogeton perfoliatus* and the floating-leaved *Potamogeton tricarinatus* (Mackay et al. 2003; Chessman and Royal 2010). In lentic systems, where light is limited primarily by water transparency, these species tolerate turbid conditions by canopy formation near the surface (*P. ochreatus*) and development of floating leaves (*P. drummondii* and *O. ovalifolia*) (Chambers 1987). However these adaptations are not advantageous where light at the water surface is limited by shade. This assemblage is also able to tolerate greater exposure to desiccation in unshaded reaches, because both *Potamogeton* and *Ottelia* produce drought-tolerant propagules (Wiegleb and Brux 1991; Jiang and Kadono 2001), enabling re-establishment from a seed bank following seasonal drying. These species occur commonly in lentic systems and are not well adapted to high flow velocity (being comparatively broad-leaved). They are likely to thrive in well-lit, low-flow conditions where sediment deposition is occurring, and may thus represent a colonising assemblage in these degraded streams. Yet despite a positive association with stream degradation, excessive growth of this plant assemblage was not observed. Excessive growth is perhaps prevented by seasonal drying (Deegan et

al. 2012), which restricts dispersal and vegetative growth required for widespread colonisation (Barrat-Segretain 1996). In addition, self-regulation of patch size may result from the diversion of flow around plant stands, created by resistance within the stands that in turn creates adjacent areas of high velocity and coarser, less stable substrate that are unsuitable for macrophyte colonisation (Sand-Jensen and Mebus 1996; Garcia et al. 2012).

### ***Macroinvertebrates***

This study found higher macroinvertebrate abundance in reaches with macrophytes compared to those without. Higher family richness in reaches with macrophytes appeared due to the capacity of plant stands to support more individuals, although the families present in plant stands also showed functional responses to plant presence (e.g. families that graze epiphytic algae) and several taxa were only found among macrophytes. Submerged macrophytes are often found to support substantially higher abundance and diversity of aquatic invertebrates in streams and rivers, and this is generally attributed to provision of habitat, food resources and refuge from predation (Heck and Crowder, 1991; Humphries et al, 1996; Lind et al. 2006; Shupryt and Stelzer 2009). In this study, higher abundance of grazers, responding to the increased productivity of epiphytic algae in macrophyte sites, accounted for most of the difference in abundance. Many other groups also had greater abundance in reaches with macrophytes and several were absent from reaches without macrophytes, including some that depend on plants for aspects of their life history (e.g. damselfly nymphs hatching from endophytic eggs). Because plants in this study did not grow in dense beds, the potential negative impacts of dense growth on macroinvertebrates (Suren and Riis 2010; Stiers et al. 2011) did not occur, so native submerged macrophyte presence in degraded reaches supported abundant and taxa-rich invertebrate communities.

While total abundance did not differ significantly, the more structurally complex *Potamogeton/Ottelia* assemblage supported greater abundance of grazers (Cyprididae, Cladocera, Curculionidae and Gastropoda) and shredders (Leptoceridae), although some taxa were more associated with *Cychnogeton* spp. Greater complexity offers protection from flow and supports periphyton growth, providing large amounts of food for grazing organisms (Warfe and Barmuta, 2006),

particularly in the unshaded conditions associated with this assemblage. Greater abundance of primary consumers in turn support larger numbers of predators (Humphries et al, 1996), and reaches with *Potamogeton/Ottelia* also had more Corixidae, Notonectidae, Leptoceridae and Odonata than those with *Cycnogeton* or without macrophytes. In contrast the strap-like leaves of *Cycnogeton* have less effect on local flow conditions (Sand-Jensen, 1998) and thus supported suspension-feeders that rely on flow (Hershkovitz and Gasith 2013; Bell et al. 2013), taxa which graze on flat surfaces such as limpets and hydroptilid caddisflies, and crustaceans that require flow for oxygen supply but also use macrophytes as a refuge from predation (Hacker and Steneck 1990). Also, because *Cycnogeton* was found in shaded reaches it may provide moist refuge habitat during the dry season, necessary for amphipods and palaemonid shrimp that have no desiccation-resistant life stages (Robson et al. 2011). Greater structural complexity is often related to higher diversity (Warfe et al. 2008; Bell et al. 2013), but the results obtained here showed no difference in family richness between the two plant assemblages. While this relationship is not always clear (Humphries et al. 1996; McAbendroth et al, 2005), the family level identification used in the present study may have been too coarse a resolution to show differences related to structural complexity. Alternatively, the river reaches studied here may have been sufficiently degraded to lose more sensitive families, limiting the capacity for macroinvertebrate richness to respond to habitat complexity (Lake et al. 2007).

Although the presence of submerged macrophytes supported macroinvertebrates in these reaches, abundance and richness metrics do not incorporate taxa sensitivity (Palmer et al. 2014). The communities present may be characterised by tolerant, opportunistic species with generalist diets which are able to thrive in degraded reaches where alternative resources are available. The use of family-level or higher classifications does not enable us to determine whether sensitive taxa were present. The loss of riparian vegetation and associated habitat and resources no doubt has negative consequences for stream biota (Allan 2004), and a shift to an invertebrate community supported by macrophytes may be indicative of altered ecosystem process and poor stream health (Bunn et al. 1999). However, in these degraded streams, the resulting invertebrate community may nonetheless provide a food source for vertebrate predators which are also of high conservation value,

particularly the highly endemic, and often threatened, native fish fauna of south-west Australia (Morgan et al. 2011).

### ***Management implications***

Although recolonisation of macrophytes is a common goal of restoration in Europe (Lorenz et al. 2012), they are rarely considered in this context in Australian streams. This study indicates the potential for positive growth response of some submerged native macrophyte species to stream degradation in seasonally-flowing streams, and demonstrates that these plant assemblages can support a more abundant and diverse aquatic fauna in reaches where riparian vegetation is degraded or absent.

Colonisation of degraded reaches by native macrophytes may compensate for loss of natural submerged habitat (*sensu* Lind et al. 2009) when riparian inputs decline as a result of vegetation clearing. While aquatic plants may be perceived as potentially invasive, this seems unlikely when restoring native species in seasonally-flowing systems, even under nutrient enriched conditions. In contrast, assimilation of nutrients from diffuse sources and stabilisation of bed sediments are potential benefits of submerged macrophyte restoration in streams (Clarke 2002). Colonisation of fine substrates by submerged macrophytes would be valuable in stabilising sediment deposits and preventing downstream transport to receiving waters (Lind et al. 2009). In degraded systems, provision of ecosystem functions by macrophytes where they naturally occur, have managed to colonise, or potentially could be planted, warrants consideration.

While large scale management intervention is necessary to improve hydrologic connectivity and water quality, and rehabilitation of riparian zones is an essential component of stream channel restoration (Palmer et al. 2014), submerged macrophytes could be considered as part of in-channel habitat enhancement. The two assemblages found in this study have different responses to light and flow, showing a variation in traits that provides options for restoration of diverse reach conditions. Their rapid seasonal growth has potential for improved biodiversity outcomes over short time periods (months), while other management actions are implemented (e.g. riparian revegetation), or could be valuable in areas where opportunities for riparian restoration are limited.

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## **Chapter 4. Potential of submerged macrophytes to support food webs in lowland agricultural streams**

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## Abstract

Submerged plants are often abundant in lowland streams in agricultural landscapes, but little is known of their role in stream ecosystems compared to riparian vegetation. We investigated the relative importance of submerged macrophytes as a basal resource of food webs in stream reaches with good and poor riparian vegetation condition, using mixing model analysis with stable carbon and nitrogen isotopes. Epilithic periphyton and terrestrial detritus were important basal resources in good condition reaches, although where macrophytes were present, they did contribute to food webs. Higher assimilation of either the macrophyte *Cyanogeton huegelii* or conspicuous epiphytes on *C. huegelii* leaves was associated with poor riparian condition. Where *Potamogeton ochreatus* and *Ottelia ovalifolia* occurred in poor condition reaches, these macrophytes contributed moderately to the food web, but were probably of greater importance as substrates for epiphytic algae. Mixing models indicated invertebrates commonly had generalist feeding strategies, feeding on the most available resource at each reach. Thus where riparian vegetation is limited, submerged macrophytes may support opportunistic consumers both directly and as a substrate for epiphytes, thereby partially compensating for the loss of allochthonous resources in lowland agricultural streams.



## Introduction

Aquatic macrophytes strongly influence physical and chemical processes in streams (Carpenter and Lodge 1986; Bornette and Puijalón 2011), and provide structural habitat and a predation refuge for aquatic fauna (Heck and Crowder 1991; Bell *et al.* 2013). They also have potential to contribute to food webs both directly in the form of fresh or detrital material (Jacobsen and Sand-Jensen 1994; Kornijow *et al.* 1995) and indirectly as a substrate for periphyton (Warfe and Barmuta 2006; Ferreira *et al.* 2011). However, river food web studies have often examined the relative importance of either terrestrial or algal inputs as the basis for stream food webs, excluding macrophytes, possibly because early research suggested low dependence of macroinvertebrates on macrophyte carbon (Hamilton *et al.* 1992; Bunn and Boon 1993).

Stable isotope studies have been valuable in comparing terrestrial detritus and algae as basal resources for food webs, because they usually have distinct isotopic signatures (Finlay 2001). The dependence of stream food webs on these two sources varies over a range of climatic and landscape factors (McCutchan and Lewis 2002, Bunn *et al.* 2003; England and Rosemond 2004). Terrestrial detritus is often important in forested streams (Power *et al.* 2013), with a shift to algal sources downstream, where slower flows rates and greater light availability increase instream productivity (Finlay 2001; Power *et al.* 2013).

Degradation of riparian vegetation in agricultural landscapes can reduce the supply of detrital material (Reid *et al.* 2008a), while increasing light availability to the stream bed thereby promoting growth of macrophytes (Canfield and Hoyer 1988). In this situation, the presence of macrophytes may provide an important alternative food source in streams, sustain in-stream productivity and consequently, faunal biodiversity. Food resources used by stream fauna can reflect availability (Vannote *et al.* 1980; St Clair 1994) and where aquatic fauna have flexible diets (e.g. Johnston *et al.* 2011), loss of riparian resources may increase the relative importance of macrophytes (Deegan and Ganf 2008).

Lowland streams can support conspicuous macrophyte growth during periods of low rainfall and flow (Watson and Barmuta 2011), as occur during spring and

summer in mediterranean-climate regions, and may then subsidise food webs (Reid *et al.* 2008b). River food webs in mediterranean-climate streams are generally thought to be based on terrestrial and algal sources rather than macrophytes (Power *et al.* 2013). However, the period of macrophyte growth in these streams corresponds with a period of relative hydrological stability, between winter flooding and summer drying, that coincides with peak biomass and diversity of aquatic fauna (Hershkovitz and Gasith 2013). At a time when freshwater macroinvertebrates may be preparing to metamorphose into aerial life stages and reproduce, or form desiccation-resistant life stages before streams dry out, macrophytes could provide an additional supply of nutritious food (Deegan and Ganf 2008; Reid *et al.* 2008b).

The development of modern mixing models has facilitated the use of stable isotopes to examine relative contributions to animal diets from multiple sources (Moore and Semmens 2008; Phillips *et al.* 2014), and recent studies have shown that macrophytes can be an important basal resource in several Australian streams (Deegan and Ganf 2008; Reid *et al.* 2008b; Watson and Barmuta 2011). This study used stable isotope analysis to examine the importance of submerged macrophytes as a basal source for stream food webs in reaches with varying riparian vegetation condition. Agricultural streams in the mediterranean-climate region of south-western Australia were sampled during late spring when macrophytes are abundant. We hypothesized that in reaches where macrophytes were present, they would contribute to stream food webs; and secondly, that the contribution of macrophytes would be greater in degraded reaches with poor riparian condition (and thus poor supply of allochthonous detritus) than in reaches with good riparian condition.

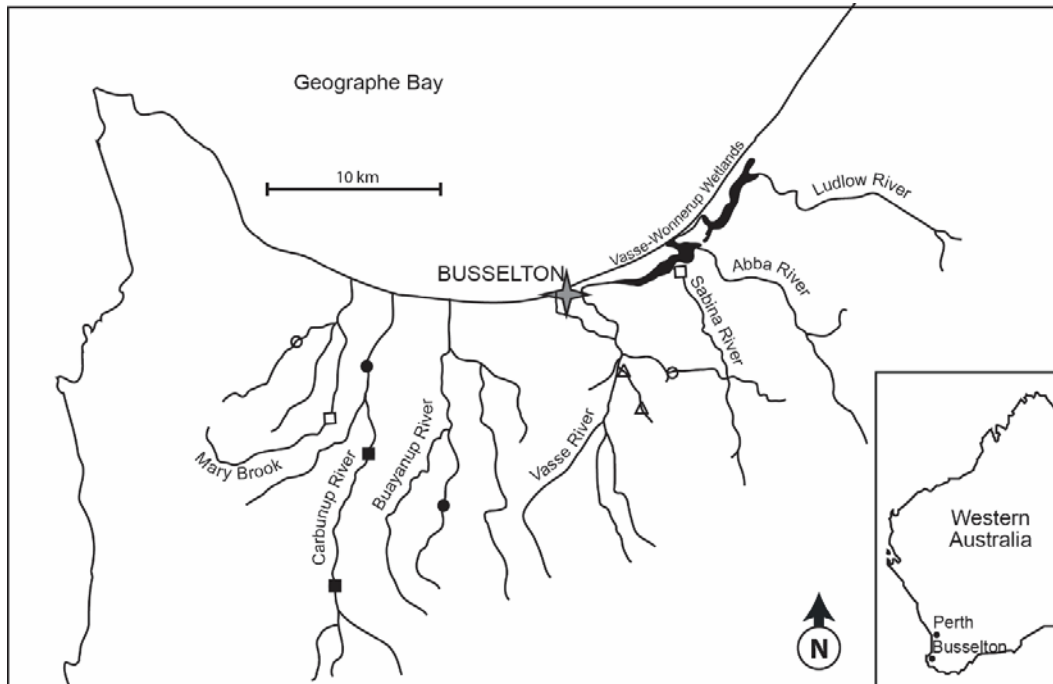
## Methods

### *Study area*

All conspicuous aquatic consumers were sampled in ten study reaches located on five seasonally-flowing lowland streams in agricultural areas of the Geographe Bay catchment, approximately 250 km south of Perth in Western Australia (Fig. 4.1, Table 4.1). The region has a mediterranean climate, with wet winters and dry summers, creating a seasonal flow pattern with peak winter-spring flows followed by low summer-autumn flows, and frequent flow cessation. All study reaches exhibited seasonal flow: streams dried to pools in summer, then dried completely in late summer to autumn. The river systems in this study are relatively short, commencing with headwaters in the lateritic Whicher Range and traversing approximately 30 km, across deep sands and sandy loams of the Swan Coastal Plain, and discharging in Geographe Bay (total catchment area approximately 2000 km<sup>2</sup>). Agriculture in the catchment comprises mainly dairy and beef cattle grazing and there has been extensive clearing and loss of riparian vegetation. The natural riparian vegetation commonly includes an over-storey of evergreen native trees (*Eucalyptus* spp., *Corymbia calophylla*, *Agonis flexuosa*), a mid-storey of small trees and large shrubs (*Melaleuca* spp. and *Taxandria* spp.) and a mixed understorey of shrubs and rushes (common emergent rushes including *Baumea*, *Juncus* and *Leptocarpus* spp.).

A pilot survey in the study area identified the occurrence of two distinct macrophyte assemblages in lowland reaches of these rivers, which were associated with different macroinvertebrate assemblages (Paice et al. in press): *Cycnogeton* spp. (*C. huegelii* (Endl.) and/or *C. lineare* (Endl.) Sond); and *Potamogeton* spp. (*P. drummondii* Benth. and/or *P. ochreatus* Raoul), often co-occurring with *Ottelia ovalifolia* (R. Br.) Rich). *Liparophyllum lasiospermum* ((F. Muell.) Tippery and Les) was common in reaches with *Cycnogeton* but was emergent and restricted to shallow areas. *Cycnogeton* is able to grow at greater depth than the other species and was both submerged and emergent in our study reaches. *Potamogeton ochreatus* is a submerged macrophyte; *P. drummondii* and *O. ovalifolia* have both submerged and floating leaves. The term macrophyte is

used here to refer to aquatic angiosperms with submerged/floating-leaves and does not include emergent rushes or Charophytes. Although often included in studies of submerged macrophytes, particularly in lentic systems, charophytes in this study (*Chara*) were inconspicuous compared to stands of macrophytes and were considered separately.



**Figure 4.1.** Location of study reaches, showing the five reach types: good riparian condition, no macrophytes (black circles); good riparian condition, *Cyanogeton huegelii* (black squares); poor riparian condition, no macrophytes (open circles), poor riparian condition, *C. huegelii* (open squares); and poor riparian condition, *P. ochreatus* and *O. ovalifolia* (triangles).

**Table 4.1.** Location and characteristics of study reaches, including riparian condition, size (m) and dominant streambed substratum. The study included two reaches of each reach type (GC: good condition, *C. huegelii*; GN: good riparian condition, no macrophytes; PC: poor riparian condition, *C. huegelii*; PPO: poor riparian condition, *P. ochreatus* and *O. ovalifolia*; and PN: poor riparian condition, no macrophytes). Good riparian condition reaches retain intact native vegetation, and poor riparian condition reaches have limited, degraded vegetation. Substratum types are F=finest, G=gravel, P=pebbles, C=cobbles, B=boulders/bedrock.

Reach type	Reach code	Latitude Longitude	River	Riparian condition	Macrophytes	Mean width	Mean depth	Substratum
GC	CARB2	33.70°S 115.18°E	Carbunup River	Good	<i>C. huegelii</i> <i>L. lasiospermum</i>	9.05	0.29	F/G/B/P
GC	CARB3	33.75°S 115.19°E	Carbunup River	Good	<i>C. huegelii</i> <i>L. lasiospermum</i>	5.6	0.25	B/F/G/P/C
GN	CARB1	33.68°S 115.29°E	Carbunup River	Good	None	8.05	0.50	P/C/F/G/B
GN	IRON2	33.77°S 115.24°E	Ironstone Gully	Good	None	7.9	0.29	G/F/P
PC	MARY2	33.70°S 115.02°E	Mary Brook	Poor	<i>C. huegelii</i> <i>L. lasiospermum</i>	3.85	0.22	G/F/B
PC	SABI1	33.68°S 115.29°E	Sabina River	Poor	<i>C. huegelii</i>	2.15	0.42	F/G
PPO	GREE1	33.70°S 115.39°E	Green Gully	Poor	<i>P. ochreatus</i> <i>O. ovalifolia</i>	8.05	0.15	F/B
PPO	GREE2	33.69°S 115.37°E	Green Gully	Poor	<i>P. ochreatus</i> <i>O. ovalifolia</i>	5.0	0.35	F
PN	SABD2	33.70°S 115.42°E	Sabina Diversion	Poor	None	3.1	0.33	F/G/P/B
PN	STAT2	33.69°S 115.14°E	Station Gully	Poor	None	4.1	0.18	F/G/P

## ***Study design***

Foreshore condition assessment was completed as part of the pilot survey using a method widely adopted in this region (WRC, 1999; Chapter 3). This classified stream reaches based on riparian vegetation condition. Reaches retaining intact native riparian vegetation were grouped as ‘good’ condition reaches, and those with limited riparian vegetation were grouped as ‘poor’ condition reaches. To test the hypotheses, the study included reaches with riparian vegetation in both good and poor condition, with and without submerged macrophytes. While *Cycnogeton* occurred in both good and poor condition reaches, the *Potamogeton/Ottelia* assemblage was found only in poor condition reaches. Given the particular species occurring in the study reaches, reach types were classified as follows:

- (i) good riparian condition, *C. huegelii* (GC);
- (ii) good riparian condition, no macrophytes (GN);
- (iii) poor riparian condition, *C. huegelii* (PC);
- (iv) poor riparian condition, *P. ochreatus* and *O. ovalifolia* (PPO); and
- (v) poor riparian condition, no macrophytes (PN) (Table 4.1).

Two reaches of each type (Table 4.1) were selected randomly from a larger set of potential sites identified in the pilot survey, and reaches with macrophytes present contained plant beds with at least 5% cover by area over a 20m reach. Sampling was undertaken in all ten reaches in late spring to early summer 2013, to coincide with maximum biomass for populations of submerged macrophytes, and prior to flow cessation. Maximum submerged macrophyte biomass occurs as a result of maximum growth rate, and so is the period where a significant contribution to the food web is most likely to occur (Cebrian and Duarte 1994). Although consumer diets can vary temporally (eg. Beatty 2006; Reid *et al.* 2008b), this study focussed on the relative importance of macrophytes during their peak growth period, and the tissues analyzed have a short metabolic turnover (Phillips *et al.* 2014), so represent diets during this period.

### ***Sample collection and preparation***

Potential food sources were sampled at each site: terrestrial riparian detritus (native rushes, trees and exotic grasses), charophytes, macrophytes (submerged and semi-emergent angiosperms) and algae (filamentous green algae, epilithon, epiphytes). Periphyton was sampled separately as epilithon and epiphytes owing to the potential for substrate to influence stable isotope results, and an interest in their comparative dietary contribution.

Epilithon, tree leaves and grass were present at all reaches, but the presence of other basal food web resources varied across reaches (Table 4.2). The two PC reaches differed in available sources: at Mary Brook, charophytes and *L. lasiospermum* were present but epiphytes were absent; Sabina River had no charophytes or *L. lasiospermum* present, but instead had conspicuous epiphytic growth on *Cycnogeton* leaves (Table 4.2). Thus separate mixing models were used for each of these reaches.

Terrestrial sources were represented by leaves of dominant native trees collected from the stream bed (cleaned to remove periphyton and sediment), and freshly cut leaf material from riparian rushes and dominant grasses. Three replicate samples were combined into one sample for analysis of each terrestrial source at each reach. Other sources were collected and analysed in triplicate at each reach. Epilithon was scraped from stream-bed stones, which were first washed gently to remove sediment. Epiphytes were scraped from macrophytes when present. All samples were placed on ice in the field for transport. Where present, samples of filamentous green algae (attached to macrophytes or woody debris), charophytes and macrophytes were cut above their substrate, and washed to remove sediment and biota. In the laboratory, samples of epilithon, epiphytes and filamentous green algae were inspected with a dissecting microscope to remove detritus particles and biota (mainly small Chironomidae and eggs). Excess water was decanted from epilithon and epiphyte samples following separation using a centrifuge. All samples were frozen for storage.

Aquatic invertebrates were collected from reaches by sweep net sampling of a 10-metre reach for two minutes. Where this did not yield sufficient individuals for

stable isotope analysis, additional collection with the sweep net and searching amongst rocks, woody debris and plants was also undertaken. Samples were live picked on site for thirty minutes and identified to family-level, different taxa were placed in separate containers of distilled water and stored on ice for transport. Following live-picking, the remaining sample was stored on ice for further sorting of small organisms in the laboratory. Sampling aimed to collect sufficient biomass for three replicate stable isotope samples of each taxon from each site, however this was not possible at all sites, so taxa with sufficient material for at least one stable isotope sample were prepared for analysis. Cleaned individuals were kept alive in separate containers for 24 hours to void gut contents, with regular inspection and removal of waste, so that unassimilated material was excluded from analysis. Leptoceridae were removed from cases to avoid ingestion of case material. Organisms were then frozen for storage. Stable isotope samples consisted of whole animals for insects; gastropods with shell removed; and muscle tissue dissected from crayfish tails. Individuals were grouped as required to provide sufficient material for analysis.

Fish were collected by placing fyke nets upstream and downstream of the sampling area prior to invertebrate and basal source sample collection. Fish samples were ideally composed of three individuals with three replicate samples per species per site. However, this number was not always obtained and single fish samples were common. Muscle tissue was dissected from fish for use in analysis.

### ***Stable isotope analysis***

Samples were dried at 60°C for 48 hours, and ground to a homogenous powder with a glass mortar and pestle in preparation for stable isotope analysis. Material from *Eucalyptus* leaves and rushes was further machine-ground in the laboratory. Stable isotope ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) and N and C content (and thus C:N ratios) were analysed using a continuous flow system consisting of an Automated Nitrogen Carbon Analyser with Sercon 20-22 mass spectrometer (SERCON, UK) (Skrzypek and Paul 2006). Raw isotopic data were normalised to the international reference scale using standards provided by International Atomic Energy Agency



( $\delta^{13}\text{C}$  - NBS22, USGS24, USGS40, USGS41, LSVEC;  $\delta^{15}\text{N}$  – IAEA-N1, IAEA-N2, USGS40, USGS41) and laboratory standards (Skrzypek *et al.* 2010).

Uncertainty associated with analyses (1 standard deviation) was not more than 0.20‰ for  $\delta^{15}\text{N}$  and 0.10‰ for  $\delta^{13}\text{C}$ .

### ***Fish stomach contents***

To provide additional information on fish diet for comparison with stable isotope analysis results, and to determine appropriate trophic levels for application of fractionation values, all fish kept for stable isotope analysis were also dissected to inspect stomach contents. Using a dissecting microscope, proportional content of each identifiable item (estimated percentage by volume) was recorded. Average results for each fish species were used to indicate potential diet for comparison to mixing model outputs.

### ***Data analysis***

Analysis of source partitioning was performed with the MixSIAR mixing model framework, using the user interface MixSIAR GUI statistical software R (Stock and Semmens 2013) to determine feasible contributions of basal resources to each consumer. MixSIAR is a Bayesian mixing model developed to incorporate advances since development of earlier models MixSIR and SIAR (Stock and Semmens 2013). The model framework takes into account uncertainty associated with variation in isotope values of both sources and consumers and in fractionation values, and concentration dependence (Moore and Semmens 2008; Stock and Semmens 2013). Separate mixing models were constructed to assess differences in food webs between good and poor condition reaches with and without macrophytes, corresponding with the five reach types described above.

All  $\delta^{13}\text{C}$  data were normalised for lipid content using formulae provided by Post *et al.* (2007), because the study included a range of consumers with a range in C:N ratios, and thus variation in  $\delta^{13}\text{C}$ -depleted lipids which may bias results (Post *et al.* 2007; Phillips *et al.* 2014). We also used concentration-dependent source data in mixing models due to differing C:N ratios of sources which contradicts the

model assumption that all sources contribute equal proportions of C and N to consumers (Phillips and Koch 2002).

Source data were pooled across reach types where values did not differ, with analysis of variance (ANOVA) to test for differences between good and poor riparian condition (2 levels, fixed) and the presence/absence of macrophytes (2 levels, fixed). Another ANOVA with the factors: source (2 levels, fixed) and reach (2 levels, random) was used to determine whether isotopic signatures differed for particular sources within reach groupings, to enable pooling prior to use in mixing models. Reducing the number of sources increases the discriminatory power of mixing models (Phillips *et al.* 2005, 2014). The same two-factor ANOVA was also used to compare means of C:N ratios for basal resources, followed by Tukey's *post hoc* test for multiple comparisons of sources. All ANOVAs were checked for homogeneity of variance (Levene's test) and normality (Shapiro-Wilk test). Heterogeneous variance was found in  $\delta^{13}\text{C}$  data for *P. ochreatus* and *O. ovalifolia*, and  $\delta^{15}\text{N}$  for epilithon and epiphytes in good condition reaches with *Cycnogeton*, and Welch's test was used to compare means in these cases.

We applied correction values for fractionation for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  from average meta-analysis values (and standard errors) provided by McCutchan *et al.* (2003), differentiated by consumer tissue sampled (McCutchan *et al.* 2003; Vanderklift and Ponsard 2003; Phillips *et al.* 2014). For whole organisms (insect and gastropod samples), values were  $0.3\text{‰} \pm 0.14$  for  $\delta^{13}\text{C}$  and  $2.1\text{‰} \pm 0.21$  for  $\delta^{15}\text{N}$ ; and for muscle tissue samples (crayfish and fish) values were  $1.3\text{‰} \pm 0.3$  for  $\delta^{13}\text{C}$  and  $2.9\text{‰} \pm 0.32$  for  $\delta^{15}\text{N}$ . Recent estimates for  $\delta^{15}\text{N}$ -fractionation from the meta-analysis of data from Australasian streams and rivers by Bunn *et al.* (2013) were considered, however substantially lower  $\delta^{15}\text{N}$ -fractionation would apply for invertebrates and result in adjusted consumer signatures outside the isospace polygon created by basal resources. A logical isospace is fundamental in calculating valid diet contributions (Fry 2013, Phillips *et al.* 2014).

Because the study focussed on comparative assimilation of basal resources (rather than specific prey items), fractionation values were multiplied by trophic level,

based on existing knowledge of likely consumer diet (obtained from Davis and Christidis (1997); and using stomach contents observations for fish diet). Herbivores and detritivores were assigned a trophic level of 1, including Gastropoda, Baetidae, Chironomidae and Simuliidae. Leptoceridae (*Triplectides australis*) were assigned a trophic level of 1.5 as shredders usually consume both tree leaves and the biofilm covering them, making them omnivores. Corixidae and crayfish were also assigned a trophic level of 1.5 due to potential for omnivory; and a trophic level of 2 was used for predatory insects (Odonata and Dytiscidae). Both fish species encountered fed mainly on insect larvae (see Results) and were assigned a conservative trophic level of 2, resulting in N-fractionation of 5.8 ‰ (similar to that recommended by Bunn *et al.* (2013) for predatory fish (5.7 ‰)).

For all mixing models, the sources used created a logical isospace polygon which encompassed signatures of consumers. However, diffuse ranges of feasible solutions were common, and such unconstrained results are difficult to interpret. To provide a meaningful benchmark for interpreting results, basal resources having a contribution range with the 95<sup>th</sup> percentile greater than 50% were considered as having high potential contribution. Median values were also used to evaluate contributions from sources with wide ranges of feasible solutions. Isospace plots were also used to visually assess resource dependence (Phillips *et al.* 2014).

## Results

### *Consumer occurrence*

Aquatic invertebrate consumers present in all reach types were: Chironomidae (*Chironomus*), Corixidae and Dytiscidae. Other common taxa were: Gastropoda (Physidae and Planorbidae), Ephemeroptera (Baetidae), Diptera (Simuliidae), Trichoptera (Leptoceridae: *T. australis*) and Odonata (Coenagrionidae, Aeshnidae and Corduliidae). Families within Gastropoda and within Odonata were grouped because they had similar isotopic signatures. A single species of freshwater crayfish (Parastacidae: *Cherax quinquecarinatus*) was present in all reach categories. Two native fish species were collected: the Western Minnow *Galaxias occidentalis* (Ogilby 1899) was present in all reach types; and the Western Pygmy Perch *Nannoperca vittata* (Castelnau, 1873) was found in all reaches except the Sabina River and poor condition reaches with no macrophytes. The two poor condition reaches with *C. huegelii*, which differed in basal resources, had several consumer taxa in common but these differed considerably in isotopic signatures, emphasising the need to analyse these reaches separately.

### *Fish diet*

Fish stomach contents varied among sites, with one type of prey item often dominant in each fish examined. Microcrustaceans (Cladocera and Ostracoda) were common prey items of *N. vittata*, while rare in *G. occidentalis*, although large numbers of Ostracoda were found in stomachs of both fish species in one good condition reach. Dytiscidae were rare in *N. vittata* but were common in *G. occidentalis*, particularly in good condition reaches and terrestrial insect parts were also found in *G. occidentalis* samples from these reaches. Insect larvae, predominantly Chironomidae, Simuliidae, Lepidoptera and Culicidae, were the most common prey item overall for both fish species (Fig. 4.2).

### *Basal Resources*

Basal resource C:N ratios in stream reaches did not differ with riparian condition or macrophyte presence. Native terrestrial sources had substantially higher and

more variable C:N ratios than all autochthonous resources and exotic grasses (Table 2). All instream resources had similar C:N ratios, and values for exotic grasses were similar. Tukey's *post hoc* tests showed that both trees and rushes had significantly higher C:N than all other sources ( $P < 0.01$ ), and other sources did not differ ( $P > 0.39$ ).

Isotope values for *C. huegelii* varied significantly with riparian condition ( $\delta^{13}\text{C}$ :  $F_{1,10} = 19.0$ ,  $P = 0.001$ ;  $\delta^{15}\text{N}$ :  $F_{1,10} = 29.9$ ,  $P < 0.001$ , Table 2), so data could not be pooled across reach types. *Liparophyllum lasiospermum* co-occurred with *C. huegelii* at most reaches (Table 1), and the two species did not differ significantly in isotope values or C:N ratio ( $\delta^{13}\text{C}$ :  $F_{1,14} = 8.79$ ,  $P = 0.097$ ;  $\delta^{15}\text{N}$ :  $F_{1,14} = 0.17$ ,  $P = 0.723$ ; C:N:  $F_{1,14} = 2.66$ ,  $P = 0.244$ ), so data were aggregated for these two species within reach type.

*Potamogeton ochreatus* and *O. ovalifolia* occurred only in poor condition reaches, and were enriched in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  compared to *C. huegelii* when growing in poor condition reaches (Table 2). These species did not differ significantly in C:N ratio ( $F_{1,8} = 2.81$ ,  $P = 0.236$ ) or isotope values ( $\delta^{13}\text{C}$ :  $F_{1,5.8} = 5.8$ ,  $P = 0.054$  (Welch);  $\delta^{15}\text{N}$ :  $F_{1,8} = 29.8$ ,  $P = 0.115$ ). Thus combining these functionally similar species was appropriate prior to mixing model analysis.

Epilithon was collected at all reaches, and values for  $\delta^{13}\text{C}$  varied substantially among reach type (Table 2). Epilithon was enriched in  $\delta^{13}\text{C}$  ( $F_{1,23} = 12.8$ ,  $P = 0.002$ ) in PPO and PN reaches. It was also  $\delta^{15}\text{N}$ -enriched in these reaches (condition x macrophytes:  $F_{1,23} = 13.9$ ,  $P = 0.001$ ). In GC reaches, epilithon and epiphytes did not differ in stable isotope values ( $\delta^{13}\text{C}$ :  $F_{1,8} = 32.08$ ,  $P = 0.111$ ;  $\delta^{15}\text{N}$ :  $F_{1,5.5} = 1.09$ ,  $P = 0.34$  (Welch)) or C:N ratio ( $F_{1,8} = 6.99$ ,  $P = 0.23$ ) and these sources were pooled for mixing model analysis.

Filamentous algae were conspicuous in PPO and PN reaches, and were enriched in  $\delta^{13}\text{C}$  relative to other in-stream producers (Table 2). Filamentous algae were enriched in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ( $F_{1,7} = 88.6$ ,  $P < 0.001$ ;  $F_{1,7} = 68.7$ ,  $P < 0.001$ , respectively) in reaches with macrophytes compared to those without.

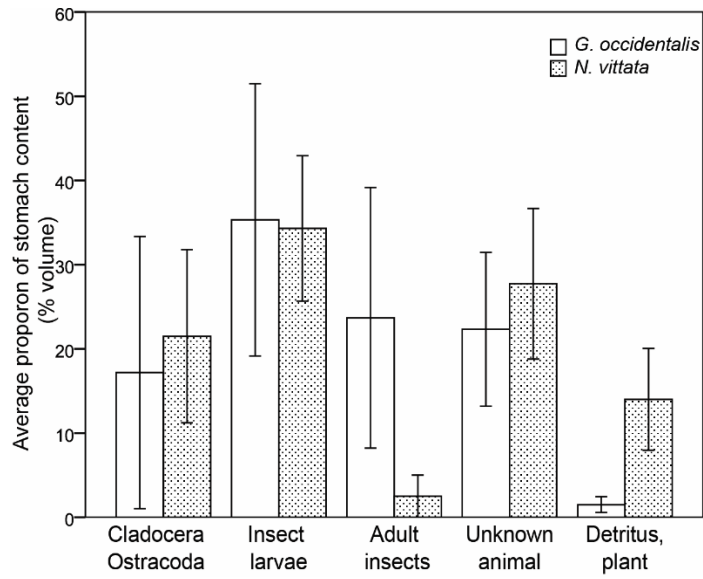
Charophytes (*Chara*) were found in all good condition reaches, PN reaches, and one PC reach (Table 3). Samples were not identified beyond genus, and while only one species occurred in each reach, species may have differed between reaches. Charophytes were depleted in  $\delta^{13}\text{C}$  in poor condition reaches relative to those in good condition ( $F_{1,7} = 57.6$ ,  $P < 0.001$ , Table 2). Replicate samples within some reaches varied in  $\delta^{15}\text{N}$ , and there was no significant difference in  $\delta^{15}\text{N}$  between reach types.

Leaves of *Corymbia calophylla* and *Agonis flexuosa* had isotopic signatures distinct from other basal resources, and varied little across reaches. Isotope values did not differ between reach condition ( $\delta^{13}\text{C}$ :  $F_{1,1} = 14.9$ ,  $P = 0.163$ ;  $\delta^{15}\text{N}$ :  $F_{1,1} = 0.06$ ,  $P = 0.607$ ; Table 2) or species ( $\delta^{13}\text{C}$ :  $F_{1,7} = 14.5$ ,  $P = 0.161$ ;  $\delta^{15}\text{N}$ :  $F_{1,1} = 0.03$ ,  $P = 0.364$ ). All data for tree leaves was therefore pooled for mixing model analysis. Native riparian rushes (*Baumea* and *Leptocarpus*) were present only in good condition reaches and showed no difference in isotopic values between species ( $\delta^{13}\text{C}$ :  $F_{1,4} = 2.7$ ,  $P = 0.177$ ;  $\delta^{15}\text{N}$ :  $F_{1,4} = 5.3$ ,  $P = 0.083$ ). All data for these plants were therefore aggregated for mixing model analyses for reaches where they occurred.

Grasses were present at all reaches, but differed in dominant species and cover. In good condition reaches, grasses were present at low density and included various exotic pasture species (*Paspalum* sp., *Phalaris* sp.), while in poor condition reaches stoloniferous perennial exotic grasses (*Pennisetum clandestinum*, *Cynodon dactylon*) formed dominant understorey components, in some cases growing within the stream channel. Isotope values of grasses were notably different between good and poor condition reaches (Table 2), with significant enrichment for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in poor condition reaches ( $F_{1,10} = 267.3$ ,  $P < 0.001$ ;  $F_{1,10} = 16.9$ ,  $P = 0.002$  respectively). Results for  $\delta^{13}\text{C}$  suggest presence of C-3 grasses in good condition reaches and C-4 grasses in poor condition reaches (O'Leary 1981). Isotope data for grasses were therefore pooled within reach condition categories for mixing model analysis.

**Table 4.2.** Carbon-nitrogen ratios for each basal resource and mean stable isotope values (%o,  $\pm$  standard deviation) for resources within each reach type (as for Table 4.1). Dashes indicate an absence of data.

Basal resource	C:N	Reach type									
		GC		GN		PC		PPO		PN	
		$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
<i>Cycnogeton huegelii</i>	12.5 ( $\pm 2.5$ )	-33.4 ( $\pm 1.5$ )	12.0 ( $\pm 1.8$ )	-	-	-29.5 ( $\pm 0.9$ )	7.1 ( $\pm 2.0$ )	-	-	-	-
<i>Liparophyllum lasiospermum</i>	16.8 ( $\pm 2.3$ )	-28.5 ( $\pm 0.8$ )	10.4 ( $\pm 0.9$ )	-	-	-28.5 ( $\pm 0.2$ )	7.4 ( $\pm 0.2$ )	-	-	-	-
<i>Potamogeton ochreatus</i>	13.2 ( $\pm 0.8$ )	-	-	-	-	-	-	-22.3 ( $\pm 0.8$ )	12.4 ( $\pm 0.3$ )	-	-
<i>Ottelia ovalifolia</i>	11.2 ( $\pm 1.9$ )	-	-	-	-	-	-	-25.0 ( $\pm 2.6$ )	11.1 ( $\pm 1.2$ )	-	-
Epilithon	13.2 ( $\pm 3.3$ )	-30.9 ( $\pm 0.3$ )	7.8 ( $\pm 0.3$ )	-30.7 ( $\pm 0.7$ )	6.1 ( $\pm 1.0$ )	-31.2 ( $\pm 1.2$ )	8.4 ( $\pm 0.6$ )	-25.5 ( $\pm 0.6$ )	9.9 ( $\pm 0.5$ )	-26.5 ( $\pm 2.2$ )	9.5 ( $\pm 0.8$ )
Epiphyte	11.3 ( $\pm 1.9$ )	-33.8 ( $\pm 1.0$ )	8.4 ( $\pm 1.2$ )	-	-	-35.6 ( $\pm 1.4$ )	10.7 ( $\pm 0.8$ )	-	-	-	-
Filamentous algae	11.7 ( $\pm 2.3$ )	-	-	-	-	-	-	-15.2 ( $\pm 0.3$ )	11.5 ( $\pm 0.3$ )	-19.7 ( $\pm 0.9$ )	9.8 ( $\pm 0.1$ )
Charophyte	13.7 ( $\pm 2.1$ )	-38.3 ( $\pm 0.3$ )	7.8 ( $\pm 0.2$ )	-38.3 ( $\pm 1.8$ )	9.4 ( $\pm 1.5$ )	-35.0 ( $\pm 0.6$ )	8.2 ( $\pm 1.3$ )	-	-	-33.6 ( $\pm 0.8$ )	7.8 ( $\pm 0.4$ )
Trees	80.3 ( $\pm 18.7$ )	-29.0 ( $\pm 1.4$ )	1.3 ( $\pm 0.9$ )	-29.4 ( $\pm 0.7$ )	1.4 ( $\pm 0.7$ )	-28.0 ( $\pm 0.7$ )	1.1 ( $\pm 0.7$ )	-27.3 ( $\pm 0.6$ )	2.4 ( $\pm 0.9$ )	-28.0 ( $\pm 0.5$ )	1.4 ( $\pm 0.9$ )
Rushes	32.9 ( $\pm 8.5$ )	-30.3 ( $\pm 0.4$ )	8.1 ( $\pm 0.9$ )	-30.5 ( $\pm 0.8$ )	7.9 ( $\pm 0.4$ )	-	-	-	-	-	-
Grass	14.6 ( $\pm 2.6$ )	-21.7 ( $\pm 0.7$ )	3.1 ( $\pm 2.4$ )	-21.4 ( $\pm 0.3$ )	3.0 ( $\pm 0.04$ )	-13.3 ( $\pm 0.7$ )	6.3 ( $\pm 2.8$ )	-13.0 ( $\pm 1.1$ )	8.5 ( $\pm 2.1$ )	-13.2 ( $\pm 1.2$ )	7.4 ( $\pm 0.3$ )



**Figure 4.2.** Stomach contents of *Galaxias occidentalis* and *Nannoperca vittata*, as average percentage of volume from samples pooled across all study reaches.



## ***Mixing model outputs***

### **Good condition, no macrophytes (GN)**

In GN reaches, rushes and epilithon had potentially high importance as food web resources (Table 4.3), however their isotopic signatures were within the isospace of other sources (Fig. 4.3a) so the mixing model found a wide range of possible contributions to consumer diets (diffuse solutions). Median values indicated most invertebrates were more reliant on rushes, but Odonata (Aeshnidae and Corduliidae) and *G. occidentalis* assimilated more epilithon (Table 4.3). Tree-leaf detritus was an important resource for Dytiscidae. Planorbid gastropods and *C. quinquecarinatus* had relatively equal contributions from all sources other than grass. *Nannoperca vittata* was depleted in  $\delta^{13}\text{C}$  and had relatively low reliance on terrestrial resources, with highest feasible contributions coming from epilithon and charophytes.

### **Good condition, *C. huegelii* (GC)**

In GC reaches, epilithon/epiphytes and rushes were isotopically similar (Fig. 4.3b), resulting in comparable feasible contributions that showed both were important resources (Table 4.3). Mixing model output suggested only a moderate contribution from macrophytes as a basal resource for most taxa, with highest contributions found for Gastropoda, Simuliidae, Odonata, *C. quinquecarinatus* and *G. occidentalis* (Table 4.3, Fig. 4.4a). Most consumers appeared dependent on both terrestrial detritus and in-stream production, although Planorbidae had greater reliance on autochthonous resources; and terrestrial resources (leaves and grass) were most important for Corixidae and Dytiscidae (Table 4.3, Fig. 4.3b).

### **Poor condition, *C. huegelii* (PC)**

In Mary Brook, macrophytes had higher potential dietary contribution for all consumers compared with good condition reaches, with the exception of Dytiscidae (Fig. 4.4b), which were again dependent on tree-leaf detritus (Table 4.3, Fig. 4.3c,d). This was the only reach in which macrophytes were a likely basal resource for Corixidae (Fig. 4.4, Table 4.3). Although model outputs were diffuse (Table 4.3), they indicated macrophytes as an important basal resource for all macroinvertebrates other than Dytiscidae, and for fish, and of moderate

importance for *C. quinquecarinatus* (Fig. 4.4b). Epilithon also potentially contributed to diets of consumers associated with macrophytes (Table 4.3). In addition to Dytiscidae, tree-leaf detritus appeared important for Baetidae and *C. quinquecarinatus* (Table 4.3).

In the Sabina River reach, importance of *C. huegelii* to diets was generally lower than in good condition reaches. Potentially high contributions were indicated for *C. quinquecarinatus* and *G. occidentalis* (Table 4.3, Fig. 4.4c), and epiphytes on macrophytes were a significant resource for most taxa (Table 4.3, Fig. 4.4). *Cherax quinquecarinatus* and *G. occidentalis* assimilated a wide range of resources, and were enriched in  $\delta^{15}\text{N}$  relative to other reaches (Fig. 4.3d). Epiphytes in this reach were also  $\delta^{15}\text{N}$ -enriched compared with good condition reaches. *Triplectides australis* had similar contributions from all sources in this reach, but a constrained high result for grass confirmed its importance and may reflect high abundance (288) of individuals encased in pieces of grass stem at this reach. Grass was of low importance for other macroinvertebrate taxa. Dytiscidae were relatively depleted in  $\delta^{15}\text{N}$  (Fig. 4.3d), and were again dependent on tree leaves as a basal resource for much of their diet (69-100%).

#### **Poor condition, *P. ochreatus* and *O. ovalifolia* (PPO)**

In PPO reaches, macrophytes had moderate ranges of potential contribution to primary consumers (Table 4.3, Fig. 4.3e). However, results were similar or lower than for macrophytes in GC reaches, and much lower than for *C. huegelii* in Mary Brook (Fig. 4.4). Epilithon was a significant resource for most consumers, particularly both fish species (Table 4.3). Dytiscidae and Corixidae had very high contributions from tree-leaf detritus. Grass was apparently a more important basal resource for invertebrates here than in other reaches, but had very low contribution for fish (Table 4.3). Filamentous algae had moderate potential contribution to several consumers (Table 4.3), and high  $\delta^{13}\text{C}$  values of consumers in these reaches also suggests assimilation of this  $\delta^{13}\text{C}$ -enriched resource (Fig. 4.3e).

#### **Poor condition, no macrophytes (PN)**

In PN reaches, potential basal resources included epilithon, filamentous algae, charophytes, native tree detritus and exotic grasses. Source isotopic signatures

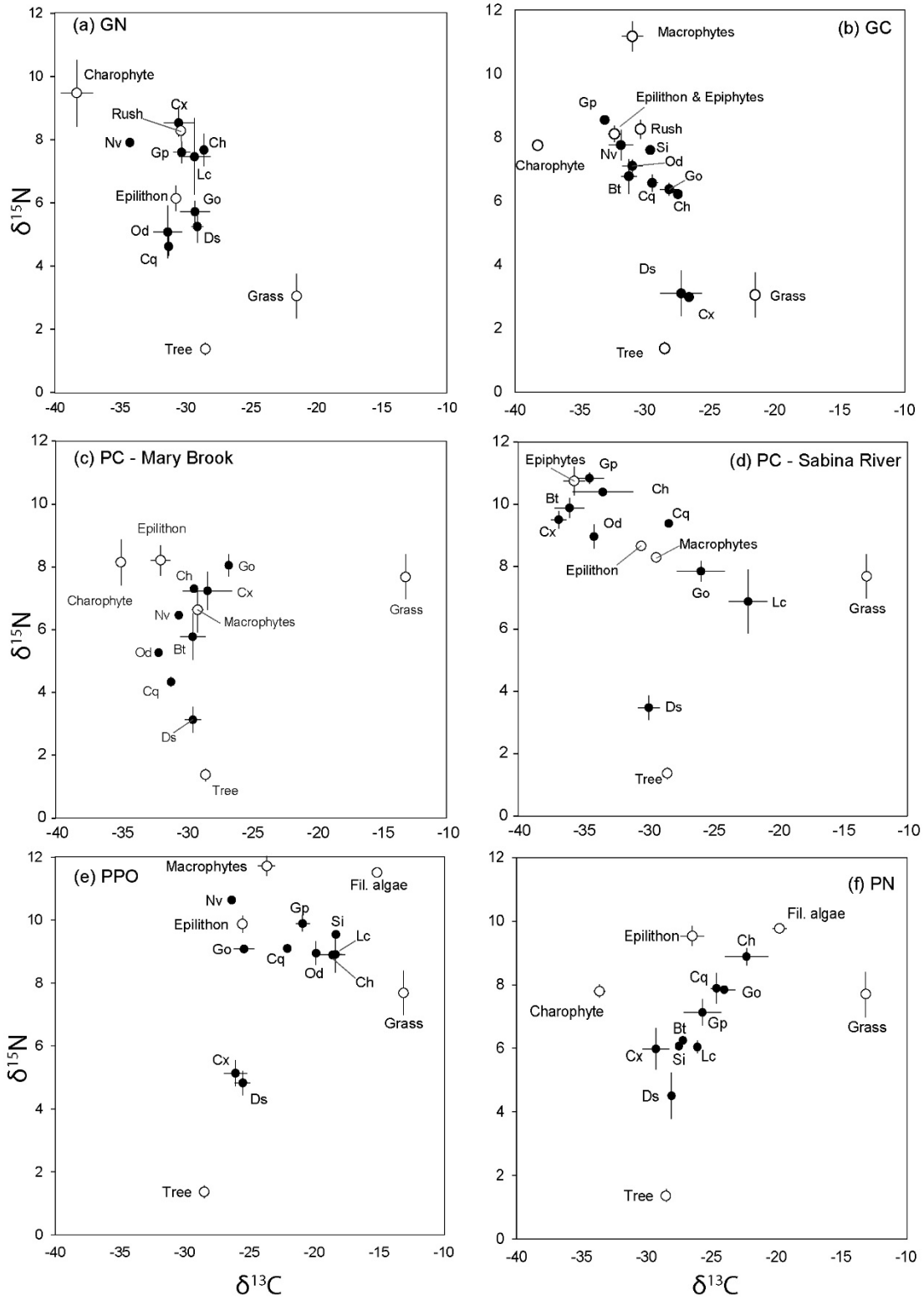
were well-separated (Fig. 4.3f) however many mixing model solutions were nonetheless diffuse (Table 4.3). Together with the location of mean consumer isotope values in the centre of the isospace polygon (Fig. 4.3f), this suggests omnivory and flexible diets among consumers. Tree detritus was a major basal resource, and more important than in poor condition reaches with macrophytes, although epilithon was also important for several taxa (Table 4.3). Chironomidae differed considerably in  $\delta^{13}\text{C}$  values between the two reaches (mean values 19.9 and 26.6 ‰) and while the mixing model used pooled data and indicated reliance on both epilithon and filamentous alga, these organisms were most likely feeding on one or the other in these reaches and this may represent variation between species.

**Table 4.3.** Feasible potential contributions of basal resources to consumers' diets in reaches varying in riparian condition and macrophyte assemblage. Ranges are 90% credible intervals (5-95 percentile ranges) with median contribution in parentheses, determined using MixSIAR mixing models. Food web resources with high potential contribution are indicated in bold type (95 percentile >50%).

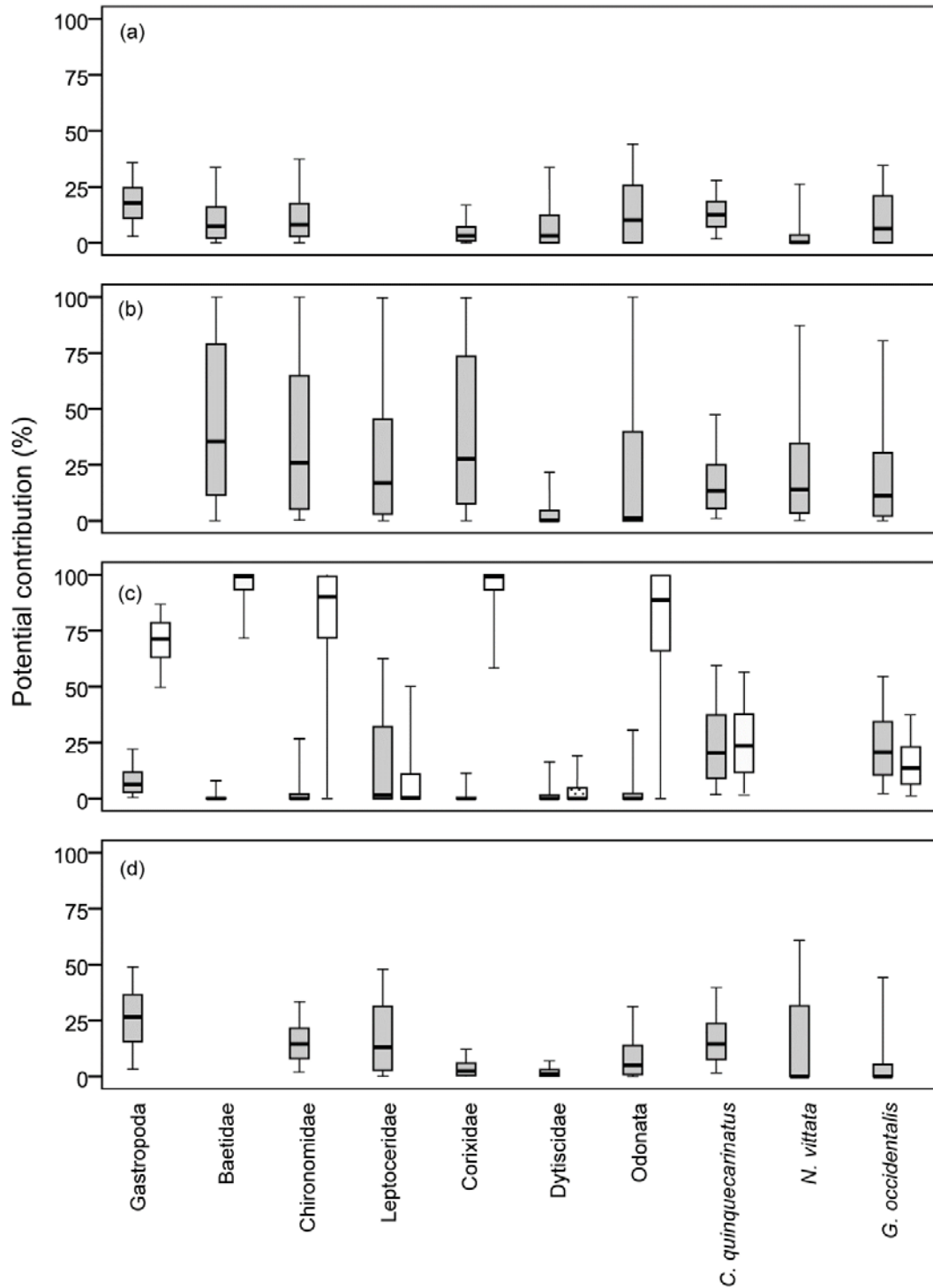
<b>Poor condition, no macrophytes</b>						
	Epilithon	Charophyte	Tree	Rush	Grass	
Gastropoda	<b>2-55 (19)</b>	3-41 (18)	1-40 (14)	<b>3-66 (30)</b>	1-27 (9)	
<i>Chironomus</i>	<b>0-95 (6)</b>	0-25 (1)	0-34 (2)	<b>0-100 (60)</b>	0-30 (8)	
<i>T. australis</i>	<b>0-99 (11)</b>	0-33 (3)	0-34 (4)	<b>0-100 (51)</b>	0-27 (5)	
Corixidae	<b>0-94 (10)</b>	0-39 (4)	0-28 (3)	<b>0-100 (59)</b>	0-19 (2)	
Dytiscidae	<b>0-99 (18)</b>	0-31 (2)	<b>0-65 (23)</b>	<b>0-71 (19)</b>	0-33 (5)	
Odonata	<b>0-100 (78)</b>	0-40 (2)	<b>0-64 (1)</b>	<b>0-85 (1)</b>	0-24 (0)	
<i>C. quinquecarinatus</i>	<b>2-56 (21)</b>	7-42 (23)	<b>3-55 (26)</b>	1-42 (12)	1-26 (9)	
<i>N. vittata</i>	<b>0-80 (26)</b>	<b>18-68 (45)</b>	0-30 (5)	1-45 (8)	0-14 (2)	
<i>G. occidentalis</i>	<b>0-100 (35)</b>	0-36 (2)	<b>0-62 (6)</b>	<b>0-76 (6)</b>	0-38 (3)	
<b>Good condition, C. huegelii</b>						
	Macrophyte	Epilithon Epiphyte	Charophyte	Tree	Rush	Grass
Gastropoda	3-36 (18)	2-43 (16)	<b>18-51 (36)</b>	1-20 (7)	1-37 (14)	0-11 (3)
Baetidae	0-34 (7)	<b>0-71 (15)</b>	0-44 (17)	0-41 (10)	<b>0-80 (16)</b>	0-24 (9)
<i>Chironomus</i>	0-37 (8)	<b>0-53 (10)</b>	0-25 (5)	<b>0-64 (12)</b>	<b>0-57 (12)</b>	<b>1-50 (26)</b>
Simuliidae	0-38 (13)	<b>0-56 (14)</b>	0-28 (7)	0-40 (11)	<b>1-79 (24)</b>	1-27 (12)
Corixidae	0-17 (3)	0-33 (6)	0-25 (7)	<b>1-89 (19)</b>	0-35 (7)	<b>1-62 (39)</b>
Dytiscidae	0-34 (3)	<b>0-58 (4)</b>	0-27 (2)	<b>0-69 (4)</b>	0-49 (3)	<b>3-77 (47)</b>
Odonata	0-44 (10)	<b>1-94 (16)</b>	0-35 (4)	0-32 (3)	<b>5-100 (26)</b>	0-16 (2)
<i>C. quinquecarinatus</i>	2-28 (12)	1-42 (15)	1-27 (11)	2-45 (19)	2-48 (19)	3-28 (15)
<i>N. vittata</i>	0-26 (0)	<b>0-100 (87)</b>	0-29 (0)	0-17 (0)	<b>0-96 (1)</b>	0-10 (0)
<i>G. occidentalis</i>	0-35 (6)	<b>0-77 (14)</b>	0-21 (1)	<b>0-72 (11)</b>	<b>0-75 (6)</b>	6-38 (19)
<b>Poor condition, C. huegelii, Mary Brook</b>						
	Macrophyte	Epilithon	Charophyte	Tree	Grass	
Baetidae	<b>0-100 (35)</b>	<b>0-72 (11)</b>	0-42 (6)	<b>0-61 (13)</b>	0-13 (1)	
<i>Chironomus</i>	<b>0-100 (26)</b>	<b>0-100 (16)</b>	<b>0-58 (8)</b>	0-49 (6)	0-17 (2)	
Corixidae	<b>0-100 (28)</b>	<b>0-95 (17)</b>	0-43 (5)	0-45 (9)	0-17 (2)	
Odonata	<b>0-100 (1)</b>	<b>0-100 (4)</b>	<b>0-100 (4)</b>	<b>0-76 (0)</b>	0-5 (0)	
Dytiscidae	0-2 (0)	0-41 (1)	0-19 (1)	<b>54-100 (83)</b>	0-3 (0)	
<i>C. quinquecarinatus</i>	1-47 (13)	1-46 (14)	<b>5-57 (31)</b>	<b>3-65 (30)</b>	0-10 (3)	
<i>N. vittata</i>	<b>0-87 (14)</b>	<b>0-81 (12)</b>	<b>0-61 (31)</b>	0-51 (17)	0-13 (3)	
<i>G. occ</i>	<b>0-80 (1)</b>	<b>0-87 (14)</b>	0-19 (1)	0-28 (5)	5-35 (20)	

**Table 4.3 continued**

<b>Poor condition, <i>C. huegeli</i>, Sabina River</b>					
	Macrophyte	Epiphyte	Epilithon	Tree	Grass
Gastropoda	0-22 (6)	<b>50-87 (71)</b>	1-36 (11)	0-15 (4)	0-7 (2)
Baetidae	0-17 (0)	<b>72-100 (99)</b>	0-36 (0)	0-11 (0)	0-3 (0)
<i>Chironomus</i>	<b>0-56 (0)</b>	<b>0-100 (90)</b>	<b>0-100 (0)</b>	0-19 (0)	0-7 (0)
<i>T. australis</i>	<b>0-63 (2)</b>	0-50 (0)	<b>0-69 (1)</b>	0-57 (4)	<b>15-58 (42)</b>
Corixidae	0-11 (0)	<b>58-100 (99)</b>	0-27 (0)	0-5 (0)	0-2 (0)
Dytiscidae	0-16 (0)	0-19 (0)	1-23 (0)	<b>69-100 (91)</b>	0-3 (0)
Odonata	0-31 (0)	<b>0-100 (89)</b>	<b>0-94 (0)</b>	0-18 (0)	0-3 (0)
<i>C. quinquecarinatus</i>	<b>2-59 (20)</b>	<b>2-57 (24)</b>	<b>2-58 (19)</b>	1-42 (13)	1-26 (10)
<i>G. occidentalis</i>	<b>2-55 (21)</b>	1-37 (14)	<b>2-54 (20)</b>	2-39 (19)	4-34 (18)
<b>Poor condition, macrophytes</b>					
	Macrophyte	Epilithon	Filamentous	Tree	Grass
Gastropoda	3-49 (27)	<b>2-62 (18)</b>	2-40 (16)	2-29 (14)	6-32 (19)
<i>Chironomus</i>	2-33 (15)	<b>1-53 (17)</b>	2-46 (17)	2-23 (11)	<b>13-52 (33)</b>
Simuliidae	2-34 (15)	<b>2-51 (18)</b>	<b>2-50 (21)</b>	2-22 (11)	<b>10-50 (29)</b>
<i>T. australis</i>	0-48 (13)	<b>0-78 (13)</b>	0-45 (4)	0-27 (7)	<b>12-59 (37)</b>
Corixidae	0-12 (2)	<b>0-55 (6)</b>	0-16 (2)	<b>39-86 (73)</b>	0-22 (8)
Dytiscidae	0-7 (1)	0-38 (3)	0-16 (3)	<b>48-81 (72)</b>	1-26 (15)
Odonata	0-31 (5)	<b>0-78 (13)</b>	<b>0-53 (16)</b>	1-37 (23)	<b>5-52 (27)</b>
<i>C. quinquecarinatus</i>	2-40 (15)	<b>2-53 (18)</b>	2-39 (16)	9-44 (28)	3-33 (16)
<i>N. vittata</i>	<b>0-61 (0)</b>	<b>0-100 (100)</b>	0-5 (0)	0-40 (0)	0-3 (0)
<i>G. occidentalis</i>	0-44 (0)	<b>0-100 (100)</b>	0-12 (0)	0-54 (0)	0-6 (0)
<b>Poor condition, no macrophytes</b>					
	Epilithon	Filamentous	Charophytes	Tree	Grass
Gastropoda	<b>1-53 (16)</b>	0-30 (11)	0-42 (10)	<b>16-70 (49)</b>	0-19 (5)
Baetidae	1-43 (12)	4-25 (9)	0-44 (1)	<b>22-76 (54)</b>	0-20 (6)
<i>Chironomus</i>	<b>1-80 (21)</b>	<b>1-79 (38)</b>	0-26 (6)	0-33 (13)	0-29 (8)
Simuliidae	<b>0-60 (12)</b>	0-29 (8)	0-56 (10)	<b>5-83 (51)</b>	0-21 (5)
<i>T. australis</i>	<b>0-65 (8)</b>	0-32 (4)	0-53 (9)	<b>1-81 (55)</b>	0-28 (6)
Corixidae	0-47 (6)	0-13 (2)	0-64 (22)	<b>12-78 (58)</b>	0-12 (2)
Dytiscidae	1-20 (7)	0-11 (3)	1-22 (6)	<b>56-86 (76)</b>	1-12 (5)
<i>C. quinquecarinatus</i>	1-47 (16)	2-46 (19)	2-42 (19)	3-46 (22)	3-32 (17)
<i>G. occidentalis</i>	1-47 (17)	2-45 (19)	2-42 (17)	3-43 (22)	5-34 (19)



**Figure 4.3.** (Opposite page) Isospace plots of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures (mean  $\pm$  SE) of sources (open circles) and consumers (filled circles) for each reach type, and separately for poor condition reaches with *C. huegelii*: (a) GN - good condition, no macrophytes; (b) GC - good condition, *C. huegelii*; (c) PC - poor condition, *C. huegelii*, Mary Brook; (d) PC - poor condition, *C. huegelii*, Sabina River; (e) PPO - poor condition, *P. drummondii* and *O. ovalifolia*; (f) PN - poor condition, no macrophytes. Consumers are labelled: Gp – Gastropoda; Ch – Chironomidae; Bt – Baetidae; Lc – Leptoceridae; Si – Simuliidae; Cx – Corixidae; Ds – Dytiscidae; Od – Odonata; Cq – *Cherax quinquecarinatus*; Go – *Galaxias occidentalis*; Nv – *Nannoperca vittata*. Consumer isotope values have been corrected for fractionation as described in text. All  $\delta^{13}\text{C}$  are lipid corrected.



**Figure 4.4.** Potential contribution ranges of macrophytes (solid grey bars) as a basal resource to consumer diets in reaches with good condition riparian vegetation and *C. huegelli* (a), poor condition riparian vegetation and *C. huegelli* without (b) and with (c) epiphytes (white bars), and with poor condition riparian vegetation and *P. drummondii* and *O. ovalifolia* (d). Boxplots show median (line), 25-75 percentile range (box) and 5-95 percentile range (whiskers).



## **Discussion**

This study included all potential basal food sources in an effort to gain understanding of the relative contribution of macrophytes in seasonally-flowing lowland streams. The results suggest that macrophytes can make a considerable contribution to these food webs both directly and through supporting epiphyte growth; particularly in reaches where riparian vegetation is in poor condition, and thus supplies little leaf litter to streams. Interpretation of results was sometimes limited by factors such as uncertainty arising from unconstrained mixing model outputs that occurs due to lack of clear isotopic distinction between sources. This is a common problem in freshwater systems where there are multiple potential sources (Phillips and Gregg 2003; Phillips *et al.* 2014). Conversely, good separation between sources did not always achieve constrained solutions, because consumer signatures were positioned centrally in the isospace polygon (Fry 2013). However, while overlapping signatures may be difficult to interpret, they may also reflect opportunistic feeding by aquatic fauna depending on resource availability (Leigh *et al.* 2010; Blanchette *et al.* 2014). Many Australian stream invertebrates are generalists (Chessman 1986; St Clair 1994), and opportunistic foraging may increase resilience in seasonally-flowing streams where availability of resources fluctuates (Leigh *et al.* 2010).

### ***Macroinvertebrates***

Our first hypothesis, that macrophytes would contribute to food webs when present in reaches with good riparian vegetation, was supported to some extent, with moderate assimilation by primary consumers and Odonata. However, they generally had lower importance than shown previously (Reid *et al.* 2008a), and terrestrial and algal sources (epilithon, epiphytes) were significant energy sources regardless of the presence of macrophytes. The Leptoceridae are an interesting trichopteran family including algal grazing, shredding and predatory species. The species collected in this study, *T. australis*, are shredders, as shown by their use of cases made of tree leaves, macrophytes or grass stems. Unfortunately, Leptoceridae were not found in good condition reaches with macrophytes, so could not be compared with other Australian studies, which have found high

assimilation of *Cycnogeton* by these caddisflies in forested reaches (Deegan and Ganf 2008; Reid *et al.* 2008b; Watson and Barmuta 2011). However, *T. australis* did have a potentially high contribution from macrophytes to their diet in all poor-condition reaches, as previously observed for reaches with low riparian detritus inputs (Deegan and Ganf 2008). High assimilation of grasses in some reaches appeared to reflect a flexible diet that depends on whichever form of coarse detrital material is most abundant at a reach (St Clair 1994). Greater assimilation of exotic grasses compared with native macrophytes may be a result of grass stems providing more suitable case materials, with both sources having a similar nutritional value, as indicated by C:N ratios.

For other macroinvertebrates, the potential contribution of *C. huegelii*/*L. lasiospermum* to diets in poor condition reaches was dependent on the presence of epiphytes. In the absence of (obvious) epiphytes, macrophyte assimilation was higher than in good condition reaches and potentially significant for all invertebrate consumers except Dytiscidae, which had strong dependence on tree detritus. These results partially supported our second hypothesis of greater assimilation of macrophytes into stream food webs when riparian vegetation is degraded, but also showed that macrophytes have an important role as substrates for epiphytic algal growth, which is assimilated in preference to macrophyte tissue by most consumers (where present).

In poor condition reaches where epiphytes were conspicuous, the contribution of macrophytes to consumer diets was lower (e.g. Sabina River), although they were potentially an important food for Chironomidae and Leptoceridae. The higher abundances of aquatic invertebrates often associated with macrophytes (Bell *et al.* 2013) can be related to epiphyte biomass (Ferreiro *et al.* 2011). Here, assimilation of  $\delta^{15}\text{N}$ -enriched epiphytes by most consumers was very high, and corresponding enrichment of consumer  $\delta^{15}\text{N}$  compared to other reaches provides further evidence that they were assimilating this resource (Bergfur *et al.* 2009). Enrichment of  $\delta^{15}\text{N}$  in periphyton is common in cleared catchments (Udy and Bunn 2001; Chessman *et al.* 2009) and can result from preferential use of  $^{14}\text{N}$  when nitrogen is present in excess (Peterson and Fry 1987). The Sabina River had the highest nitrogen concentrations of all streams included in this study (owing to diffuse agricultural

sources) with a long term median value of 2700  $\mu\text{g/L}$  (DoW (2010); more than 2.5 times local ecosystem protection guidelines (ANZECC and ARMCANZ 2000). Epilithon and tree detritus also had notably lower contribution to consumer diets in the presence of epiphytes. However, although macrophytes and their epiphytes were important, consumers were not dependent on them, and also assimilated other basal resources (epilithon, charophytes, tree detritus), indicating the potential for flexible, generalist feeding depending on resource availability (St Clair 1994, Leigh *et al.* 2010, Blanchette *et al.* 2014).

While *P. ochreatus* and *O. ovalifolia* were likely contributors to the diet of most consumers in poor condition reaches, epilithon was the most important basal resource in these reaches, particularly for native fish. Lower assimilation of macrophytes in this assemblage than of *C. huegelii/L. lasiospermum* is consistent with the findings of Watson and Barmuta (2011), that *Cycnogeton* was preferred over *Potamogeton* in both stable isotope and feeding trial results in another Australian river system. However in Europe, *Potamogeton* can be an important food source (Jacobsen and Sand-Jensen 1994), particularly for aquatic snails (Elger *et al.* 2002). Although epiphytes were not apparent on the *P. ochreatus* and *O. ovalifolia* assemblage sampled here, morphologically complex assemblages such as this can provide important substrate for epiphytes (Warfe and Barmuta 2006; Ferreiro *et al.* 2011). The assimilation of grass by several invertebrates was surprising, given that aquatic invertebrates are usually thought to avoid C-4 plants (Clapcott and Bunn 2003; Jardine *et al.* 2013). Lower contributions for tree detritus may reflect its relative scarcity (Vannote *et al.* 1980; Reid *et al.* 2008a) and indicate opportunistic feeding by consumers (St Clair 1994; Blanchette *et al.* 2014).

The two invertebrate predators in this study showed very different patterns of resource dependence. Basal resources assimilated by Odonata reflected the primary consumers collected, indicating that they were prey for the odonates. In contrast, adult Dytiscidae showed consistent dependence on terrestrial detritus, indicating that they were preying on consumers that we did not collect which were feeding on tree leaf-litter (for example, detritivorous Chironomidae dwelling in leafpacks). In particular, constrained results in poor condition reaches indicated

high dependence of Dytiscidae on tree litter, despite likely lower availability of this resource compared to good condition reaches. Similar dependence on tree litter was also indicated for Corixidae in some reaches. Some research in the Australian dry tropics has also shown consistent importance of terrestrial detrital inputs as a source of carbon for Dytiscidae at varying levels of canopy cover (Blanchette *et al.* 2014). Allochthonous detritus has also been shown to be the main food source for a range of primary consumers at low riparian cover in alpine areas (McCutchan and Lewis 2002). An alternative explanation is that the fractionation values applied to these beetles were incorrect. While the lower  $\delta^{15}\text{N}$  fractionation values provided by Bunn *et al.* (2013) did not seem applicable for this study (because they did not create a logical isospace), it is possible that they were more applicable for this family.

### ***Crayfish***

The freshwater crayfish *C. quinquecarinatus* appeared to be a flexible omnivore in these streams. It showed moderate assimilation of macrophyte material regardless of reach condition, but had similar and overlapping contributions from a range of basal resources in all sampled reaches. In PC reaches, it did not show the high dependence on epiphytes observed for other taxa, but did have a high contribution from macrophytes. Variation in  $\delta^{15}\text{N}$  between reach types was apparent for *C. quinquecarinatus*, and although this may indicate trophic flexibility (Beatty 2006), in this study it more likely reflects general enrichment of basal resources (Johnston *et al.* 2011), indicated by  $\delta^{15}\text{N}$  values for macrophytes, epiphytes and filamentous algae in poor condition reaches. Others have found preferences by *Cherax* spp. for macrophytes, (Bunn and Boon 1993, Thorp *et al.* 1998), even when potentially feeding at a higher trophic level (Reid *et al.* 2008b).

### ***Native fish***

In good condition reaches, native fish species were highly dependent on epilithon, both in the presence and absence of macrophytes. While epilithon continued to be important in poor condition reaches, assimilation of macrophyte material was greater than in good condition reaches. Results for fish were not always consistent

with those for primary consumers, however this is not surprising given the variability in isotopic values of potential prey items. Galaxiid fishes are opportunistic feeder, with diet dependent on prey availability (McHugh *et al.* 2012), but which include drift-feeding at the water surface on terrestrial insects, whereas pygmy perch are mainly benthic feeders. Stomach contents in the present study indicated that although *G. occidentalis* and *N. vittata* diets varied, they were distinct because *Galaxias*' diet included nekton and terrestrial insects whereas *Nannoperca* consumed benthic detritus. Consistent importance of macrophytes and their epiphytes in poor condition reaches may indicate that these fish were feeding on prey within macrophyte habitat, as well as the variable consumption of these resources (and epilithon) by prey. The dependence by fish on in-stream resources indicated by our study contrasts with the dependence on allochthonous resources reported by Reid *et al.* (2008b) for *Nannoperca australis* and *Galaxias olidus* in forested reaches. However, Reid *et al.* (2008b) also showed that *Cycnogeton* was an important carbon source for *N. australis* during summer, perhaps reflecting increased availability of this resource, or increased time spent by this small fish amongst complex habitat in declining pools.

### ***Conclusions and management implications***

Given the demonstrated importance of allochthonous detritus and algae as carbon sources in rivers and streams elsewhere (Finlay 2001; McCutchan and Lewis 2002; Bunn *et al.* 2003), we did not anticipate macrophytes to be the dominant basal resource in our study, but did hypothesize that they would contribute to food webs and might provide an alternative resource when supplies of allochthonous detritus were limited owing to degraded riparian zones. We found support for this hypothesis, both in terms of direct contribution of macrophyte material, and through the provision of substrate for epiphytes. There is increasing acceptance of periphyton as a key resource for river food webs (DeLong and Thorp 2006; Jardine *et al.* 2013) and this was apparent in our study. Where isotopic signatures were distinct, epiphytes were equally or more important than epilithon as a food resource, suggesting that plant surfaces provide valuable substrate for biofilm growth.

Our results indicate opportunistic feeding by consumers in degraded reaches, which is likely to be particularly important for survival in seasonally-flowing lowland streams with degraded riparian vegetation. The presence of macrophytes in streams with reduced riparian-detritus inputs may provide an alternative resource in these streams because there are taxa able to switch between different food sources. Indeed, only one family (Dytiscidae) in this study showed no flexibility in assimilated diet.

This study highlights the importance of macrophytes in food webs of streams with degraded riparian vegetation, and suggests they may be valuable in reach-scale stream restoration. However, submerged macrophytes are rarely considered in stream restoration, which often focuses on stream channel modification and riparian revegetation (Palmer *et al.* 2014). This is despite their potential to support ecosystem processes in addition to food resources, particularly structural habitat (Warfe and Barmuta 2006; Strayer and Malcom 2007). While riparian vegetation is considered vital for healthy stream metabolism (Bunn *et al.* 1999, Davies 2010), revegetation takes decades for tree growth to provide the services of mature native stands (Becker and Robson 2009). Submerged macrophytes grow rapidly, so are able to provide a seasonally-important resource for many taxa in a short time-frame (months) that may complement longer term revegetation goals. Even where pre-disturbance conditions did not include macrophytes as significant stream components, they may be valuable in achieving functional ecosystem restoration provided there is acceptance of an alternative recovery endpoint for restoration (Lake *et al.* 2007).

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## **Chapter 5. Outcomes of submerged macrophyte restoration in a shallow impounded, eutrophic river**

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## Abstract

Restoration of submerged macrophytes in eutrophic shallow freshwater ecosystems is rarely undertaken without additional measures to improve water clarity.

Increasing water clarity is extremely difficult to achieve in some eutrophic waters, so this study trialled the establishment of macrophytes directly into a turbid, phytoplankton-dominated system. The submerged macrophyte *Vallisneria australis* grew successfully in five 48-m<sup>2</sup> protective exclosures, from transplants attached to steel mesh for anchorage in flocculent sediments. Plant growth, water quality, and zooplankton and macroinvertebrate richness and abundance were measured and compared with open water control plots throughout the growing season. *V. australis* grew well despite poor water quality (total phosphorus 44 -1400 µg L<sup>-1</sup>; total nitrogen 650 -14000 µg L<sup>-1</sup>; chlorophyll *a* 1.6-770 µg L<sup>-1</sup>; turbidity 3-207 NTU), attaining 85-100% cover after six months. Water quality was not improved within macrophyte meadows and zooplankton grazing was not enhanced. Richness and abundance of macroinvertebrates increased and additional native macrophyte species colonised the exclosures. Co-dominance of phytoplankton and macrophytes was achieved in exclosures, with beneficial outcomes for biodiversity. Rapid destruction of macrophyte meadows by waterbirds on removal of protective cages indicated the need for continued protection for long-term establishment of submerged macrophytes.

**Key words:** macrophytes, freshwater invertebrates, phytoplankton, restoration, transplantation, water quality.



## Introduction

Submerged macrophytes are important components of aquatic ecosystems, providing structural habitat and food sources for animals (Heck and Crowder, 1991; Diehl and Kornijów, 1998), and maintaining a clear-water regime in shallow lentic systems (Davis et al., 2010) through nutrient uptake, enhanced sedimentation and reduced resuspension (Van Donk and van de Bund, 2002) and provision of habitat for herbivorous zooplankton (Timms and Moss, 1984). Nutrient enrichment generally leads to the decline of submerged macrophytes, primarily due to light limitation caused by excessive growth of epiphytic algae (Phillips et al., 1978) and phytoplankton (Jupp and Spence, 1977). The result is a turbid, phytoplankton-dominated regime which presents a serious management problem in terms of public health, aesthetics and loss of biodiversity.

Owing to their significance in ecosystem processes, recolonisation of submerged macrophytes is frequently a goal of restoration, but one which is generally achieved following initial improvement in water clarity. The most prominent example is biomanipulation in cool temperate lakes (Ozimek et al., 1990; Meijer et al., 1994; Sondergaard et al., 2007) whereby increased water clarity allows recolonisation of submerged vegetation, which then stabilises a clear-water state (Van Donk and van de Bund, 2002). Macrophyte establishment through transplantation is less common, but where herbivory limits natural re-establishment, protected plantings can be successful (e.g. Sondergaard et al., 1996; Lauridsen et al., 2003). In subtropical China, submerged macrophyte transplants have been incorporated in multi-faceted restoration trials (Chen et al., 2009, Ye et al., 2011), but large-scale restoration has been problematic under conditions of low transparency and high nutrients (Qin, 2013). While transplantation is successful in less degraded systems (Carter and Rybicki, 1985; Moore et al., 2010), there are few examples of restoration of submerged macrophytes in phytoplankton-dominated shallow systems without additional actions to improve water clarity.

Management of excessive phytoplankton growth logically targets nutrient loading reduction. However, significant reductions may be difficult to achieve in developed catchments and may not lead to ecosystem recovery owing to release of nutrients from sediments within turbid, phytoplankton-dominated regimes (Mortimer, 1942;

Marsden, 1989). In Australia, biomanipulation is often not feasible owing to potential impacts on protected small fish species either through direct removal or introduction of predatory species (Sierp et al., 2009), and there are few short-to-medium term restoration actions available to improve water clarity. Under these circumstances, the capacity to establish macrophytes directly into eutrophic systems may be beneficial.

We planted submerged macrophytes in a shallow, impounded reach of the eutrophic Vasse River in south-western Australia, without concomitant water clarity improvement, to evaluate the potential for caged submerged plants to be used in restoration. The method used aimed to overcome light limitation by planting a species tolerant of low light and high nutrient conditions, and assisted colonisation by using protection from waterbird disturbance and providing a substratum for root anchorage in the flocculent sediment. The questions were: (1) can submerged macrophytes be established in turbid, nutrient rich waters when protected from herbivorous waterfowl; (2) what influence do submerged macrophytes have on the aquatic invertebrate assemblage in this degraded system; and (3) does the presence of submerged macrophytes improve water quality in terms of reduced nutrient concentrations, turbidity and phytoplankton growth?

## **Materials and Methods**

### ***Study site***

The lower reach of the Vasse River in south-western Western Australia (33° 38.901' S; 115° 20.675' E) is a shallow section of variable width (10-30 m) in Busselton, which is impounded to maximise water levels during summer. Diversion of flow from the upper 90% of the catchment in this mediterranean climate, has resulted in an elongated wetland with maximum depth around 2m, and negligible summer flow. A thick layer of flocculent sediment covers the river bed, exceeding one metre depth in the thalweg. High nutrient concentrations support extensive cyanobacterial blooms during summer and autumn (Novak and Chambers, 2014), impacting aesthetic and recreational values. Due to its prominent location in Busselton, this is a significant management issue driving considerable past investment in restoration, including sediment remediation and removal, foreshore

revegetation and management of diffuse nutrient sources. However, none of these actions have yet improved water quality. Submerged macrophytes have not been observed in the lower Vasse River, despite numerous studies and management actions undertaken since 2000.

Seasonal changes in water quality were observed during the experiment. Mean conductivity increased from 0.7 mS cm<sup>-1</sup> in October 2011 to 2.2 mS cm<sup>-1</sup> in March 2012, while mean pH increased from 6.9 to 8.9. Monthly average water temperature ranged from 19.3 °C in October to 26.8 °C in February. A range of phytoplankton groups was present during November and December 2011, with population densities reaching over 20 000 cells mL<sup>-1</sup> in December (unpublished data, Department of Water, 2013), which is sufficient to cause water discolouration. From January to March only cyanobacteria were present, with an extremely high average cell density of 760 000 cells mL<sup>-1</sup> (unpublished data, Department of Water, 2013).

### ***Study species***

*Vallisneria australis* S.W.L. Jacobs & Les is a perennial, rooted submerged macrophyte with long ribbon-like leaves growing from a basal cluster. It is widely distributed in the eastern states of Australia (Sainty and Jacobs, 1994) and naturalised in Western Australia, with four known populations in the south-west region (DPAW, 2013), and tolerates low light conditions (Blanch et al., 1998). In pilot studies in this system using a range of submerged macrophyte species, *V. australis* was the only species to survive in pot trials at the very high nutrient and phytoplankton levels (Novak and Chambers, 2014), and grew successfully in small (1-m<sup>2</sup>) meadows with waterbird protection. With the highly degraded nature of the river, macrophyte dominance, even of a naturalised species, would be preferable to the toxic cyanobacterial blooms currently present. However, *V. australis* did not flower during this trial, all biomass of the species was removed when cages were taken out at the end of the study, and subsequent checks found no evidence of the species over the following two growing seasons.

## ***Experimental Design***

Ten plots (five treatment (macrophyte) plots and five control (bare sediment) plots, (interspersed and allocated randomly) were positioned along a two-kilometre reach of the lower Vasse River in October 2011. Each treatment plot included plants in 48m<sup>2</sup> (6m x 8m) protective cages with steel mesh fencing (mesh size 50mm x 75mm) covered with bird netting (mesh size 20mm). Cages prevented disturbance by water birds and large fish but allowed passage of small fish and invertebrates and maintained connectivity with surrounding water. Control plots were marked open water sites of the same size as the treatment plots. Controls comprising cages without plants were not used because of potential for natural colonisation of macrophytes in protected areas (Lauridsen et al., 2003), which would conflict with research aims to compare water quality and invertebrates in the presence and absence of plants. Control plots of plants without cages were not considered possible based on pilot trials of small meadows which indicated protection was essential to survival. Therefore treatments comprised the cage, netting and the macrophytes, with any independent effect of cages accepted as part of the restoration regime. Furthermore, the netting and cages were removed at the end of the experiment to confirm their role in sustaining the macrophyte beds (see below).

*Vallisneria* transplants were of about thirty ramets of variable length (90-410 mm) held together with wire. Six transplants were attached with wire uniformly to each of eight 1.2m x 2.4m steel mesh grids (mesh size 100mm). Grids were placed on the sediment surface uniformly throughout each treatment plot. Depth varied because plots were located on a gentle slope, and depth within plots ranged from 0.81-1.26m at the time of transplantation.

## ***Sampling***

Initial planting created cover of 1% (48 transplants within 48m<sup>2</sup>, each transplant within an area 0.01m<sup>2</sup>). Visual estimates of percentage plant cover (basal cover) were made each month, excluding November, when small plant size, high water depth and turbidity prevented estimation. Because volumetric estimates of biomass would be correlated with decreasing depth, basal cover estimates were used to

measure growth over time. Depth measurements were made monthly at the four corners of each plot.

Epiphyte load was sampled in December and February, with shoot samples cut at the sediment surface from five randomly-selected 100 x 100 mm quadrats. The native submerged macrophyte *Potamogeton crispus* unexpectedly colonised two macrophyte plots, and similar sampling for epiphyte load on this species was done for these plots. Samples were gently pre-washed to remove any sediment and then epiphytes were carefully scraped and washed from *V. australis* leaves into clean water; and removed from *P. crispus* leaves by shaking for one minute in water owing to the more complex morphology and fragility of this species. Epiphytes were filtered onto pre-weighed glass fibre filters, and both epiphytes and plants were dried at 70°C for 72 hours to determine epiphyte load per unit dry weight of plant material.

Flow in the study reach was negligible from late spring until the winter rains and as sampling aimed to detect localised changes associated with macrophytes, water was sampled monthly within macrophyte and control plots. Sampling in macrophyte plots was done from a temporary aluminium plank to minimise disturbance. At each plot, depth-integrated sub-samples were taken from four random points using a 45 mm internal diameter clear plastic tube, and combined for analysis of nutrients, chlorophyll *a* and turbidity. Standard analytical methods were used to determine total phosphorus and total nitrogen (Valderrama, 1981), filterable reactive phosphorus (Johnson, 1982), nitrate plus nitrite (Johnson, 1983), ammonium-nitrogen (Switala, 1993), and chlorophyll *a* (APHA, 1995) as an indicator of phytoplankton biomass. Turbidity was analysed *in situ* (Hach 2100P Turbidimeter). *In situ* measurements of temperature, dissolved oxygen, conductivity, salinity and pH were recorded near the surface and bottom at one location within each plot (YSI 556 MPS multiparameter probe).

Aquatic invertebrates were sampled monthly from each plot by three replicate 3-metre sweeps (D-frame net, mesh size 250 µm) from random start-points. Samples were preserved in ethanol and stored at < 5 °C prior to identification and counting. Taxa present in very large numbers were counted using volumetric subsampling. Invertebrates were identified to family level with the exception of Copepoda,

Cladocera, Collembola, Clitellata, Hydracarina and Hirudinea, which were not identified further. Fish in sweep samples were identified and counted: native species were returned immediately to the water and introduced species were killed in an ice slurry.

### ***Data analysis***

*Vallisneria* growth (percentage cover, arcsin transformed), was analysed over time using a single factor repeated-measures analysis of variance (ANOVA) with sample date as a main effect (six levels, random). Epiphyte load (g per g leaf biomass, dry weight) on *V. australis* and *P. crispus* was compared over time for each species using repeated measures ANOVA with sample trip as a repeated-measure factor (two levels, random: December and February) and plot as between-subjects factor (random, five levels for *V. australis*, two levels for *P. crispus*). Comparisons of epiphyte load between the two species and sample dates were analysed for the two sites where they co-occurred using repeated measures ANOVA with sample trip (two levels, random: December and February) as the repeated-measures factor and species (two levels, fixed) as the between-subjects factor. Plant growth and epiphyte data met assumptions of normality and homogeneity of variance, as indicated by Shapiro-Wilk and Levene's tests respectively; and Mauchly's test confirmed equal variances between different levels of measurement (sphericity).

Repeated measures ANOVA was used to examine differences between macrophyte and control plots for each water quality variable and invertebrate abundance and richness data: with plot type as the between-subjects factor (two levels, fixed: macrophyte and control) and sample date as the repeated-measures factor (six levels, random). Abundances of Copepoda and Cladocera were analysed separately because of their potential as phytoplankton grazers. Levene's test verified homogeneity of variance and water quality and abundance data were transformed ( $\log_{10} + 1$ ) to meet assumptions of normality. Where Mauchly's test indicated sphericity was not met, outcomes of Wilk's Lambda exact multivariate test were used. Complete ANOVA results are presented in Appendix 1 (Online Resource 1). Spearman's correlation coefficient was used to identify variables correlated with chlorophyll *a* levels because this was the primary indicator of phytoplankton

growth, which is dependent on nutrient availability and is often the main cause of turbidity in eutrophic waters.

Invertebrate abundances differed greatly between treatment and control plots, so rarefaction curves were constructed (EcoSim: Gotelli and Entsminger, 2001) to compare richness between treatments, independent of the effect of abundance. Differences in invertebrate assemblage composition between macrophyte (plant plus cage) and control plots (no plants and no cage) were analysed by ANOSIM (analysis of similarity) using data for each plot (3 sweeps averaged; log-transformed) on each sample date. SIMPER (similarity percentages) was used to identify the families responsible for the differences between treatment and control plots and the Bio-Env procedure determined whether patterns in the invertebrate assemblage were associated with patterns in the water quality variables. All multivariate analyses were completed using software package PRIMER-E 6 (Plymouth Routines in Multivariate Ecological Research: Clarke and Warwick, 2001).

## **Results**

### ***Macrophyte growth***

*Vallisneria* transplants were not visible for two months owing to poor water clarity. By December water levels had dropped to 0.54-1.02m and transplants were visible at or near the water surface in all plots, with 100% survival and mean cover of 13% (Figure 5.1a). Rapid growth followed, with mean cover increasing to over 50% in January, when decreases in water level were followed by a further increase in mean cover to 86.5% (range 70-100%) in March (Figure 5.1a). Not surprisingly, growth was highly significant over time ( $F_{1,4} = 45.3$ ,  $P < 0.001$ ). Although water level continued to drop during the study period, with final depth range of 0.0-0.38m (Figure 5.1a), rapid initial growth, resulting in leaves reaching the surface, suggests transplants would be likely to continue to grow at depths of at least 1m.

Other native submerged macrophyte species were found in some plots during the study. Small amounts of hornwort *Ceratophyllum demersum* L. were observed in caged plots from January to March (1- 4% cover). *Potamogeton crispus* grew in the two most downstream caged plots from December to March, with decreasing cover

over time (Figure 5.1a). *P. crispus* was also present in nearby unprotected areas during December only, including one control site.

Protective cages were removed at the end of the experiment, following sampling in March, and meadows were completely destroyed by waterbirds within four days (zero plants remaining within plots). Both herbivorous and predatory waterbirds were observed feeding within macrophyte meadow areas (Table 5.1).

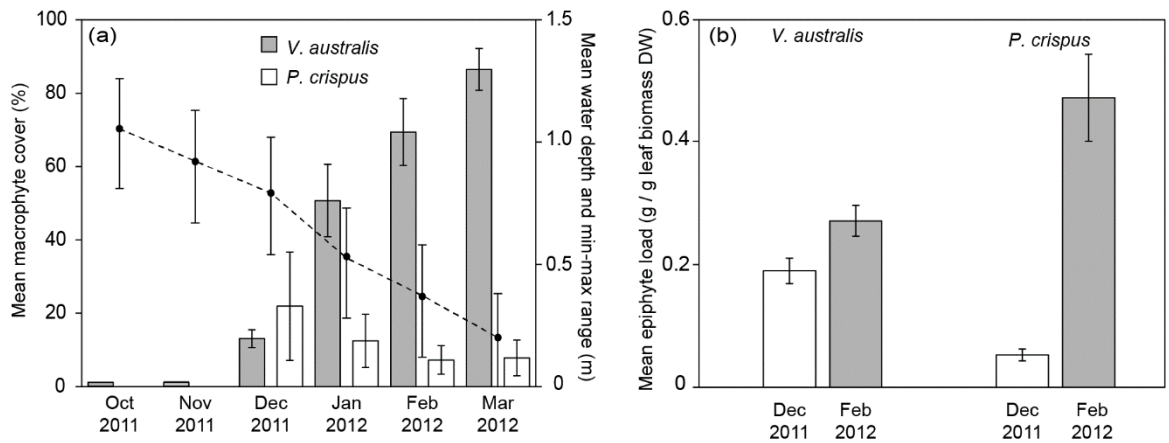
**Table 5.1.** Bird species observed in vicinity of macrophyte plots during the study and following cage removal. Species known to consume macrophytes are marked \* (from Jones et al., 2009).

Group and scientific name	Common name
<i>Chenonetta jubata</i> *	Australian wood duck
<i>Anas superciliosa</i>	Pacific black duck
<i>Anas rhynchotis</i> *	Australasian shoveler
<i>Oxyura australis</i> *	Blue-billed duck
<i>Cygnus atratus</i> *	Black swan
<i>Poliiocephalus poliocephalus</i>	Hoary-headed grebe
<i>Biziura lobate</i> *	Musk duck
<i>Anhinga melanogaster</i>	Darter
<i>Phalacrocorax melanoleucos</i>	Little pied cormorant
<i>Phalacrocorax sulcirostris</i>	Little black cormorant
<i>Egretta novaehollandiae</i>	White-faced heron
<i>Nycticorax caledonicus</i>	Nankeen night heron
<i>Egretta garzetta</i>	Great egret
<i>Platalea flavipes</i>	Yellow-billed spoonbill
<i>Fulica atra</i> *	Eurasian coot

## ***Epiphytes***

Between December and February, mean epiphyte load on *V. australis* decreased slightly (Figure 5.1b), but loads decreased in the two most upstream plots and increased at other plots downstream (sampling date x plot interaction:  $F_{4,20} = 6.62$ ,  $P = 0.001$ ). Epiphyte load on *P. crispus* leaves was significantly higher in February compared with December (Figure 5.1b.  $F_{1,8} = 28.12$ ,  $P = 0.001$ ). For the two sites where *V. australis* and *P. crispus* occurred together, epiphyte load increased over time for both species, but there was a significant interaction between sampling date and species ( $F_{1,18} = 10.1$ ,  $P = 0.005$ ) because epiphyte loads were higher on *V. australis* in December but higher on *P. crispus* in February.





**Figure 5.1** (a) Growth of *V. australis* transplants (grey bars) and naturally colonising *P. crispus* (white bars) in caged plots. Plot depth change (hatched line) is also shown. Transplants were not visible until December and results in (a) show transplant cover of 1% for October and November. (b) Mean epiphyte load on *V. australis* and *P. crispus*. Results for *P. crispus* in both (a) and (b) reflect only the two caged plots where it occurred. Bar values are mean with standard error. Depth values are mean with min-max range.

### **Water quality**

Total phosphorus (TP) increased to extremely high levels during the study ( $F_{5,4} = 14.80$ ,  $P < 0.001$ ), but did not differ between macrophyte and control plots ( $F_{1,8} = 6.62$ ,  $P = 0.591$ ) (Figure 5.2a). Concentrations at the start of sampling were more than three times the local guideline for ecosystem protection ( $65\mu\text{gL}^{-1}$ ; Australian and New Zealand Environment and Conservation Council [ANZECC] and Agriculture and Resource Management Council of Australia and New Zealand [ARMCANZ], 2000) and increased to an order of magnitude higher than this guideline in March. Results were also well above the nominal minimum threshold for phytoplankton dominance in temperate shallow lakes ( $150\mu\text{gL}^{-1}$ ; Scheffer and Jeppesen, 1998), which has also been shown to apply in south-western Australia (Novak and Chambers 2014). Filterable reactive phosphorus (FRP) concentrations decreased over time in all plots ( $F_{5,4} = 43.08$ ,  $P = 0.001$ ) (Figure 5.2b), and did not differ between macrophyte and control plots ( $F_{1,8} = 0.04$ ,  $P = 0.843$ ). FRP contributed 53% of TP in October 2011 but only 2% in March 2012, reflecting an increasing proportion of particulate phosphorus within phytoplankton cells (c.f. Figure 5.2e).

Total nitrogen (TN) increased markedly over time ( $F_{5,4} = 157.52$ ,  $P < 0.001$ ) to levels well above the ecosystem protection guideline ( $1200\mu\text{gL}^{-1}$ ; ANZECC and ARMCANZ, 2000) from January onwards (Figure 5.2c), and was significantly higher in macrophyte than control plots ( $F_{1,8} = 7.10$ ,  $P = 0.029$ ). Most nitrogen was in particulate form, especially at higher concentrations towards the end of the study. The highest levels of ammonium-nitrogen ( $\text{NH}_4\text{-N}$ ) and nitrate plus nitrite ( $\text{NO}_{3/2}\text{-N}$ ) occurred during October, with concentrations of both then falling to very low levels by December (Figure 5.2d,  $\text{NH}_4\text{-N}$ :  $F_{5,4} = 2957.0$ ,  $P < 0.001$ ;  $\text{NO}_{3/2}\text{-N}$ :  $F_{5,4} = 5700.0$ ,  $P < 0.001$ ). Ammonium-nitrogen increased at the two most downstream macrophyte plots in March.

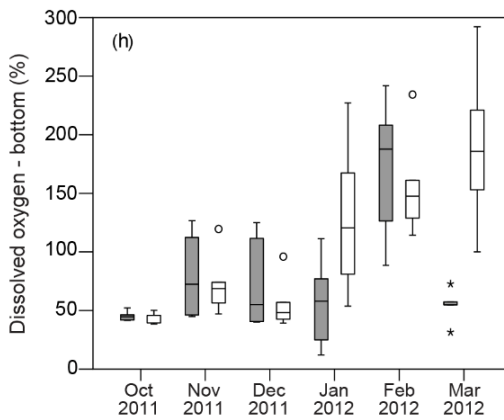
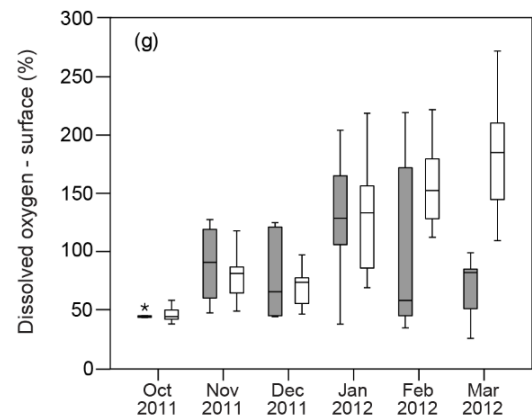
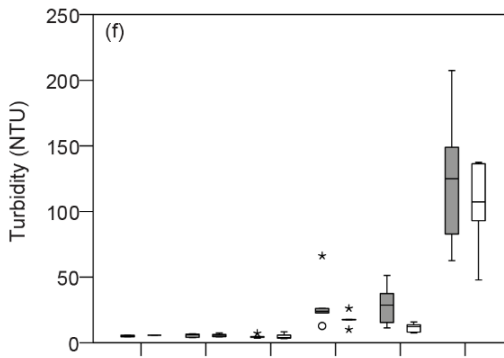
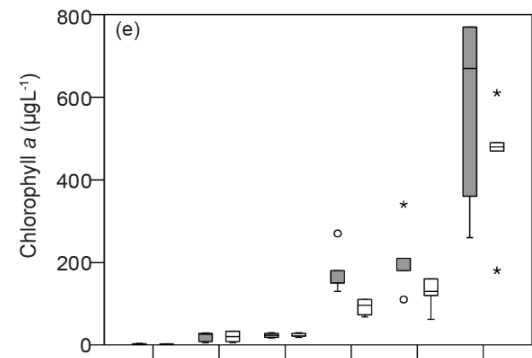
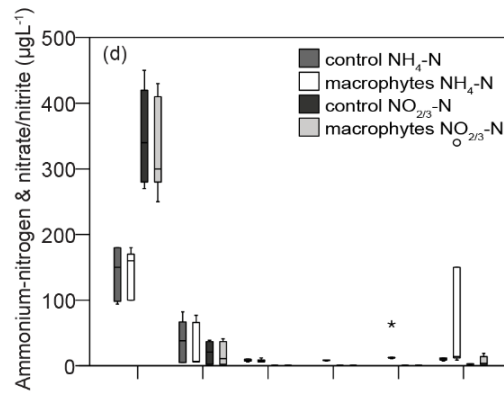
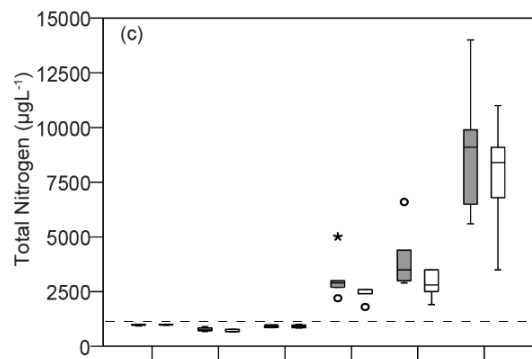
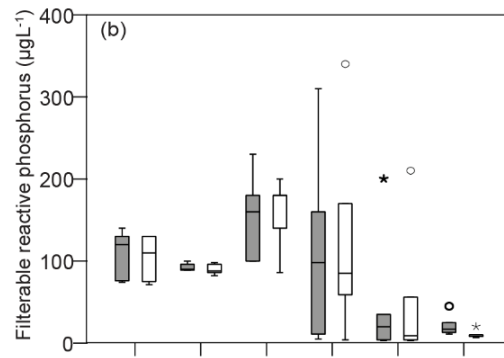
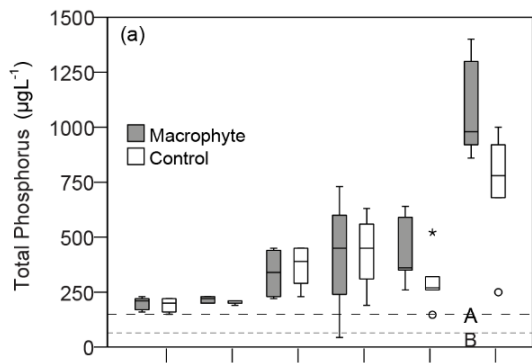
Chlorophyll *a* concentrations greatly exceeded the ecosystem protection level from January onwards ( $5\mu\text{gL}^{-1}$ ; ANZECC and ARMCANZ, 2000) (Figure 5.2e).

Although chlorophyll *a* levels differed little between macrophyte and control plots early in the experiment, concentrations increased from January to March in macrophyte plots more than in control plots (plot type x sample date interaction:  $F_{5,4} = 8.19$ ,  $P = 0.032$ ) (Figure 5.2e). There was strong positive correlation of chlorophyll *a* with both TP and TN (TP:  $r = 0.69$ ,  $P < 0.01$ ; TN:  $r = 0.82$ ,  $P < 0.01$ ).

Turbidity was generally low from October to December, when all samples were below the lowland rivers ecosystem protection guideline (10 NTU; ANZECC and ARMCANZ, 2000); but increased rapidly over time from January ( $F_{5,4} = 334.2$ ,  $P < 0.001$ ) (Figure 5.2f). This increase was associated with the cyanobacterial bloom, indicated by a strong correlation with chlorophyll *a* ( $r = 0.85$ ,  $P < 0.01$ ). Turbidity was significantly higher in macrophyte plots than controls ( $F_{1,8} = 7.10$ ,  $P = 0.017$ ).

Daytime dissolved oxygen (DO) generally increased during the study and varied considerably within both plot types (Figure 5.2g, h). Surface DO did not differ between plot types until March, when concentrations were substantially lower in macrophyte plots, but there was no significant effect of plot type or sample date (respectively:  $F_{1,8} = 2.7$ ,  $P = 0.139$ ;  $F_{5,4} = 4.58$ ,  $P = 0.083$ ) (Figure 5.2g). In bottom waters, DO levels were lower in macrophyte plots in January and March (plot type x sample date interaction:  $F_{5,4} = 20.5$ ,  $P = 0.006$ ).

**Figure 5.2** (opposite page) Comparison of total phosphorus (a), filterable reactive phosphorus (b), total nitrogen (c), ammonium-nitrogen ( $\text{NH}_4\text{-N}$ ) and nitrate plus nitrite ( $\text{NO}_{3/2}\text{-N}$ ) (d), chlorophyll *a* (e), turbidity (f) and dissolved oxygen in surface (g) and bottom (h) waters in macrophyte and control plots. Boxplots show median (line), 25-75<sup>th</sup> percentile range (box); 5-25<sup>th</sup> and 75-95<sup>th</sup> percentile range (error bars), outliers (★) and extremes (○). Reference lines in (a) are phytoplankton dominance threshold (A) (Scheffer and Jeppesen, 1998) and local lowland river ecosystem protection guideline (B) (ANZECC and ARMCANZ, 2000). Reference line in (c) is ecosystem protection guideline (ANZECC and ARMCANZ, 2000).

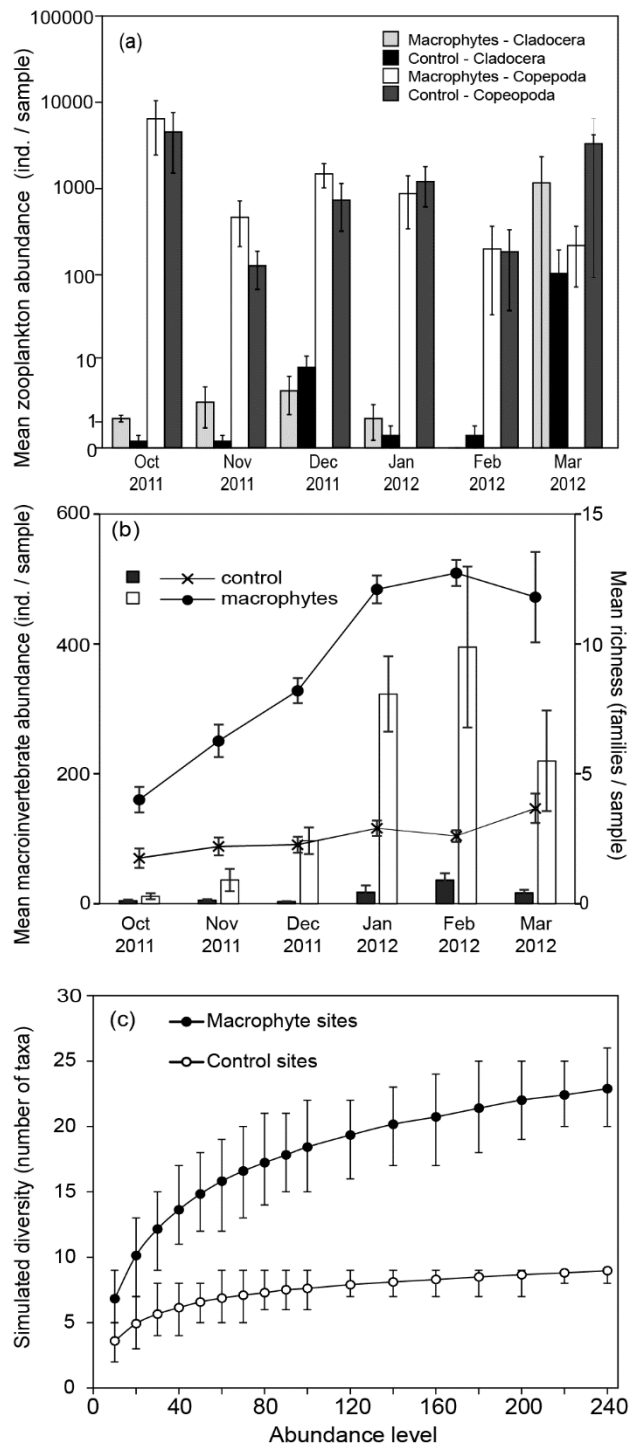


## ***Invertebrates***

Cladoceran abundance was very low in all plots from October to February (Figure 5.3a) and did not differ between plot types ( $F_{1,8} = 0.003$ ,  $P = 0.958$ ) or over time (sample date effect:  $F_{5,4} = 0.262$ ,  $P = 0.186$ ). Greater mean abundance in March was due to high numbers in one sample from each plot type. Copepoda were much more abundant than cladocerans and were consistently present throughout the study (Figure 5.3a), although abundance was variable with no difference between macrophyte and control plots ( $F_{1,8} = 0.003$ ,  $P = 0.955$ ).

Macroinvertebrate abundance was very low in control plots throughout the study, whereas abundance in macrophyte plots increased substantially over time until February (Figure 5.3b) and was significantly higher than in controls ( $F_{5,40} = 4.35$ ,  $P = 0.003$ ). Family-level richness was also higher in macrophyte plots compared with controls ( $F_{5,40} = 8.48$ ,  $P < 0.001$ ) and showed a similar increase over time (Figure 5.3b). Twenty-four taxa were identified from control plots, compared with thirty taxa from macrophyte plots. All taxa present in control plots were also found in macrophyte plots. Importantly, higher taxon richness in macrophyte stands was not solely due to higher abundances (Figure 5.3c).

Macroinvertebrate assemblage composition also differed substantially in the presence and absence of macrophytes ( $R = 0.25$ ,  $P < 0.001$ ), and average dissimilarity between macrophyte and control plots was also high at 67.7%. Taxa with the greatest contribution to dissimilarity due to higher abundance in macrophyte plots were Coenagrionidae larvae, gastropods (Physidae, Planorbidae and Lymnaeidae), Palaemonidae, Chironomidae, Clitellata, Notonectidae, Cyprididae and Corixidae (Appendix 2, Online Resource 2). Invertebrate composition in macrophyte plots was associated with nitrogen (TN, N-anions and  $\text{NH}_4$ ) and total phosphorus ( $p_w = 0.39$ ) but in control plots, invertebrate composition was most related to chlorophyll *a* and total phosphorus ( $p_w = 0.23$ ).



**Figure 5.3** Comparison of zooplankton abundance (a) and macroinvertebrate abundance (b: bars) and family-level richness (b: lines) in macrophyte and control plots; and rarefaction curve of diversity (family-level richness) and abundance (c). Note log scale used in a. Error bars in a and b are +/- standard error, error bars in c are +/- standard deviation.

## ***Fish***

Fish species observed in invertebrate sweep samples from macrophyte plots included 17 native Swan River Goby (*Pseudogobius olorum* Sauvage), two native Nightfish (*Bostockia porosa* Castelnau) and three small introduced Goldfish (*Carassius auratus* L.). Introduced Eastern Gambusia (*Gambusia holbrooki* Girard) were present in sweep samples from both plot types, but abundance was significantly higher in macrophyte plots ( $F_{1,8} = 42.6$ ,  $P < 0.001$ ). Abundance of Eastern Gambusia increased over time ( $F_{5,4} = 15.5$ ,  $P = 0.010$ ), but remained relatively low in control plots (maximum mean of  $6.5 \pm 0.80$  std. error in March) compared with macrophyte plots, in which mean abundance increased to  $25.8 (\pm 15.0 \text{ SE})$  in January and  $98.7 (\pm 44.6 \text{ SE})$  in March.

## **Discussion**

### ***Macrophyte establishment***

This restoration trial successfully created, in flocculent sediments, dense meadows of *V. australis*, which were sustained under turbid conditions when protected from waterbird disturbance. Light is a key factor limiting submerged macrophyte growth and high turbidity is known to prevent macrophyte recolonisation (Scheffer and van Nes, 2007) and successful revegetation (Carter and Rybicki, 1985). Unlike other transplantation studies (Sondergaard et al., 1996; Lauridsen et al., 2003; Chen et al., 2009), this study did not use additional measures to improve water clarity. Light limitation was overcome by the ability of *V. australis* to tolerate low light levels, including its growth response of leaf elongation and recruitment in low-light conditions (Blanch et al., 1998). The initial transplant length of up to 0.41m provided a canopy with some exposure to light, and rapid growth allowed *V. australis* to reach the surface of the water within two months, before onset of the seasonal algal bloom and resulting increase in turbidity. Plants continued to grow rapidly during January at depths up to 0.73m in conditions of very high turbidity, because of the cyanobacterial bloom, with colonisation of new substrata to extents of more than 50%, which continued in the turbid conditions, reaching 70 to 100% after six months. Rapid growth of *V. australis* may also have limited epiphyte load by providing new leaf surfaces. Although final maximum depth of growth

demonstrated in this study was only 0.38m, and decreasing water levels compensated for increasing turbidity (Moore et al., 2010; Bucak et al., 2012), leaves of *V. australis* transplants had elongated to the surface at up to 1.02m depth, which would likely support continued growth regardless of further drop in water levels. Furthermore, increasing cover of *V. australis* over time contrasted with that of *P. crispus*, which was unable to colonise new substrata despite declining water levels.

Protection from waterbird feeding and disturbance was essential to successful establishment of macrophyte meadows, demonstrated by complete loss of macrophytes because of grazing and disturbance by waterbirds within four days of cage removal. Previous studies have also demonstrated damage by waterbirds to macrophyte transplants and the need either for ongoing protection, or establishment of stands large enough to sustain grazing pressure (Carter and Rybicki, 1985; Lauridsen et al., 2003; Moore et al., 2010). However, these examples came from restored lakes that were not under eutrophic, turbid conditions. Macrophytes in nutrient-enriched conditions are more palatable to birds (Bakker et al., 2014), and high bird density can reduce macrophyte development in eutrophic waters (Marklund et al., 2002). Although there are no counts of waterbird densities in the lower Vasse River, waterfowl were common during this study and the nearby Vasse-Wonnerup Wetlands support over 30,000 waterbirds during summer (Lane et al., 2007). Waterbirds would easily prevent natural development of submerged macrophytes in this system. While the absence of submerged vegetation is generally attributed to light limitation in phytoplankton-dominated regimes, this study demonstrates the additional potential importance of top-down control by herbivory in shallow waters within feeding range of birds.

The protective cages also excluded large goldfish, which may uproot plants while foraging and prevent their establishment (Morgan and Beatty, 2007). Although their contribution to the destruction of meadows would be negligible in comparison to birds, they may limit natural macrophyte colonisation.



### ***Water quality***

Nutrient concentrations increased to extremely high levels during the study and were generally similar in macrophyte meadows and open water controls. Thus, despite negligible flow during the study period, meadows were not large enough to influence water quality independently of external water exchange. Dissolved nutrient fractions declined rapidly at the start of the growing season, while TN and TP increased in correlation with chlorophyll *a*, suggesting nutrients were bound within algal cells. Phytoplankton was the main cause of high turbidity from January to March and both chlorophyll *a* and turbidity were higher in macrophyte plots than controls over that period. Although trapping of particulates and associated sedimentation can improve water clarity, in this study cyanobacterial colonies were trapped within macrophytes, possibly contributing to higher TN and turbidity. We also noted higher TP and lower DO in macrophyte plots during the final two months of this study, when high plant density resulting from shallow conditions may have reduced oxygenation by restricting mixing and benthic primary production, causing release of phosphorus (Mortimer, 1942; Boros et al., 2011).

Given the absence of top-down control by zooplankton (see below), the basis of water quality improvement through this restoration method is to promote sedimentation and nutrient uptake, which are known to limit nutrient availability in waterbodies with a clear-water, macrophyte-dominated regime. Sedimentation was apparent in our macrophyte plots, and nutrient uptake was likely, but despite this, extremely high nutrient availability continued to support excessive phytoplankton growth.

### ***Zooplankton***

Abundance of grazing zooplankton was not enhanced by submerged macrophytes. Copepoda, which have limited potential for grazing on phytoplankton (Boon et al., 1994), was the most abundant zooplankton group. Larger cladocerans, which exert more grazing pressure on phytoplankton (Brooks and Dodson, 1965), were rarely found in high abundance. This is consistent with studies in subtropical waters where high densities of small omnivorous fish prevent development of large cladoceran populations (Beklioglu et al., 2007; Brucet et al., 2012). Macrophyte meadows in

our study had high abundance of predatory invertebrates (Coenagrionidae, Notonectidae, Corixidae) and the omnivorous *G. holbrooki*. Such aggregation of fish and macroinvertebrates within submerged vegetation can limit their value as zooplankton refuge (Meerhoff et al., 2006; González Sagrario and Balserio, 2010). Interestingly however, greatest cladoceran abundance occurred in March, when fish density was highest, so it is possible that *G. holbrooki* had little effect (Ho et al., 2011). Nonetheless, this introduced species is widespread in south-western Australia and several native fish species that consume zooplankton do have a preference for cover (Morgan et al., 2011), which may limit potential refuges within submerged macrophytes.

The dominance of cyanobacteria also restricts top-down control of phytoplankton, as large colonies of *Microcystis* sp are difficult for zooplankton to ingest (Boon et al., 1994), and at very high density (January to March average: 515,000 cells per mL) *Microcystis* may release harmful levels of toxic microcystins (Rohrlack et al., 2001). Although *Daphnia* has been shown to resist toxicity and suppress cyanobacteria (Chislock et al., 2013), chronic exposure may prohibit the development of substantial populations in natural systems (Dao et al., 2010). Prevalence of small zooplankton and cyanobacteria is common in eutrophic waters in warmer regions (Romo et al., 2004; Domis et al., 2013), and in this study limited any potential for grazing pressure on phytoplankton growth.

### ***Macroinvertebrates***

The creation of submerged macrophyte meadows substantially increased macroinvertebrate abundance and richness compared with open water controls. This result is not surprising, with vegetated aquatic systems known to support much greater biomass and diversity of invertebrates (Dvorak and Best, 1982; Heck and Crowder, 1991). However, while fish communities within macrophytes may limit macroinvertebrate populations in some shallow subtropical systems (Bruce et al., 2012), this was not observed in our study. Plant structure and associated increased surface area and niche diversity may offer greater potential for predator avoidance than open water with bare substrata (Diehl and Kornijów, 1998). Additional primary food sources (the macrophytes themselves, epiphytic algae, and decomposing material from these sources) (Kornijów et al., 1995) within

macrophyte stands doubtless also had a positive effect on macroinvertebrates. Correlation of macroinvertebrate assemblages with chlorophyll *a* in controls and the absence of this correlation in macrophyte plots may reflect alternative food sources provided by macrophytes. Organisms consuming these varied food sources in turn support a more complex food web within macrophyte stands (Dvorak and Best, 1982). Gastropods were a major component of the macroinvertebrate community within meadows from an early stage. They probably ate algal epiphytes and may have controlled epiphyte biomass on macrophyte leaves (Jones and Sayer, 2003). Many studies have shown the important influence of submerged macrophytes on macroinvertebrates, a phenomenon now evident in newly planted stands, even under poor water quality. The restored plants attracted families that were absent from control plots, indicating the restoration of biodiversity (and its supporting ecosystem processes) that were otherwise absent from this degraded system. Protection by cages from predacious waterbirds may have contributed to increased abundances of macroinvertebrates, although this was confounded because macrophytes could not be established without protection. However, submerged macrophytes are well-known to support increased abundance and diversity of macroinvertebrates compared with open water (Dvorak and Best, 1982; Shupryt and Stelzer, 2009), and long-term research in the nearby Vasse-Wonnerup Wetlands has also found extremely high densities of macroinvertebrates amongst aquatic vegetation (Chambers et al., 2013) despite very high concurrent waterbird densities (Lane et al., 2007), so plants would likely have supported an abundant and diverse macroinvertebrate community, regardless of bird predation.

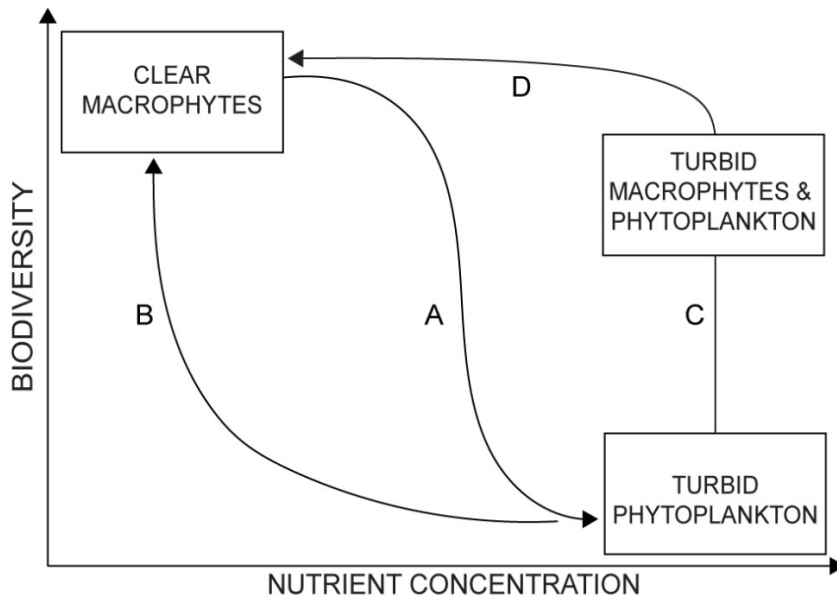
### ***Future use of submerged macrophytes in restoration***

While the feasibility of growing submerged macrophytes in turbid, eutrophic conditions was demonstrated in this study, a corresponding improvement in water quality was not. Rather, an alternative turbid regime with co-dominance of macrophytes and phytoplankton occurred, which supported increased richness and abundance of aquatic macroinvertebrates. Increased native biodiversity is often a primary goal in the restoration of aquatic systems, so inclusion of submerged macrophytes should be considered as a component in their recovery. At the scale of this experiment, water quality was not improved over that observed in control plots,

but the effects of larger macrophyte beds on reach-scale water quality over a longer period is worth investigation. Although potential for top-down control of phytoplankton through this approach appears limited in this mediterranean-climate system, it may be an advantage if applied in cool-temperate shallow lakes. In addition, submerged macrophytes do have potential to maintain water clarity through other mechanisms regardless of climate (Scheffer and van Ness, 2007; Vanderstukken et al., 2011), and particularly in warmer systems given their potential occurrence throughout the year (Beklioglu et al., 2007). Therefore their transplantation in some degraded systems may provide improved water quality outcomes.

While ongoing reduction of nutrient inputs is a priority for management, inclusion of this approach presents an alternative pathway for restoration of shallow, eutrophic waters (Figure 5.4). Nutrient enrichment is responsible for degradation (Figure 5.4 - A), however reduction of nutrient inputs can be difficult and restoration of water clarity and biodiversity hindered by feedback mechanisms associated with the phytoplankton-dominated regime (Figure 5.4 – B). Protected transplants of macrophyte species tolerant of low light availability (Figure 5.4 – C) can improve biodiversity in a relatively short period, and potential beneficial mechanisms of macrophytes (sedimentation and nutrient uptake) may support longer term actions to reduce nutrient concentrations (Figure 5.4 – D).

Attachment of transplants to steel mesh was a simple, low-cost and effective method of establishment within loose, flocculent sediments. However, future implementation of this approach requires long-term protection from grazers to maintain biodiversity and increase the potential for improved water quality. Thus there must be consideration of structural endurance and potential vulnerability during flood events; and public acceptance of the ongoing presence of cages would be necessary. Where limited options for restoration are available, this approach may be useful to establish a regime of co-dominance as an important step in the restoration pathway towards a clear, macrophyte-dominated system.



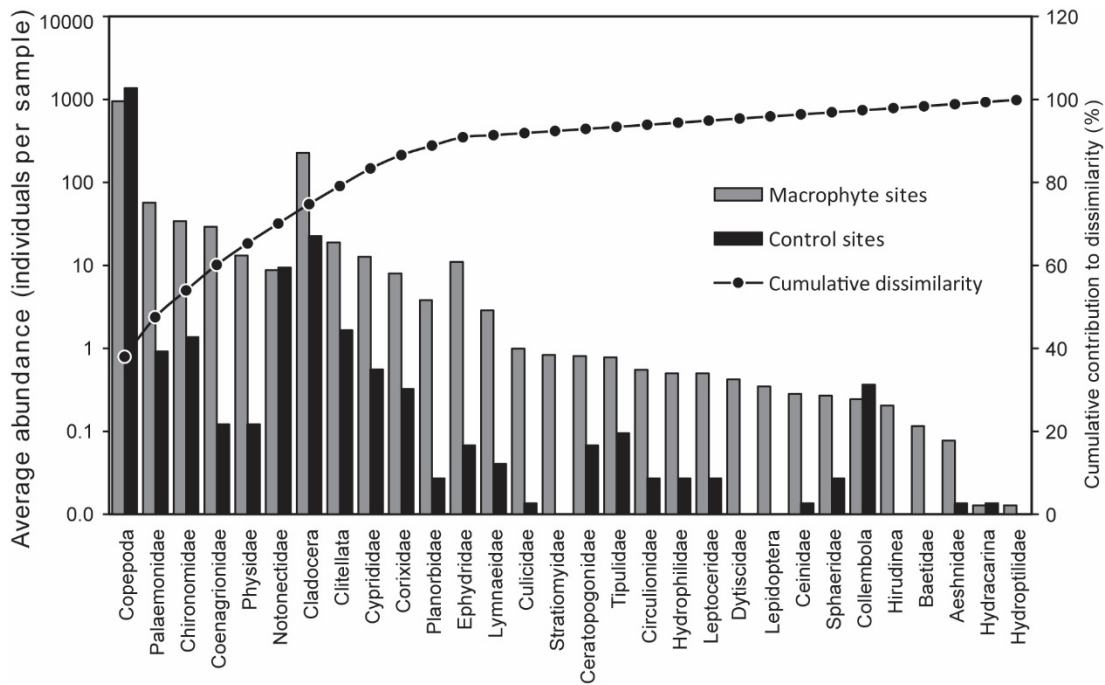
**Figure 5.4** Potential pathways of degradation (A) and restoration (B, C) in shallow lentic systems associated with nutrient enrichment. Submerged macrophytes withstand nutrient enrichment until a threshold is reached beyond which plants and associated diverse invertebrate community are lost (A), with the end point a turbid, phytoplankton dominated regime with low biodiversity. Restoration via nutrient reduction alone has a lengthy transition pathway due to feedback mechanisms within the turbid regime (B). Protected macrophyte transplantation (C) can achieve co-dominance of macrophytes and phytoplankton with increased biodiversity despite high nutrient concentrations. Over a longer period, potential beneficial nutrient cycling processes together with nutrient loading reduction facilitate establishment of a clear, macrophyte-dominated regime (D). The restoration pathway C, D presents a faster recovery than B, and achieves biodiversity outcomes in the short term.



Appendix 5.1 cont.

Turbidity	Sample date	5,5	334.2	<0.001	M/C	0.74 (0.01)	0.74 0.03	0.67 0.05	1.32 0.07	1.22 0.07	2.02 0.06
	Date x plot type	5,40	1.50	0.357	M	0.71 (0.02)	0.73 0.05	0.67 0.06	1.42 0.09	1.40 0.10	2.06 0.09
					C	0.76 (0.02)	0.76 0.05	0.67 0.06	1.23 0.09	1.04 0.10	1.99 0.09
	Plot type	1,8	8.98	0.017	M	1.16 0.02					
					C	1.08 0.02					
Dissolved oxygen (surface)	Sample date	5,5	4.58	0.083	M/C	0.66 (0.02)	0.86 0.05	0.81 0.07	0.97 0.08	0.98 0.08	0.98 0.06
	Date x plot type	5,40	4.49	0.085	M	0.65 (0.02)	0.86 0.07	0.82 0.07	0.98 0.10	0.84 0.12	0.76 0.09
					C	0.66 (0.02)	0.86 0.07	0.80 0.07	1.02 0.10	1.11 0.12	1.20 0.09
	Plot type	1,8	2.70	0.139	M	0.82 0.05					
					C	0.94 0.05					
Dissolved oxygen (bottom)	Sample date	5,5	41.65	0.002	M/C	0.62 (0.02)	0.79 0.05	0.71 0.06	0.76 0.10	1.09 0.04	0.93 0.05
	Date x plot type	5,40	20.50	0.006	M	0.63 (0.02)	0.80 0.07	0.76 0.08	0.55 0.14	1.10 0.06	0.66 0.07
					C	0.62 (0.02)	0.77 0.07	0.67 0.08	0.97 0.14	1.08 0.06	0.19 0.07
	Plot type	1,8	3.13	0.115	M	0.75 0.05					
					C	0.88 0.05					
Cladocera abundance	Sample date	5,5	2.62	0.186	M/C	0.21 (0.03)	0.20 (0.10)	0.61 (0.16)	0.17 (0.09)	0.05 (0.05)	0.90 (0.46)
	Date x plot type	5,40	2.48	0.199	M	0.35 (0.05)	0.32 (0.14)	0.46 (0.22)	0.23 (0.13)	0.00 (0.07)	0.74 (0.64)
					C	0.08 (0.05)	0.07 (0.14)	0.76 (0.22)	0.10 (0.13)	0.10 (0.07)	1.05 (0.64)
	Plot type	1,8	0.003	0.958	M	0.35 (0.12)					
					C	0.36 (0.12)					
Copepoda abundance	Sample date	5,5	7.35	<0.001	M/C	3.16 (0.31)	2.04 (0.22)	2.83 (0.14)	2.62 (0.25)	1.34 (0.37)	1.58 (0.45)
	Date x plot type	5,40	0.97	0.448	M	3.34 (0.44)	2.28 (0.31)	3.08 (0.20)	2.48 (0.36)	1.31 (0.53)	1.15 (0.64)
					C	2.98 (0.44)	1.18 (0.31)	2.58 (0.20)	2.75 (0.36)	1.38 (0.53)	2.00 (0.64)
	Plot type	1,8	0.003	0.955	M	2.27 (0.27)					
					C	2.25 (0.27)					
Macro. abundance	Sample date	5,5	18.23	<0.001	M/C	0.79(0.13)	1.05 (0.12)	1.25 (0.07)	1.78 (0.10)	2.00 (0.10)	1.70 (0.10)
	Date x plot type	5,40	4.35	0.003	M	0.91 (0.18)	1.37 (0.17)	1.95 (0.09)	2.48 (0.14)	2.51 (0.14)	2.26 (0.15)
					C	0.67 (0.18)	0.73 (0.17)	0.56 (0.09)	1.07 (0.14)	1.48 (0.14)	1.17 (0.15)
	Plot type	1,8	213.34	<0.001	M	1.91 (0.05)					
					C	0.94 (0.05)					
Invertebrate richness	Sample date	5,4	15.63	<0.001	M/C	2.00 (0.31)	3.13 (0.35)	4.10 (0.29)	6.05 (0.30)	6.37 (0.27)	5.90 (0.92)
	Date x plot type	5,4	8.48	<0.001	M	2.25 (0.43)	4.07 (0.50)	5.93 (0.41)	9.20 (0.43)	10.13 (0.39)	8.13 (1.29)
					C	1.75 (0.43)	2.20 (0.50)	2.27 (0.41)	2.90 (0.43)	2.60 (0.39)	3.67 (1.29)
	Plot type	1,8	88.38	<0.001	M	6.62 (0.30)					
					C	2.56 (0.30)					

**Appendix 5.2** Dissimilarity of aquatic invertebrate assemblages in macrophyte and control plots showing comparison of abundance of all taxa in macrophyte and control plots (bars) and cumulative percentage contribution to dissimilarity between plot types (line).





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## Chapter 6. General Discussion

Submerged macrophytes in seasonally-flowing streams provide important ecosystem functions supporting biodiversity, as they do in many aquatic ecosystems, and their presence in these streams is associated with a combination of processes known from both lotic and lentic environments. Submerged macrophytes were relatively common in these streams, and supported a more diverse aquatic fauna regardless of whether they were remnant populations, populations that had colonised degraded reaches, or transplanted meadows. The plants clearly provided structural habitat for aquatic fauna, and supported stream food webs both directly and indirectly as substrate for epiphytes. Although not demonstrated directly in this study, submerged macrophytes have the potential to stabilise sediments and influence nutrient cycling, contributing to improved water quality. This chapter provides a detailed synthesis of the findings of this research, in terms of the three main research objectives (summarised in Table 6.1):

- (iv) the drivers of submerged macrophyte distribution and relationships between macrophytes and environmental factors;
- (v) their contribution to ecosystem processes; and
- (vi) their capacity to improve biodiversity and water quality in degraded reaches.

This is followed by discussion of the implications of the research for conservation and restoration of stream environments.

## **Drivers of submerged macrophytes distribution in seasonally-flowing agricultural streams.**

### ***Primary determinants of distribution***

In contrast to perennial streams, flow velocity was not a primary factor determining macrophyte distribution in these seasonal streams. Rather, adequate duration of flow and disconnected, lentic summer pools, to allow sufficient time for growth, was required for macrophyte presence. While physical effects of high velocity often limit macrophyte establishment in perennial streams (Riis and Biggs 2003, Bornette et al. 2008, Franklin et al. 2008), this is less important in smaller lowland seasonal streams, where growth occurs outside times of peak streamflow. Riparian shading is also known to limit distribution and biomass of macrophytes, but while this is a direct relationship in perennial systems (Canfield and Hoyer 1988, Julian et al. 2011), this study shows that increased growth due to light availability is mediated by periodic drying in seasonal streams. Distinct distributions of the two macrophyte assemblages in relation to light was also evident, showing how variation in traits amongst different species enables successful growth in both high light availability (resulting from cleared and degraded riparian vegetation), and low light availability (caused by shading and phytoplankton blooms). Occurrence of *Cycnogeton* spp. in areas of riparian shade shows that this factor does not always limit growth and suggests potential for widespread distribution of this species in naturally vegetated streams in the study area, and indeed it was common amongst the few reaches with intact riparian vegetation. Its flexible strap-leaf morphology is also resistant to high velocity conditions (O'Hare et al. 2007), which were historically more common, and the current distribution probably reflects remnant populations. As a consequence of the seasonal flow conditions in these systems, macrophyte establishment is influenced by a combination of factors known from lotic and lentic environments. This has implications for their growth response to altered conditions arising from land use change, and in their potential establishment for stream restoration.

### ***Effects of stream degradation on distribution***

Reduced streamflow was evident from historical streamflow data (Figure 2.2) as a result of regionally declining rainfall, drainage modifications, impoundment and abstraction. Extensive sediment deposition was also evident in these streams, typical of lowland agricultural streams (Gurnell et al. 2006), and exacerbated by erosion and sediment mobilisation in unstable channels. While low flows and fine sediments provide stable conditions that can enhance macrophyte growth (O'Hare et al. 2007, Suren and Riis 2010), and supported *Potamogeton/Ottelia* colonisation in degraded reaches in the study area, it is also likely to exacerbate seasonal drying. A reduction in the flow period or duration of summer pools, beyond that required for successful completion of their annual life cycle, would clearly limit macrophyte establishment.

In contrast to the drying effects of declining flows, impoundment of streams can limit natural sediment consolidation during seasonal drying (Davis et al. 2010). Excessive build-up of sediments with high organic matter content in larger, more permanent, stream pools and impoundments can limit root development and provide poor substrate for anchorage (Raun et al. 2010). Furthermore, flocculent organic material provides nutrients for phytoplankton. Both these negative effects of excessive organic sediments were evident in the Vasse River site. While permanent presence of water is advantageous for macrophyte growth, unconsolidated sediments arising from this permanence are restrictive. Impoundments for abstraction purposes are common in mediterranean-climate regions (Kondolf et al. 2013), and contrasting effects of water permanence and organic sediment loads will both influence macrophyte growth.

The overriding limitation of the hydrological regime also mediates growth response to increased light availability. A positive response to reduced riparian shading was only found for the *Potamogeton/Ottelia* assemblage, which appeared to colonise open reaches with high light availability. Other Australian research has associated several *Potamogeton* species with low riparian canopy cover (Mackay et al. 2003, Chessman and Royal 2010). While reduced canopy increases macrophyte biomass in perennial systems (Julian et al. 2011), this is restricted where seasonal flows limit time available for expansion of the plant bed and is likely to be a key factor

preventing excessive growth in seasonal streams. While the *Cycnogeton* spp. assemblage has potential for enhanced growth from reduced shade in degraded streams, it is unlikely to respond positively if this is associated with increased drying.

Although nutrient enrichment is sometimes correlated with macrophyte growth (Chambers 1987, Mebane 2014), no association was found in the study area, concurring with other Australian studies (Chessman and Royal 2010; Mackay et al. 2010). Conversely, conditions of nutrient enrichment, combined with periodic low flow and pooling, are conducive to enhanced growth of phytoplankton and epiphyte growth (Hilton et al. 2006), which can negatively affect macrophyte growth (Jupp and Spence, 1977, Phillips et al., 1978). These conditions may occur widely in mediterranean-climate catchments, particularly in impoundments, although in shallow reaches light availability may not be limited by high turbidity (Bucak et al. 2012). The restoration trial study area was an example of the negative effects of high nutrient concentrations, where high turbidity owing to phytoplankton growth appeared to inhibit macrophyte presence. This is a key example of the application of macrophyte ecology in lentic waters to seasonal streams.

In lentic environments within seasonal streams, nutrient enrichment and the establishment of a turbid-phytoplankton dominated regime can cause macrophyte loss, but pressures from waterbird disturbance, impacts of exotic fish species, and invasive plant species may also contribute. It is difficult to know whether these factors act as causal mechanisms for the loss of macrophytes, or as feedback mechanisms preventing re-establishment. Growth of *P. crispus* in exclosures during the restoration trial and destruction of plant following cage removal clearly showed disturbance by waterbirds as an additional pressure limiting establishment of macrophytes (although perhaps *P. crispus* species does grow in this system but we cannot see it in the turbid waters). Seasonal pools and impoundments may provide dry-season refuge habitat for birds, which may potentially limit macrophyte distribution. This impact would be exacerbated in degraded catchments by increased palatability of plants in nutrient-enriched conditions (Marklund et al., 2002, Bakker et al., 2014) and possibly by contraction of available refuge habitat. The presence of feral goldfish in the Vasse River also has potential to adversely affect macrophyte growth through uprooting and bioturbation resulting from their

benthic foraging (Morgan and Beatty 2007). Carp were observed in a pool within an agricultural drain (pers. obs). The prevalence of these fish is unknown in this catchment, however carp are well-known to devastate aquatic vegetation (Crivelli 1983). Certainly these particular individuals would have died when this pool dried out, had I not removed them, but even their occasional presence in a pool or impoundment may have serious impacts of macrophytes. Invasion by exotic macrophytes was excluded from this study and, while not associated with macrophyte distribution in the surveyed area, would doubtless impact on macrophyte communities elsewhere.

The presence of complex interacting factors (Chapter 3, Fig. 3.1) makes it difficult to determine the response of macrophyte communities to changed conditions in degraded waters. I have shown that the pattern of flow predominantly affects the capacity of plants to sustain a presence in seasonal streams. Periodic drying limits the potential for successful growth and reproduction, and low flow conditions increases the potential effect of algal growth. Light availability to macrophytes is often dependent on shading in lotic waters, and turbidity in lentic waters, but there is potential for both shading and turbidity to affect macrophytes in seasonally-flowing streams, which represent a combination of these environments. Substrate characteristics and interactions with birds, fish (and in some systems terrestrial grazers) and exotic plants are also potentially influential factors which were not included in the conceptual model (Figure 3.1). It is important to recognise the application of existing studies from both lotic and lentic systems to seasonally-flowing streams, where little research has been done. Further studies of biotic interactions in these systems would be valuable, particularly given the variable results often reported for temperate systems.

### ***Feasibility of establishing macrophytes for restoration***

Where suitable conditions for macrophyte establishment occur, their presence may be limited by lack of propagation material owing to limited mechanisms for dispersal other than hydrochory (Nillson et al. 2010), and loss of upstream plant populations (Riis and Sand-Jensen 2001, Riis et al. 2009). Altered stream conditions present new opportunities for colonisation by different species, as found for *Potamogeton/Ottelia*. This may also be the case for *Cycnogeton* and other native

macrophyte species not found in the survey, but is restricted by a lack of propagule dispersal into these locations. Where natural colonisation pathways are limited, transplantation could be used to facilitate recolonisation. Transplantation can be used to restore macrophyte populations (Riis et al. 2009), although very few examples exist in practice for lentic systems (Riis et al., 2009, Larned et al. 2006).

The variety of traits found amongst submerged macrophyte species present options for their use in restoration under a range of conditions, as found here in relation to shading and turbidity (Table 6.1). Riis et al. (2009) provided some recommendations for macrophyte transplantation, suggesting their use in relatively shallow (< 1 m), unshaded sites with flow velocity less than  $0.4 \text{ ms}^{-1}$ . However, morphological variation among species, and morphological plasticity within species may allow transplantation to a broader range of sites with conditions outside these limits. For example, some species would be more suited to shaded sites, such as *Cycnogeton* spp. Its morphology is also resistant to high flow rates, and while maximum base flow rates in this study were less than  $0.4 \text{ ms}^{-1}$ , this assemblage clearly withstands higher velocities during episodic rainfall events. In contrast, *Potamogeton* and/or *Ottelia ovalifolia* could be used in shallow sites exposed to direct sunlight. Successful establishment requires understanding of autecology to determine appropriate species for a given location (Barrat-Segretain 1996), with surveys such as that undertaken here helpful in achieving this.

Turbidity is primary barrier to establishment of submerged macrophytes in eutrophic environments, both in lentic systems and in impoundments and slow-flowing streams and rivers in agricultural catchments (Hilton et al. 2006). Shallow lakes are the most common systems for which macrophyte establishment is a restoration goal. This is generally achieved through recolonisation following improvement in water clarity (Sondergaard et al., 2007). However increasing water clarity can be difficult, particularly when nutrient sources are diffuse and biomanipulation is not an option. Turbid conditions can be overcome by using macrophyte species adapted to low light, as was observed for *Vallisneria australis* in turbid waters during the restoration trial, and providing protection from waterbirds. *Cycnogeton* spp. have a similar growth form, which facilitates growth in conditions of higher turbidity as well as shade (Middelboe and Markager 1997, Blanch et al. 1998). While growth of the *Potamogeton/Ottelia* assemblage was

limited by shade, traits such as surface canopy formation (*P. ochreatus*) and floating leaves (*P. drummondii* and *O. ovalifolia*) would assist tolerance of turbidity (Chambers 1987), as observed for *Potamogeton crispus* in the restoration trial site. The trial also demonstrated a method which overcomes anchorage problems in flocculent sediments, common in eutrophic waters. In addition, the attachment to steel mesh outside the water and subsequent placement makes this approach suitable for use at depths where physical planting is logistically difficult.

Prevailing conditions in degraded reaches may be suitable for establishing species which did not originally grow at a particular site, but which could provide reach-scale ecological benefits. This was seen in both the colonisation of artificial drains by *Potamogeton/Ottelia*, and in the use of the naturalised *V. australis* in the lower Vasse River restoration trial. There is potential for translocation of macrophyte species outside their historical distribution range into now-suitable degraded reaches to achieve ecological objectives. This idea of assisted colonisation is being increasingly considered within the context of altered future distribution patterns resulting from climate change (Hoegh-Guldberg et al. 2008, Webber and Scott 2012), mainly for fauna populations (eg. Olden et al 2011). Obviously, species selection for restoration is fundamental and translocation to new areas must be cautious (Seddon 2010). Although non-native aquatic plants have capacity to provide ecological functions (Strayer et al. 2003, Theel et al. 2008, Schlaepfer et al. 2011), their use is not justified where there is potential for excessive growth and associated adverse ecological effects, the risk of which may be greater in systems with increased availability of light and nutrients (Canfield et al. 1988, King and Buckney 2000, Hastwell et al. 2008, Mebane et al. 2014). However, appropriate species for an altered landscape may not be limited only to known locally-occurring native species (Riis et al. 2009). While distinct geographical translocations present higher invasion risk (Hastwell et al. 2008, Quinn et al. 2011), species from within the same broad regional distribution may be considered. In addition, locally-native species from different environments may be suitable and present a low risk: for example, restoration in seasonal pools and impoundments of seasonally-flowing systems using species from downstream or adjacent lentic environments.

## **Contribution of submerged macrophytes to ecosystem processes in seasonally-flowing, agricultural streams.**

### ***Abiotic interactions***

As known for a range of aquatic systems, this research showed a positive effect of submerged macrophytes on aquatic fauna (Table 6.1). The survey results suggested higher invertebrate richness in reaches with macrophytes was due to the capacity of plant stands to support more individuals, indicating importance as physical habitat. This function is not surprising, as structural habitat within macrophytes is well-known to increase niche heterogeneity and availability (Downing 1991, Warfe et al. 2008, Strayer and Malcolm 2007) and provide refuge from flow and predation (Heck and Crowder 1991, Warfe and Barmuta 2004). Greater abundance for several taxa in the presence macrophytes reflected their dependence on plants for aspects of their life history (e.g. damselfly nymphs hatching from endophytic eggs). In some degraded reaches in the study area, macrophytes are likely to have been the dominant structural habitat present. In the Geographe Bay catchment, an extensive drainage network has resulted in many artificial watercourses and channelisation and clearing of natural streams. The *Potamogeton/Ottelia* assemblage has clearly colonised some of these reaches, providing habitat and resources in an environment which would otherwise have limited capacity to support aquatic fauna.

The effect of plant presence was more profound in the restoration trial, where controls provided negligible habitat and few resources. This demonstrated that newly established plant beds have potential to provide significant habitat in a short period of time, with rapid colonisation of the newly planted stands by a diverse invertebrate assemblage, despite extremely poor water quality. Systems with a turbid, phytoplankton-dominated regime have little ecological value especially where dominated by toxic cyanobacteria. Previously the effect of macrophytes in such conditions has not been tested because it is generally assumed that they cannot be grown. However, when macrophytes are established in these waters, a regime of co-dominance was shown to be beneficial to aquatic fauna.

In addition to direct effects on habitat, submerged macrophytes interact with other ecosystem processes. Interactions with physical factors conditions of flow and substrate are common in perennial streams (Sand-Jensen 1998; Franklin et al. 2008)



and likely to act similarly during the flow period in seasonal streams. Trapping of particulate material in transplanted plant beds indicated potential for sedimentation, and the association of plant presence with fine sediments in the survey may indicate both colonisation of these sediments and accumulation owing to localised effects of macrophytes on flow. Differences in oxygen and nutrient concentrations in transplanted meadows compared with open water suggests an effect of the plants, as has been found in shallow lentic systems (Wigand et al. 1997; Barko and James 1998). Substantially higher grazer abundance and overall greater invertebrate richness and abundance also suggests that macrophytes may influence trophic relationships. So, although no water quality improvement was associated with transplanted macrophytes in this research, their capacity to influence these ecosystem processes was evident. Enhanced biodiversity was found despite poor water quality, and may indicate improved conditions for aquatic fauna.

As the native macrophyte assemblages found in these agricultural streams did not grow excessively in response to increased light and nutrients (Table 6.1), there was no apparent risk to stream condition from excessive accumulation of organic matter (Bunn et al. 1999, Stiers et al. 2011, Schultz and Dibble 2012). Non-native macrophyte species present a greater risk of invasion (Hastwell et al. 2008, Quinn et al. 2011), although in streams and rivers with low levels of disturbance (i.e. where seasonal drying or flooding do not limit macrophyte growth), there is potential for a positive growth response to increased resources by any macrophyte species (Riis and Biggs 2001). The use of a naturalised rather than a native species in the restoration trial did result in rapid dense growth of plants, and although there was some indication of lower oxygen levels in plant beds at high densities, this did not appear to negatively affect aquatic fauna.

### ***Trophic interactions***

The top-down effects on phytoplankton described in classic theory of alternative regimes (Moss et al. 1990, Scheffer et al. 1993) were not apparent in this research, with no difference in abundance of grazing zooplankton in response to macrophyte presence. However, there was evidence of functional response of the invertebrate community, with macrophytes supporting a higher abundance of grazing taxa in both the survey and the restoration trial. A substantial increase in grazer abundance in transplanted macrophyte meadows suggests a direct contribution of macrophytes and/or epiphytes to the food web, supporting higher abundance and richness of other macroinvertebrates.

The stable isotope study adds support to other recent Australian studies demonstrating the contribution of macrophytes as a basal resource for stream food webs (Reid et al. 2008, Watson and Barmuta 2011). While the present study confirms current theory that detritus and algae are key resources (Finlay 2001, Power 2013), it contradicts assertions that macrophytes are an unpalatable food source (Bunn et al. 1999). During their peak seasonal occurrence, macrophytes and associated epiphytes were assimilated by a range of primary and secondary consumers, including native fish and freshwater crayfish, reflecting opportunistic diets. Opportunism may be an important adaptation in seasonally variable systems (Blanchette et al. 2014) and provide resilience in altered landscapes (Chapter 4). Macrophytes and epiphytes have a much lower C:N ratio than terrestrial detritus (Deegan and Ganf 2008; this study), which is more similar to that of consumers, and these in-stream resources therefore provide a more nutritious food resource (Elser et al. 2000). High availability of this nutritious food can support opportunistic consumers during the critical period when streams are drying out and aquatic macroinvertebrates prepare to metamorphose, reproduce, or form desiccation-resistant life stages. Furthermore, greater assimilation in reaches with degraded riparian vegetation (Table 6.1) highlights their importance as an alternative food source when allochthonous detritus is limited.

## **The usefulness of stable isotope analyses (SIA) in freshwater ecology**

The stable isotope study in this thesis showed many of the potential pitfalls of using SIA in investigating stream food webs, and leads one to question whether SIA is useful and appropriate in these systems. However, SIA has been used successfully in many studies, leading to advances in understanding stream ecology. For example, the relative importance of autochthonous versus allochthonous carbon contributions to food webs has long been debated. The shift from allochthonous-driven food webs in forested headwaters to autochthonous sources in lowland reaches (Finlay 2001) has been challenged by showing significance of algal carbon sources in headwaters (Brito et al. 2006; Delong and Thorp 2006) and conversely, the potential importance of terrestrial carbon in lowland streams (Reid et al. 2008). However, the inconsistency of conclusions from SIA studies also highlights strong variation in food webs at regional and global scales (Dudgeon et al. 2010) and it is clear that greater importance of one is never at the exclusion of the other. Furthermore, these studies have ignored other potential basal resources.

The development of modern mixing models, allowing inclusion of additional sources, such as macrophytes (e. g. Reid et al. 2008; Watson and Barmuta 2011; Chapter 4), has produced studies showing potential for assimilation of a mixture of basal resources. Opportunistic, flexible diets are common for many stream consumers across a range of climate types: Mediterranean (Chapter 4), northern temperate (England and Rosemond 2004; Leberfinger et al. 2011), and wet-dry tropics (Leigh et al. 2010; Blanchette et al. 2014). Although the pictures derived from SIA are not always clear-cut, this may reflect the reality of dietary flexibility for many freshwater animals. The nature (e. g. seasonal availability) and extent (e. g. different taxa) of this opportunism deserves further research to provide insights into ecosystem responses to changing landscapes and climate. Although SIA will likely continue as a useful tool for this research, its application will continue to be challenging in lotic waters. We must ensure that conclusions of flexible diets are real and not an inevitable result of confounding factors implicit in these studies.

Best practice for implementation of mixing models (Phillips et al. 2014) and ongoing development of methods that further separate isotopic signatures are valuable for future research. The use of additional isotopes, such as sulfur ( $\delta^{34}\text{S}$ )

and hydrogen ( $\delta^2\text{H}$ ) (Peterson and Fry, 1987; Cole et al. 2011) and isotope tracers may resolve problems of overlapping signatures (Hall et al. 2000; Dodds et al. 2014; Middelburg et al. 2014). The use of surrogate primary consumers to address variation in primary resources can be useful (Anderson and Cabana 2007) but is not always practical (Jardine et al. 2014), and it may be more valuable to use correlation over large spatial scales (Rasmussen 2010; Jardine et al. 2014). Greater understanding of metabolic turnover rates to improve synchronisation of the signatures of sources and consumers in isotope studies (Anderson and Cabana 2007; Jardine et al. 2014) are also valuable.

Given the complexity of using SIA in stream food web studies, the wide variation in reported results and the likelihood of dietary opportunism, it is reasonable to question the role of these analyses in future research. Future application will benefit from the hindsight of existing research and ongoing development of improved methods. Ideally, pilot analyses to predict source variability and overlap, and to ensure that all potential sources are included (i. e. that consumers fall within the isospace of sources, Phillips et al. 2014), will inform researchers whether SIA will answer their hypotheses prior to large investment (Fry 2006). This is often not practical within funding and time constraints, but targeted effort to understand isotopic variability is essential for SIA studies. If this cannot be adequately included, then there is a significant risk that the investment in SIA will be wasted. Complementary studies, such as gut content analysis and feeding trials, will continue to be valuable components of food web studies research, because they can provide additional evidence that strengthens conclusions arising from SIA. Researchers are becoming more aware that SIA is only one tool for understanding resource use in stream ecosystems, and it is clear that we must use *a healthy dose of realism* (Jardine et al. 2014) in its application and interpretation.

## **Capacity for submerged macrophytes to improve biodiversity and water quality in degraded waters.**

### ***Biodiversity***

Existing knowledge demonstrating that submerged macrophytes support increased biodiversity of aquatic fauna, through provision of habitat and food resources also applies to seasonally-flowing streams (Table 6.1). While accepted for many perennial streams in which macrophytes are common (Phillips 2003, Shupryt and Stelzer 2009), this is now also evident for plant beds colonising degraded reaches of seasonally-flowing streams and for transplanted meadows, which have potential to compensate for lost natural submerged habitat and food resources.

The use of invertebrate abundance and taxa richness as measures of biodiversity is somewhat limited (particularly family-level richness used here), because it does not incorporate species-level sensitivities (Palmer et al. 2014). Macrophytes may simply support greater abundance of tolerant, opportunistic taxa with generalist diets, allowing them to thrive in degraded reaches. It is acknowledged that further identification of invertebrate taxa may have provided more insight into biodiversity supported by macrophytes, both existing and in response to restoration. Family-level (and lower) taxonomy used here was adequate for the research aims of the thesis, however species level data would have enabled better assessment of endemism of the invertebrate communities.

High endemism of aquatic fauna in south-western Australia is attributed to low levels of in-stream primary production (Davies and Stewart 2013), and it may be argued that a shift to an invertebrate community supported by macrophytes and epiphytes represents altered ecosystem processes characteristic of poor stream health (Bunn et al. 1999). However, this assumes that submerged macrophytes were rare in uncleared lowland streams of the region. Unfortunately, there are now relatively few uncleared lowland streams in the Geographe Bay catchment to show whether macrophytes were common there or not. Although present in some reaches with good riparian vegetation, there was no opportunity to sample uncleared lowland streams within native forest in the present study because these conditions were not encountered. So the role of native macrophytes in streams in very good

condition remains unknown, and further research is needed across the region more broadly to establish a baseline condition for such streams.

### ***Water quality***

In terms of water quality, potential benefits were not obvious (Table 6.1). This research did not assess potential assimilation of nutrients by macrophyte beds within streams, and did not find water quality improvement at a local scale within transplanted beds. Nonetheless, research in some perennial rivers does show that water quality may be improved in streams through increased retention of particulate organic matter and increased uptake of nutrients (Svendsen and Kronvang 1993, Clarke 2002, Julian 2011). The opposite scenario has been demonstrated, where substantial deterioration of water quality has occurred following macrophyte removal (Bicudo et al. 2007). Furthermore, macrophytes are well-known to promote water clarity in shallow lentic systems (Scheffer et al. 1993; Davis et al. 2010) through sedimentation, reduced resuspension and nutrient uptake (Van Donk and van de Bund, 2002) and enhancing phytoplankton grazing through providing refuge for herbivorous zooplankton (Timms and Moss, 1984). Trapping of particulates was observed in the restoration trial, but net improvement in water quality was limited by the extremely degraded nature of the site (eg. substantial internal nutrient load; dominance of unpalatable cyanobacteria), the scale of the experiment and the short time-frame (one season only). The potential remains for these beneficial functions to occur in restored macrophyte beds in lentic environments within seasonal systems. Further research involving greater coverage and a longer time period would inform whether this is a worthwhile approach to water quality improvement, and if protection from bird disturbance needs to remain indefinitely (indeed, the provision of food for higher order consumers would be a positive outcome).

This role is relevant to spring and summer pools in seasonal systems, however, due to connectivity with in the flow season, potential undesirable effects of sedimentation in macrophyte beds include decreased channel capacity and intermittent export of accumulated nutrients in plant beds. In this impoundment the presence of macrophytes is likely to reduce the risk of downstream transport nutrient-rich sediments, which are currently unstable. Many stream and drainage channels in agricultural catchments are unstable and have eroded beyond their

natural capacity (and in this region, declining rainfall has reduced necessary capacity), with a mobile sediment load. Restoration efforts which slow the transport of sediment and nutrients would be favourable in these conditions, including the establishment of macrophyte beds.

## **The role of submerged macrophytes in conservation and restoration in agricultural catchments**

Agricultural development in the Geographe Bay catchment has substantially altered its rivers at landscape and reach scales. There are limited areas of intact riparian vegetation remaining, and often these remnants are narrow, and floodplains inevitably include areas of farmland. Even within reaches classified as good-condition, these are influenced by broader impacts of altered flow regimes, sedimentation and nutrient enrichment. With no pre-settlement records of stream ecosystems, there is no historical reference state with which to compare current conditions. The streams in the study area are relatively small compared to temperate rivers in other parts of the world, and would have been heavily shaded, even in the lowland reaches. Macrophytes are typically more common in wider, well-lit lowland temperate rivers at a global scale (Bornette and Puijalon 2011), but the streams here also support macrophytes, with *Cyanogeton* spp. common in the few good-condition reaches remaining. The provision of habitat and food resources by existing submerged macrophytes suggests they are worthy of conservation in these streams, whether occurring as remnant populations or as colonising stands in degraded reaches.

This thesis shows that existing knowledge demonstrating that submerged macrophytes support increased biodiversity of aquatic fauna, through provision of habitat and food resources also applies to seasonally-flowing streams (Table 6.1). While accepted for many perennial streams in which macrophytes are common (Phillips 2003, Shupryt and Stelzer 2009), this is now also evident for plant beds colonising degraded reaches of seasonally-flowing streams and for transplanted meadows, which have potential to compensate for lost natural submerged habitat and food resources. Rapid seasonal growth of macrophytes means these biodiversity outcomes are achieved in a short time period. Furthermore, colonisation of mobile sediment deposits in streams may stabilise the stream bed and promote

sedimentation within plant stands, preventing transport to receiving waters (Lind et al. 2009).

Despite recognition of their ecological importance in many river systems, submerged macrophytes are rarely included in restoration projects. Perhaps this is due to their inconspicuous presence in some river systems, or perceptions that their establishment will cause problematic excessive growth. A recent global analysis of river restoration projects found the most common goals were biodiversity (33%), channel stability (22%), riparian habitat (18%), water quality (14%), and in-stream habitat (11%) (Palmer et al. 2014). The most commonly implemented restoration approaches to achieve these are physical channel modification to improve habitat heterogeneity and riparian vegetation rehabilitation (Palmer et al. 2014). Less common at the global scale, management of environmental flows is often important for restoration of river ecosystems (Arthington and Pusey 2003, Roni et al. 2008), particularly in warmer-climate regions where humans have greater reliance on water abstraction (Bernhardt and Palmer 2011, Kondolf et al. 2013). The analysis of Palmer et al. (2014) did not identify any projects for which establishment of submerged macrophyte communities was either an action or a goal for river restoration, nor have they been included in other assessments of river restoration outcomes (Roni et al. 2009, Feld et al. 2011). Yet, there is potential for their use to achieve all the restoration goals mentioned above (except riparian habitat).

The definition of successful restoration is strongly influenced by perspective (Palmer et al. 2014). Where the restoration goal is a return to pre-disturbance conditions, restoration of submerged plant communities is desirable for many systems where they have historically been widespread. Examples include cool temperate lakes (Moss 1990; Sondergaard et al. 2007); and many unshaded lowland streams with conspicuous aquatic plant communities that are recognised for maintaining aquatic fauna (Pedersen et al. 2007, Riis et al. 2009, Lorenz et al. 2012). For stream reaches that historically have been heavily shaded with low autochthonous production, establishing submerged macrophytes does not constitute a return to pre-degraded conditions, but may be an acceptable improvement in some situations. Restoration of pre-disturbance conditions is often prevented by catchment-scale pressures such as poor water quality and changes to hydrology and



connectivity, which prevent restoration of sensitive taxa (Hughes et al. 2005, Kail et al. 2012), regardless of the approach used.

In practice, restoration does not dictate that conditions of historical reference states are achieved, but that some improvement occurs (Aronson et al. 2014). Indeed, in the current state of global climate change, such reference states may not be appropriate (Davies 2010). Where there is acceptance of a recovery endpoint that differs from pre-disturbance conditions (Lake et al. 2007), acknowledging the limitations in some disturbed ecosystems, then the perspective changes to bringing some ecological value back to degraded waterways. In this 'remediation' approach to restoration, establishing submerged macrophytes may be useful in achieving functional ecosystems.

In poorly studied agricultural catchments, such as the area studied here, there may be no baseline for submerged macrophyte distribution, and their occurrence may be as remnant populations (e.g. *Cycnogeton*) or as colonists of degraded conditions (e.g. *Potamogeton/Ottelia*). Their role in supporting aquatic fauna in agricultural landscapes is important regardless of historical conditions, and although the faunal assemblage may differ to that originally found, it does potentially include species of high conservation value, such as fish. So, where restoration goals focus on biodiversity outcomes, these plants can provide important seasonal habitat and food resources. Where restoration goals focus on water quality, submerged macrophytes have an uncertain contribution in seasonal streams. Shallow lakes theory suggests positive outcomes for seasonally lentic reaches but the overriding aims of restoration may be protection of water quality in receiving waters, and further research would be needed to understand whether macrophyte beds in the catchment can contribute to this.

While loss of sensitive invertebrate taxa is important from a conservation viewpoint (Allan 2004), and notwithstanding the limitations in taxonomy used in the research here, an invertebrate community which differs from that of pre-disturbed conditions may nonetheless provide a food source for vertebrate predators which are of high conservation value. For example, native fish species are also mostly endemic to south-western Australia (Morgan et al. 2011). Macrophytes and epiphytes were consistently an important basal resource for fish in degraded streams in the study

area, and invertebrate taxa with high abundance in the presence of macrophytes were common prey items. The generalist, opportunistic diets shown by invertebrates and fish would provide resilience in these seasonal systems, where availability of food resources fluctuates naturally (Blanchette et al. 2014). Although not included in the stable isotope study, the potential of macrophytes as resource for waterbirds through supporting invertebrate prey was evident by the presence of diving birds at the conclusion of the restoration trial, when protective cages were removed. In degraded stream reaches, the presence of macrophytes may benefit higher-order predators regardless of an altered macroinvertebrate assemblage, by supporting high abundance of prey.

Rehabilitation of riparian zones is frequently implemented to achieve water quality and biodiversity objectives (Roni et al. 2008, Feld 2011, Palmer et al. 2014). It is important for bank stability (Allan 2004) and has been shown to increase sediment and nutrient retention for many systems (Correll 2005, Newbold et al. 2010), although the potential for nutrient assimilation appears limited in the deep sands characteristic of the Swan Coastal Plain (O'Toole 2014). Increased biodiversity (as measured by aquatic fauna indices) results from improved habitat and restoration of beneficial processes (Orzetti et al. 2010, Palmer et al. 2014). Interestingly, it has been suggested that impacts on submerged vegetation resulting from riparian zone revegetation and subsequent shading may have negative effects on aquatic fauna (Zeffernan 2014). Rehabilitation of riparian vegetation is very common in southwestern Australia, where it is considered vital for healthy stream metabolism (Bunn et al. 1999, Davies 2010), but there has been no evaluation of its effectiveness in achieving biodiversity objectives here. It is likely to take many years for riparian revegetation projects to achieve a pre-disturbance macroinvertebrate assemblage (Becker and Robson 2008, Orzetti et al. 2010), if indeed this is possible in the context of broader-scale degradation. There is great potential for submerged macrophytes as a complementary tool to achieve short-term biodiversity outcomes while riparian revegetation projects mature. If the barrier to use of these plants in restoration is concern over their proliferation, this may be overcome by using them in conjunction with riparian plantings (Bunn 1998, Zeffernan 2014), so that over time the important ecological processes reliant on riparian vegetation may become re-established, while the macrophytes provide interim short-term recovery.

## Conclusion

As has been found for a range of freshwater environments (Carpenter and Lodge 1986, Bornette and Pujalon 2011), this thesis demonstrates that submerged macrophytes can provide important ecosystem services in seasonally-flowing streams, and their growth in degraded reaches within an agricultural catchment can compensate for loss of natural habitat and food web resources (Table 6.1). Given their widely-accepted influence on physical, chemical and biological processes in both perennially-flowing and lentic systems, this is perhaps not surprising. However, they have previously received little attention in these systems, both research and restoration of stream ecosystems generally has been focussed on riparian vegetation (Bunn et al. 1999, Allan 2004, Davies 2010, Feld 2011). The seasonal provision of habitat and resources by submerged plant communities has clear benefits for aquatic fauna, which may be particularly important in relation to the life cycle of many organisms in mediterranean-climate systems. The ecological importance of these plant assemblages in degraded reaches found in this research shows that their presence in agricultural streams is beneficial, whether as remnant or colonising populations, and that they should therefore be considered in reach-scale restoration in agricultural landscapes. Understanding of the important ecological functions provided by submerged macrophytes in perennial streams, and shown here also for seasonal streams, has not translated into restoration ecology. This contrasts with the situation for cool-temperate shallow lakes, where restoration is strongly guided by ecological theory that includes these plants. For lotic systems, a focus of restoration ecology on riparian zones and in-stream physical habitat may be missing the potential for short term outcomes through the use of macrophytes. Seasonally lentic environments in drier climate streams present an opportunity to apply shallow lake theory in managing water quality and biodiversity. The limiting effect of seasonal drying on macrophyte growth reduces the risk of excessive growth, even where resources are in excess, removing these concerns. Successful establishment of species suited to prevailing environmental conditions can provide beneficial outcomes for biodiversity in a short time-frame, complementing longer-term actions of riparian zone rehabilitation and catchment management.

**Table 6.1** Summary of outcomes for specific research questions in relation to each component of the overall thesis aim to investigate the importance of submerged macrophytes in seasonally-flowing stream ecosystems in a mediterranean-climate agricultural landscape.

<p><b>Research questions from studies in Chapters 3, 4 and 5.</b></p> <p><b>(i) Drivers of distribution and relationships with environmental factors</b></p>	<p><b>(ii) Contribution to ecosystem processes</b></p>	<p><b>(iii) Capacity to improve biodiversity and water quality in degraded waters</b></p>	<p>3.1 What environmental variables are associated with macrophyte occurrence?</p> <p>Two morphologically contrasting macrophyte assemblages found with distinct distribution related to riparian shading, owing to different light requirements.</p>	<p>Colonisation of sediment deposits suggests potential to stabilise sediment load, benefiting downstream receiving waters.</p> <p>Provided water regime is adequate, variable species traits present opportunities for restoration of different species suited to prevailing conditions, but providing similar ecosystem services.</p>	<p>Both macrophyte assemblages were associated with poor riparian condition, however: <i>Cynogeton</i> spp. assemblage associated with shady conditions; likely present as remnant populations; while <i>Potamogeton/Oelia</i> assemblage colonised exposed, degraded sites with low riparian cover and sediment deposits.</p>	<p>3.2 Is the presence of submerged macrophytes associated with stream degradation?</p> <p>Excessive growth was not observed, and is probably limited by water regime. Occurrence in degraded reaches indicates potential establishment in other degraded reaches.</p>	<p>3.3 Do reaches with native macrophytes support a more abundant and diverse aquatic invertebrate assemblage than those without?</p> <p>Significantly greater macroinvertebrate abundance was found in reaches with submerged macrophytes than those without. Higher family richness was found, owing to greater abundance.</p> <p>Submerged macrophytes may compensate for lost habitat and resources in degraded reaches, and may provide substitute ecosystem services where riparian vegetation is lost.</p>
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4.1 Do macrophytes contribute to food webs in these streams?		<p>Macrophytes did contribute to food webs in good condition reaches.</p> <p>Epilithon and terrestrial detritus were more important.</p> <p>Macrophytes were a consistent basal resource for fish and freshwater crayfish across sites.</p>	<p>Assimilation of multiple basal resources, including macrophytes, indicated generalist feeding strategies for most macroinvertebrate consumers, so macrophytes are a viable resource in degraded reaches.</p>
4.2 Is the contribution of macrophytes to food webs is greater in degraded reaches than those in good condition?		<p>Assimilation of <i>Cycnogeton</i> was higher in poor condition reaches with no obvious epiphyte growth.</p> <p>Conspicuous epiphytes were a preferred food source where present.</p> <p><i>Potamogeton ochreatus</i> and <i>Ottelia ovalifolia</i> contributed moderately to the food web in degraded reaches.</p>	<p>Aquatic macrophytes may support opportunistic consumers in degraded streams, both directly and as a substrate for epiphytes.</p>
5.1 Can submerged macrophytes be established in turbid, nutrient rich waters when protected from herbivorous waterfowl?	<p>Top-down control by herbivory was important in limiting submerged macrophytes in this system.</p>		<p><i>Vallisneria australis</i> achieved 85-100% cover in exclosures after six months despite poor water quality.</p> <p>Additional native macrophyte species colonised the exclosures.</p>
5.2 What influence do submerged macrophytes have on the aquatic invertebrate assemblage in this degraded system?		<p>Increased abundance of grazers indicated that presence of macrophytes provided an alternative food resource.</p>	<p>Richness and abundance of macroinvertebrates increased rapidly in response to transplants.</p> <p>Zooplankton abundance was not affected.</p>
5.3 Does the presence of submerged macrophytes improve water quality in terms of reduced nutrient concentrations, turbidity and phytoplankton growth?			<p>Water quality was not improved within macrophyte meadows. This may be due to substantial internal nutrient load, dominance of unpalatable cyanobacteria, the scale and short time frame of the experiment. There was evidence of particulate material trapped within meadows.</p>

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