Impact of willows on aquatic invertebrate communities

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Cover image: A sudden influx of willow leaves during autumn creating a flush of organic pollution − Sixth Creek, Mt. Lofty Ranges, South Australia.

Photo: Wahizatul A. Azmi

DECLARATION

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Wahizatul A. Azmi

Dated: June 2011

I dedicate this thesis to my beloved husband, Md. Yuzeiry Md. Yasin and my sons, Erfan Kheiry and Ezlan Kheiry.

ABSTRACT

Exotic willows (*Salix* spp.) have invaded the riparian zones of many Australian streams, but the impact of willows and their removal on aquatic invertebrate communities are poorly understood. In the Mount Lofty Ranges, South Australia, willows have aggressively invaded riparian zones of many freshwater streams, often affect stream morphology and erosion, leading to water quality problems and suppress growth of native vegetation. We hypothesized that aquatic invertebrate diversity and abundance would be reduced in streams with willows present and after they have been removed. We also investigated whether willow leaves could provide a useful food source by comparing feeding preference, growth rates and survivorship of five dominant aquatic invertebrates. Also, we investigated the potential habitat value created by willow roots for aquatic invertebrates and whether shade (willow canopies) can influence the invertebrate assemblages.

Our findings suggest that the presence of willows was clearly associated with a reduction in taxon diversity. However, the abundance of invertebrates was significantly higher in sites with willows due to the high abundance of the introduced hydrobiid snail (*Potamopyrgus antipodarum*). The establishment of this snail under willows should be considered a serious threat as it may be in resource competition with native invertebrates. Lower invertebrate diversity and taxa numbers were observed where willows were removed and the site not revegetated. This reduction in diversity and change in composition of aquatic invertebrate communities may be due to loss of habitat, changes in water quality, or may depend on the prior history of willow invasion. Taxa responsible for the significant differences among sites when riparian vegetation is changed from the original vegetation to willows, or when willows are removed, were also identified. We found that changes in the pattern of invertebrate assemblages seemed to be influenced by differences in season, habitat quality, food availability and water quality.

Feeding preference experiments where eucalypt and willow leaves were compared revealed that willow leaves are a source of food for some native invertebrates [e.g., *Dinotoperla evansi* (Plecoptera: Gripopterygidae), *Tasmanocoenis tillyardi* (Ephemeroptera: Caenidae) and *Lingora aurata* (Trichoptera: Conoesucidae)], and may influence their growth rates and survivorships. In habitat preference experiments, we found willow roots supported significantly higher and more diverse aquatic invertebrate assemblages than an artificial substrate of aluminium wire mesh of different sizes. These findings revealed that willow roots provide a better habitat and a variety of microhabitats

for invertebrate colonisation. However, the introduced hydrobiid snails were strongly associated with willow root habitats compared with other invertebrates. In experiments of the effect of shade, we found that increased light as a result of willow removal and revegetation resulted in lower invertebrate abundance, although there were higher taxa numbers and diversity. This increase in sites lacking a riparian canopy (i.e., open canopy), may be due to an increase in the availability of quality food through reduced shading, which in turn increases the long term invertebrate community diversity, productivity and abundance.

Careful management of restoration programs to remove willows and to revegetate the sites is highly recommended, particularly in small streams such as those in this study. Many aspects need to be considered before willows are removed and revegetation programs carried out. These include: the impact of willows including their canopies and root masses, and that of the revegetation to replace willows. Aquatic invertebrates are potential bioindicators in the ecological success of willow control and revegetation programs, and should be considered as an important component during monitoring of such programs.

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CHAPTER 1:   General Introduction

This chapter will introduces the importance of riparian vegetation to the aquatic ecosystems, with particular reference to the effect of willows (*Salix* spp.) on the diversity and composition of aquatic invertebrate communities. The impact of willow leaves on the feeding, survival and growth of aquatic invertebrates, effect of willow roots as a habitat, and the effect of shade (willow canopies) on invertebrate communities are covered in the individual results chapters that follow.

1.1 Introduction

The importance of riparian vegetation as an essential component of stream ecosystem processes has long been recognized (e.g., Walker 1993, Rios & Bailey 2006). Riparian vegetation is very important in determining the structure, function and stability of stream ecosystems. Its presence at the boundary between terrestrial and aquatic ecosystems plays an important role in the maintenance of channel morphology, solar energy transmission, water quality, and therefore maintains a level of stability (McKie & Cranston 2001). Various researchers have warned that removal or modification of riparian vegetation along a stream can have a significant impact on the physical and biological components of the aquatic ecosystem (e.g., Quinn *et al.* 1992 & 1994; Bunn *et al.* 1999a; Walsh *et al.* 2007; Jayawardana & Westbrooke 2010). Removal or modification of riparian vegetation may initiate erosion of the streambed and often results in the high input of organic matter and sedimentation (Naiman *et al.* 1988). It may also contribute to elevated temperature, water quality problems, and destruction of habitats, which in turn; will lead to the deterioration of aquatic fauna abundance (Bothwell *et al.* 1993; Allan 1995; Gutierrez-Rodriguez & Williamson 1999; Kelly *et al.* 2003; Reid *et al.* 2008).

One of the ways in which the ecological function of riparian vegetation can be disrupted is by the invasion of exotic species. As with all riparian vegetation, exotic willows play an important role in controlling stream ecosystems by helping the maintenance of stream integrity. However, the extensive spread of willows along Australian watercourses has raised concerns over possible ecological effects on both riparian and aquatic ecosystems. Willows are presumed to be responsible for a decline in native biodiversity and are generally seen as a serious threat to Australian freshwater ecosystems. Various ecologists have speculated that changes between native Australian vegetation and exotic willow species will affect channel morphology and bank erosion (Bunn *et al.* 1993; Ladson *et al.* 1997), water quality (Gregory *et al.* 1991; Wallace *et al.* 1995), primary and secondary production (Lester *et al.* 1994a) and the density of aquatic

fauna (Glova & Sagar 1994; Jayawardana *et al.* 2006). As a consequence, willows are now being poisoned, removed from river banks, and the banks replanted with native vegetation. However, such programs have accelerated bank erosion, leading to water quality problems and allowing exotic weeds to become established. Research on the effects of willows on freshwater ecosystems is needed in order to predict the potential short and long term effects of willow on the physico-chemical and biological components of aquatic ecosystems.

The effects of willows on the ecological aspects could have direct or indirect affects on aquatic invertebrate community structure. In this thesis, I examine various ecological aspects of aquatic invertebrates relating to the invasion of exotic willows in two freshwater streams (Sixth and Deep Creeks) in the Mount Lofty Ranges, South Australia (SA). The main questions addressed in this thesis are:

1) Do willows have an impact on aquatic invertebrate diversity and abundance?
2) What happens to the invertebrate communities when willows are removed?
3) Does the removal of willows affect invertebrate communities, and how?
4) Do revegetation and original vegetation sites support different invertebrate communities, and why?

This chapter provides a review of previous literature and in particular, focuses on the impact of willows on aquatic invertebrate communities, thus providing a background of the research presented in this thesis.

1.2   Effect of willows on aquatic invertebrate communities

Willows (*Salix* spp.) are seen as a serious weed in Australian aquatic ecosystems. Since the early 1900s, the riparian zones of many Australian streams became dominated by the exotic willows. They were mainly used by the European settlers in boat navigation, erosion control, bank stabilization, and as ornamental trees (Ladson *et al.* 1997; Schulze & Walker 1997; Holland & Davies 2007). Since then, as more willows have become established, further changes in the hydrologic regime, water quality problems, decline in native species and establishment of other exotic weeds have been reported (e.g., Merigiano 1997; Patten 1998). Willows spread 'naturally' and grow best particularly when they are near water and after floods (Schulze & Walker 1997), where their brittle branches break off easily, spread freely and became established further downstream.

It is estimated that willows have invaded ~30,000 km of the 68,000 km river frontage (e.g., River Murray) in Victoria (Ladson *et al.* 1997) and over one-third of the 830 km of

the River Murray in South Australia is now dominated by the weeping willow (*S. babylonica*) (Schulze & Walker 1997). More than 32 different taxa (species, varieties, cultivars and hybrids) of willows have been identified in Australia (Cremer 1995) and most are listed as 'Weeds of National Significance' (excluding *S. babylonica, S. x calodendron* and *S. x reichardtii*) (ARMCANZ 2001). There has been some research on the impact of willows on aquatic invertebrate abundance, stream channel morphology and stream production especially in Australia and New Zealand (Walker 1993; Glova & Sagar 1994; Lester *et al.* 1994a; Ladson *et al.* 1997; Schulze & Walker 1997; Read & Barmuta 1999; Stohlgren *et al.* 1999; McKie & Cranston 2001; Jayawardana *et al.* 2006; Jayawardana & Westbrooke 2010), however, many of the views expressed remain largely untested due to inconclusive and/or contradictory findings.

A study in Central Otago streams, New Zealand by Lester *et al.* (1994a) revealed that lower densities of macroinvertebrates were found in stretches densely lined with *S. fragilis*. This was further supported by Lester *et al.* (1996), who found lower densities of benthic macroinvertebrates in willow sections in Heeney Creek, New Zealand. However, Schulze and Walker (1997) found only minor differences in the composition of littoral macroinvertebrates between sites lined with willow (*S. babylonica*) and river red gum (*Eucalyptus camaldulensis*) in the River Murray at Blanchetown, South Australia. Jayawardana *et al.* (2006) found no consistent differences in species richness and abundance in willow habitats in Birch Creek, Victoria.

In several studies, the impact of willow invasions was found to have positive effects on the aquatic invertebrates by providing a food supply or habitat for some species to exploit. Glova and Sagar (1994) found greater species richness and diversity in benthic invertebrate communities in reaches lined with willows than in reaches with bare banks in three New Zealand streams. Parkyn and Winterbourn (1997) found high densities of invertebrates colonizing willow leaves in a small South Island stream in New Zealand. Interestingly, Lester *et al.* (1994b) found that willow leaves were used as a food source by a caddisfly larva in willow lined reaches of two Central Otago streams in New Zealand. In forest streams in Tasmania, Yeates and Barmuta (1999) found three macroinvertebrates (*Notolina* sp., Trichoptera: Leptoceridae; *Koornonga* sp., Ephemeroptera: Leptophlebiidae; *Physastra gibbosa,* Mollusca: Planorbidae) had a strong preference for green willow leaves over senescent willow, green and senescent eucalypt leaves.

Willows have quite different characteristics when compared with native riparian vegetation and each of these characteristics has possible environmental impacts. Willows are deciduous and most leaves fall in autumn over a short period of time (Schulze &

Walker 1997). This massive leaf drop can lead to high nutrient pulses and reduced water quality (Gregory *et al.* 1991). By contrast, native eucalypts drop leaves year round, have relatively slow decomposition rates and are available as a long-term food resource for the invertebrates.

Dense willow canopies, especially in spring and summer, can decrease light intensity and water temperatures (Lester *et al.* 1994a). As a result, these may be a decline in stream primary and secondary production, suppression of the indigenous understorey on the banks and in the water, and also reduction in dissolved oxygen concentration (Lester *et al.* 1994a; Quinn *et al.* 1994).

Dense willow root mats can grow into the stream channel, trapping silt and layering new roots over old roots, building up the streambed and creating a broad shallow stream (Bunn *et al.* 1999a; Ladson *et al.* 1997). These modifications lead to increased flooding, changing channel morphology and sediment conditions (Bunn *et al.* 1993; Lester *et al.* 1994a; Ladson *et al.* 1997), which in turn may affect habitat availability for aquatic invertebrates, fish, small mammals and birds (Collier *et al.* 1995; Schulze & Walker 1997; Greenwood *et al.* 2004).

Willow leaf litter decomposes rapidly in water (Pidgeon & Cairns 1981; Schulze & Walker 1997), and may not provide a suitable food source (Yeates & Barmuta 1999) or habitat for aquatic animals (Lester *et al.* 1994a). Many studies have demonstrated that leaves and bark of some European willow species contain high concentrations of phenolics (e.g., cyanidins, delphinidins, leucoanthocyanidins and phenolglycosides) (Rowell-Rahier 1984; Julkunen-Tiitto 1985) and tannins (Haapala *et al.* 2001), which are deterrents to aquatic invertebrates. Other impact of willows include the use of water by willows, where willows are also presumed to use more water compared with native vegetation such as river red gums. A study by Benyon and Doody (2006) revealed that willows growing in waterways utilise 3-4 $MLyear^{-1}ha^{-1}$ more water than river red gums growing on stream banks.

To date, very little is known about the effect that the removal of willows has on aquatic invertebrates. Although the impact of willows on Australian aquatic systems has been researched previously, no comprehensive study has investigated community responses to the effect of willows removal and any subsequent revegetation on aquatic invertebrates. It is also not known whether willow leaves can provide a useful food source or whether willow roots have any habitat value for aquatic invertebrates in Australian streams. Further, little is known about the impact of willow canopies on invertebrate assemblages

and how the invertebrates might respond to different shade levels following management programs to remove willows and subsequent revegetation.

Wide-scale willow removal programs are currently underway in Australia. Local councils are commencing programs to remove and poison the willows from accessible river banks (e.g., Victorian Catchment Management Authorities). In many cases, efforts have been made to restore the native vegetation. However, this may take some time and such programs run the risk of accelerating bank erosion or allowing other exotic weeds to become established. Until now, there are little or no specific policies on willow management in most local Catchment Management Authorities.

How the aquatic invertebrate communities responded to the removal of willows and subsequent revegetation is currently unknown. Therefore, the main aim of this study was to determine the impact of willows and their removal and subsequent revegetation on aquatic invertebrate communities. The outcomes from this research may provide information useful for the development of site specific willow management strategies to minimise detrimental short term impacts and accelerated recovery of the riparian and aquatic ecosystems. This research is necessary to improve the decision making process as to whether willows should left in place, removed or controlled, and whether revegetation is a warranted.

1.3   Aquatic invertebrates as bioindicators

Studies of aquatic invertebrates of freshwater river and stream ecosystems have frequently examined the species-habitat relationship with regard to the water quality of the habitat. Some species are known to have particular requirements for nutrients, water quality, substrate components and the structure of vegetation. Once these are defined, the presence of a particular species in a habitat indicates that the given determinants or parameters are within the tolerance limits of the species (Hellawell 1986).

Indicator species are those taxa known to be particularly sensitive to specific environmental factors, so that changes in their incidence or abundance may directly reflect an environmental change (New 1984). The concept of biological indicators using aquatic invertebrates is based on their diversity, abundance and distribution in relation to the physical and chemical conditions of the habitat. Data provided by indicator organisms can be used to estimate the degree of environmental impact and its potential dangers for other living organism (Kovacs 1992). Biological monitoring, whereby living organisms are used

to estimate the water quality or its chemical contents, is important in determining the health of an aquatic ecosystem (Gruber & Diamond 1988). Physicochemical monitoring of a water body is known to be insufficient to fully characterize its status or reliably detect adverse impacts. However, it has been recognized as a vital component of an integrated assessment utilizing physicochemical and biological measure for assessing a waterway's condition (Hellawell 1986).

Aquatic invertebrates are the most diverse and abundant group in any aquatic faunal community and have proven useful as biological indicators of both recent and long-term environmental conditions. As they play an important role in the aquatic food web, changes in the abundance, diversity and composition of invertebrate assemblages may indirectly alter in-stream food webs and may have important impacts on higher-order consumers in the riparian zone. Aquatic invertebrates have been used to assess water quality of streams/rivers for ambient monitoring and for evaluating the effectiveness of pollution control measures throughout the world (New 1984; Hellawell 1986). In Australia, predictive models using aquatic invertebrates have been developed for most states and territories under the Australian River Assessment System (AUSRIVAS) (Davies 2000). AUSRIVAS is a nationally standardised approach to biological assessment of stream condition that provides an assessment of stream condition based on the ratio of the number of taxa collected (observed) at a site and the number predicted (expected) by a multivariate model. Since aquatic invertebrates play a considerable role in aquatic ecosystems, the current reseach would further increase the knowledge surrounding the potential of aquatic invertebrates as indicators for an early detection and evaluation of stream health in willows management strategies.

1.4   Thesis outline

In this thesis, each chapter is presented as a research journal article, followed by a discussion of the potential impacts of willows removal based on the findings present in that chapter. The term "we" is used throughout the thesis as some chapters are planned to be published as co-authored papers.

Chapter 2 investigates the differences in taxon richness and abundance when willows are present, removed or revegetated and how they compare with original vegetation. We examined the taxon richness and abundance in four different treatments (willows present, willows removed, revegetation and original vegetation) in three different habitats (pool, riffle and edge) during all seasons (spring, summer, autumn and winter) at each site (Sixth

and Deep Creeks). The results of this chapter provided an indication of the changes in invertebrate communities that take place when riparian vegetation changes from original vegetation to willows vegetation; and when willows are removed.

Chapter 3 deals with feeding preference experiments where five dominant aquatic invertebrate species found in the two creeks [*Potamopyrgus antipodarum* (Gastropoda: Hydrobiidae), *Physa acuta* (Gastropoda: Physidae), *Dinotoperla evansi* (Plecoptera: Gripopterygidae), *Tasmanocoenis tillyardi* (Ephemeroptera: Caenidae) and *Lingora aurata* (Trichoptera: Conoesucidae)] were chosen to determine their feeding preference, survivorship and growth rates when offered a diet of leaves of crack willow (*Salix fragilis*) or white gum (*Eucalyptus viminalis*), the latter being a common native species along both creeks. The results indicate that willow leaves are a suitable food source for some invertebrate species studied (*D. evansi*, *T. tillyardi* and *L. aurata*) and there is also some impact on their growth rates and survival.

Chapter 4 outlines the results of a manipulative field experiment designed to investigate differences in colonisation by aquatic invertebrates on natural substrate (willow roots) and artificial substrate (wire mesh). Wire mesh was used after an initial pilot study involving several different artificial substrates (see Appendix VI). The response of the invertebrate communities to different levels of substrate complexity after two colonisation periods (30 days and 90 days) was investigated. We found that willow roots have habitat value for some of the native invertebrates, especially for the introduced hydrobiid snail (*P. antipodarum*). We conclude that invertebrate communities utilise the willow roots as habitat and there is the potential for disruption of these communities when willows are removed.

Chapter 5 also reports results of a manipulative field experiment, where we investigated the effect of shade (canopy) on aquatic invertebrate communities to simulate what happens after willows are removed and prior to revegetation programs. Two separate experiments (natural experiment and manipulative experiment) investigating differences in diversity and patterns of abundance were conducted from early November to December 2009. The experiment using natural willow shade was conducted at Sixth Creek, whereas the manipulative experiment using shade cloth was carried out at Deep Creek. Each site consisted of three different shade levels: (1) fully shaded, (2) partly shaded and (3) open canopy. Increased light resulted in lower invertebrate abundance but higher taxa numbers and diversity. More diverse and richer invertebrate fauna was found in treatments lacking canopies, and was possibly due to the effects of increased light resulting in an increase in

the amount of high quality of food sources to the invertebrates. We suggest that complete clearing of willows or other vegetations in single step should be avoided, and partial canopies would provide a viable alternative when removing the willows.

A summary of the conclusions from the main findings and their implications for the broader context (future research) of the impact of willows on various aspects of aquatic invertebrate biodiversity is provided in Chapter 6.

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CHAPTER 2: Exotic willows and their impact on the diversity and abundance of aquatic invertebrate communities
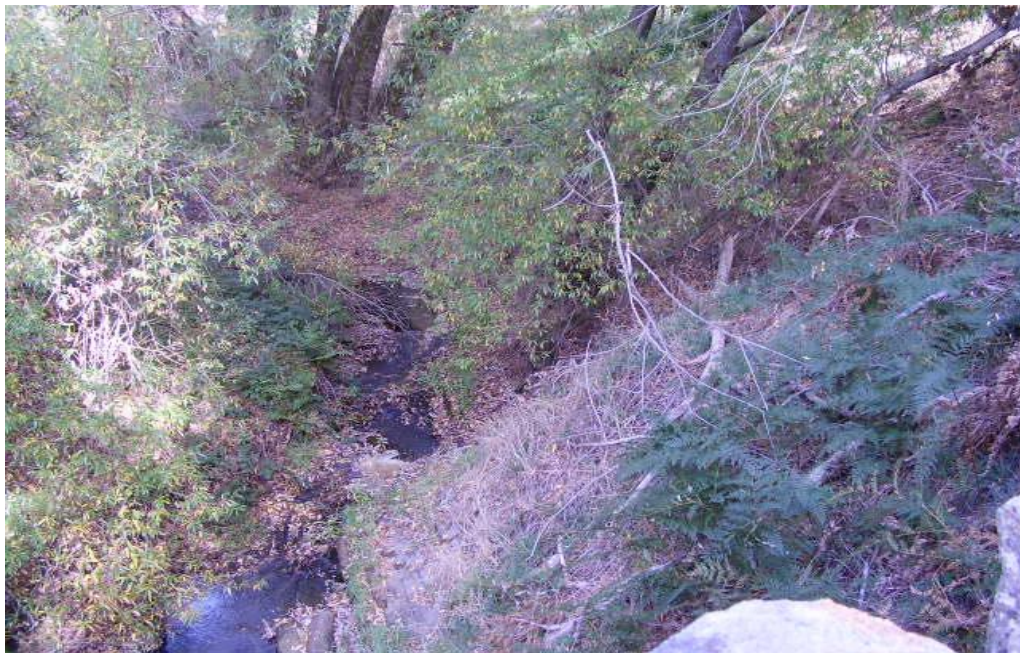
2.0   Abstract

The impact of willows, their removal and subsequent revegetation on aquatic invertebrate communities were examined in two freshwater streams (Sixth and Deep Creeks) in the Mount Lofty Ranges, South Australia. We hypothesized that lower abundance, taxon diversity and changes in functional feeding groups would occur where willows were present and where willows have been removed. Unexpectedly, invertebrate abundance was significantly higher when willows were present in both sites. The introduced hydrobiid snail (*Potamopyrgus antipodarum*) was the most dominant taxon overall and was significantly more abundant under willows in both streams. More than half of the total abundance under willows was contributed by scrapers (mostly *P. antipodarum*) as willow roots are presumed to provide a more stable habitat from high currents and have increased food availability compared with other vegetation. Where willows were removed and not revegetated, there were lower invertebrate taxa numbers and diversity in both streams. The removal of willows influenced not only loss of habitat, but also an increase in light intensity, decline in both water quality (e.g., high conductivity and total dissolved solids) and food availability. This situation is reversed when the riparian canopy is revegetated. However, the presence of willows was clearly associated with a reduction in taxa diversity. A slightly more diverse fauna was recorded in the original vegetation (51 taxa) compared with both revegetated sites (49 taxa), and sites with willows removed (47 taxa), but was lower with willows present (39 taxa). The richer and more abundant fauna in the original vegetation and revegetation sites is probably due to a sparse, open canopy which permits higher primary productivity and favours more diverse growth of aquatic plants, shrubs and grasses. Besides *P. antipodarum,* planorbiid snails (*Isidorella* sp.), baetid mayfly nymphs (*Offadens* sp.), elmid larvae (*Simsonia leai*), hydrobiosid caddisfly nymphs (*Taschorema evansi*) and dragonfly nymphs (Coenagrionidae) were significantly more abundant under willows in Sixth Creek. Blackfly larvae (*Austrosimilium furiosum*) and amphipods (*Austrochiltonia australis*) were significantly more abundant under willows in Deep Creek. In contrast, the freshwater snails (e.g. *Glyptophysa* sp., lymnaeid snails) and leptophlebiid mayfly nymphs (*Koornonga inconspicua*) were significantly more abundant in sites lacking willows. Our findings conclude that the presence of willows reduces taxa numbers and diversity. Removal of willows without subsequent revegetation resulted in lower taxa diversity and abundance of the aquatic invertebrates. We suggest that large scale willows removal may need special management considerations in order to reduce the impact on aquatic invertebrate communities.

Keywords: aquatic invertebrate communities, willows, diversity, abundance, revegetation.

2.1   Introduction

Exotic willows (*Salix* spp.) are a naturalized component of the flora of Australia and have invaded thousands of kilometers of watercourses (Schulze & Walker 1997; Read & Barmuta 1999). They were widely introduced by European settlers during the early 19th century mainly for erosion control, bank stabilization and navigation (Ladson *et al.* 1997). Their high invasion rate has raised concerns because they are presumed to be responsible for a decline in native biodiversity and are generally seen as a serious threat to Australian freshwater ecosystems. Various ecologists have speculated that changes between native Australian vegetation and exotic willow species will affect channel morphology and bank erosion (Bunn *et al.* 1993; Ladson *et al.* 1997), water quality (Gregory *et al.* 1991; Wallace *et al.* 1995), primary and secondary production (Lester *et al.* 1994a) and aquatic fauna densities (Glova & Sagar 1994; McKie & Cranston 2001; Jayawardana *et al.* 2006).

Willows are deciduous and drop all their leaves over a short period of time in late autumn (Schulze & Walker 1997). As a result, they provide a sudden influx of organic material (Gregory *et al.* 1991) that may release large quantities of nutrients into aquatic systems which could potentially threaten aquatic organisms. The leaf material breaks down rapidly in water (Pidgeon & Cairns 1981; Schulze & Walker 1997), and may not provide a suitable food source (Yeates & Barmuta 1999) or habitat for aquatic animals (Lester *et al.* 1994a). Also, leaves and bark of willows contain cyanidins, delphinidins, leucoanthocyanidins and phenolglycosides which are deterrents to herbivores (Rowell-Rahier 1984). The dense canopy of willows can decrease water temperature and light availability in streams and rivers. This may reduce primary and secondary production (Lester *et al.* 1994a), decrease the dissolved oxygen concentration and affect different life stages of invertebrates (Nebeker *et al.* 1996) and fish (Penczak 1997). As well as the impact of the leaves, the fibrous willow root mats which spread into the bed of watercourses can trap sediment, reduce water flow and aeration, and lead to increased sedimentation and flooding (Bunn *et al.* 1993; Ladson *et al.* 1997).

The impact of willows on aquatic invertebrate communities has been researched previously, but there are inconclusive and/or contradictory findings. In streams in Central Otago, New Zealand, Lester *et al.* (1994a) found lower densities of benthic macroinvertebrates in reaches lined by *S. fragilis*. In the Murray River at Blanchetown, South Australia, Schulze and Walker (1997) found only minor differences in the

composition of littoral macroinvertebrates between sites lined with willow (*S. babylonica*) and river red gum (*Eucalyptus camaldulensis*). They concluded that willows may influence stream invertebrates through altering food supplies (through decreasing sunlight penetration and fallen leaves during autumn) or habitat (by reducing the size of substrate particles and flow rates). Jayawardana *et al.* (2006) found no consistent differences in species richness and abundance in willows and *Phragmites australis* habitats in Birch Creek, Victoria. In contrast, some studies have suggested that willows may support high numbers of aquatic invertebrates by providing a food supply or habitat for some species to exploit. Glova and Sagar (1994) found greater species richness and diversity in benthic invertebrate communities in reaches lined by willows than in reaches with bare banks in three New Zealand streams. Interestingly, Yeates and Barmuta (1999) reported in forest streams in Hobart, Tasmania, that three macroinvertebrates (*Notolina* sp., Trichoptera: Leptoceridae; *Koornonga* sp., Ephemeroptera: Leptophlebiidae; *Physastra gibbosa,* Mollusca: Planorbidae) strongly preferred to feed on green willow leaves over senescent willow, green and senescent eucalypt leaves.

Most studies have only compared communities of aquatic invertebrates in willow and non-willow/native vegetation sites or reaches with bare banks. No study has been carried out to investigate the community response to potential effects of the removal of willows and subsequent revegetation on aquatic invertebrates. Specifically, our research questions were: are there any differences in taxa richness and abundance when willows are present, removed or revegetated and how do they compare with original vegetation? To determine the impact of willows, their removal and subsequent revegetation on aquatic invertebrate communities, we tested the following hypotheses:

1) lower abundance, taxon diversity and functional feeding groups will occur in treatments in where willows were present and removed; and

(2) higher abundance, taxon diversity and functional feeding groups will occur in original vegetation and revegetation treatments.

Outcomes of these hypotheses would give an insight as to whether willows should be retained, removed or controlled, and whether revegetation is warranted.

2.2 Materials and Methods

2.2.1 *Study sites*

Sixth Creek is a freshwater stream located at 34^o52'N and 138^o45'E. It flows north to meet the Torrens River at Castambul, NE of Adelaide in the Mount Lofty Ranges, South Australia (Fig. 2.1). It is a fifth order stream with a total length of ~18 km and drains an area of ~46 km^2. It ranges from 1−4 m in width, 0.5−4.0 m in depth and the water velocity is slow to fast. The upper reaches of the stream are heterogeneous with a coarse bed substrate, fine roots, leaf packs, woody debris, and overhanging vegetation. Some reaches are totally exposed to sunlight with fast flowing water (mainly in the winter and spring), while others are canopied with large trees and shrubs and have slow moving water. Most plants in the riparian zone are introduced weeds, including willows (*Salix* spp.), ash (*Fraxinus* spp.), blackberries (*Rubus fruticosus*), and hawthorns (*Crataegus* spp.), although in some reaches these have been removed by landowners. The middle and lower parts of the stream are relatively deep (2.5−4.0 m) and mostly covered by forest canopy, with grasses and shrubs along the stream edge.

Deep Creek is also located in the Mount Lofty Ranges NE of Adelaide at 34^o56'N and 138^o46'E (Fig. 2.1). This small fourth order stream is approximately 8.5 km long, is 2.0−4.5 m in width, 0.5−2.5 m in depth, is slow to sometimes fast flowing and the catchment area is ~13 km^2. The stream bed is almost debris free, mainly comprising sand, pebbles, cobbles and small boulders. Aquatic vegetation is mainly *Carex* sp., *Juncus* sp. and *Rorrippa* sp. The major riparian vegetation in the study area consists of willows, *Eucalyptus viminalis, E. obliqua, Acacia retinoides* and *A. melanoxylon.* Generally, the upper reaches of the stream are exposed to direct sunlight where the water is deep and slow moving and the bed mostly sandy. The lower reaches are partly cleared and dominated by willows.

The climate in the Mount Lofty Ranges catchment area is hot Mediterranean. The average annual rainfall in the catchment areas is approximately 900 mm, with average maxima of 25^0C (soaring into the 40s) during dry summers and with maximum temperatures averaging 12^0 to 15^0C during cool, wet winters. Both study catchments include a mixture of rural residential, horticulture, orchards and grazing land. The description, physico-chemical characteristics and photos of each treatment in Sixth and Deep Creeks are presented in Table 2.1 and Figure 2.2.
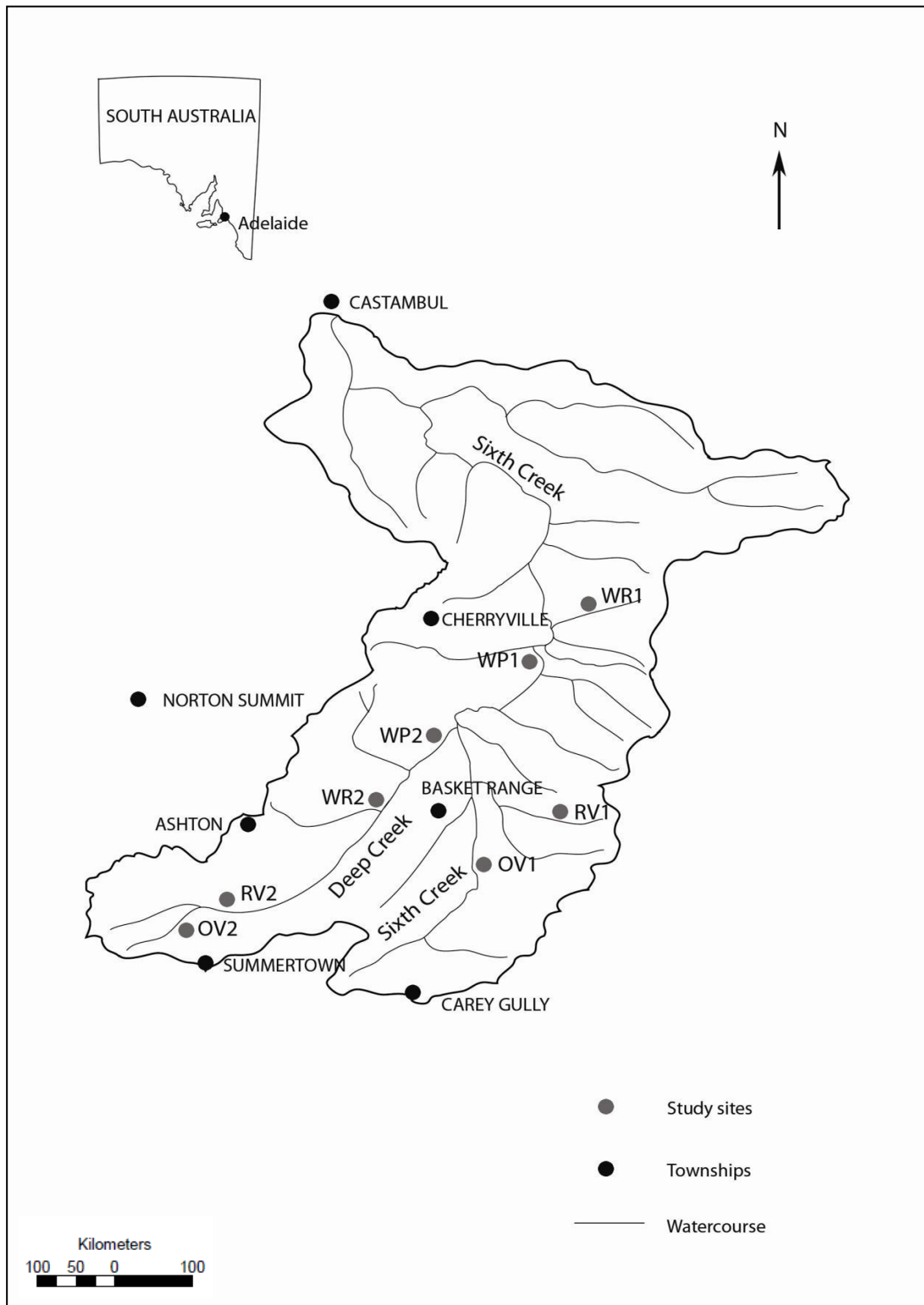
Fig. 2.1 Location of study sites in the Mount Lofty Ranges catchment area, South Australia (Notes: WP = Willows present; WR = Willows removed; RV = Revegetation; OV = Original vegetation; 1 = Sixth Creek; 2 = Deep Creek).

Table 2.1 Summary of description and physico-chemical characteristics (bimonthly measurements) of each treatment in Sixth and Deep Creeks.

Site	Treatment	Altitude (m)	Depth (m)	Width (m)	Dissolved oxygen (mg/L)	Conductivity (µS/cm)	pH	Total dissolved solid (ppm)	Water temperature (^{0}C)	Flow rate	Dominant substrate type
Sixth Creek	Willows present (WP1)	380.0	1.15±0.62	3.50±0.85	9.30±1.16	577±75.54	8.41±0.33	345±23.35	20.60±2.61	Slow−Medium	Willow roots/organic
	Willows removed (WR1)	350.0	0.45±0.45	4.00±0.90	9.57±2.10	515±79.51	8.13±1.23	356±23.07	20.85±2.31	Medium−Fast	Mud/organic
	Revegetation (RV1)	345.5	0.50±0.37	1.75±0.95	10.81±2.11	240±47.66	8.15±1.07	275±31.13	18.60±2.98	Slow−Fast	Wood debris/Cobble
	Original vegetation (OV1)	363.3	0.35±0.55	1.80±0.76	10.98±2.13	253±60.55	8.05±1.11	270±22.74	19.30±2.23	Slow−Fast	Cobble /Boulder
Deep Creek	Willows present (WP2)	375.0	1.20±0.29	2.50±0.65	9.07±1.55	440±55.69	8.15±1.38	334±29.97	21.60±1.98	Slow−Medium	Willow roots/organic
	Willows removed (WR2)	422.5	0.35±0.55	2.50±0.80	9.50±1.55	425±45.56	8.25±0.99	323±25.02	22.60±2.59	Medium−Fast	Mud/organic /cobble
	Revegetation (RV2)	497.3	0.55±0.35	1.95±0.55	9.48±1.85	271±45.22	8.77±1.29	212±26.09	18.60±1.89	Slow−Fast	Cobble /Boulder
	Original vegetation (OV2)	345.5	0.55±0.40	1.95±0.75	10.76±1.26	197±44.45	8.22±1.39	255±20.65	19.80±1.62	Slow−Fast	Cobble /Boulder

Fig. 2.2 Photos of each treatment in Sixth and Deep Creeks (Notes: WP = Willows present; WR = Willows removed; RV = Revegetation; OV = Original vegetation; 1 = Sixth Creek; 2 = Deep Creek).

Each study stream had four different treatments (Fig. 2.2):

(1) willows present (WP) – ~60% of the riparian area dominated by willows and ~40% covered by ash, herbs and shrubs at both Sixth Creek (WP1) and Deep Creek (WP2).

(2) willows removed (WR) – banks more or less bare, except for grasses at Sixth Creek (WR1); at Deep Creek, much of the ground was bare and ~20% were covered by grasses and small shrubs (WR2).

(3) revegetation (RV) – at Sixth Creek (RV1), willows were removed in 1997 and revegetated mainly with *Juncus* spp. (rush), *Carex* spp. (tussock sedge), *Rorrippa* spp. (watercress) and *E. viminalis* (white gum or manna gum); at Deep Creek (RV2), willows were removed in 2004 and revegetated mainly with *Acacia melanoxylon*, *A. retinoides,* and *E. viminalis*.

(4) original vegetation (OV) – ~50% of the canopy comprising mainly *E. viminalis, E. obliqua* (messmate stringybark)*, A. retinoides*, *A. melanoxylon* and ~50% various shrubs and herbs, many of which are introduced weeds (periwinkle, soursob, blackberries and bamboo) at Sixth Creek (OV1); at Deep Creek (OV2), ~60% were dominated by native species included *E. viminalis, E. obliqua, A. melanoxylon, Juncus* spp.*, Carex* spp. and the rest are much denser, species-rich ground carpet of exotic grasses and small shrubs.

2.2.2   *Sampling methodology*

Sampling of the aquatic invertebrate communities was carried out every two months from September 2007 to November 2008 to examine any differences in diversity and patterns of seasonal abundance at each site and across treatments. We followed a slightly modified AUSRIVAS (Australian River Assessment System) methodology for South Australian protocol (http://ausrivas.canberra.edu.au/Bioassessment/Macroinvertebrates). In the AUSRIVAS methodology, invertebrates are sampled over the period of 6–12 months and sampling requires only one or two visits per year. In this study, we sampled the invertebrates intensively (every two months) in order to determine the effect of seasonality on taxon diversity and abundance. We included some additional measurements for basic water chemistry such as total dissolved solids (ppm) and flow rate (m/s). We also simplified the type of bedform into three categories of major habitats; pool, riffle and edge. Some of the physical measurements were not included such as bed compaction, sediment angularity, type of bars, channel shape and physical barriers to local fish passage; as those

measurements were not necessary for this study. Sampling for each treatment covered ~100 m^2 in area and within each treatment, three different habitats (pools, riffles and edges) were surveyed. Each habitat was replicated 10 times to obtain reasonable estimates of population density (Lenat 1988). A total of 1440 samples were collected during this study to provide representative coverage of the sites.

Aquatic invertebrates were collected using an aquatic net with the size of sampler of 40 x 40 cm frame, 60 cm long net of 250 μm mesh. Big stones in swift water were hand-lifted and checked for invertebrates. The invertebrates and the content of each sample (net) was transferred into properly labeled plastic containers, preserved in 80% ethanol and taken back to the laboratory for analysis. Samples were washed in white trays and screened through 1 mm and 250 μm sieves. The invertebrates were initially sorted into functional feeding groups (Pennak 1978; Merritt & Cummins 1996; Gooderham & Tsyrlin 2002) and then identified as far as possible using keys of Williams (1980), McCafferty (1981), Merritt and Cummins (1996), Watts (1998), Gooderham and Tsyrlin (2002), Dean *et al.* (2004), and Theischinger and Hawking (2006).

Stream depth and width were measured *in situ*. General descriptions of the density and general composition of riparian vegetation, physical characteristics of water bodies and type of substrate were recorded. Dissolved oxygen (mg/L) and water temperature (^{0}C) were measured with YSI-57 Oxygen meter, pH recorded using PH-200 pH meter, conductivity (μS/cm), and total dissolved solid (ppm) were measured with COM-100 combo meter. Three readings at each site were taken to allow the means to be recorded for each parameter. Canopy cover of the riparian zone was estimated based on the openness of sites and categorized into three groups; 1 = shaded (80−100%); 2 = partly-shaded (30−80%); 3 = open (<30%). Water velocity was determined using Hydroprob flow-meter. Data were converted to the following nominal categories for analysis; 1 = fast flowing (>0.35 m/s); 2 = slow flowing (0.10−0.35 m/s); 3 = stagnant (<0.10 m/s).

2.2.3 *Data analysis*

The 10 replicate subsamples from each habitat (pool, riffle and edge) were pooled at each site for each sampling occasion. This was done in order to provide more robust assessment of the effect of treatments during all seasons in each site on species diversity and abundance. Besides that, we did not test annual variation as no data for individual years were tested; thus pooled data is recommended for the data analysis. Two-way ANOVAs

were used to evaluate differences in invertebrate distribution at different sites, treatments, seasons and habitats. Data were log(x+1) transformed to ensure normality in calculations of means and ANOVAs. Where there were significant differences, posterior pairwise comparisons (*t*-test) were employed to describe which means were most alike (or different) and to test the equality of means for each pair of variables. We used the same model of two-way ANOVAs to estimate differences in the abundance of functional feeding groups among sites, treatments, seasons and habitats. The abundance of functional feeding groups was assigned to the most common feeding habit. The ANOVAs analyses were performed using the Prism 5.00 statistical program (PRISM 2007).

Two-way factorial permutation-based nonparametric MANOVA (PerMANOVA; Anderson & ter Braak 2003) was used to analyse differences in the community composition between sites, treatments, seasons and habitats. The Sorensen Bray-Curtis test was used as a distance measure and 4999 permutation of raw data units were used for each multivariate analysis. Principal component analysis (PCA) was used to determine differences among taxa assemblages and to illustrate the pattern of variation for the most highly species associated ($P < 0.05$) with the effects of treatments and habitats (Anderson & ter Braak 2003). Two-way cluster analysis using Sorenson's Coefficient method was used to sort species into groups to reveal the degree of association between generic composition and the effects of treatments/habitats (Krebs 1989; Kovach 1999). However, not all taxa are included in the cluster analysia. Taxa such as bivalves, amphipods, ostracods and hirudineas were excluded due to their lower number in the samples. We also used the detrended correspondence analysis (DCA) to identify the species assemblage ordination which could then be related to measure how much the species distribution differed along environmental variables (Jongman *et al.* 1995). All the non-parametric multivariate analyses were conducted using the statistical program of PC-ORD version 5.13 developed by McCune and Mefford (2006). Spearman Correlation Analysis was also used to investigate the influences of physico-chemical parameters on the abundance of invertebrates and taxa richness at each site. This analysis was done using the SPSS 15.0 statistical package (SPSS 2006).

2.3   Results

2.3.1   *Effect of treatments*

A total of 76,683 individuals from 51 taxa in 42 families of aquatic invertebrates were collected from Sixth and Deep Creeks from September 2007 to November 2008. Significantly more aquatic invertebrates were collected in Sixth Creek (40,864 individuals) compared with Deep Creek (35,819 individuals) ($P < 0.001$) (Table 2.2; Fig. 2.3a). The effect of treatments on invertebrate total abundance varied significantly among habitats in each site and during different seasons (Table 2.2). Total abundance was significantly greater in sites with willows present than in other treatments for both streams (29,741 individuals) (Fig. 2.3a). Where willows were removed, slightly lower numbers of aquatic invertebrates (13,050 individuals) were recorded. In revegetation and original vegetation sites, fewer numbers of aquatic invertebrates were found (15,753 individuals and 18,139 individuals). Further analysis using the *t*-test showed that the total number of individuals in the willows present treatment in Sixth Creek was significantly greater at $P < 0.001$ compared with other treatments for both streams. However, no significant differences in total abundance were detected for other treatments.

In terms of taxon richness, in both streams there was a slightly more diverse and richer fauna was recorded in original vegetation (51 taxa) compared with both revegetation (49 taxa) and sites with willows removed (47 taxa), but was lower in sites with willows present (39 taxa) (Fig. 2.3b). Based on PerMANOVAs results, the effect of treatments on invertebrate community assemblages differed significantly over seasons ($P < 0.001$), but did not vary over different sites or habitats (Table 2.3).

Table 2.2 Results of two-way ANOVAs on total abundance and taxon richness of aquatic invertebrates collected from Sixth and Deep Creeks (d.f. = degree of freedom; MS = mean squares; *** $P < 0.0001$; ** $P < 0.001$; *$P < 0.01$).

Source	d.f.	Taxon richness			Total abundance		
		MS	*F*	*P*	MS	*F*	*P*
Treatment (Tre)	3	19.53	2.551	0.092	6850.00	1998.000	<0.0001***
Site (Si)	1	6.25	0.024	0.880	609.50	177.800	<0.0001***
Season (Se)	3	20.61	2.693	0.081	6583.00	110.900	<0.0001***
Habitat (Ha)	2	1074.00	7.388	0.008*	644.90	19.500	0.0002 **
Tre x Si	3	40.74	0.108	0.953	223.80	65.260	<0.0001***
Tre x Se	9	9.75	1.274	0.322	4134.00	0.491	<0.0086*
Tre x Ha	6	37.80	0.125	0.991	744.70	22.520	<0.0001***
Se x Si	3	1840.00	7.179	0.012*	1239.00	20.880	0.0004 **
Se x Ha	6	386.60	2.658	0.071	1685.00	81.970	<0.0001***

Table 2.3 Results of two-way factorial PerMANOVAs on aquatic invertebrate community assemblages collected from Sixth and Deep Creeks (d.f. = degree of freedom; MS = mean squares; *** $P < 0.0001$; ** $P < 0.001$; *$P < 0.01$)

Source	d.f.	MS	*F*	*P*
Treatment (Tre)	3	0.875	5.826	0.0002 ***
Site (Si)	1	0.206	1.081	0.3496
Season (Se)	3	4.001	20.999	0.0002 ***
Habitat (Ha)	2	0.589	3.173	0.0004 ***
Tre x Si	3	0.538E-02	0.217	1.0000
Tre x Se	9	0.264	1.760	0.0002 ***
Tre x Ha	6	0.142	0.621	0.9994
Se x Si	3	0.152	0.800	0.7286
Se x Ha	6	0.184	0.990	0.4650
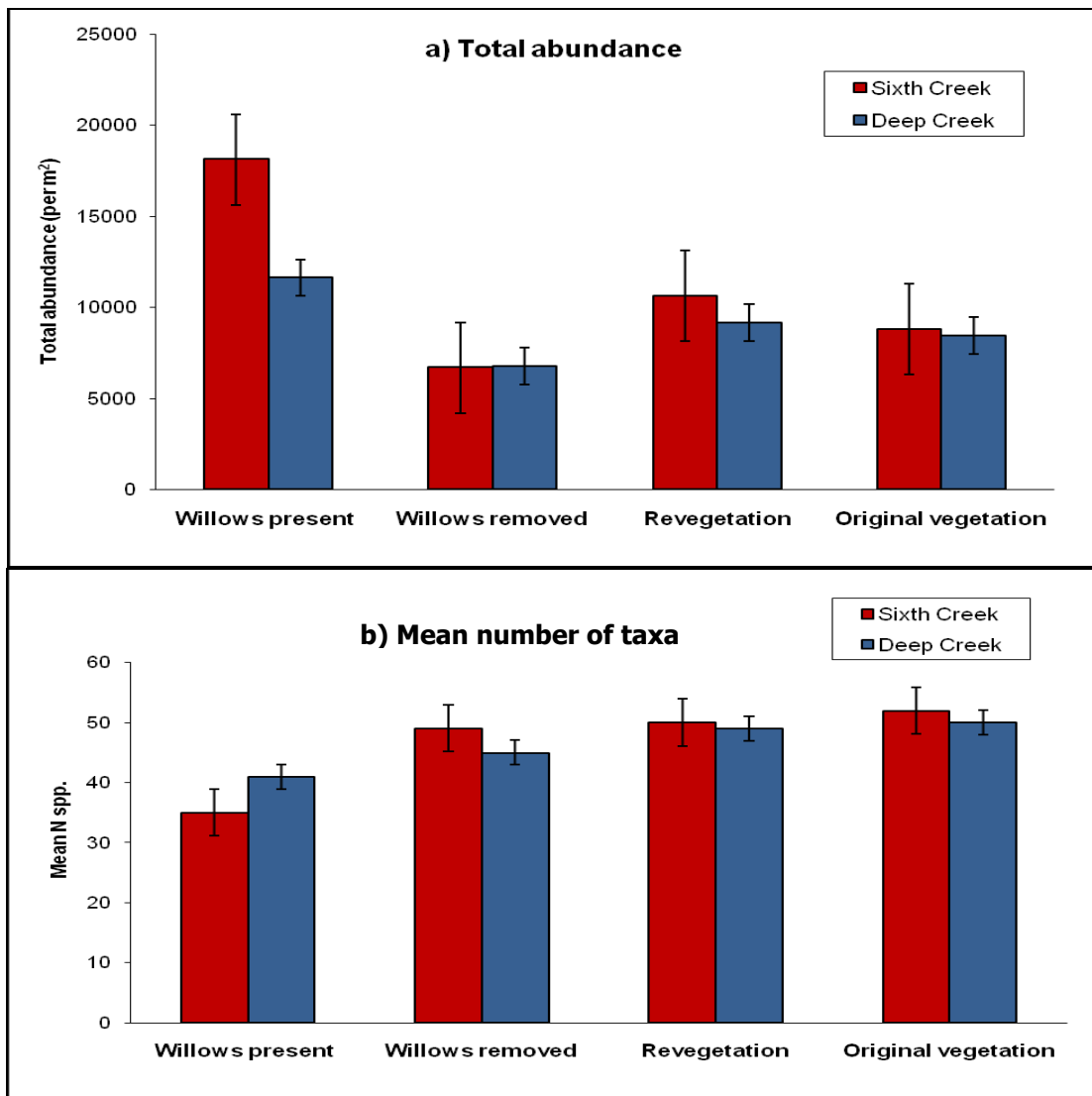
Fig. 2.3 Total abundance (a) and mean number of taxa (b) of aquatic invertebrate communities in four different treatments in Sixth and Deep Creeks.

Snails (Gastropoda) were the most dominant invertebrate, comprising almost half of the total abundance at each site (48.1%). They were numerous in all treatments in both streams. Plecoptera were the second most abundant invertebrate comprising 18.2% in Sixth Creek and 17.3% in Deep Creek, and Diptera were the third highest in both streams, although more were found in Deep Creek (14.6%) compared with Sixth Creek (10.6%). Similar trends in total abundance were observed in both streams for Trichoptera, Ephemeroptera and Coleoptera. However, fewer Odonata and Hemiptera were recorded in Sixth Creek than Deep Creek, but slightly more bivalves were found in Sixth Creek (1.2%) than in Deep Creek (0.8%). A number of minor groups, Oligochaeta, Amphipoda, Arachnida, Ostracoda, Hirudinea and Decapoda, were represented by less than 1.0% of the total abundance at each site. Collembola and Isopoda were only found in Sixth Creek.

The PCA case score plots indicate that the invertebrate community assemblages were divided into three main groups (Fig. 2.4). Revegetation (RV1 and RV2) and original vegetation (OV1 and OV2) samples from both sites were grouped together as Group 1. Group 1 was characterized by high relative abundances of physid snails (*Physa acuta*), planorbiid snails (*Isidorella* sp., *Glytophysa* sp.), lymnaied snails and gripopterygid stonefly nymphs (*Dinotoperla evansi*) (Fig. 2.4). Group 2 comprised mainly samples of willows present (WP1 and WP2) and willows removed (WR1 and WR2) from both sites (Fig. 2.4). The most abundant species found in these treatments were hydrobiid snail (*Potamopyrgus antipodarum*), leptophlebiid mayfly nymphs (*Illiesoperla mayii*), caenid mayfly nymphs (*Tasmanocoenis tillyardi*), and coenagrionid damselfly nymphs. Interestingly, riffle habitat samples WP1, WP2 and WR2 were clearly separated from the other groups (Group 3). This group was characterized by high abundance of elmid beetle larvae (*Simsonia leai*), conoesucid caddisfly nymphs (*Lingora aurata*) and hydrobiosid caddisfly nymphs (*Taschorema evansi*).
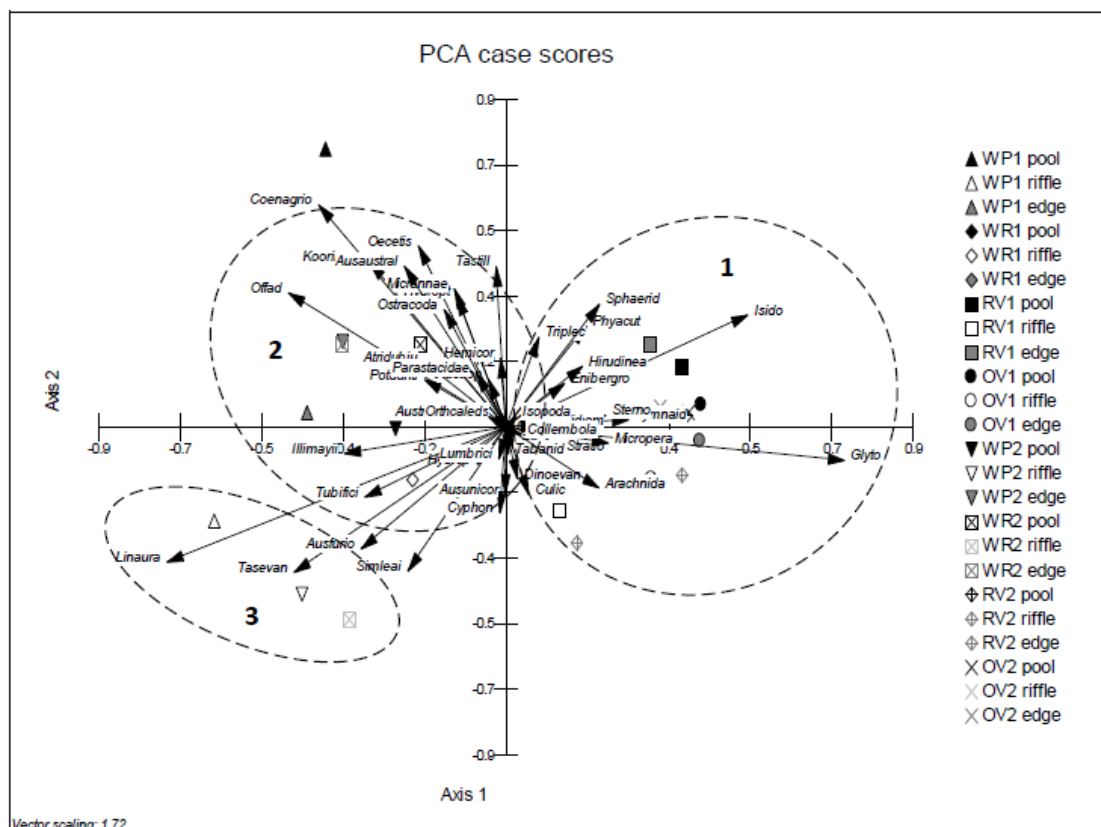
Fig. 2.4 Principal component analysis (PCA) case scores showing taxa assemblages associated with treatments in each habitat. (Note: the three dotted circles represent three main groups which indicate the degree of association between invertebrates, treatments and habitats). See Appendix I for taxa codes.

The introduced New Zealand hydrobiid snail (*P. antipodarum*) was the most dominant taxon overall and was significantly more abundant under willows in both streams (26,433 individuals). Other commonly collected taxa included the introduced physid snails (*Ph. acuta*), gripopterygid stonefly nymphs (*D. evansi*), midge larvae (Chironomidae), mayfly nymphs (*T. tillyardi*) and water bugs (*Enithares bergrothi*) which were mostly abundant under willows treatments in both streams, but this pattern was not statistically significant. Planorbiid snails (*Isidorella* sp.), baetid mayfly nymphs (*Offadens* sp.), elmid beetle larvae (*Si. leai*), hydrobiosid caddisfly nymphs (*Ta. evansi*) and damselfly nymphs (Coenagrionidae) were significantly more abundant under willows in Sixth Creek. The abundance of blackfly larvae (*Austrosimilium furiosum*) and amphipods (*Austrochiltonia australis*) was significantly greater when willows were present in Deep Creek. The freshwater snails *Glyptophysa* sp. and Lymnaiedae, and *Koornonga inconspicua* (Leptophlebiidae) were significantly more abundant in sites lacking willows.

Aquatic invertebrates showed marked variation in functional feeding groups among the different treatments in both streams (Fig. 2.5a-d). Using the criteria of Pennak (1978), Merritt and Cummins (1996) and Gooderham and Tsyrlin (2002), of a total of 51 taxa, approximately one third (21 taxa) were categorized as collector-gatherers. Other taxa were considered as predators (18), scrapers (9), or shredders (10) and only four taxa were categorized as collector-filterers (see Appendix I for details). It should be noted that some of the taxa can be included into two or three functional feeding groups (e.g. *Oecetis* sp., *Si. leai, Paramerina* sp.). Scrapers were about twice as abundant under the willows in Sixth Creek compared with Deep Creek and were the most abundant group in each treatment (Fig. 2.5a-d). Scrapers were dominated by *P. antipodarum* in all treatments in both streams ($P < 0.001$). The abundance of collector-gatherers was also significantly greater in willow sites than in other treatments in both sites ($P < 0.05$). However, the abundance of predators, shredders and collector-filterers appeared unrelated to all treatments in both streams.
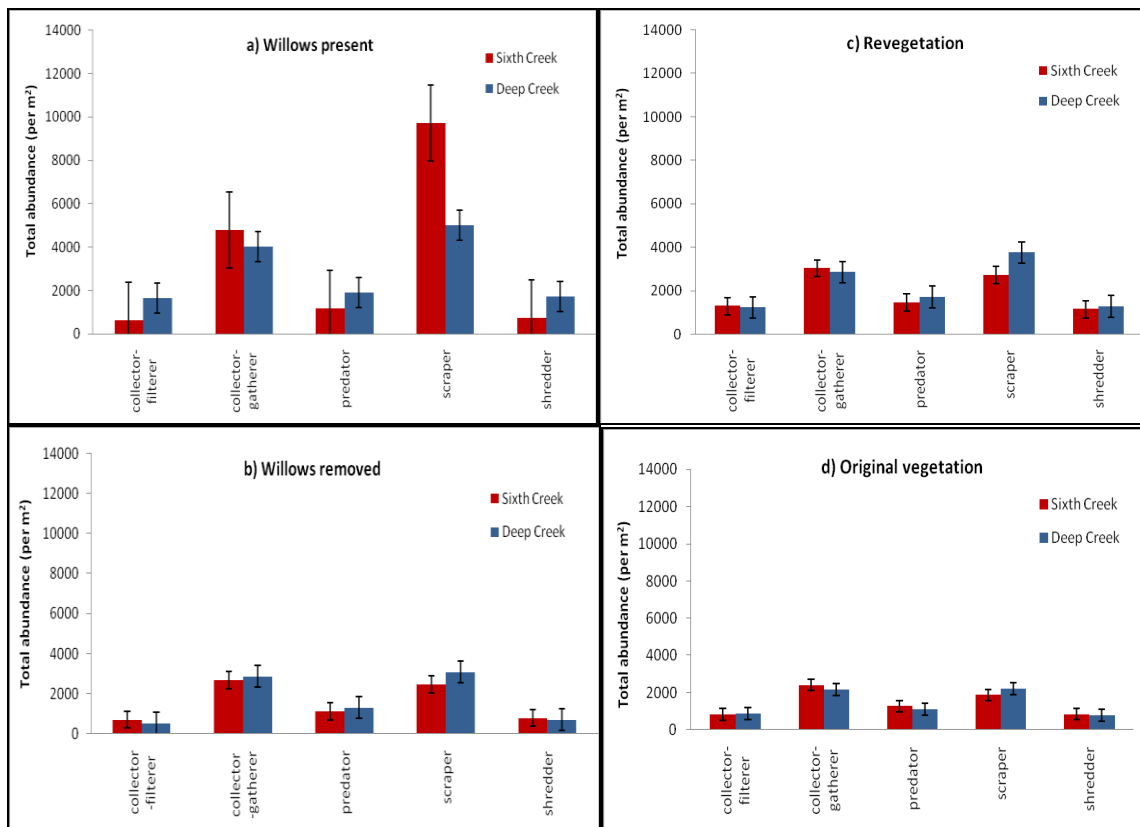
Fig. 2.5 Total abundance of functional feeding groups of aquatic invertebrates in four different treatments in Sixth and Deep Creeks (Note that *Potamopyrgus antipodarum* contributed more than half of the total abundance of scrapers; Fig. 2.5a).

2.3.2 *Seasonal patterns*

There were significant differences among different seasons for the total abundance of invertebrates across different treatments, habitats and sites ($P < 0.001$) (Table 2.2). However, seasonal differences in taxa richness were not significant even though invertebrates showed some seasonal differences with summer, autumn and spring have richer fauna than winter. Relatively high numbers of aquatic invertebrates (23,224 individuals and 51 taxa) were collected during summer in both streams. However, aquatic communities decreased to 18,983 individuals and 48 taxa in autumn and 16,732 individuals and 34 taxa in winter. Numbers recovered slightly in spring with 17,744 individuals and 47 taxa. PerMANOVA analysis shows that the effect of seasons on aquatic invertebrate assemblages in both streams varied significantly during different seasons and treatments (Table 2.3).

Generally, most taxa of the major taxonomic groups showed similar seasonal patterns of abundance (Fig. 2.6a-b) with high numbers during summer and autumn, and lower numbers in winter and spring. There was a significant increase in the abundance of *P. antipodarum, Isidorella* sp., *Glytophysa* sp., *Ph. acuta* and lymnaieds in summer and autumn compared with winter and spring. *Potamopyrgus antipodarum* was relatively abundant in both streams during summer and autumn, but their abundance fluctuated more in Sixth Creek compared with Deep Creek. Similarly, beetles had a significantly greater diversity in summer in both streams. Some dystiscid (*Platynectes decempunctatus* and *Sternopriscus* spp.), elmid (*Si. leai*) and hydrophilid beetle populations increased significantly in summer. Larvae of the stonefly *D. evansi* were significantly more abundant in both streams only in winter. Their populations peaked in July but declined to much lower densities in summer. Seasonal patterns in the total numbers of Diptera, especially chironomids, were similar in both streams with peak abundances in spring and summer (Fig. 2.6a-b). There was a negative correlation between chironomids and hydrobiid snails as the densities of chironomids were only high when the introduced hydrobiid snails were at low levels. The numbers of Ephemeroptera, Trichoptera and some species of odonate larvae (e.g., *Austroaeschna unicornis*) increased markedly during spring and early summer but were very low in winter.

Some functional feeding groups were particularly abundant at certain seasons. However, no significant differences were detected between seasons and abundance of functional feeding groups at any sampling site. Scrapers (mostly *P. antipodarum*) were the most abundant group in most seasons. Collector-gatherers (mostly ephemeropterans, plecopterans and trichopterans) were most abundant in spring and summer. In summer, predators, shredders and collector-filterers were significantly greater in number under the willows site in Deep Creek than Sixth Creek. In autumn, most of the groups were greater in abundance in Deep Creek compared with Sixth Creek, except for scrapers, which were greater in Sixth Creek. Most groups were significantly lower in winter, except for scrapers and collector-gatherers which were quite abundant in both streams.
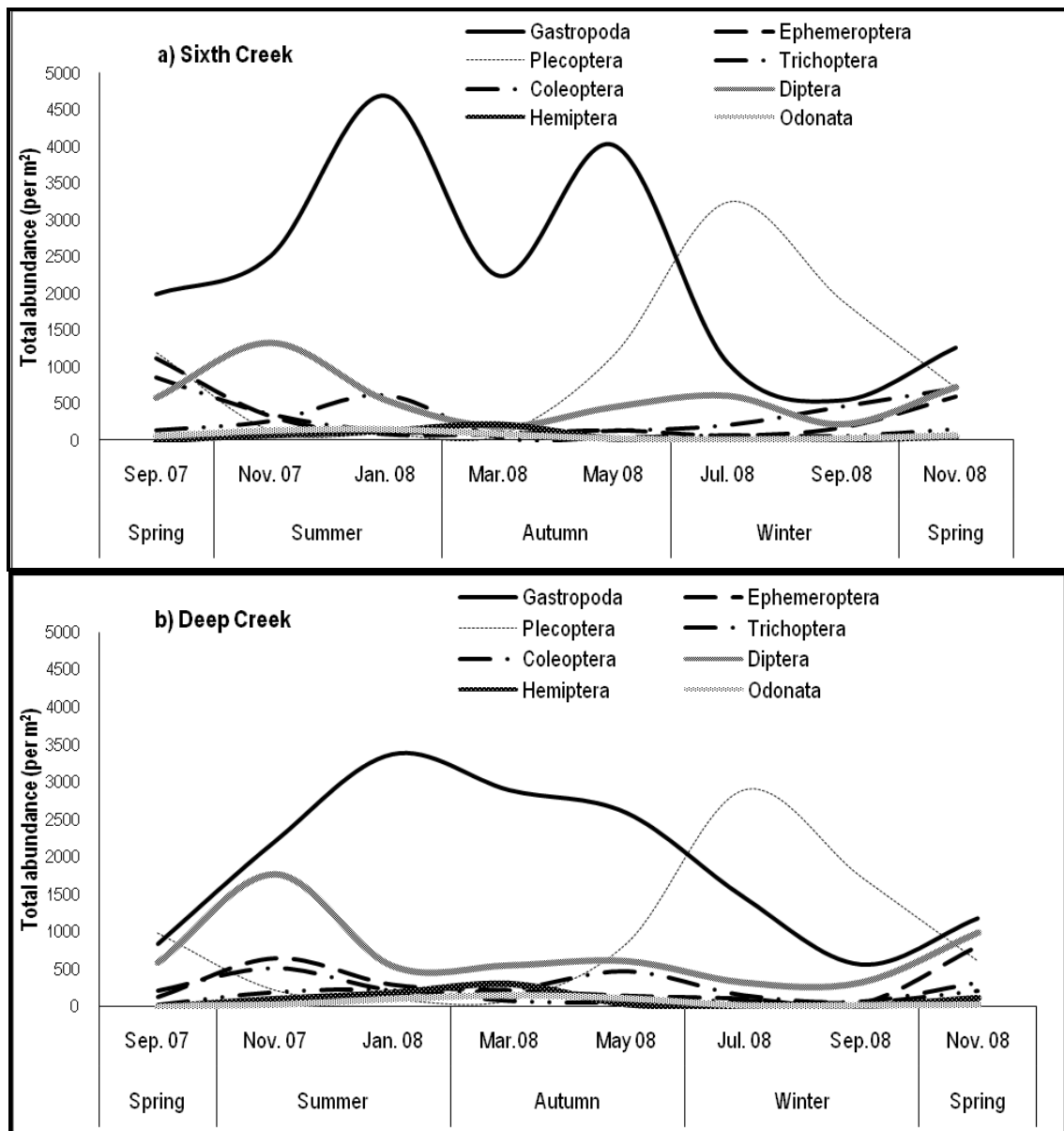
Fig. 2.6 Seasonal abundances of major groups of aquatic invertebrates in (a) Sixth and (b) Deep Creeks (Note that only major groups with more than 100 individuals are included).

2.3.3 *Effect of habitats*

In terms of habitat preference, there were significant differences in total abundance of invertebrates among riffles, edges and pools over different treatments, seasons and sites ($P < 0.001$) (Table 2.2). Further analysis using *t*-test showed that higher abundances of invertebrates were found under willows in Sixth Creek in pools and riffles than in the open edge habitat ($P < 0.01$). However, there were no significant differences in total abundance in the willows present site in Deep Creek, and even more invertebrates were found in

riffles than in pool and edge habitats. Where willows have been removed, the abundance of invertebrates was significantly reduced in both streams, but no difference was observed among riffle, edge and pool habitats for each stream. Similarly, habitat had a significant effect on total abundance in revegetation and original vegetation treatments in both streams. More invertebrates were collected in riffle habitat in revegetation sites than in pool and edge habitats. In contrast, invertebrates were more likely to be found in edges in original vegetation sites than in both pool and riffle habitats in both streams.

The effect of habitats on taxa richness and invertebrate assemblages did not show significant variation among treatments within different seasons and sites (Tables 2.2 and 2.3). Larger and richer invertebrate communities were collected in each habitat in original vegetation and revegetation treatments in Sixth Creek. A similar pattern was also observed in Deep Creek, where high numbers of taxa were collected particularly in edge and riffle habitats in the site with original vegetation. Quite low taxon diversity was observed in all habitats where willows were removed in both streams. The taxon richness was drastically reduced in each habitat where willows were present in both streams, especially in the riffle habitat. In terms of invertebrate assemblages, the riffle habitat of treatments samples of WP1, WP2, WR1 and WR2 were grouped together (Group 3) and clearly separated from other habitats of other treatments (Fig. 2.4).

In contrast, the two-way cluster analysis indicated two main groups (Fig. 2.7). Pool, riffle and edge samples of revegetation (RV1 and RV2) and original vegetation (OV1 and OV2) treatments forming one group (Group 1). All habitat samples of willows present (WP1 and WP2) and edges samples of willows removed (WR1 and WR2) forming the other group (Group 2). In Group 1, pools, riffles and edges samples of revegetation (RV1 and RV2) and original vegetation (OV1 and OV2) treatments were well separated from pools and riffles of willows removed samples (WR1 and WR2) (Fig. 2.7). Relatively higher abundance of hydroptilid caddisfly nymphs and coenagrionid damselfly nymphs were found in the samples of pools and riffles of WR1 and WR2. In Group 2, riffle samples of WP1 and WP2 were well separated from other habitats of WP1 and WP2 treatments (Fig. 2.7). Riffle samples of WP1 and WP2 were grouped together as *Si. leai*, *L. aurata* and *Ta. evansi* were abundant in that particular habitat. Also, higher densities of *Cyphon adelaidae*, *I. mayii* and tubificids were detected in the riffles of WP1 and WP2 than in other habitats.
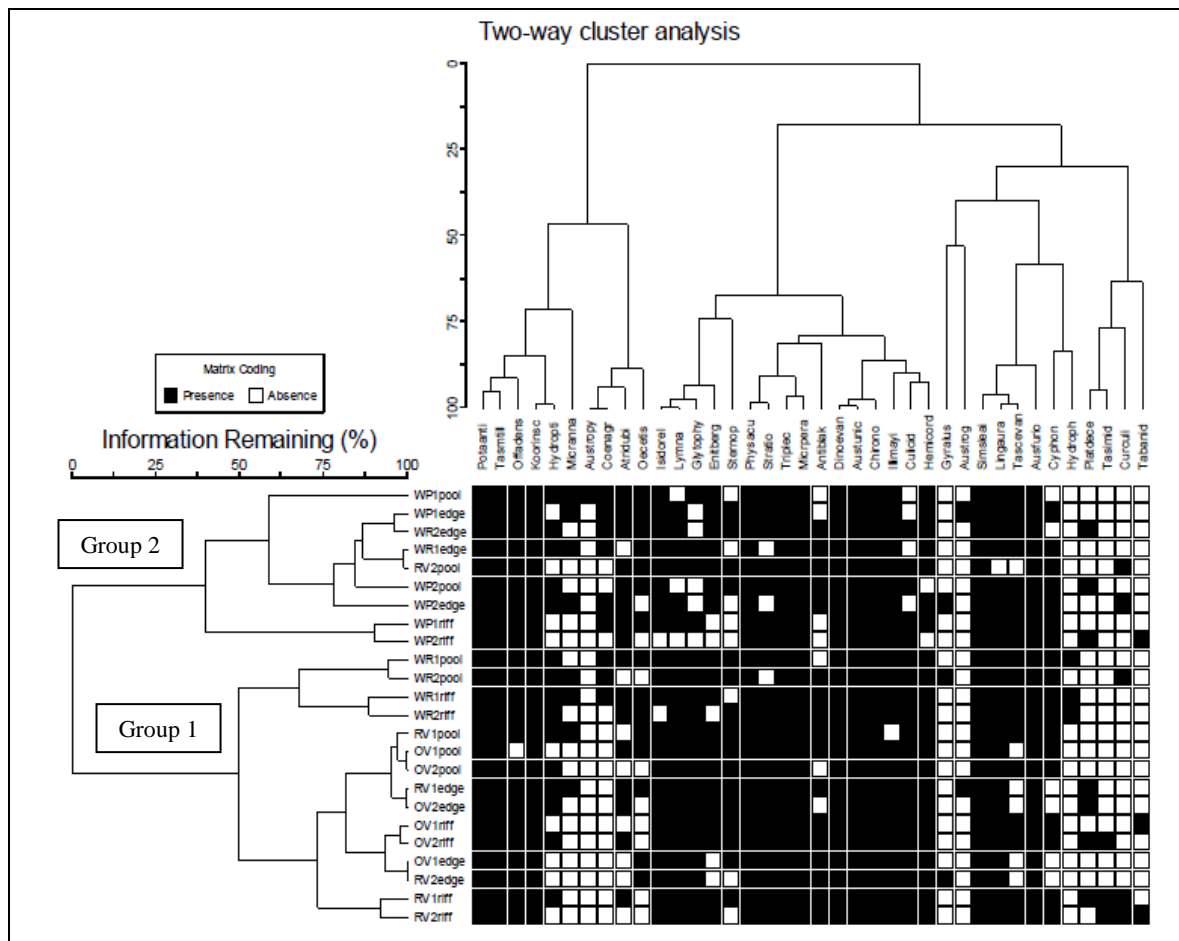
Fig. 2.7 Dendrogram using Sorensen's Coefficient method for two-way clustering analysis of generic composition in pool, riffle and edge habitats in each treatment. See Appendix I for taxa codes.

2.3.4 *Influences of physico-chemical parameters*

Generally, the physico-chemical parameters recorded were similar in both streams (see Table 2.1 & Appendix III for more details). Only conductivity, total dissolved solids and shade coverage were significantly different between treatments, seasons and sites. No significant differences were detected among treatments in any season for stream depth and width, dissolved oxygen, pH, water temperature and flow rate.

With pH ranging from 7.2 to 10.0, the water in both streams was range from circumneutral to alkaline. There were minor seasonal changes, although the pH was lower in Deep Creek than Sixth Creek. Dissolved oxygen content was quite variable (8.05–14.60 mg/L) in both streams. In summer, dissolved oxygen levels were lower in Sixth Creek than in Deep Creek. In winter, dissolved oxygen levels were two times greater in Sixth Creek than in Deep Creek. The water flow, especially where willows were present in both

streams, was slow flowing to almost still in most seasons. However, no significant differences between treatments and seasons were detected for flow rates in both streams, although the water flow was lower in summer than other seasons. Correspondingly, the electrical conductivity (477–669 µS/cm) and total dissolved solids (248–345 ppm) were significantly higher in autumn in both streams. Shade cover was significantly higher in summer where willows were present, but much lower in autumn and winter in both streams.

Based on Spearman Correlation Analysis (Table 2.4), depth and width of the streams were positively correlated with total invertebrate abundance, whereas lower invertebrate abundance was found in fully shaded area. In contrast, the taxon richness was greater in more turbid water, with high conductivity, total dissolved solids, water temperature, and flow rates in both streams. Five dominant species (*P. antipodarum, T. tillyardi, D. evansi, L. aurata* and chironomids) were chosen in order to understand the effect of physico-chemical parameters (Table 2.4 & Fig. 2.8). Depth and width of streams, dissolved oxygen and shade cover showed no correlation with the most dominant species. *Potamopyrgus antipodarum, T. tillyardi* and chironomids flourished in water of high conductivity and total dissolved solids, whereas *D. evansi* and *L. aurata* were reduced in water of high conductivity, total dissolved solids and water temperature (Fig. 2.8). *Tasmanocoenis tillyardi* and chironomids were significantly more abundant in slow flowing water, but *D. evansi* and *L. aurata* were significantly more abundant in fast flowing water.

Further analysis using DCA shows that only four parameters were significantly influenced to the invertebrate assemblages and species variation (Fig. 2.9). Axis 1 represents increased water width, pH and total dissolved solids, whereas Axis 2 shows increased dissolved oxygen. However, water depth decreased along Axis 2. Similar results were found in DCA, where *D. evansi* and *L. aurata* increased along Axis 1. The two dominant species were highly correlated to the width of water, total dissolved solids and pH. Other species which show positive correlations to these parameters were *Ta. evansi, Si. leai, Au. furiosum* and tubificids. However, some species such as *Micronecta annae, Oecetis* sp., coenagrionids and *Austropyrgus* sp. decreased along Axis 2. It shows that the deeper the water and the lower the dissolved oxygen, the fewer of these invertebrates they were.

Table 2.4 Spearman's-rho correlation analysis between physico-chemical variables, total abundance, taxon richness and five dominant aquatic invertebrate species in Sixth and Deep Creeks (**Correlation is significant at the 0.01 level; *Correlation is significant at the 0.05 level).

	Total abundance	Taxon richness	*Potamopyrgus antipodarum*	*Dinotoperla evansi*	*Tasmanocoenis tillyardi*	*Lingora aurata*	Chironomidae
Depth (m)	0.258**	0.038	0.074	0.201**	0.019	0.045	-0.036
Width (m)	0.230**	-0.057	0.039	-0.070	-0.032	0.040	0.073
Dissolved oxygen (mg/L)	-0.059	0.054	-0.021	0.122	-0.053	0.040	-0.121
Conductivity (μS/cm)	0.108	0.385**	0.333**	-0.405**	-0.190**	-0.163*	0.212**
pH	0.064	0.053	0.053	0.470	-0.111	0.201**	-0.372**
Total dissolved solid (ppm)	0.108	0.402**	0.332**	-0.420**	-0.214**	-0.142*	0.233**
Water temperature (^{0}C)	-0.016	0.295**	0.060	-0.640**	-0.273**	-0.191**	0.446**
Shade cover (%)	-0.224**	-0.055	-0.257**	0.198**	-0.129	-0.390	-0.046
Flow rate	0.023	0.324**	0.141	0.630**	0.185*	0.360**	-0.240**
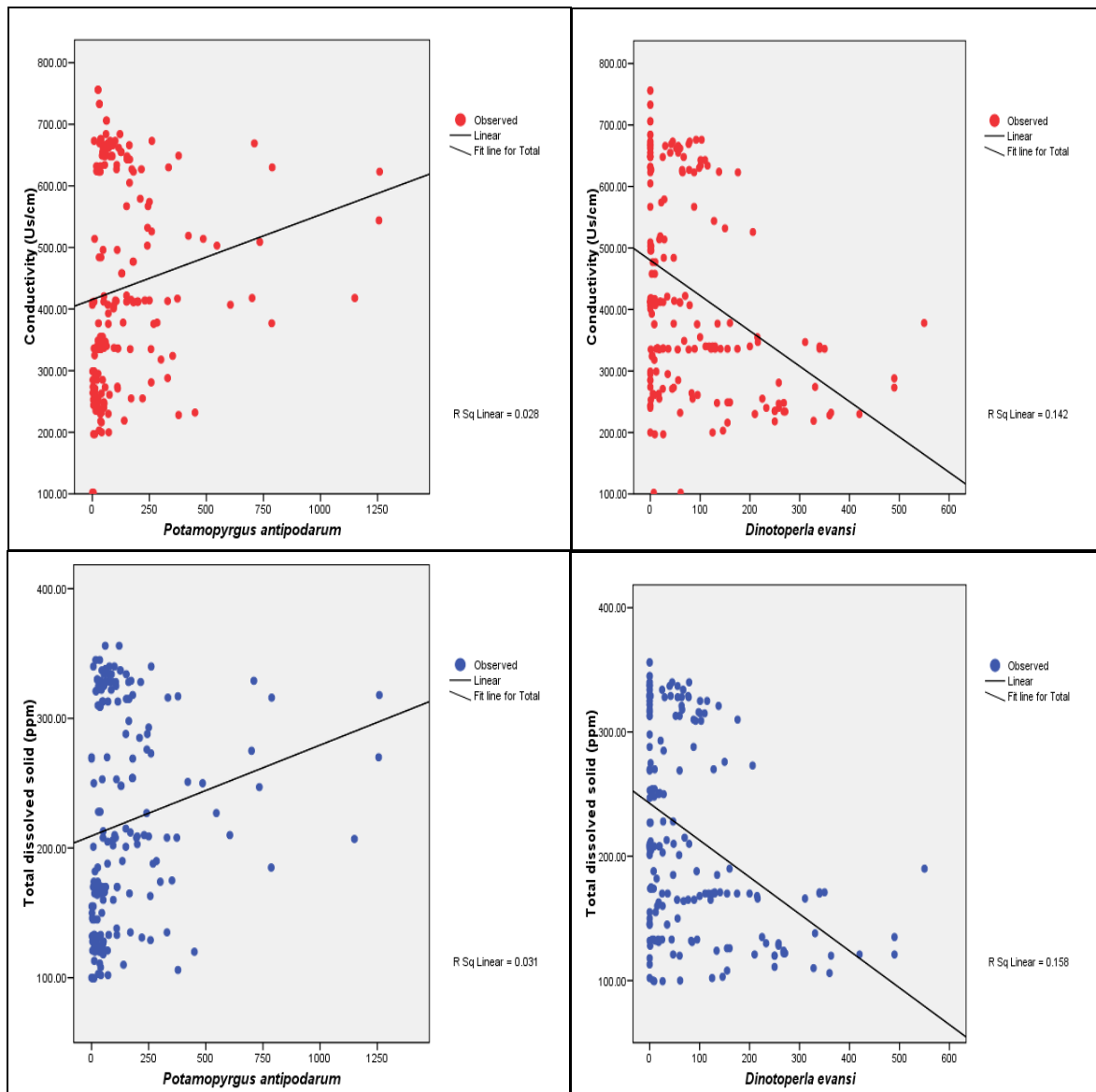
Fig. 2.8 Partial regression plots between densities of two dominant taxa and two water quality parameters. (Note: *P. antipodarum* and *D. evansi* were chosen as examples as both species showed strong correlation to water of high conductivity and total dissolved solids).
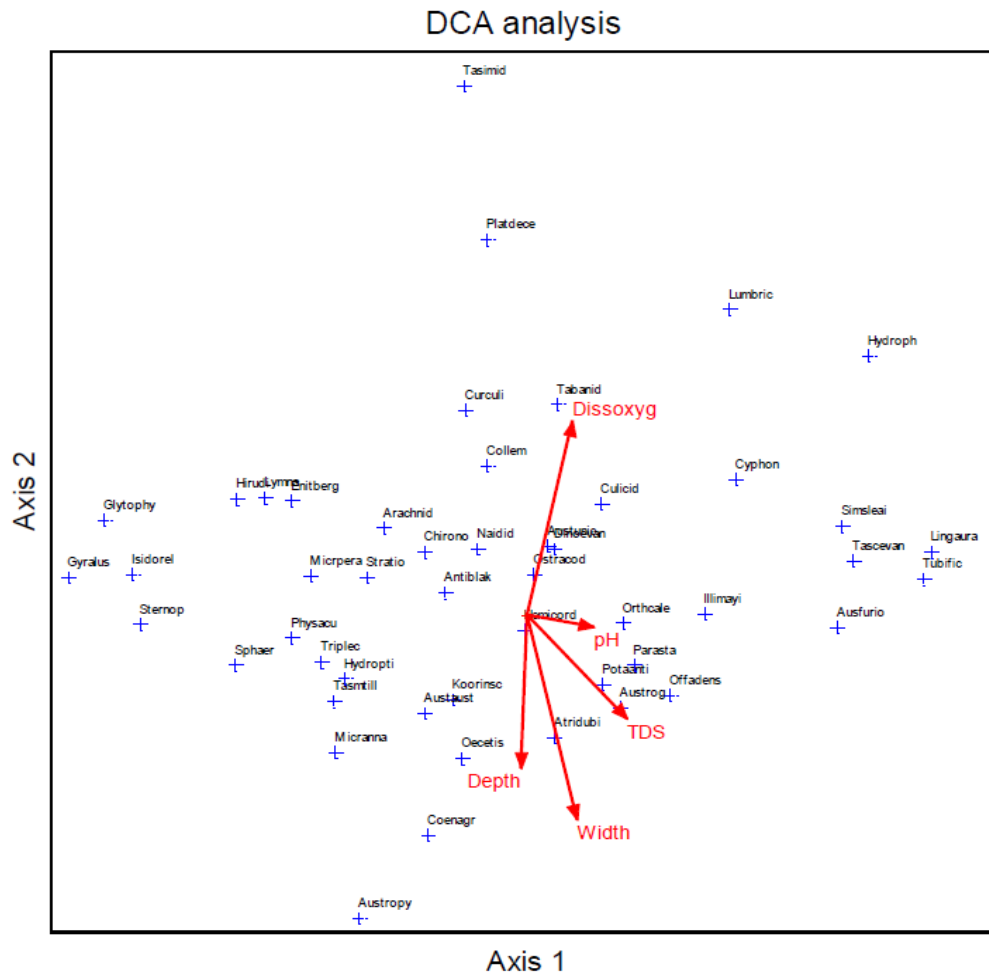
Fig. 2.9 Detrended correspondence analysis (DCA) plots showing taxa assemblage ordinations which related to some environmental variables. (Note: Dissoxyg = dissolved oxygen; TDS = total dissolved solids; Width = stream width; Depth = stream depth). See Appendix I for species codes.

2.4 Discussion

Overall, our results indicate that the presence of willows along the riparian zones of Sixth and Deep Creeks had a negative impact on the taxon diversity of the aquatic invertebrate communities. Initially, we hypothesized that lower abundance, taxon diversity and fewer functional feeding groups would occur in those treatments where willows were present and when they had been removed. Unexpectedly, invertebrates were significantly more abundant in both sites where willows were present, whereas, lower abundance and taxon diversity were recorded in the willows removed treatment at both sites.

More invertebrates were collected in Sixth Creek than in Deep Creek (Fig. 2.3a). This may be due to water availability as the former creek is permanent and flows all year round, whereas the latter is more ephemeral and dries up in some years (K. Warner, pers. comm.). Water balance, temperature tolerances, respiratory system and mode of movement of aquatic invertebrates are all adaptations for living underwater (Merritt & Cummins 1996). When Deep Creek dries up, the aquatic invertebrate communities face the challenge to survive and this would presumably contribute to lower taxon abundance and richness in Deep Creek. However, taxon richness of the aquatic invertebrate communities in Sixth and Deep Creeks were similar and no consistent differences were detected (Table 2.2). Interestingly, treatments had a significant effect on the total abundance, taxon richness and invertebrate community assemblages across different habitats, seasons and sites (Tables 2.2 & 2.3; Fig 2.3a-b).

This suggests that there may be some physical or chemical mechanism(s) that might explain these differences in invertebrate assemblages in the different treatments. We suspect that willows may provide more habitat heterogeneity, especially for the scrapers (mostly gastropods), than other treatments. The distribution and abundance of aquatic invertebrates has long been known to be associated with the heterogeneity of habitats (e.g. McKie & Cranston 2001; Rios & Bailey 2006; Walsh et al. 2007). A study by Hawkins *et al.* (1982) showed that habitats with soft organic bottoms and rooted aquatic plants represent high habitat heterogeneity in stream. High heterogeneity refers to physically complex substrate types which consist of leaves, gravel or cobble, macrophytes, moss and wood; which generally support more taxa than structurally simple substrates such as sand and bedrock (Hawkins *et al.* 1982; Collier *et al.* 1995).

We found the introduced hydrobiid snails were the most dominant taxon with about 50% of the total abundance under willows in both streams (Figs 2.5 & 2.6). Some New Zealand studies have shown that willows may have positive effects on invertebrates by providing a stable habitat among tree roots for large numbers of *P. antipodarum* (Winterbourn 1970) or trichopteran shredders (*Pycnocentria forcipata*) (Linklater & Winterbourn 1993). However, *P. antipodarum* has become a pest species in many parts of the world (e.g. Zaranko *et al.* 1997) and has rapidly invaded Australian freshwater systems (Ponder 1988). Several authors speculate that the establishment of this species may disrupt the physical characteristics of invaded ecosystems (Ponder 1988; Zaranko *et al.* 1997). A study in the western United States reported that the invasion of this snail may impact the food chain of native trout and concluded that *P. antipodarum* is a potential competitor with native aquatic species of the streams (Richards *et al.* 2004). However, to date, little research has documented decreases in native invertebrate populations in Australian streams where *P. antipodarum* has invaded. Thus, this study is important to understand the possible indirect impact of willow presence on the reduction of native invertebrates mediated by a strong competition for habitat or food between *P. antipodarum* with native invertebrates.

In terms of taxon richness, a reduction in taxon number and diversity in both streams was clearly associated with the presence of willows (Fig. 2.3a-b). Similar findings have been reported by Pidgeon and Cairns (1981), Lester *et al.* (1994a), Schulze and Walker (1997) and Read and Barmuta (1999). However, New Zealand streams are often heavily cleared for pasture and retain little of the original riparian vegetation. The authors suggest a number of factors which result in a lower diversity of aquatic invertebrates being supported by willows than native vegetation, include decreased primary production due to shading, increased sedimentation, reduced water flow, and leaching of inhibitory chemicals from leaves, barks or roots. Detritus from willow leaves may also contribute to the lower densities, as willow detritus only provide a food source for a relatively short period of time and may not be fully utilized by aquatic invertebrates (Lester *et al.* 1994a). Jayawardana and Westbrooke (2010) found greater abundance of shredders in willow habitats in three Victorian streams. They suggested that the vegetation changes can bring about changes in material cycle and energy flow within these streams.

However, some taxa such as planorbiid snails (*Isidorella* sp.), baetids nymphs (*Offadens* sp.), elmid larvae (*Si. leai*), trichopterans (*Ta. evansi*), damselfly nymphs (Coenagrionidae),

blackfly larvae (*Au. furiosum*) and amphipods (*Aus. australis*) were significantly more abundant in the willow treatments in both streams in this study (Fig. 2.4). The high abundance of these taxa (mostly collector-gatherers and shredders) under willows are possibly due to elevated levels of coarse willow detritus. Their increase may also be due to an increase in coarse particulate organic matter (CPOM) in the shaded sites as we observed that water under willows had a higher CPOM content during summer and autumn samplings. Lester *et al*. (1994a) also found that organic standing stocks were greater in willowed reaches than a native woodland reach in autumn. Willows may therefore enhance invertebrate abundance by contributing readily processed litter and stimulating production through nutrients from leaf leachates (Glova & Sagar 1994; Lester *et al*. 1994a). Willow leaves also have been observed to be more palatable than most native leaves and support many taxa during autumn when willows shed most of their leaves (Yeates & Barmuta 1999; Lester *et al*. 1994b; Parkyn & Winterbourn 1997).

Higher invertebrate diversity was observed in both original vegetation and revegetation sites in both streams (Fig. 2.3b). Taxon diversity and richness in these sites were the highest compared with other treatments, indicating that original vegetation and revegetation treatments provide suitable habitat for a diverse community of aquatic invertebrates. The richer fauna in the original vegetation and revegetation sites is probably due to a sparse, open canopy which permits higher primary productivity and favours a more diverse growth of aquatic plants. Some native snails (*Glyptophysa* sp. and Lymnaiedae) were significantly more abundant in sites lacking willows (Fig. 2.4). River red gum (*E. camaldulensis*) and white gum (*E. viminalis*) dominate these sites, and the presence of these snails may be due to the presence of diatoms and microflora on decaying leaves of these eucalypts as found by Schulze and Walker (1997). Most predator taxa (e.g. dragonfly nymphs) had higher densities in these treatments (Fig 2.5c-d). More diverse growth of riparian/aquatic vegetation and more complex substrate types (gravel/cobbles, macrophytes) are thought to provide additional sources of food and shelter (Collier 1995) in original vegetation and revegetation treatments. Increases in habitat heterogeneity increase taxon richness and abundance of invertebrates and may provide suitable habitat for reproduction, protection from predators, and food supply for many taxa (Minshall 1984). Furthermore, leaves of native species (especially *Eucalyptus*) are often only consumed by invertebrates after a period of conditioning in the stream. As eucalypts drop leaves continually, they provide a more or less continuous food resource for aquatic

invertebrates which have presumably adapted to this pattern of leaf drop (Schulze & Walker 1997). This could explain why a more diverse and richer fauna was generally observed in both original vegetation and revegetation sites compared with willows.

In contrast, slightly lower numbers of invertebrates were recorded in sites where willows had been removed in both streams (Fig. 2.3a-b). The sudden removal of willows without subsequent revegetation and little plant growth, apart from a few weeds, led to a reduction in the abundance and diversity of aquatic invertebrate communities, and may be due to loss of habitat and also to decreasing substrate size. Degradation of the substrate can be devastating to invertebrate communities, as decreased substrate size is generally associated with a decrease in invertebrate abundance (Minshall 1984). Each habitat sampled in willows removed treatments included areas of slow moving water with patches of fine sand and silt. Most pollution tolerant invertebrates such as oligochaetes, snails and midge larvae, can survive in these areas (Hellawell 1986). The high abundance of these taxa indicates strongly that they are tolerant of or have adapted to live in stressed environments. This finding supports that removal of willows without revegetation has had a detrimental impact on taxon diversity. This could also result in accelerating bank erosion allowing more organic pollution-tolerant taxa to be established.

Seasonal changes significantly influenced the total abundance and invertebrate community assemblages, but no statistically significant differences were detected between taxon richness and season even though the invertebrate densities showed seasonal patterns of abundance (Tables 2.2 & 2.3). Clearly, there is a richer invertebrate fauna in summer and autumn than winter and spring, probably because the streams experienced more disturbed conditions due to increased water flow during the latter two seasons. Rainfall events which could initiate washout and catastrophic drift could result in a decline in invertebrate richness during winter and spring.

A similar seasonal pattern was observed for total abundance with higher numbers recorded during summer and autumn (Fig. 2.6a-b). This could be due to elevated stream temperatures. It is known that this may affect physiological processes such as growth rate, attainment of sexual maturity, reproduction and associated behaviour (Hellawell 1986).

During summer, dense willow canopies reduce the penetrability of light to banks and watercourses. Presumably, lower light intensity can reduce primary production by diminishing algal growth which in turn may reduce secondary production and change invertebrate

communities (Lester *et al.* 1994a; Schulze & Walker 1997). Dense shade does not allow the light required for the production of high quality food for invertebrates (Bunn *et al.* 1993). In autumn, when willows provide little shade, water temperature and epilithic biomass are usually higher than in summer or spring. Fallen willows leaves and fibrous root mats also trap silt and reduce the water velocity to a series of nearly isolated pools where dissolved oxygen levels were very low in autumn. We found willow-lined reaches had lower dissolved oxygen only during summer and autumn (see Appendix III). However, dissolved oxygen within this range does not seem to influence the distribution of the invertebrates.

The massive fall of willow leaves in autumn can lead to high nutrient pulses, and sudden changes of water temperature due to higher light intensity may also reduce the invertebrate community composition and abundance (Lester *et al.* 1994a). Interestingly, no such relationships were observed in this study. We found significant increases in the abundance of gastropods, chironomids and beetles in summer, but their numbers were lower in other seasons (Fig. 2.6a-b). Amphipods, ostracods and hydracarina were also abundant in summer. The high amount of organic matter and high water temperature during the low summer flows also favoured some predators (e.g., *Austroaeschna unicornis*). Organic nutrients probably have a direct link with food supply in the aquatic food chain that leads to numerical increases in some prey taxa such as chironomid, Ephemeroptera and mosquito larvae.

The most obvious seasonal difference was the change in the degree of shading during the year which was reflected in the differences in relative abundance of functional feeding groups (Fig. 2.5a-d). We found most feeding groups were less abundant during autumn in both streams. However, quite high numbers of some collector-gatherers and shredders such as *D. evansi, L. aurata* and *T. tillyardi, w*ere recorded during autumn. A similar pattern of seasonal influence on relative abundance of functional feeding groups was also observed during spring, but more collector-gatherers were found in spring compared with other groups. Consistently, collector-filterers also increased in numbers as they may benefit from an increase in fine particulate organic matter caused by the activities of shredders and collector-gatherers. Filterers (e.g., chironomids and blackfly larvae) and some collector gatherers (e.g., oligochaetes and amphipods) were more abundant in willow sites in shaded areas. It has been suggested that this is a result of more 'clean' attachment sites being available in shaded areas (Casey & Ladle 1976). Usually, a lower abundance was found in shaded areas due to lower availability of quality food such as algae and algal detritus (e.g., Hawkins *et al.* 1982; Behmer

& Hawkins 1986). Thus, we suspect that less competition among invertebrate communities occurred in shaded areas, leading to a higher abundance of filterers and collector-gatherers in shaded willow sites.

Generally, abundance of all taxa was lowest in winter due to heavy rainfall and flooding which probably washed them downstream. However, we found *D. evansi* nymphs were significantly more abundant only in winter in both streams (Fig. 2.6a-b). Their population peaked in winter, especially in July, and declined to their lowest density in summer. This is probably due to the emergence of many nymphs in summer since many young nymphs were collected during this season. Nymphal exuviae of this species were found hanging on marginal vegetation along the streams during summer.

We also found a negative correlation between chironomids and *P. antipodarum* in many seasons (Fig. 2.6a-d). There may have been food competition between *P. antipodarum* and chironomids as more fine particulate organic matter were observed during summer and late autumn. However, any relationship that might exist is unclear as there is no evidence to indicate that *P. antipodarum* has any direct impact on chironomid assemblages.

There were significant differences in total abundance among pool, riffle and edge habitats across different treatments, seasons and sites (Tables 2.2 & 2.3). However, habitats themselves had no effect on species richness and invertebrate community assemblages (Tables 2.2 & 2.3). Species assemblages in pools, riffles and edges of revegetation (RV1 and RV2) and original vegetation (OV1 and OV2) treatments were clustered together (Figs 2.7 & 2.9) due to similar habitat availability, food sources and physico-chemical characteristics. As a generalisation, more Ephemeroptera, Plecoptera and Trichoptera were collected in open riffle and edge habitats under original vegetation and revegetation treatments in both streams. Their nymphs and larvae require high oxygen levels, often associated with fast running water (Merritt & Cummins 1996). However, no significant correlation was detected between the invertebrate abundance and dissolved oxygen (Table 2.4).

In contrast, under willows treatment (WP1 and WP2), higher numbers of *P. antipodarum* were found in pools than in either riffle or edge habitats. Their high numbers in pools is because they show a preference for still and slow moving water. The diversity of all gastropods (scrapers) tended to be higher in slow (pools and edges) than fast currents (riffles) as slow moving water provides a stable habitat for scrapers and increased food availability. Besides, the dense shallow root mats of willows are presumed to provide more stable shelters

for many invertebrates from high currents compared with other treatments. Similar results were found by Jayawardana *et al.* (2006) where amphipods and *P. antipodarum* were commonly associated with willow root habitats. Suter (1990) suggested that concentrated leaf matter, rapid breakdown of leaves and limited flushing of organic material under willows, favours certain pollution tolerant invertebrates such as Oligochaetes, *Chironomus* sp., *Heterias* sp. and *Ph. acuta*. Willows not only provide habitat availability, but also food availability and thereby the species composition it supports.

Riffle habitat samples in willows sites and where willows had been removed (WP1, WP2 and WR2) were grouped together (Fig. 2.4) because of the highly abundant of elmid beetle larvae (*Si. leai*), conoesucid caddisfly nymphs (*L. aurata*) and hydrobiosid caddisfly nymphs (*Ta. evansi*). However, it is hard to explain the effect of riffle habitat on the taxa assemblages due to seasonal inconsistencies of total abundance especially in the WR2 treatment. Invertebrate abundance and taxon richness in each habitat in the willows removed treatment (WR) decreased markedly from summer to winter. This was probably due to loss of habitat due to several rainfall events, and leaf input (a potential food source) may consequently have been largely exported downstream. However, there was an increase in invertebrate abundance in pools and edges during spring in sites where willows have been removed. Presumably, there were more stable habitats (e.g., leaves, gravel or cobble, and macrophytes) and more suitable food in spring as conditioning of leaves to increase their palatability occurred during winter.

Usually, a large reduction in the extent of the riparian vegetation results in water temperatures which are higher in summer and lower in winter. Variations in temperature affects physiological processes resulting in changes in growth rate, reproduction, and behaviour (Merrit & Cummins 1996). Warmer water temperatures can lead to earlier emergence and high temperatures may result in lower dissolved oxygen concentrations (Resh *et al.* 1995). We also found that the sites where willows were removed were more turbid in summer and autumn, with higher conductivity and greater organic matter (see Appendix III). The total dissolved solids (248–345 ppm) were significantly higher in autumn compared with other seasons. Also, the removal of willows not only initiates erosion of the stream bed which reduces the stream's depth, water flow and water quality, but may also disrupt the invertebrate communities that utilize willow roots as habitat.

In conclusion, willows may support high numbers of aquatic invertebrates by providing a more stable and complex habitat and also providing a suitable food source (e.g., by producing

greater surface for colonisation by biofilms on willow leaves and roots). However, removal of willows without any subsequent revegetation resulted in lower species richness and abundance of aquatic invertebrates, suggesting that taxon richness and abundance will only recover when the riparian canopy is reinstated by suitable revegetation efforts.

In this study, taxa responsible for the significant differences observed in sites where willows are present or removed were identified. Taxa such as *P. antipodarum,* planorbiid snails (*Isidorella* sp.), baetid mayfly nymphs (*Offadens* sp.), elmid larvae (*Si. leai*), hydrobiosid caddisfly nymphs (*Tas. evansi*), dragonfly nymphs (Coenagrionidae), blackfly larvae (*A. furiosum*) and amphipods (*Au. australis*) were significantly more abundant under willows vegetation. However, only two species of native freshwater snails (*Glyptophysa* sp., lymnaeid snails) and leptophlebiid mayfly nymphs (*K. insconspicua*) were significantly more abundant in sites lacking willows.

This present study has provided information on the changes in aquatic invertebrate communities that might take place when the original riparian vegetation changes to willow; and when willows were removed without revegetation. It is also inferred that large scale willows removal may require special management considerations particularly in small and shallow streams to reduce the impact on aquatic invertebrates. These would include of site specific willow management strategies which consider variety of factors such as the ecological characteristics of the water body (e.g., invertebrates and fish assemblages, primary productivity, etc.) and the physico-chemical conditions of the water body (e.g., bank stability, water temperature, flows, depth and size, etc.)

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CHAPTER 3: Feeding preference, survival and growth of aquatic invertebrates on crack willow (*Salix fragilis*) and white gum (*Eucalyptus viminalis*) leaves
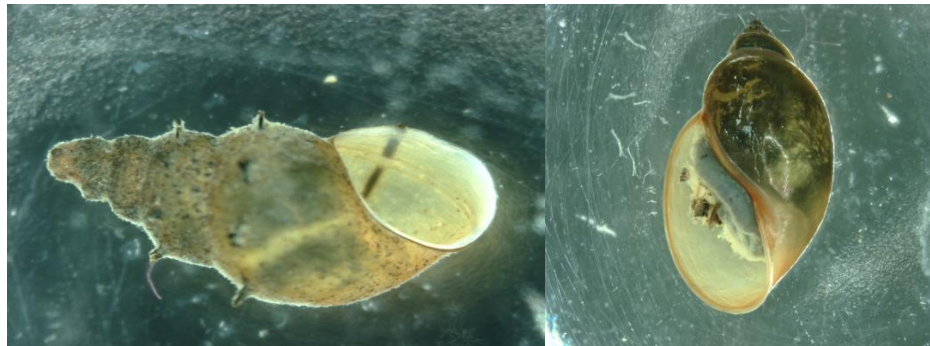
Potamopyrgus antipodarum
(Gastropoda: Hydrobiidae)

Physa acuta
(Gastropoda: Physidae)
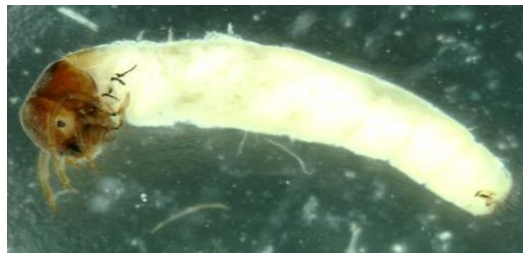
Lingora aurata
(Trichoptera: Conoesucidae)

Tasmanocoenis tillyardi
(Ephemeroptera: Caenidae)

Dinotoperla evansi
(Plecoptera: Gripopterygidae)

3.0   Abstract:

This study was initiated to investigate whether willow leaves can provide a food source for introduced and native aquatic invertebrates in Australian streams. Five dominant aquatic invertebrates commonly found in both willow and non-willow sites in the Mount Lofty Ranges, South Australia were used to determine whether they exhibit similar feeding preferences, survivorship and growth rates when offered a diet of leaves of crack willow (*Salix fragilis*) and white gum (*Eucalyptus viminalis*). The three native species of shredders investigated: *Dinotoperla evansi* (Plecoptera: Gripopterygidae), *Tasmanocoenis tillyardi* (Ephemeroptera: Caenidae) and *Lingora aurata* (Trichoptera: Conoesucidae), were fed diets of leached willow, senescent willow, leached eucalypt and senescent eucalypt leaves, and showed preference for leached willow leaves. The degree of feeding preference for all shredders differed significantly between leached willow and both leached and senescent eucalypts, but not with senescent willow. However, introduced hydrobiid snails (*Potamopyrgus antipodarum*) and physid snails (*Physa acuta*) did not show any preference. Growth rates of each species (except *P. antipodarum)* were significantly greater on leached and senescent willow than on the other leaf types. Survivorships were similar, but the order of survival was as follows: leached willow > senescent willow > leached eucalypt > senescent eucalypt. The preference of the invertebrates for leached willows compared with other food sources was not correlated with carbon and nitrogen contents. In terms of nutritional value, leached eucalypts had the highest carbon and nitrogen contents. However, the C/N ratio of leached willows was the highest which suggests the leaves were probably more palatable than other leaf types. Willow leaves may provide a suitable food source for native aquatic invertebrates studied, although this source is only available for a short period compared with native eucalypt detritus which falls continuously throughout the year.

Keywords: Willows, eucalypts, leached, senescent, feeding preference, survivorship, growth.

3.1   Introduction

Willows (*Salix* spp.) are one of the worst invaders of riparian zones in southern Australia and are considered a weed of national significance (ARMCANZ 2001). The presence of riparian willows is associated with a reduction in biodiversity and is presumed to be responsible for a decline in aquatic biota. The impact of willows on aquatic invertebrate abundance, stream channel morphology and stream production have been widely researched especially in the southern hemisphere (Walker 1993; Glova & Sagar 1994; Lester *et al.* 1994a; Ladson *et al.* 1997; Schulze & Walker 1997; Read & Barmuta 1999; Stohlgren *et al.* 1999; Jayawardana *et al.* 2006; Azmi & Jennings in prep., see Chapter 2). The majority of published research in Australia has focused on willow leaf breakdown rates in aquatic ecosystems (Collier & Winterboum 1986; Parkyn & Winterbourn 1997; Schulze & Walker 1997; Yeates & Barmuta 1999).

To date, little research has been carried out on the impact of nutrient value of willow leaves on feeding preference, survivorship and growth rates of aquatic invertebrates, although several authors provide evidence that willows are an important food source for many aquatic invertebrates (Hanlon 1981; Collier & Winterbourn 1986; Glova & Sagar 1994; Lester *et al.* 1994b). Feeding experiments by Hanlon (1981) found that the snail *Potamopyrgus jenkinsi* ingested partially decayed willow leaves (*S. fragilis*) and grew significantly faster on this diet than when fed harder leaves of other tree species (poplar and oak). Collier and Winterbourn (1986) suggested that the faster breakdown of willow leaves in 3 mm mesh bags in two streams in Christchurch, New Zealand could be in part the result of feeding activities of the snails *P. antipodarum* and *Physa acuta.* Lester *et al.* (1994b) revealed that *Olinga feredayi* (Trichoptera: Conoesucidae) preferred crack willow leaves (*S. fragilis*) to periphyton, probably because of lower phenolic contents in willow leaves. They concluded that willow leaves could provide a preferred food source for New Zealand macroinvertebrates.

However, some studies suggested that willows may not provide a sustained food source for the invertebrates. Willows drop their leaves over a short period of time in autumn and they break down faster than native species (e.g., Yeates 1994; Schulze & Walker 1997). Lester *et al.* (1994a) confirmed that willow detritus only provided a food source for a relatively short period of time and may not be fully utilized by aquatic invertebrates.

Pidgeon and Cairns (1981) reported that *S. babylonica* leaves had virtually disappeared after 4 weeks and supported lower densities of invertebrates than *Eucalyptus blakelyi* leaves in a Tasmanian river. They suggest that the slow break down of eucalypt leaves may have several advantages for invertebrate detrivores. As eucalypts drop leaves continually, they contribute a persistent, low-level input of nutrients that may enhance the resistance of ecosystems to disturbance (Pidgeon & Cairns 1981; Schulze & Walker 1997). Thus, native eucalypts are thought to provide a more or less continuous food resource throughout the year which have presumably adapted to this pattern of leaf drop. Collier and Winterbourn (1986) also supported this view and showed the response by invertebrates to inputs of willow leaves may be delayed as the leaves need to become 'conditioned' to increase their palatability. Lester *et al.* (1994a) suggested that willows reduced the production of high quality detritus derived from algae and replaced it with detritus of lower quality from willow leaves, which may have contributed to lower invertebrate densities at willow sites in their study. Feeding trial experiments by Schulze and Walker (1997) showed that common atyid shrimp (*Paratya australiensis*) preferred microorganism-colonized river red gum (*E. camaldulensis*) leaves over willow leaves. They suggested that leaf biofilms were of relatively high density and the diversity of diatoms on river red gum leaves generally attracted greater densities of aquatic invertebrates.

Different types of leaves have different physical structures as well as differences in their nutritional value. Leaf structure plays an important role in determining feeding preference and leaf toughness has been implicated as a deterrent to consumption by terrestrial herbivores (Ohmart *et al.* 1987; Chergui & Pattee 1993). As pointed by Chergui and Pattee (1993) and Lester *et al.* (1994b), willow detritus becomes more attractive to invertebrates after it has been submerged for some time (i.e. conditioned). Lester et al. (1994a) found rapid leaching of polyphenolics including tannins when leaves were submerged in stream water. The authors suggested that palatability may have been enhanced from the leaching of compounds in leaves, or an increase in microbial colonists, rather than softness. Generally, willow leaves are rich in nitrogen and soft in texture, whereas eucalyptus leaves are poor in nitrogen and hard in texture (Ohmart *et al.* 1987; Lester *et al.* 1994a). In willows present sites, where eucalyptus is absent from the riparian vegetation, willow leaves decompose faster, while eucalyptus leaves require a conditioning period (Schulze & Walker 1997). Thus, both species need some conditioning, but the processing of eucalyptus leaves shows more spatial differences than that of willow leaves.

In the feeding preference study by Lester *et al.* (1994b), *Olinga feredayi* (Trichoptera: Conoesucidae) preferred 56-day willow leaves to the periphyton (a mixture of algae, cyanobacteria, heterotrophic microbes, and detritus), and 7-day and 28-day incubated leaves. They suggested this was probably because submerged leaves have a lower phenolic content and a thinner cuticle and epidermis. In contrast, Yeates and Barmutta (1999) suggested that leaf structure was not the main factor on preference as invertebrates had access to softer internal leaf tissues during preference trials. Green leaves may have been a better food source because of the noticeably thicker biofilm that it supported and they may also retain higher levels of nutrients than leached leaves. Biofilm is composed of algae, bacteria, fungi and organic matter embedded in a mucilaginous matrix which covers the surface of structures such as stones or plant leaves and stalks (Graca *et al.* 2001). However, there is some evidence that green material not only contains more nutrients but also more potentially toxic secondary compounds such as phenolics and tannins (Rowell-Rahier 1984). For example, Julkunen-Tiitto (1985) pointed out that green leaves of several species of willows (*S. viminalis, S. phylicifolia, S. myrsinifolia* and *S. aquatica*) have a high phenolic content. Irons *et al.* (1988) found that the concentration of condensed tannins in willow leaves was negatively correlated with leaf consumption of detritivores. Thus, it is expected that green leaves will be avoided because they have higher levels of secondary compounds than senescent and leached leaves (Julkunen-Tiitto 1985; Irons *et al.* 1988).

Our initial observations indicated that both leached and senescent leaves of willows are fed upon by various aquatic invertebrates found in Sixth and Deep Creeks in the Mount Lofty Ranges of South Australia, and so we designed a study to investigate whether green, leached or senescent leaves of willows and eucalypts are more or less preferred by range of aquatic invertebrates found in these creeks.

In Sixth and Deep Creeks, introduced willows are a dominant component of the riparian zone. In this study, leaves of exotic crack willows (*S. fragilis*) and native white gums (*E. viminalis*), both species being common in the two creeks, were used to compare the feeding preference of five dominant aquatic invertebrate species. We also examined whether these invertebrates are able to discriminate between leaf species and between leached and senescent leaves, and whether leaf species and leaf state affect the feeding preference, survivorship and growth of these invertebrates.

3.2   Materials and Methods

3.2.1   *Study sites and species studied*

Five dominant species of aquatic invertebrates which are abundant under both willows and non-willows in Sixth and Deep Creeks, Mount Lofty Ranges, South Australia (Fig. 2.1) were used for this study: two snails, *Potamopyrgus antipodarum* (Gastropoda: Hydrobiidae), *Physa acuta* (Gastropoda: Physidae), and three shredder species, *Dinotoperla evansi* (Plecoptera: Gripopterygidae), *Tasmanocoenis tillyardi* (Ephemeroptera: Caenidae) and *Lingora aurata* (Trichoptera: Conoesucidae). Each species differs in the way they consume the leaves. *Potamopyrgus antipodarum* and *Ph. acuta* both have scraping mouthparts and rasp material from the surface of the leaves, whereas *D. evansi*, *T. tillyardi* and *L. aurata* are shredders and browsers.

The invertebrates used in feeding preference, survivorship and growth experiments were collected on several occasions between May and June 2008 from Sixth and Deep Creeks (Fig. 2.1). Sixth Creek is a fifth order stream, has a total length of ~18 km and drains an area of ~46 km^2. It is located at an elevation of around 380 m at latitude 34°52'N and longitude 138°45'E. It flows north to meet the Torrens River at Castambul in the Mount Lofty Ranges, South Australia. It ranges 1−4 m in width, depth is between 0.5−4.0 m and the water velocity is slow to fast. The stream shaded by a diverse canopy of riparian trees including willows (*Salix* spp.), ash (*Fraxinus* spp.), eucalypts (*E. viminalis* and *E. obliqua*), blackberries (*Rubus fruticosus*) and hawthorns (*Crataegus* spp.). Deep Creek, also located in the Mount Lofty Ranges at around 350 m elevation with latitude 34°56'N and longitude 138°46'E. This small fourth order stream is approximately 8.5 km length, is 2.0−4.5 m in width, 0.5−2.5 m deep and is slow to sometimes fast flowing. The catchment area of Deep Creek is ~13 km^2 and major riparian vegetation in the study area consists of willows (*Salix* spp.), *E. viminalis, E. obliqua, Acacia retinoides* and *A. melanoxylon.*

Aquatic invertebrates were collected using an aquatic net with a 40 x 40 cm frame, 60 cm long net of 250 μm mesh. Each sample containing invertebrates was brought back to the laboratory where they were acclimatized for 1-2 days prior to start of experiments. About 25% of the water from each sample was removed and the water was replaced with the same amount of freshly collected stream water from either Sixth Creek or Deep Creek. Every 10 minutes, about 200 mL of stream water was added to each sample and this process was repeated for

about an hour. After an hour, the invertebrates were sorted and were placed into plastic feeding trays (supplied by Australian Entomological Supplies Pty. Ltd.) partially filled with freshly collected stream water from Sixth and Deep Creeks (Fig. 3.1). Each tray consisted of 8 cells, with individual cell dimension of 6.0 cm long x 6.0 cm wide x 2.54 cm depth. Twentyfour hrs before the start of each experiment, they were placed in a controlled temperature room at 15^0C to acclimatize them to experimental conditions. No food was supplied during this period.

Fig. 3.1 Feeding preference experiments were conducted in plastic feeding trays (44.45 cm long x 20.9 cm wide x 2.54 cm depth), partially filled with freshly collected stream water.

3.2.2   *Feeding preference experiments*

The experimental designs followed those of Yeates and Barmuta (1999), Friberg and Jacobsen (1994) and Graca *et al.* (2001). Fresh green and senescent leaves of *S. fragilis* and *E. viminalis* were collected from randomly selected trees at Sixth and Deep Creeks in late summer. White gums (*E. viminalis*) were also used as this species dominates the original vegetation in each creek. Prior to use in feeding experiments, leached leaves were prepared by soaking the green leaves for 2 days in four changes of running tap water. Senescent leaves were prepared by conditioning the leaves in loosely packed nylon bags (30 cm x 15 cm, 3 mm mesh) submerged in the creeks for 2 weeks to allow entry of invertebrates and biofilms that would normally be associated with decomposing leaves. Leaves were then air-dried and stored in polyethylene

bags until needed. In this study, green, leached and senescent leaves of each plant were tested for feeding preference experiments. However, due to insufficient numbers being available of *Ph. acuta* and all species of shredders, only *P. antipodarum* were fed on green leaves of willows and eucalypts.

Leaves were cut into 14 mm diameter disks so that the mid-rib of the leaf is included in the centre of each disk (average disk weights = $\pm$ 1 mg). Using disks eliminates the possibility of invertebrates using leaves as habitat. Pairs of the following foods were offered to each invertebrate: green willow (GW); leached willow (LW); senescent willow (SW); green eucalypt (GE); leached eucalypt (LE); and senescent eucalypt (SE). Approximately 0.80 $\pm$ 0.05 g (wet weight) of one food was added to one half of the container, and the same amount of another food was added to the other half. Overall, there were 180 replicates for *P. antipodarum* (6x5 diet combinations x 1 sp. X 6 repetitions), 288 replicates for other invertebrate species (4x3 diet combinations x 4 spp. X 6 repetitions), and 6 controls to determine weight change due to decomposition with no invertebrates present. All treatments were conducted at 15^0C in a constant temperature room, the approximate temperature of the stream at time of collection, and in a 12:12 light-dark photo-period. Feeding was allowed to progress for 14 days or until the animal died. Invertebrates were removed and leaf disks were oven-dried for 3 days at 60^0C, and weighed to the nearest 0.01 mg.

To determine which leaf is most preferred, a modified version of the Chesson-Manly selection index was used (Chesson 1983; Manly 1995). Food preference was measured by: $\beta_i = \alpha_i / K\sum_{j=1} \alpha_j$, where β_i = preference for food type i; α_i = proportion of food type j consumed; and the denominator represents the sum of all proportions of the K food types consumed. The average proportion of food type i consumed is measured by: $c_i - f_i$, where c_i and f_i are the means natural of natural logs of the amounts of dry weight lost in control (c_i) (i.e., decomposition), and feeding trials (f_i). If $\beta_i = 1/K$, it indicates that all food types were consumed equally; if $\beta_i \geq 1/K$, it indicates selection for food type i; and if $\beta_i \leq 1/K$, it indicates avoidance for food type i. The corrected t distribution recommended by Manly (1995) was used to compute confidence intervals of β_i. ANOVA and multiple contrast tests were also used to detect differences among the food consumption (mg of leaf dry mass) of each invertebrate species for leaf species and leaf structures. Data were $\log_e(x+1)$ transformed to ensure normality in calculations of means and ANOVAs. All analyses were tested using SPSS statistical software (SPSS 2006).

3.2.3 *Survival and growth experiments*

For these experiments, the same five aquatic invertebrate species were used to determine whether leaf species and leaf states affect their survivorship and growth rates. Leached and senescent leaves of *S. fragilis* and *E. viminalis* were offered to each invertebrate species in the form of 14 mm diameter leaf disks. For each invertebrate species, six replicates were used for each treatment and each replicate contained one invertebrate. The experiment was conducted in a constant temperature room at 15^0C and 12:12 light-dark photo-period. Food and filtered fresh stream water were replaced weekly during the experiments. Initial shell height of each snail species and body length of each shredder species were measured to the nearest 0.01 mm with Vernier callipers or under a dissecting microscope. All invertebrates were maintained for 28 days or until death and again measured at the end of the experiment. However, experiments for the shredders were shortened to 18 days due to insufficient number of nymphs surviving. After checking for normality and equal variances of data, the effect of leaf species and leaf states on survival and growth was analyzed by one-way ANOVA and if significant, Tukey's Significant Difference (HSD) test was applied to determine which leaf species and leaf structures differed significantly from one another. Survival was considered as the percentage still alive at the end of the experiments. Data from different leaf species and leaf states were lumped together and tested for differences in survival (%) among treatments using one-way ANOVA and Tukey's HSD test. All statistical analyses were conducted with SPSS statistical software (SPSS 2006).

3.2.4 *Total carbon and nitrogen analyses*

A sample of each of the food types was air-dried and placed in a drying oven at 60^0C for three days. After weighing, the samples were ground and passed through a 1 mm sieve. Total carbon and nitrogen were determined by high temperature combustion of 0.03−0.05 g sample in an atmosphere of oxygen using an elemental analyzer model Leco CNS-2000. Carbon was converted to CO_2 and determined by infrared detection. Nitrogen was determined as N_2 by thermal conductivity detection (Matejovic 1997). Spearman Correlation Analysis was then used to investigate whether C/N ratios had any effect on feeding, survival and growth of each invertebrate species. This analysis was done using the SPSS 15.0 statistical package (SPSS 2006).

3.3 Results

3.3.1 *Feeding preference*

Not all invertebrates consumed all food types offered. Overall consumption was considerably lower for hydrobiid snails (*P. antipodarum*) and physid snails (*Ph. acuta*) than other invertebrate species (Fig. 3.2). The strength of preference measured by the Chesson-Manly Index for *P. antipodarum* and *Ph. acuta* was below 0.25, indicating that both species avoided all food types. None of the food items was completely consumed in any of the experiments. *Potamopyrgus antipodarum* clearly avoided green eucalypts when given a choice between green, leached and senescent leaves of willows and eucalypts. However, the difference between food consumption (leaf dry mass) for both snail species did not differ significantly among leaf species (*P. antipodarum*, $F_{1,23} = 0.011$, $P = 0.880$; *Ph. acuta*, $F_{1,23} = 0.405$, $P = 0.991$) and leaf states (*P. antipodarum*, $F_{3,23} = 0.071$, $P = 0.975$; *Ph. acuta*, $F_{3,23} = 0.299$, $P = 0.825$) (Table 3.1).

In contrast, the shredders (*D. evansi*, *T. tillyardi* and *L. aurata*) reacted differently to all the food types offered. All species showed clear and consistent preferences, with all shredders preferring leached willow over other leaf types (Fig. 3.2). When given a choice between willow and eucalypt leaves, all shredders preferred willow leaves, with the consumption of leached leaves being significantly higher than senescent leaves (Table 3.1). The shredders would feed on the entire surface of the leached willow leaves until the leaf skeleton remained. However, when the shredders were offered a choice of leached versus senescent eucalypt leaves, all shredders appeared to avoid both offered food items. The ANOVA and Tukey's HSD values show that food consumption for all species of shredders were highly significant: *D. evansi*, $F_{3,23} = 79.733$, $P < 0.001$; *T. tillyardi*, $F_{3,23} = 77.058$, $P < 0.001$; and *L. aurata*, $F_{3,23} = 30.329$, $P < 0.001$.

During the experiments, *D. evansi* and *T. tillyardi* were clearly the most mobile species. The behaviour of both species were unpredictable, as in some cases, the nymphs began feeding immediately after been placed in the food trays whereas others tried to crawl out of the trays. The nymphs of these species frequently swim fast and generally spent a few minutes exploring the tray before settling down to feeding. Conversely, most *L. aurata* nymphs were motionless and there was no obvious indication that they tested the available food items before feeding.
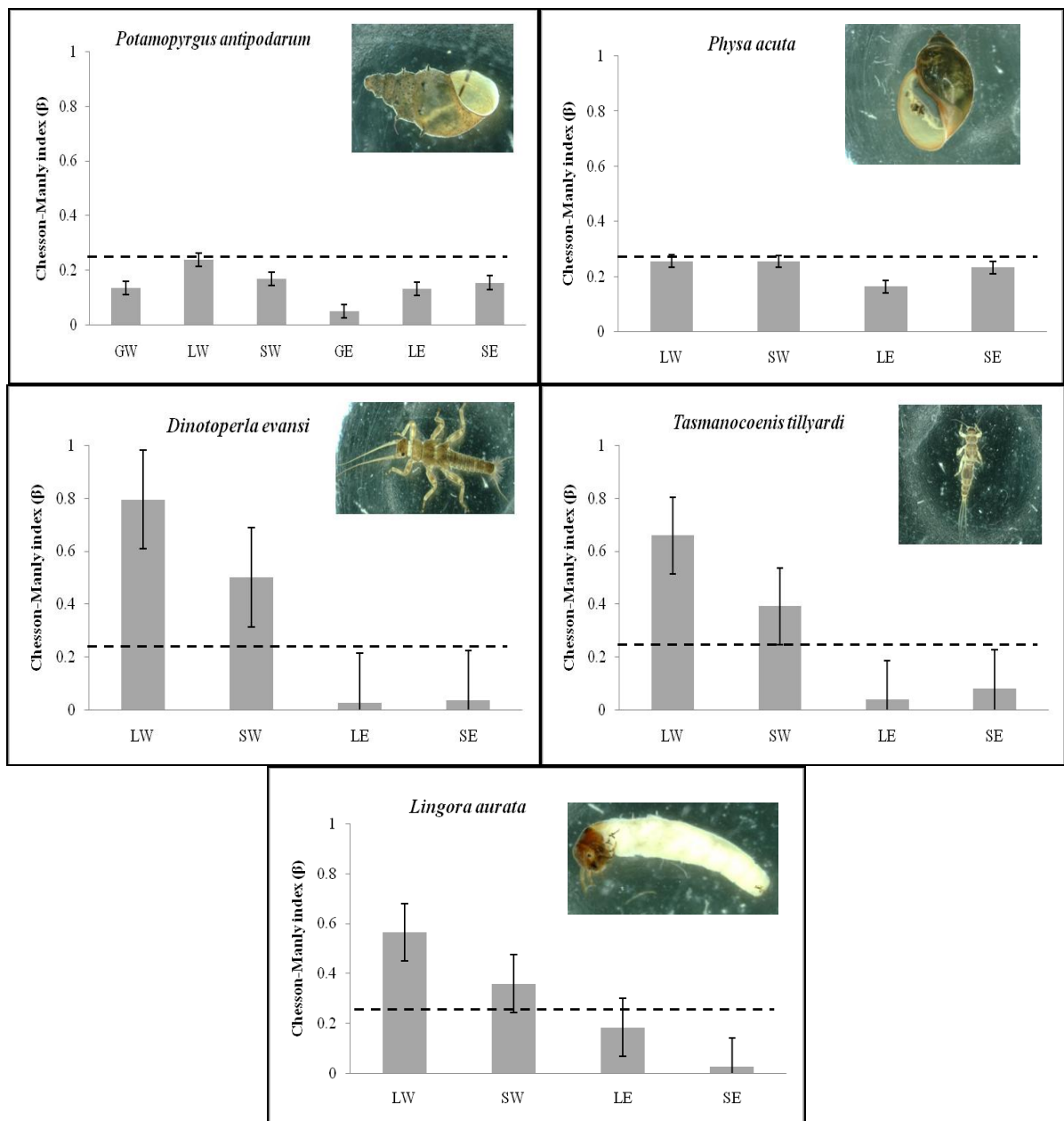
Fig. 3.2 Feeding preference for the different leaf types of five dominant invertebrates as indicated by the Chesson-Manly index, β (mean values $\pm$ SE; $n = 30$). The dashed line refers to the 95% confidence intervals which indicate preference or avoidance of the food type. Note that GW and GE were only tested for *P. antipodarum.* (GW = green willow; LW = leached willow; SW = senescent willow; GE = green eucalypt; LE = leached eucalypt; SE = senescent eucalypt).

Table 3.1 F values for one-way ANOVAs on the effect of leaf species and leaf states on food consumption, survival and growth of each invertebrate species (d.f. = degree of freedom; $*$ $P < 0.05$; $**$ $P < 0.01$; $***$ $P < 0.001$).

Effect	Species	Variables	d.f.	F
Leaf species	*P. antipodarum*	Food consumption	1	0.011
		Survival	1	2.250
		Growth	1	1.836
	Ph. acuta	Food consumption	1	0.405
		Survival	1	9.000*
		Growth	1	5.390**
	D. evansi	Food consumption	1	247.141***
		Survival	1	0.233
		Growth	1	99.649***
	T. tillyardi	Food consumption	1	225.513***
		Survival	1	0.184
		Growth	1	209.065***
	L. aurata	Food consumption	1	94.890***
		Survival	1	0.267
		Growth	1	18.044***
Leaf states	*P. antipodarum*	Food consumption	3	0.071
		Survival	3	0.667
		Growth	3	0.195
	Ph. acuta	Food consumption	3	0.299
		Survival	3	3.000
		Growth	3	4.159*
	D. evansi	Food consumption	3	79.733***
		Survival	3	0.267
		Growth	3	61.067***
	T. tillyardi	Food consumption	3	77.058***
		Survival	3	0.167
		Growth	3	191.089***
	L. aurata	Food consumption	3	30.329***
		Survival	3	0.300
		Growth	3	86.684***

3.3.2 Survival and growth

Survival of each species during the growth experiments was quite high on all food items offered. ANOVA test revealed there were no significant differences among any of leaf types offered, except that *Ph. acuta* showed higher survivorship when fed on willow leaves (Table 3.1). Generally, survivorship of the snails was significantly higher on all food items than was the case for the three shredders species (Table 3.2). Even though the snails did not consume the offered food, most of the snails survived until the end of the experiments. For the shredders, survival was consistently higher on leached and senescent willow leaves than leached and senescent eucalypts leaves (Table 3.2). However, we suspect some of the shredders were stressed as some of them lost their gills or caudal filaments during the initial body length measurement process. As a result, most nymphs of each species could not survive very long and most of them died in less than 18 days.

Table 3.2 Survival (%) of each invertebrate species fed on leached and senescent leaves of willows and eucalypts. (n = number of individuals surviving at the final experiment date).

Invertebrate species	Leaf species and food type				n	Duration (days)
	Salix fragilis		*Eucalyptus viminalis*			
	Leached	Senescent	Leached	Senescent		
P. antipodarum	100	100	83.33	100	23	28
Ph. acuta	100	100	83.33	83.33	22	28
D. evansi	100	83.33	66.67	50	18	18
T. tillyardi	83.33	83.33	50	50	16	18−14
L. aurata	100	66.67	66.67	50	17	18−14

Growth rates of *P. antipodarum* did not differ among any of the leaf species or leaf states (Table 3.1). However, significantly higher growth of *Ph. acuta* was observed when they were fed leached willow, even though they clearly avoided all food items offered (Fig. 3.3). We observed that the snails were often seen feeding underneath the leaf disks, presumably feeding on the biofilms that attached on the abaxial surface of the leaves. All species of shredders grew significantly on different food types offered: *D. evansi,* $F_{3,23}$ = 61.067, $P < 0.001$; *T. tillyardi,* $F_{3,23}$ = 191.089, $P < 0.001$; and *L. aurata,* $F_{3,23}$ = 86.684, $P < 0.001$ (Fig. 3.3). The effect of leaf species was significantly different, but leaf states had no effect on their growth. ANOVA, followed by Tukey's HSD tests showed that shredders fed a diet of leached and senescent willows grew more than the shredders fed on leached and senescent eucalypts. However, there were no differences between leached and senescent eucalypts (Table 3.1 & Fig. 3.3).
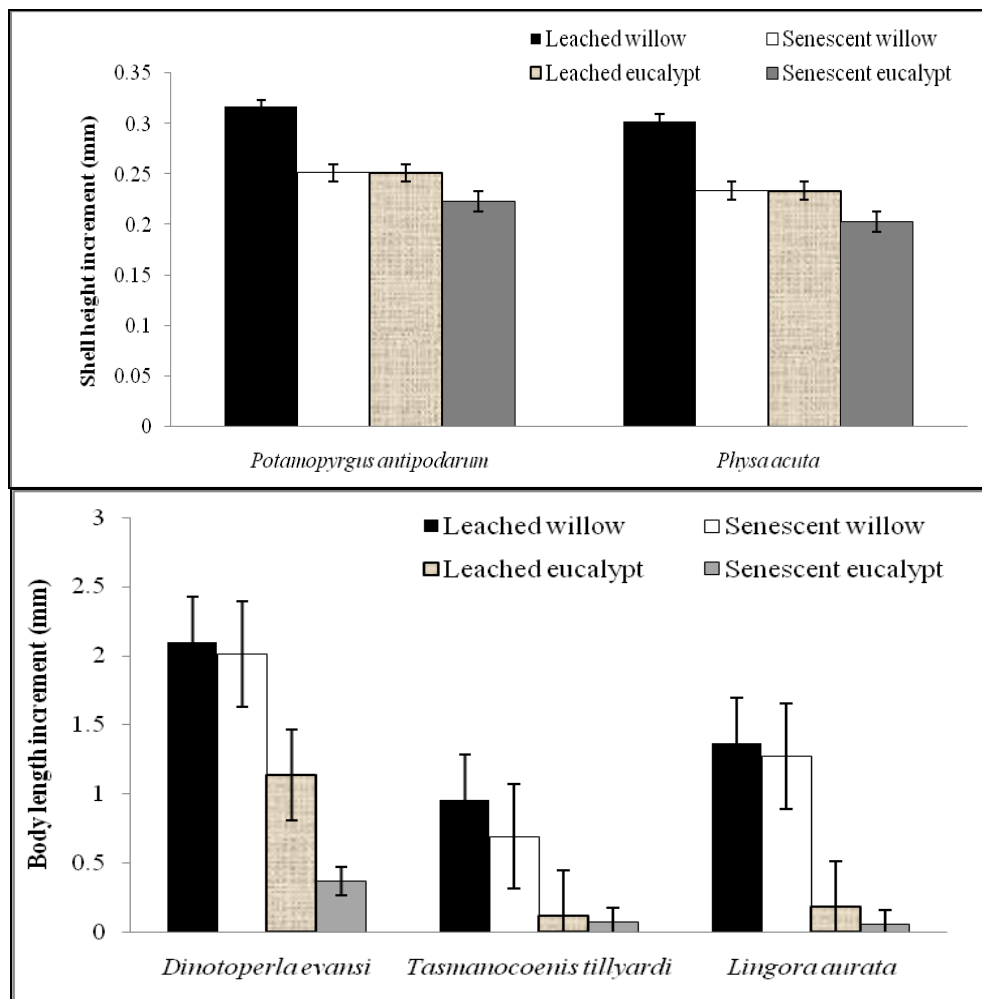
Fig. 3.3 Growth rates (mean $\pm$ SE) of five dominant invertebrates fed with four different food types for 28 days. Note that *D. evansi, T. tillyardi* and *L. aurata* were only fed for 18 days as most of the nymphs did not survive for the experimental period.

3.3.3   *Nutritional value of leaves*

There were higher carbon and nitrogen contents in leached eucalypt leaves compared with other food items (51.6% and 1.72%, respectively) (Fig. 3.4). However, total carbon and nitrogen contents did not show significant differences among leaf species and leaf states. The values of C/N ratios were different for each leaf species and leaf state (Fig. 3.4). Leached eucalypts which was rich in nitrogen, had a low C/N ratio of 28.91, whilst senescent eucalypts had the highest C/N ratio of 37.75. Although both leached and senescent willows had low carbon and nitrogen contents, the C/N ratios of leached willows and senescent willows were relatively high, with 37.74 and 31.44 respectively.
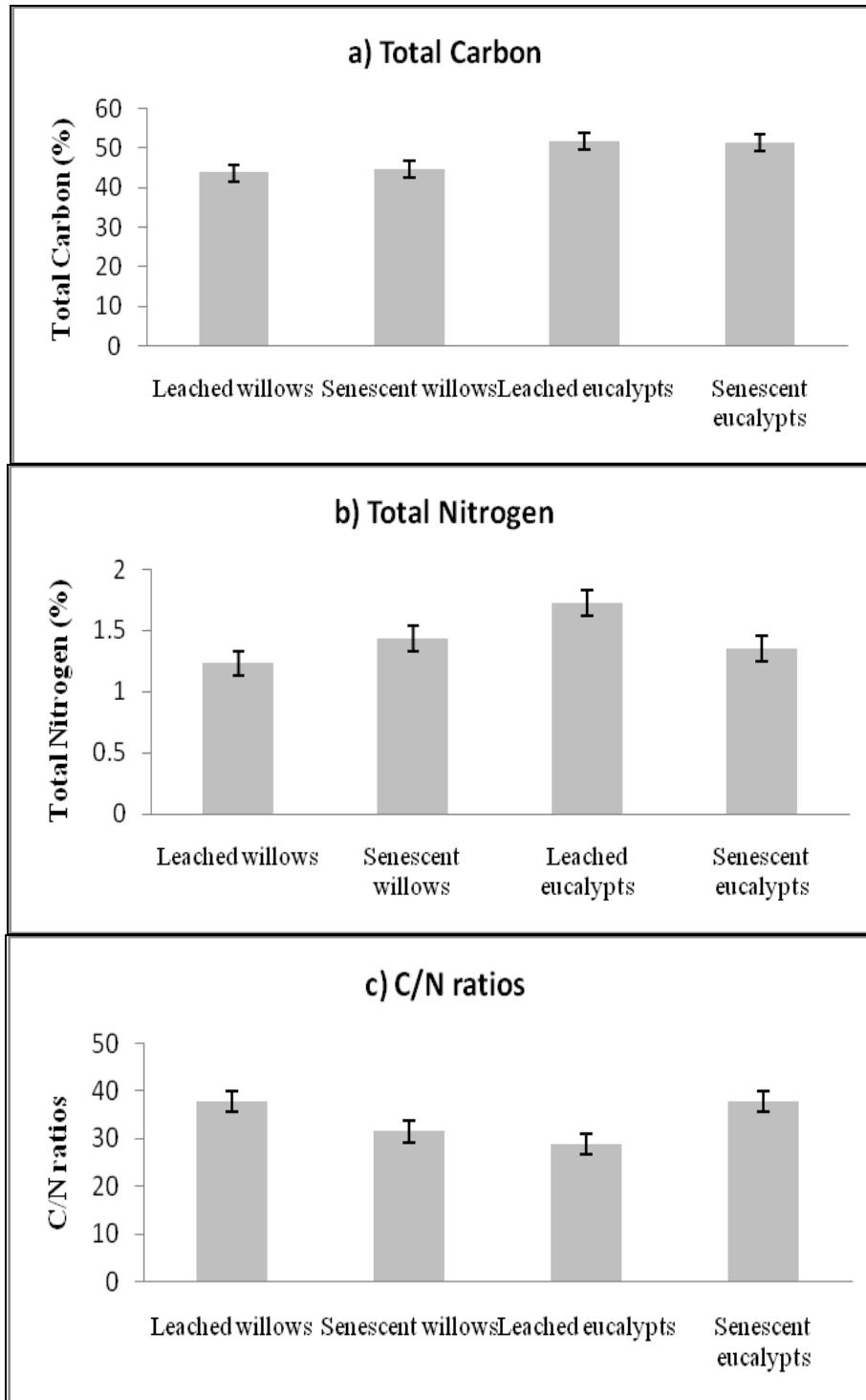
Fig. 3.4 Total carbon (a), nitrogen (b) and C/N ratios (c) for each leaf species and leaf states (Note: Bar represents standard error)

3.4   Discussion

We expected that native eucalypt leaves would be preferred and would be a better food source than exotic willow leaves. However, the five species of invertebrates preferred leached willow leaves over senescent willow, leached eucalypt and senescent eucalypt. The two snail species did not show any preference even though we used leaf disks which presumably give good access without having to break through the cuticle and epidermis. We observed that most of the snails preferred to feed on the abaxial leaf surface of leached leaves compared with senescent leaves of both willow and eucalypt. Green leaves were leached by soaking for 2 days in four changes of running tap water. We suspect that leached willow leaves are more attractive as they supply additional nutrition from increased microbial conditioning (Collier & Winterbourn 1986) or perhaps, from the removal of toxic secondary compounds which affect the invertebrates feeding directly (Lester *et al.* 1994b).

In the case of *P. antipodarum,* we found that the snails clearly avoided green leaves when given a choice between leached or senescent leaves of either willow or eucalypt (Fig. 3.2). This suggests that even though the green leaves presumably contain more nutrients (Yeates & Barmuta 1999), the snails are possibly avoiding the higher levels of potentially toxic secondary compounds (Rowell-Rahier 1984). Green willow leaves (*S. viminalis, S. phylicifolia, S. myrsinifolia* and *S. aquatica*) have been shown to have high concentrations of phenolics (Julkunen-Tiitto 1985) and tannins (Nyman & Julkunen-Tiitto 2000). Hanlon (1981) found that the European species *Potamopyrgus jenkinsi* ingested partially decayed leaves of *S. fragilis* and grew significantly faster on this diet than when fed harder leaves of other tree species. Another study by Collier and Winterbourn (1986) found that *P. antipodarum* and *Ph. acuta* were the most abundant colonists in the breakdown process, and they suggested that leaves of *S. babylonica* were most palatable to snails when 35-45% of initial leaf biomass remains.

In this study, the survivorship and growth rates of *Ph. acuta* were significantly greater when fed leached willow than the other leaf types (Table 3.2 & Fig. 3.3). Leaf consumption and growth by invertebrates have been shown to be positively associated to the nitrogen content of leaves; whereas tannins and other phenolic compounds have been shown to be negatively associated with leaf consumption and growth (Irons *et al.* 1988).

We found that leached leaves of willows and senescent eucalypts have the highest C/N ratios (Fig. 3.4), which is commonly associated with lower carbon content, but higher nitrogen content. Usually, leaves that have higher nitrogen content would have lower lignin content which has contributed to lower leaf toughness (Ohmart *et al.* 1987). Leached willows are easier to pierce by the shredders and should be more palatable than leached and senescent eucalypts. However, carbon content in senescent eucalypts was very high which indicates that the leaf decomposition may take a longer time and are probably less palatable for the invertebrates (Chauvet 1987). Generally, the invertebrates tested prefer leached willows than senescent eucalypts as probably at that stage of leaf decomposition, the C/N ratios of leached willows make the leaves more palatable. As well, leaching compounds from the leaves can increase microbial mass which are favoured by many aquatic invertebrates, especially the three shredder species studied.

As suggested by Barlocher and Kendrick (1974), leaf toughness and higher concentrations of protein-phenolic complexes in the green leaves of willow and eucalypt might be responsible for a lower fungal colonisation and nitrogen availability. Interestingly, the preferences of the shredders for leached willows compared with other food items was not correlated with carbon and nitrogen contents and therefore, there may be other factors at work. For example, food selection is not determined exclusively by concentrations of macronutrients but also maybe affected by specific fungal attractants and repellents (Graca *et al*. 2001).

The effects of leaf state were significantly different for both food consumption and growth of all species (Table 3.1). The shredders discriminated among food types offered, and showed an overall preference for leached leaves over senescent leaves. The strength of feeding preference for all shredders differed significantly between leached willow and both leached and senescent eucalypts, but not with senescent willow. Growth rates of each species were significantly greater on leached and senescent willow than on the other leaf types (Fig. 3.3). However, survivorships were similar, but the order of survival on growth was as follows: leached willow > senescent willow > leached eucalypt > senescent eucalypt.

Generally, the preference of shredders depends on leaf toughness, nutrient content and the presence of plant secondary compounds. Since shredders studied in this experiment are very mobile (except *L. aurata)*, they should be able to search for appropriately conditioned leaves and be highly selective. Leaf toughness can be a physical barrier for invertebrate feeding as

harder leaves are probably more difficult to pierce than soft ones (Ohmart *et al.* 1987) as in the case where shredders preferred 'soft' willow rather than 'hard' eucalypt leaves.

In terms of nutrient content, leached willow may have been a better food source than senescent willow because of the noticeably thicker biofilms that it supported. Even though leached eucalyptus contained the highest carbon and nitrogen contents (Fig. 3.4), we noticed that leached willow leaves rapidly accumulated a much thicker slimy microbial biofilm than the other leaf types. Although conditioned leaves may have already lost more nutrients by leaching than recently leaves, microbial colonization is expected to increase food quality of fresh leaves by enhancing then biochemically and by softening their tissues. Irons *et al.* (1988) concluded that leaves preferred in laboratory selection experiments are those containing a higher nutrient content, especially nitrogen. Leached leaves have been shown to have the highest proportion of nitrogen and the lowest phenolic contents (Irons *et al.* 1988), which are more likely to be responsible for the preferences observed. Secondary compounds that are known to remain active after leaf senescence, may be toxic and interfere with digestion or give a bitter taste, acting as a feeding deterrent (Irons *et al.* 1988).

We observed that most shredders appear distressed when exposed to senescent eucalypt leaves and tried to crawl out of the feeding trays. Similar to our results, Canhoto and Graca (1995) found that the larvae of *Tipula lateralis* did not grow when feeding on leaves of *E. globulus*. They suggested that leaf utilization by *T. lateralis* seems to be negatively correlated with cuticle thickness and polyphenolic content, which has been implicated as a deterrent to the invertebrates.

Another study by Abelho and Graca (1996) reported that streams running through monocultures of eucalypt plantations had a lower abundance of invertebrate taxa compared with mixed deciduous forests. They suggested that the lower abundance of invertebrates was related to the low 'quality' of eucalypt leaves (Canhoto & Graca 1999), but may also be due to increased tannin levels in the water. A study by Gehrke *et al.* (2005) found that litter of river red gum (*E. camaldulensis*) became hypoxic, with decreased pH and contained up to 30 mg l^{-1} tannin and lignin. They suggested that the presence of toxic leachates and low oxygen availability in flooded river red gum forests may make these habitats unsuitable as nursery areas for native fish.

In summary, willows are a food source to aquatic invertebrate communities in Sixth and Deep Creeks, and have the potential to influence the diversity and abundance of scrapers (snails) and shredders. Therefore, changes in riparian vegetation may have the potential to affect the assemblages of aquatic invertebrates in both streams studied. Willow leaves are a suitable food source for the native shredder species studied (*D. evansi, T. tillyardi* and *L. aurata*) and that differences in leaf states influence not only food preference and consumption, but also growth rates and survival. Future research should focus on the nutritional value of the leaves, phenolic composition and the biofilms which develop on them. Also, observation of gut contents might allow us to understand more on the feeding processing of the invertebrates.

In conclusion, willow leaves may represent a good food source, but it is only available as a short-term resource, especially for the shredders. Large scale removal of willows might have negative short-term impacts on the aquatic invertebrate communities especially those which utilize the willows leaves as their food sources.

CHAPTER 4: Comparison of willow roots and artificial substrates as habitat for aquatic invertebrate communities

4.0   Abstract

A field experiment was conducted at Deep Creek in the Mount Lofty Ranges (South Australia) to investigate colonisation of willow roots and artificial aluminium wire mesh by aquatic invertebrates. Two different types of substrate with differing degrees of complexity were used. Willow roots with (1) only primary roots left intact; (2) lateral and primary roots left intact; and (3) no roots removed, and aluminium wire mesh of three different mesh sizes: (1) coarse (15 mm), (2) medium (9 mm), and (3) fine (1.5 mm), were allowed to be colonised *in situ* for 30 and 90 days. Aquatic invertebrates were significantly more abundant on willow roots than on the aluminium wire mesh substrates. However, there was no significant difference in taxon richness. Habitat complexity and period of colonisation are important determinants of invertebrate community structure on each substrate type. Scrapers (primarily gastropods) often constituted the greatest abundance on willow roots. The introduced hydrobiid snail, *Potamopyrgus antipodarum,* was significantly more abundant especially on the most complex of willow root structures and after a longer period of colonisation (90 days). Our findings suggest that willow roots have habitat value for aquatic invertebrates, especially the introduced hydrobiid snail, and decrease in structure associated with willow roots resulted in a lower abundance of invertebrates. Carefully management of willow removal should take into account the sudden removal of willow roots which may disrupt the invertebrate communities which utilise the roots as habitat. It is also evident that a long period of colonisation is needed to obtain a stable invertebrate community, especially during revegetation efforts.

Keywords: willow roots, aluminium wire mesh, abundance, richness, aquatic invertebrates.

4.1   Introduction

Since the early 1900s, thousands of kilometres of the riparian zones of many south-eastern Australia have become dominated by the exotic willow trees (Holland & Davies 2007). They have been mainly used for erosion control, bank stabilization, boat navigation and as ornaments (Ladson *et al.* 1997; Holland & Davies 2007). Since then, as more willows have become established, greater changes in hydrologic regimes, problems with water quality, decline in native species and establishment of exotic weeds have been reported (e.g., Merigiano 1997; Patten 1998; Jayawardana & Westbrooke 2010).

The ability of willow roots to spread into river and stream banks can have long term effects on channel morphology. Willow roots trap silt and layer new roots over old roots in stream channels, building up the streambed and creating a broad shallow stream (Bunn *et al.* 1999a; Ladson *et al.* 1997). They can be associated with altering bank morphology, changing channel depth and width, flow and sediment conditions (Bunn *et al.* 1993; Lester *et al.* 1994a; Ladson *et al.* 1997). Willow roots are also presumed to be an important component responsible for a decline in native biodiversity by decreasing habitat heterogeneity for fish, aquatic plants and aquatic invertebrates, especially in the southern hemisphere (e.g., Collier *et al.* 1995; Schulze & Walker 1997). Willows are now often being poisoned, removed from accessible river banks and the areas replanted with native vegetation. However, such programs have accelerated bank erosion, leading to water quality problems and allowing exotic weeds to become established.

The effect of the removal of willows on the aquatic ecosystem is largely unknown, although in Chapter 2, we found slightly lower numbers of aquatic invertebrates where willows had been removed. The removal of willows directly destroys the thick mats of willow roots. Thick willow roots mats can play an important role in the formation and maintenance of habitat for aquatic fauna. Adult fish have been reported to use the roots as a habitat source (Pusey *et al.* 1998), whilst exposed roots have been used as a spawning substrate and larval habitat (Pusey *et al.* 2001a; 2001b). However, little is known about the value of willow roots as suitable habitat for aquatic invertebrate communities. Some studies suggest that willows may support high numbers of aquatic invertebrates by providing suitable habitat. For example, Glova and Sagar (1994) found greater species richness and diversity in benthic invertebrate communities in reaches lined by willows in three New Zealand streams. Azmi and Jennings

suggested that dense shallow willow root mats provide more stable shelters for the invertebrates, protecting them from high currents (see Chapter 2). Similar results were recorded by Jayawardana *et al.* (2006), who found *Potamopyrgus antipodarum* was commonly associated with willow root habitats in Birch Creek, Victoria. Willow root mats have greater surface texture, area and provide a variety of microhabitats for invertebrates colonisation. The rough-fibrous texture of willow roots is known to affect colonisation by aquatic invertebrate communities (Minshall 1984; Mackay 1992), and provides a suitable habitat for invertebrate reproduction and protection from predators (Korinkova 1971; Lester *et al.* 1994a). In terms of food resources, no study has examined the potential value of willow roots as a food supply for aquatic invertebrates.

In this study, the principal objective was to compare differences in taxon richness and abundance of aquatic invertebrate communities between natural willow roots and artificial aluminium wire mesh substrates. Artificial substrates have been used to sample benthic macroinvertebrates in similar habitats in lotic systems (e.g., Souter & Williams 2001; Souter 2004; White & White 2005). However, artificial substrates do not collect a representative sample of the zoobenthos on the natural substrates, but rather select for mobile, drift-prone species on hard substrata (Rosenberg & Resh 1993). While artificial substrates do have limitations; they are very useful tools in ecological studies if the limitations are understood.

Boulton and Boon (1991) used a 'rock-bag' sampling technique to avoid loss from accidents, high flows and vandalism. However, rock-bag substrates (with the bags containing gravel or clay brick to weigh them down) may be unacceptable as numerous rock-bag substrates in the stream may alter flow characteristics, impede vertical migration from the sediments by invertebrates, trap detritus and even attract vandals.

In this study, we chose aluminium wire mesh as an artificial substrate in an attempt to mimic microhabitats found in willow roots because it is manufactured in various mesh sizes, is easy to manipulate and when placed on the bottom of a stream, is relatively stable in currents. Generally, heavier or bottom-sited artificial substrates are recommended because they are considered more reliable than artificial floating substrates, which tend to be selectively colonised by beetles, mayflies and caddisflies (Crossman & Cairns 1974). Furthermore, artificial floating substrates (e.g., leaf packs/bags) are usually positioned near the stream bed and often float for a time before sinking to the bottom, which may affect the representation of invertebrate communities (Boulton & Boon 1991). Although Souter (2004) used bricks to

weigh down his artificial substrates, we used metal stakes to hold down the artificial substrates which will also avoid the possibility of invertebrates using bricks as habitat.

We investigated differences in aquatic invertebrates colonisation on two different substrate types (willow roots vs. aluminium wire meshes), each with three different habitat complexities and for colonisation periods of 30 and 90 days. Previous studies (e.g., Mode & Drewes 1990; Casey & Kendall 1996), found that natural substrates usually supported higher densities and diversity of aquatic invertebrates than artificial substrates. Casey and Kendall (1996) also found that the quantity of organic material and epilithic cover was greater on natural substrates than artificial substrates. We predicted that willow roots would be preferred over artificial aluminium wire mesh substrates, and with a greater abundance and taxon richness of aquatic invertebrates, as natural willow roots provide both more heterogeneous microhabitats and organic material for invertebrates colonisation.

We also predicted that a more diverse and richer invertebrate community would establish on more complex habitats especially on the willow roots, and that abundance and taxon richness would be higher after 90 days than 30 days of colonisation. Using the data obtained, we suggest that it might be possible to infer whether it is better to remove the whole willow tree or just the canopy during any willow removal operation, and whether willow roots should be left *in situ* until the riparian canopy is reinstated by suitable revegetation.

4.2 Materials and Methods

4.2.1 *Site description*

In situ experiments were conducted in Deep Creek in the Mt. Lofty Ranges, South Australia (Fig. 2.1). Deep Creek is located at around 350 m elevation with latitude $34^0 56$'N and longitude $138^0 46$'E (see Chapter 2 for more details on site description). Some stretches of the creek are totally open and exposed to sunlight with fast flowing water mainly in the winter and spring, while other parts are highly canopied with large trees and the water is slow moving. The components of substratum are heterogeneous with a coarse bed substrate, fine roots, leaf packs and woody debris.

In Chapter 2, I described the physico-chemical characteristics of the stream in detail. During the study period, the mean water temperature ranged from $15.70–23.60^0$C, pH ranged from 8.15–9.84, dissolved oxygen ranged from 10.81–16.25 mg/L, conductivity ranged from 271–422 µS/cm and total dissolved solids ranged from 102–275 ppm. Major aquatic invertebrates in the stream are the introduced hydrobiid snail (*Potamopyrgus antipodarum*), introduced physid snail (*Physa acuta*), gripopterygid stonefly (*Dinotoperla evansi*), caenid mayfly (*Tasmanocoenis tillyardi*), and conoesucid caddisfly (*Lingora aurata*). Other species present are the water bug (*Enithares bergothi*), blackfly larvae (*Austrosimilium furiosum*), lephtophlebiid mayfly (*Koornonga insconspicua*) and chironomids, which are often very abundant (Azmi & Jennings, see Chapter 2).

4.2.2 *Experimental designs*

A pilot study was conducted in October 2008 (spring season) as most investigators agree that experiments using artificial substrates should be conducted during the warmer seasons when animals are more active (e.g., Mathooko & Mavuti 1992). In a preliminary experiment, five different substrates (willow roots, aluminium wire mesh, bamboo sticks, coconut husks and plastic loofahs) were tested for 14 days in Deep Creek to examine colonisation by aquatic invertebrates. However, no significant differences were observed in taxon richness or taxon diversity among the different substrates (see Appendix V). The lack of colonisation was observed, perhaps reflecting the short exposure time (14 days). Hilsenhoff (1969) showed that exposure for at least 25 days was necessary to obtain a stable population of invertebrates. In

consequence, all the cages were left undisturbed for at least 1-3 months to allow colonisation by microbes and invertebrates.

The final experiment was a 2 x 3 x 2 level factorial design, with 4 replicates per sub-class. Treatments were two types of substrate (willow roots vs. aluminium wire mesh), six different habitat complexities and two periods of colonisation (30 and 90 days). Fresh willow roots were collected from Deep Creek, and air dried at ambient temperatures for 2-3 weeks. There were three different habitat complexities for the willow root treatments: (1) all fine and lateral roots were cut down with only primary roots left intact (WR1); (2) only fine roots were cut down, and lateral and primary roots were left intact (WR2); and (3) no roots were cut down (WR3). For the artificial substrate, aluminium wire mesh of three different mesh sizes ranging from: (1) coarse (15 mm; AS1), (2) medium (9 mm; AS2), and (3) fine (1.5 mm; AS3), were used as the three treatments. The use of aluminium mesh of varying sizes has been used by Rosenberg and Resh (1993) to determine the effects of habitat complexity on aquatic invertebrates. Replicate of the substrates were constructed from bundles of willow roots or aluminium wire mesh of 10−15 cm long and 5−10 cm diameter (total surface area 50−150 cm^2) (Fig. 4.1). Each replicate was placed in a galvanized wire mesh cage (mesh size: 3 mm, 15 cm X 10 cm) to avoid being washed away from high flows. As most larger invertebrates quickly leave the cages when it was removed, we decided to wrap the each substrate with nylon onion bags before placing in the cages. Thus, all the invertebrates could be retrieved without the invertebrates escaping.

There were four replicates of each treatment and a total of 48 cages were placed randomly in a uniform section of Deep Creek with an open canopy. Cages were anchored by metal stakes and marked to facilitate recovery.
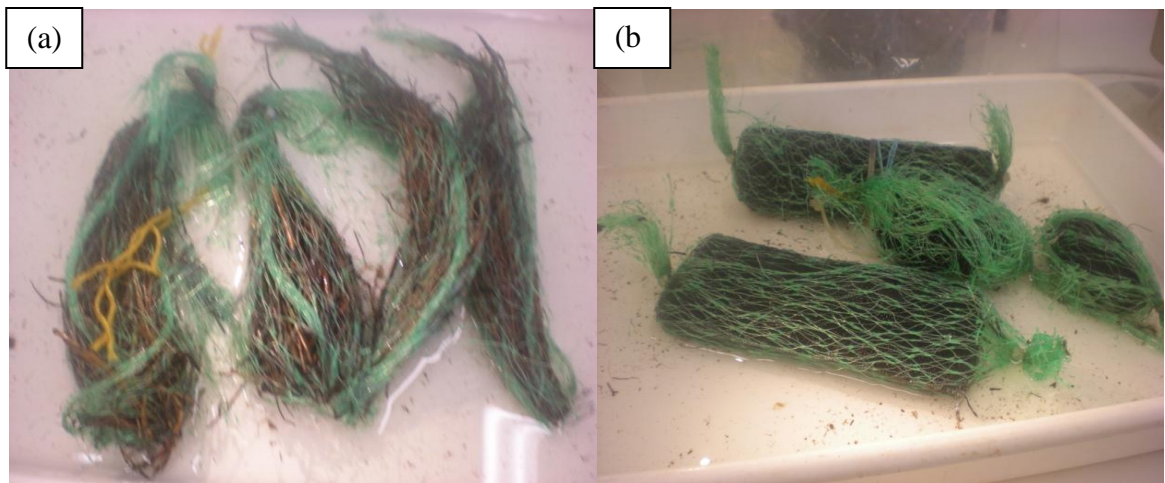
Fig 4.1 Willow roots (a) and aluminium wire mesh (b) bundles in onion bags.

Cages were collected twice to observe variations in colonisation by invertebrates. Between 1200 and 1700 h on 19th December 2008 (Day 30) and again on 21st February 2009 (Day 90), four cages of each different substrates complexity were randomly selected and removed from the stream (n = 24 cages). For each cage, samples were collected by placing a fine-meshed net (250 μm) directly downstream of the cage and quickly placing the cage into the net. The cage and net contents were transferred to individual plastic bags filled with 80% ethanol and returned to the laboratory for analysis.

The cage substrate was washed carefully to remove any attached sediment particles and invertebrates into 250 μm mesh screen, fixed with 80% ethanol for sorting and identification. All invertebrates were sorted into functional feeding groups (Pennak 1978; Merritt & Cummins 1996; Gooderham & Tsyrlin 2002) (see also Chapter 2), and then identified to the lowest possible taxon, and the number of individuals per species for each treatment was counted for further analysis.

4.2.3 *Analysis of findings*

Two-way ANOVAs were used to analyse differences in community distribution between substrate type, habitat complexity and days of colonisation. Data were log(x+1) transformed to ensure normality in calculations of means and ANOVAs. Pair-wise comparisons, using Bonferroni *post hoc* tests were used to compare means to determine between which treatments were different. The same model of two-way ANOVAs was applied to estimate differences in the abundance of functional feeding groups among types of habitat complexity and days of

colonisation. All of the analyses were performed using the Prism statistical program (PRISM 2007). Non-parametric multivariate analysis of variance was used to analyse differences in the invertebrate community assemblages among substrate type, habitat complexity and days of colonisation, using two-way factorial permutation-based nonparametric MANOVA (PerMANOVA; Anderson & ter Braak 2003). The Sorensen Bray-Curtis test was used as a distance measure and 4999 permutation of raw data units were used for each analysis. The Monte Carlo test was used to investigate maximum indicator value for taxon abundance on willow roots and aluminium wire mesh substrates. Detrended correspondence analysis (DCA), which ordinates both species and samples simultaneously was used to identify the taxon assemblage ordination which could be then related to measure how much the taxon distribution differed along types of substrate (Jongman *et al.* 1995). One-way and two-way cluster analysis using Sorenson's Coefficient method was used to sort taxa into groups to reveal the degree of associations between generic composition and the effects of habitat complexity and days of colonisation (Krebs 1989; Kovach 1999). This method sort cases in groups or cluster, so that the degree of association is strong between members of the same cluster and weak between members of different clusters. PerMANOVA, DCA and clustering analyses were performed using the statistical program of PC-ORD version 5.13 developed by McCune and Mefford (2006).

4.3 Results

4.3.1 *Effect of substrate type*

A total of 9935 individuals from 29 species belonging to 25 families of aquatic invertebrates were identified from the different treatments of substrate types in Deep Creek (December 2008 to February 2009). There were significant differences in the total abundance between willow roots and aluminium wire mesh substrates treatments ($F_{1,47} = 15.275$, $P < 0.001$) (Table 4.1). Total abundance was significantly higher on willow root substrates (7564 individuals) compared with aluminium wire mesh substrates (2371 individuals) (Fig. 4.2). However, there was no significant difference in taxon richness of aquatic invertebrates between substrates ($F_{1,47} = 14.771$; $P = 0.2462$) (Table 4.1 & Fig. 4.3).

Table 4.1 Results of two-way ANOVAs on total abundance and taxon richness of aquatic invertebrates at different substrate type, habitat complexity and periods of colonisation (df = degree of freedom; MS = mean squares; ***$P < 0.0001$; *$P < 0.05$).

Source	df	Taxon richness			Total abundance		
		MS	F	P	MS	F	P
Substrate type (Sub)	1	0.441	14.771	0.2462	145.960	15.275	<0.0001***
Habitat complexity (Hab)	2	9131.001	9.124	0.3900	1060.000	4.270	<0.0001***
Days (Day)	1	3498.000	3.495	0.0697	10630.000	42.820	0.0461*
Sub x Hab	2	0.12492E-01	1.098	0.3818	0.145	1.482	0.2626
Sub x Day	1	0.21867E-01	0.2656	0.6470	0.83280E-01	0.695	0.4636
Hab x Day	2	1765.000	1.764	0.1453	332.800	1.344	0.2696
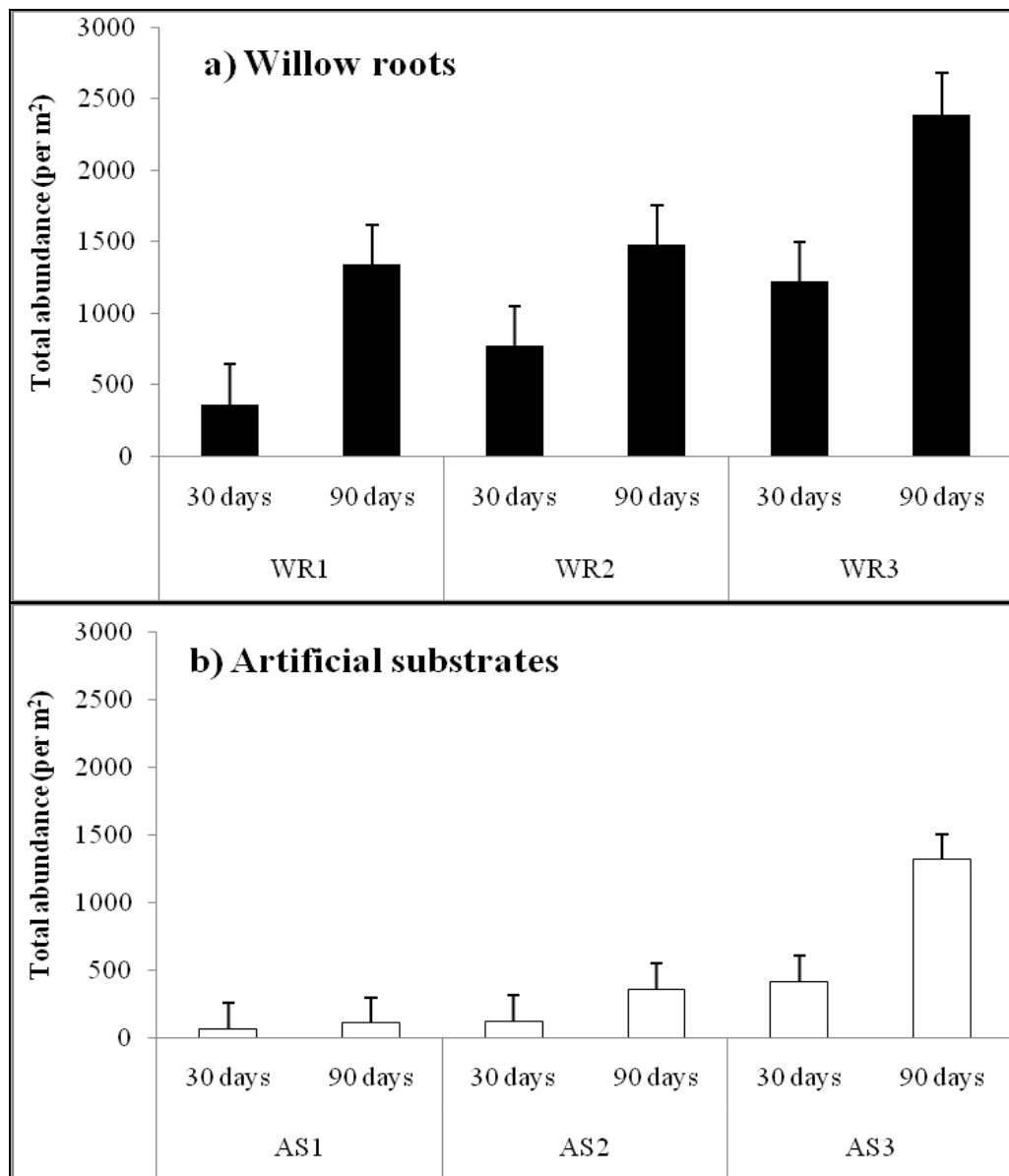
Fig. 4.2 Total abundance of aquatic invertebrate communities in different substrates, habitat complexity and periods of colonisation (Note: WR = willow roots; AS = Aluminium wire mesh substrates; Error bars = standard error).
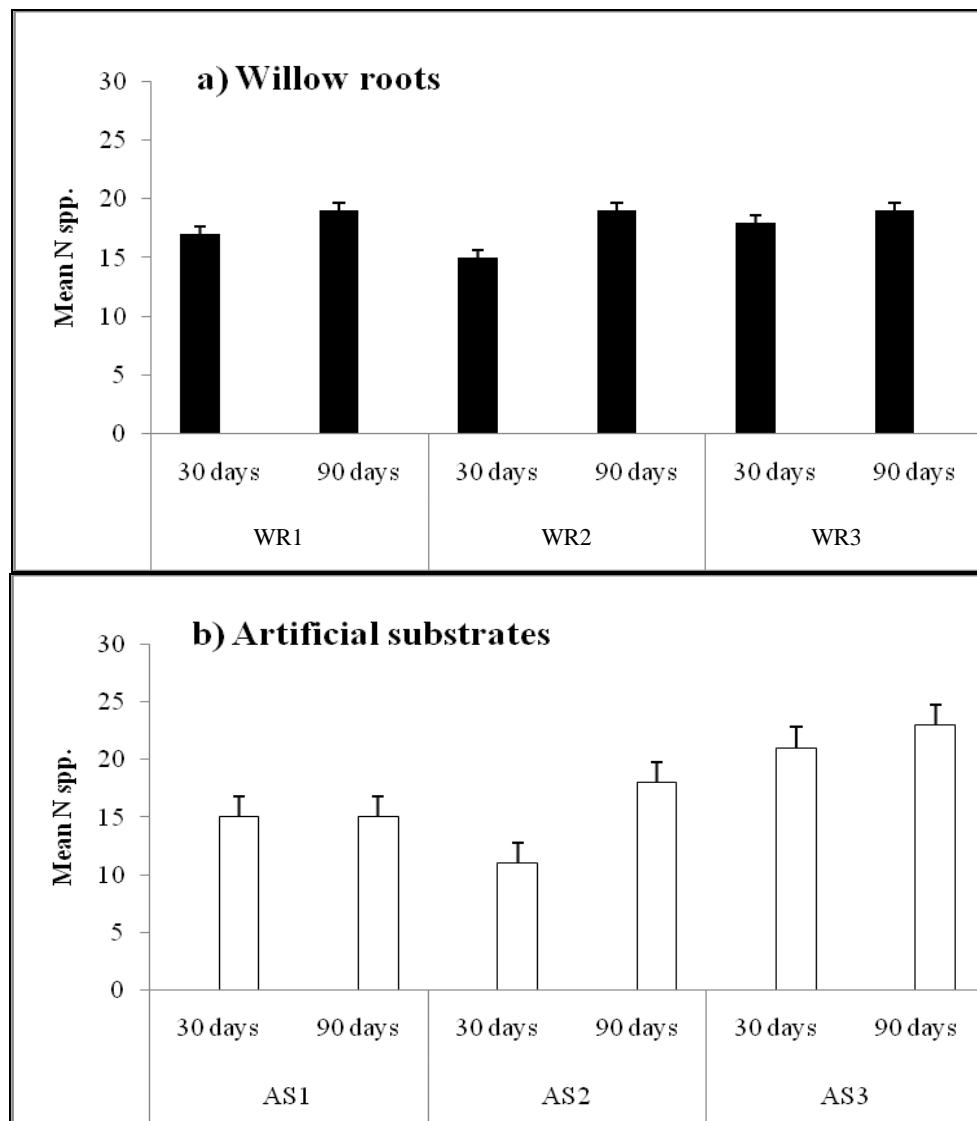
Fig. 4.3 Mean number of species of aquatic invertebrate communities in different substrates, habitat complexity and periods of colonisation (Note: WR = willow roots; AS = Aluminium wire mesh substrates; Error bars = standard error).

Similar trends in relative abundance (%) for the major taxonomic groups of aquatic invertebrates were observed on both substrates (Fig. 4.4). Gastropoda (mostly the introduced hydrobiid snail, *P. antipodarum*) was the most dominant group, making up about half of the total abundance, but more were collected on willow roots than aluminium wire mesh substrates. Plecoptera was the second most abundant group, while Trichoptera and Ephemeroptera were third and fourth highest on both substrates. More Coleoptera, Diptera and Oligochaeta were recorded on willow roots than on aluminium wire mesh substrates. However, slightly more Odonata were found on aluminium wire mesh than on willow roots.

Other groups such as Bivalvia, Ostracoda and Hirudinea were uncommon on both substrates. However, the effect of substrate type on invertebrate community assemblages did not vary significantly among habitat complexity or for colonisation periods (Table 4.2).
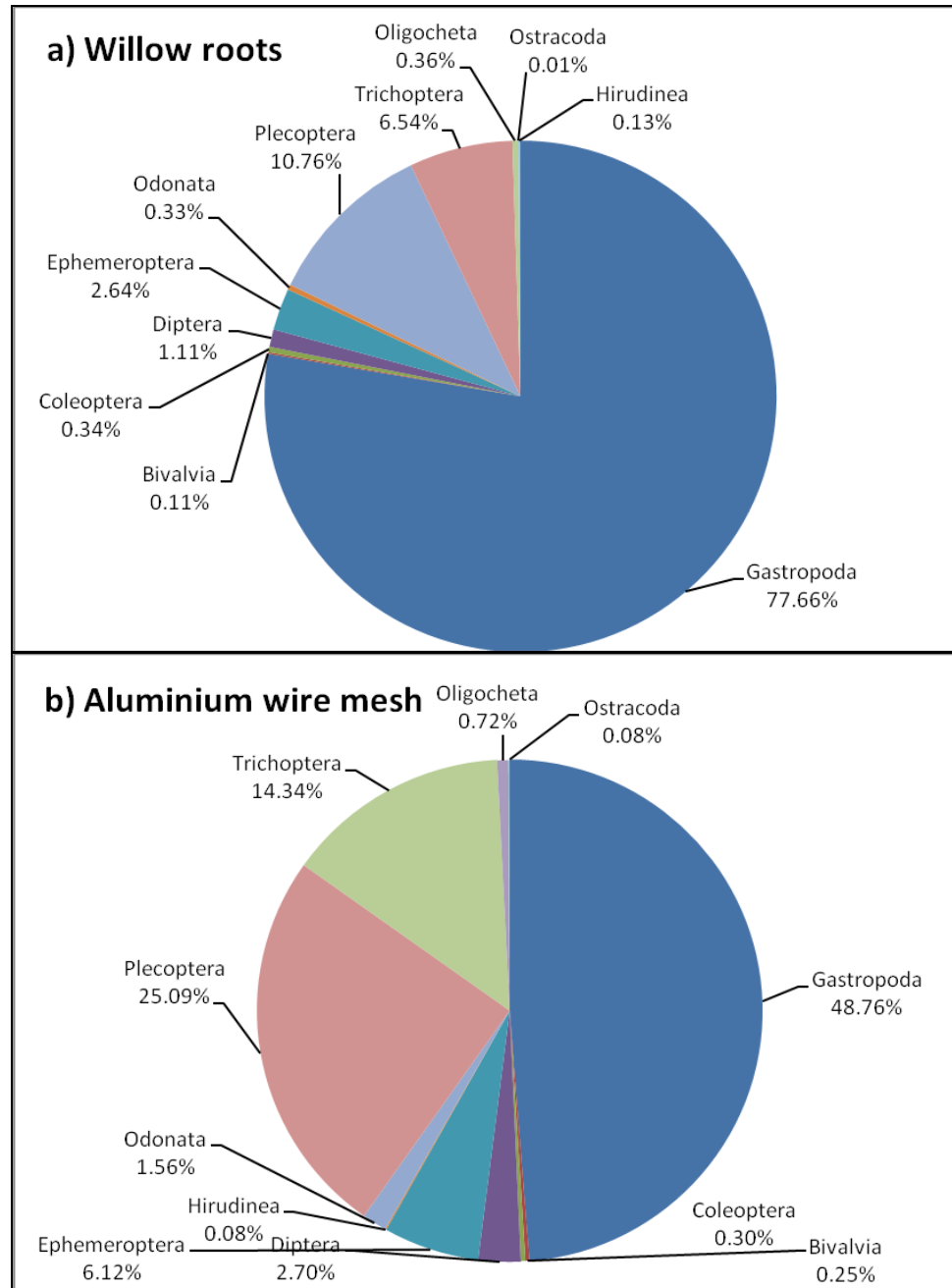
Fig. 4.4 Relative abundance of major aquatic invertebrate groups collected from willow roots (a) and aluminium wire mesh substrates (b).

Table 4.2 Results of two-way factorial PerMANOVAs on aquatic invertebrate community assemblages at different types of substrate, substrate complexity and periods of colonisation (df = degree of freedom; MS = mean squares; *$P < 0.05$).

Source	df	MS	*F*	*P*
Substrate type (Sub)	1	0.6859	7.0808	0.0082*
Habitat complexity (Hab)	2	0.2222	2.2938	0.1186
Days of colonisation (Day)	1	0.2203	1.6031	0.2070
Sub x Hab	2	0.1832	1.8911	0.1768
Sub x Day	1	0.72530E-01	0.5279	0.6112
Hab x Day	2	0.43524E-01	0.1969	0.9736

Of the 29 taxa found, 13 taxa were classified as collector-gatherers, 7 species as shredders and 8 taxa each as predators and scrapers. Only 4 taxa were identified as collector-filterers. However, some of the taxa can be included into two or three functional feeding groups (e.g. *Oecetis* sp., *Simsonia leai*) (Pennak 1978; Merritt & Cummins 1996; Gooderham & Tsyrlin 2002) (see Table 4.3). Scrapers were dominated by the introduced hydrobiid snails, *P. antipodarum,* in all treatments and were significantly greater on willow roots than on the aluminium wire mesh substrates ($P < 0.0001$) (Fig. 4.5). There were clear differences among other feeding groups, with the shredders and collector-gatherers colonising rapidly on the willow roots, while predators and collector-filterers colonised more slowly on both types of substrates. Shredders (e.g., *D. evansi, L. aurata*) were more abundant and more variable on aluminium wire mesh substrates than on the willow roots. However, the abundance of predators (e.g., chironomids, Hemicorduliidae) was greater on the aluminium wire mesh substrates compared with willow roots ($P < 0.01$). Collector-gatherers and collector-filterers were in very low numbers in all substrates (Fig. 4.5).

Table 4.3 List of aquatic invertebrates, their functional feeding groups and taxon abundance in relation to the type of substrates and substrate complexity (Note: scr = Scraper; c-g = Collector-gatherer; c-f = Collector-filterer; shr = Shredder; prd = Predator; ++++ = Very abundant (> 100 individuals); +++ = Abundant (50-99); ++ = Common (10-49 individuals); + = Rare (1-9 individuals); - = Absent (none); bold font = $p < 0.05$).

Class / Order	Family / Species	Functional feeding groups	Type of substrates					
			Willow roots			Artificial substrates		
			WR1	WR2	WR3	AS1	AS2	AS3
Mollusca								
Gastropoda	*Potamopyrgus antipodarum*	scr	++++	++++	++++	++++	++++	++++
	Isidorella sp.	scr	++	+	++	+	++	+
	Glytophysa sp.	scr	-	-	++	-	+	-
	Physa acuta	c-g	++	++	++	+	++	+
	Lymnaea sp.	c-g	-	-	-	+	-	-
	Sphaerium sp.	c-f	-	+	+	-	-	+
Insecta								
Plecoptera	*Dinotoperla evansi*	shr	++++	++++	++++	++	++	++++
	Illiesoperla mayii	shr	++	++	++	+	+	++
Ephemeroptera	*Koornanga inconspicua*	c-g	++	++	++	+	+	++
	Tasmanocoenis tillyardi	c-g	++	+	+	++	+	++
	Offadens sp.	c-g / scr	+	-	-	+	-	-
Coleoptera	*Simsonia leai*	scr / c-g	+	+	+	+	-	-
	Cyphon adelaidae	scr	+	-	+	-	-	-
	Lingora aurata	shr / c-g	+	++	++	+	+	+++
	Taschorema evansi	shr / c-g	+	+	+	+	+	-
	Oecetis sp.	c-g / shr / prd	++	+	++	++	++	+
	Hydroptilidae	shr	++	++++	++++	++	++	+++
	Atriplectides dubius	c-g / scr / prd	+	+	++	+	+	+

Table 4.3 (continued).

Class / Order	Family / Species	Functional feeding groups	Type of substrates					
			Willow roots			Artificial substrates		
			WR1	WR2	WR3	AS1	AS2	AS3
Insecta								
Diptera	*Procladius* sp.	prd	+	+	+	+	+	++
	Polypedilum sp.	c-g / scr / c-f / shr	+	+	+	+	+	+
	Paramerina sp.	prd	+	+	+	-	-	-
Odonata	Hemicorduliidae	prd	+	-	+	+	+	-
	Pseudagrion sp.	prd	+	+	+	+	++	++
	Austroaeschna unicornis	prd	-	-	-	-	-	+
	Austrochiltonia australis	prd	+	-	-	-	-	-
Annelida	Tubificidae	c-g	-	+	++	-	-	+
Oligochaeta	Naididae	c-g / prd	-	+	+	+	+	+
Ostracoda	Undetermined	c-g	-	-	-	+	-	-
Hirudinea	Undetermined	prd	-	+	-	-	-	+
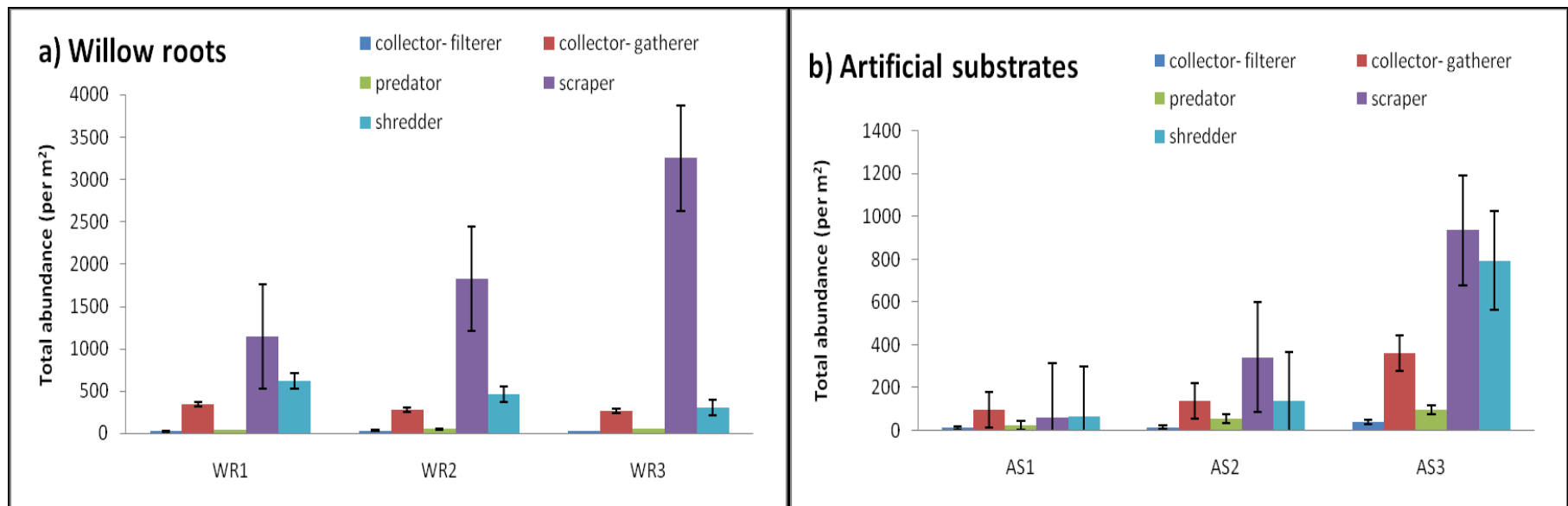
Fig. 4.5 Total abundance of functional feeding groups of aquatic invertebrates in six different habitat complexity for both periods of colonisation (Note: WR = Willow roots; AS = Aluminium wire mesh substrate; habitat complexity of each substrate type increased as the numbers were increased; WR1<WR2<WR3; AS1<AS2<AS3).

4.3.2 *Effect of habitat complexity*

The total abundance of aquatic invertebrates varied significantly from one habitat complexity to another ($F_{2,47}$ = 4.270, P < 0.0001) (Table 4.1). Further analysis using Bonferroni multiple comparison tests showed that the total abundance of invertebrates on aluminium wire mesh substrates (AS3, AS2 and AS1) was significantly lower than on willow roots (WR3, WR2 and WR1) at P < 0.01. However, habitat complexity did not influence the invertebrate community assemblages for different periods of colonisation ($F_{2,47}$ = 0.1969, P = 0.9736) (Table 4.2).

More invertebrates were found on soft, fibrous willow roots (WR1, WR2 and WR3) than on firm, smooth artificial aluminium wire mesh (AS1, AS2 and AS3) (Fig. 4.2). Invertebrates were more abundant on intact willow roots (WR3; 3874 individuals) compared with roots where fibrous fine roots had been removed (WR2; 2246 individuals and WR1; 1444 individuals). On aluminium wire mesh, quite high numbers of invertebrates were recorded on the fine aluminium wire mesh (AS3; 1727 individuals), although lower numbers were found with medium and coarse mesh sizes (AS2; 474 individuals and AS1; 170 individuals).

There were no significant differences between taxon richness and habitat complexity ($F_{2,47}$ = 9.124; P = 0.3900) (Table 4.1). Similarly, no interaction for taxon richness was detected between habitat complexity and periods of colonisation ($F_{2,47}$ = 1.764; P = 0.1453) (Table 4.1). AS3 (fine size of aluminum wire mesh) was found to support the most diverse invertebrate community as 23 taxa were identified (Fig. 4.3, Table 4.3). A total of 18 taxa were found on AS2 (medium mesh size) and 15 taxa on AS1 (coarse mesh size). Willow roots (WR3, WR2 and WR1) were represented by 19 taxa in each treatment.

Both habitat complexity and periods of colonisation significantly affected the number of scrapers, shredders and predators (Table 4.4). Scrapers were much more abundant on soft, fibrous willow roots (WR3, WR2 and WR1) than on the firm, smooth aluminium wire mesh. Scrapers were significantly more abundant on willow roots on aluminium wire mesh (Fig. 4.5, Table 4.3). Of the eight taxa of scrapers recorded, *P. antipodarum* was the dominant taxon in all willow root treatments, indicating to a strong preference to use willow roots. Shredders were dominated by *D. evansi, L. aurata* and hydroptilid caddisfly larvae. *Dinotoperla evansi* and hydroptilids were found on willow root treatments WR2 and WR3, whereas *L. aurata,* which have, in general, similar feeding habits, occurred mostly on AS3. Higher numbers of predators were recorded on AS3, especially the damselfly nymphs (*Pseudagrion* sp.) and the

chironomids. Of the three common taxa of predatory chironomids, *Procladius* sp. were found more on AS3, whereas *Polypedilum* sp. and *Paramerina* sp. appeared to be more on willow roots. However, their abundance was not statistically significant (Table 4.3; Fig. 4.5). Collector-gatherers (e.g., *K. inconspicua* and *T. tillyardi*) were more abundant on all aluminium wire mesh treatments, although no significant differences were detected. Collector-filterers, like the bivalves and chironomids, were very low in numbers in most treatments, but more of them were collected on AS3. However, based on the Monte Carlo test, only the abundance of caenid mayflies (*T. tillyardi*), atriplectidid caddisflies (*Ariplectides dubius*.) and oligochates (Tubificidae) were significantly higher on willow roots than aluminium wire mesh substrates (see Appendix IV).

Table 4.4 Results of two-way ANOVAs on functional feeding groups of aquatic invertebrates at different types of habitat complexity and days of colonisation (df = degree of freedom; MS = mean squares; *** $P < 0.001$; ** $P < 0.001$; *$P < 0.01$).

Functional feeding group	Source	df	MS	*F*	*P*
Collector-filterer	Habitat complexity (Hab)	2	5.48	0.773	0.5758
	Days (Day)	1	120.30	16.950	0.0002***
	Hab x Day	2	14.00	1.970	0.1064
Collector-gatherer	Habitat complexity (Hab)	2	2217.00	0.981	0.4429
	Days (Day)	1	21720.00	9.606	0.0038**
	Hab x Day	2	1834.00	0.811	0.5494
Predator	Habitat complexity (Hab)	2	112.30	2.824	0.0299*
	Days (Day)	1	1576.00	39.630	<0.0001***
	Hab x Day	2	129.00	3.244	0.0161*
Scraper	Habitat complexity (Hab)	2	17140.00	3.609	0.0095**
	Days (Day)	1	156600.00	32.980	<0.0001***
	Hab x Day	2	167000.00	35.270	<0.0001***
Shredder	Habitat complexity (Hab)	2	5397.00	16.210	<0.0001***
	Days (Day)	1	40660.00	122.100	<0.0001***
	Hab x Day	2	9569.00	28.740	<0.0001***

Taxa abundances and taxa assemblage ordinations showed marked variation in relation to the types of substrate and habitat complexity (Fig. 4.6). The most abundant taxon was *P. antipodarum* which was significantly more abundant on all willow root treatments, especially on WR3. It was followed by gripopterygid stonefly nymphs (*D. evansi*) and hydroptilid caddisfly nymphs, which were also significantly more abundant on willow root treatments. Other major taxa, such as lephtophlebiid mayfly nymphs (*K. inconspicua*), caenid mayfly nymphs (*T. tillyardi*), conoesucid caddisfly nymphs (*L. aurata*) and physid snails (*Ph. acuta*) were very commonly found on all types of substrate complexity. In willow root treatments, the coenagrionid damselfly nymphs (*Pseudagrion* sp.) and leptocerid caddisfly nymphs (*Oecetis* sp.) appeared to be particularly uncommon, but more were found on aluminium wire mesh substrates (AS3 and AS2). Other taxa (e.g., *Si. leai, Isidorella* sp.) were considered as minor since less than 49 individuals of each taxon were recorded. The least common taxa were telephebiid dragonfly nymphs (*Austroaeschna unicornis*), lymnaeid snails, ostracods and amphipods, each represented by less than 5 individuals throughout the study.
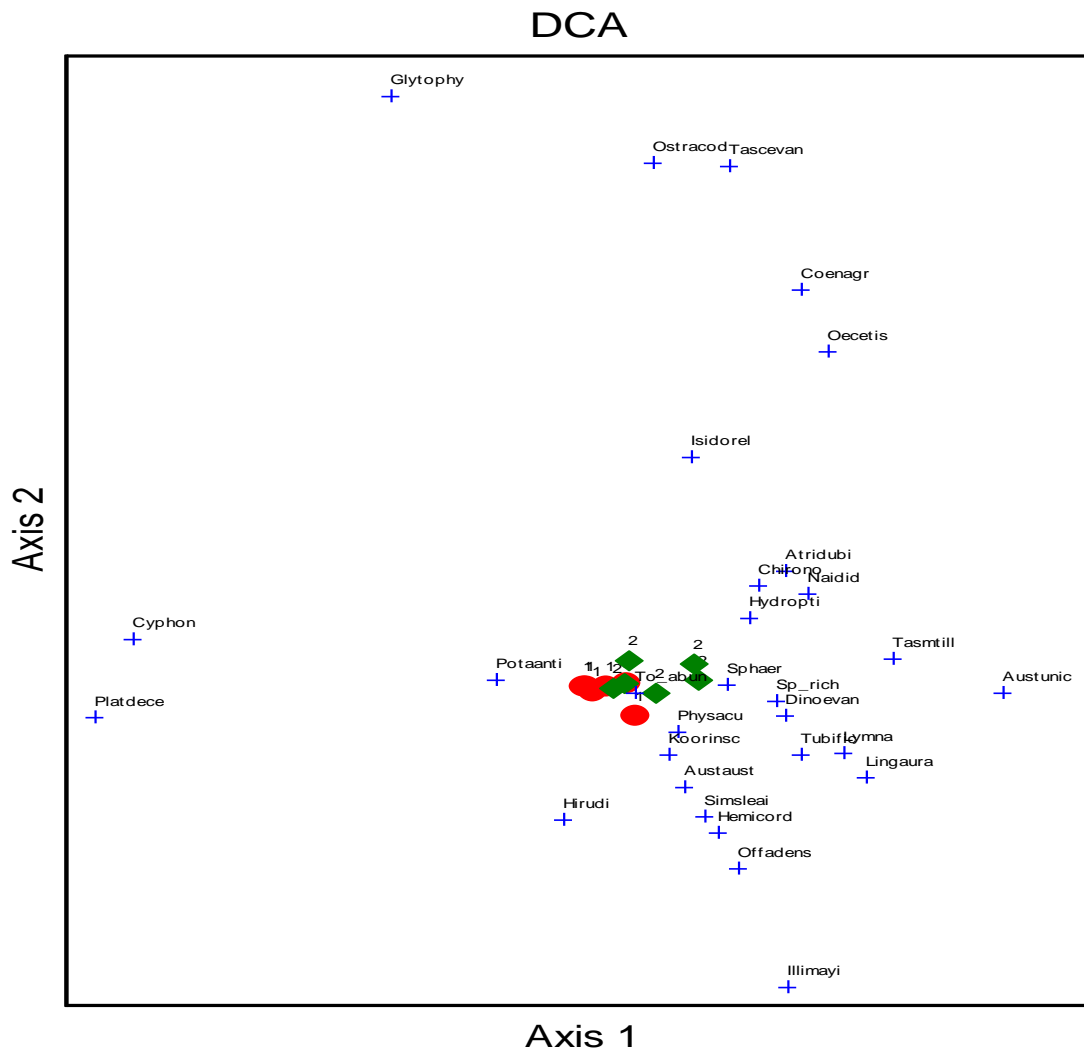
Fig. 4.6 Detrended correspondence analysis (DCA) plots showing taxon assemblage ordinations which related to the types of substrate. ●= willow root habitats; ◆= aluminium wire mesh substrates. Axis 1 = increased habitat complexity of aluminium wire mesh; Axis 2 = increased habitat complexity of willow roots. Not all taxa are included: taxa such as bivalves, amphipods and hirudineas were excluded due to their lower number in the samples. See Appendix I for taxa codes.

4.3.3 *Effect of periods of colonisation*

There were significant differences in the total abundance of aquatic invertebrates between periods of colonisation ($F_{1,47} = 42.820$, $P < 0.05$) (Table 4.1). As predicted, more invertebrates were found after 90 days than after 30 days of colonisation (Fig. 4.2). After 90 days, invertebrates were markedly higher on WR3 with 2397 individuals, and considerably higher in other willow root treatments (WR2 = 1477 individuals; WR1 = 1339 individuals). Relatively high levels of abundance occurred on AS3 with 1316 individuals, but abundance was drastically reduced on AS2 (355 individuals) and AS1 (105 individuals). The highest abundance after 30 days was observed on WR3 with 1220 individuals, followed by WR2 and WR1, with 769 individuals and 362 individuals respectively. However, significantly lower total abundances were recorded in aluminium wire mesh substrates after 30 days of colonisation. The total abundance on AS3 was the highest (411 individuals) and the abundance declines on AS2 (119 individuals) and reached its lowest level on AS1 (66 individuals) (Fig. 4.2).

In terms of taxon richness, a slightly richer and more diverse fauna was found after 90 days than 30 days. However, habitat complexity and days of colonisation did not influence the taxon richness of the invertebrate fauna ($F_{2,47} = 1.764$; $P = 0.1453$) (Table 4.1). The fine aluminium wire mesh substrate (AS3) had a richer fauna than other treatments with 23 taxa recorded after 90 days (Fig. 4.3). Interestingly, only 19 taxa colonised each of the willow root treatments by 90 days, although many of them were highly abundant (Table 4.3). Again, the fine aluminium wire mesh substrate (AS3) recorded the richest fauna after 30 days with 21 taxa, whereas other treatments were considerably lower with the lowest diversity being on AS2 with only 11 taxa.

Sorensen's Coefficient method for one-way and two-way clustering analysis was used to examine differences in habitat complexity and time of colonisation, and is represented by the dendrograms (Fig. 4.7). The importance of habitat complexity on the composition of the invertebrates is illustrated by one-way clustering analysis (Fig. 4.7a) in which all samples from WR3 and AS3 fall into Cluster A. *Platynectes decempunctatus, Au. unicornis* and *Cyphon adelaidae* were only found on more complex habitats (WR3 and AS3; Cluster A). Cluster B contains the samples from less complex habitats (WR2, WR1, AS2 and AS1). However, AS1 was well separated from the other samples in Cluster B, probably due to its lowest total abundance and taxon richness compared with other samples.
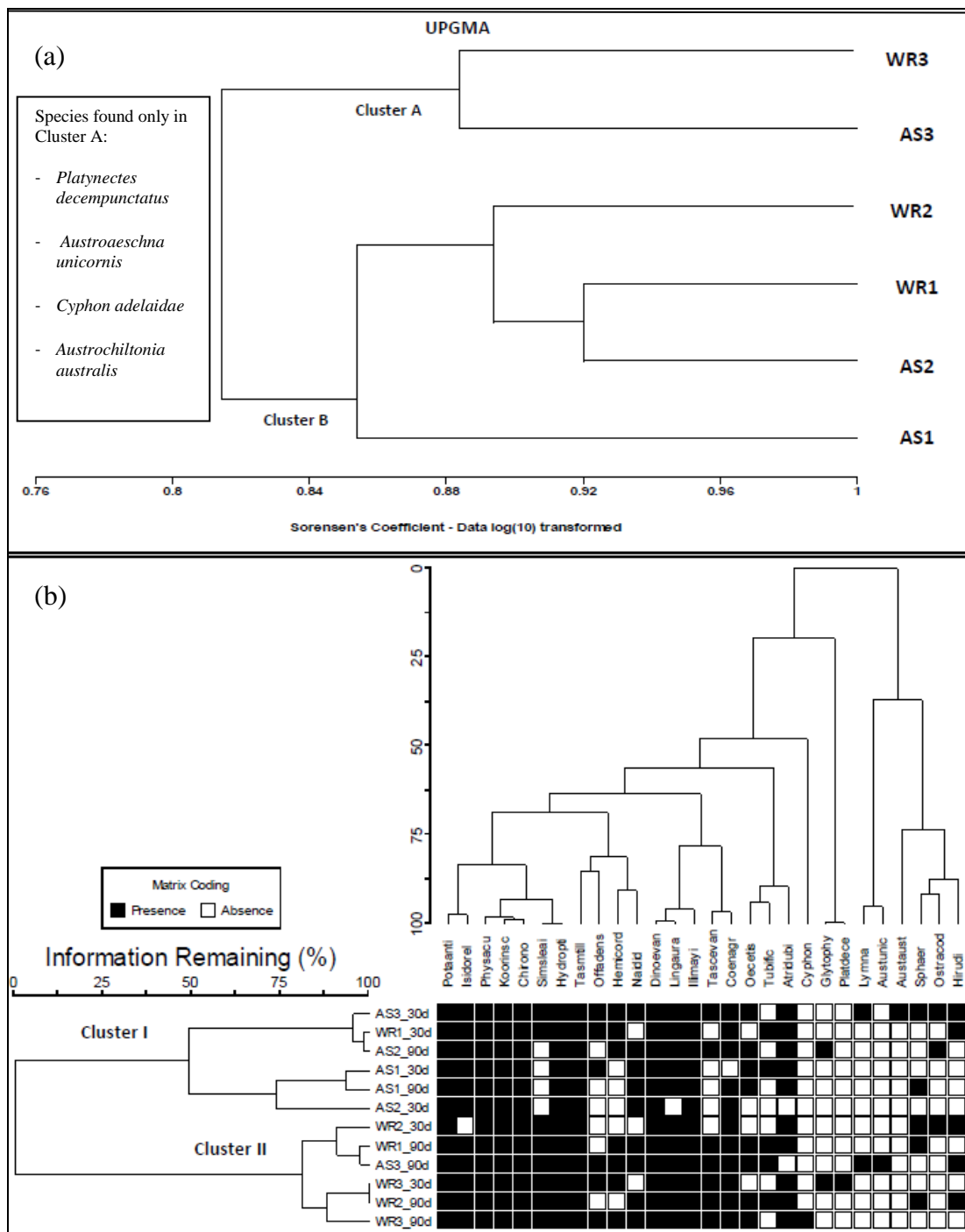
Fig. 4.7 Dendrograms using UPGMA Sorensen's Coefficient method for one-way (a) and two-way (b) clustering analysis of individuals and generic composition in different substrates, habitat complexity and days of colonisation. (WR = Willow roots; AS = Artificial substrates; 30d = 30 days of colonisation; 90d = 90 days of colonisation; Note that not all taxa are included. Taxa with fewer than 5 individuals, such as bivalves and amphipods, were excluded. See Appendix I for species codes.

The interaction between habitat complexity and periods of colonisation, however, differ with the dendogram of two-way cluster analysis (Fig. 4.7b) in that two main groups were found. Most samples from the aluminium wire mesh substrates (AS1, AS2 and AS3) of each colonisation period (30 and 90 days; except for AS3) fall into Cluster I together with the sample least complex of willow root habitats after 30 days (WR1). The remaining samples, willow root treatments (WR1, WR2 and WR3) from each colonisation period (30 and 90 days; except for WR1), and the sample of fine mesh size of aluminium wire substrate (AS3) after 90 days form the other group (Cluster II).

Generally, invertebrates among the treatments were similarly distributed as the average Sorenson similarity coefficient values were medium to high, ranging from 0.50 to 0.95. Sorenson's coefficient is an index of community similarity, ranges from 0 to 1 with higher values indicating a higher degree of similarity (Jongman *et al.* 1995; Krebs 1989). Thus, a coefficient of 1 means complete overlap of taxa between two communities and 0 means no overlap. In this study, similarity was determined within types of habitat complexity (i.e., WR1, AS1, etc.) and between days of colonisation (30 and 90 days). The samples from willow roots (WR1, WR2 and WR3) are not more distinct from the samples of aluminium wire mesh substrates (AS1, AS2 and AS3) (Fig. 4.7a). This was due to the similar distribution of certain dominant taxa among all treatments (e.g., *P. antipodarum, Ph. acuta, D. evansi, T. tillyardi*).

However, Cluster I was well separated from Cluster II (Fig. 4.7b). This separation is mainly due to the presence/absence of several taxa. For example, baetid mayfly nymphs (*Offadens* sp.), elmids beetle larvae (*S. leai*), conoesucid caddisfly nymphs (*Taschorema evansi*) and tubificids were absent from most samples in Cluster I. Whereas, some taxa such as telephebiid dragonfly nymphs (*Au. unicornis*) and scirtid beetle larvae (*Cy. adelaidae*) were only present in Cluster II. In terms of periods of colonisation, most taxa were more abundant on all substrates after 90 days than after 30 days. The more complex willow root habitats (WR2 and WR3) and aluminium wire mesh habitat (AS3) exhibited a greater similarity to each other in terms of frequent taxa composition than the less complex habitats (WR1, AS1 and AS) after both 30 and 90 days.

4.4 Discussion

Overall, willow roots supported significantly higher total numbers of aquatic invertebrates by providing a better habitat than the aluminium wire mesh substrates (Table 4.1; Fig. 4.2). In addition, more taxa were recorded on willow roots than on aluminium wire mesh substrates, although the differences were not significant (Table 4.1; Fig. 4.3). No consistent differences were detected in the mean number of taxon among the treatments tested. Thus, our first hypothesis was only partially supported: the abundance of aquatic invertebrates was higher on willow roots than on aluminium wire mesh, but this was not the case for taxon richness or taxa differences.

Willow roots have habitat value for aquatic invertebrate communities. Willow root mats are fine, rough, fibrous in texture and extend into the water column, and provide a suitable habitat for invertebrate reproduction, protection from predators, and food supply (Karinkova 1971). Willow roots can be colonised by beneficial bacteria and naturally occurring food organisms (biofilms), and these biofilms and epilithics material (including algae, bacteria, fungi, detritus, sand and silt) are known to affect colonisation by aquatic invertebrate communities (Minshall 1984; Mackay 1992). Davoudi (2011) reported that willow root is recommended as an effective technique for preventing superficial sliding in Iran.

In our study, most taxa were greater in number on rough, fibrous substrates than on smooth, firm substrates, indicating that artificial aluminium wire mesh is less suitable as a site of attachment and less able to provide a food source than natural willow roots. We noticed willow roots accumulated a much thicker slimy microbial biofilm and epilithics material compared with on aluminium wire mesh. To date, although there have been no studies on the amount of periphyton growth or detritus accumulation on willow roots or aluminium wire mesh substrates, Casey and Kendall (1996) found that densities of benthic macroinvertebrates and the quantity of organic material were greater on a natural substratum than on artificial substrate comprising particles of natural rock or clay brick. They suggested that the development of an invertebrate community on a substrate is linked to both the mobility of different species and the accumulation of food sources, i.e., periphyton and organic detritus, on the substrates. Other investigators who have studied artificial substrates (e.g., Crossman & Cairns 1974; Souter & Williams 2001; Souter 2004) did not measure organic material (e.g., biofilms, epilithics material, etc.) on the surface of the substrates. This study which mimics the

management situation where the willows are sprayed with herbicides or have the tops removed, with the roots left in the soil, shows that lower abundance and diversity of the invertebrates on decaying roots would be expect than those of living roots. In future, investigators should consider the potential effect of the natural epilithic cover of substratum particles might have on colonisation by invertebrates when choosing the type of artificial substrates.

In terms of relative abundance of the major taxonomic groups, we found the gastropods were significantly greater ($P < 0.0001$) on willow roots than on aluminium wire mesh, although this was primarily due to a single species, *P. antipodarum,* which contributed more than half of the total abundance. Jayawardana *et al.* (2006) also found that willow root habitats facilitate the high abundance of *P. antipodarum*. This alien species has rapidly invaded Australian freshwater systems, and potentially impacts on native invertebrate assemblages include domination of preferred habitat, physical covering of egg sites and egg masses, and attraction of fish predators (e.g., Ponder 1988; Bowler 1991). Other commonly collected taxa in our experiment, mostly on all substrates, included stonefly nymphs (*D. evansi*), mayfly nymphs (*T. tillyardi*), caddisfly nymphs (hydroptilids) and midge larvae (Chironomidae). However, no significant differences and no correlation in relative abundance were detected.

In most cases, the abundance of the functional feeding groups was greater on willow roots treatment. Scrapers (primarily *P. antipodarum*) were the most dominant colonist on willow roots. This was probably a result of the physical heterogeneity and quantity of organic material provided by willow roots compared with the aluminium wire mesh. Azmi and Jennings (see Chapter 2) supported this finding as they found *P. antipodarum* were highly associated with willow root habitats. Similarly, the high numbers of shredders observed on willow roots suggest that most colonists are drawn from the drift, and include those which disperse from low or high flows over short distances, by actively swimming or crawling over the substratum (Mackay 1992). In this study, ephemeropterans (Leptophlebiidae and Caenidae) are strong swimmers and crawlers, as are plecopterans such as *D. evansi* and *I. mayii,* and were more abundant on willow root habitats (Table 4.3). Predator species were also more abundant on willow roots, probably due to prey availability, and refuge requirements. In contrast, other functional feeding groups such as collector-gatherers and collector-filterers, showed no consistent differences in abundance among substrate types. It might be expected that they would not be influenced by either structural heterogeneity or the presence of organic material,

but for most feeding groups, abundance was consistent with the prediction that invertebrate assemblages would be greater on the willow roots than on the aluminium wire mesh.

The hypothesis that habitat complexity would have a significant influence on the total abundance of aquatic invertebrate assemblages was supported (Table 4.1). Slightly more invertebrates were found on soft, fibrous willow roots especially where none of the willow roots were cut (WR3; Fig. 4.2). When the structure of willow roots were less complex (i.e. more fibrous fine and lateral willow roots were removed), we found that the invertebrates were significantly less abundant. This suggests that willow roots are an adequate refuge for the invertebrates, but major decreases in structural complexity results in a lower abundance.

The firm, smooth aluminium wire mesh substrates had significantly fewer aquatic invertebrates than willow roots (Fig. 4.2). However, the attempt to mimic the structure of willow roots provides interesting findings into the effect of habitat complexity. Among the aluminium wire mesh substrates, invertebrates were more abundant on the fine mesh size (AS3) than on either medium (AS2) or coarse (AS1) mesh sizes. Although more species and a greater diversity was also found on the fine mesh (AS3; 23 taxa), these differences were not significant. The complex habitat provided by aluminium wire mesh could support a diverse invertebrate fauna, and implies that the fine aluminium wire mesh (AS3) provided a better habitat than the other aluminium wire mesh treatments. However, further testing is necessary to confirm this. Not all invertebrate communities on the aluminium wire mesh had a high diversity, and it is therefore not always possible to correlate total number of species with habitat complexity. The low diversity of invertebrates on medium (AS2) and coarse (AS1) aluminium mesh might indicate a poorer microhabitat for the invertebrates compared with willow roots.

There may be several reasons that might explain the differences observed in invertebrate abundance and taxon richness between the artificial and natural systems. The differences in total abundance and species richness were probably caused by several factors, such as physical heterogeneity of the substrate, quantity of organic material, and biofilms. The high numbers of invertebrates on the willow roots was probably caused by greater surface area and physical characteristics of these substrata. However, more richness and diverse of invertebrates were found on fine aluminium wire mesh (AS3) indicating that artificial substrates may have greater impact on uncommon taxa. Indeed, a greater number of uncommon taxa such as the native lymnaied snails, baetid mayfly nymphs (*Offadens* sp.), telephebiid dragonfly nymphs

(*Au. unicornis*) and caenid amphipods (*Austrochiltonia australis*) were present on the fine aluminium wire mesh (AS3). Although not tested, further research should focus on the biofilms as the presence of organic materials might be responsible for the occurrence of these uncommon taxa on fine aluminium wire mesh (AS3).

One-way cluster analysis showed that habitat complexity is probably the main factor in determining the distribution of invertebrates (Fig. 4.7a). The importance of the effect of habitat complexity on the composition of the invertebrates is illustrated by the fact that all samples from less complex habitats (WR1, WR2, AS2 and AS1) fall into Cluster A, and those with high habitat complexity (WR3 and AS3) were grouped together in Cluster B. This indicate that substrate complexity is important, as more taxa were found in significantly greater densities on rough, fibrous and/or more complex substrates (WR3 and AS3) than smooth and less complex of substrates (WR1, WR2, AS2 and AS1). Four uncommon taxa were found exclusively on these more complex habitats. They were the dragonfly nymph (*Au. unicornis*), amphipod (*Aus. australis*), scirtid beetle (*Cy. adelaidae*) and dystiscid beetle (*Platynectes decempunctatus*) (Fig. 4.7a).

Physical heterogeneity alone did not necessarily lead to the differences we observed in the fauna between natural willow roots and aluminium wire mesh. Several observers have suggested that organic material affects colonisation by some invertebrate taxa as it provides either a source of food or a suitable habitat (Suter 1990; Casey & Kendall 1996; Souter & Williams 2001). The fine sediment we observed on willow roots probably included a natural biofilm and fine detritus that would provide food for some invertebrates especially the scrapers. Willows are an important food source for many aquatic invertebrates (Hanlon 1981; Collier & Winterboum 1986; Lester *et al.* 1994a), and Jayawardana *et al.* (2006) found more scrapers (*Ph. acuta, P.antipodarum*) and organic pollution-tolerant taxa (*Cura* sp., *Megadrilii* sp.) under willow roots, when willows had a higher coarse particulate organic matter (CPOM) content. We found the three native shredders (*D. evansi, T. tillyardi* and *L. aurata*) preferred willow leaves over eucalypt leaves in feeding, growth and survival experiments (see Chapter 3). We suspect that willow roots are attractive to some invertebrates and possibly supply additional nutrition (e.g. Collier & Winterbourn 1986), or may lack the harmful secondary compounds which directly affect the invertebrate feeding observed on leaves (Lester *et al.* 1994b).

Differences in willow root habitat complexity may also influence the refuge selection behaviour of organisms. Levin *et al.* (2000) suggested that habitat may affect the frequency of interspecific or intraspecific behavioural interactions of the organisms. We suspect that reduced habitat complexity may modify the effect on invertebrate functional feeding groups by providing fewer refugia. We found more scrapers on fibrous, fine willow roots than on the aluminium wire mesh (Fig. 4.5). Scrapers are probably colonists of willow roots, as they provide a more stable and better habitat from high currents. The low number of other functional feeding groups may be due to competitive interactions, where there might be competition, especially between the scrapers and collector-filterers, for fine particulate organic matter on the willow roots. However, for the aluminium wire mesh, more shredders were found on fine (AS3) compared with medium (AS2) and coarse (AS1) mesh sizes. This is supported by the high abundance of some drift organisms such as *D. evansi, L. aurata* and hydroptilid caddisfly nymphs, which were highly associated with more complex habitats during high stream flows in winter and spring.

Other functional feeding groups such as collector-gatherers (e.g., *K.inconspicua* and *T. tillyardi*) and collector-filterers (e.g., bivalves and chironomids) were greater in number on all willow root treatments, although no significant differences were detected (Table 4.3; Fig. 4.5). Predators such as chironomids and odonate nymphs were consistently associated with willow roots. Therefore, this study not only supports the hypothesis that habitat complexity is an important factor influencing the distribution of aquatic invertebrates, but it also indicates the relatively large degree of change in the faunal composition and functional feeding groups in the stream.

There were significant higher abundances of aquatic invertebrates on all treatments (WR3, WR2 and WR1) after 90 days of colonisation compared with 30 days of colonisation (Table 4.1, Fig. 4.2). This support the third hypothesis and previous generalisations by other workers concerning the influence of colonisation periods on aquatic invertebrate communities (e.g., Bologna & Heck 1999; Gore *et al.* 2001; Becker & Robson 2009). It is suggested a long colonisation period is crucially important to obtain a stable invertebrate community because it allows development of biofilms and epilithics material, known to affect colonisation by aquatic invertebrates. However, a stable invertebrate community and colonisation dynamics is complex and generally not well understood (Rosenberg & Resh 1993). Generally, short colonisation periods (< 2 weeks) on artificial substrates cannot represent a stable community

because the habitat will still be changing relatively rapidly. Casey and Kendall (1996) found that the macroinvertebrate assemblages colonising both artificial and natural substrate types were higher after 29 days of exposure. In another study, the greatest number of taxa and individual on the perspex artificial substrates occurred after 35 days of exposure, with a second peak in total individual after 56 days (Boothroyd and Dickie 1989). Hilsenhoff (1969) suggested that at least 25 days was necessary for the artificial substrates to be placed in stream channels in order to obtain a stable population of invertebrates. We found species composition was more diverse and abundant after 90 days than after 30 days of colonisation on all substrates (Fig 4.7b). Some species such as baetid mayfly larvae (*Offadens* sp.), elmid beetle larvae (*S. leai*), conoesucid caddisfly nymphs (*Tas. evansi*) and tubificids were more abundant on complex habitats (WR2, WR3 and AS3) after 90 days of colonisation, probably due to the long exposure time and food source availability (e.g., biofilms and organic material).

In terms of taxon richness, we expected a greater invertebrate diversity would occur on the natural willow roots, which are expected to provide more microhabitats for reproduction, protection from predators and food supply. Various authors have suggested that certain features of the vegetation (including the roots) play a role in the regulation of faunal distribution and their assemblage is dependent on the composition and structure of vegetation (e.g., Bologna & Heck 1999; Flory & Milner 1999).

A study by McKie and Cranston (2001) in some streams of Canberra shows that macroinvertebrate assemblages varied taxonomically and functionally with both wood species and riparian vegetation composition. They found two specialist feeding groups responded clearly to riparian vegetation: wood gougers were most common in forested streams, and algal grazers in more open streams. They suggested that the importance of sampling appropriate substrates when assessing questions of this type – if seeking shifts in functional organisation, the substrates on which the feeding groups of interest occur must be sampled.

We found a more diverse and richer invertebrate fauna on fine aluminium wire mesh (AS3) after 90 days of colonisation (25 taxa) (Fig. 4.3), suggesting that aluminium wire mesh substrates may provide suitable habitats as well as food availability, but that a longer period of colonisation is essential to obtain this richer and more diverse invertebrate community. The greatest abundance of invertebrates was found on WR3 after 90 days of colonisation (2397 individuals) (Fig. 4.2). However, habitat complexity and periods of colonisation did not influence invertebrate community assemblages (Table 4.2). Consistent patterns in total

abundances and taxa richness on each substrate type were found after each colonisation period. The classification of the samples obtained by one-way and two-way cluster analyses (Figs. 4.7a & 4.7b), shows a relationship with habitat complexity and days of colonisation. The results not only support the hypothesis that habitat complexity is an important factor influencing the distribution of aquatic invertebrates in a stream, but also indicate that the period of colonisation may change the faunal composition (i.e., the longer the period, the better the colonisation).

For habitat complexity, the relative abundance of taxon was similar for most willow root treatments, except for the least complex willow roots (WR1) after 30 days of colonisation. This suggests that willow roots tend to be selectively colonised by certain taxa, which may explain why the most complex willow roots (WR3) had a lower richness compared with fine size of aluminium wire mesh (AS3). Generally, gastropods (primarily scrapers) constituted the greatest abundance in willow root treatments. The introduced hydrobiid snails, *P. antipodarum,* were significantly more abundant, especially on more complex habitat structures and over a longer period of colonisation (e.g., WR3 after 90 days). This may help explain why willow root treatments were grouped together. The rapid colonisation and utilisation of much or all of the available space by *P. antipodarum* on the willow roots had excluded other invertebrate species that might utilise the willow roots as a refuge. Besides that, there is a possibility that strong predation by vertebrates (e.g. fish) had consequently increased the need by *P. antipodarum* to utilise the willow roots as a refuge.

The abundance of shredders and predators showed significant differences among the degree of habitat complexity and days of colonisation (Table 4.3). Of the shredders, *D. evansi, T. tillyardi* and hydroptilid caddisflies were numerically dominant on all substrate types, but their density was affected by the interaction between habitat complexity and days of colonisation. The only possible taxon preference noted was the colonisation of dragonfly nymph (*Au. unicornis*) and scirtid beetle larvae (*Cy. adelaidae*) on aluminium wire mesh, and their complete absence on the willow roots. Future research should concentrate on colonisation by individual species, and species interactions, which might lead to a greater insight into colonisation patterns.

Overall, soft, fibrous willow roots have habitat value for aquatic invertebrates by providing a better surface texture and area, and variety of microhabitats, for invertebrate colonisation. This study also indicates that artificial willow roots (fine aluminium wire mesh) can be used to stimulate invertebrate community, and may be particularly useful especially in sites where willows have been removed. In the large scale removal of willows, especially in a small stream, at least some of the willow roots could be left, as the sudden removal of roots may disrupt the communities, including many native invertebrates, which utilise the roots as habitat. As a relatively long period of colonisation is needed to obtain a complex and stable invertebrate community approaching what might be considered 'normal', further examination of the amount of detritus and periphyton growth on willow roots and aluminium wire mesh should be carried out to decide which of these substrates is better for invertebrate colonisation during revegetation efforts. However, the most serious threat may be the resource competition between exotic *P. antipodarum* and native invertebrates.

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CHAPTER 5: Impact of shade on aquatic invertebrate communities

5.0   Abstract

The impact of shade on the abundance and distribution of aquatic invertebrates under willows or artificial canopies (shade cloth) was examined in two separate trials at Sixth and Deep Creeks in Mount Lofty Ranges, SA. At each study site we tested three different levels of shade; (1) fully shaded (c. 90-100%), (2) partly shaded (c. 70%) and (3) open canopy (0%). After a 28 day colonisation period, we found a significantly higher abundance of aquatic invertebrates in fully or partly shaded willow treatments compared with artificial shade. The effect of willow shade on invertebrate total abundance varied significantly among levels of shade, where greater abundance was found in fully and partly shaded treatments. A similar trend in total abundance was observed under artificial shade, but the difference was not statistically significant. Treatments without shading (open canopy) had a lower abundance of invertebrates than fully and partly shaded treatments in both willow and artificial shade trials. However, greater taxon richness was found in treatments lacking a riparian canopy (i.e., open canopy) in both trials. Even though the relationship of light intensity on relative abundance was not analysed due to limited data, we observed that invertebrate abundance decreased as light intensity increased. These changes in taxon richness may have been influenced by higher quantity and quality of food or perhaps higher temperatures compared with the fully and partly shaded treatments. In terms of management, complete removal of willows should be considered carefully, and we recommend that removal operations be carried out in small sections, especially during the summer months when the impact on aquatic invertebrate communities is likely to be greatest.

Keywords: willow shade, artificial shade, aquatic invertebrates, abundance, taxon richness.

5.1   Introduction

The significant role of riparian vegetation in stream ecosystem structure and function has long been recognized (e.g., Quinn *et al.* 1992 & 1994; Bunn *et al.* 1999a; Mokany *et al.* 2008), and various authors have warned that its removal may initiate erosion of the streambed, resulting in a high input of organic matter and increased sedimentation (e.g., Naiman *et al.* 1988; Quinn *et al.* 1992 & 1994), and can lead to increased water temperatures (Lynch *et al.* 1984; Quinn *et al.* 1992) and the consequential decrease in dissolved oxygen levels (Hellawell 1986). The interaction between higher water temperatures and lower dissolved oxygen may affect all physiological processes of different life stages of invertebrates (Bothwell *et al.* 1993, Helmuth *et al.* 2006) and fish larvae (Gutierrez-Rodriguez & Williamson 1999).

As with all riparian vegetation, willow canopies play an important role in controlling stream ecosystems by contributing to the maintenance of stream integrity. A reduction in willow canopies can increase the degree of daily fluctuations in water temperature due to higher light incidence and increased illumination (Allan 1995, Kelly *et al.* 2003). Removal of canopies may also contribute to sediment scouring and deposition, and to decreasing habitat heterogeneity for fish, aquatic plants and invertebrates (Collier *et al.,* 1995; Schulze & Walker 1997), which in turn, will lead to the deterioration of aquatic communities. These effects are probably most obvious in small and shallow streams as they are particularly sensitive to modifications in riparian vegetation (Quinn *et al*. 1994).

As willows are seen as a serious weed in Australian aquatic ecosystems, wide-scale willow removal programs are currently underway. Results from previous studies suggest that changes in riparian cover near aquatic environments influence the structures of aquatic fauna communities. Most findings indicate that total abundance of macroinvertebrates increased with a decrease in canopy cover (e.g., Hawkins *et al.* 1986). In fact, Hawkins *et al.* (1986) suggested that effect of canopy was more important than substrate character in influencing total abundance and guild structure. Another study by Sabater *et al.* (1998) found that macroinvertebrate community had higher density and biomass in the logged section (open canopy) than in the forested section in Ter River, Barcelona (NE Spain). Bojsen and Jacobsen (2003) reported similar findings at twelve sites located in an area of fragmented rainforest in the Ecuadorian Amazon, where total macroinvertebrate density increased with decreasing canopy cover, and with increasing periphyton biomass. By contrast, Kelly *et al.* (2003) found

that sites with less canopy shading had a lower biomass of total invertebrates, in particular mayflies and stoneflies, and reduced invertebrate community diversity compared with a heavily shaded reach of a stream in Vancouver Island, British Columbia. This was interpreted as being due to the riparian shading moderating the effects of ultraviolet radiation on benthic communities, mainly through impacts on invertebrates, but with indirect effects on algae.

There has been little research on the impact of shading, especially on Australian aquatic invertebrates, and in particular, little is known about what effects the removal of willows might have on aquatic invertebrates and how they might respond to different shade levels following the removal of willows and any subsequent revegetation. A study by McKie and Cranston (2001) in streams near Canberra demonstrated that both form (closed or open) and composition of the riparian strip can influence the diversity and structure of macroinvertebrate assemblages. They found gougers favoured native eucalyptus, whereas gatherers were most abundant on sticks with greatest surface complexity. Another study done by Reid et al. (2003) on streams flowing through agricultural floodplains in south-eastern Australia showed that standing stocks of benthic detritus were consistently very low where the streamside canopy cover was below ~35%. They suggested that canopy cover should be restored along cleared agricultural streams because allochthonous detritus is a major source of food and habitat for aquatic ecosystems.

In this study, we wanted to examine the impact of willow removal on aquatic invertebrate communities, particularly during periods of high light intensity (summer season). Two different field trials were conducted to investigate differences in aquatic invertebrate distributions under two different shade types (willow shade and artificial shade) and levels following a 28 day colonisation period.

There were two main objectives in this study: (1) to compare aquatic invertebrate abundance and taxon richness under three different natural willow shade levels (fully shaded, partly shaded and open site) (Sixth Creek) and (2) to compare aquatic invertebrate abundance and species richness under artificial shade in a creek without willows (Deep Creek).

We predicted that a richer invertebrate fauna would occur in shaded treatments (fully and partly shaded) than in the open canopy treatment (representing riparian removal). We also predicted that increased light intensity in the open canopy treatment would result in a lower abundance and taxon richness, where higher light intensities and water temperatures would occur. This is due to several mechanisms created by the lack of or removal of the canopy

cover, the most fundamental being an increase in light intensity and water temperature. Butler (1984) found that higher water temperatures contribute to a reduction in the overall growth potential for aquatic invertebrates. Allan (1995) also noted that high temperatures may result in lower dissolved oxygen concentrations, affecting biotic community production. Quinn *et al.* (1994) noted that some invertebrate species were absent from unshaded pasture streams in New Zealand, possibly due to of high water temperatures.

Data on the aquatic invertebrate communities might indicate whether complete removal of willows is the best option, and whether willow removal operations should be conducted over summer months when the impact of higher light intensities and water temperatures on aquatic invertebrates is likely to be greatest.

5.2   Materials and Methods

Two separate trials (natural and manipulative trials) investigating differences in taxon richness and patterns of abundance at each site were conducted from early November 2009 to December 2009 (late spring). The trial using natural willow shade was conducted at Sixth Creek, whereas the manipulative trial using shade cloth was carried out at Deep Creek. Each site consisted of three different shade levels: (1) fully shaded, (2) partly shaded and (3) open canopy.

The climate in the Mount Lofty Ranges catchment area is hot Mediterranean with average annual rainfall of approximately 900 mm. The average maximum temperature during hot, dry summers is 25^0C (soaring into the 40s) with maximum temperatures averaging 12^0 to 15^0C during cool, wet winters. The streambeds of both streams were almost debris free, mainly comprising sand, pebbles, cobbles and small boulders. Mean current velocity was also similar, and ranged from fast flowing (>0.55 m/s) to slow flowing (0.10−0.55 m/s). Both creeks traverse a landscape comprising of rural residential, horticulture, orchards and grazing land. Azmi and Jennings (see Chapter 2) have described the physico-chemical characteristics of both streams in detail.

5.2.1   *Impact of natural willow shade on aquatic invertebrates*

This trial was conducted in Sixth Creek, Mount Lofty Ranges, South Australia, that located at an elevation of around 380 m at 34^o52'N and 138^o45'E (see Chapter 2 for details). Three 40 m long study sites were selected to represent contrasting degrees of overhead canopy; complete willows canopy (60−70% canopy), a partial willow canopy (30−40% canopy) and no canopy (0−10% canopy). They were located in close proximity to minimize physico-chemical differences other than shading. At the time of the trials, mean water temperature at Sixth Creek ranged from 23.50–26.50^{0}C, pH 8.2−10.0, dissolved oxygen content 10.98–13.30 mg/L, electrical conductivity 577–669 μS/cm and total dissolved solids 248–345 ppm.

The fully shaded site was bordered on both sides by thick, mature willows and provided complete shade for most of the day. The partly shaded site was bordered on both sides with partial willow canopies, but large openings which allowed variable shading at different times of the day. The open canopy site was completely unshaded on both sides. Sampling for each shade level treatment covered ~100 m^2 in an area and within each treatment, four sample replicates were collected.

Aquatic invertebrates were collected using an aquatic net with a 40 x 40 cm frame, 60 cm long net of 250 µm mesh from an area ~100 m^2 in each of three sections representing different levels of shade. Organisms dislodged in the sampling process and trapped in the net were included with the sample. They were transferred into labelled plastic containers, preserved in 80% ethanol and taken back to the laboratory for analysis. Invertebrates were sorted, counted and identified to the lowest taxon level possible, and then grouped into functional feeding groups (see Chapter 2 for methodology).

5.2.2   *Impact of artificial shade on aquatic invertebrates*

The second site, Deep Creek is also located in the Mount Lofty Ranges at around 350 m elevation with 34^o56'N and 138^o46'E. A section of creek with an open canopy was chosen to allow us to conduct an experiment in which shade levels were artificially manipulated by using shade cloth of different mesh sizes. Shade levels were artificially increased by 30−70% to represent the various canopy level conditions found at the Sixth Creek site (see 'Experimental Designs' below for details). During the trials, mean water temperature ranged

from 23.60–25.50^{0}C, pH 8.7−9.4, dissolved oxygen content 10.48–13.26 mg/L, electrical conductivity 630–675 µS/cm and total dissolved solids 275–334 ppm.

Two different mesh sizes were used to represent different shade levels; (1) fully shaded - 70% shade cloth (i.e., 70% of light is blocked) and (2) partly shaded - 30% shade cloth (i.e., 30% of light is blocked] (Fig. 5.1a & b). One section without a riparian canopy was chosen to represent what is essentially zero shade (Fig 5.1c). Shade levels were artificially manipulated using monofilament shade cloth (SARLON®). A 1.0 x 1.0 m section of cloth was anchored to four 45 cm metal stakes and hammered into the creek bed until standing about 25 cm above the water level. In the case of no canopy, there were just the four metal stakes positioned. Each shade treatment was replicated four times and each replicate was placed randomly within a 40 m section at Deep Creek.

After 28 days exposure, the invertebrate were collected from each 1.0 m^2 treatment on 10 December 2009 between 1000 h to 1700 h. The invertebrates were sampled using similar materials and methods with the willow shade experiment. Invertebrates were sorted, counted and grouped into functional feeding groups and then identified to the lowest taxon level (see Chapter 2 for methodology).
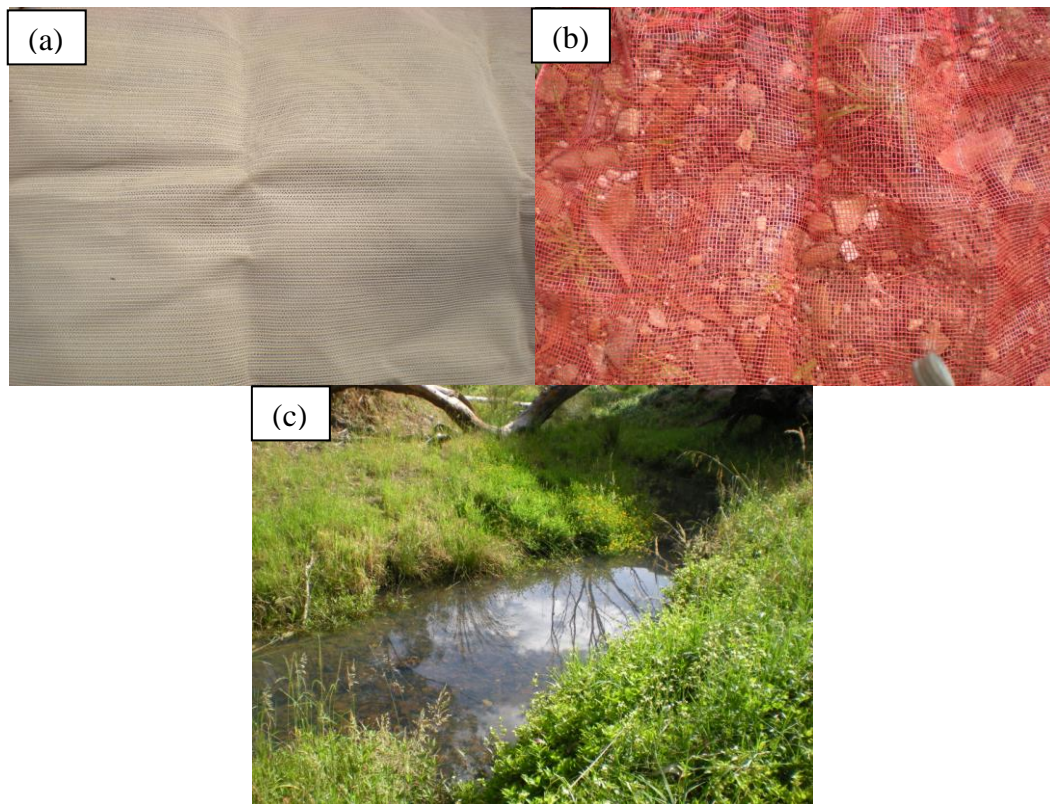
Fig. 5.1 Shade cloth of different mesh sizes; 70% (a) and 30% (b), representing artificial shade for fully and partly shaded treatments, and (c) typical section without a canopy at Deep Creek representing an open canopy.

5.2.3 *Water temperature and light intensity*

Light intensity was estimated based on the openness of sites and categorized into three nominal categories for analysis; 1 = low (fully shaded); 2 = moderate (partly shaded) and 3 = high (open canopy). Water temperature (^{0}C) of each shade level treatment was measured in the field with COM-100 combo meter at the beginning and end of the experiments. Three readings were taken to allow means to be recorded for each treatment. However, due to limited data on water temperatures throughout the trials, the correlation between water temperature and light intensity could not be analysed. Further, the light intensity between natural shade provided by willows and artificial shade provided by shade cloth could not be distinguished as we only had estimated data. Therefore, any correlation between levels of shade and invertebrate abundance or taxon richness could not be tested.

5.2.4 *Analysis of findings*

One-way ANOVAs (PRISM 2007) were used to evaluate differences in distribution of invertebrates at different shade levels in each experiment. All data were log (x+1) transformed to improve the normality and homogeneity of the variances. Where there were significant differences, posterior pairwise comparisons (*t*-test) was employed to describe which means were most alike (or different) and to test the equality of means for each pair of variables.

Similar one-way ANOVA models as described above were used to estimate differences in the abundance of functional feeding groups among shade levels in each trial.

Sorenson's Coefficient method of two-way cluster analysis was used to sort invertebrate taxa into groups. This analysis has been used by other workers to reveal the degree of association between generic composition and the effects of shade levels (Krebs 1989; Kovach 1999). Clustering analyses were performed using the statistical program of PC-ORD version 5.13 (McCune & Mefford 2006). Taxa (e.g., ostracods, amphipods and hirudineas) that were found with only one or zero individuals per each sample were excluded from the data.

5.3   Results

Generally, water temperatures (^{0}C) recorded were similar in willow (Sixth Creek) and artificial shade (Deep Creek) treatments, with average maximum temperature 23.50−26.50^{0}C and with minimum temperatures averaging 16.60−18.50^{0}C. Within the willow and artificial shades, the fully and partly shaded treatments exhibited similar water temperatures, with a maximum difference of 1.0−2.5^{0}C between them. However, the open canopy sites recorded an average increase of 45−52% in ambient temperature compared with fully and shaded sites respectively. However, the influence of water temperatures on taxa assemblages could not be analysed as the readings for water temperature were not taken regularly throughout the experiments.

5.3.1   *Impact of natural willow shade on aquatic invertebrates*

A total of 7,794 individuals of 46 taxa belonging to 35 families of aquatic invertebrates were identified under willows in Sixth Creek from November to December 2009. There was a significant difference in the total abundance with shade levels ($F_{2,11}$ = 10.440, P < 0.0001) (Table 5.1). A considerable variation within replications between treatments was detected (Table 5.2). Mean total abundance for each replicate was quite higher in partly shaded treatment (686.5 individuals/replicate) and fully shaded treatment (663 individuals/replicate) compared with open canopy treatment (517.5 individuals/replicate). Further analysis using *t*-test verified that the open canopy treatment had a significantly lower abundance of invertebrates than did the fully and partly shaded treatments (P < 0.001) (Fig. 5.2a).

In terms of taxon richness, the open canopy supported the most diverse invertebrate communities compared with fully and partly shaded treatments (Fig. 5.2b). Mean number of taxon was greater in open canopy treatment (26.3 taxa/replicate), followed by fully shade (24 taxa/replicate) and partly shaded treatments (22.5 taxa/replicate) (Table 5.2). However, no significant difference was detected between mean taxon richness and shade levels ($F_{2,11}$ = 0.415, P = 0.6342) (Table 5.1).

Table 5.1 Results of one-way ANOVAs on total abundance and taxon richness of aquatic invertebrates at different willow shade levels (at Sixth Creek) (df = degree of freedom; MS = mean squares; *** $P < 0.0001$).

Source	df	Taxon richness			Total abundance		
		MS	F	P	MS	F	P
Willow shade (Sixth Cr.)	2	0.2661	0.415	0.6342	0.1531	10.440	<0.0001***
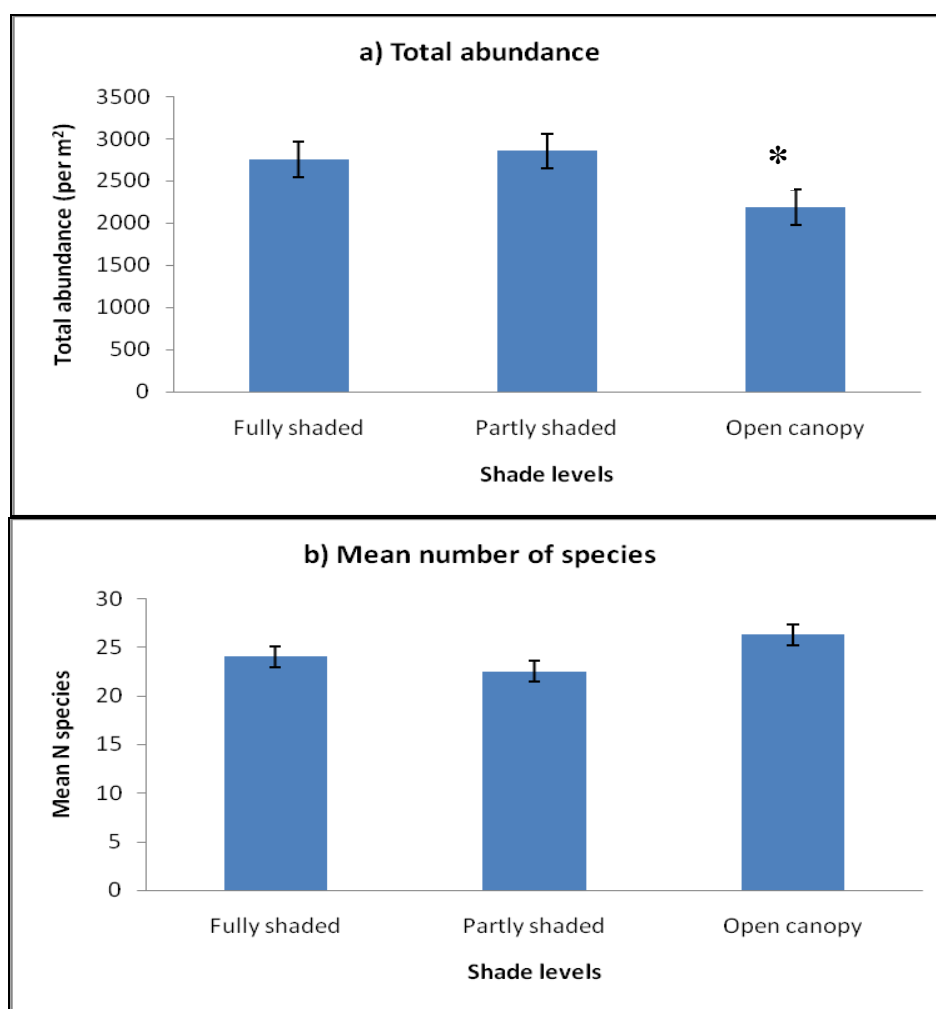
Fig. 5.2 Total abundance (a) and mean number of taxa (b) of aquatic invertebrate communities in three different willow shade levels at Sixth Creek (Note: * $P < 0.001$).

Table 5.2 Summary statistics for mean total abundance and mean number of taxon under three different willow shade levels.

Variable	Levels of shade	Mean	SD	Minimum	Maximum
Total abundance	Fully shaded	663.0	42.09	631	732
	Partly shaded	686.5	38.38	686	764
	Open canopy	517.5	47.14	486	643
Number of taxon	Fully shaded	24.0	4.08	18	27
	Partly shaded	22.5	2.08	20	25
	Open canopy	26.3	2.36	23	28

Gastropods were the most dominant invertebrate group, comprising almost half of the total abundance at each shade level in willow shade (Table 5.3 & Fig. 5.3). Their high abundance was due mostly to a very high percentage of the introduced hydrobiid snail (*Potamopyrgus antipodarum*) in all treatments. More *P. antipodarum* were collected in the partly shaded treatment than other treatments. Trichoptera, primarily *Lingora aurata*, were the second most abundant invertebrate group, especially in the fully shaded treatment. Diptera (mostly chironomids) were the third highest and was also quite abundant in fully shaded treatment, followed closely by Ephemeroptera, Plecoptera and Coleoptera. The two most abundant ephemeropteran taxa [*Koornanga inconspicua* (Leptophlebiidae) and *Tasmanocoenis tillyardi* (Caenidae)] were mostly found in the fully and partly shaded treatments. The plecopterans, mostly *Dinotoperla evansi* (Gripopterygidae), were also found more in the fully shaded treatment. In contrast, the coleopterans (mostly Elmidae; *Simsonia leai*) preferred the partly shaded treatment compared with fully shaded or open canopy treatments. A number of minor groups, such as Oligochaeta, Amphipoda, Hemiptera, Bivalvia, Arachnida, Ostracoda and Hirudinea were represented by less than 5.0% of the total abundance at each shade level treatment.

Table 5.3 Composition of aquatic invertebrates in different willow shade level treatments.

Class/Order	Family	Species	Willow shade		
			Fully shaded	Partly shaded	Open canopy
Mollusca / Gastropoda	Hydrobiidae	*Potamopyrgus antipodarum*	1050	1408	1221
		Austropyrgus sp.	3	0	2
	Planorbiidae	*Isidorella* sp.	1	0	0
		Glytophysa sp.	1	1	0
	Physidae	*Physa acuta*	73	24	37
	Lymnaeidae	*Lymnaea* sp.	0	0	2
Mollusca / Bivalvia	Sphaeriidae	*Sphaerium* sp.	14	2	14
Insecta / Plecoptera	Gripopterygidae	*Dinotoperla evansi*	175	91	82
		Illiesoperla mayii	14	13	34
Insecta / Ephemeroptera	Caenidae	*Tasmanocoenis tillyardi*	93	119	78
	Leptophlebiidae	*Atalophlebia* sp.	0	0	3
		Koornanga inconspicua	102	105	87
	Baetidae	*Offadens* sp.	43	46	46
Insecta / Trichoptera	Conoesucidae	*Lingora aurata*	601	442	170
	Hydrobiosidae	*Taschorema evansi*	3	12	10
	Leptoceridae	*Oecetis* sp.	13	9	0
		Triplectides sp.	3	0	4
	Hydroptilidae	Undetermined	1	3	15
Insecta / Coleoptera	Elmidae	*Simsonia leai*	99	153	66
	Scirtidae	*Cyphon adelaidae*	0	4	0
	Curculionidae	Undetermined	2	4	0
	Dystiscidae	*Antiporus blakei*	0	3	0
		Sternopriscus sp.	2	5	3
Insecta / Diptera	Chironomidae	*Procladius* sp.	67	95	63
		Podonominae sp.	45	61	28
		Paramerina sp.	40	24	43
		Polypedilum sp.	30	7	3
	Culicidae	*Culex sp.*	6	0	5
	Simuliidae	*Austrosimulium furiosum*	61	50	0
	Stratiomydae	Undetermined	11	13	3
Insecta / Hemiptera	Notonectidae	*Enithares bergrothi*	4	3	1
	Corixidae	*Micronecta annae*	1	0	1
	Veliidae	*Microvelia peramoena*	1	6	6

Table 5.3 continues.

Class/Order	Family	Species	Willow canopy		
			Fully shaded	Partly shaded	Open canopy
Insecta /	Hemicorduliidae	*Hemicorduliidae* sp.	5	7	10
Odonata	Coenagrionidae	Undetermined	61	17	10
	Gomphidae	*Austrogomphus* sp.	0	0	1
	Libellulidae	*Orthetrum caledonicum*	0	0	1
	Telephebiidae	*Austroaeschna unicornis*	22	9	5
Crustacea /	Ceinidae	*Austrochiltonia australis*	2	3	2
Annelida /	Tubificidae	Undetermined	2	5	6
Oligochaeta	Naididae	Undetermined	1	2	8
Collembola		Undetermined	0	10	3
Ostracoda		Undetermined	4	4	8
Hirudinea		Undetermined	1	0	1
Arachnida		Undetermined	0	2	2
		TOTAL	**2652**	**2746**	**2070**
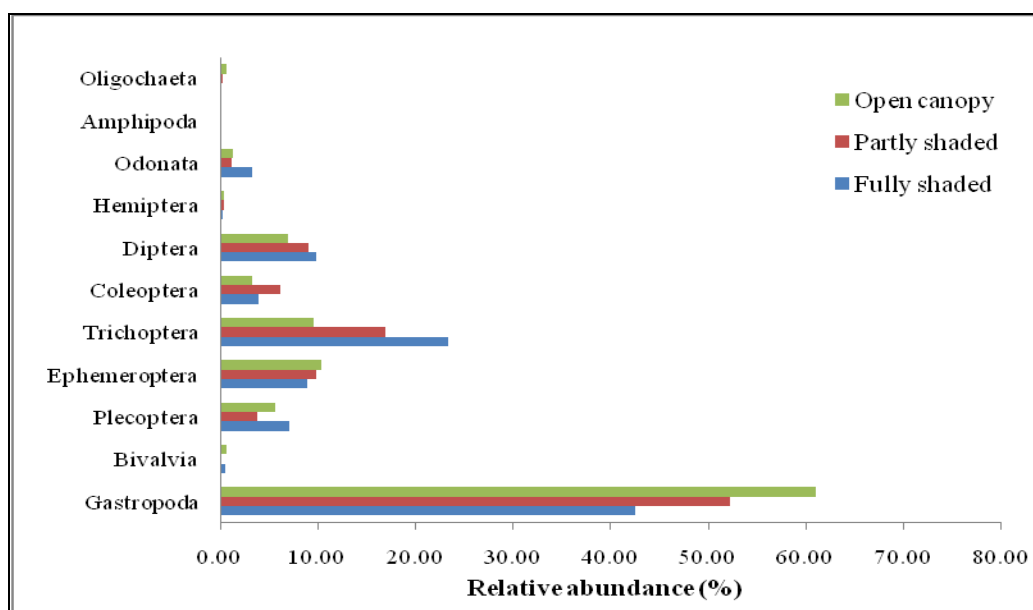
Fig. 5.3 Relative abundance of major aquatic invertebrate groups collected from three different willow shade levels.

A marked variation in abundance based on functional feeding groups in different shade levels was observed in willow shade (Fig. 5.4). However, no significant differences were detected among shade levels on functional feeding group assemblages. In the fully shaded treatment, scrapers, collector-gatherers and shredders were the three most abundant functional feeding groups. Scrapers were dominated by *P. antipodarum,* and collector-gatherers and

shredders were mostly *D. evansi, T. tillyardi, K. inconspicua, L. aurata* and *S. leai.* These three feeding groups made up more than 70% of the total abundance and were greater in fully and partly shaded treatments than in the open canopy treatment (Fig. 5.4). In partly shaded treatment, more collector-gatherers (20 spp.) were collected than the scrapers (8 spp.) In open canopy treatment, again the collector-gatherers were about three times more abundant than other feeding groups in each shade type, due mainly to ephemeropterans and trichopterans. Collector-filterers (mainly blackflies and bivalves) and predators (mainly chironomids and odonate nymphs), appeared unrelated to shade levels in willow shade.
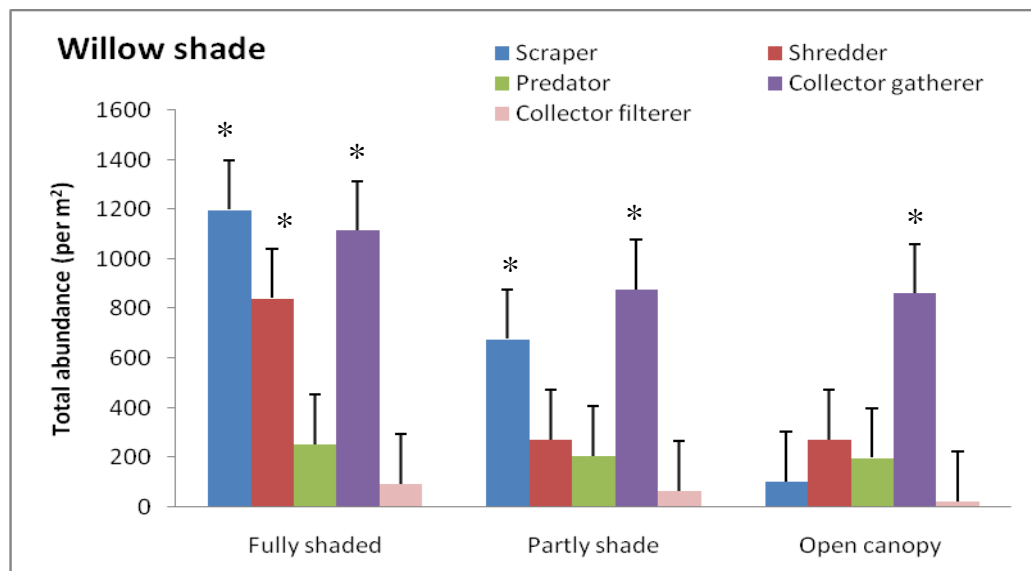
Fig. 5.4 Total abundance of functional feeding groups of aquatic invertebrates in three different willow shade levels (Note: * $P < 0.001$).

The classification between samples of shade levels was obtained by two-way clustering analysis and represented in the dendograms (Fig. 5.5). In the case of willow shade in Sixth Creek, there were two main groups (Cluster I and Cluster II) (Fig. 5.5). All samples from fully shaded treatment (SC-full1, SC-full2, SC-full3 & SC-full4) and one sample from partly shaded treatment (SC-half2) fall into Cluster I. Cluster II on the other hand, contains all samples from open canopy treatment (SC-open1, SC-open2, SC-open3 & SC-open4), plus three samples from partly shaded treatment (SC-half1, SC-half3 & SC-half4).

The interaction among shade levels and species assemblages under willow shade was clearly illustrated. Across the samples, the fully shaded treatments were composed of similar

taxa, particularly leptocerid caddisfly (*Oecetis* sp.), odonate nymphs (coenagrionids, telephebiids; *Aus. unicornis*) and chironomids (*Polypedilum* sp.). These species were commonly found in fully shaded site under willows. The composition of invertebrates in partly shaded treatments was similar to the open canopy treatments, and was composed of chironomids (*Orthocladiinae*), lymnaeids snail and some beetle larvae (e.g., *Cyphon adelaidae, Antiporus blakei, Platynectes decempunctatus*).
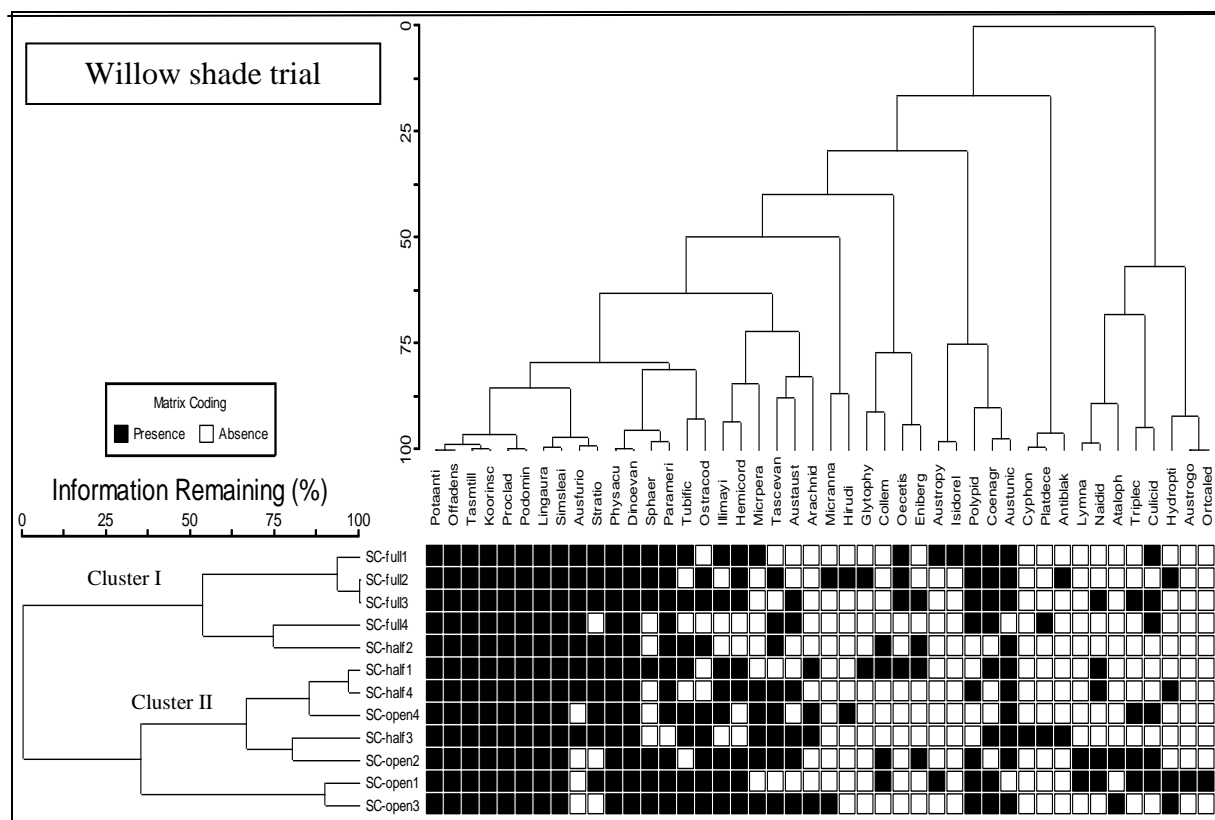
Fig. 5.5 Dendrograms using UPGMA Sorensen's Coefficient method for two-way clustering analysis of individuals and generic composition in different willow shade levels (Note: SC = Sixth Creek; full = fully shaded; half = partly shaded; open = open canopy). Note that not all taxa are included; see Appendix I for species codes.

5.3.2 *Impact of artificial shade on aquatic invertebrates*

Total abundance under artificial shade in Deep Creek was considerably low with only 3003 individuals (Fig. 5.6a). Mean total abundance was higher in fully shaded (254.8 individuals/replicate) and partly shaded treatments (240 individuals/replicate), but very low in open canopy treatment (192 individuals/replicate) (Table 5.4). However, no significant difference in the total abundance among shade levels were detected ($F_{2,11}$ = 2.3675, P = 0.5414) (Table 5.5). Open canopy treatment contributed a more diverse and richer invertebrates compared with both fully and partly shaded treatments (Fig. 5.6b). An average number of 19.8 taxa per replicate were recorded in the open canopy treatment, 18 taxa under fully shaded treatment and only 16 taxa under partly shaded treatment (Table 5.4). Similarly, there was no significant difference in mean taxon richness among the shade levels ($F_{2,11}$ = 0.883, P = 0.3472) (Table 5.5).
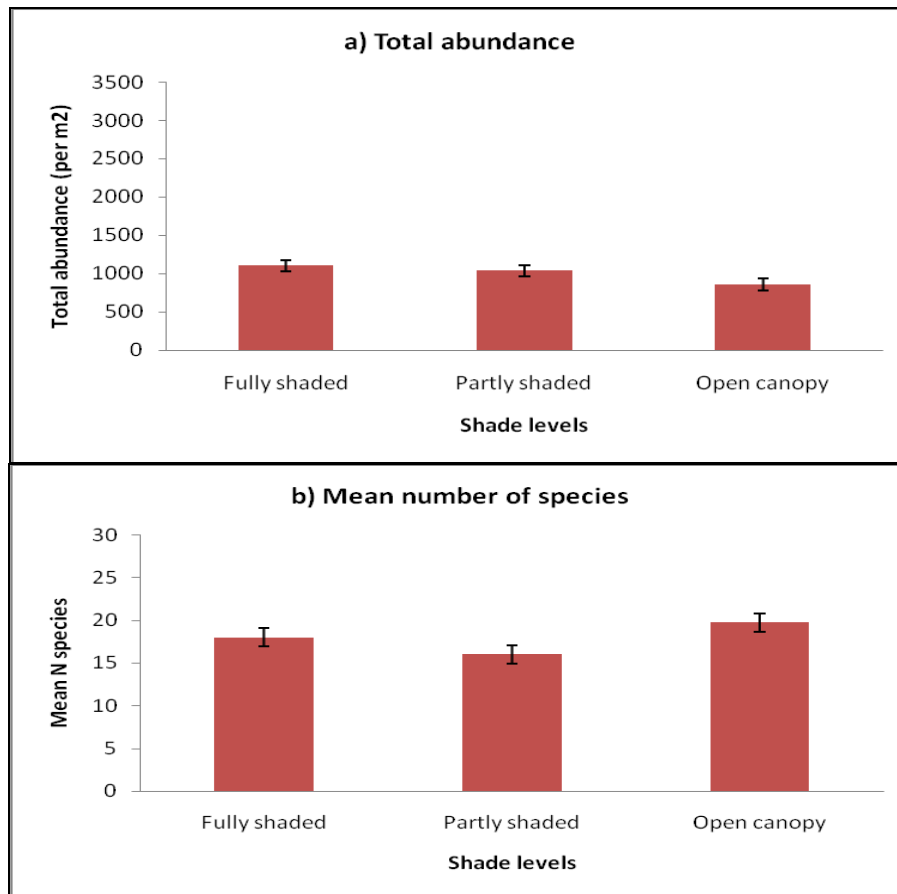
Fig. 5.6 Total abundance (a) and mean number of taxon (b) of aquatic invertebrate communities in three different artificial shade levels at Deep Creek.

Table 5.4 Summary statistics for mean total abundance and mean number of taxon under three different artificial shade levels.

Variable	Levels of shade	Mean	SD	Minimum	Maximum
Total abundance	Fully shaded	254.8	58.05	217	340
	Partly shaded	240	56.32	188	344
	Open canopy	192	51.11	191	283
Number of taxon	Fully shaded	18	2.71	16	22
	Partly shaded	16	2.16	14	19
	Open canopy	19.8	3.21	17	23

Table 5.5 Results of one-way ANOVAs on total abundance and taxon richness of aquatic invertebrates at different artificial shade levels (at Deep Creek) (df = degree of freedom; MS = mean squares; *** $P < 0.0001$).

Source	df	Taxon richness			Total abundance		
		MS	F	P	MS	F	P
Artificial shade (Deep Cr.)	2	0.12955E-01	0.883	0.3472	0.1087	2.3675	0.5414

A similar trend for species composition and relative abundance (%) of the aquatic invertebrate groups was observed in artificial shade (Table 5.6 & Fig. 5.7). Gastropoda (primarily *P. antipodarum*) was the most dominant group, but more were collected in the open canopy and partly shaded treatments than in full shade. Plecoptera (Gripopterygidae; mostly *D. evansi*) made up the second highest of total abundance and were numerous in the fully shaded treatment. Diptera (chironomids; *Procladius* sp.) and Ephemeroptera (Caenidae; *T. tillyardi*) contributed the third and fourth highest. However, slightly more dipterans were found in partly shaded treatment, whereas more ephemeropterans were recorded in fully shaded treatment. Trichoptera (Coenosucidae; *L. aurata*), Coleoptera (Elmidae; *S. leai*), Hemiptera (Veliidae; *Microvelia peramoena*) and Odonata (Telephebiidae; *Austroaeschna unicornis*) were abundant in the open canopy treatment. Bivalvia, Amphipoda, Oligochaeta and Hirudinea were very low in each shade level. Each of these minority group contributed less than 5.0% of the total relative abundance.

Table 5.6 Composition of aquatic invertebrates in different artificial shade level treatments.

Class/Order	Family	Species	Artificial shade		
			Fully shaded	Partly shaded	Open canopy
Mollusca / Gastropoda	Hydrobiidae	*Potamopyrgus antipodarum*	317	585	440
		Austropyrgus sp.	9	0	0
	Planorbiidae	*Isidorella* sp.	4	0	17
		Glytophysa sp.	2	17	16
	Physidae	*Physa acuta*	41	58	69
	Lymnaeidae	*Lymnaea* sp.	1	3	11
Mollusca / Bivalvia	Sphaeriidae	*Sphaerium* sp.	9	14	15
Insecta / Plecoptera	Gripopterygidae	*Dinotoperla evansi*	333	68	63
		Illiesoperla mayii	6	11	5
Insecta / Ephemeroptera	Caenidae	*Tasmanocoenis tillyardi*	63	16	13
	Leptophlebiidae	*Atalophlebia* sp.	13	0	2
		Koornanga inconspicua	12	0	1
	Baetidae	*Offadens* sp.	2	3	0
Insecta / Trichoptera	Conoesucidae	*Lingora aurata*	17	0	0
	Hydrobiosidae	*Taschorema evansi*	2	1	1
	Leptoceridae	*Oecetis* sp.	11	0	3
		Triplectides sp.	3	0	5
	Tasimidae	Undetermined	1	0	0
	Atriplectidae	*Atriplectides dubuis*	1	0	0
Insecta / Coleoptera	Elmidae	*Simsonia leai*	3	8	17
	Scirtidae	*Cyphon adelaidae*	4	3	0
	Curculionidae	Undetermined	2	0	1
	Dystiscidae	*Antiporus blakei*	1	2	3
		Sternopriscus sp.	0	1	1
Insecta / Diptera	Chironomidae	*Procladius* sp.	87	129	57
		Podonominae sp.	16	0	0
		Paramerina sp.	9	0	0
		Polypedilum sp.	6	0	1
	Culicidae	*Culex sp.*	2	7	0
	Simuliidae	*Austrosimulium furiosum*	8	9	0
	Stratiomydae	Undetermined	4	0	0
Insecta / Hemiptera	Notonectidae	*Enithares bergrothi*	8	4	0
	Corixidae	*Micronecta annae*	5	0	0
	Veliidae	*Microvelia peramoena*	3	6	14

Table 5.6 continues.

| Class/Order	Family	Species	Willow canopy		
			Fully shaded	Partly shaded	Open canopy
Insecta /	Hemicorduliidae	*Hemicorduliidae* sp.	0	1	4
Odonata	Gomphidae	*Austrogomphus* sp.	2	0	0
	Libellulidae	*Orthetrum caledonicum*	0	0	1
	Telephebiidae	*Austroaeschna unicornis*	5	5	5
Crustacea /	Ceinidae	*Austrochiltonia australis*	4	0	0
Annelida /	Tubificidae	Undetermined	0	0	0
Oligochaeta	Naididae	Undetermined	3	9	4
Collembola		Undetermined	0	5	5
Ostracoda		Undetermined	4	6	9
Hirudinea		Undetermined	2	1	4
Arachnida		Undetermined	0	4	1
		TOTAL	**1019**	**960**	**768**
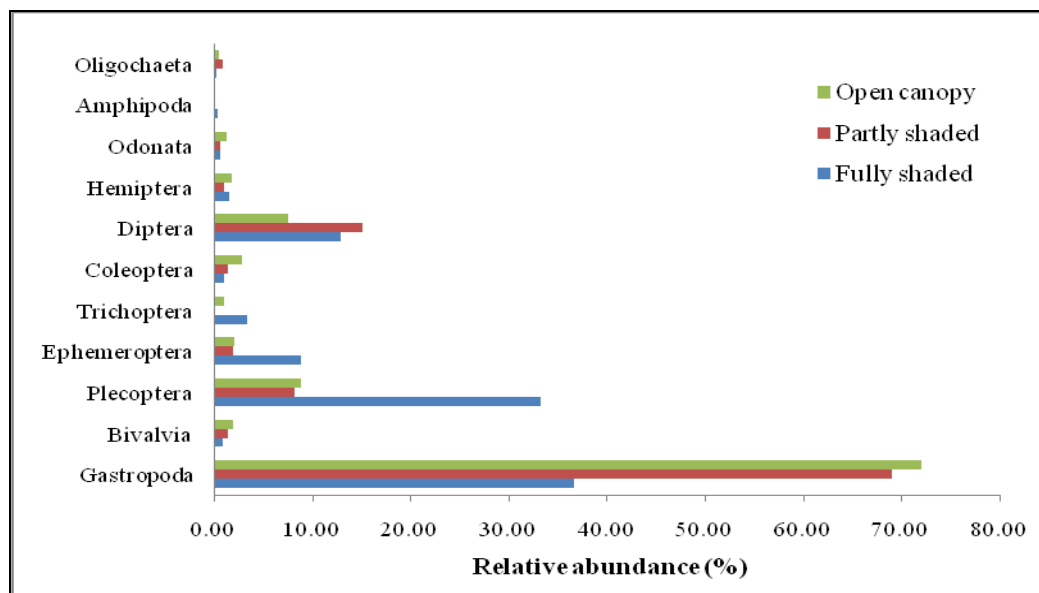
Fig. 5.7 Relative abundance of major aquatic invertebrate groups collected from three different artificial shade levels.

In terms of functional feeding groups, scrapers and shredders were significantly higher in the full shaded treatment than in other treatments (Fig. 5.8). In partly shaded treatment, the abundance of predators (e.g., chironomids, odonates) was greater than other feeding groups, but the difference was not statistically significant. In the open canopy treatment, the abundance of collector-gatherers (e.g., *D. evansi, T. tillyardi, K. inconspicua*) was significantly higher than other feeding groups. Collector-filterers (e.g., Culicidae, Sphaeridae) were very low numbers in all shade levels in artificial shade.
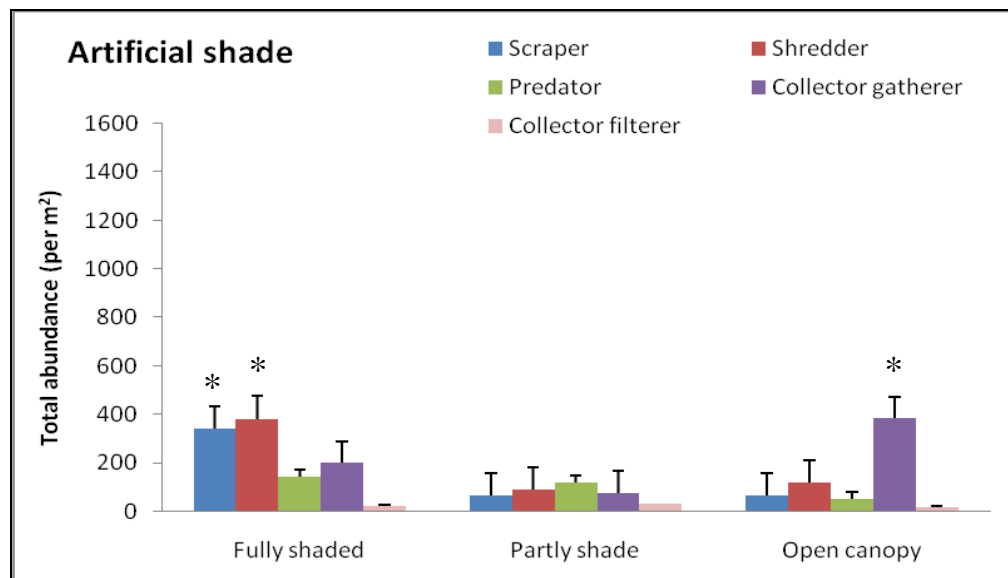
Fig. 5.8 Total abundance of functional feeding groups of aquatic invertebrates in three different artificial shade levels (Note: * $P < 0.001$).

The analysis of classification on species assemblages in different shade levels was unclear as most of the samples were mixed with one another (Fig. 5.9). There were two main groups of samples (Cluster I and Cluster II), but Cluster II was mostly composed of samples from open canopy treatment (DC-open2 & DC-open3) and partly shaded treatment (DC-half1, DC-half3 & DC-half4). One sample of partly shaded treatment (DC-half2) was well separated from Cluster II due to the presence of caddisfly nymphs (*Taschorema evansi*) and scirtid beetle larvae (*Cy. adelaidae*) which were also found under fully shaded treatment (DC-full2). For most taxa, there was a greater abundance at each fully and partly shaded treatments than open canopy treatment. The only difference was the presence of multiple native taxa [hydrobiid snail (*Austropyrgus* sp.), gomphid nymph (*Austrogomphus* sp.), atriplectid

caddisfly larvae (*Atriplectides dubius*) and ceinid amphipod (*Austrochiltonia australis*)] that were only found in the fully shaded treatment (DC-full2 & DC-full3).
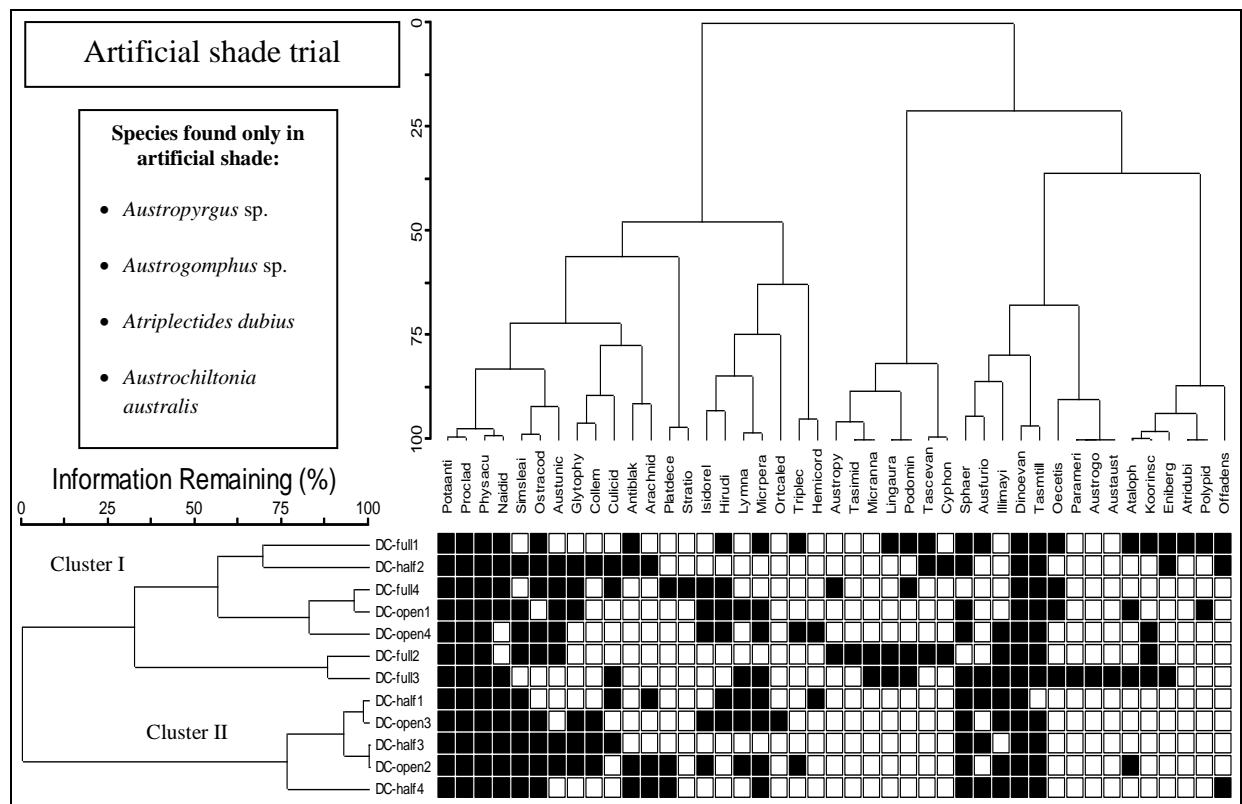
Fig. 5.9 Dendrograms using UPGMA Sorensen's Coefficient method for two-way clustering analysis of individuals and generic composition in different artificial shade levels (Note: DC = Deep Creek; full = fully shaded; half = partly shaded; open = open canopy). Note that not all taxa are included; see Appendix I for species codes.

5.3 Discussion

Willow canopies play an important role in regulating light intensity and spectral qualities of light (Van Kraayenoord *et al.* 1995; Bunn *et al.* 1999b, Kelly *et al.* 2003). Removal of willow canopies can increase the exposure of aquatic organisms to ultraviolet radiation which are harmful to attached algae and invertebrates (Bothwell *et al.* 1993, Kelly *et al.* 2003) and some fish larvae (Gutierrez-Rodriguez & Williamson 1999). Several hypotheses have been documented to explain differences in invertebrate abundance associated with riparian canopies. Riparian canopies are presumed to be responsible in controlling and determining water temperatures. Thus, reduction in willow canopies through the removal of willow can result in greater mean summer water temperatures, lower winter water temperatures and an increase in the degree of daily fluctuations of water temperature (Lynch *et al.* 1984; Quinn *et al.* 1992). Aquatic invertebrates associated with freshwater are poikilothermic, that is they are unable to control their body temperatures, and therefore, are highly dependent on ambient temperatures (Helmuth *et al.* 2006). Departures from the normal temperature pattern, particularly caused by the sudden removal of riparian canopies or seasonal changes, could disturb all physiological processes. Variation in temperatures could cause accelerated or retarded, growth and abnormal timing of life cycles, and this is revealed in changes in growth rate, attainment of sexual maturity, reproduction and behaviour of many aquatic organisms (Helmuth *et al.* 2006).

In our trials, we hypothesised that the abundance and taxon richness of aquatic invertebrates would be lower under open canopies with greater amounts of light. Treatments without shading (open canopy) had a lower invertebrate abundance than fully and partly shaded treatments in both willow and artificial shade trials (Figs. 5.2a & 5.6a). However, contrary to our expectations, taxon richness was greater and more diverse in open canopy treatment in both willow and artificial shade trials (Figs. 5.2b & 5.6b).

The highly abundance of aquatic invertebrates in fully and partly shaded treatments, particularly in willow shade trial was primarily due to a single species, *P. antipodarum,* which contributed more than half of the total abundance. The high abundance of this taxon under willows is possibly due to the habitat availability and organic material provided by the willows. Chapters 2 and 4 supported this finding as we found *P. antipodarum* were highly associated with willow habitats. A similar result was found by Jayawardana *et al.* (2006)

where *P. antipodarum* were commonly found under willow root habitats in Birch Creek, Victoria.

In contrast, slightly lower numbers of invertebrates were found in the open canopy treatment in both willow and artificial shade trials (Figs. 5.2a & 5.6a). The two trials point to the possibility that light intensity is an important factor in determining the abundance and distribution of species assemblages, particularly in small and shallow streams like Sixth and Deep Creeks. Higher light intensity probably has a direct link with the water temperature that leads to decreases in the invertebrate abundance. As pointed by Resh *et al.* (1995) and Helmuth *et al.* (2006) warmer water temperatures can affects physiological processes (e.g., growth rate, reproduction, behaviour etc.) and high temperatures may result in lower dissolved oxygen. A study by Kelly *et al.* (2003) found that sites with less canopy shading had lower biomass of total invertebrates and reduced invertebrate community diversity compared to the heavily shaded reach of a stream in Vancouver Island, British Columbia. Their results were similar to our study which indicated that a reduction in riparian shading may effects on invertebrate community abundance.

However, in terms of taxon richness, we found that higher invertebrate richness was observed in open canopy treatment in both willow and artificial shade experiments (Figs. 5.2b & 5.6b). The greater diversity and taxon richness in open canopy treatment may have been influenced by higher quality food available compared with the fully and partly shaded treatments. Hawkins *et al.* (1982) found that decreased in shade levels can lead to increased light levels, which in turn appeared to increase the amount of high-quality food available to consumers. Hawkins *et al.* (1982) studied six Oregon streams affected by logging and found greater abundance of most invertebrates in clear-cut areas. They suggested that the food availability in less riparian canopy stream was more important than substrate in influencing invertebrate abundance and guild structure.

This phenomenon has also been observed in other studies, where some researchers found that removal of shade could directly increase primary production and indirectly increase secondary production and invertebrate densities. Towns (1981) found three species of chironomids were more abundant in unshaded segments in artificially shaded segments of a New Zealand stream. Behmer and Hawkins (1986) found abundance and production of macroinvertebrates was greater at open site than shaded site of a stream in the Wasatch Mountains, Utah. They suggested that greater abundance and production of most invertebrate

taxa at the open site is probably associated with either higher quality food (algae and algal detritus) or a phototactic attraction to sunlit areas. Kelly *et al.* (2003) found sites with less canopy shading had greater algal accrual, decreased biomass of total invertebrates, mayflies, and stoneflies, and reduced invertebrate community diversity compared to the heavily shaded reach in three reaches of a British Columbia coastal stream that differed in the degree of shading by riparian canopy (a full canopy, a partial canopy, and no canopy).

We observed that the open canopy treatments in both Sixth and Deep Creeks were slightly warmer ($23.5–26.5^0$C) and had more nutrients (organic matter) than the fully and partly shaded treatments. This is an indication that availability of quality food (e.g., microbial, diatoms, algae) might be influencing the composition of invertebrate assemblage in streams lacking a riparian canopy. A study done by Mokany *et al.* (2008) found that increasing light increased the biomass of filamentous algae (metaphyton) which increased the overall productivity of the ecosystem, and shifted the invertebrate community. They suggested light may have a potentially strong indirect effect and may impact the communities through altered bottom-up structuring forces.

However, it appears that willows canopy has a positive effect on invertebrate abundance as fully and partly shaded sites in willow shade had a greater abundance of invertebrates than did the open canopy site (Fig. 5.2a). Besides the effect of lower light intensity, availability of food source under willows might be one of the reasons of the increased abundance. Lester *et al.* (1994a) suggested that willows may enhance invertebrate abundance by contributing readily processed litter and stimulating production through nutrients from leaf leachates. Thus, further experimentation looking at the impact of shade (see above), would also benefit by monitoring the algae and other found resources, which might lead to a greater insight into the pattern of abundance and distribution of the invertebrates.

When we compared the relative abundance of major aquatic invertebrate groups from different shade levels in both creeks (Figs. 5.3 & 5.7), we found that the gastropods were the most dominant invertebrate. The introduced hydrobiid snails were the most abundant taxon in all treatments, especially in willow shade, making up more than 60% of the community. This suggests that *P. antipodarum* actually benefited from the presence of willow trees, possibly through cooler summer water temperatures and from the provision of a stable shelter among the willow roots (see Chapters 2 and 4).

Interestingly, several native taxa such as hydrobiid snails (*Austropyrgus* sp.), gomphid nymph (*Austrogomphus* sp.), caddisfly nymphs (*Atriplectides dubius*) and ceinid amphipods (*Austrochiltonia australis*) were only found in fully shaded treatment under artificial shade (Fig. 5.9). In our earlier surveys (see Chapter 2 for details), we found that *Austropyrgus* sp. were only recorded in sites lacking willows, whereas *Austrogomphus* sp. were only found under willows in both Sixth and Deep Creeks. However, the differences were not statistically significant. Such differences in invertebrate abundance and species richness among shade levels in each creek could occur for a number of reasons, such as differences in physico-chemical characteristics or perhaps differences in light intensities. As Sixth and Deep Creeks have different physico-chemical characteristics (see Chapter 2 and Appendix III), further experiments on the impact of shade should be conducted in the same stream and situation, to enable valid comparisons. Characteristics such as temperature, light intensity and dissolved oxygen should be regularly monitored to enable various reasons for observed differences to be determined. It should be noted that in the current study, we were only comparing invertebrate abundance and taxon richness among shade levels in a single creek, not between creeks.

A few major differences within different functional feeding groups were observed among shade levels (Figs. 5.4 & 5.8) in each creek, as the invertebrate communities responded to shade levels with significant changes in the proportion of functional feeding groups being observed. More scrapers, collector-gatherers and shredders were collected under willow shade than artificial shade. In general, scrapers, collector-gatherers and shredders were significantly more abundant in fully and partly shaded treatments under willow shade. The increase of these dominant feeding groups in fully and partly shaded treatments, might be attributed to the corresponding availability of suitable habitat created by willow root mats or an in increase of food source (see Chapters 2, 3 and 4). Their increase in willow shade treatment is probably due to an increase in coarse particulate organic matter (CPOM) as we observed bottom under willows had a higher CPOM content particularly during summer and autumn.

In artificial shade trial, we found scrapers and shredders were more abundant in the full shaded treatment than in other treatments (Fig. 5.8). Scrapers were expected to be most strongly affected by shading and to be most abundant in shaded sites, however, did not exhibit a marked difference in abundance in partly shaded and open canopy treatments. Lower abundance of shredders was also observed under either partly shaded or open canopy treatments. This could be related to lower biofilms and CPOM contents in the treatments

observed, as shredders use both living macro-algae and coarse detritus as food sources (Hawkins *et al,* 1982). Although the abundance of functional feeding groups was generally lower in artificial shade trial, we found the collector-gatherers were significantly higher in the open canopy treatment than in other treatments. Predators, in contrast, were greater in the partly shaded treatment (Fig. 5.8), but the difference was not statistically significant. Predator abundance may be influenced indirectly by an observed increase in the abundance of prey taxa, especially collector gatherers and collector filterers. For future research, investigators should also consider the effect of food sources (e.g., microbial, diatoms, algae, CPOM contents, etc.) in relation to effects of light intensity which might have on functional feeding group abundance or assemblage pattern.

During our field trials, we found that water temperatures were on average 8^0C warmer in open canopy treatments vs. fully and partly shaded treatments. Treatments without shading had higher maximum water temperatures ($23.5-26.5^0$C) than shaded treatments ($16.6-18.5^0$C). However, the effect of water temperature on species assemblages could not be analysed due to limited data. Further, the influence of light intensity between invertebrate abundance or species richness and shade levels for both trials also could not be tested, as we did not measure the light intensity regularly during the study period. Therefore, future research should measure water temperature and the degree of light intensity quantitatively for each replicate, so that explanation of their assemblage patterns can be determined accurately.

The cluster analyses on taxa level assemblages provided partial support for our first and second hypotheses (Figs. 5.5 & 5.9). Only samples from willow shade in Sixth Creek were clearly grouped, as it indicates that taxa assemblages in the partly shaded treatment are not more distinct from the samples from the open canopy treatment (Fig. 5.5). This was due to a similar distribution of certain taxa such as chironomids (Orthocladiinae), lymnaeids snail and some beetle larvae (e.g., *Cy. adelaidae*, *An. blakei, Pla. decempunctatus*) in partly shaded and open canopy treatments.

However, taxa assemblages under artificial shade in Deep Creek were not clearly illustrated as most samples from different shade levels were mixed with each other (Fig. 5.9). Samples from the open canopy treatment were not clearly separated from those partly and fully shaded treatments, which could not fully support our hypothesis on species assemblages at different shade levels. Interestingly, some of uncommon taxa were only found in sites with artificial shade. For examples, native freshwater snails (*Austropyrgus* sp.), gomphid nymph

(*Austrogomphus* sp.), caddisfly nymph (*Atri. dubius*) and ceinid amphipod (*Aust. australis*) were only found at the fully shaded site for artificial shade in Deep Creek. This could be due to differences in physico-chemical characteristics and habitat availability in the two creeks, which has been explained before in the above paragraph.

In conclusion, a more diverse and richer invertebrate fauna was found in treatments lacking canopies, possibly due to the effects of increased light which results in an increase in the amount of high quality of food sources to the invertebrates. As we found a richer and most diverse invertebrate fauna but lower in abundance in open canopy treatments in both creeks, we would suggest that complete clearing a stream of a willows or other vegetation should be avoided and partial canopies would be a recommended alternative when conducting the removal of willows. Future research could concentrate on individual taxon such as *P. antipodarum,* so that explanation of their patterns can be sought. Future research should also measure a number of environmental variables such as water temperature, light intensity and dissolved oxygen throughout the study, and also microbial, diatoms or algae abundance, as these provide a source of food for many aquatic invertebrates. Measuring these environmental variables would aid data in interpretation and is strongly recommended. Future research should also be conducted in the same stream to minimise variation among treatments, and so that data interpretation and the identification of factors (other than the effect of study sites) which may affect species richness and abundance of invertebrate community could be explained accurately. The inclusion of these should allow for an accurate assessment of the impact of shade on aquatic invertebrate community by such experiments is to be achieved.

Even though the causes of the detected relationships between the removal of willow canopies and the distribution and abundance of invertebrates is not yet clear, the effect of shade remains a strong candidate. Willows removal without revegetation and the consequent increase in light intensity and water temperature may have short and long term effects on the distribution and abundance of aquatic invertebrates particularly in small and shallow streams. This may be potentially greater if removal is done over the summer months when higher light intensities and water temperatures occur. Large scale removal of willows may therefore need special management considerations, especially the timing to reduce possible impacts on aquatic invertebrate communities.

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CHAPTER 6: General Discussion and Conclusions

The current understanding on the impact of willows (*Salix* spp.) on aquatic invertebrate communities has increased and enables us to predict the community changes resulting from management programs to remove willows and subsequent revegetation. Willows support significantly different invertebrate communities from those in 'natural' conditions and such differences depend on seasonal changes, and are influenced by many habitat and water quality parameters. This present study allows us to incorporate research outcomes in various willow management decisions such as whether to willows should remain in place, be removed or controlled, and whether subsequent revegetation is warranted.

6.1   Impact of willows and their removal on aquatic invertebrate communities

The presence of exotic willows in the Australian watercourses is not a new problem. In the last 20 years, several research papers have been published covering the impact of willows in hydrological regimes, the problems associated with water quality, the decline in native aquatic fauna densities, and the establishment of exotic weeds (see, for example, Gregory *et al.* 1991; Lester *et al.* 1994a; Glova & Sagar 1994; Wallace *et al.* 1995; Ladson *et al.* 1997; Jayawardana *et al.* 2006; Jayawardana & Westbrooke 2010). However, the impacts of the removal of willows and any subsequent revegetation have on aquatic invertebrates are usually not clear and in at least the popular press, are sometimes controversial. As well, the nutritional value of willow leaves as a food source, the habitat value created by willow root masses, and the influence of the willows canopy on aquatic invertebrates are poorly understood.

We have provided information on invertebrate community changes where willows are present, removed or revegetated and how these communities compare with original vegetation (see Chapter 2). A reduction in taxon diversity was clearly associated with the presence of willows in the Sixth Creek. Interestingly, the abundance of invertebrates was significantly higher under willows than other treatments in both creeks (Fig. 2.3). This interesting outcome is puzzling as initially we predicted there would be a lower abundance in treatments where willows were present. However, it appears that the high abundance of invertebrates under willows was primarily caused by the introduced hydrobiid snail (*P. antipodarum*) which was the most dominant taxon overall and was significantly more abundant under willows in both creeks (Fig. 2.5). We found the snails have a close association with willow root habitats. The willow root masses provide a stable habitat, protected from high currents and increase the

availability of food (by biofilms and algal detritus). However, the establishment of the pest species *P. antipodarum* under willows vegetation should be considered a serious threat. It is a pest in many parts of the world (Ponder 1988; Zaranko *et al.* 1997) and the most serious threat may be resource competition between *P. antipodarum* and native invertebrates, which also may have effects down on the trophic chain.

Where willows were removed and the area was not revegetated, both lower invertebrate taxa numbers and diversity were observed (Fig. 2.3). The sudden removal of willows influenced the abundance and diversity of aquatic invertebrates by not only loss of habitat, but also increased light intensity, poorer water quality and less food being available (see Appendix III). The removal of willows with no subsequent revegetation (apart from a few weeds) also has the potential to initiate erosion of streambed which was strongly associated with a decrease in invertebrate abundance and taxon richness. Pollution tolerant invertebrates such as oligochaetes, gastropods and chironomids were highly abundant in sites where willows have been removed (Fig. 2.4). This suggests that the removal of willows without subsequent revegetation can have a detrimental impact on taxon diversity and could result in risk of accelerating bank erosion, which could then allow more organic pollution-tolerant taxa to be established, particularly the chironomids (e.g., Chironominae), oligochaetes (e.g., Tubificidae, Naididae) and gastropods (e.g., Hydrobiidae, Physidae) (Figs. 2.4 & 2.7).

The pattern of community assemblages is reversed when the riparian canopy is revegetated (see Figs. 2.4 & 2.7). This may well be the most important effect of revegetation efforts on the invertebrate communities. A slightly more diverse and abundant fauna was recorded in both sites of original vegetation or revegetated than in sites where willows present or removed (Fig. 2.3). This is probably due to a sparse, open canopy that permits higher primary productivity and favours a more diverse growth of aquatic vegetation, in turn providing heterogeneity of habitats for aquatic fauna communities. The heterogeneity of habitats (e.g., moss, rooted plants, filamentous algae and floating macrophytes) provided by diverse aquatic vegetation greatly affects the invertebrate community as more diverse invertebrates were found in each habitat (pools, riffles and edges) in both original vegetation and revegetation sites (Fig. 2.7). Floating aquatic vegetations generally support more taxa and serve as important habitat especially to those clinging nymphs/larvae like the Ephemeroptera, Plecoptera, Odonata and some aquatic Coleoptera. Some native freshwater snails (e.g., *Glytophysa* sp. and lymnaieds), beetle larvae (e.g., *Cyphon adelaidae*) and odonate nymphs (e.g., *Austroaeschna unicornis*)

were found in sites lacking of willows (see Chapter 2). Thus, it suggests that revegetation is a good decision for biodiversity conservation as many native invertebrates depend on the protection of existing native vegetation in order to persist, and also the provision of a suitable habitat for reproduction, protection from predators and food supply through revegetation.

The impact of willows was seasonal, as indicated by significant interactions and variations between seasons and invertebrates abundance (Fig. 2.6). Differences in both abundance and diversity of aquatic invertebrates between spring, summer and autumn were not obvious, but were greatest in winter when deciduous willows had dropped their leaves while the evergreen native riparian retain theirs. The most important water quality parameters affecting the invertebrate communities were water depth and width, turbidity, conductivity, total dissolved solids and flow rates (Table 2.4). Dominant taxa, particularly *P. antipodarum,* caenid mayfly nymphs (*Tasmonocoenis tillyardi*) and chironomids, were more abundant in more turbid water with high conductivity, total dissolved solids and temperatures in both creeks. However, native invertebrates such as stonefly nymphs (*Dinotoperla evansi*) and caddisfly nymphs (*Lingora aurata*) were less abundant in water of high conductivity, total dissolved solids and temperature. When the taxa that were responsible for the significant differences between willow sites and non-willow sites were examined, hydrobiid snail was the most prominent amongst those which were very dense in willow sites. Importantly, there was also an absence of some native invertebrates from revegetation and original vegetation sites even though they occurred under willows (e.g., Gomphidae: *Austrogomphus* sp.). These results are important considerations in the practical implementation of willows management, and may provide a conceptual basis for the analysis, assessment and prediction of biological conditions and should be incorporated into Catchment Management Authorities policies on willows control. For example, the potential use of *P. antipodarum* as indicator species can be suggested to identify short term effects of invaded willows streams. The presence and abundance of *P. antipodarum* also can be used to indicate the health of willows invaded aquatic systems, whether they are classified as non-impact, slightly impact, moderately impact or severely impact.

Willow leaves may be an important source of food for some native invertebrates and may influence their growth rates and survivorships (see Chapter 3). We investigated the feeding preference, survivorship and growth rates of five dominant aquatic invertebrate species: *P. antipodarum*, *Physa acuta*, *D. evansi*, *T. tillyardi* and *L. aurata* using diets of leaves of crack

willows (*S. fragilis*) and white gums (*E. viminalis*). We found the three native taxa of shredders (*D. evansi*, *T. tillyardi* and *L. aurata*) preferred leached willow leaves over those of senescent willow, leached eucalypt and senescent eucalypt (Fig. 3.2). However, the scrapers (*P. antipodarum* and *Ph. acuta*) did not show any preference (Fig. 3.2). We presumed possibly the scrapers which usually consumed biofilms (bacterial, fungal, algal) on the leaves did not affect the amounts of weight loss in all proportions of offered food diet. Thus, no significant differences of weight loss were detected among diet of leaves offered.

Shredders that were fed a diet of leached and senescent willow leaves clearly grew more than the shredders fed on leached and senescent eucalypt leaves (Fig. 3.3). Similarly, survivorships of each taxa of shredders were significantly greater on leached and senescent willow leaves than on the other leaf types (Table 3.2). Since shredders are very mobile, we presumed they should be able to search for appropriately conditioned leaves and be highly selective. It seems reasonable to assume that the shredders preferred 'soft' willow rather than 'hard' leaf species (eucalypt).

In terms of nutrient content, leached willow supported a noticeably thicker biofilms providing a better food source than senescent willow leaves. Even though the highest carbon and nitrogen contents was found in leached eucalypt leaves (Fig. 3.4) and would potentially provide the best nutritional source, we noticed that leached willow leaves rapidly accumulated a much thicker slimy microbial biofilm than all other leaf types. Leached leaves have been shown by Irons *et al.* (1988) to have the highest proportion of nitrogen and the lowest phenolic contents which are more likely to be responsible for the preference observed in our experiments. Secondary compounds which are known to remain active after leaf senescence, may be toxic and interfere with digestion or give a bitter taste, acting as a feeding deterrent (Irons *et al.* 1988). We suspect that generally the invertebrates tested prefer leached willows than senescent willows as probably at that stage of leaf decomposition, the C/N ratios of leached willows make the leaves more palatable. As well, leaching compounds from the leaves can lead to an increase in microbial populations favoured by many aquatic invertebrates, especially the shredders taxa studied.

In conclusion, willow leaves may provide a suitable food source for aquatic invertebrates and that differences in leaf states influence not only their food preference and consumption, but also growth rates and survival. Therefore, any management decisions need to take account the fact that removal of willows may represent a reduction in the quantity and quality of food

available for some invertebrates (especially shredders), at least until the riparian canopy is re-established. Future research should also focus on the nutritional value of the leaves, phenolic contents and the biofilms which develop on them.

Willow roots have habitat value for aquatic invertebrate communities (see Chapter 4). We designed a simple field experiments to investigate differences in colonisation by aquatic invertebrates on two different types of substrates and with different structural complexities (willow roots vs. aluminium wire mesh) after two colonisation periods (30 days and 90 days). We found willow roots supported significantly higher numbers of aquatic invertebrates by providing a more stable habitat than on artificial substrate (wire mesh of different sizes) (Fig. 4.2). We also found greater species richness and diversity on willow roots than on the artificial substrates (Fig. 4.3). These findings revealed that willow roots do have greater surface texture, area and variety of microhabitats for invertebrates colonisation. The rough-fibrous texture of willow roots also provides a suitable habitat for invertebrate reproduction, protection from predators and food supply (Korinkova 1971; Lester *et al.* 1994a).

In terms of habitat complexity differing in both hardness and texture, more invertebrates were found on soft, fibrous willow roots than on firm, smooth aluminium wire mesh (Fig. 4.2). When the willow roots were less complex (by removal of fine and lateral roots with only primary roots left intact), the abundance of invertebrate was significantly reduced. It was apparent that the lower abundance of invertebrates on less complex of willow roots was influenced by reduced heterogeneity and availability of habitats. Also, the length of colonisation period appears to be an important determinant of the resulting invertebrate community structure on both substrate types. There were significant increases in the abundance of aquatic invertebrates on both willow roots and aluminium wire mesh after 90 days compared with 30 days of colonisation (Fig. 4.2). The longer colonisation period was important to obtain 'stable' invertebrate communities because it allowed development of biofilms and epilithics material which is known to affect colonisation by aquatic invertebrates (Minshall 1984; Mackay 1992). Generally, at least 25 days was necessary for the artificial substrates to obtain a 'stable' population of invertebrates (Hilsenhoff 1969) and the greatest number of taxa and individual on the artificial substrates usually occurred after 35 days of exposure (Boothroyd & Dickie 1989).

Of all of the functional feeding groups, the scrapers (primarily *P. antipodarum*) were more abundant especially on the more complex willow root structures and after a longer period of

colonisation (Fig. 4.5). The abundance of all scrapers combined may be a good indicator of the abundance of all invertebrates that are likely to have a preference for willow root habitat. The fine sediment on the willow roots include a natural biofilm and fine detritus that would provide food for some invertebrates, especially the scrapers. Other authors (e.g., Hanlon 1981; Collier & Winterbourn 1986; Lester *et al.* 1994a) provide some evidence that willows are an important food source for many aquatic invertebrates. Willow roots are also have similar nutritional value as willow leaves, as they supply additional nutrition from increased microbial conditioning (Collier & Winterbourn 1986) or perhaps, from the removal of harmful secondary compounds as found in submerged willow leaves which affect invertebrates feeding directly (Lester *et al.* 1994b).

This study suggests that willow roots have habitat value for aquatic invertebrates, but mainly for the introduced *P. antipodarum,* and total removal of willow roots may disrupt the invertebrate communities which utilise the roots as habitat. The artificial willow roots (fine size of aluminium wire mesh) can be used as an alternative to stimulate invertebrate community, especially in the sites where willows had been removed. This study also suggests that it is inappropriate to remove the whole willow tree during a de-willowing operation and willow roots should be left *in situ* until the riparian canopy is reinstated by suitable revegetation efforts. Longer period of colonisation is needed to obtain a 'stable' invertebrate community, which appears that a considerable time period is required during revegetation efforts.

The removal of the willows canopy and revegetation programs may affect aquatic invertebrate community assemblages (see Chapter 5). We investigated the differences in the total abundance and species richness of aquatic invertebrates in two different experiments: (1) natural willow shade at Sixth Creek, and (2) artificial shade, using shade cloth at Deep Creek. Each study site had three different levels of shade; (1) fully shaded, (2) partly shaded and (3) open canopy and the experiments ended after a 28 day of colonisation period. Three sections at Sixth Creek representing different levels of willow canopies were chosen to contrast degree levels of shade; whereas shade levels for artificial treatment were manipulated using different mesh sizes of shade cloth to represent stream segments with contrasting degrees of overhead canopy.

Initially, we hypothesized that open canopy treatment (e.g., from the removal of willow canopy) would increase the exposure of aquatic invertebrates to high light intensity, which

would directly influence invertebrate community structure. Several reasons have been advanced to explain differences in invertebrate abundance associated with a reduction in riparian canopies (e.g., McKie & Cranston 2001; Rios & Bailey 2006; Walsh *et al.* 2007). These authors suggest that higher light intensities and water temperatures resulting from a reduction in riparian canopies could disturb physiological processes of many aquatic organisms, which in turn could indirectly lead to further changes at higher trophic levels such as fish, birds and small mammals.

Interestingly, we found fully and partly shaded treatments under willows in Sixth Creek had significantly higher abundance of aquatic invertebrates (Figs. 5.2 & 5.6). All treatments with an open canopy (no shading) had a lower abundance of invertebrates than all shaded treatments (fully and partly shaded). The greater abundance under willow canopies in Sixth Creek may have been influenced by cooler summer stream temperatures or possibly the greater availability of willow leaf litter, used especially by the gastropods as a source of food (Lester *et al.* 1994a; Schulze & Walker 1997). Again, we found the introduced hydrobiid snails were the most abundant taxon in all treatments, especially under willows. In addition to the direct impact of willow canopies, the riparian willows may have benefited the hydrobiid snails in other ways. *Potamopyrgus antipodarum* may benefit from a more stable habitat in the willow roots with protection from fast water currents (see Chapter 4) or from availability of food source (e.g., biofilms, epilithic materials) provided by willow leaves (see Chapter 3).

Taxa assemblages in partly shaded willows in Sixth Creek are not more distinct from the samples from open canopy treatment (Fig. 5.5). This was due to similar distribution of certain taxa in partly shaded and open canopy treatments under willows in Sixth Creek such as chironomids (Orthocladiinae), lymnaeids snail and some beetle larvae (e.g., *Cyphon adelaidae*, *Antiporus blakei, Platynectes decempunctatus*). Interestingly, some of uncommon taxa were only found under artificial shade in Deep Creek such as native freshwater snails (*Austropyrgus* sp.), dragonfly nymphs (*Austrogomphus* sp.), caddisfly nymphs (*Atri. dubius*) and amphipods (*Aust. australis*). However, there was no clear pattern of taxa assemblages under artificial shade as result from two-way clustering analysis could not indicate the pattern of species assemblages at different shade levels (Fig. 5.9). Such differences in invertebrate abundance and taxon richness among shade levels in each creek could probably be influenced by physico-chemical variables or perhaps differences in light intensities, as Sixth and Deep

Creeks have different ecological and physico-chemical characteristics (see Chapter 2 for details).

Unexpectedly, we found more a diverse and richer community in open canopy treatments in both experiments. This result suggests that an increase in light intensities following willows removal may increase the diversity and richness of the invertebrate communities. Similar findings have been observed by some other researchers such as McKie & Cranston (2001), Walsh *et al.* (2007) and Jayawardana & Westbrooke (2010), who found abundance and diversity of invertebrates was greater in open sites compared with shaded sites. We suggest that increased light intensities may increase water temperatures and in-stream primary productivity and therefore, increasing secondary productivity which in turn, leads to an increase in invertebrate abundance. However, there is little evidence in the literature showing a direct link between the removal of a willow canopy and aquatic invertebrates, and there is still potential for both short and long term effects such as changes in growth rate, attainment of sexual maturity, reproduction and behaviour of many aquatic organisms to occur if stream temperatures increase (Hellawell 1986; Helmuth *et al.* 2006).

We suggest that observed differences in invertebrate communities between willow and artificial shade may be explained by a combination of environmental variables such as water temperature, light intensity and dissolved oxygen. Measuring these environmental variables would aid data in interpretation on the abundance and diversity of aquatic invertebrate communities. Although we found higher densities of invertebrates under willow canopies, possible long term water temperature changes may negatively affect stream productivity, especially existing native invertebrates. Light intensity plays an important role in influencing the invertebrate communities by reducing primary productivity, while higher species richness under open canopy treatment was possibly due to the effects of increased light which in turn may result in an increase in the amount of high quality of food. Also, the presence of microbial, diatoms or algae should be included in the study and is strongly recommended, as these provide a source of food for many aquatic invertebrates.

Large scale willow removal programs may need to consider the effect of increased light on the impact on aquatic invertebrate communities. Future research should also be conducted in the same stream, so that the data interpretation and identification factors (other than the effect of study sites) which may affect the species richness and abundance of invertebrate community could be identified and explained accurately.

6.2   Conclusions and Further Research Recommendation

Overall, willows have a significant impact on taxon richness and abundance, and on the composition of aquatic invertebrate communities. Removal of willows without subsequent revegetation leads to lower species numbers and diversity. Predictions based on invertebrate assemblages rather than separate taxa can be used as an indication of community changes resulting from management programs to remove willows and subsequent revegetation. If the type of invertebrate assemblage predicted does not match the one actually found (e.g., absence of some native invertebrates under willows), it might be then used as an indicator or detection for the impacts of willows threat. Even revegetation efforts sometimes cannot guarantee that conditions will be suitable for the native invertebrates. Thus, this study is important as it assesses the current status of the diversity of aquatic invertebrates in streams where willows are present, and compares this with situations where willows have been removed as well as with the original or 'natural' vegetation and with revegetation.

Willow leaves especially leached leaves have been shown to be a suitable food source for some native invertebrate taxa, particularly *D. evansi*, *T. tillyardi* and *L. aurata*. However, it is only available as a short-term resource during and shortly after leaf fall. Future research should focus on the nutritional value of the willow leaves, their phenolic content and the biofilms and natural epilithic organisms which develop on them. There should also be composed with other leaf types found in these streams (Sixth and Deep Creeks) to determine their nutritional value as a food source for the invertebrates. Whether willow leaves are a preferred food source for other native invertebrates should also be investigated.

Willow roots have been demonstrated to support high numbers of aquatic invertebrates by providing a variety of microhabitats. We suggest that during large scale removal of willows, especially in a small stream, might be advisable to leave the willow roots intact to reduce the impact on the invertebrates which utilise the willow roots as their habitats. Fine size of aluminium wire mesh also could be used as artificial willow roots to obtain a 'stable' invertebrate community after 30 days of colonisation period, particularly after the removal of willows. Further research is suggested to determine the effect of willow roots removal in larger rivers systems, where the impact on aquatic invertebrate communities might be greater.

This study also revealed that open canopy (no shading) following the removal of willows and subsequent revegetation programs increases both light intensity and water temperature,

and resulted in lower invertebrate abundance. This impact on aquatic invertebrate communities is likely to be major especially if removal is done over the summer months when higher light intensity and water temperature occur. However, the removal of willows may also increase primary and secondary productivity, which in turn increases the long term invertebrate community diversity, productivity and abundance. Outcomes from this study indicate that complete removal of willow canopies should be carefully considered, and that possible long term effect of willow canopies on the existing invertebrate community should be further investigated. Even though dense willows in a stream lead to a reduction in taxon diversity and richness, until they are replaced by native vegetation with similar shade, the invertebrate will be reduced. Thus, total stream cut should be avoided during revegetation effort, and moderate concentrations of willows are suggested to maintain balance stream productivity.

In general, this current study provides basic principles and information for the management of existing areas of willows and subsequent revegetation programs. This study includes consideration of the treatment of willow root masses, willow canopies and the type of vegetation which should be established in place of willows. This study hopefully will be used by Catchment Management Authorities in developing specific policies on willows management across Australia. This study also showed the potential use of aquatic invertebrates as indicators for the early detection and evaluation of the health of stream environments in willows management strategies.

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Appendix I List of species of aquatic invertebrates collected from Sixth and Deep Creeks. (Note: scr = Scraper; c-g = Collector-gatherer; c-f = Collector-filterer; shr = Shredder; prd = Predator; Species codes were used in Chapters 2, 4 and 5).

Class / Order	Family / Subfamily	Species	Species code	Feeding habit
Mollusca				
Gastropoda	Hydrobiidae	*Potamopyrgus antipodarum*	Potaanti	scr
		Austropyrgus sp.	Austropy	scr
	Planorbiidae	*Isidorella* sp.	Isidorel	scr
		Glytophysa sp.	Glytophy	scr
		Gyralus sp.	Gyralus	scr
	Physidae	*Physa acuta*	Physacu	c-g
	Lymnaeidae	*Lymnaea* sp.	Lymna	c-g
Bivalvia	Sphaeridae	*Sphaerium* sp.	Sphaerid	c-f
Insecta				
Plecoptera	Gripopterygidae	*Dinotoperla evansi*	Dinoevan	shr
		Illiesoperla mayii	Illimayi	shr
Ephemeroptera	Leptophlebiidae	*Koornanga inconspicua*	Koorinco	c-g
	Caenidae	*Tasmanocoenis tillyardi*	Tasmtill	c-g
	Baetidae	*Offadens* sp.	Offadens	c-g/scr
Coleoptera	Elmidae	*Simsonia leai*	Simsleai	scr/c-g
	Scirtidae	*Cyphon adelaidae*	Cyphon	scr
	Dystiscidae	*Antiporus blakei*	Antiblak	prd
		Platycnetes decempunctatus	Platdece	prd
		Sternopriscus sp.	Sternopr	prd
	Hydrophilidae	Undetermined larvae	Hydroph	c-g/prd/shr
Trichoptera	Conoesucidae	*Lingora aurata*	Lingaura	shr/c-g
	Hydrobiosidae	*Taschorema evansi*	Tascevan	shr/c-g
	Leptoceridae	*Oecetis* sp.	Oecetis	c-g/shr/prd
		Triplectides sp.	Triplec	c-g/shr/prd
	Hydroptilidae	Undetermined larvae	Hydropti	shr
	Atriplectididae	*Atriplectides dubius*	Atridubi	c-g/scr/prd
	Tasimidae	Undetermined larvae	Tasimid	shr

Appendix I (continued).

Class / Order	Family / Subfamily	Species	Species code	Feeding habit
Diptera	Chironomidae /			
	Tanypodinae	*Procladius* sp.	Proclad	prd
	Podominae	Undetermined larvae	Podomin	c-g
	Tanypodinae	*Paramerina* sp.	Parameri	prd
	Chironominae	*Polypedilum* sp.	Polypedi	c-g/prd/shr/ c-f/scr
	Simuliidae	*Austrosimulium furiosum*	Austfuri	c-f
	Stratiomydae	Undetermined larvae	Stratio	c-g
	Tabanidae	Undetermined larvae	Tabanid	c-g/prd
	Culicidae	Undetermined larvae	Culicid	c-f
Hemiptera	Notonectidae	*Enithares bergrothi*	Enitberg	prd
	Corixidae	*Micronecta annae*	Micranna	prd
	Veliidae	*Microvelia peramoena*	Micrpera	prd
Odonata	Hemicorduliidae	Undetermined nymph	Hemicord	prd
	Coenagrionidae	Undetermined nymph	Coenagr	prd
	Gomphidae	*Austrogomphus* sp.	Austrogom	prd
	Telephebiidae	*Austroaeschna unicornis*	Austunic	prd
	Libellulidae	*Orthetrum caledonicum*	Orthcale	prd
Crustacea				
Amphipoda	Ceinidae	*Austrochiltonia australis*	Austaust	c-g
Annelida				
Oligochaeta	Tubificidae	Unidentified	Tubifici	c-g
	Lumbricidae	Unidentified	Lumbrici	c-g
	Naididae	Unidentified	Naidid	c-g / prd
Ostracoda	Ostracoda	Unidentified	Ostracod	c-g
Collembola	Collembola	Unidentified	Collem	c-g
Arachnida	Hydracarina	Unidentified	Arachnid	prd
Hirudinea	Hirudinea	Unidentified	Hirudin	prd
Decapoda	Parastacidae	Unidentified	Parastac	c-g

Appendix II List of species of aquatic invertebrates recorded at each treatment in Sixth and Deep Creeks (see Chapter 2).

(Note: √ = Present; X = Absent).

Class / Order	Family / Subfamily	Species	Willows Present		Willows Removed		Revegetation		Original Vegetation	
			WP_1	WP_2	WR_1	WR_2	RV_1	RV_2	OV_1	OV_2
Mollusca										
Gastropoda	Hydrobiidae	*Potamopyrgus antipodarum*	√	√	√	√	√	√	√	√
		Austropyrgus sp.	X	X	X	X	√	√	√	√
	Planorbiidae	*Isidorella* sp.	X	√	√	√	√	√	√	√
		Glytophysa sp.	X	X	√	√	√	√	√	√
		Gyralus sp.	X	X	X	X	X	√	X	√
	Physidae	*Physa acuta*	√	√	√	√	√	√	√	√
	Lymnaeidae	*Lymnaea* sp.	√	√	√	√	√	√	√	√
Bivalvia	Sphaeridae	*Sphaerium* sp.	√	√	√	√	√	√	√	√
Insecta										
Plecoptera	Gripopterygidae	*Dinotoperla evansi*	√	√	√	√	√	√	√	√
		Illiesoperla mayii	√	√	√	√	X	√	√	√
Ephemeroptera	Leptophlebiidae	*Koornanga inconspicua*	√	√	√	√	√	√	√	√
	Caenidae	*Tasmanocoenis tillyardi*	√	√	√	√	√	√	√	√
	Baetidae	*Offadens spp.*	√	√	√	√	X	X	√	X
Coleoptera	Elmidae	*Simsonia leai*	√	√	√	√	√	X	√	√
	Scirtidae	*Cyphon adelaidae*	X	√	√	√	√	√	√	√

Appendix II (continued).

Class / Order	Family / Subfamily	Species	Willows Present		Willows Removed		Revegetation		Original Vegetation	
			WP_1	WP_2	WR_1	WR_2	RV_1	RV_2	OV_1	OV_2
Coleoptera	Dystiscidae	*Antiporus blakei*	X	√	√	√	√	√	√	√
		Platycnetes decempunctatus	X	X	√	X	X	X	X	X
		Sternopriscus sp.	X	√	√	√	X	√	√	√
	Hydrophilidae	Undetermined larvae	X	X	√	√	X	X	X	X
	Curculionidae	Undetermined larvae	X	√	X	X	X	√	√	√
Trichoptera	Conoesucidae	*Lingora aurata*	√	√	√	√	√	X	√	√
	Hydrobiosidae	*Taschorema evansi*	X	√	√	X	X	X	X	√
	Leptoceridae	*Oecetis* sp.	√	X	√	X	X	√	√	√
		Triplectides sp.	√	√	√	√	√	√	√	√
	Hydroptilidae	Undetermined larvae	X	X	√	√	√	X	√	√
	Atriplectididae	*Atriplectides dubius*	√	√	X	X	X	√	√	X
	Tasimidae	Undetermined larvae	X	X	X	X	X	√	X	√
Diptera	Chironomidae / Tanypodinae	*Procladius* sp.	√	X	√	√	X	√	√	√
	Podominae	Undetermined larvae	√	√	X	X	√	X	X	√
	Tanypodinae	*Paramerina* sp.	√	√	√	√	X	X	X	X
	Chironominae	*Polypedilum* sp.	√	√	X	√	√	X	√	√
	Simuliidae	*Austrosimulium furiosum*	√	√	√	√	√	√	√	√
	Stratiomydae	Undetermined larvae	√	√	√	√	√	√	√	√
	Tabanidae	Undetermined larvae	√	√	√	√	√	√	√	√
	Culicidae	Undetermined larvae	X	√	X	X	X	X	X	X

Appendix II (continued).

Class / Order	Family	Species	Willows Present		Willows Removed		Revegetation		Original Vegetation	
			WP_1	WP_2	WR_1	WR_2	RV_1	RV_2	OV_1	OV_2
Hemiptera	Notonectidae	*Enithares bergrothi*	√	√	√	√	√	√	√	√
	Corixidae	*Micronecta annae*	√	X	√	√	√	√	√	√
	Veliidae	*Microvelia peramoena*	√	X	√	√	X	X	√	X
Odonata	Hemicorduliidae	Undetermined larvae	√	√	√	√	√	√	√	√
	Coenagrionidae	Undetermined larvae	√	X	√	√	√	√	√	√
	Gomphidae	*Austrogomphus* sp.	√	√	√	√	X	X	X	X
	Telephebiidae	*Austroaeschna unicornis*	√	√	√	√	√	√	√	√
	Libellulidae	*Orthetrum caledonicum*	X	X	X	√	X	√	X	√
Crustacea										
Amphipoda	Ceinidae	*Austrochiltonia australis*	√	√	X	√	√	X	√	√
Annelida										
Oligochaeta	Tubificidae	Unidentified	X	X	√	√	X	X	X	X
	Lumbricidae	Unidentified	X	X	X	√	X	X	X	X
	Naididae	Unidentified	√	√	√	√	√	√	√	√
Ostracoda		Unidentified	√	√	√	√	√	√	√	√
Collembola		Unidentified	√	√	√	X	√	√	√	√
Arachnida	Hydracarina	Unidentified	√	√	√	√	√	√	√	√
Hirudinea		Unidentified	√	√	√	√	√	√	√	√
Decapoda	Parastacidae	Unidentified	X	X	X	X	√	√	√	√

Appendix III Raw physico-chemical data for each sampling event in Sixth and Deep Creeks. [Notes: TDS = total dissolved solids. Data for flow rates were converted to the following nominal categories; 1 = fast flowing (>0.35 m/s); 2 = slow flowing (0.10−0.35 m/s); 3 = stagnant (<0.10 m/s)].

Site / Date of sampling	Treatment	Site code	Depth (m)	Width (m)	Dissolved oxygen (mg/L)	Conductivity (µS/cm)	pH	TDS (ppm)	Water temperature (°C)	Flowrate (m/s)	Shade cover (%)
Sixth Creek (22/09/07)	Willows present	D001WP1P	3.00	3.50	9.30	377	8.98	285	12.50	3	90
		D001WP1R	0.05	3.00	11.80	378	9.26	290	11.10	1	70
		D001WP1E	1.50	2.00	11.73	376	9.12	288	11.00	2	95
	Willows removed	D001WR1P	0.50	2.80	10.57	402	8.60	200	14.60	3	0
		D001WR1R	0.30	3.00	13.84	497	9.54	200	14.00	1	0
		D001WR1E	0.20	2.80	11.19	400	9.18	202	14.00	2	0
	Revegetation	D001RV1P	0.50	2.00	11.60	336	9.10	170	11.60	3	30
		D001RV1R	0.30	1.50	12.22	340	8.98	170	12.50	1	70
		D001RV1E	0.45	1.50	11.00	336	9.02	171	12.20	2	70
	Original vegetation	D001OV1P	1.00	1.50	12.30	348	8.90	175	11.80	3	60
		D001OV1R	0.50	0.80	12.68	325	8.78	173	12.00	1	50
		D001OV1E	0.45	1.00	12.05	330	8.65	170	11.80	2	50
Deep Creek (22/09/07)	Willows present	D001WP2P	0.70	3.50	10.45	271	8.75	173	10.50	3	30
		D001WP2R	0.25	2.50	12.70	273	8.87	171	10.20	1	30
		D001WP2E	0.60	3.50	10.20	264	8.67	172	10.30	2	30
	Willows removed	D001WR2P	0.60	2.50	10.05	255	9.03	171	12.60	3	30
		D001WR2R	0.15	1.00	13.96	263	8.82	173	10.00	1	25
		D001WR2E	0.35	2.00	12.24	261	8.71	177	10.30	2	25

Appendix III (continued).

Site / Date of sampling	Treatment	Site code	Depth (m)	Width (m)	Dissolved oxygen (mg/L)	Conductivity (µS/cm)	pH	TDS (ppm)	Water temperature (°C)	Flowrate (m/s)	Shade cover (%)
Deep Creek (22/09/07)	Revegetation	D001RV2P	0.25	2.00	10.05	197	8.50	168	11.00	3	50
		D001RV2R	0.15	1.50	12.68	188	8.90	170	11.60	1	50
		D001RV2E	0.15	1.00	11.30	190	8.88	165	10.98	2	45
	Original vegetation	D001OV2P	1.00	2.50	11.50	285	8.19	150	8.19	3	40
		D001OV2R	0.25	1.50	11.60	240	8.05	118	8.80	1	50
		D001OV2E	0.20	1.50	11.70	268	8.40	138	8.90	2	50
Sixth Creek (25/11/07)	Willows present	D060WP1P	0.70	4.00	9.93	509	8.56	247	23.30	3	90
		D060WP1R	0.13	2.50	11.46	318	8.37	274	22.70	1	100
		D060WP1E	0.50	3.00	10.10	519	8.58	261	20.00	2	100
	Willows removed	D060WR1P	0.55	2.00	10.11	553	8.25	228	26.00	3	20
		D060WR1R	0.25	2.00	11.50	540	8.05	218	25.80	1	0
		D060WR1E	0.35	3.00	10.04	543	8.46	213	25.80	2	0
	Revegetation	D060RV1P	0.58	3.00	10.81	258	8.44	148	25.60	3	20
		D060RV1R	0.25	2.00	13.30	248	8.28	128	24.80	1	30
		D060RV1E	0.25	1.00	12.27	277	8.24	154	19.70	2	30
	Original vegetation	D060OV1P	0.60	1.00	10.98	279	8.32	168	23.30	3	50
		D060OV1R	0.35	1.50	14.50	330	8.50	178	25.60	1	35
		D060OV1E	0.35	1.00	13.98	265	8.45	166	24.50	2	40
Deep Creek (25/11/07)	Willows present	D060WP2P	0.60	4.00	11.02	524	8.30	275	21.40	3	80
		D060WP2R	0.30	3.00	12.70	535	8.15	263	20.30	1	90
		D060WP2E	0.30	3.50	10.93	509	8.56	247	23.30	2	90

Appendix III (continued).

Site / Date of sampling	Treatment	Site code	Depth (m)	Width (m)	Dissolved oxygen (mg/L)	Conductivity (µS/cm)	pH	TDS (ppm)	Water temperature (^{0}C)	Flowrate (m/s)	Shade cover (%)
Deep Creek (25/11/07)	Willows removed	D060WR2P	0.50	2.50	11.86	435	9.29	274	25.70	3	30
		D060WR2R	0.30	0.80	13.50	439	8.53	264	26.10	1	30
		D060WR2E	0.20	3.00	11.63	437	8.68	282	26.30	2	30
	Revegetation	D060RV2P	0.25	2.00	12.07	303	8.28	127	25.60	3	80
		D060RV2R	0.15	2.00	13.26	314	8.34	150	20.10	1	90
		D060RV2E	0.20	2.50	12.50	396	8.31	153	20.30	2	80
	Original vegetation	D060OV2P	0.35	3.00	13.50	311	8.45	178	24.60	3	80
		D060OV2R	0.25	3.00	14.80	325	8.50	189	34.80	1	80
		D060OV2E	0.15	2.50	13.20	310	8.38	178	23.30	2	80
Sixth Creek (11/01/08)	Willows present	D120WP1P	0.85	3.50	9.52	623	8.66	418	21.80	3	70
		D120WP1R	0.50	1.50	16.55	630	8.52	416	21.70	1	70
		D120WP1E	0.45	2.00	9.85	567	8.45	388	23.30	2	50
	Willows removed	D120WR1P	0.53	2.00	9.50	695	8.34	355	24.60	3	0
		D120WR1R	0.10	1.50	11.71	699	8.27	355	21.70	1	0
		D120WR1E	0.25	2.00	9.03	685	8.42	350	21.40	2	0
	Revegetation	D120RV1P	0.40	1.70	11.20	328	8.34	234	23.60	3	30
		D120RV1R	0.05	0.35	11.59	366	8.43	228	23.60	1	30
		D120RV1E	0.05	1.00	11.57	355	8.35	237	22.60	2	40
	Original vegetation	D120OV1P	0.50	2.00	10.87	299	8.34	155	21.70	3	30
		D120OV1R	0.35	1.00	11.30	285	8.24	150	20.60	1	30
		D120OV1E	0.35	1.00	11.25	295	8.56	145	21.80	2	30

Site / Date of sampling	Treatment	Site code	Depth (m)	Width (m)	Dissolved oxygen (mg/L)	Conductivity (μS/cm)	pH	TDS (ppm)	Water temperature (°C)	Flowrate (m/s)	Shade cover (%)
Deep Creek (11/01/08)	Willows present	D120WP2P	0.60	2.50	9.57	612	8.59	308	22.10	3	70
		D120WP2R	0.35	1.00	12.85	613	8.46	310	23.60	1	60
		D120WP2E	0.40	2.50	10.28	614	8.36	310	21.00	2	80
	Willows removed	D120WR2P	0.50	3.00	9.07	412	9.37	301	20.60	3	30
		D120WR2R	0.10	0.25	13.98	407	9.25	370	20.50	1	40
		D120WR2E	0.30	1.00	10.14	411	8.92	369	20.20	2	30
	Revegetation	D120RV2P	0.35	1.10	11.33	269	8.27	229	19.70	3	60
		D120RV2R	0.30	0.80	12.74	273	8.34	240	18.70	1	60
		D120RV2E	0.30	1.00	12.24	362	8.32	213	19.10	2	60
	Original vegetation	D120OV2P	0.45	2.00	10.55	218	8.22	201	20.00	3	75
		D120OV2R	0.30	1.80	11.70	245	8.16	211	19.80	1	60
		D120OV2E	0.30	1.00	11.40	235	8.16	205	19.90	2	60
Sixth Creek (14/03/08)	Willows present	D180WP1P	0.85	3.50	9.52	623	8.66	318	22.50	3	50
		D180WP1R	0.50	2.00	16.55	630	8.52	316	23.30	1	70
		D180WP1E	0.45	2.30	9.85	567	8.45	288	21.70	2	50
	Willows removed	D180WR1P	0.53	2.00	9.50	648	9.25	334	23.60	3	0
		D180WR1R	0.15	1.50	11.71	666	8.27	328	24.60	1	0
		D180WR1E	0.30	1.50	9.33	655	8.42	327	22.60	2	0
	Revegetation	D180RV1P	0.40	1.70	11.20	295	8.34	145	21.70	3	30
		D180RV1R	0.05	0.35	11.59	299	8.43	155	23.60	1	30
		D180RV1E	0.05	1.00	11.57	285	8.35	150	21.40	2	30

Appendix III (continued).

Site / Date of sampling	Treatment	Site code	Depth (m)	Width (m)	Dissolved oxygen (mg/L)	Conductivity (µS/cm)	pH	TDS (ppm)	Water temperature (^{0}C)	Flowrate (m/s)	Shade cover (%)
Sixth Creek (14/03/08)	Original vegetation	D180OV1P	0.50	2.00	10.65	285	8.55	165	19.80	3	40
		D180OV1R	0.40	1.50	11.65	256	8.70	145	19.30	1	50
		D180OV1E	0.40	1.50	10.78	399	8.45	140	19.80	2	50
Deep Creek (14/03/08)	Willows present	D180WP2P	0.60	2.50	9.57	412	8.58	308	20.10	3	70
		D180WP2R	0.50	1.00	12.85	413	8.46	308	20.60	1	60
		D180WP2E	0.40	2.50	10.28	414	8.36	310	20.00	2	80
	Willows removed	D180WR2P	0.50	2.00	9.50	412	9.37	329	23.30	3	35
		D180WR2R	0.10	0.25	13.98	407	9.25	340	21.80	1	40
		D180WR2E	0.35	1.50	10.14	411	8.92	337	22.60	2	30
	Revegetation	D180RV2P	0.75	2.50	10.87	269	8.27	129	19.30	3	70
		D180RV2R	0.15	0.80	12.74	273	8.34	140	20.80	1	60
		D180RV2E	0.50	1.00	12.24	262	8.32	113	21.10	2	60
	Original vegetation	D180OV2P	0.50	2.00	9.98	276	8.31	151	19.95	3	60
		D180OV2R	0.45	1.50	13.30	271	8.29	145	20.10	1	65
		D180OV2E	0.40	1.50	12.88	270	8.35	144	19.00	2	60
Sixth Creek (14/05/08)	Willows present	D240WP1P	0.85	4.00	12.08	649	8.71	317	13.30	3	45
		D240WP1R	0.30	2.00	14.49	627	8.49	329	13.80	1	40
		D240WP1E	0.50	2.50	14.11	605	8.41	298	13.50	2	40
	Willows removed	D240WR1P	0.20	2.00	9.41	632	8.33	345	13.78	3	0
		D240WR1R	0.20	1.50	12.30	682	8.15	356	12.50	1	0
		D240WR1E	0.20	1.50	10.85	649	8.27	322	12.60	2	0

Appendix III (continued).

Site / Date of sampling	Treatment	Site code	Depth (m)	Width (m)	Dissolved oxygen (mg/L)	Conductivity (µS/cm)	pH	TDS (ppm)	Water temperature (^{o}C)	Flowrate (m/s)	Shade cover (%)
Sixth Creek (14/05/08)	Revegetation	D240RV1P	0.20	2.50	10.03	244	7.77	145	12.98	3	50
		D240RV1R	0.20	2.00	13.66	285	7.29	132	12.30	1	70
		D240RV1E	0.20	2.00	12.57	274	7.77	146	13.50	2	50
	Original vegetation	D240OV1P	0.35	3.00	10.88	275	7.90	145	12.95	3	50
		D240OV1R	0.20	2.50	14.10	267	7.68	139	12.70	1	45
		D240OV1E	0.20	2.00	13.11	278	7.70	148	13.30	2	50
Deep Creek (14/05/08)	Willows present	D240WP2P	0.60	4.00	6.57	418	8.44	207	11.10	3	30
		D240WP2R	0.35	2.00	8.08	414	8.02	209	12.60	1	40
		D240WP2E	0.30	3.00	7.90	412	7.88	209	11.50	2	30
	Willows removed	D240WR2P	0.40	2.50	9.64	733	11.25	325	12.80	3	20
		D240WR2R	0.30	0.55	13.57	706	9.32	338	12.40	1	30
		D240WR2E	0.25	2.00	12.91	756	10.22	330	12.90	2	25
	Revegetation	D240RV2P	0.20	2.00	9.48	401	7.22	206	12.20	3	45
		D240RV2R	0.20	1.30	13.86	406	7.98	202	13.60	1	50
		D240RV2E	0.20	1.30	11.73	393	7.89	205	12.70	2	50
	Original vegetation	D240OV2P	0.25	2.50	10.57	368	7.56	230	12.30	3	40
		D240OV2R	0.20	2.00	12.56	350	7.77	267	13.35	1	55
		D240OV2E	0.20	2.00	11.50	348	7.75	256	13.60	2	45
Sixth Creek (17/07/08)	Willows present	D300WP1P	0.60	3.50	12.40	274	9.31	138	10.20	3	0
		D300WP1R	0.30	3.50	14.56	288	9.07	135	9.80	1	0
		D300WP1E	0.25	3.50	12.45	281	9.56	129	10.60	2	0

Appendix III (continued).

Site / Date of sampling	Treatment	Site code	Depth (m)	Width (m)	Dissolved oxygen (mg/L)	Conductivity (µS/cm)	pH	TDS (ppm)	Water temperature (°C)	Flowrate (m/s)	Shade cover (%)
Sixth Creek (17/07/08)	Willows removed	D300WR1P	0.60	2.50	15.50	216	9.32	108	12.10	3	0
		D300WR1R	0.50	0.80	18.30	203	9.82	103	10.30	1	0
		D300WR1E	0.25	2.00	16.75	218	9.39	111	10.70	2	0
	Revegetation	D300RV1P	0.60	1.00	11.75	248	8.77	124	10.50	3	10
		D300RV1R	0.35	0.65	13.30	230	8.08	121	9.50	1	10
		D300RV1E	0.45	0.75	9.98	235	8.68	120	10.10	2	10
	Original vegetation	D300OV1P	0.55	2.00	13.80	239	8.70	130	10.10	3	30
		D300OV1R	0.45	2.00	15.90	230	8.68	128	11.25	1	35
		D300OV1E	0.45	1.50	13.90	235	8.75	130	10.30	2	30
Deep Creek (17/07/08)	Willows present	D300WP2P	0.50	0.50	10.87	219	8.84	110	10.00	3	0
		D300WP2R	0.25	0.25	11.77	228	8.03	106	9.70	1	0
		D300WP2E	0.25	0.25	9.75	232	8.98	120	9.50	2	0
	Willows removed	D300WR2P	0.40	2.00	15.50	247	8.84	128	10.30	3	5
		D300WR2R	0.35	2.50	17.70	232	7.65	120	10.00	1	5
		D300WR2E	0.50	3.00	16.34	255	8.90	135	11.50	2	5
	Revegetation	D300RV2P	0.45	1.50	10.60	249	9.07	126	10.30	3	5
		D300RV2R	0.35	2.00	13.33	234	8.98	122	9.40	1	5
		D300RV2E	0.35	2.00	11.45	240	8.90	130	9.80	2	5
	Original vegetation	D300OV2P	0.40	2.00	11.20	220	8.03	116	9.80	3	10
		D300OV2R	0.35	2.00	12.50	230	8.15	110	9.70	1	10
		D300OV2E	0.30	1.50	11.45	225	8.10	115	9.36	2	10

Appendix III (continued).

Site / Date of sampling	Treatment	Site code	Depth (m)	Width (m)	Dissolved oxygen (mg/L)	Conductivity (µS/cm)	pH	TDS (ppm)	Water temperature (°C)	Flowrate (m/s)	Shade cover (%)
Sixth Creek (04/09/08)	Willows present	D360WP1P	3.00	2.60	11.80	378	9.26	190	11.10	3	0
		D360WP1R	0.10	2.00	11.73	376	9.12	188	11.00	1	0
		D360WP1E	1.70	3.50	9.30	377	8.98	185	12.50	2	0
	Willows removed	D360WR1P	0.45	2.70	13.84	297	9.54	109	12.50	3	0
		D360WR1R	0.35	2.80	11.19	300	9.18	102	12.00	1	0
		D360WR1E	0.30	2.90	10.57	202	8.60	100	11.60	2	0
	Revegetation	D360RV1P	0.60	1.50	12.22	240	8.98	170	14.00	3	40
		D360RV1R	0.30	1.00	10.00	236	9.02	171	12.20	1	50
		D360RV1E	0.35	1.75	11.60	236	9.10	170	14.60	2	50
	Original vegetation	D360OV1P	0.38	1.70	11.59	266	8.43	128	13.60	3	40
		D360OV1R	0.30	1.00	11.70	255	8.35	137	12.60	1	40
		D360OV1E	0.05	0.50	11.57	248	8.34	134	13.60	2	50
Deep Creek (04/09/08)	Willows present	D360WP2P	0.70	2.50	12.70	273	8.87	121	10.20	3	0
		D360WP2R	0.13	3.00	10.20	264	8.67	132	10.30	1	0
		D360WP2E	0.60	3.00	10.45	271	8.75	133	10.50	2	5
	Willows removed	D360WR2P	0.55	1.00	13.96	263	8.82	133	10.00	3	5
		D360WR2R	0.35	2.00	12.24	261	8.71	135	10.30	1	5
		D360WR2E	0.15	2.50	10.06	255	9.03	131	12.60	2	5
	Revegetation	D360RV2P	0.55	2.00	11.25	255	8.78	168	13.30	3	30
		D360RV2R	0.25	1.50	11.10	240	8.99	170	13.78	1	30
		D360RV2E	0.30	1.50	11.05	247	8.85	166	14.00	2	30

Appendix III (continued).

Site / Date of sampling	Treatment	Site code	Depth (m)	Width (m)	Dissolved oxygen (mg/L)	Conductivity (μS/cm)	pH	TDS (ppm)	Water temperature (^{0}C)	Flowrate (m/s)	Shade cover (%)
Deep Creek (04/09/08)	Original vegetation	D360OV2P	0.33	1.30	12.20	273	8.34	240	12.80	3	30
		D360OV2R	0.22	0.80	12.24	262	8.32	213	12.70	1	40
		D360OV2E	0.25	1.00	11.30	269	8.27	229	13.30	2	40
Sixth Creek (08/11/08)	Willows present	D420WP1P	1.30	4.00	9.17	532	9.34	276	13.60	3	50
		D420WP1R	0.50	1.50	11.41	574	9.38	293	10.30	1	40
		D420WP1E	0.30	1.50	10.25	579	9.27	385	10.40	2	40
	Willows removed	D420WR1P	0.40	1.50	9.30	632	9.34	276	13.60	3	20
		D420WR1R	0.30	0.90	11.07	626	9.99	273	13.50	1	20
		D420WR1E	0.25	0.95	9.28	644	9.56	270	13.80	2	20
	Revegetation	D420RV1P	0.50	3.50	10.50	427	8.68	228	13.20	3	40
		D420RV1R	0.35	1.00	14.25	424	8.84	221	13.20	1	50
		D420RV1E	0.45	1.00	10.76	434	8.88	225	13.50	2	45
	Original vegetation	D420OV1P	0.50	2.50	11.21	431	8.90	245	12.30	3	45
		D420OV1R	0.35	2.00	12.76	421	8.56	213	12.90	1	50
		D420OV1E	0.35	2.00	12.00	420	8.78	220	13.10	2	40
Deep Creek (08/11/08)	Willows present	D420WP2P	0.45	4.00	9.07	615	9.36	312	13.40	3	40
		D420WP2R	0.50	2.50	11.39	622	9.46	315	13.90	1	30
		D420WP2E	0.30	3.00	10.98	621	9.87	313	13.60	2	40
	Willows removed	D420WR2P	0.45	1.50	11.78	617	9.39	308	13.30	3	35
		D420WR2R	0.25	0.50	12.12	612	9.39	303	13.10	1	40
		D420WR2E	0.25	2.00	11.63	607	9.40	310	13.70	2	40

Appendix III (continued).

Site / Date of sampling	Treatment	Site code	Depth (m)	Width (m)	Dissolved oxygen (mg/L)	Conductivity (µS/cm)	pH	TDS (ppm)	Water temperature (^{0}C)	Flowrate (m/s)	Shade cover (%)
Deep Creek (08/11/08)	Revegetation	D420RV2P	0.25	1.50	10.98	476	8.20	209	12.20	3	40
		D420RV2R	0.15	0.95	13.68	443	8.29	215	12.30	1	45
		D420RV2E	0.15	0.95	11.45	423	8.24	210	12.00	2	50
	Original vegetation	D420OV2P	0.30	1.50	10.87	450	8.88	275	11.89	3	50
		D420OV2R	0.25	1.50	11.70	445	8.60	270	12.00	1	50
		D420OV2E	0.20	1.00	11.00	431	8.45	270	11.95	2	50

Appendix IV Monte Carlo test of significance of observed maximum indicator value for taxon abundance on willow roots and aluminium wire mesh substrate (See Appendix I for species codes; $P < 0.05$).

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       ----------------------------------------------

                  Indicator    groups
     Column      Maxgrp  Value  Mean   S.Dev     p *
       --------------------------------------------------
       Austropy      1     1.1    2.1    5.64   1.0000
       Isidorel      1    42.3   36.0   13.45   0.2793
       Glytophy      1    31.7   29.7   14.15   0.5587
       Gyralus       1     1.6    2.9    5.88   1.0000
       Physacu       0    53.1   53.0    3.20   0.7317
       Lymna         1    45.5   37.8   12.88   0.2613
       Dinoevan      1    50.6   48.5    9.76   0.5663
       Illimayi      0    41.6   34.8   13.18   0.3499
       Koorinsc      0    36.2   42.4   11.36   0.6725
       Tasmtill      1    68.8   45.5   11.81   0.0326*
       Offadens      1    36.0   31.4   14.18   0.5403
       Simsleai      1    37.8   48.1    9.89   1.0000
       Cyphon        0    21.9   22.2    9.95   0.4365
     Antiblak        1    12.7   18.0   10.03   1.0000
     Platdece        1     5.3    8.9    9.22   1.0000
       Sternop       1    13.2   18.5    9.79   1.0000
       Hydroph       0    31.8    4.1    7.55   0.0664
       Curculi       1     3.2    5.7    8.14   1.0000
       Lingaura      1    32.0   41.2   11.65   1.0000
       Tascevan      1    17.9   31.5   14.34   1.0000
       Oecetis       1    16.4   21.5   10.42   1.0000
       Triplec       0    70.0   36.9   12.70   0.0792
       Hydropti      1    14.8   20.0   10.07   1.0000
       Tasimid       1     1.6    2.9    6.29   1.0000
       Atridubi      0    57.5   16.9   10.02   0.0354*
       Chirono       1    56.0   52.1    2.26   0.1414
       Ausfurio      0    34.6   44.4   11.85   1.0000
       Stratio       1    27.5   27.9   12.43   0.5631
       Tabanid       1     2.1    3.9    7.23   1.0000
       Culicid       1    21.7   24.9   10.80   0.5941
       Enitberg      1    19.0   23.5   11.15   1.0000
       Micranna      1     8.5   13.4   10.26   1.0000
       Micrpera      0    36.1   42.7   11.53   1.0000
       Hemicord      0    45.4   29.6   13.71   0.2354
       Coenagr       1    15.9   20.9   10.16   1.0000
       Austrog       1     1.1    2.0    5.40   1.0000
       Austunic      0    34.9   44.1   11.73   1.0000
       Orthcale      0    31.3    4.8    7.81   0.0732
       Austaust      0    17.6   28.9   13.23   1.0000
       Tubific       0    60.4   12.8    9.94   0.0160*
       Lumbric       1     2.6    5.1    8.37   1.0000
       Naidid        0    66.5   40.3   11.93   0.1234
       Sphaer        1    60.8   43.8   11.67   0.2789
       Ostracod      0    44.7   30.5   14.52   0.2675
       Collem        1    10.1   15.4   10.34   1.0000
       Arachnid      1    17.1   30.4   14.14   1.0000
       Hirudi        1    23.3   25.8   11.29   0.5845
       Parasta       1     2.1    3.9    7.14   1.0000
       ----------------------------------------------------
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Appendix V Taxa abundances in relation to the types of substrate and substrate complexity (Notes: ++++ = Very abundant (> 100 individuals); +++ = Abundant (50-99); ++ = Common (10-49 individuals); + = Uncommon (1-9 individuals); - = Absent (none); WR = Willow root; AS = Aluminium wire mesh; * = $P < 0.05$). (See Chapter 4 for details).

Class/Order	Family	Species	Type of substrate					
			Willow roots			Aluminum wire mesh		
			WR1	WR2	WR3	AS1	AS2	AS3
Mollusca								
Gastropoda	Hydrobiidae	*Potamopyrgus antipodarum*	++++	++++*	++++*	++++	++++	++++*
	Planorbiidae	*Isidorella* sp.	++	+	++	+	++	+
		Glytophysa sp.	-	-	++	-	+	-
	Physidae	*Physa acuta*	++	++*	++*	+	++	+
	Lymnaeidae	*Lymnaea* sp.	-	-	-	-	-	+
Bivalvia	Sphaeridae	*Sphaerium* sp.	-	+	+	-	-	+
Insecta								
Plecoptera	Gripopterygidae	*Dinotoperla evansi*	++++*	++++*	++++*	++*	++*	++++*
		Illiesoperla mayii	++*	++*	++*	+*	+*	++*
Ephemeroptera	Leptophlebiidae	*Koornanga inconspicua*	++	++	++*	+*	+*	++*
	Caenidae	*Tasmanocoenis tillyardi*	++*	+*	+*	++*	+*	++*
	Baetidae	*Offadens* sp.	-	-	-	-	-	+
Coleoptera	Elmidae	*Simsonia leai*	+	+*	+*	+	-	-
	Scirtidae	*Cyphon adelaidae*	+*	-	+	-	-	-

Appendix V (continued).

Class/Order	Family	Species	Type of substrate					
			Willow roots			Aluminum wire mesh		
			WR1	WR2	WR3	AS1	AS2	AS3
Trichoptera	Conoesucidae	*Lingora aurata*	+	++	++*	+	+	+++*
	Hydrobiosidae	*Taschorema evansi*	+	+	+	+	+	-
	Leptoceridae	*Oecetis* sp.	++	+	++*	++	++	+
	Hydroptilidae	Undetermined	++	++++*	++++*	++	++	+++*
	Atriplectididae	*Atriplectides dubius*	+	+	++*	+	+	+
Diptera	Chironomidae	*Procladius* sp.	+	+	+	+	+	++
		Polypedilum sp.	+	+	+	+	+	+
		Paramerina sp.	+	+	+	-	-	-
Odonata	Hemicorduliidae	Undetermined	+	-	+	+	+	-
	Coenagrionidae	*Pseudagrion* sp.	+	+	+	+	++	++
	Telephebiidae	*Austroaeschna unicornis*	-	-	-	-	-	+
Crustacea								
Amphipoda	Ceinidae	*Austrochiltonia australis*	-	-	-	-	-	+
Annelida	Tubificidae	Undetermined	-	+	++	-	-	+
Oligochaeta	Naididae	Undetermined	-	+	+	+	+	+
Ostracoda		Undetermined	-	-	-	-	+	-
Hirudinea		Undetermined	-	+*	-	-	-	+

Appendix IV Pilot study comparing willow roots and four artificial substrates as habitat for aquatic invertebrate communities.

A pilot study was conducted between 14 until 28 October 2008 (spring season) to compare the differences in colonisation by aquatic invertebrates using natural willow roots and four artificial substrates. Our main objective was to investigate various artificial substrates that might be suitable for a study comparing willow roots and artificial substrates as habitat for aquatic invertebrate communities.

This experiment was conducted in Deep Creek in the Mt. Lofty Ranges, South Australia (Fig. 2.1). Azmi and Jennings (unpublished) have described the study site in detail (refer to Chapter 2). Five different substrates (willow roots, aluminium wire mesh, bamboo sticks, coconut husks and plastic loofahs) were tested to examine colonisation differences (Fig. 1). The experimental design used various techniques modified from Hilsenhoff (1969), Souter and Williams (2001), Souter (2004); and White & White (2005). Each substrate was constructed such that they were 10−15 cm long and 5−10 cm diameter (total surface area 50−150 cm^2) and were packed inside nylon onion bags (mesh size: 2x2 mm) to avoid loss of samples. Rocks obtained from the locality were placed in the bottom of each sample bag to act as ballast.

There were four replicates of each substrate and in total 20 bags were placed randomly at a depth of approximately 1 m in a section of Deep Creek. Each bag was anchored by tying them to the trees on the bank or to metal stakes hammered into the edge of the stream. All bags were left undisturbed for 14 days. Between 1200 and 1700 h on 28 October 2008, the bags were collected by placing using a 250 µm aquatic net. They were transferred individually to plastic bags containing 80% ethanol and returned to the laboratory for analysis. Invertebrates were sorted, counted and identified to the lowest taxonomic level using keys of Williams (1980), McCafferty (1981), Merritt and Cummins (1996), Watts (1998), Gooderham and Tsyrlin (2002), Dean *et al.* (2004), and Theischinger and Hawking (2006).

Differences in invertebrate abundance and species richness among substrate types were compared using one-way ANOVA. Data were log($x+1$) transformed to ensure normality in calculations of means and *post-hoc* comparisons were undertaken using Tukey HSD test. All of the analyses were performed using Prism statistical program (PRISM 2007).

Fig. 1 Photos of five substrate types tested in a trial experiment to compare willow roots and artificial substrates as a habitat for aquatic invertebrates.

A total of 679 individual aquatic invertebrates were collected from the five different substrate types in Deep Creek. Total abundance was significantly greater on natural willow roots (235 individuals) than on other substrate types ($F_{4,19}$ = 15.539, $P < 0.001$) (Fig. 2a). There were significant differences in species richness of aquatic invertebrates, as a slightly greater mean number of species was recorded on willow roots than on artificial substrates. ($F_{4,19}$ = 0.539, $P < 0.05$). Of 679 individuals, 11 species were recorded on willow roots and few on the artificial substrates (Fig. 2b).
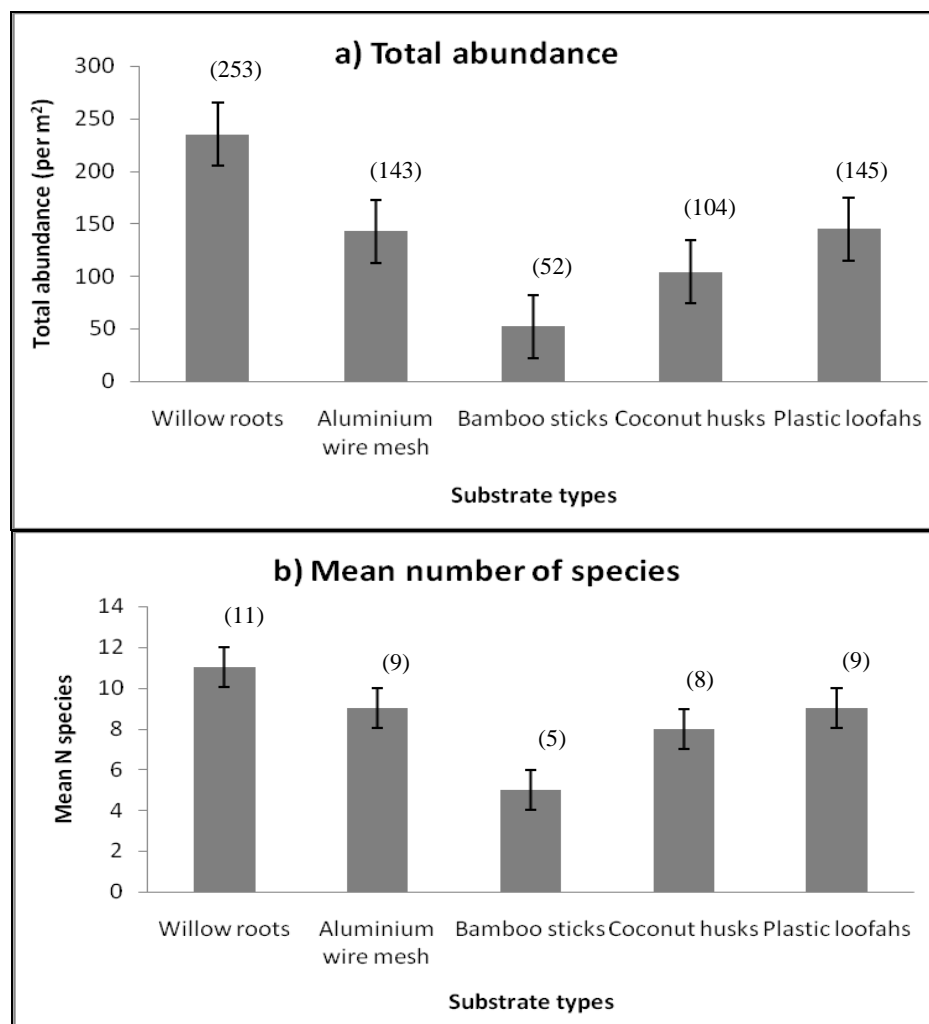
Fig. 2 Total abundance (a) and mean number of species (b) of aquatic invertebrate communities collected in five different substrate types. The numbers in ( ) are the actual numbers recorded.

We suspect that 14 days of time exposure was not enough to allow sufficient time for colonisation. For example, Hilsenhoff (1969) showed that exposure for at least 25 days was necessary to obtain a stable population of invertebrates. Thus, we decided to modify any future experimental design and leave the substrates undisturbed for at least 1-3 months to allow colonisation by microbes and invertebrates. We also found that containing samples within nylon onion bags were not stable as they were fragile and easy to wash away from high flows. Future experimentation should use more robust container such as galvanized wire mesh cages to avoid loss of samples. As most of invertebrates leave the sample when it is removed, we decided to wrap each replicate substrate with nylon onion bags before placing into the cages. Thus, all the invertebrates could be retrieved without the invertebrates escaping.

Artificial substrate have usually been used rather than natural substrates like coconut husks (e.g., Souter & Williams 2001; Souter 2004; White & White 2005), however, there are both advantages and disadvantages in each case. We decided on aluminium wire mesh as an artificial substrate in the final experiment because they are generally intended to mimic aquatic vegetation (various mesh sizes make them easier to manipulate as aquatic vegetation), and being heavier, when they are placed on the bottom of a stream, they are more stable in currents. Results from this pilot study support the hypothesis that natural substratum usually supported higher densities and diversity of aquatic invertebrates than on an artificial substratum. Thus, we predict willow roots would be the most preferred substrate as they provide more heterogeneity of microhabitats for invertebrate colonisation. We also found that habitat complexity is an important factor influencing the distribution of aquatic invertebrates in a stream, and days of colonisation may change the faunal composition. Therefore, we decided to include habitat complexity and days of colonisation as other treatments besides the type of substrate in our final experimental design. The expected outcomes would indicate whether the removal of the whole tree or just the canopy during a de-willowing operation was an option, and whether willow roots should be left until the riparian canopy is reinstated by suitable revegetation efforts.