The Role of Habitat Structure in a Freshwater Food Web

By

Danielle M.Warfe, B.Sc.(Hons)

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Declaration

This thesis contains no material which has been accepted for a degree or diploma by the University of Tasmania or any other institution, except by way of background information which is duly acknowledged in the text. To the best of my knowledge, this thesis contains no material previously published or written by another person, except where due acknowledgement is made in the text.

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Abstract

Habitat structure refers to the nature of the physical structure that provides an environment for biotic communities. Much of the research in marine and freshwater systems notes the importance of habitat in community organisation (for example, fish predators are commonly less effective as habitat structure increases), but few studies have specifically described the mechanisms by which it influences trophic interactions and thereby community structure. My research investigated the role of macrophyte structure in trophic interactions and community structure in the macrophyte beds of a lowland river.

One of the problems in assessing the role of habitat structure is the confusion over the definition, and therefore the measurement, of habitat structure, particularly in a way that allows comparison between different habitats and systems. I defined habitat structure as a combination of the qualitative and quantitative components of structure, so where macrophytes provide the habitat, this refers to their shape and density. While macrophyte density is relatively straightforward to quantify, macrophyte shape is more problematic which has lead to a variety of system-specific measures. I tested nine different indices of habitat complexity to determine which would best describe plant shape and best relate to the macroinvertebrate distribution on different macrophytes. I found a high degree of intercorrelation and redundancy between the structural indices such that they could be organised into two suites: one describing the interstitial space and the surface rugosity at coarse scales, the other describing the "whole plant" attributes of surface area and plant volume and the surface rugosity at fine scales. In particular, there were two indices which fell into both suites, an index of refuge space from predation, and the surface rugosity at 5 × magnification. Both these indices were also the most highly related to macroinvertebrate abundance and taxon richness, so I suggest they should be incorporated in the development of a broadly applicable index of macrophyte shape.

As macroinvertebrates responded to the refuge role of macrophytes, I tested if differences in both macrophyte density and macrophyte shape had any effect on the prey-capture success of two predators, the southern pygmy perch and a predatory damselfly. I used two predators to address the impacts of multiple predators; if habitat structure can mediate the outcomes of predator-prey interactions, then it may also

affect the outcomes of predator-predator interactions. I tested predator success in three macrophyte shapes at each of five macrophyte densities in a tank experiment. Surprisingly, there was no effect of plant density, but plant shape was important as fewer prey were captured, by each predator in isolation and by both predators combined, in the most structurally complex plant. This indicated that a more structurally complex plant can negatively affect the prey-capture success of predators, and also that macrophyte shape can mediate the outcomes of predator interactions.

The implications of this laboratory experiment prompted a field experiment to determine if the influence of macrophyte shape on fish predator success translated to field conditions and affected the macroinvertebrate and periphyton communities in macrophyte beds. I conducted a two-factorial, repeated measures, randomised complete block experiment using floating cages in existing macrophyte beds. I tested the factors of macrophyte shape (three types) and the presence or absence of fish predators using the native southern pygmy perch. I ran the experiment for eight months, sampling the macroinvertebrate and periphyton communities at 2, 6, 10, 26 and 30 weeks. Macrophyte shape had strong, consistent effects on both the macroinvertebrate and periphyton communities; both were most abundant on the most structurally complex plant. In contrast, pygmy perch affected only a subset of the macroinvertebrate community and had minor indirect effects on the periphyton composition. Contrary to expectations though, pygmy perch had their strongest effects on vulnerable invertebrate herbivores in the most structurally complex plant.

I concluded that in this system, macrophyte shape has a stronger influence than macrophyte density on trophic interactions, and constitutes a clear regulating influence on the macroinvertebrate and periphyton communities such that it precludes the conditions most likely to reveal strong effects of fish predation.

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Chapter 1: General Introduction

In this thesis, I explore the role of macrophytic plant structure in a freshwater community. Specifically, I examine how plant structure can be measured, how it mediates predator-prey and predator-predator interactions, and how it influences the effects of a fish predator on macroinvertebrate and periphyton community structure.

Introduction

"Habitat structure" refers to the physical structures in space which support plant and animal communities (McCoy and Bell 1991). It can refer to abiotic structures such as the size and arrangement of stones on a stream bed (e.g. Flecker and Allan 1984), the crevices and pits on stones (e.g. Downes et al. 1998), and the holes and fissures found on marine rocky shores (e.g. Menge et al. 1985). It can also refer to living structures, for example: mosses (e.g. Robson and Barmuta 1998), trees (e.g. Lawton 1983) and coral reefs (e.g. Bradbury et al. 1984), or any surface upon which organisms live. In vegetated aquatic systems such as lakes, lowland rivers, estuaries and marine littoral zones, habitat structure is provided by vascular macrophytes and macroalgae (Heck and Crowder 1991). In lowland rivers in particular, macrophyte beds can form an important link between the main channel and floodplain food webs, and at a larger scale, between riverine and terrestrial food webs (Davies and Humphries 1996, Dettmers et al. 2001). The importance of macrophytes as habitat in aquatic systems is demonstrated by the diverse and abundant communities they support, often many magnitudes greater than unvegetated areas (Crowder et al. 1998). Macrophytes provide more food resources, because there is more space available for food attachment and collection, and more refuges from predation than unvegetated areas (Crowder et al. 1998, Diehl and Kornijow 1998).

While there is little doubt in the literature regarding the importance of habitat structure (Heck and Orth 1980, Orth et al. 1984), much of this importance has been bestowed in an *a posteriori* fashion to explain results. Thus the mechanisms by which habitat structure mediates trophic interactions, and hence community structure, remain obscure (McCoy and Bell 1991). There are numerous studies that have specifically attempted to elucidate the role of habitat structure in community dynamics (e.g. Robinson 1981, Flecker and Allan 1984, Mattila 1992, Martin-Smith 1993, Bourget et al. 1994), yet we seem to be no closer to making generalisations regarding its role. For

example, Flecker and Allan (1984) manipulated the spatial refugia available to stream macroinvertebrates (by presenting loose stones and gravel compared to cemented stones and gravel), but found the variety of predatory fish in the stream had no impact on either the macroinvertebrate abundance or diversity. Mattila (1992) varied the size, number and arrangement of artificial reeds, corks and natural macrophytes, and found amphipod and isopod survival (from predation by perch and ruffe) increased with habitat complexity. Unfortunately, given the number of treatment combinations, it is not clear what constituted "complexity". While these studies provide an insight into aspects of habitat structure that may, or may not, influence community structure, they do not allow quantitative generalisations regarding its role, and highlight the two reasons for this gap in our knowledge: a lack of consensus in defining habitat structure, and therefore, a lack of consistency of its measurement (Beck 2000).

Definition of habitat structure

Habitat structure has been referred to in most systems, but is often termed differently depending on the system. For instance, on geological substrates in rivers or on rocky shores it has been referred to as "surface roughness" (Sanson et al. 1995), "substrate heterogeneity" (Menge et al. 1985) or "topographical complexity" (Walters and Wethey 1996), while on plant substrates it has been called "architecture" (Lawton 1986) or "habitat complexity" (Stoner and Lewis 1985, Floater 2001) to name just a few. These various definitions are implicitly scaled to the organisms or processes under investigation, as the terms referring to stony substrates imply two-dimensional surfaces (with three-dimensional irregularities such as fissures and crevices) upon which organisms move about, whereas the terms referring to vegetation imply three-dimensional structures within which organisms move about. Therefore, these terms are system-specific and do not permit simple comparison between studies, making it difficult to form generalisations about the role of habitat structure in community dynamics (Beck 1998).

McCoy and Bell (1991) addressed this problem by attempting to define habitat structure in such a way that researchers could be specific about what they were studying, yet would be able to compare across different systems. They identified two components of habitat structure, "complexity" and "heterogeneity", both of which are scale-dependent (e.g. Cooper et al. 1997, Crowl et al. 1997). According to their definition, "complexity" of structure refers to the absolute abundance or density of a

structure, and "heterogeneity" to the variation in structure (McCoy and Bell 1991). Earlier, Stoner and Lewis (1985) made the distinction between these two components of habitat structure when they noted that crustacean assemblages were influenced by both quantitative (density) and qualitative (species) attributes of seagrass meadows.

Unfortunately, McCoy and Bell's (1991) choice of words is unwieldy, and does not seem to have been adopted widely by other researchers. In the context of the research presented in this thesis, where macrophytes provide the habitat structure, their abundance could be referred to as "complexity" (sensu McCoy and Bell 1991); however, I prefer to refer to it as "density" which is a more descriptive term less likely to be confused with other aspects of their structure.

"Heterogeneity" (sensu McCoy and Bell 1991) would refer to differently shaped macrophytes, although here confusion can arise due to scale. "Heterogeneity" implies qualitative differences of structure within a patch or macrophyte bed, whereas at the scale of individual plants it is a less useful term. For example, when comparing a reed-like macrophyte to a macrophyte with highly dissected leaves, referring to them as having a "less" or "more heterogeneous" structure (respectively) implies that they are made up of a number of structural elements which are mostly the same (in the "less heterogeneous" structure) or mostly different in the "more heterogeneous" structure (cf. definitions of spatial heterogeneity used by Cooper et al. 1997). Clearly, these plants have completely different shapes rather then differing degrees of heterogeneity in the common-sense uses of the word; one is a simple reed with no extra structural elements like leaves or nodes, while the other has many identical structural elements. As this thesis is focussed at the scale of individual plants, I prefer to describe their shape as lying along a continuum of "structural complexity" which is a more descriptive term than McCoy and Bell's "heterogeneity". Thus a plant having many leaves which in turn are dissected into many leaflets has a "structurally complex" shape, while a plant with no leaves and a single stem (like a reed) has a "structurally simple" shape.

Measurement of habitat structure

While there are many studies of habitat structure, very few have considered the distinction between its quantitative and qualitative components, despite the fact that Stoner and Lewis (1985) and McCoy and Bell (1991) have drawn attention to this

distinction. Gilinsky (1984) assessed the role of bluegill sunfish predation and habitat structure (or spatial heterogeneity as she termed it) on macroinvertebrate community structure, but measured habitat structure as simply the presence or the absence of artificial macrophytes. Diehl (1992) assessed the impacts of fish density and habitat structure on a benthic macroinvertebrate community, but also measured habitat structure as the presence or absence of macrophytes. Other researchers have measured habitat structure using biomass (Cyr and Downing 1988a) and tend to use it as an index of surface area (Stoner and Lewis 1985, Attrill et al. 2000). However, the presence or absence of macrophyte structure, or its biomass, does not give any insight into the relative effects of macrophyte density versus structural complexity.

Many studies investigating the role of habitat structure in aquatic systems have measured it as macrophyte density (Savino and Stein 1982, Gotceitas and Colgan 1989, Nelson and Bonsdorff 1990, Swisher et al. 1998). For example, Crowder and Cooper (1982) compared the amount of macroinvertebrate prey consumed by bluegill sunfish in low, intermediate and high densities of natural macrophytes. As with Diehl's (1992) experiment, the natural vegetation was dominated by one macrophyte species but up to seven other macrophyte species were also present; thus macrophyte shape may have been confounded with macrophyte density.

Some researchers have assessed habitat structure with different shapes of structure, but each at a single density thereby failing to make the distinction between the quantitative and qualitative components of habitat structure (eg. Leber 1985, Persson and Eklov 1995). For example, Coull and Wells (1983) investigated the effectiveness of blenny preying on epifaunal communities in eight different types of structure: stones, gravel, plastic bottle brush, and five different algal species. While they measured the surface area to volume ratio (SA:V) of each structure – finding predator success was lowest in the structure with the highest SA:V ratio (*Corallina officinalis*) – this may have been confounded with the density of each structure.

In reviewing these studies, the role of habitat structure on trophic interactions and species distributions is difficult to discern because the separate components of density and shape are so often confounded. The studies of Downes et al. (1998) and Beck (2000) represent the only two investigations of habitat structure which have explicitly and experimentally distinguished the quantitative and qualitative components of habitat structure. Downes et al. (1998) manipulated the roughness, number of pits and

crevices, and macroalgae on brick surfaces in a stream, finding that macroinvertebrate abundance and species richness increased with both the type and density of structure. In contrast, they found that macroalgae responded to the surface roughness but not the number of pits and crevices. Beck (2000) compared the distribution of marine intertidal gastropods on five artificial substrates on which he varied the depth of pits and their density, finding that the type of structure consistently influenced the gastropod abundance and richness, but not all species were affected by the density of pits. These two studies clearly illustrate that both the density and the type of habitat structure can have independent effects on the fauna living on them. At present, no such comparison between these two components has been conducted on the density and shape of macrophyte structure.

To properly determine the role of macrophyte structure on its associated macroinvertebrate assemblage, both the density and shape of the plant should be quantified. While the measurement of macrophyte density is relatively straightforward, the measurement of macrophyte shape is more problematic (which may explain why macrophyte density is a far more common measure of habitat structure). Some researchers have attempted to quantify the differences in macrophyte shape by measuring the surface area to volume ratio (e.g. Coull and Wells 1983) and the interstitial space to plant volume ratio (Hacker and Steneck 1990), but this gives no indication of the shape. Others have derived indices of shape from specific plant characteristics such as the number and length of leaves and stems (e.g. Stinson and Brown 1983) or the number and height of structural components (e.g. Raizer and Amaral 2001), but these indices do not allow for comparisons between different habitats. As Beck (2000) has noted, an ideal measure or index of habitat structure should allow comparison between different habitats, give an idea of the shape of the habitat, and be logistically easy to determine. I further explain and explore the issue of quantifying macrophyte shape in Chapter 2.

Habitat structure and predator-prey interactions

Despite the great variation in the measurement of habitat structure, and the associated difficulty comparing between studies, there still appear to be some common patterns emerging. Much of this work has focussed on trophic interactions, particularly predator-prey interactions and the role of habitat structure in providing refuges.

Since the late 1970s, there has been a relatively large amount of research conducted on predator-prey interactions in vegetated habitats. A common pattern is the increase in abundance and diversity of macroinvertebrates, or marine epifauna, with increasing vegetation biomass or density (Heck and Wetstone 1977, Heck and Orth 1980, Crowder and Cooper 1982, Gilinsky 1984, Stoner and Lewis 1985, Dean and Connell 1987, Diehl 1988, Carlisle and Hawkins 1998, Kornijow and Moss 1998). Patterns of macroinvertebrate abundance on different species of macrophytes are less clear, although it has been suggested macrophytes of a more complex morphology, with more finely divided leaf structure, should support a greater abundance and diversity of macroinvertebrates (Heck and Orth 1980). Rooke (1986) compared the macroinvertebrate assemblages on eight different macrophytes and found greater abundances of macroinvertebrates on the more structurally complex macrophytes (showing more leaf dissection) than on plants with a simple morphology, but he also found individual species differ in their distributions leading to different communities on these macrophytes. Furthermore, macroinvertebrate communities on macrophytes have also been shown to vary with changing water levels (Humphries 1996), season (Chilton 1990), and predation by fish (Crowder and Cooper 1982).

While fish predators have been shown to alter macroinvertebrate community composition in vegetated systems (Heck and Crowder 1991, Crowder et al. 1998), their effectiveness at capturing prey commonly declines as habitat structure increases (Heck and Wetstone 1977, Coen et al. 1981, Heck and Thoman 1981, Savino and Stein 1982, Ryer 1988, Gotceitas and Colgan 1989, Nelson and Bonsdorff 1990, Bettoli et al. 1992, Diehl 1992, Swisher et al. 1998). So common is this pattern that Nelson (1979) postulated a "threshold" of macrophyte density beyond which the prey-capture success of fish predators markedly declines. Despite some corroborating studies (e.g. Coen et al. 1981, Heck and Thoman 1981, Gotceitas and Colgan 1989), this is now seen as too simplistic a description of the role of habitat structure (Nelson and Bonsdorff 1990).

In all these studies, macrophyte structure has been measured as density and has ignored differences in shape. Some research has used differently shaped macrophyte species, showing predator success can vary between species, but only at one density, thus confounding the quantitative and qualitative components of habitat structure (Coull and Wells 1983, Leber 1985, Persson and Eklov 1995). The only study to test

the effects of both macrophyte density and shape on predator effectiveness, shows that prey-capture success of juvenile pinfish (*Lagodon rhomboides*) was highest on the most structurally complex macrophyte (Stoner 1982). Stoner (1982) suggested prey were less concealed, and therefore at a greater risk of fish predation, by the many fine leaves of shoalgrass (*Halodule wrightii*) than broader-leaved macrophytes, a finding which has been supported by Edgar (1983).

Clearly, these patterns will depend on the behaviour of both predator and prey. For example, ambush predators such as seahorses are unaffected by increased macrophyte density (James and Heck 1994), and Savino and Stein (1982) showed largemouth bass (*Micropterus salmoides*) can shift to an ambush foraging mode with increasing macrophyte density. Larger, more active macroinvertebrates are more likely to be targeted by visual predators (Crowder and Cooper 1982, Leber 1985, Main 1987), and the risk of predation to prey will depend on their ability to perceive this risk and conceal themselves in the available habitat (Welborn and Robinson 1987, Ryer 1988, Diehl and Kornijow 1998). If the available refuge from fish predation is a limited resource, this can lead to competitive interactions between prey, potentially altering dynamics with predators. Coen et al. (1981) showed competition for refuge between two caridean shrimp prey resulted in the loser being more vulnerable to pinfish predation. However, these patterns assume the presence of a single predator – a situation unlikely in natural circumstances.

Prey can make themselves more vulnerable to one type of predator in their escape responses to a second predator (Soluk and Collins 1988, Swisher et al. 1998). The impact of multiple predators may not necessarily be obtained by simply summing the impacts of individual predators in isolation; this is known as non-additivity (Losey and Denno 1988, Soluk and Collins 1988, Van Buskirk 1988, Martin et al. 1989, Soluk 1993, Morin 1995, Crowder et al. 1997). If habitat structure can influence predator-prey interactions, then it might be able to mediate predator-predator interactions and hence the combined impacts of multiple predators. Or, the presence of habitat structure may result in the prey's responses to one predator making it more vulnerable to a second predator. In the only published study to have investigated the effects of habitat structure on multiple predators, Swisher (1998) showed the combined impact of bluegill sunfish (*Lepomis macrochirus*) and dragonfly larvae (*Erythemis simplicicollis*) was greater at low macrophyte densities, thereby exceeding

additivity because prey were more easily detected by bluegills as they escaped dragonflies. As the density of macrophytes increased, bluegills were less able to detect the prey and the combined impact of both predators became additive (Swisher et al. 1998). At the time of writing, there were no published studies investigating the effects of both macrophyte density and shape on the impact of multiple predators.

Habitat structure and food-web community dynamics

Despite the plethora of studies on habitat structure and predator-prey interactions, there are surprisingly few that have looked at the implications for community structure and food-web dynamics (Diehl and Kornijow 1998). It is only relatively recently that the focus in aquatic vegetated habitats has shifted from pairwise predator-prey interactions to multifactorial food-web dynamics (Crowder et al. 1998). This comes with the realisation that species interactions can have a multitude of direct and indirect effects throughout the rest of the food web (Crowder et al. 1998).

Perhaps the most dramatic of these indirect effects is the trophic cascade, where the effects of a top predator cascade down the separate trophic levels of a community to alter the biomass of the primary resource (Polis et al. 2000). Carpenter et al. (1987) provide a classic, exciting example of a top predator regulating the structure of a pelagic lake community, and many studies have attempted to replicate this result in a variety of systems such as rivers (Power 1990), grasslands (Beckerman et al. 1997) and vegetated dunes (Spiller and Schoener 1993). Mixed success from these studies suggests that a trophic cascade requires the conditions of defined trophic levels and strong links between these levels. The generality of trophic cascades remains controversial (Strong 1992, Pace et al. 1999), and depends to a large degree on how a trophic cascade is defined (Polis et al. 2000). Polis (1999) distinguishes between species-level (where only one or a few plant species are affected by indirect predator effects) and community-level cascades (where the biomass of the primary resource is substantially altered throughout the system), and suggests that the majority of documented trophic cascades are at the species-level and therefore constitute a set of indirect effects within a community rather than a trophic cascade. True community cascades have been shown in pelagic lake systems (Carpenter et al. 1987) and terrestrial monocultures (Halaj and Wise 2001), where the habitat in which these interactions occur is relatively uniform and the community simple, comprising a few separate trophic levels with strongly interacting members. In systems with more

structurally complex habitats, there are more complex, diverse, and weakly-interacting communities which are less amenable to being split into the discreet trophic levels necessary for a community-level trophic cascade (Strong 1992).

If fish predators are more likely to target large, active members of the prey community, or subsets of the prey community, and their effectiveness is likely to decline with increasing habitat complexity, the logical hypothesis that follows is that fish predators will have stronger, potentially cascading effects on the prey community in structurally simple rather than more structurally complex habitats (Power 1992). Power (1992) found the functional significance of fish in streams varies with substrate heterogeneity at small scales, with fish having no effects on a relatively complex gravel substrate (where there are more refuges from predation) compared to a boulder-bedrock substrate. While significant variation in fish impact has been found between vegetated and unvegetated areas in aquatic systems (Crowder and Cooper 1982), there is as yet no research comparing different morphologies of vegetation offering different levels of protection.

Research strategy and expectations

This research was organised into three parts: the measurement of habitat structure, investigating the influence of habitat structure on multiple predator effects, and investigating the indirect effects of habitat structure on community structure through its direct effects on predation. The first part is an empirical critique of the methods of quantifying habitat structure. The second part was conducted in the laboratory to determine specific effects of habitat structure on trophic interactions without the noise inherent in natural communities. The third part was a field experiment, necessary to determine the relevance of laboratory results and allow extrapolation to the instream community, and gain a greater understanding of the interconnectedness of all things (Adams 1987, Ford 2000).

The habitat structure under investigation was provided by three species of macrophytes common to the Macquarie River, Tasmania, Australia. I used plastic imitations of these plants to test the effects of plant morphology and eliminate any potentially confounding effects of senescence and secondary chemicals. This allowed for consistent and quantifiable sampling units. They represented the reed *Eleocharis*

sphacelata, the water ribbon *Triglochin procera*, and the water milfoil, or foxtail, *Myriophyllum variifolium* (see Chapter 2, Figure 1, and Chapter 4 for details).

Chapter 2 compares different indices of habitat structure as measured for each macrophyte, to determine which best quantifies plant shape in a manner relevant to the macroinvertebrates using it. I expected the reed *Eleocharis* to have the most simple structure, *Myriophyllum* to be the most structurally complex macrophyte with its high degree of leaf-dissectedness, and the tufted, strap-like *Triglochin procera* to be intermediate between the two.

Chapter 3 describes a laboratory experiment testing the separate components of macrophyte shape and density on multiple predator effects. The prey-capture success of two predators (damselfly larvae and pygmy perch) was tested in each macrophyte, at each of five densities. Given damselfly larvae are potential prey items of, and competitors with, pygmy perch, both their separate and combined impacts were assessed, as I expected damselflies to capture less prey in the presence of pygmy perch. I expected more prey to be consumed by each predator at low macrophyte densities and in the structurally simple macrophyte. I also expected the combined impact of both predators to be positively non-additive at lower densities and in the structurally simple macrophyte, i.e. more prey would be consumed than expected by adding the individual effects of each predator alone.

Based on the results from the laboratory experiment, Chapters 4 and 5 describe a multifactorial field experiment investigating the influence of macrophyte shape on the direct and indirect effects of fish predators on the macrophyte-associated macroinvertebrate and periphyton communities. If pygmy perch had strong effects, I expected to find their direct and indirect effects greater in the structurally simple macrophyte, *Eleocharis*, where theoretically, there are no refuges and fish can capture more prey and potentially have cascading effects on lower trophic levels (consumers and producers). Correspondingly, I expected little, if any, effects of fish in the most structurally complex macrophyte, *Myriophyllum*, due to the presence of more refuges.

Chapter 6 provides a synthesis and discussion of the results in the context of habitat structure and food-web ecology.

Chapter 2: The Quantification of Habitat Structure.

Introduction

While there is little doubt in the literature regarding the importance of habitat complexity, the mechanisms by which it influences invertebrate communities, and the trophic interactions therein, remain obscure (McCoy and Bell 1991). One reason for this is the lack of consistency in the measurement of habitat structure (Beck 2000, Downes et al. 2000). Habitat structure incorporates both the type of structure and the density of structure. However, even when the same aspect of habitat structure is defined, it is often measured in a manner that is specific to the system under investigation: for example, the number of different plant structures (e.g. Stinson and Brown 1983) or the degree of branching in a plant (e.g. Edgar 1983). This does not allow for comparisons between systems and has therefore made it difficult to ascertain the effects of habitat structure on invertebrate distributions, and to identify the aspects of structure to which they are responding (Beck 1998, Bartholomew et al. 2000, Beck 2000).

A useful index of habitat structure should be relatively simple to measure, and be independent of specific structural components in order to be used within and between habitats to allow meaningful comparisons between studies (Beck 1998). Beck (1998) addressed this issue by using four different indices to measure the structural complexity of rocky substrates between habitats and compared their effectiveness as they related to the distribution of gastropods; he found the fractal dimension of the substrate was most highly correlated with gastropod distribution. Carleton and Sammarco (1987) measured the surface irregularity of plating coral substrata with five different geomorphological measures. They found that while vector dispersion was the best descriptor of surface irregularity, no single index stood out as a good predictor of coral settlement, although this may have been partly due to over 90% of corals settling underneath, rather than on, the experimental plates. To date, no such study comparing several different measures of habitat structure has been conducted in a system where three-dimensional freshwater macrophytes, rather than two-dimensional surfaces, provide the habitat structure.

Ecologists know that the structure of aquatic plants influences macroinvertebrate (and epifaunal) distributions, because differently shaped macrophytes support different

macroinvertebrate communities (Rooke 1986, Cyr and Downing 1988b, Chilton 1990, Humphries 1996). However, invertebrate abundance and diversity have often been related to broad habitat descriptors such as plant presence or biomass (e.g. Dvorak and Best 1982, Stoner and Lewis 1985, Cyr and Downing 1988a) which do not distinguish the specific aspects of plant morphology to which invertebrates may be responding, and so the predictive power of these descriptors is low (Lawton 1986, Hacker and Steneck 1990, Gee and Warwick 1994b). Furthermore, macroinvertebrates are more likely to be making choices between habitats at the scale of individual plants or plant parts (Davenport et al. 1996), and therefore may not respond to coarse-scale measures such as plant presence and biomass. If plant shape influences the distribution of macroinvertebrates and is to be used as a tool describing that distribution, illustrating its role as a component of habitat structure, then it should be quantified at the relevant scale (McCoy and Bell 1991, Beck 1998).

The structural indices that have been used to relate macrophyte structure to macroinvertebrate distributions can be grouped into three categories: those that measure the physical attributes of the plant, those that measure the amount of interstitial space associated with the physical structure of the plant, and the fractal dimension of the plant. Each of these categories is considered in turn.

There is a variety of indices that have been used to describe the structural complexity of macrophytes. For example, the degree of folding (which measures the amount of folds on a surface and is determined with integral calculus) has been shown to be a good indicator of amphipod distributions on artificial substrates (Jacobi and Langevin 1996). However, I chose not to use this measure here as firstly, it was determined from artificial substrata very dissimilar from natural substrata, and secondly, it was considered to provide no new information about a habitat compared to other measures because it required too many simplifying assumptions (S. Wotherspoon, School of Mathematics and Physics, University of Tasmania, *pers. comm.*). The indices that are most often used to describe the physical attributes of plant structure are surface area and plant volume (e.g. Coull and Wells 1983, Edgar 1983), although plant biomass has also been used as an index of surface area (e.g. Stoner and Lewis 1985, Attrill et al. 2000). Macrophytes with a more structurally complex morphology (e.g. those with a higher degree of leaf-dissection) are hypothesised to have a greater surface area, and a relatively lower plant volume, and therefore support greater macroinvertebrate

abundances (Heck and Wetstone 1977, Heck and Orth 1980). However, Sher-Kaul et al. (1995) measured the surface area of six macrophytes, while holding biomass constant, and found that plants with a dissected morphology do not necessarily offer the largest surface area per unit biomass. The indices of surface area and plant volume were used here to test this hypothesis and to determine if surface area in particular was a sufficiently good descriptor of macrophyte structure as it relates to the distribution of macroinvertebrates between plant types.

Some researchers have noted that where aquatic plants provide the habitat structure, invertebrates respond to the interstitial space as well as the physical structure (Hacker and Steneck 1990, Schmid 2000). Hacker and Steneck (1990) compared the interstitial volumes of four macroalgae (two of which were artificial), and found that plants with an intermediate proportions of interstitial volume (relative to plant volume) provide optimal habitats for the amphipod *Gamarellus angulosus* as there was enough space to accommodate their body volume, yet enough substrate on which to cling. However, measuring the overall interstitial volume of a plant gives no indication of its shape and how it may be split or partitioned among macrophyte structural elements, and assumes all the interstitial space is equally available to the epifauna. Dibble et al. (1996) developed an index of interstitial space (ISI) which took into account the structural complexity of macrophytes, yet was an independent measure of a plant's structure. The ISI measures the frequency and size of interstitial spaces between structural components along vertical and horizontal axes, such that a greater number of smaller gaps indicates a more structurally complex plant shape. Bartholomew et al. (2000) went one step further and incorporated the potential role of macrophyte structure as a prey refuge in their structural index. More structurally complex plants are hypothesised to provide more prey refuges because the increased structural complexity impedes the ability of fish predators to find and capture prey, thus there are more spaces which are safe from predation (Heck and Crowder 1991). Bartholomew et al. (2000) therefore divided the average interstitial space size, *Sp*, by the predator size, *Pr*, to develop an index, Sp/Pr, which measured the amount of interstitial space for prey where they were safe from predation. Obviously this index would vary with the size of the predator under investigation, but it is dimensionless and gives an indication of the amount of available interstitial space (Bartholomew et al. 2000). I used three indices to measure the amount of space associated with

macrophyte structure: the indices of Dibble et al. (1996) and Bartholomew et al. (2000), together with a third index I developed which measured the absolute amount of space unavailable to a fish predator but available to macroinvertebrate prey, the fish-free volume (FFV). If macroinvertebrates are tracking refuge space, and a more complex plant shape provides more refuge space, then the absolute amount of FFV should relate to the distribution of macroinvertebrates between plants.

Many natural objects have irregular surfaces and cannot be sufficiently described by Euclidean geometry (Sugihara and May 1990). Fractal geometry describes the measurement of an object where that measurement depends on scale, such that where a smaller "ruler" is used, the measurement picks up the addition of detail at greater magnification (Schmid 2000). Thus it is independent of the nature of the habitat and is related to the scale at which the habitat is viewed (Lawton 1986, Gee and Warwick 1994b, Schmid 2000). Mathematical fractals display scale-invariance, or self-similarity, in that they show the same amount of detail or roughness at all scales. By contrast, natural fractals differ because natural structures are usually truncated at certain scales (Avnir et al. 1998, Schmid 2000).

The fractal dimension (D) of an object is estimated from the surface of that object, and as it increases, indicates the surface becomes more convoluted, departing from a two-dimensional surface and approaching a three-dimensional object. D has been shown to be a relatively good indicator of structural complexity, with more complex habitats having a higher D and supporting more animals, for both rocky substrates (Beck 1998, 2000, Schmid 2000) and plants (Morse et al. 1985, Shorrocks et al. 1991, Williamson and Lawton 1991, Jeffries 1993, Gee and Warwick 1994a). If the structural complexity of a habitat is scale-dependent, then measuring the fractal dimension at different scales should prove useful. If there is no difference in D between scales, then the dimensionality is the same; the plant is self-similar and truly fractal. If there are differences in D between different scales, then the dimensionality depends on scale suggesting small animals perceive the habitat differently from larger animals and thus the plant is not fractal. Therefore, I measured the fractal dimension of the macrophytes studied here, at each of four scales, to determine if it could describe the macrophyte structural complexity at different scales, and if it related to the distribution of macroinvertebrates.

Given that differently shaped macrophytes support different macroinvertebrate communities, this research was motivated to find an index of macrophyte structural complexity that quantified their shape (thereby allowing the comparison of different macrophyte habitats between systems) and consistently related to the distribution of associated macroinvertebrate communities. I compared three differently shaped macrophytes, *Eleocharis sphacelata*, *Triglochin procera*, and *Myriophyllum variifolium* (Figure 1), which could immediately and intuitively be ranked as structurally simple to structurally complex respectively. *Myriophyllum* can be perceived to have a complex structure because of its finely divided leaf structure, giving it many structural components. Whereas *Eleocharis* can be perceived to be structurally simple because it is a stem with no projections or finer-scale structure. *Triglochin*, with its numerous long flat leaves, can be perceived as having a structure of intermediate complexity. I measured these macrophytes with a range of indices of habitat structure that measured different attributes of the habitat: the physical structure (surface area and plant volume), the interstitial space (the ISI, Sp/Pr and FFV), and the fractal dimension. I assessed the strength of association between these indices and macroinvertebrate abundance and taxon richness to determine if any were consistently better at explaining the macroinvertebrate distribution on these plants. This would allow me to evaluate their usefulness as a quantitative measure of macrophyte structure to compare within and between systems.

Methods

The invertebrate data for this chapter were obtained from a field experiment conducted in the Macquarie River, Tasmania (147°28′E, 41°57′S) and explained in more detail in Chapter 4. The experiment and sampling protocol were designed to assess the effects of macrophyte structure and fish predation on the macroinvertebrate community, but as this current chapter is focussed on the measurement of macrophyte structure, the effects of fish predation are addressed elsewhere (Chapters 4 and 5).

The macrophytes

Artificial plants were used to prevent changes in plant shape over time, and to ensure consistent and quantifiable sampling units. They also prevented any effects of secondary plant chemicals on the periphyton quality, thereby ensuring macroinvertebrates were responding to differences in macrophyte shape only in their

distribution. They represented analogues of three macrophytes common in the Macquarie River; the spikerush *Eleocharis sphacelata*, the water ribbon *Triglochin procera* and the water milfoil *Myriophyllum variifolium* (Figure 1). (Macrophytes and their artificial imitations are hereafter referred to by their genus name only.) *Eleocharis* is comprised of cylindrical stems with no leaves and was designated, *a priori*, as structurally "simple" whereas *Myriophyllum* has many whorls of finely-dissected leaves and was considered structurally "complex". *Triglochin* has long strap-like leaves arising from a tuft and was considered "intermediate" in its structure.

The simple macrophyte was constructed of green electrical conduit (7 mm diameter), cut into 280 mm lengths and sealed with neutral silicon to resemble stems of *Eleocharis*. Four stems comprised a sampling unit (or "plant") for measurements of habitat structure. Lengths of green packing strap (12 mm width) were used to represent *Triglochin*. A plant consisted of 18 lengths of packing strap, 3 of each length 100, 130, 170, 200, 260, and 280 mm, held together at the base with epoxy-resin to form a tuft. Commercially produced plastic aquarium plants (Tetra Secondnature©) were used to represent *Myriophyllum*. A plant comprised four stems of whorled leaves with a high degree of leaf-dissectedness to specifically imitate *Myriophyllum* spp. (Tetra Secondnature, Blacksburg, Virginia, USA., *pers. comm.*).

The macrophytes were given ten weeks colonisation by periphyton and macroinvertebrates, after which 36 plants of each type were sampled on each of seven sampling occasions over a 30-week period. The plants and their associated fauna were scooped out with a 250 μm mesh net, preserved with 5% formalin and returned to the laboratory. The macrophytes were cleaned and the macroinvertebrates were picked out of the periphyton and identified to genus where possible to obtain the total macroinvertebrate abundance and taxon richness.

Indices of structural complexity

Seven indices of habitat structure were measured for each plant: the surface area and plant volume described physical attributes of macrophyte structure; the fish-free volume, interstitial space index, and the interstitial space size / predator size index described the interstitial spatial attributes of macrophyte structure; and the fractal dimension. Each index is explained in turn and, unless otherwise mentioned,

measurements were not replicated as the artificial imitations of each macrophyte were identical in structure.

Surface area (SA) – I initially attempted to measure surface area using the detergent method of Harrod and Hall (1962); however, arbitrary decisions regarding the concentration of detergent in the solution, and a relatively high standard error in the final weights (up to 33% of the mean), led me to abandon this method as unreliable. Thus, surface area was measured geometrically with vernier calipers. The surface area of *Eleocharis* was obtained with the formula $2r\pi h$ (i.e. treating each stem as a cylinder) where r denotes the radius and h the height, and multiplied by 4 (the number of stems making up a "plant"). The surface area of the individual "leaves" of *Triglochin* were calculated using the formula $2lw$, where l is the length and w the width of each leaf (multiplied by 2 to account for both sides of the leaf), and then added to give the surface area of the entire plant. The depth of each *Triglochin* leaf was 1 mm and therefore would not have changed the final surface area value.

Myriophyllum consisted of two large stems and two small stems, each with leaves and leaflets of 1 mm depth and width, but slightly different lengths. Leaflets varied in length from 5-9 mm on the small stems and 6-16 mm on the large stems, with the smaller leaflets at the leaf apices. Ten leaflets from the same position on ten leaves were measured to obtain the average leaflet length at that position, and the surface area of a leaflet calculated with the formula $2lw$. Surface area values for the leaflets were added to give the surface area of a leaf, and then multiplied over the number of leaves per stem. This was repeated for leaves from both large and small stems, and the surface area of the cylindrical main stems was also calculated (using $2r\pi h$), and all values added to give the total surface area of the plant.

Plant volume (PV) – The plant volume was determined geometrically in the same manner as surface area and confirmed by the amount of water each plant displaced.

Fish-free volume (FFV) – The fish-free volume (FFV) was also determined geometrically and was effectively a measurement of the amount of space available to macroinvertebrates but unavailable to pygmy perch, *Nannoperca australis*, the fish predator used in the laboratory and field experiments. The minimum size of pygmy perch used in the experiments was 30 mm with a gill-to-gill width of 5 mm (see Chapters 3 and 4). Assuming pygmy perch could only access spaces they could fit

their head into, any space under 5 mm wide was considered to be FFV. The low complexity macrophyte, *Eleocharis*, had no FFV, and this was confirmed by direct observations of pygmy perch under laboratory conditions.

The FFV in *Triglochin* comprised the wedge-shaped spaces between leaves at the base of the tuft, each of which was measured geometrically using the formula $lwd/2$ which measured the length (l), width (w) and depth (d) of an oblong and divided by 2 as if the oblong was sliced diagonally to produced a wedge (Figure 2a). The volumes were added to provide the FFV per plant, and calculated for five plants to obtain an average FFV per *Triglochin* plant.

The four stems comprising *Myriophyllum* (including leaves) were treated as cylinders (Figure 2b); the volume of each "cylinder" was calculated with the formula $\pi r^2 h$, where r denotes the radius of the cylinder (the stem and the leaves) and h the height, and added to provide the overall volume. The PV was then subtracted from this value to give the FFV for *Myriophyllum*.

Interstitial Space Index (ISI) – The interstitial space index was based on the index of habitat structure developed by Dibble (1996) and is calculated with the formula $I_{hv} = (f_h/l_h) + (f_v/l_v)$, where f_h is the mean number of gaps between structural elements along horizontal axes, l_h is the mean length of those gaps, f_v is the mean number of gaps along vertical axes, and l_v is the mean length of the vertical axis gaps. Gaps along 3 horizontal and 3 vertical axes (each at least 3 cm apart) were counted and measured using a ruler against 4 unmagnified photographs of each macrophyte. A greater I_{hv} value indicates a higher frequency of gaps and a smaller gap length, and thereby indicates a greater structural complexity.

Interstitial Space Size/Predator Size (Sp/Pr) – This dimensionless measure was developed by Bartholomew (2000) to determine the extent to which structure interferes with a fish predator's ability to move through the habitat. The gaps between structural elements were measured along 3 horizontal axes (at least 3 cm apart) on 4 unmagnified photographs of the plants and averaged, giving the mean interstitial space size. This value was then divided by the predator size measured as the minimum width across the gills (5 mm, as for the FFV) to give the Sp/Pr index. Only gaps along the horizontal axis were measured as this is perpendicular to the predator's normal orientation and direction of motion and represents the plane in which the predator is

most likely to be impeded by macrophyte structure (Bartholomew et al. 2000). Values below 1 indicate the predator's movement through the habitat is impeded by structural elements, implying a greater structural complexity, and values above 1 indicate the interstitial space is large enough to allow free movement through the habitat.

Fractal Dimension (D) – The fractal dimension of each macrophyte was estimated, at each of 4 scales, based on the method of Morse (1985) and Jeffries (1993) which used the Kolmogorov or box-counting dimension (Schmid 2000). This dimension is based in the log-log relation between the side-length of grid squares and the edge of the plant. The macrophytes were suspended upside down to recreate their position in the water column. Four black-and-white photographs were taken, with very shallow depths of field (5 mm), at 5 mm intervals "through" the plants, at each scale. Scale 1 was the smallest scale at 6.5 × magnification, Scale 2 was at 5 × magnification, Scale 3 at 2.5 × magnification, and Scale 4 was at 1 × magnification (i.e. unmagnified) and thereby the largest scale. This method yielded 4 two-dimensional "slices" of each plant at each scale. Grid squares of 100 mm, partitioned into 2^n squares (where n was 2, 4, 6, 8 or 10, and the square size was therefore 50, 25, 12.5, 6.25 or 3.125 mm respectively), were progressively placed over each photograph, and the number of squares (per grid) in which the plant was in focus was counted. The log of this number was plotted against the log of the number of squares along the respective grid edge (2, 4, 8,16, or 32 squares respectively), and the slope of the resulting regression line was D, the fractal dimension (also called the Kolmogorov dimension). A separate D was calculated for each scale to determine how D varied with scale as I did not assume self-similarity in D.

Data Analysis

Each index of habitat structure was obtained from the same three plant types. Principal Components Analysis (PCA) was conducted on the structural indices to assess their interrelationships; the indices which are more highly correlated with each other will map onto the same component and would allow me to assess whether there was any redundancy amongst them. I used orthogonal rotation on the loadings which helps interpretation by maximising the high correlations and minimising the low correlations of each index with the principal components (Tabachnick and Fidell 2001). By plotting each macrophyte in principal component space I could judge how

they differed from each other, and which index, or combination of indices, best described them.

Single factor analysis of variance (ANOVA) was conducted on each of the total number of macroinvertebrates and the total number of taxa to determine if they varied with macrophyte shape, and planned pairwise comparisons (*Myriophyllum* v. *Triglochin*, and *Triglochin* v. *Eleocharis*) were also conducted.

If the macroinvertebrate assemblage is affected by the structural complexity of the habitat, there should be significant correlations between the structural indices and macroinvertebrate abundance and taxon richness. Furthermore, the index with the most significant correlations should provide the best measure of habitat structure that most strongly affect macroinvertebrates (Beck 1998). Each structural index was correlated with macroinvertebrate abundance and taxon richness, and site was treated as independent for this analysis, so there were six correlations for each structural index. Each site was sampled a different number of times which meant 3 sites had 60 replicate samples, and 3 had 24 replicate samples. The time factor was not considered independent here because the abundance and taxon richness of the macroinvertebrate assemblage at any one time was likely to depend on the abundance and richness at the previous time (Underwood and Anderson 1994). Due to the large number of tests performed, the level of significance was reduced to $p < 0.01$ to prevent the occurrence of Type I errors. Data were log-transformed where necessary, to meet assumptions of normality, and all analyses were performed using SYSTAT Version 9 (Wilkinson 1999).

Results

Each complexity index differed between macrophytes except for D_3 (the fractal dimension at Scale 3) which was the same for *Eleocharis* and *Triglochin* (Table 1). This indicates each index was capable of measuring differences in structure between the macrophytes. The fractal dimension did not increase linearly with scale, as Scale 2 (at 5 × magnification) had the lowest D (Figure 3), which indicates a lack of self-similarity. Significant differences between the fractal dimension of each macrophyte only appeared at the largest scale (Scale 4, no magnification) where *Myriophyllum* had a greater fractal dimension than either *Triglochin* or *Eleocharis*. The ISI value for *Myriophyllum* was nearly 40 times greater than that for *Eleocharis* or *Triglochin*,

indicating many small interstitial spaces, and therefore many structural elements, on this plant. An Sp/Pr value under 1.0 for *Myriophyllum* suggested these structural elements potentially impede the mobility of pygmy perch.

The Principal Components Analysis reduced the structural indices to just two components which explained 100% of the variation (Table 2) and indicates the indices are highly correlated with each other. This was expected given the indices were derived from the same three plant shapes. Component 1 is most strongly correlated with the indices measuring the interstitial space of the plants (ISI, FFV and Sp/Pr) and the fractal dimension at Scales 3 and 4 (at 2.5 and 1 × magnification respectively), while Component 2 is most strongly correlated with SA, PV and the fractal dimension at Scales 1 and 2 (6.5 and 5 × magnification respectively). The plot of each macrophyte in Principal Component space (Figure 4) shows that *Myriophyllum* loads highly on PC1 and low on PC2, indicating that it has greater amount of interstitial space and fish-free volume, a lower Sp/Pr index, and a higher fractal dimension at Scales 3 and 4 relative to the other macrophytes. By contrast, *Eleocharis* loads highly on PC2 and low on PC1, indicating it affords little refuge from predation and is best described by surface area, plant volume and a low degree of surface rugosity at finer scales.

The abundance of macroinvertebrates significantly varied according to macrophyte shape ($F_{2,246} = 49.733$, $p<0.001$; Figure 5a). *Myriophyllum* supported 48% more macroinvertebrates than *Triglochin* ($F_{1,246} = 30.063$, $p<0.001$), which supported 38% more macroinvertebrates than *Eleocharis* ($F_{1,246} = 19.594$, $p<0.001$). Likewise, the taxon richness was also significantly affected by macrophyte shape ($F_{2,246} = 53.238$, $p<0.001$; Figure 5b). There were 13% more taxa on *Myriophyllum* than *Triglochin* ($F_{1,246} = 7.766$, $p<0.006$), and 29% more taxa on *Triglochin* than *Eleocharis* ($F_{1,246} = 51.439$, $p<0.001$).

The structural indices D_2 and Sp/Pr had the highest number of significant correlations with macroinvertebrate abundance and taxon richness, although SA, PV and D_1 also had six significant correlations with taxon richness (Table 3). D_2 and Sp/Pr can therefore be considered to best measure the habitat structure as it influences macroinvertebrate abundance, while the intercorrelated indices of Component 2 best measure the habitat structure as it influences taxon richness. D_4 had the lowest number of significant correlations with both macroinvertebrate abundance and

richness, and thus did not describe the habitat in a way which related to the macroinvertebrate assemblage despite being highly correlated with the Component 1 indices.

Discussion

This research was designed to determine how best to quantify macrophyte shape, and to elucidate the aspects of macrophyte structure which influence the distribution of an instream macroinvertebrate assemblage. I measured three morphologically different macrophytes with a variety of indices to determine which index was most strongly related to the distribution of associated macroinvertebrates and may therefore be used as a predictive tool.

Indices of structural complexity

I measured each plant with indices relating to their physical structure (surface area and plant volume), their fractal dimension, and their interstitial space attributes (ISI, FFV and Sp/Pr) to determine which best described the habitat as it related to the distribution of the macroinvertebrate community. Each index (apart from D_2) was capable of separating the macrophytes, and the values of each mirrored my *a priori* classification of structural complexity. For example, *Myriophyllum*, the macrophyte I considered most structurally complex, consistently had a higher fractal dimension indicating a higher degree of surface rugosity, although contrary to expectations, its surface area was very similar to that of *Triglochin*, which supports the view that a dissected morphology does not necessarily provide more surface area (Cyr and Downing 1988b, Sher-Kaul et al. 1995). *Eleocharis*, the plant I considered least structurally complex, had the highest Sp/Pr value which indicated it had the largest gaps and thus the least amount of structure likely to impede the mobility of pygmy perch. D_2 was the same for both *Eleocharis* and *Triglochin*, suggesting these plants had the same degree of surface rugosity at 5 × magnification.

There was a high degree of intercorrelation between the indices, as shown in the principal components analysis, which summarised them into just two components. When the macrophytes were plotted in principal components space, I found *Myriophyllum* was best described by PC1, which comprised the indices ISI, FFV, Sp/Pr, $D3$ and $D4$. The fractal dimension of *Myriophyllum* increased as spatial scale became coarser, which indicates that at larger scales, the surface is perceived to have

more protrusions into three-dimensional space and larger animals would perceive it differently from smaller animals. The interstitial space is the space between structural elements, therefore, should be associated with a greater degree of surface rugosity. Given the highly dissected leaf structure of *Myriophyllum*, with many structural elements, it makes sense that the combination of these indices best described its shape.

Similarly, it made sense that *Eleocharis* separated strongly from *Myriophyllum* on PC2, which comprised the indices SA, PV, *D*1 and *D*2. The surface area and plant volume describe the physical structure without any indication of structural elements, and the lower fractal dimension at finer scales indicates a relatively smooth surface. Thus, the combination of these indices best described a smooth surface and shape lacking finer structural elements, i.e. *Eleocharis*.

Triglochin was intermediate between *Eleocharis* and *Myriophyllum*. It differed from these two macrophytes in that its fractal dimension did not vary with scale, which indicated a degree of self-similarity over the scales measured. Unlike the relatively smooth surface of *Eleocharis*, the packing strap used to construct *Triglochin* had a rough dimpled surface; this provided a degree of surface rugosity at the scale of the leaf surface which shared a similar fractal dimension to the whole plant. However, *Triglochin* did provide some refuge for macroinvertebrates from pygmy perch predation, and had a similar surface area to *Myriophyllum*, which explains its closer affinity to *Myriophyllum* than *Eleocharis* in principal component space.

Although the three macrophytes are separated on PC1, they are more clearly separated by considering both principal components. This suggests that there are two suites of variables with a high degree of intercorrelation (and redundancy) within each suite: one suite measuring the refuge "volumes" and rugosity at coarser spatial scale, the other measuring "whole plant" attributes (SA and PV) and rugosity at finer spatial scales. This suggests firstly, many measures of structural complexity are likely to be highly correlated. Secondly, as few as two carefully selected, relatively independent measures would suffice to quantitatively describe the structural complexity of macrophytes. While he did not assess the collinearity between four indices of habitat structure on a rocky shore, Beck (2000) also concluded that multiple indices should be used to incorporate the many correlated features of habitat structure.

Macroinvertebrate distribution and indices of structural complexity

The distribution of macroinvertebrates varied with macrophyte shape, as predicted, and the Sp/Pr and fractal dimension at Scale 2 best described the habitat structure that influenced this distribution.

The greater abundance and richness of macroinvertebrates on *Myriophyllum* than *Triglochin*, and on *Triglochin* than *Eleocharis*, supports my *a priori* classification of each macrophyte and also the many studies showing macroinvertebrates, marine epifauna and terrestrial arthropods are more abundant and diverse in more structurally complex habitats (Robinson 1981, Orth et al. 1984, Heck and Crowder 1991, Schneider and Mann 1991, Jeffries 1993, Gee and Warwick 1994a, Jacobi and Langevin 1996, Crowder et al. 1998, Diehl and Kornijow 1998, Raizer and Amaral 2001). For example, Rooke (1986) compared the macroinvertebrate fauna on eight different macrophyte species, and found consistently greater abundances on the highly dissected leaf forms, a *Myriophyllum* and *Ranunculus* species. Raizer and Amaral (2001) assessed the spider communities on the emergent parts of aquatic macrophytes, and found the most abundant and diverse community on the most structurally complex plant, *Eichornia azurea*. I explore this pattern further in Chapter 4, and here I will discuss the relationship between the structural indices and the distribution of macroinvertebrates between macrophytes.

The Sp/Pr was one of the most highly correlated indices with macroinvertebrate distribution as both total macroinvertebrate abundance and taxon richness were greater in the habitat with a lower Sp/Pr, *Myriophyllum*. This supports the findings of Hacker and Steneck (1990) who investigated the distribution of amphipods on marine macroalgae, and found that the interstitial space associated with the plant morphology was as important as the structure itself. The Sp/Pr index was designed to measure the degree to which structure impedes the movement of a fish predator, the implication being that as the average space size decreases, the more the predator's movement becomes hindered by structure (Bartholomew et al. 2000). While Bartholomew et al. (2000) found it to be a good indicator of amphipod survival, it may also be a measure of the amount of refuge available to the entire prey community rather than a single prey taxon. Despite pygmy perch having only weak effects on subsets of the macroinvertebrate community (Chapter 4), their macroinvertebrate prey may have been able to assess the risk of predation and accordingly select the "safest" habitat

with the greatest amount of refuge space (Bell and Westoby 1986), which in this case corresponds to *Myriophyllum*. *Myriophyllum* also supported a greater biomass of periphyton (Chapter 5) and macroinvertebrates may have been tracking food resources rather than refuge availability. However, Miller (2002) and Schneider and Mann (1991) have shown that invertebrates can still select structurally complex macrophytes even in the absence of periphyton, which suggests refuge availability is an important factor in their distribution.

The Sp/Pr, ISI and FFV indices all measured the space associated with each macrophyte, yet the ISI and FFV indices were not as correlated with the macroinvertebrate distribution because they each lacked information that was incorporated in the Sp/Pr index. Unlike the Sp/Pr, the FFV index does not specifically incorporate information about the number of structural elements, which in the Sp/Pr is inferred from the size of interstitial spaces (the smaller the average space size, the more frequently it is split by structural elements). The FFV is based on the assumption that space is unavailable to pygmy perch because parts of the structure are impeding their access, and therefore a greater overall amount of fish-free volume implies a more structurally complex habitat. It appears macroinvertebrates are responding to the refuge space associated with the actual structure of the macrophyte, rather than the refuge space *per se*. This agrees with the PCA results, where Component 1 (ISI, FFV, Sp/Pr, D_3 and D_4) described the interstitial space and the surface rugosity of macrophyte shape, and thus best described *Myriophyllum*, the plant with the greatest macroinvertebrate abundance and richness.

While the ISI incorporated habitat structure, measuring the size and frequency of interstitial gaps associated with macrophyte shape, it did not incorporate any information about the predator. Thus, unlike the Sp/Pr, it was not a measure of refuge space. Sanson et al. (1995) developed a method to describe the surface structure of stone substrates, which modelled the amount of refuge space available to prey at risk from predators of various sizes, illustrating that refuge availability can be much more accurately determined by scaling space to the size of relevant predators. By scaling the average interstitial gap size to the predator of interest, in this case pygmy perch, the Sp/Pr specifically incorporated information about how the habitat was used (Bartholomew et al. 2000). It thus proved one of the best descriptors of the habitat structure in a way that was relevant to the macroinvertebrate distribution.

The other good descriptor of habitat structure for macroinvertebrate abundance and taxon richness was D_2, the fractal dimension at Scale 2 (5 × magnification). Fractal dimension has been shown to be a relatively good indicator of habitat structural complexity, with more complex habitats having a higher D and supporting more animals (Morse et al. 1985, Shorrocks et al. 1991, Williamson and Lawton 1991, Jeffries 1993, Gee and Warwick 1994a, Beck 1998, Schmid 2000). The fractal dimension is generally seen as an effective method of describing habitat structure because it is independent of the nature of the habitat and is related to the scale at which the habitat is viewed (Morse et al. 1985, Lawton 1986, Gee and Warwick 1994b, Schmid 2000). This may explain why the fractal dimension at Scale 4 was by far the worst descriptor of macrophyte structure in relation to the macroinvertebrate distribution, because it is at a scale too "coarse" to be perceived by macrophyte-associated macroinvertebrates. If macroinvertebrates are responding to their immediate vicinity, then they are not likely to perceive structural complexity at the scale of the entire plant (Davenport et al. 1996).

Against expectations, surface area was one of the worst descriptors of habitat structure as it related to macroinvertebrate abundance. This contrasts with the many studies which have shown that as plant surface area increases, so does animal abundance (Dvorak and Best 1982, Stoner and Lewis 1985, Parsons and Matthews 1995, Parker et al. 2001). Much of the surface area may not have been directly available to macroinverterbates because it left them vulnerable to predation from pygmy perch. In *Eleocharis* there was no surface area available as a refuge from predation and in *Triglochin*, "safe" surface area was only available at the base of the tuft, in the spaces between leaves. In *Myriophyllum*, however, the entire inside surfaces of the leaves (i.e. half the total surface area) provided a surface free from the risk of predation. The absolute surface area may therefore not be a good representation of usable habitat.

Other researchers have also found no relationship between macroinvertebrate density and macrophyte surface area, and have suggested the fauna are responding to different aspects of structure (Brown et al. 1988). Jeffries (1993) constructed artificial macrophytes with varying structural complexities, measured by the fractal dimension, but with the same surface area. He found higher macroinvertebrate abundance and richness as the fractal dimension increased, thereby illustrating that structural

complexity can be separated from surface area and can influence the distribution of macroinvertebrates.

However, surface area was one of the best descriptors of macroinvertebrate taxon richness; the number of species increased with the amount of surface area. This supports the positive relationship between surface area and species richness (Dean and Connell 1987), and suggests that surface area may indeed be a good predictor of macroinvertebrate diversity. Parker et al. (2001) also found species diversity was related to surface area, but after controlling for the surface area of sea grasses and seaweeds, found plant architecture also influenced epifaunal community structure. In an experiment designed to test the mechanisms for generating this relationship, Douglas and Lake (1994) manipulated the number of grooves on bricks, while holding surface area constant, and found that the species richness of stream macroinvertebrates was best explained by habitat diversity (as represented by grooves). If two habitats have similar surface areas, but the greater structural complexity of one habitat creates a greater variety of resources, then that habitat tends to support a greater taxon richness (O'Connor 1991, Douglas and Lake 1994, Schmid 2000). My results also showed that macroinvertebrate richness was not solely dependent on surface area.

I found five of the nine structural indices I measured to be highly correlated with taxon richness; SA, PV, D_1, D_2, and Sp/Pr. Interestingly, the first four of these indices were highly correlated with Component 1 in the PCA, which suggests macroinvertebrate taxon richness is strongly related to the "whole plant" attributes of surface area and plant volume, and rugosity at finer spatial scales. These attributes best described *Eleocharis*, thus taxon richness should be greatest on this macrophyte. However, the macroinvertebrate taxon richness was also strongly related to Sp/Pr, suggesting it is also affected by refuge availability, and there was no refuge on *Eleocharis*. *Myriophyllum* had the greatest amount of refuge and it also supported a greater biomass of periphyton which may have resulted in it supporting a greater taxon richness. Yet, in offering artificial macrophytes with and without periphyton, it has been shown that many species choose a plant by its shape regardless of the presence of periphyton (Schneider and Mann 1991, Miller 2002). It appears the taxon richness of the macroinvertebrate community is responding to a variety of aspects of macrophyte structure.

Reliability of structural indices

I found a combination of indices best described the plant shapes, and also related to macroinvertebrate abundance and richness, but not the same combinations. This is partly due to the collinearity between these indices, but it may also be due to the methods of obtaining them.

Apart from SA, PV and FFV, each structural index was derived from a two-dimensional representation of the macrophytes rather than the three-dimensional structures they are. While Beck (1998) found D to be the best descriptor of gastropod distribution, he measured the D of a rocky shore, a two-dimensional surface where it is relatively simple to obtain a profile of surface topography, and therefore the method is reliable. Attempts to apply measures of two-dimensional structure to the three-dimensional structure of plants have proven problematic and generally involve reducing the plant to a two-dimensional structure; for example, by squashing it between glass plates (e.g. Hacker and Steneck 1990, Davenport et al. 1996), or taking photographic "slices" of the plant as I have done here (e.g. Morse et al. 1985, Jeffries 1993). By measuring macrophyte structure in a two-dimensional plane, these indices may not give a faithful representation of the three-dimensional structure. Ideally, stereoscopic imaging or three-dimensional computer animation would provide the most realistic representations of macrophyte morphology, that could then be measured by three-dimensional extensions of the Kolmogorov grid method, but these methods are presently too expensive for most researchers.

Using two-dimensional representations of plants appeared to be most problematic in determining the fractal dimension. In particular, there were anomalous results of the fractal dimension at Scale 2 (5 × magnification) which indicated the photographic "slice" method of representing macrophyte shape may not be adequate. At this scale the fractal dimension of both *Eleocharis* and *Triglochin* was below 1.00 whereas the fractal dimension of a line or curve must, by definition, lie in the range $1 \leq D \leq 2$ (Schmid 2000). Although taking photographic "slices" of a plant and using the Kolmogorov dimension has been a recommended method for three-dimensional plants (Sugihara and May 1990, Schmid 2000), Jeffries (1993) has ackowledged it can never fully describe three-dimensional structure. Morse et al. (1985) estimated the fractal dimension of plant parts using photographs, but they specifically selected plant parts and a focal length to obtain a two-dimensional photograph. While I deliberately used

a shallow depth of field, it was impossible to obtain a photograph which was both two-dimensional and fully in focus; leaves and stems obstructed other parts of the plants. Furthermore, all plant parts were utilised by macroinvertebrates and should be included in an assessment of fractal dimension, particularly as plant parts can vary widely in fractal dimension (Shorrocks et al. 1991).

At Scale 2 the fractal dimension of *Myriophyllum* was 1.02, which often indicates a low degree of surface rugosity and therefore a relatively smooth surface. On the other hand, if a surface is considered as an edge folding back on itself to form a plane, then a D of 1.02 indicates a lot of edge. The photographic "slices" of *Myriophyllum* were through many leaves and leaflets, thus there were many edges counted but the fractal dimension gives no indication of this different representation of "edge" or shape. Likewise, at D_3 (2.5 × magnification), *Triglochin* and *Eleocharis* both had a D of 1.10, which indicates they had the same degree of surface rugosity at this scale, but raises questions about the usefulness of an index that does not distinguish between two clearly different shapes (B.J. Downes, Dept. Geography, University of Melbourne, *pers. comm.*) and further strengthens the case for using multiple indices to describe plant shape.

Conclusions

A useful index of habitat structure should have the capacity to measure different structures, to allow comparisons between different habitats, and to relate to the animals occupying those habitats. This research is the first to compare different indices of macrophyte structural complexity, and I found no single index effectively described both the shape of the macrophytes and explained the distribution of the associated macroinvertebrate fauna. For this reason, I shall not be using any of these individual indices in the following chapters, rather I shall refer to the plants by their names.

One reason for not finding a single index of structural complexity is that I used structures very different in shape. By experimentally manipulating some attributes of plant structure while holding others constant, more information can be gleaned about the specific effects of particular structural attributes. For example, Jeffries (1993) kept surface area constant and found the fractal dimension of artificial macrophytes significantly influenced the distribution of macroinvertebrates. It would be intriguing

to be able to hold the fractal dimension constant, and vary the amount of refuge space between plants, particularly as prey refuge is common explanation for greater macroinvertebrate abundances on more structurally complex plants.

While there is some suggestion the methods for obtaining a faithful representation of three-dimensional structure are not yet adequate, the high degree of collinearity between the indices I used points to a solution of using multiple indices. Rather than finding a single index of macrophyte structure, I found combinations of indices best described the macrophyte shape and the macroinvertebrate distribution. However, they were different combinations: *Myriophyllum* was best described by indices that measured the interstitial prey refuge and the surface rugosity at larger scales (Component 1), whereas *Eleocharis* was best described by the indices measuring "whole plant" attributes and the physical surface at finer scales (Component 2). While Sp/Pr loaded onto Component 1 and D_2 loaded onto Component 2, they still had relatively high correlations with the opposite component, suggesting they described structural aspects of both *Myriophyllum* and *Eleocharis*. Given *Triglochin* was not more strongly described by one component than the other suggests its structure may also be best described by Sp/Pr and D_2. Furthermore, these two indices were the most strongly related to macroinvertebrate abundance, and among those most strongly related to taxon richness. As they described the surface rugosity and the refuge space of the habitat, this suggests that macroinvertebrates are responding to a combination of these structural attributes, and thus these two indices should be included in the development of a useful index of macrophyte structural complexity.

Table 1: Structural Index values for each macrophyte.

Structural Index	*Eleocharis*	*Triglochin*	*Myriophyllum*
Surface Area (cm^2; SA)	246	821	862
Plant Volume (cm^3; PV)	43	20	17
Fish-Free Volume (cm^3; FFV)	0	10	396
Interstitial Space Index (ISI)	0.50	0.47	18.27
Interstitial Space size / Predator size (Sp/Pr)	2.59	1.83	0.42
Fractal Dimension (*D*)			
D_1 (6.5×magnification)	0.94	1.10	1.17
D_2 (5×magnification)	0.86	0.94	1.02
D_3 (2.5×magnification)	1.10	1.10	1.32
D_4 (1×magnification)	1.27	1.09	1.58

Table 2: The absolute values of correlations between structural indices and the components (after rotation) in the Principal Components Analysis. Higher correlations indicate stronger a stronger association between the index and the Component.

Structural Index	Component 1	Component 2
D_4 (1×magnification)	1.000	0.030
D_3 (2.5×magnification)	0.913	0.408
Interstitial Space Index (ISI)	0.913	0.408
Fish-Free Volume (cm^3; FFV)	0.904	0.428
Interstitial Space size / Predator size (Sp/Pr)	0.717	0.698
D_2 (5×magnification)	0.588	0.809
D_1 (6.5×magnification)	0.397	0.918
Plant Volume (cm^3; PV)	0.212	0.977
Surface Area (cm^2; SA)	0.167	0.986
Eigenvalues	4.571	4.429
% variance explained	50.791	49.209

Table 3: The range of absolute values of correlation coefficients (over 6 sites) and the number of significant correlations ($p < 0.01$) between each structural index and the dependent variables macroinvertebrate abundance and taxon richness.

	Macroinvertebrate abundance		Taxon richness	
Structural Index	Range of correlation coefficients	Number significant correlations	Range of correlation coefficients	Number significant correlations
Surface area	0.315 – 0.664	3	0.557 – 0.795	6
Plant volume	0.321 – 0.685	3	0.566 – 0.809	6
Fish-free volume	0.313 – 0.761	4	0.490 – 0.731	5
Interstitial space index	0.311 – 0.755	4	0.481 – 0.720	5
Space size / Predator size	0.348 – 0.811	5	0.573 – 0.841	6
Fractal dimension				
D_1	0.344 – 0.756	4	0.591 – 0.853	6
D_2	0.352 – 0.802	5	0.592 – 0.863	6
D_3	0.310 – 0.754	4	0.480 – 0.721	5
D_4	0.221 – 0.585	1	0.219 – 0.486	0

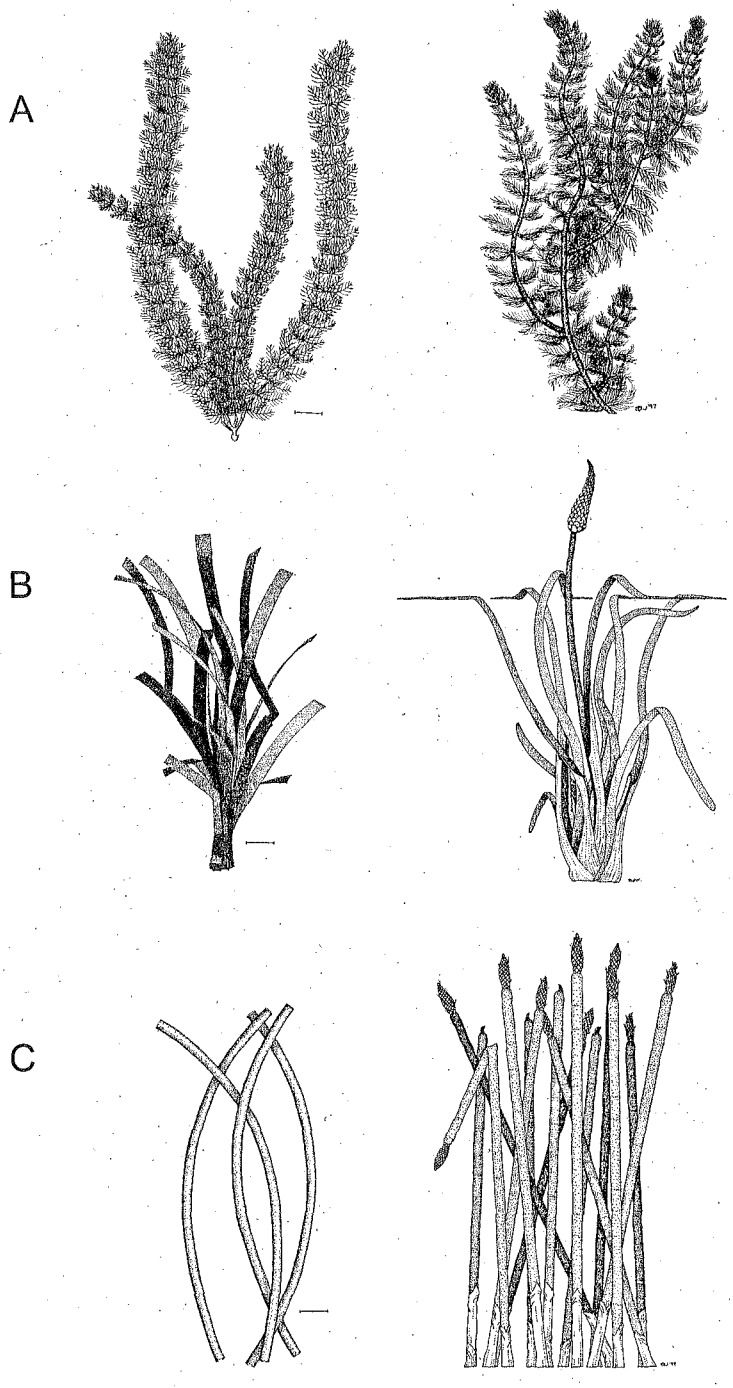

Figure 1: The three macrophytes common to the Macquarie River (on the right), and their artificial analogues (on the left) used in the field experiment and to measure habitat structural complexity. Only the imitation macrophytes are drawn to scale (scale bar = 2 cm). *Myriophyllum variifolium* (A) represented the most complex habitat structure according to *a priori* classification, *Triglochin procera* (B) represented an intermediate level of habitat structural complexity, and *Eleocharis sphacelata* (C) represented the most simple habitat structure.

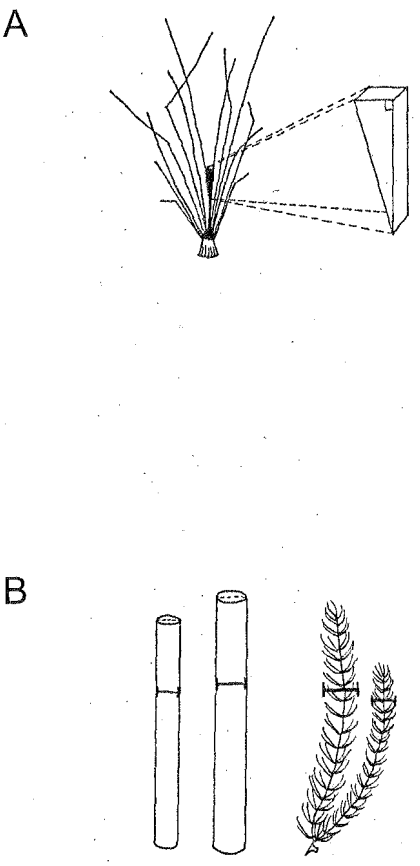

Figure 2: Schematic diagram illustrating how the fish-free volume (FFV) of *Triglochin* (A) and *Myriophyllum* (B) was obtained. The spaces at the base of the *Triglochin* leaves were assumed to form a wedge shape, the volume of which was calculated from the formula for an oblong and divided by 2. The depth of the oblong was always 12 mm (the leaf width) and the width was always 5 mm, which was the smallest space that could accommodate a pygmy perch. The width and length of the *Myriophyllum* stems were used to calculate their volume as if they were cylinders, as the gaps between the leaflets were smaller than 5 mm and therefore could not be accessed by pygmy perch. The volume of the stems, calculated geometrically with vernier calipers, was substracted from this figure to give the volume of the fish-free space associated with *Myriophyllum*.

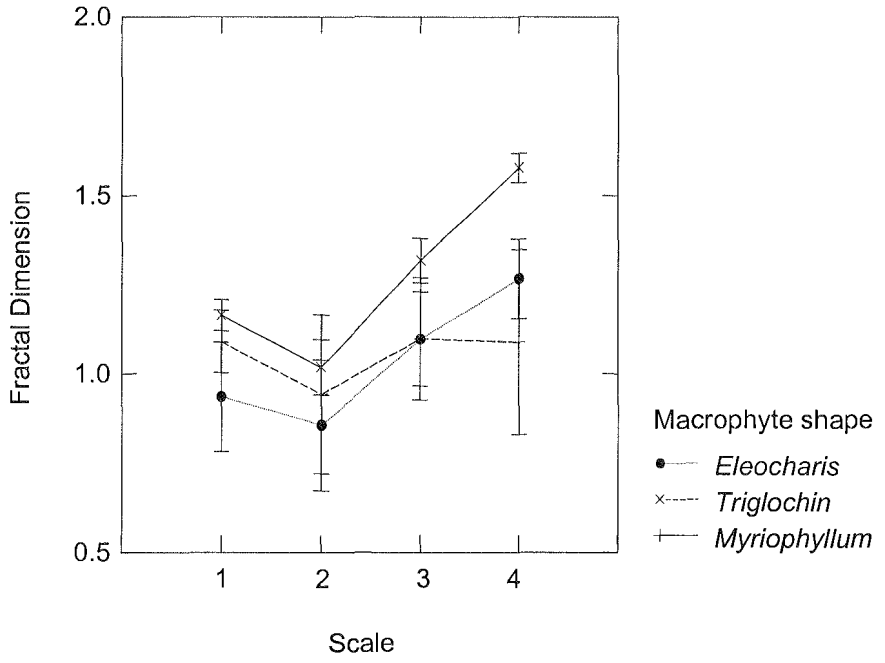

Figure 3: The fractal dimension of each macrophyte at Scale 1 (6.5 × magnification), Scale 2 (5 × magnification), Scale 3 (2.5 × magnification) and Scale 4 (1 × magnification) (n=4). Error bars are standard error about the mean.

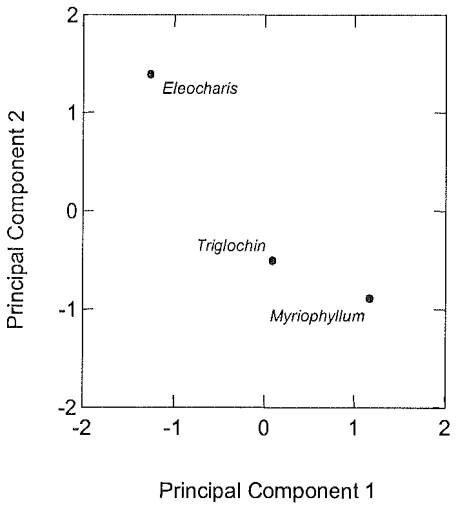

Figure 4: Principal component plot showing the separation of each macrophyte along components 1 and 2.

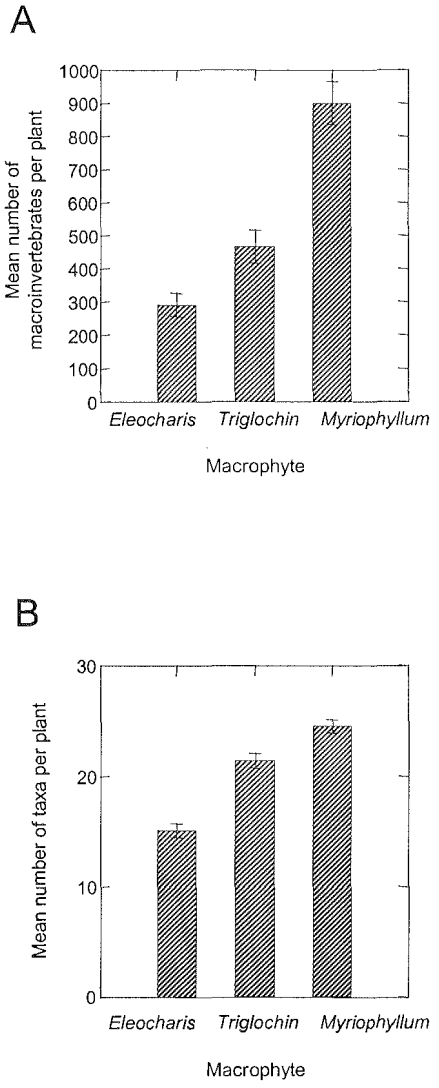

Figure 5: The number of macroinvertebrates per plant (A) and taxa per plant (B) on each macrophyte (n=249). Error bars are one standard error about the mean.

Chapter 3: The Effects of Habitat Structure on Multiple Predators

Introduction

In vegetated aquatic systems, the foraging success of fish predators has commonly been shown to decrease with increasing habitat structure (Nelson 1979, Crowder and Cooper 1982, Savino and Stein 1982, Stoner 1982, Diehl 1988, Gotceitas and Colgan 1989, Nelson and Bonsdorff 1990, Bettoli et al. 1992, Persson and Eklov 1995, Swisher et al. 1998). However, habitat structure has usually been measured simply as macrophyte density, which ignores differences in macrophyte shape. Some studies have tested different shapes, but have only used the one density of each (e.g. Leber 1985, Persson and Eklov 1995), confounding the independent structural components of shape and density. To properly understand the role of habitat structure in mediating trophic interactions, the effects of the quantitative and qualitative aspects of structure need to be separated (McCoy and Bell 1991, Beck 2000).

Furthermore, prey are usually at risk from more than one predator at any one time, and, given the variability present in predator behaviours and prey responses, the effects of predators in isolation may not give an accurate picture of the overall effects of predation on prey (Soluk 1993, Sih et al. 1998). Investigations of multiple predator effects have shown that their impacts may be non-additive; i.e. their combined impact may not necessarily be obtained by simply summing the impacts of individual predators in isolation (Losey and Denno 1988, Soluk and Collins 1988, Van Buskirk 1988, Martin et al. 1989, Soluk 1993, Morin 1995, Crowder et al. 1997). Two types of non-additive effects have been documented: positive and negative. Negative non-additivity occurs when fewer prey are eaten by mutiple predators than would be expected by adding together their individual predator impacts (Soluk and Collins 1988). This implies a reduction in predation risk for the prey due to negative interactions between the predators (Sih et al. 1998), and includes intraguild predation where one predator is not only a competitor but also a prey item of another predator (Polis 1989). Positive non-additivity occurs where more prey are consumed by both predators in combination than would be expected from their separate effects, and usually indicates facilitation, where one predator increases the vulnerability of prey to another predator (Losey and Denno 1988, Soluk and Collins 1988). These facilitative

interactions often result from prey behaviour; in their response to one predator, prey can become more vulnerable to predation by a second, usually differently-foraging, predator and therefore the prey's predation risk is greater in the presence of multiple predators (Soluk 1993, Sih et al. 1998, Swisher et al. 1998). Soluk and Collins (1988) suggested that mayfly larvae move out from underneath stones in the presence of stonefly predators, thereby increasing their risk of predation from fish predators (mottled sculpins), and indicating the habitat structure may play a role in these interactions. Indeed, Swisher et al. (1998) found an enhanced predation risk from bluegill sunfish and libellulid dragonfly larvae but only at low levels of macrophyte density; less habitat structure made the prey's escape response to the dragonfly larvae more easily detectable by the visually-feeding bluegill sunfish.

This experiment was motivated from observations of macrophytes beds in a lowland river in Tasmania. These beds are structurally diverse, and have a diverse community of macroinvertebrates, of which a coenagrionid damselfly, *Ischnura heterosticta tasmanica*, is an abundant invertebrate predator (Chapter 4). The macrophyte beds also support large populations of the southern pygmy perch, *Nannoperca australis*, a small native fish which feeds on epiphytic macroinvertebrates, including *I.h. tasmanica* (D.M. Warfe pers.obs., Humphries 1995). If macrophyte density can decrease a predator's foraging success (Crowder and Cooper 1982, Swisher et al. 1998), then it is also reasonable to predict a reduction in predator success as macrophyte shape becomes more structurally complex. Furthermore, if habitat structure can mediate pairwise predator-prey interactions, then it is reasonable to expect it to influence the combined impacts of multiple predators (Sih et al. 1998). This experiment investigated these expectations by testing the following hypothesis: that macrophyte shape and density are separate components of habitat structure and have independent effects on the individual and combined impacts of two predator species. Specifically, I hypothesised that: 1) more prey would be consumed by both a fish predator and a coenagrionid damselfly predator at low macrophyte densities regardless of macrophyte shape; 2) in three different macrophyte shapes, more prey would be consumed in the structurally simple shape than the most structurally complex shape; and 3) the combined impact of both predators would be greater at lower macrophyte densities and in the simplest shape.

Methods

The predators

The southern pygmy perch, *Nannoperca australis*, is found throughout southeastern Australia in lakes, shallow wetlands and lowland rivers where it occurs in patches of dense macrophyte growth (Humphries 1995). Individuals reach up to 80 mm in length and consume macroinvertebrates associated with macrophytes, such as amphipods, ostracods, chironomids and mayflies (Humphries 1995). Pygmy perch were collected by backpack electrofishing (Model 12-B POW, Smith-Root Inc.) and sweep-netting in macrophyte beds in the Macquarie River (147°28′E, 41°57′S), a slow-flowing lowland river in the midlands of Tasmania, Australia.

The second predator was a coenagrionid damselfly, *Ischnura heterosticta tasmanica*, which is a common member of the macrophyte-associated community (Davies and Humphries 1996, Humphries et al. 1996). Preliminary field sampling showed it to be one of the most numerous invertebrate predators in the macrophyte beds of the Macquarie River, and gut analyses revealed a diet of predominantly chironomids, chydorids, and mayflies (Appendix A). Damselflies were collected by sweep-netting in macrophytes. While neither damselflies nor pygmy perch are the top predators of the system (introduced brown trout, *Salmo trutta*, and redfin perch, *Perca fluviatilis*, are also present), they are potential competitors, sharing the same habitat and food resources, and also intraguild predators as pygmy perch can prey on damselflies (D.M. Warfe pers. obs., Humphries 1995).

Pygmy perch and damselflies were held in laboratory aquaria stocked with macrophytes under a regime of 12 hours light:12 hours dark, at water temperatures of 13-15°C (which represented median water temperatures in the Macquarie River, Humphries 1995). The experimental aquaria were kept under the same conditions throughout the experiment. Macroinvertebrates were collected to supplement a live food supply of *Daphnia* spp. and mosquito larvae for both predators. Mosquito larvae, *Anopheles* sp., collected from local ponds were used as prey because both predators ate them readily and they were representative of the mobile prey common in the diets of these predators.

Fifty-eight pygmy perch were collected and each fish was randomly allocated to a treatment combination. Individual fish were used in more than one but less than five

trial runs, but not on consecutive days. The average length of pygmy perch used in the trials was 37.46 ± 5.65 mm, and a positively significant body length:weight regression ($F_{(1,18)} = 256.653$, $p<0.001$) estimated the average wet biomass of fish used was 1.37 g. Visual analysis of residuals versus fish identity indicated there were no anomolies arising from fish identity and that all fish displayed the same patterns of consumption.

Five hundred and fifty damselflies were collected in total and, like the pygmy perch, each individual was used in more than one but less than five trials. Damselflies were sorted into size groups before each trial and randomly picked from each group so that the ten individuals used in any one treatment covered the range of damselfly sizes (9-18 mm length, 2.2-4.3 mm head width). Each group had an average body length of 13.94 ± 3.36 mm, average head width of 2.15 ± 0.43 mm, and total wet weight of approximately 0.5 g. Final instar larvae were not used.

The predator treatments were split into two separate factors, "Damselflies" and "Pygmy Perch", each with two levels, "Absent" and "Present". So the range of predator treatments applied were one with no predators ("Damselflies Absent / Pygmy Perch Absent"), one with 10 damselflies only ("Damselflies Present / Pygmy Perch Absent"), one with 1 pygmy perch only ("Damselflies Absent / Pygmy Perch Present"), and finally one with both predators together ("Damselflies Present [10] / Pygmy Perch Present [1]").

The macrophytes

The two predator factors were crossed with two other factors representing the separate components of habitat structure: "Shape" of macrophytes (3 levels) and "Density" of macrophytes (5 levels; Table 1). Artificial imitations of three macrophytes, varying in shape and common to the Macquarie River (Humphries 1996), were constructed to allow quantifiable differences in density to be achieved and to control against influences of secondary chemicals and autogenic change in the plants. Wooden dowling (9.6 mm diameter, 280 mm height) was used to represent the macrophyte shape of low complexity, *Eleocharis sphacelata*, which has a simple, cylindrical reed structure. Plastic aquarium plants (Tetra Second Nature Plantastic©, 280 mm height) were used to represent the macrophytes of intermediate and high structural complexity. *Triglochin procera*, a tufted plant with long strap-like leaves, represented the intermediate level of habitat structure and *Myriophyllum variifolium*, which has

highly dissected leaves, represented the plant of the greatest structural complexity (see Chapter 2). Macrophyte densities were based on the field densities found in surveys of macrophyte beds in the Macquarie River (D.M. Warfe, *unpubl.data*), and the highest density level corresponded to the highest density observed in the field.

Thus the experiment was a fully-crossed 4 factorial design: 2 damselfly treatments × 2 pygmy perch treatments × 5 macrophyte densities × 3 macrophyte shapes. Each treatment combination was randomly allocated to separate PVC (polyvinylchloride) tanks (350 × 200 × 280 mm height, 15 L) equipped with a polystyrene base and an airstone. Seven replicates of each treatment combination were conducted as follows. Plants were added to each tank according to macrophyte shape and density treatments. The tanks were then filled with a mixture of tap water and fresh river water (collected from local streams), and 25 mosquito larvae were introduced into each tank. Pilot trials showed that this was more prey than the predators could eat during the experimental period. Fifteen minutes later damselflies were added to their respective tanks, and 15 minutes after that, the pygmy perch were added. 24 hours after the introduction of the fish, the predators were removed, the plants were removed and rinsed (collecting any stray damselflies and mosquito larvae), and the remaining mosquito larvae were counted. I also recorded qualitative observations of mosquito, damselfly and pygmy perch behaviour from both experimental and holding tanks during the experiment.

Data analysis

A fully-crossed 4-factorial ANOVA with 5 planned comparisons was carried out on the number of prey consumed. No transformation of the data was necessary because plots of residuals and normal probability showed no violations of the assumptions of the ANOVA. For macrophyte density, planned linear and quadratic contrasts were carried out across the five levels. Planned comparison were also conducted on macrophyte shape, *Eleocharis* was contrasted with *Triglochin* and *Myriophyllum*, and *Triglochin* was contrasted with *Myriophyllum*. Simple effects tests were conducted for any significant interactions.

To test whether the number of prey consumed by both predators combined was additive, the amount consumed by each predator alone was incorporated into an additive-consumption model developed by Soluk (1993, Swisher et al. 1998):

$$C_{pd} = N_{prey}(P_p + P_d - P_pP_d)$$

where C_{pd} is the predicted combined consumption for the intial prey density (N_{prey}), and P_p and P_d are the probabilities of prey being consumed by pygmy perch or damselflies, respectively, over a 24 hour period. This model takes into account that the predicted combined consumption cannot exceed the initial prey density. The predicted combined consumption values were compared to the actual combined consumption values using a paired *t*-test for each macrophyte shape. A significant difference between the predicted and actual values indicated a non-additive effect of both predator species when they were present together. All analyses were carried out using SYSTAT Version 9 (Wilkinson 1999).

Results

Behavioural observations

Mosquito larvae appeared to move near and remain around the macrophytes, regardless of their shape, although they were never seen inside the highly-dissected leaves of *Myriophyllum*. They displayed the same behaviour upon contact from either predator, swimming away rapidly, although prey capture by both damselflies and pygmy perch was usually successful on the first attempt.

Damselflies used macrophyte structure as a perch from which to capture the prey, but used each shape differently. In the structurally simple macrophyte, *Eleocharis*, they would perch on the side of a stem and move around to the other side if a pygmy perch was nearby. Likewise, on *Triglochin* (the tufted plant of intermediate structural complexity) they perched on one side of a leaf, moving to the other side, or occasionally another leaf, on sighting the pygmy perch. On *Myriophyllum*, damselflies would perch on the outside of the leaflets to capture prey, but would move to the inside, next to the main stem, if a pygmy perch was near. They were rarely observed to move back to the outside of the leaflets during the trials.

Pygmy perch also appeared to use the macrophyte structure, generally remaining close to the plants except to dart out and capture prey. In the trials with high macrophyte density, they were difficult to see, but were observed to swim around more within areas of thick macrophyte density in the holding tanks.

Statistical analyses

Macrophyte density had no significant effect ($F_{(4,300)} = 1.046$, $p>0.383$; Figure 3a) on the number of mosquito larvae consumed; 45% ± 3.8 (mean ± standard error) of them were consumed at each density level.

Macrophyte shape significantly affected the number of prey consumed ($F_{(2,300)} = 5.311$, $p<0.005$; Figure 3b). Planned comparisons showed that 9% more prey were consumed in *Eleocharis* than in *Myriophyllum* ($F_{(1,300)} = 10.530$, $p<0.001$) but only 5% more than in *Triglochin* which was not significant ($F_{(1,300)} = 3.559$, $p>0.060$). Four percent more prey were consumed in *Triglochin* than in *Myriophyllum*, and this was also not significant ($F_{(1,300)} = 1.845$, $p>0.175$).

Both the damselfly and pygmy perch treatments were significant; however, there was also a significant interaction between the two factors ($F_{(1,300)} = 41.856$, $p<0.001$; Figure 4). When both predators were absent, 1% ± 0.3 of prey were missing which indicated the significant differences observed in the other predator treatments were real and due to predator consumption rather than missing larvae. In the absence of pygmy perch, damselfly larvae consumed 40% of the prey ($F_{(1,150)} = 425.139$, $p<0.001$). When pygmy perch were present, only 12% more prey were consumed by damselflies ($F_{(1,150)} = 9.468$, $p<0.002$).

None of the three-way interaction terms were significant (all $p>0.189$), nor was the four-way interaction significant ($F_{8,300} = 0.935$, $p<0.488$).

Both predators combined consumed 10% less than that predicted by the additive-consumption model ($t_{(104)} = 2.044$, $p<0.043$), which indicated negative non-additivity. Separate *t*-tests for each macrophyte shape showed that this negative non-additivity only occurred in *Myriophyllum* ($t_{(34)} = 2.207$, $p<0.034$; Figure 5). The combined consumption of both predators was additive in both *Triglochin* ($t_{(34)} = 0.467$, $p>0.643$) and *Eleocharis* ($t_{(34)} = 0.739$, $p>0.465$).

Discussion

This experiment tested two separate components of habitat structure – macrophyte density and macrophyte shape – on the foraging success of two freshwater predators. As predicted, these components had separate and independent effects on predators; macrophyte density had no effect on the number of prey eaten by either predator,

whereas macrophyte shape not only influenced the number of prey consumed by each predator alone, but also their combined impact. These results support the arguments proffered by McCoy and Bell (1991) and Beck (2000), that the independent effects of shape and density must be separated in order to gain a more thorough understanding of how trophic interactions are mediated by the environment in which they occur.

Macrophyte density

Contrary to my expectations, macrophyte density did not affect the number of prey captured by either predator, regardless of macrophyte shape. Most studies that have tested the influence of habitat structure on predator success have measured it as macrophyte density, and have found that predator success declines as density increases (Nelson 1979, Heck and Thoman 1981, Crowder and Cooper 1982, Savino and Stein 1982, Stoner 1982, Folsom and Collins 1984, Gilinsky 1984, Gotceitas and Colgan 1989, Nelson and Bonsdorff 1990, Bettoli et al. 1992, Lipcius et al. 1998, Swisher et al. 1998). A common explanation is that predator mobility is impeded by the structure (Stoner 1982, Diehl 1988, Heck and Crowder 1991), so pygmy perch may simply be too small to be physically hampered by macrophytes, as has been suggested for the similarly-sized pinfish, *Lagodon rhomboides* (Stoner 1982).

There is also evidence that predators can shift their mode of foraging as the habitat structure becomes more dense. Savino and Stein (1989) found that while largemouth bass (*Micropterus salmoides*) were less efficient at higher macrophyte densities, they were still able to feed by shifting their mode of foraging. Likewise, James (1994) found that the lined seahorse (*Hippocampus erectus*) shifted from a searching mode of foraging to an ambush strategy as the seagrass habitat became more dense. At low densities of macrophytes, pygmy perch adopted a sit-and-wait strategy, remaining motionless except to dart out and capture prey. This is possibly due to their risk of predation by piscivorous fish such as brown trout and redfin perch, both present in the Macquarie River, and such trade-offs between predation risk and foraging have been documented for other small fish (Crowder and Cooper 1982, Werner et al. 1983, Persson and Eklov 1995, Jacobsen et al. 1997). At higher macrophyte densities pygmy perch were too small to be hampered by the habitat structure and adopted a searching strategy, moving amongst plant stems and consuming prey as they encountered them.

By changing their foraging strategy with habitat structure, pygmy perch could consume a similar amount of prey regardless of macrophyte density.

However, this does not explain why damselflies were also unaffected by macrophyte density and suggests that prey behaviour contributed to this pattern. Had I used a prey species that was epiphytic, such as cased caddisfly larvae, I may have found an effect of macrophyte density on the number of prey consumed. However, preliminary investigations of caddisfly prey showed this would have required much longer experimental times to detect an effect. Furthermore, not only are mosquito larvae present in the Macquarie River, but they were deliberately chosen to represent relatively active prey such as amphipods, ostracods and some chironomids consumed by pygmy perch and damselflies. Numerous studies have shown that prey can alter their behaviour depending on their risk of predation (Werner et al. 1983, McIntosh and Townsend 1996, Beckerman et al. 1997) and mosquito larvae have been shown to select habitats on the basis of macrophyte density and shape, occurring at greater abundances on dense *Myriophyllum* (Orr and Resh 1991, 1992). My results suggest that while mosquito larvae may be able to perceive higher macrophyte density as a better refuge from predation, they may move around more within that habitat, negating the effect of refuge so their risk of predation was unaltered as density increased. Macrophyte shape rather than density significantly influenced the risk of predation by pygmy perch and damselflies.

Macrophyte shape

Macrophyte shape affected the ability of both damselfly larvae and pygmy perch to find and capture prey; significantly fewer prey were consumed in the most structurally complex macrophyte, *Myriophyllum*, than in structurally simple macrophyte, *Eleocharis*. It is commonly reported that differently shaped macrophyte species support different macroinvertebrate assemblages (Stoner and Lewis 1985, Cyr and Downing 1988b, Chilton 1990, Humphries 1996), which, given predator efficiency can be lower in more complex structures, may be partly due to differential effects on predator success (Coull and Wells 1983, Leber 1985, Diehl 1988). For example, Diehl and Kornijów (1998) tested the foraging efficiencies of 3 fish in 3 different structures; bare sand, imitation *Potamogeton* at 140 stems/m^2, and imitation *Chara* at 900 stems/m^2. They found that all fish were less effective in *Chara*, but as *Chara* has a

more complex and dissected form than *Potamogeton*, it is not possible to ascertain whether these fish were negatively affected by macrophyte shape, density, or a combination of the two (Beck 2000).

In one of the few studies to investigate the separate effects of macrophyte shape and density on the foraging efficiency of fish predators, Dionne and Folt (1991) found that shape had far more effect on prey capture rates than density. Pumpkinseed sunfish, *Lepomis gibbosus*, caught more cladoceran and damselfly prey in the simpler, straight-stemmed *Scirpus* than in the leafy *Potamogeton*. Prey can be more readily detected in macrophytes with simple leaves (or no leaves), thus more complex plants act as a prey refuge by making it more difficult for predators to locate prey and hence easier for prey to avoid capture (Heck and Orth 1980, Main 1987, Ryer 1988). This would also explain why the damselfly larvae showed the same pattern, consuming fewer prey in *Myriophyllum* because prey were harder to detect amongst the highly dissected leaves. In an experiment using *Elodea*, *Ceratophyllum* and *Myriophyllum*, Walsh (1995) also found that damselfly larvae captured significantly fewer rotifer prey in *Myriophyllum*. The greater structural complexity of *Myriophyllum* provided more refuge for prey, however, she suggested damselflies also had more difficulty perching on *Myriophyllum*, thereby reducing their encounter rate and ability to capture prey (Walsh 1995). In my experiment, damselflies appeared to have no difficulties perching, possibly because the plastic imitations are more rigid than natural *Myriophyllum* plants. Instead, the highly dissected leaves of *Myriophyllum* may make prey detection more difficult for visually-feeding damselfly and pygmy perch predators, and therefore provide an effective prey refuge.

However, plants considered structurally complex due to a fine-leaf structure can actually prove less effective as a prey refuge as large and broad leaves can allow prey to hide more effectively from predators (Edgar 1983). Stoner (1982) found that while fewer prey were captured by pinfish (*Lagodon rhomboides*) with increasing seagrass density, prey were more readily detected and captured in the fine leafy seagrass species than the simple wide-bladed forms. Clearly, the morphology of different macrophyte species can have significant effects on the ability of predators to find and capture prey, and hence on their refuge value to prey, but these effects are likely to depend to some degree on predator and prey behaviour.

Multiple Predator Effects

Facilitative interactions between multiple predators seem to be more common than negative interactions in the literature, and tend to occur because the avoidance behaviour displayed by a prey species to a predator species makes it more vulnerable to another predator species (Losey and Denno 1988, Martin et al. 1989, Soluk and Richardson 1997, Sih et al. 1998, Swisher et al. 1998). Negatively non-additive interactions can occur when there is interference between multiple predators (Soluk and Collins 1988, Soluk 1993), or when a prey's avoidance behaviour makes it less vulnerable to both predators, thus precluding any direct interference between them (Crowder et al. 1997).

My hypothesis that habitat structure would not only influence the effects of each predator in isolation but also their combined impact was supported – the amount of prey consumed depended on the presence of a second predator species. However, like the effects of habitat structure on the foraging success of individual predators, the success of both predators combined was not mediated by macrophyte density, but by macrophyte shape alone. Pygmy perch and damselflies had an additive impact in the plants of low and intermediate structural complexity (*Eleocharis* and *Triglochin*), but fewer prey were consumed than expected in the most structurally complex *Myriophyllum*. Damselfly larvae consumed 28% less prey when they were in the presence of pygmy perch than when they were alone, indicating the non-additivity observed was due to a negative interaction between the predators.

Pygmy perch are not only competitors of damselfly larvae, but are also intraguild predators (sensu Polis 1989), in that they also prey on damselflies (D.M. Warfe pers. obs., Humphries 1995). One explanation for the negatively non-additive effects of two predators is that one predator reduces the abundance of the other (Morin 1995). Over the course of the experiment, 25 of a total possible 900 damselflies were never recovered from the treatments with both predators, and were presumed consumed by the fish. While the number of damselflies eaten would be unlikely to have a noticeable impact on overall prey consumption, it does show they are at risk from predation by pygmy perch and may therefore possess some predator avoidance behaviour. Thus the negative non-additivity displayed by these predators may have arisen through behaviour modification rather than direct consumption.

Many odonates modify their behaviour accordingly in the presence of predators (Pierce 1988, McPeek 1998). Damselflies have been shown to exhibit predator avoidance behaviour by hiding behind stems (Heads 1985), reducing their movements in the presence of fish predators (Koperski 1997), and even being able to assess the relative risk of predation conferred by different macrophyte species and modify their behaviour accordingly (Dionne et al. 1990). In *Eleocharis* and *Triglochin*, damselflies perched on a stem or leaf (respectively) would move to the other side when a pygmy perch was nearby, thus they were hidden but still able to capture prey. In *Myriophyllum*, however, damselflies perched on the outside of the leaflets would move to the inside, next to the main stem, and therefore were unable to capture prey swimming past. Thus their predator avoidance strategy reduced their ability to capture prey in this particular macrophyte shape.

This negative interaction in a structurally complex habitat contrasts with the results of Swisher et al. (1998), who found bluegill sunfish (*Lepomis macrochirus*) and libellulid dragonfly larvae (*Erythemis simplicicollis*) had a positively non-additive impact at low densities of artificial *Ceratophyllum demersum*, which became additive at higher densities. The mayfly prey escaped dragonfly attacks by swimming away, which made them more vulnerable to bluegill predation at low densities where they could be easily detected, and hence there was a facilitative interaction between these predators at low macrophyte densities. Indeed, Swisher et al. (1998) predicted that the synergistic effects of multiple predators would be more apparent in lower macrophyte densities where it is easier to find and capture escaping prey. My experiment found almost opposite results; the impact of two predator species was additive in a structurally simple habitat, but became negatively non-additive in a structurally complex habitat. However, it must be remembered that the results of Swisher et al. (1998) were for macrophyte density, while mine were contingent on macrophyte shape.

Given that both macrophyte shape and density contribute to habitat structure in vegetated systems, the results from both studies could be combined to predict a relationship between habitat structure and the impact of multiple predators (Figure 4). From this proposed model, several hypotheses can be generated: 1) the effects of multiple predators should be additive at intermediate levels of habitat structure (both density and structural complexity); 2) the effects of multiple predators should be

positively non-additive at low levels of habitat structure where prey are easier to detect and their avoidance behaviour can increase their risk of predation; and 3) the effects of multiple predators should be negatively non-additive at high levels of habitat structure where the habitat structure interferes with the success of each predator in isolation, and also mediates the outcomes of predator-predator interactions, thus affording a greater refuge from predation in this habitat.

Conclusions

This experiment illustrates the importance of testing both the quantitative and qualitative components of habitat structure in order to understand the mechanisms by which it may mediate trophic interactions. Furthermore, not only does habitat structure influence the outcome of predator-prey interactions, but also the outcome of predator-predator interactions and thereby the combined impact of multiple predators. It appears the effects of predation in the field, and therefore the functional significance of predators in food webs, may depend on the type of habitat available, and that strong predatory effects may be more tightly coupled with structurally simple habitats (Power 1992). To explore this hypothesis, and to determine if my laboratory results had any relevance to the dynamics occurring in a natural community, I designed a field experiment testing the effects of macrophyte shape and pygmy perch predation, with the expectation that pygmy perch would have the greatest direct and indirect effects in the structurally simple macrophyte.

Table 1: Densities of each artificial macrophyte used in the experiment, based on field densities, expressed as density of stems per tank (d/tank) and per m^2 (d/m^2). *Triglochin procera* was quantified by tufts rather than stems, and each tuft comprised 18 leaves of varying lengths (see text).

Macrophyte	*Eleocharis sphacelata*		*Triglochin procera*		*Myriophyllum variifolium*	
Density level	d/tank	d/m^2	d/tank	d/m^2	d/tank	d/m^2
1	0	0	0	0	0	0
2	13	186	1.5	22	11	157
3	39	557	4.5	64	33	472
4	65	929	7.5	107	55	786
5	91	1300	10.5	150	77	1100

Figure 1: The southern pygmy perch, *Nannoperca australis*. Photograph courtesy of Jean Jackson and Brett Mawbey, Inland Fisheries Service, Tasmania.

Figure 2: The coenagrionid damselfly, *Ischnura heterosticta tasmanica*. Photograph courtesy of Simon Talbot, University of Tasmania.

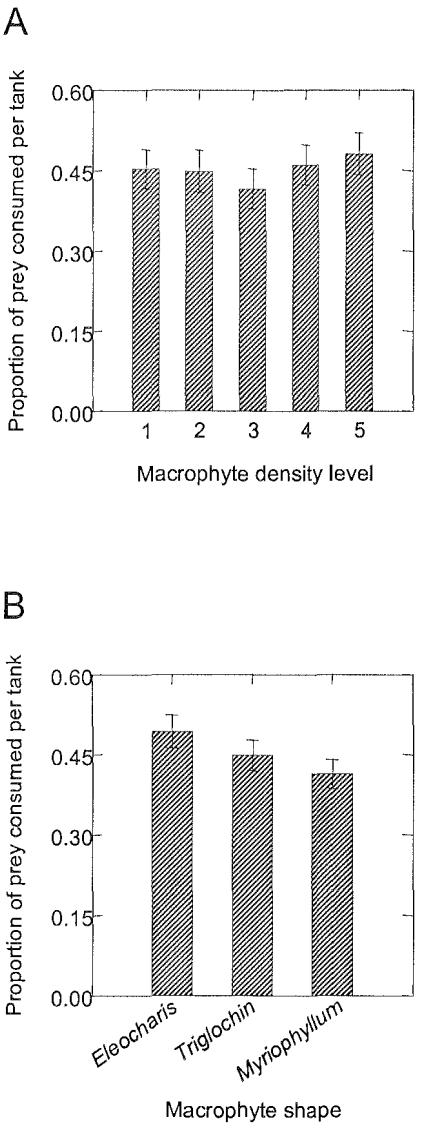

Figure 3: The proportion of prey consumed per tank at each level of macrophyte density (A), and in each macrophyte shape (B). Error bars are the standard error about the mean.

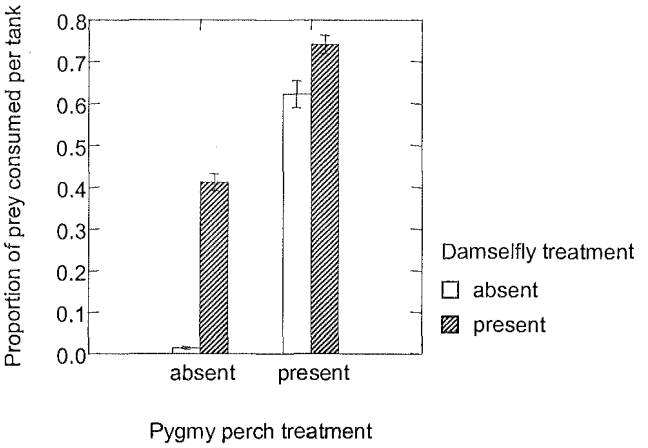

Figure 4: The proportion of prey consumed (per tank) by pygmy perch in treatments where damselfly larvae were absent and present. Error bars are the standard error about the mean.

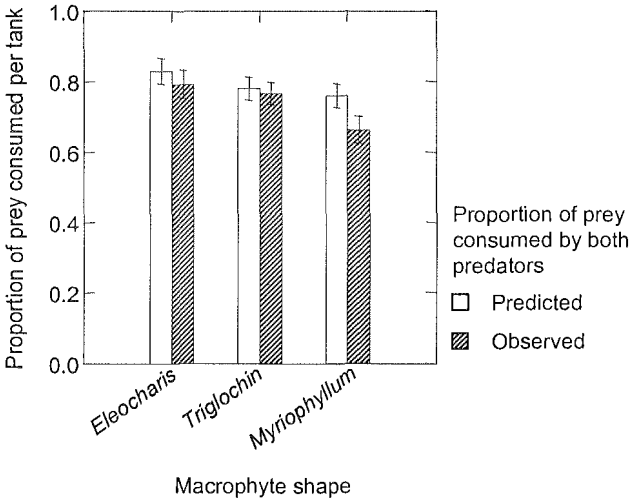

Figure 5: The predicted and observed proportion of prey consumed (per tank) by both pygmy perch and damselfly larvae combined, in each macrophyte shape. Error bars are the standard error about the mean.

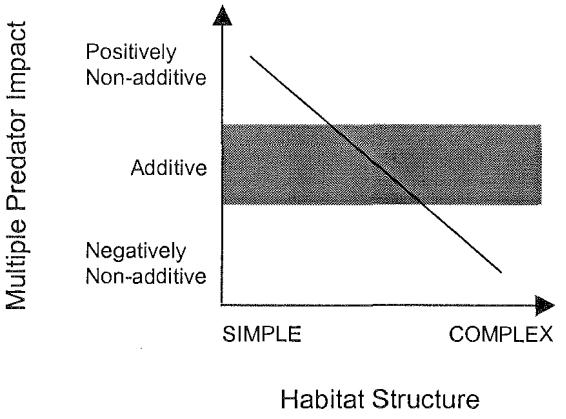

Figure 6: Hypothesised relationship between habitat structural complexity and the impact of multiple predators.

Chapter 4: The Effects of Habitat Structure and Fish Predation on Macroinvertebrate Community Structure.

Introduction

Habitat structure can be defined as the arrangement of physical objects in space that provide the environment in which an organism lives (McCoy and Bell 1991). In vegetated aquatic systems, habitat structure is often provided by macrophytes, and their importance as a habitat is evidenced by the greater abundance, often in orders of magnitude, of macroinvertebrates than in unvegetated areas (Heck and Crowder 1991). The abundance and richness of the macroinvertebrate community appears to be proportional to the density, or biomass, of freshwater macrophytes (Crowder and Cooper 1982, Stoner and Lewis 1985). However, the relationship is less clear when referring to the type rather than the density of structure. Different macrophyte species tend to support different epiphytic communities (Rooke 1986, Cyr and Downing 1988b, Chilton 1990, Humphries 1996), but whether this is due to macrophyte morphology, and why, remains unclear (Stoner and Lewis 1985).

The role of habitat structure as a refuge from predation is a common explanation and habitat structure has often been shown to negatively affect the ability of predators to find and capture prey (Heck and Thoman 1981, Crowder and Cooper 1982, Savino and Stein 1982, Stoner 1982, Gotceitas and Colgan 1989, Nelson and Bonsdorff 1990, Bettoli et al. 1992, Persson and Eklov 1995, Swisher et al. 1998). However, these studies have only compared the presence and absence of habitat structure, or the density of structure, on predator success. Very few have assessed the shape of the structure, and while they suggest macrophyte shape can also affect predator success, have been confounded with the density of the structure (e.g. Leber 1985, Persson and Eklov 1995).

Fish predators can have large impacts on their prey communities, the most striking of which are evidenced by trophic cascades where a predator can have strong indirect effects cascading down the trophic levels of a community (Carpenter et al. 1987, Pace et al. 1999). Yet these strong predator effects have generally been observed in systems with relatively little habitat structure. If habitat structure can influence the effects of predators, then it might thereby influence how strongly a community is regulated by

predation. In other words, the importance of predation might depend on the habitat in which it occurs (Power 1992).

I conducted a multifactorial caging experiment to determine the relative importance of habitat structure and fish predation in structuring an epiphytic macroinvertebrate community (effects on the periphyton community are addressed in Chapter 5). My specific hypotheses were: 1) macrophyte structural complexity affects the abundance, richness and composition of the macroinvertebrate assemblage; 2) predation by pygmy perch affects the abundance, richness and composition of the macroinvertebrate community; and 3) the effects of pygmy perch predation are mediated by the habitat in which they occur. I was expecting an interaction between predation and plant shape such that on the structurally simple macrophyte, where there are fewer refuges, predators would consume more prey than on the more structurally complex plant where there are more refuges.

Methods

Study site

The experiment was conducted in the macrophyte beds, on a property called Fosterville (147°28′E, 41°57′S), on the Macquarie River, a slow-flowing lowland river in Tasmania. The Macquarie River rises in the Eastern Tiers of Tasmania at an elevation of 575 m, and drains an area of 3,765 km^2 over its 155 km length before it joins the South Esk River in the state's north; Fosterville is 85 km from the source, at an elevation of 170 m. The catchment receives an annual rainfall of 300-500 mm and the discharge at Fosterville ranges from a daily average of 1.5 $m^3 s^{-1}$ to 20 $m^3 s^{-1}$ annually, with most of its flow occurring between winter and mid-spring (Davies and Humphries 1996). The river is partially regulated, with river storages augmenting the low flows during the summer irrigation season; however, large floods and winter flows are essentially natural.

The Macquarie River drops 350 m over 30 km from its source, and over the remaining length it is a slow-flowing, low-gradient, sinuous channel with few riffles, long narrow relatively deep runs, and large deep (up to 30 m) pools known as broadwaters (Davies and Humphries 1996). The riparian vegetation of this lowland section is highly disturbed by agriculture and dominated by introduced plants such as willow, gorse and hawthorn, with native grasses and remnants of dry sclerophyll woodland.

The margins of runs and pools support abundant and diverse macrophyte beds up to 20 m wide. While up to 30 macrophyte species have been recorded, the beds are dominated by *Myriophyllum* spp., *Vallisneria gigantea*, *Potamogeton* spp., *Carex* sp., *Juncus* spp., *Eleocharis sphacelata*, and *Triglochin procera* (D.M.Warfe *unpubl. data*, Humphries et al. 1996). These macrophyte beds are stable, highly productive, and relatively rare in Tasmanian rivers (Davies and Humphries 1996). They provide habitat for numerous endemic and restricted-distribution species such as the freshwater mussel (*Velesunio mortoenicus*), the burrowing crayfish (*Engaeus nulloporious*) and the red spinner mayfly (*Atalophlebia australis*); as such these beds have high conservation and recreational fishery values (Davies and Humphries 1996). These macrophyte beds also support large populations of the southern pygmy perch, *Nannoperca australis*, which is rarely found in open water and was used as the predator in the experiment. This fish is native to south-eastern Australia, reaches 75-80 mm in length and feeds on epiphytic invertebrates (Humphries 1995).

Experimental design

The experiment was a fully-crossed, two-factorial, repeated measures, randomised complete block design with both factors fixed. The first factor was macrophyte shape comprising three levels of structural complexity: low, medium and high, as represented by the three species of macrophyte *Eleocharis sphacelata, Triglochin procera* and *Myriophyllum variifolium* respectively. The second factor was predator treatment which had four levels represented by different types of cages: control, predator access, predator enclosure, and predator exclosure cages. One complete set of the 12 factor combinations was placed in macrophyte beds at each of six sites in the river; "sites" thus formed the blocks. The blocks were placed over 1.5 km, and each was separated by at least 200 m, in areas of negligible flows with extensive macrophyte growth (often extending to the centre of the main channel). Each site encompassed 40 m of the littoral zone on both sides of the river, where there was sparse riparian vegetation of tea-tree and willow typical of this stretch of river. The cages were placed in macrophyte beds at least 2 m apart from each other, and anchored to trees or stakes on the riverbank by rope.

I expected the river height to vary over the planned course of the experiment, so I used floating cages to ensure the volume of water per cage was standardised over

time. The floating cages were constructed of black plastic oyster mesh (mesh size 6 mm, NYLEX), 500 mm long × 500 mm wide × 300 mm high, attached to a square upper frame (500 mm × 500 mm) of sealed polyvinylchloride piping (75 mm diam) which acted as the float. The cage floors were also constructed of oyster mesh for plant attachment, and the tops were left open. Each cage had removeable panels (300 × 240 mm) on two opposing sides to allow colonisation by periphyton and macroinvertebrates.

Plastic imitations of three macrophytes species were used to provide consistent and quantifiable sampling units over the duration of the experiment (see Chapter 2, Figure 1). These species were chosen because they were numerically dominant in the macrophyte beds at Fosterville and had demonstrably different shapes that could be ranked from least to most structurally complex as described in Chapter 2. Moreover, these differences in plant shape influenced the foraging success of pygmy perch under laboratory conditions (Chapter 3). Because the laboratory experiment described in Chapter 3 showed the foraging success of pygmy perch was unaffected by macrophyte density, the macrophyte densities used were based on median field densities found in surveys of natural macrophyte beds at Fosterville (D.M.Warfe, *unpubl.data*).

Green electrical conduit (7 mm diam) represented the reed *Eleocharis sphacelata*, the plant of low structural complexity. The conduit was cut into 280 mm lengths, lightly sandpapered to remove the smooth surface, sealed at both ends with neutral silicon, and attached to the base of the cage with a length of twist-tie set into the silicon, at a density of 220 stems/cage (880 stems/m^2).

Green packing strap (12 mm width) was used to represent the water ribbon *Triglochin procera*, the macrophyte of intermediate structural complexity. Each plant consisted of 18 lengths of packing strap, 3 of each length 100, 130, 170, 200, 260, and 280 mm with the longer ones towards the centre, held together at the base with epoxy-resin to form a tuft, and attached to the cage base at a density of 25 plants/cage (100 plants/m^2) by a length of twist-tie set into the epoxy-resin.

Plastic aquarium plants (Tetra Secondnature®, Blacksburg, Virginia, USA) were used to represent the macrophyte of high structural complexity, *Myriophyllum variifolium*. The plants comprised four stems of whorled leaves and had a high degree of leaf

dissectedness to specifically imitate *Myriophyllum* spp. (Tetra Secondnature, *pers.comm.*). They were attached to the cage bases with twist-tie, at a density of 45 plants/cage (180 plants/m^2).

There were four levels of the predator treatment factor. The cage control lacked sides and the PVC frame, consisting of just the plants and the base weighted to 300 mm depth, and was designed to control for cage effects. The predator access cage had the two panels removed to allow fish predators free access – this was designed to control for predator enclosure effects. (During the colonisation period and experiment, pygmy perch were often observed in the access cages.) The predator enclosure cage had the panels replaced and three pygmy perch (30-60 mm length) enclosed within it, which were matched by size across enclosures to establish a similar size distribution in each cage. This density was based on estimates of field densities from pygmy perch surveys in the Macquarie River, where often a few fish would be caught in a single sweep through the macrophyte beds (D.M. Warfe pers.obs., Humphries 1995). It was considered a low-to-medium density initially, although the confinement of fish over the duration of the experiment probably corresponded to a relatively high density. The predator exclosure treatment was a closed cage with no fish. Thus, if pygmy perch had an effect on macroinvertebrates, and pygmy perch were present in the control, access and enclosure cages, then I expected to see differences in macroinvertebrate assemblage in the exclosure cages.

The experiment included two starting times to control for the timing of the initiation of the experiment as the algal and invertebrate communities appeared to differ depending on season (D.M. Warfe *pers.obs.*). This design distinguishes between temporal variation (patterns throughout time) and seasonal variation (patterns at different times) (Underwood and Anderson 1994). It resembles the "staircase design" recommended by Walters et al. (1988) to avoid confounding treatment effects with any peculiarities that prevailed at the time the experiment was started. Three blocks commenced in mid-summer (24 January 2000) and ran for eight months, and 3 in mid-winter (7 July 2000), running concurrently for the remaining two months (Figure 1). These were referred to as the summer-start and winter-start blocks respectively. The experiment was initially designed to run for a year; unfortunately, a one-in-ten-year flood (Chris MacGeorge, Bureau of Meteorology, Tasmania, *unpubl. flow data*) in late August 2002 terminated the experiment at 31 weeks. The cages were full of

woody and macrophyte debris washed down the river, and many were stranded metres from the water, some even in trees. The pygmy perch were not retrieved so I was also unable to gather body size and weight data.

Sampling was conducted at 2 weeks (7 February), 6 weeks (6 March), 10 weeks (3 April), 26 weeks (24-25 July) and 30 weeks (21-22 August). The 16-week jump between sampling times was not considered a problem as I was interested in the consistency of predation patterns over time rather than the effect of time itself. Given the staggered time-of-start design, the 3 summer-start blocks were sampled on the first 3 dates, and all 6 blocks on the remaining two dates. Thus, the winter-start blocks were sampled at 2 and 6 weeks after their initiation and concurrently with the summer-start blocks at 26 and 30 weeks (although they are referred to as having being sampled at 26 and 30 weeks after the overall experimental initiation). All cages, and plants, were colonised by periphyton and macroinvertebrate for 10 weeks prior to the application of the predator treatments, and the cage walls were scrubbed monthly to prevent periphyton accumulation on the mesh and restricting water circulation (cage controls also received simulated scrubbing disturbance).

Plants were randomly sampled, untied at the base and scooped out with a 250 μm mesh net, and immediately replaced with a new plant. Plants were assigned random coordinates at the beginning of the experiment such that no plant would be sampled twice. Four stems of the low complexity macrophyte, *Eleocharis*, were considered an "individual" plant to roughly equate (by overall canopy volume in the water column) to the four-stem structure of *Myriophyllum*, for the purposes of sampling. All samples and attached periphyton and macroinvertebrates were preserved with 5% formalin.

Upon return to the laboratory, the plants were gently cleaned of periphyton and macroinvertebrates with forceps and a toothbrush under running water over a 250 μm sieve. The macroinvertebrates were picked from the periphyton and identified to genus where possible (see Appendix B for identification keys for both macroinvertebrates and periphyton). The periphyton was frozen for compositional and biomass analyses, the details of which are presented in Chapter 5.

A total of 77 macroinvertebrate taxa were identified which – if I were to conduct individual analyses on each taxon would significantly increase my chances of obtaining Type I errors – required *a priori* decisions to be made regarding choosing

and lumping of taxa (see Appendix C for a complete list of taxa). As I was interested in the effects of macrophyte shape and predator treatment on the overall community structure rather than individual taxa, I analysed the total abundance, taxon richness, and community composition of the macroinvertebrate assemblage.

Habitat complexity and fish predators can commonly affect the size-structure of a macroinvertebrate assemblage (Strayer 1991) so the total abundance was also split into size classes: < 5mm, 5-10 mm, 10-15 mm, 15-20 mm, and above 20 mm. Invertebrates in the <5 mm size class were not split into further size divisions for two reasons. Firstly, the smallest pygmy perch used in the experiment were 30 mm long, and the smallest measurement across the mouth of a 30 mm fish (the width) was 5 mm, so I assumed that potential prey in spaces < 5 mm wide would not be available. Secondly, animals < 5 mm had to be picked and teased from the periphyton and as periphyton can provide some refuge from fish predation (Power 1992, Johnson et al. 1996), and pygmy perch do not graze on periphyton (Humphries 1995), I assumed these prey were unavailable to pygmy perch. Ostracods proved the only exception due to their high mobility and this taxon was analysed in a separate group (see below).

I was also interested in potential indirect or cascading effects on the rest of the community, including the periphyton, so I grouped taxa on the basis of trophic status and their vulnerability to pygmy perch predation. Invertebrate predators formed one group and over 92% consisted of the coenagrionid damselfly, *Ischnura heterosticta tasmanica*. Other odonates (*Austrogomphus guerini*, *Austroaeshna* sp., *Aeshna* sp. and *Austrolestes analis*), naucorid hemipterans (*Naucoris congrex*) and dytiscid adults (*Antiporus* sp.) comprised the remainder. While tanypod larvae were relatively common in the macroinvertebrate samples, they were under 5 mm in length and embedded in the periphyton, and were therefore not considered at risk from pygmy perch predation for the aforementioned reasons.

The second group, 'vulnerable invertebrate herbivores', consisted of ostracods, the amphipod *Austrochiltonia australis*, and the red spinner mayfly *Atalophlebia australis*. These taxa were chosen by classifying all taxa according to their relative vulnerability to pygmy perch predation. Herbivore vulnerability was assessed by adapting Rader's (1997) scheme for classifying invertebrate availability to visually feeding salmonids. As Rader's scheme was developed for drifting invertebrates, I devised four traits that were more appropriate to macrophyte-dwelling invertebrates as

follows (see also Table 1). "Abundance" denoted the average total abundance of each taxon collected over the duration of the experiment, as I assumed a more abundant taxon would be more likely to be preyed upon by a generalist fish predator such as pygmy perch (Humphries 1995). "Body size" was split into two categories, under and over 5 mm (as measured by body length), and taxa were scored according to the largest size observed during the experiment, as larger invertebrates have been shown to be targeted by fish predators (Diehl 1992). "Mobility/exposure" distinguishes between taxa that tended to be relatively immobile and hidden within periphyton versus those taxa which tended to be active swimmers and thus more exposed to visually-feeding pygmy perch. Active macroinvertebrates have also shown to be preferentially targeted by fish predators (Crowder and Cooper 1982, Fuller and Rand 1990) and taxa were scored for this trait based on personal observations and the published literature (Williams 1980, Hawking and Smith 1997, Gooderham and Tsyrlin 2002). The fourth trait quantifies the frequency of occurrence of the taxon in the gut contents of pygmy perch. This was quantified using Humphries' (1995) analysis of the gut contents of 365 pygmy perch from the Macquarie River over one year. The average percentage contribution of prey to the gut contents used here were determined from fish over 30 mm in size (Humphries 1995). For each trait, low scores indicated low vulnerability and high scores indicated high vulnerability to predation. Scores for each trait were added for each taxon, the total scores ranked, the three highest ranking taxa forming the group 'vulnerable invertebrate herbivores'(see Appendix D).

Data analyses

Both multivariate and univariate analyses were used to assess effects of habitat complexity and fish predation on the macroinvertebrate community. Details regarding the periphyton analyses will be discussed in Chapter 5.

Analyses of the community structure of macroinvertebrates were conducted with non-parametric multivariate analysis of variance (NP-MANOVA, Anderson 1999). This model has not been developed for repeated measures analysis so separate tests were performed for three sampling dates: 2, 26 and 30 weeks. One of the control cages was dragged from the river by stock on both the 6 and 10 week sampling events, thus creating a missing cell in these data sets and precluding analysis using this method

(M.A. Anderson, Auckland University, *pers. comm.*). As it was a control cage, its absence was considered to have no effect on patterns of pygmy perch predation which, if present, would arise in the enclosure vs. exclosure comparisons. The data were fourth-root transformed (stronger than log transformation, Downing 1979) prior to computing Bray-Curtis dissimilarities, and post-hoc comparisons were conducted for significant effects and interactions. Non-metric multidimensional scaling (MDS) was performed on the data from all sampling events to provide a visual representation of the separation of treatments on the basis of community composition differences, and IndVal Version 2.0 (Dufrene and Legendre 1997) was used to determine which taxa characterised community differences between treatments. IndVal derives indicator species from within-species patterns of abundance and occurrence between sample units, thus an indicator species is present in most sample units, but characterises only a few (Dufrene and Legendre 1997).

The univariate analyses were conducted with general linear models and tested the effects of macrophyte shape and pygmy perch predation on the total macroinvertebrate abundance, the taxon richness, and the invertebrate predator and the vulnerable invertebrate herbivore groups. These analyses also tested the within-subjects repeated-measures and the time-of-start effects. Given the design, there were two types of univariate analyses conducted: the "summer-start" analyses refer to the repeated measures tests on the 3 summer-start blocks that were initiated in January and ran for the full 30 weeks; there were 5 sampling events, or repeated measures, in these tests. These summer-start tests therefore allowed an assessment of effects throughout time. The "winter-start" analyses refer to the sampling events at 26 and 30 weeks, and encompass both the 3 winter-start blocks initiated in July, and the 3 summer-start blocks continuing from January (Figure 1). These winter-start tests therefore allowed a test of the timing of the initiation of the experiment, hereafter referred to as the "time-of-start" factor. The data were log-transformed where necessary (to meet the assumptions of normality for these tests), and planned comparisons were conducted on the main effects of macrophyte shape (*Eleocharis* v. *Triglochin*, and *Triglochin* v. *Myriophyllum*) and predator treatment (control v. access, access v. predators enclosed, and predators enclosed v. predators excluded) where they were significant. The univariate analyses were conducted using SYSTAT Version 9 (Wilkinson 1999) and SAS Version 6.12 (SAS Institute Inc. 1990).

Analyses of the macroinvertebrate size classes were abandoned for two reasons. Firstly, macroinvertebrates under 5 mm comprised over 99% of the total macroinvertebrate abundance and therefore displayed the same patterns as the total macroinvertebrate abundance. Secondly, macroinvertebrates in the invertebrate and vulnerable herbivore groups included those in the remaining size classes and therefore showed similar patterns to these groups. Thus I concluded that the groupings analysed here were sufficient to determine effects of pygmy perch predation on community structure in this system.

Results

Multivariate analyses

Non-parametric MANOVA at 2 weeks showed macrophyte shape significantly affected macroinvertebrate community composition ($F_{2,24} = 5.037$, $p<0.001$). *Eleocharis* supported a different community from those on *Triglochin* ($t_2 = 2.267$, $p<0.001$) and *Myriophyllum* ($t_2 = 2.863$, $p<0.001$). Macrophyte shape also significantly affected community composition at 26 weeks ($F_{2,60} = 8.008$, $p<0.001$), where the community on *Eleocharis* differed from that on *Triglochin* ($t_2 = 2.273$, $p<0.001$) and *Myriophyllum* ($t_2 = 3.816$, $p<0.001$), and the communities on *Triglochin* and *Myriophyllum* differed from each other ($t_2 = 2.089$, $p<0.001$). At 30 weeks, the communities on each macrophyte were again different from one another ($F_{2,60} = 8.905$, $p<0.001$): the *Eleocharis* community was different from the *Triglochin* ($t_2 = 2.279$, $p<0.001$) and *Myriophyllum* ($t_2 = 4.136$, $p<0.001$) communities, which also differed from each other ($t_2 = 2.189$, $p<0.001$). At no point were there any significant interactions between the main effects (all $p>0.604$), nor any significant effects of predator treatment on the macroinvertebrate community (all $p>0.076$).

MDS of the data from all sampling events (including 6 and 10 weeks) resulted in a two dimensional solution with a stress of 0.107. Ordination plots clearly showed a greater clustering of samples on *Myriophyllum* than *Eleocharis*, with samples on *Triglochin* showing an intermediate separation (Figure 2). There was a clear separation of samples between seasons on all macrophytes: the cages at 2, 6 and 10 weeks (summer-start cages only) clustered together and did not overlap with any cages sampled at 26 and 30 weeks. Furthermore, in the winter samples there was a

separation between the summer-start and winter-start cages indicating an effect of the time-of-start of the experiment (Figure 2).

IndVal showed the communities on *Triglochin* and *Eleocharis* were characterised by the absence rather than the presence of taxa. The highest indicator value for *Eleocharis* was Ceratopogonidae at 22%, and for *Triglochin* was the freshwater limpet *Ferrissia tasmanica* at 18.5%. *Myriophyllum* had 26 significant indicator taxa, the highest indicator values belonging to the amphipod *Austrochiltonia australis* at 74.4%, and the coenagrionid damselfly *Ischnura heterosticta tasmanica* at 70% (Table 2).

Univariate analyses

Total macroinvertebrate abundance – Overall, the total abundance of macroinvertebrates increased over time and was highest on *Myriophyllum*, the most structurally complex plant shape (Figure 3a). The summer-start analysis revealed a significant interaction between macrophyte shape and time ($F_{8,80} = 3.492$, $p<0.005$). While the fauna was consistently most abundant on *Myriophyllum*, it was generally higher on *Triglochin* than *Eleocharis*, except at 6 and 10 weeks (Figure 3a). There was no effect of predator treatment on macroinvertebrate abundance in the summer-start analysis ($F_{3,20} = 0.548$, $p>0.655$).

The winter-start analysis showed a significant interaction between macrophyte shape and the time-of-start ($F_{2,92} = 8.68$, $p<0.001$) as macroinvertebrate abundance was greater in the summer-start blocks than the winter-start blocks, but the magnitude of this effect varied with macrophyte shape (Figure 3b). The difference between summer- and winter-start blocks was greatest for *Myriophyllum* (58.0%) and least for *Eleocharis* (31.2%). A significant overall effect of predator treatment on macroinvertebrate abundance ($F_{3,92} = 5.45$, $p<0.002$; Figure 3c) was due to the highest abundance in the predator access cages; 47.2% more than in the cage controls ($F_{1,92} = 15.71$, $p<0.001$), and 32.3% more than in the predator enclosures ($F_{1,92} = 7.37$, $p<0.01$). This may be due to cage effects as abundance did not differ between predator enclosures and exclosures. Finally, there was a significant overall effect of time in the winter-start analysis ($F_{1,92} = 22.05$, $p<0.001$), with 42.6% more macroinvertebrates at 30 weeks than 26 weeks regardless of time-of-start.

Taxon richness – Taxon richness was strongly affected by macrophyte shape and showed some changes over time, but was unaffected by predator treatment. In the summer-start analysis, the pre-planned comparisons showed that taxon richness was greatest on *Myriophyllum* at all times, with 9.3% (all $p<0.05$) fewer taxa on *Triglochin* and 34.9% (all $p<0.001$) fewer taxa on *Eleocharis* ($F_{2,20} = 54.661$, $p<0.001$; Figure 4a). Likewise, there were more taxa on *Myriophyllum* than either *Triglochin* (18.7%; all $p<0.001$) or *Eleocharis* (44.9%; all $p<0.001$) in the winter-start analysis ($F_{2,92} = 99.66$, $p<0.001$; Figure 4c). Taxon richness in the summer-start blocks showed a 20% decrease after 10 weeks which coincided with the end of summer ($F_{4,80} = 14.825$, $p<0.001$; Figure 4b). The winter-start analysis revealed a significant interaction between time and time-of-start ($F_{1,92} = 9.90$, $p<0.002$); taxon richness showed a sharper increase between weeks 26 and 30 for the winter-start blocks while there was little change between these dates for the summer-start blocks (Figure 4d). Predator treatment had no effect on taxon richness in either the summer-start ($F_{3,20} = 0.353$, $p>0.787$) or winter-start ($F_{3,92} = 1.12$, $p>0.344$) analyses.

Invertebrate predators – Invertebrate predators were affected by all treatment factors but generally occurred at low abundances, averaging <1% and never contributing more than 6% to the total macroinvertebrate abundance. The summer-start analysis showed invertebrate predator abundance varied significantly over time ($F_{4,80} = 14.673$, $p<0.001$), peaking at 6 weeks after which it steadily declined (Figure 5a). There was also a significant interaction between macrophyte shape and predator treatment in the summer-start analysis ($F_{6,20} = 3.596$, $p<0.02$). In *Myriophyllum*, there were more invertebrate predators, and they were more abundant in the control and access cages than the fish enclosures and exclosures, suggesting a cage effect, but these differences were not apparent in the other two plant-types (Figure 5b). There were no significant effects involving predators in the winter-start analysis ($F_{3,92} = 2.13$, $p>0.102$); overall abundances were much lower in the winter-start than the summer-start blocks, with the greatest differential on *Myriophyllum* (Figure 5c) which was responsible for the significant interaction between macrophyte shape and time-of-start ($F_{2,92} = 9.48$, $p<0.001$).

Vulnerable invertebrate herbivores – Vulnerable invertebrate herbivores (amphipods, ostracods and the red spinner mayfly) averaged 8% of the total invertebrate abundance, but contributed up to 52% in some treatment combinations. Their

abundance varied over time, and was affected by both macrophyte shape (being consistently greater on *Myriophyllum*), and predator treatment. The summer-start analysis showed a significant interaction between macrophyte shape and predator treatment ($F_{6,20} = 5.772$, $p<0.001$; Figure 6a); vulnerable herbivores were twice as abundant in the fish exclosures than the enclosures, but only in *Eleocharis* and *Myriophyllum*. In *Triglochin*, no such pattern was seen, and herbivores were slightly more abundant in the access cages (Figure 6a). However, both these main effects interacted separately with time (macrophyte shape: $F_{8,80} = 2.374$, $p<0.02$; predator treatment: $F_{12,80} = 2.175$, $p<0.04$). The changes over time were most striking in *Myriophyllum* where herbivores decreased in abundance until 10 weeks (summer's end) before increasing again (Figure 6b). The same trend was observed in each predator treatment except the pygmy perch enclosures where herbivores did not increase in abundance after 10 weeks (Figure 6c).

Similar patterns of vulnerable herbivore abundance were observed in the winter-start analysis. A significant interaction between macrophyte shape and predator treatment ($F_{6,92} = 2.77$, $p<0.02$) again showed the strongest predator effect in *Myriophyllum*; there were fewer herbivores in the pygmy perch enclosures (Figure 7a). There was no predator treatment effect in *Eleocharis*, and in *Triglochin* there were more herbivores in the access treatment. A significant interaction between macrophyte shape and time-of-start ($F_{2,92} = 3.87$, $p<0.03$) showed that while herbivores were consistently more abundant in the summer-start than the winter-start blocks, this difference was much greater in *Myriophyllum* than either *Triglochin* or *Eleocharis* (Figure 7b). The abundance of herbivores also increased by 38% between 26 and 30 weeks ($F_{1,92} = 6.25$, $p<0.02$) and this was independent of any treatment factors.

In summary, the total macroinvertebrate abundance and taxon richness, the abundance of invertebrate predators and the abundance vulnerable invertebrate herbivores, were all strongly affected by plant shape; all were greater on the structurally complex *Myriophyllum*, followed by *Triglochin*, and the structurally simple *Eleocharis*. All groups varied over time, and all showed significant time-of-start effects, occurring at lower abundances in the winter-start than in the summer-start blocks, although these differences were often most pronounced for the most complex plant, *Myriophyllum*. Finally, despite some suggestion of cage effects on the total macroinvertebrate

abundance and invertebrate predators, there were clear effects of fish predators on vulnerable macroinvertebrate herbivores.

Discussion

This experiment tested the degree to which habitat structure can influence community structure through its effects on predation. My expectations that pygmy perch effects would be greatest in the structurally simple habitat and smallest in the most structurally complex habitat were not supported. Habitat structure, as represented by the macrophyte shape, was a much stronger force shaping these communities than the effects of predation by pygmy perch. I will address the effects of macrophyte structure and fish predation separately, and then their interactive effects in the ensuing discussion.

Habitat Structure

Multivariate and univariate analyses showed that all the community variables analysed were significantly influenced by macrophyte structural complexity. The composition, total abundance and taxon richness of macroinvertebrates were all greatest on *Myriophyllum*, as were the community subsets of invertebrate predators and vulnerable invertebrate herbivores. The persistence of these patterns through both space and time clearly shows that plant shape is an important regulator of macroinvertebrate community structure in the Macquarie River.

Each macrophyte was characterised by a different community, and in particular, *Myriophyllum* was characterised by amphipods, chironomids, coenagrionid damselflies, lymnaeid gastropods, mayflies and caddisflies. These taxa represent a fairly typical community to be found on macrophytes in freshwater systems (Bayly and Williams 1981, Chilton 1990, Humphries 1996), and it is likely they characterised the *Myriophyllum* community because they were so much more abundant on this plant. Given most of these taxa were also herbivorous, it is possible they characterised *Myriophyllum* due to the greater periphyton biomass it supported (Chapter 5). The *Eleocharis* community was characterised by ceratopogonid larvae, and freshwater limpets characterised the *Triglochin* community as the strap-like leaves of this plant were probably a more suitable substrate for limpets than the highly-dissected leaf structure of *Myriophyllum*.

Different macroinvertebrate communities on different macrophyte species have often been attributed to habitat structure – a more structurally complex habitat will support a more diverse and abundant community (Heck and Orth 1980, Crowder and Cooper 1982, Orth et al. 1984, Rooke 1986, Heck and Crowder 1991, McCoy and Bell 1991, Humphries 1996, Crowder et al. 1998), and my results support this contention. A structurally complex habitat provides more surface for food collection and attachment (therefore greater food resources), and more space that acts as a refuge from predation (Heck and Wetstone 1977, Heck and Crowder 1991, Crowder et al. 1998, Diehl and Kornijow 1998). Implicit in this argument is that surface area is greater in a more complex habitat (Heck and Orth 1980). Although it has proved difficult to disentangle the separate elements of surface area and plant architecture, it has been shown that macroinvertebrates can be more abundant where there is more surface area (Krecker 1939, Dvorak and Best 1982, Parker et al. 2001), and can respond to surface area over any architectural features of the habitat (Stoner and Lewis 1985, Dean and Connell 1987).

However, surface area alone is an insufficient explanation of the patterns found in this experiment. While there was an obvious difference in morphology between the macrophytes, in terms of the number and type of leaves, the plants of medium (*Triglochin*) and high (*Myriophyllum*) complexity had almost identical surface areas (820 cm^2 and 860 cm^2 respectively; see Chapter 2), yet there was a consistently higher abundance and diversity of macroinvertebrates on *Myriophyllum*. When Parker et al. (2001) corrected for surface area, epifaunal abundance was greater on more structurally complex seaweeds, and Kershner and Lodge (1990) showed that snails colonised more structurally complex substrates when surface area was held constant. At a larger scale, Brown et al. (1988) found macroinvertebrates were related to the structural heterogeneity of macrophyte beds rather than the surface area of macrophytes themselves. Jeffries (1993) tested the influence of macrophyte structural complexity while holding surface area constant and found that complexity had a strong effect on total abundance and diversity of macroinvertebrates. Thus, it appears the shape of the habitat rather than surface area *per se* can be responsible for the abundance and diversity of macroinvertebrate communities.

Absolute surface area is not equivalent to available surface area, and macroinvertebrates may be responding to the proportion of surface area that affords

them a refuge from predation. This would explain the predator refuge function of structurally complex macrophytes (Crowder and Cooper 1982). On *Eleocharis*, there is no surface area inaccessible to pygmy perch, and therefore no refuge from predation. On *Triglochin*, macroinvertebrates can hide from predators by moving from one side of the strap-like leaf to another, but they are still exposed to predation. The base of the leaves – where they form a tuft – is inaccessible to pygmy perch due to their size, and thus forms the only absolute refuge from predation on *Triglochin*. This is not a large proportion of the overall surface area of the plant. On *Myriophyllum*, only macroinvertebrates on the outside of the leaflets are at risk of predation because pygmy perch cannot fit through the gaps to get to the inside, the predator-free surface on the inside of the leaflets comprises over half the plant's surface area. The structure of *Myriophyllum* therefore affords the greatest amount of surface area on which macroinvertebrates are safe from pygmy perch predation.

The greater abundance and richness of macroinvertebrates on *Myriophyllum* supports the hypothesis of more available (or predator-free) surface area and therefore a lesser risk of predation on this plant. It is possible that the perception of predation risk was enough to cause a behavioural response by prey – such that they occurred in a "safer" environment (Pierce 1988, Forrester 1994). Such adaptive behaviour in response to the threat of predation has been shown for both fish (Werner et al. 1983, Jacobsen et al. 1997) and invertebrates (Welborn and Robinson 1987, Forrester 1994, McIntosh and Townsend 1996, Clark and Messina 1998). However, this argument assumes prey are capable of assessing and responding to the risk of predation, which then assumes predators would have an impact in habitats offering less refuge; mostly, this was not the case in this experiment.

Fish predation

The effect of pygmy perch predation on the macroinvertebrate community was almost as weak as the effect of habitat structure was strong: pygmy perch had no effect on the composition, abundance or richness of the overall macroinvertebrate community. However, there were predator treatment effects on parts of the community: invertebrate predators and vulnerable herbivores.

The lack of a predator effect on the abundance, richness and composition of the macroinvertebrate community was unexpected, as fish predators have commonly been

found to negatively influence their macroinvertebrate prey (Crowder and Cooper 1982, Diehl 1992), sometimes creating strong indirect effects on other parts of the community (Martin et al. 1992, Power 1992). However, the evidence from vegetated littoral systems is equivocal in its support for the strong regulatory role of fish predators. Some studies have found fish predator effects to be negligible in vegetated systems (Thorp and Bergey 1981, Gilinsky 1984, Johnson et al. 1996, Pierce and Hinrichs 1997) which has led to the suggestion that fish predation may not be as important a regulator of macroinvertebrate community structure as the type of habitat in which it occurs (Leber 1985, Carlisle and Hawkins 1998).

There are two reasons why predation by pygmy perch may not structure the macroinvertebrate community. Firstly, this macrophyte-associated community in the Macquarie River is very diverse and speciose which allows the effects of predation to travel many indirect routes through the food web (Strong 1992). This diversity is not conducive for simple, sharply defined trophic levels, and therefore precludes the strong effects of predators sometimes seen (Polis 1999). It is likely the presence of structurally complex macrophytes supports this diversity, as I have found here, and thereby prevents strong trophic links (Power 1992, Strong 1992). Secondly, pygmy perch are omnivorous, consuming prey from different trophic levels (Humphries 1995) as do many benthivorous fish (Morin 1984, Diehl 1988). Omnivory, by its very nature, does not encourage clear separation between trophic levels, and therefore could also explain the lack of predator effects on the overall community assemblage in this system (Polis 1991).

Nevertheless, fish predators are still able to affect subsets of the community (Diehl 1988, Polis et al. 2000), and predator treatment effects were observed for invertebrate predators and vulnerable invertebrate herbivores (the latter is discussed later in the next section, *Fish Predation and Habitat Structure*).

Predator treatment effects were found for invertebrate predators; however, I suspect they were due to cage artifacts rather than any impacts of pygmy perch. Invertebrate predators were more abundant in the control and access cages than the enclosure and exclosure cages, which indicated an effect of the closed cages. In caging experiments, too fine a mesh size can overestimate predator effects by preventing sufficient immigration of prey (Englund 1997), so I used a relatively large mesh size to allow as much prey movement as possible. While this may underestimate predator effects,

pygmy perch were enclosed in cages for eight months (and were intended to be enclosed for a year), which I considered would translate to a very great predation pressure on the prey community, and I wanted to prevent overexploitation of the prey community during this time.

It is possible the access cages granted access to fish other than pygmy perch, such as brown trout (*Salmo trutta*) and redfin perch (*Perca fluviatilis*) which are also present in the Macquarie River and may have encouraged invertebrate predator abundances by disturbing other prey on the plants. If this was the case, then the access cages confounded pygmy perch access with the access of larger fish, and thus were not an effective control for the enclosure of pygmy perch. However, the control cages also permitted access by fish other than pygmy perch but did not show the same patterns of macroinvertebrate abundance, therefore this reason can be discounted.

Finally, another cause of cage effects may have been due to the build-up of periphyton on the cage sides, which despite scrubbing monthly, was slightly greater on the access cages (Chapter 5). The closed cages may have reduced the amount of light reaching the sides, hence there was less periphyton growth on the cage walls. The lower plant density in the *Triglochin* treatments may have also allowed for more periphyton growth (by allowing more light), leading to the slight cage effect on vulnerable invertebrate herbivores on this plant. Alternatively, the open cages may have experienced more disturbance by the movement of fish in and out of them, which may have actually stimulated more periphyton growth.

While I cannot be sure about the causes of the cage effects, where they were present there were no differences between the closed cages (the pygmy perch enclosures and exclosures) therefore I am confident the observed effects of pygmy perch on vulnerable invertebrate herbivores were real and not experimental artifacts.

Fish predation and habitat structure

It has often been shown that the foraging success of fish predators decreases as habitat structure increases (Heck and Thoman 1981, Crowder and Cooper 1982, Savino and Stein 1982, Stoner 1982, Leber 1985, Gotceitas and Colgan 1989, Nelson and Bonsdorff 1990, Bettoli et al. 1992, Persson and Eklov 1995, Swisher et al. 1998). While most of these studies have only measured habitat structure by macrophyte density, those that have tested macrophyte shape have found similar effects (Chapter

2, Coull and Wells 1983, Diehl 1988, Dionne and Folt 1991). These studies maintain the argument that fish predation should be a strong regulator of invertebrate community structure in vegetated systems, yet the results from this experiment do not support this contention; pygmy perch did not structure the overall macroinvertebrate community. Generally, fish predators have their greatest impacts where there is no habitat structure, such as pelagic lake systems (Carpenter et al. 1987) or unvegetated areas of the littoral zone (Diehl 1992). That the introduction of habitat structure, and increasing its structural complexity, can markedly reduce predator success suggests that the effects of predation depend on the habitat in which they are occurring.

Pygmy perch may not have influenced the overall macroinvertebrate community but they did have a negative impact on a subset of the community; the abundance of vulnerable herbivores was consistently greater in the predator exclosure cages than the predator enclosures. These invertebrates were classified as vulnerable due to their larger size, greater mobility and exposure, and increased likelihood of appearing in pygmy perch guts, and the significant impact on their abundance by pygmy perch supports this assessment. It also corroborates previous research showing fish predators, when faced with a diverse array of prey, tend to target large, active prey (Mittelbach 1981, Leber 1985, Main 1987, Lodge et al. 1988, Heck and Crowder 1991, Strayer 1991). The impact of pygmy perch on vulnerable invertebrate herbivores varied with macrophyte shape, but not in the manner I was expecting.

Pygmy perch affected the abundance of vulnerable invertebrate herbivores in *Eleocharis*, the structurally simple macrophyte, but unexpectedly, had their greatest impact in the most structurally complex plant shape, *Myriophyllum*; there were significantly more vulnerable invertebrate herbivores on *Myriophyllum* when pygmy perch were excluded. This may be due to there being simply more prey on *Myriophyllum* (particularly as these vulnerable taxa were also among the taxa that characterised the *Myriophyllum* community) and therefore more opportunity for pygmy perch to capture prey, but then I would expect greater predation effects in more structurally complex habitats to be a common pattern in the literature.

In the only published study to find a similar result, Stoner (1982) assessed the effectiveness of a fish predator in three different seagrass species in the laboratory and found the pinfish, *Lagodon rhomboides*, captured the most prey in *Halodule wrightii*, the seagrass he considered the most structurally complex with many narrow leaves

and the highest surface area to volume ratio. He suggested the amphipod prey were less able to conceal themselves from predation compared to broader-leaved seagrasses, a suggestion also postulated by Edgar (1983) for seaweeds. *Myriophyllum* had a finely-dissected leaf structure with very narrow leaflets which may have made prey more easy to detect, but unlike the seagrass *Halodule*, prey moving to the other side of the leaf were protected from predation because the leaves of *Myriophyllum* face inwards to the stem and create a space inaccessible to pygmy perch. Thus, rather than increasing the exposure of prey, the intricate structure of *Myriophyllum* should theoretically reduce prey exposure if prey are utilising this space. It is possible that the vulnerable macroinvertebrates may not have utilised this space inside the *Myriophyllum* leaves, staying on the outside and therefore exposing themselves to pygmy perch predation. But then I would have expected to see little difference in the abundance of these herbivores between macrophyte shapes as there was little, if any, refuge space available on *Eleocharis* and *Triglochin*.

The greatest effect of predation in the most structurally complex macrophyte may be due to the possibility that pygmy perch were able to perceive the more complex habitat and change their predation strategy accordingly (Savino and Stein 1982, Ryer 1988). Savino (1982) observed the piscivorous largemouth bass, *Micropterus salmoides*, switched from a searching strategy to an ambush strategy as their movement became impeded by increasing artificial macrophyte density, and their bluegill sunfish prey shifted their behaviour from schooling to dispersing amongst plant stems. James and Heck (1994) found predatory success of seahorses was unaffected by the density of artificial seagrasses because the seahorse shifted from an ambush strategy to a search strategy with increasing density. Personal observations of pygmy perch suggest they shift their foraging strategies in a similar way (Chapter 3). In the Macquarie River, pygmy perch are at risk of predation by piscivorous trout, *Salmo trutta*, and redfin perch, *Perca fluviatilis*, and in a simple habitat forage as an ambush predator with little movement. In a more complex habitat where they have cover from piscivorous predators, they adopt a searching strategy (Chapter 3). As has been shown for other small fish, their small size prevents the habitat structure being an impediment to their movement (Gilinsky 1984), thus, by increasing their movement with structural complexity, pygmy perch may have been able to find and capture more prey in *Myriophyllum*. This result is further discussed in Chapter 6.

Temporal and seasonal variation

The major patterns of the effects of macrophyte shape and pygmy perch predation on macroinvertebrate community structure were consistent, persisting through time and space. The summer-start analyses gave an indication of changes in macroinvertebrate structure over a 30 week period, while the winter-start analyses allowed the timing of the experimental initiation to be assessed.

The macroinvertebrate taxon richness, and the abundance of invertebrate predators and herbivores, all showed a decrease at around 10 weeks after initiation, after which they began to gradually increase. This increase in abundance over winter may reflect the fact that peripyhton biomass (and dissolved oxygen concentrations, Humphries 1995) also increased over time (Chapter 5), possibly reflecting organic enrichment due to agricultural runoff in the catchment (Davies and Humphries 1996).

The dip in macroinvertebrate abundance at 10 weeks(Tabachnick and Fidell 2001, Quinn and Keough 2002) corresponded to late summer in Tasmania and probably reflected insect emergence patterns. Seasonal effects, such as insect emergence, have been noted in the structure of macroinvertebrate communities (Mittelbach 1981, Brown et al. 1988), including those of the Macquarie River (Humphries 1996). Humphries (1995) noted that while pygmy perch consumed amphipods and ostracods year-round, the red-spinner mayflies were less common in pygmy perch guts over the winter months, reflecting their seasonal abundance. Interestingly, the vulnerable invertebrate herbivores in the fish enclosures showed decreased abundance at 10 weeks as in all other treatments, but differed by not increasing again. This suggests that while pygmy perch may have only weak effects on abundances, they may exert enough pressure on vulnerable herbivore populations to affect their resilience.

These potential seasonal effects, combined with personal observations of community structure and periphyton growth in the Macquarie River at different seasons, provided the impetus for incorporating "time-of-start" into the analysis. Seasonal differences in community structure are often considered to be obvious, yet are rarely taken into account in the design field experiments investigating community dynamics (Walters et al. 1988, Underwood and Anderson 1994). This design was used to control for potential confounding effects of season and distinguish seasonal responses from treatment responses (Hurlbert 1984, Walters et al. 1988), and also provided a control

for the time-dependence inherent in repeated-measures designs (Underwood and Anderson 1994). Underwood and Anderson (1994) submerged replicate panels at different seasons in an investigation of the recruitment and succession of intertidal fouling communities, and found that the season of submersion had a strong effect on the resultant communities. Likewise, Nandakumar (1996) repeated a 4 month experiment in each season to test the interaction between experimental initiation and season on subtidal community succession, finding striking differences in the dominance hierarchy of sessile organisms. In both these studies, the season of experimental initiation had a significant effect on the outcome of competitive interactions and therefore successional patterns. Studies that have considered time-of-start effects have been conducted on successional processes, and on marine subtidal, sessile communities, where season often has strong effects on the recruitment and settlement of marine organisms (Keough 1983). Thus, the design used here represents the first occasion where temporal and seasonal effects have been explicitly compared in a freshwater macroinvertebrate community.

While the abundances of macroinvertebrates were consistently lower in the winter-start than the summer-start blocks, the effects of macrophyte structure and pygmy perch predation were still consistent, albeit dampened. This corroborates the results of Thorp and Bergey (1981) who repeated an experiment investigating the relative impacts of indirect predator effects and thermal stress in three consecutive seasons, but found no seasonal differences in community responses to predation. However, their conclusions were based on informal comparisons rather than statistical tests of season. In my experiment, time-of-start effects were at their strongest in the structurally complex *Myriophyllum* and had their smallest effects in *Eleocharis*, possibly because the *Myriophyllum* community was characterised by more insect taxa which emerged before winter. This suggests that not only might habitat structure influence predator effects, but it may also influence the strength of seasonal effects on macroinvertebrate community composition.

Conclusions

In this system, macrophyte structural complexity clearly regulates macroinvertebrate structure, having a much stronger influence on community composition than predation, and its influence is consistent through time and between seasons. The

system here is physically complex and the community diverse, both of which can act to buffer effects of predation. Diehl (1988) has noted that while predators can affect subsets of the prey community, the structure of the habitat affects the entire prey community. While this was true for the Macquarie River community, pygmy perch only affected a subset of the entire macroinvertebrate community, these effects were unexpectedly strongest in the most structurally complex macrophyte. This was unexpected because the literature suggests more structurally complex plants support larger and more diverse macroinvertebrate communities by providing more refuge from predation. Here, it seems the most structurally complex macrophyte actually provided the least refuge. If a small fish like pygmy perch is not impeded by a more complex structure, and they shift their foraging strategy with increasing complexity as it becomes more difficult to detect prey, then that structurally complex habitat, by supporting more animals, may increase the encounter rate of predators with prey, and therefore may not provide a refuge from predation.

Table 1: Categories and scores used to classify invertebrates according to their vulnerability to predation by pygmy perch. Higher scores indicate greater vulnerability. Scores were calculated for all 77 taxa sampled during the experiment, the total scores were ranked and the three highest ranked taxa were grouped as vulnerable herbivores for univariate repeated-measures analyses (see Appendix C). These taxa were ostracods, the amphipod *Austrochiltonia australis*, and the red spinner mayfly *Atalophlebia australis*. The traits of abundance, body size, mobility/exposure and occurrence in pygmy perch guts are explained in the text.

Score	0	1	2	4
abundance	under 250	over 250	over 500	over 1000
body size	always under 5 mm	over 5mm		
mobility/exposure	relatively immobile, embedded in periphyton	limited movement over leaves, within periphyton	crawls over stems and leaves	active swimmers between stems and leaves
occurrence in pygmy perch guts	under 2.5%	under 5%	between 5-10%	over 10%

Table 2: Indicator taxa characterising the macroinvertebrate community on each macrophyte as determined by IndVal (significance is at $p<0.01$, apart from Ceratopogonidae which is not significant but has the highest indicator value for *Eleocharis*). IV is the indicator value (%).

Eleocharis		*Triglochin*		*Myriophyllum*	
Taxon	**IV**	**Taxon**	**IV**	**Taxon**	**IV**
Ceratopogonidae	22.00	*Ferrissia*	18.48	*Austrochiltonia*	74.38
		Aeshna	8.27	*Ischnura*	69.90
				Chironomini	60.78
				Naididae	60.68
				Physastra	60.28
				Tanytarsiini	59.53
				Copepoda	56.86
				Tanypodinae	56.08
				Tricladida	55.78
				Orthocladiinae	55.30
				Ostracoda	53.42
				Hydra	51.48
				Tasmanocoenis	50.78
				Hellyethira	45.69
				Atalophlebia	44.40
				Nemertea	38.69
				Notalina	38.60
				Ecnomus	38.44
				Centroptilum	38.01
				Orobatida	36.43
				Astigmata	21.59
				unidentified mites	18.82
				Sisyridae	15.88
				Leptocerus	15.75
				Hydromidae	15.16
				Naucoris	7.25

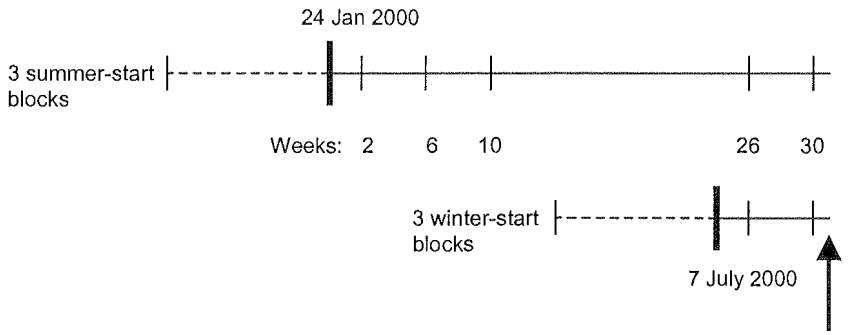

Fig 1: Schematic diagram illustrating the experimental design. The broken lines indicate the colonisation period, the bold ticks indicate the initiation of the experiment for the summer-start and winter-start blocks, and the remaining ticks indicate sampling events at 2, 6, 10, 26 and 30 weeks. The arrow indicates the flood event in August 2000. The summer-start analyses were conducted on the 3 summer-start blocks and had 5 sampling events over 30 weeks. The winter-start analyses encompassed both the winter-start blocks, and the 3 summer-start blocks continuing from January, and therefore tested a total of 6 blocks at 26 and 30 weeks.

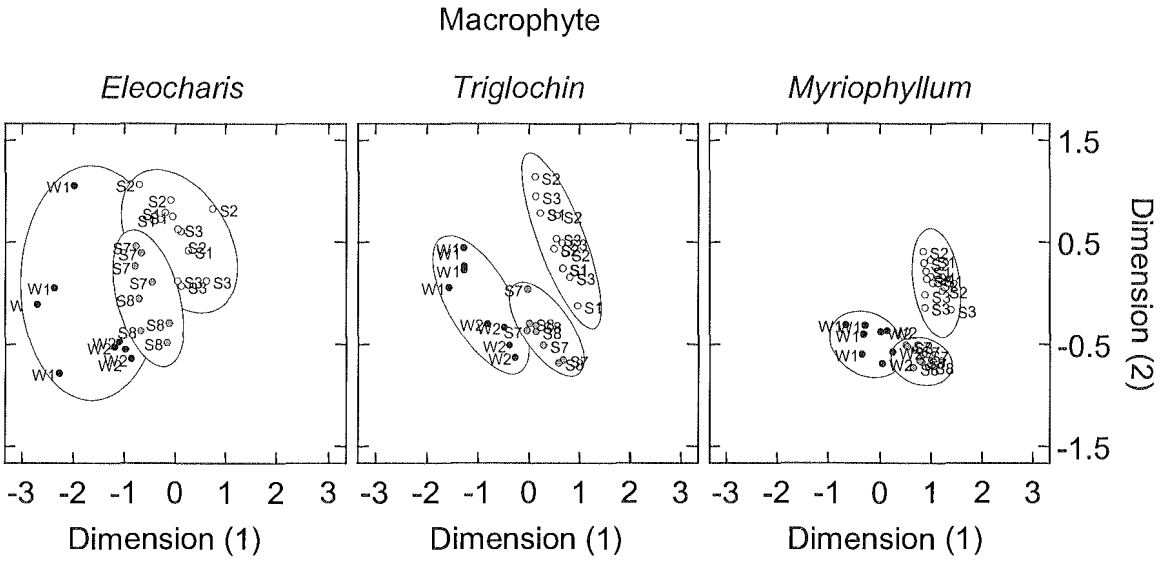

Figure 2: MDS plots showing the separation of macroinvertebrate communities, on each macrophyte and at each sampling event, along dimensions 1 and 2. The open circles represent the summer-start cages sampled at 2 weeks (S1), 6 weeks (S2) and 10 weeks (S3). The grey circles represent the summer-start cages sampled in winter, at 26 and 30 weeks (S7 and S8 respectively), and the black circles represent the winter-start cages sampled in winter (W1 and W2 respectively).

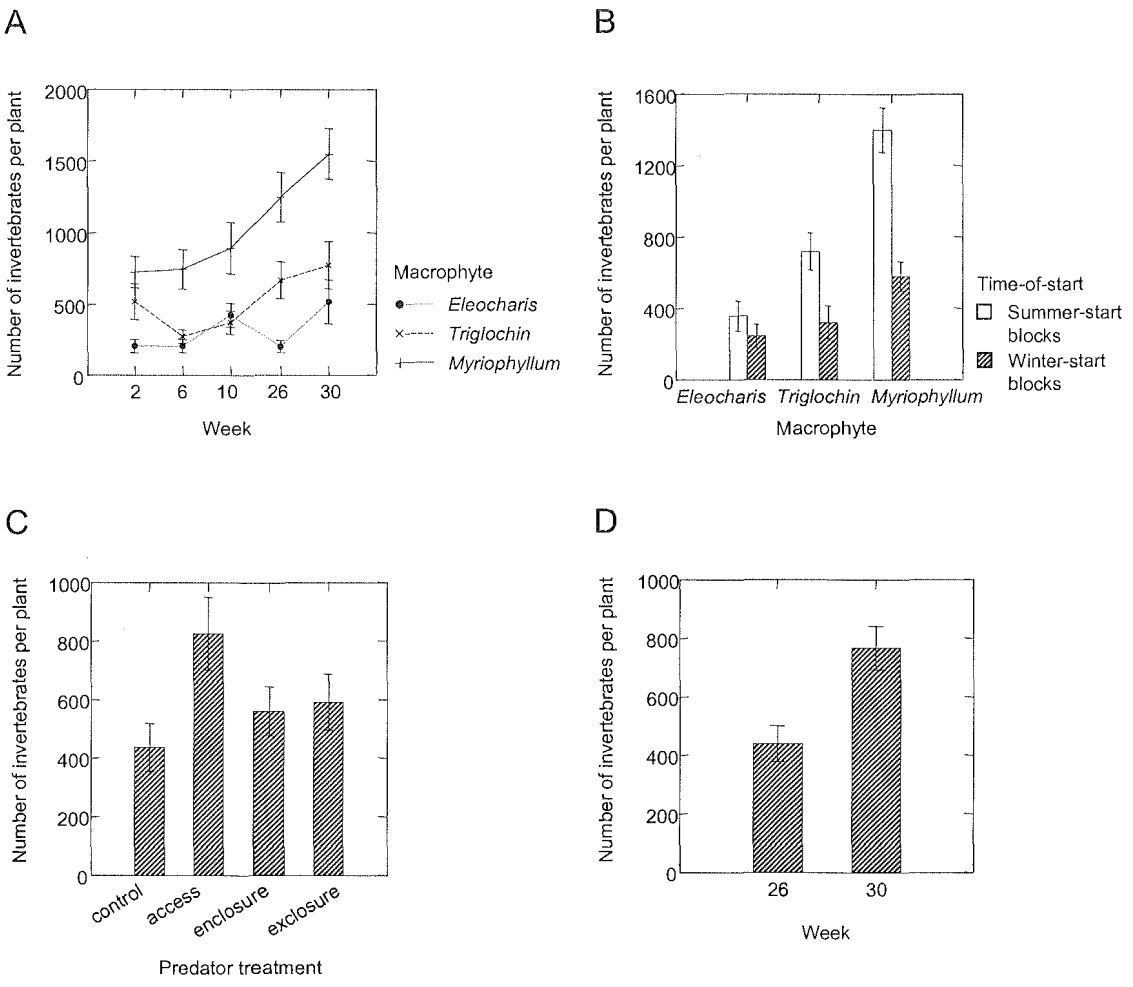

Figure 3: The total abundance of macroinvertebrates per plant on each macrophyte over time in the summer-start analysis (A); on each macrophyte in the winter-start analysis (B); in each predator treatment in the winter-start analysis (C); and at 26 and 30 weeks in the winter-start analysis (D). Error bars are one standard error about the mean.

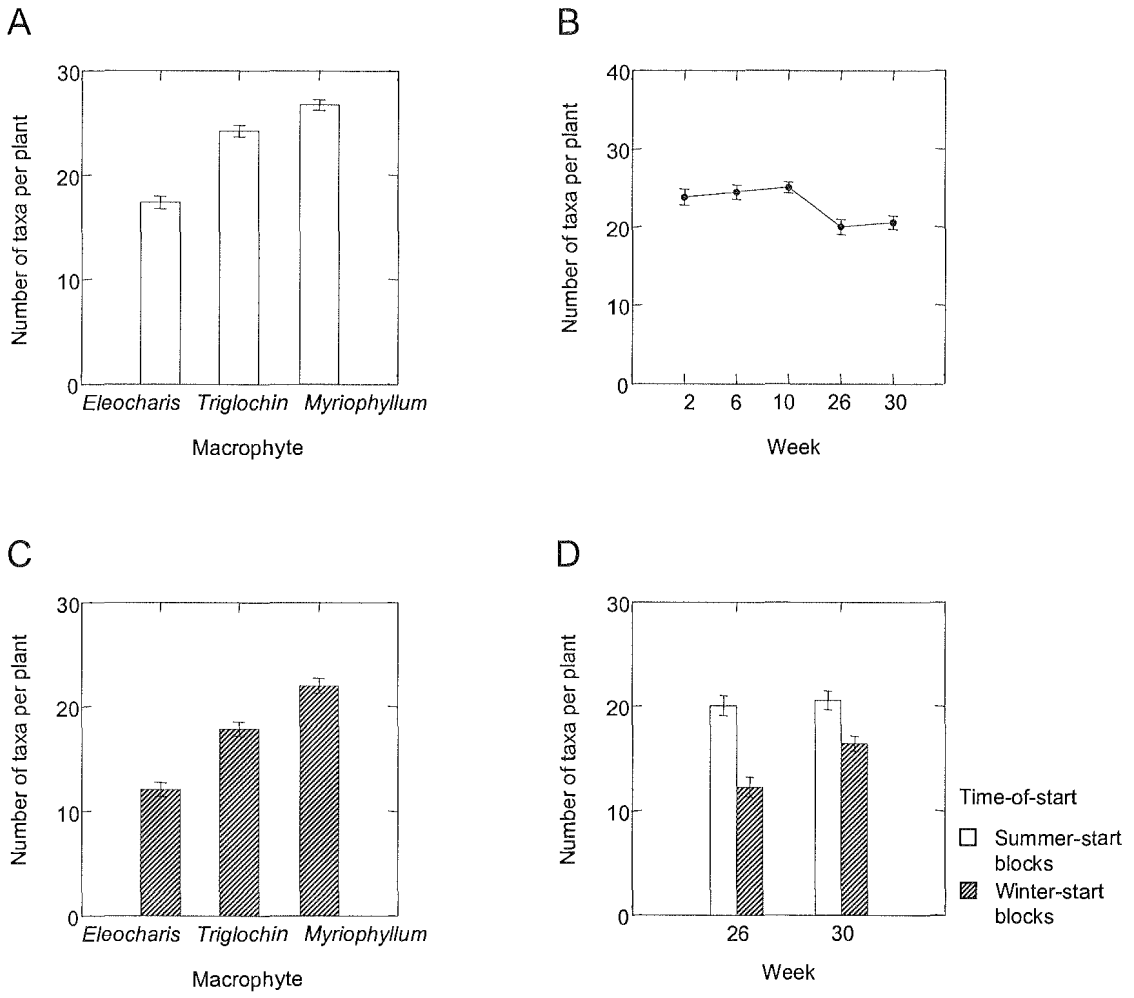

Figure 4: The total number of macroinvertebrate taxa per plant on each macrophyte in the summer-start analysis (A); over time in the summer-start analysis (B); on each macrophyte in the winter-start analysis (C); and at 26 and 30 weeks in the winter-start analysis (D). Error bars are one standard error about the mean.

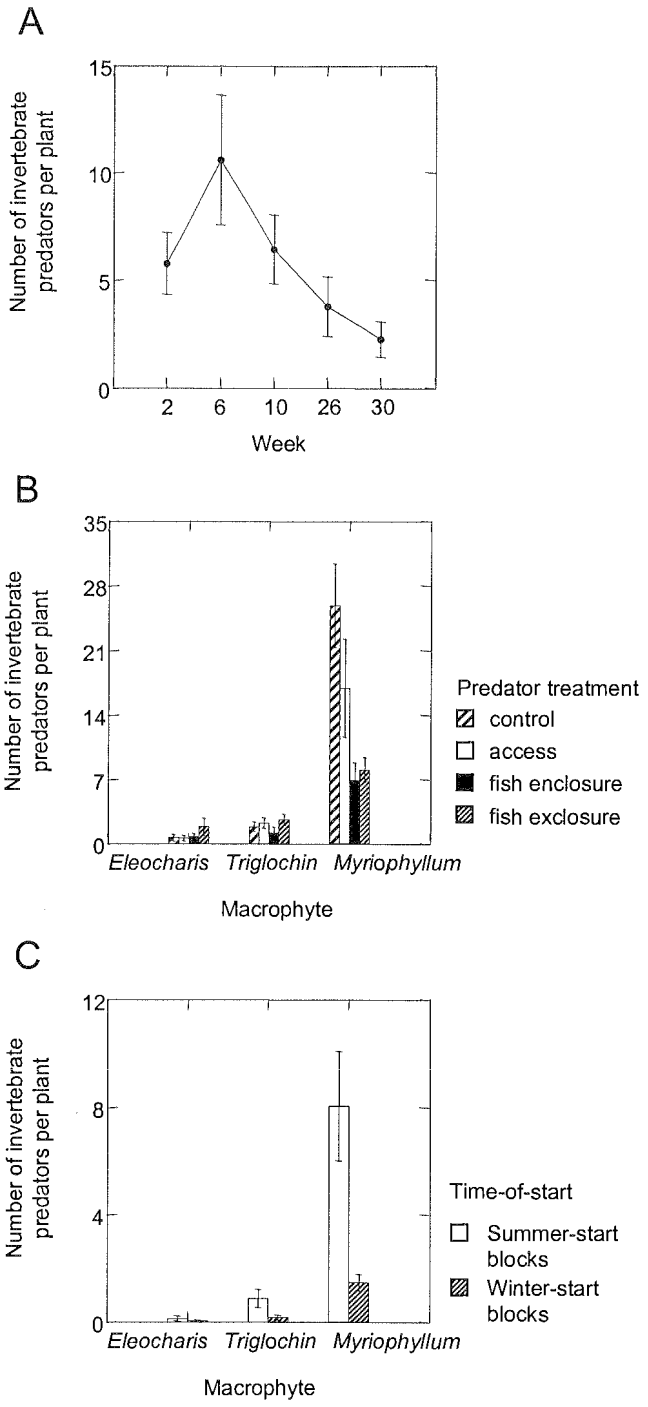

Figure 5: The abundance of invertebrate predators per plant over time in the summer-start analysis (B); on each macrophyte shape and predator treatment in the summer-start analysis (A); and on each macrophyte in the winter-start analysis (C). Error bars are one standard error about the mean.

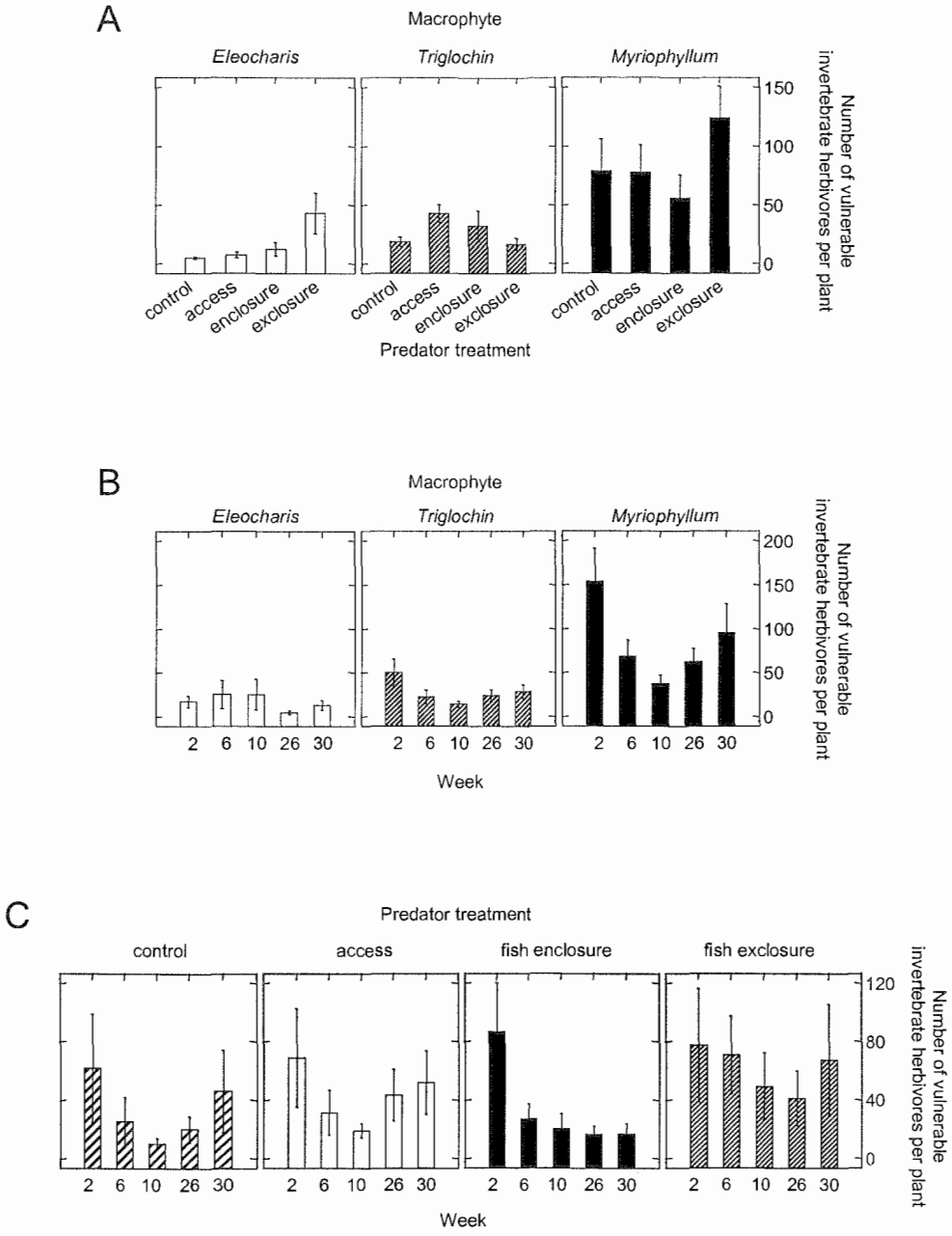

Figure 6: The abundance of vulnerable invertebrate herbivores per plant in the summer-start analysis, on each macrophyte in each predator treatment (A); on each macrophyte over time (B); and in each predator treatment over time (C). Error bars are one standard error about the mean.

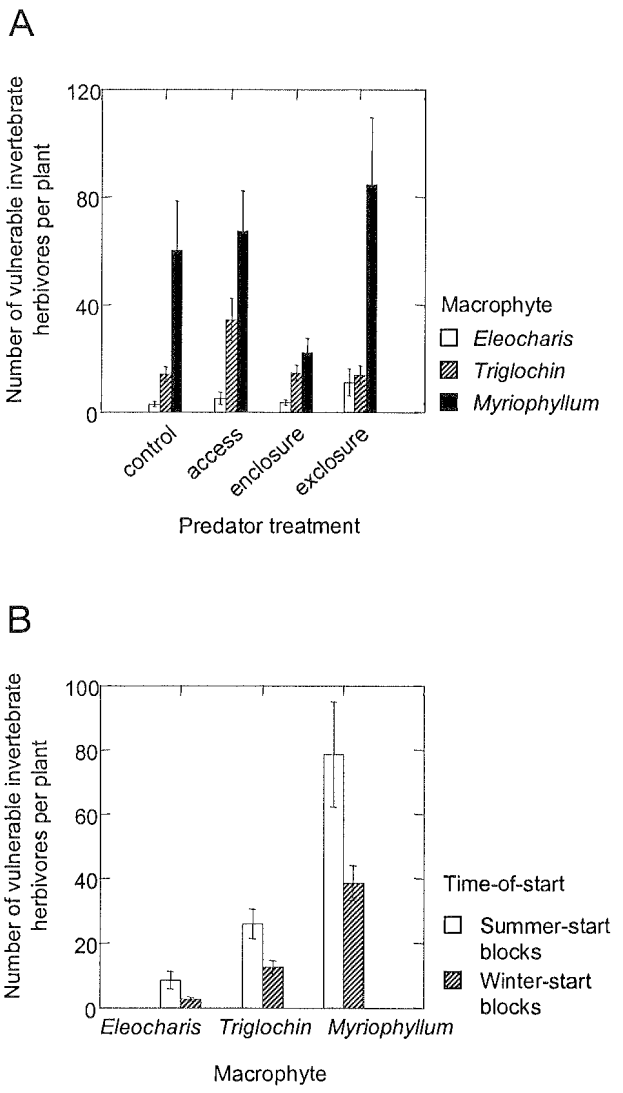

Figure 7: The abundance of vulnerable invertebrate herbivores per plant in the winter-start analysis, on each macrophyte shape in each predator treatment (A), and on each macrophyte shape in the summer-start and winter-start blocks (B). Error bars are one standard error about the mean.

Chapter 5: The Effects of Habitat Structure and Fish Predation on Periphyton Community Structure

Introduction

Predators can indirectly affect primary productivity through direct effects on their consumer prey (Heck and Crowder 1991, Martin et al. 1992, Spiller and Schoener 1993, Halaj and Wise 2001). The most striking example of the strength of these indirect effects are trophic cascades, where the effects of a top predator cascade down the trophic levels of a food web to alter the primary productivity (Polis et al. 2000). Trophic cascades have most often been shown in systems with relatively simple trophic and physical structure, such as pelagic lake systems (Carpenter et al. 1987, Power 1990), and their generality remains contentious (Strong 1992, Pace et al. 1999, Polis et al. 2000).

The littoral zone in freshwater and marine systems generally supports abundant macrophyte growth which in turn provides habitat structure for periphyton and macroinvertebrates (Carpenter and Lodge 1986, Heck and Crowder 1991, Jeppesen et al. 1998). Recent studies in littoral zones indicate fish predators can regulate the abundance of snail grazers and thereby indirectly influence the periphyton community (Bronmark et al. 1992, Martin et al. 1992, McCollum et al. 1998). It is also apparent that fish predators can be less effective at capturing prey as the habitat structure becomes more dense (Nelson 1979, Crowder and Cooper 1982, Heck and Crowder 1991, Swisher et al. 1998). Therefore, if fish predators can indirectly affect periphyton communities through their effects on grazers, and their effects on grazers are stronger in simple habitats without physical refuge from predation, then strong indirect effects of fish are more likely to be observed in structurally simple habitats (Power 1992).

However, in littoral zones, habitat structure has most often been measured as either the presence or the abundance of macrophytes which does not take into account the broad array of different macrophyte morphologies. Not only can differently shaped macrophytes support different macroinvertebrate communities (Rooke 1986, Cyr and Downing 1988b, Humphries et al. 1996), but they may also influence the biomass and composition of their periphyton communities through the creation of different microenvironments (Gregg and Rose 1982, Bronmark 1989). Moreover, if

macrophyte shape can also influence biotic interactions, then these community differences may at least partly result from the influence of macrophyte morphology on the indirect effects of fish predators.

I conducted a multifactorial caging experiment which addressed these expectations by comparing the relative impacts of macrophyte shape and indirect effects of fish predators on the macrophyte-associated periphyton community (effects on the macroinvertebrate community are addressed in Chapter 4). I specifically tested the following hypotheses: 1) that macrophyte shape affects the biomass and composition of the associated periphyton community, 2) that pygmy perch predation indirectly affects the biomass and composition of the periphyton community, and 3) the indirect effects of pygmy perch are mediated by the habitat in which they occur. I was expecting an interaction between fish predation and macrophyte shape such that, if pygmy perch were a strong top-down force in this community, they would have stronger effects on their herbivorous prey, and therefore stronger indirect effects on the periphyton, in the structurally simple macrophyte where there is less refuge. Thus, I also expected weak, if any, indirect effects in the most structurally complex macrophyte.

Methods

The study site and experimental design are described in Chapter 4. I tested the effects of macrophyte shape (of which there were three; *Eleocharis*, *Triglochin* and *Myriophyllum*) and predator treatment. There were four predator treatments: control cages, access cages, pygmy perch enclosures and pygmy perch enclosures. Thus, if pygmy perch were having indirect effects on the periphyton, and they were present in the control, access and enclosure cages, then I expected the composition and/or the biomass of the periphyton to differ in the exclosure cages.

Preliminary work showed no difference in the composition of periphyton between real and artificial plants, and the artificial plants used in the experiment were given a 10 week colonisation period before applying the predator treatments. Plants were sampled from summer-start blocks at 2, 6, 10, 26 and 30 weeks, and on the last two dates, the winter-start blocks were also sampled. After collection and preservation with 5% formalin, samples were returned to the laboratory and the plants were gently cleaned of periphyton and macroinvertebrates with forceps and a toothbrush under

running water over a 250 μm sieve. This mesh size may have resulted in the loss of some periphyton components, however I was only interested in broad compositional patterns within the periphyton community. After the macroinvertebrates had been picked from the periphyton, the periphyton samples were washed and frozen until compositional and biomass analyses.

The periphyton composition was analysed by a method based on that developed by O'Connor (1993) which estimates the proportion of each item in the periphyton. Each sample was thawed and shaken vigorously for 60 seconds, and 1 mL was pipetted onto a Sedgwick-Rafter cell. At 40 × magnification, a field of view comprised 12 cell squares, and one of a total 13 periphyton components (Table 1) was recorded as being the dominant item in each square (see Appendix B for identification keys used). Earlier identification at higher magnifications showed that broad periphyton components could be separated and identified at 40 × magnification. Initially, the periphyton was sorted to finer taxonomic levels, but many taxa had too many zeroes for analysis of variance tests. Six randomly chosen fields of view were recorded per 1 mL of periphyton, and 5 mL were analysed from each sample. Preliminary sampling showed that a periphyton sample was relatively uniform and the majority of samples comprised no more than 10 mL, thus 5 separate 1 mL subsamples gave results indistinguishable from larger volumes. The number of cell squares in which each item was dominant was added and expressed as a proportion of the total number of cell squares per sample (12 squares per field of view × 6 fields of view per subsample × 5 subsamples = 360 cell squares per sample).

The collection and storage methods used for the periphyton samples precluded chlorophyll *a* analysis, so an estimate of periphyton biomass was obtained from the ash-free dry weight (AFDW) of each sample. The samples were dried to constant weight at 50°C for 48 hours, and then ashed in a muffle furnace at 500°C for two hours.

Data Analysis

As for the macroinvertebrate community, both multivariate and univariate analyses were used to assess effects of habitat complexity and fish predation on the periphyton community. Likewise, both summer-start analyses (testing effects throughout time on the 3 summer-start blocks) and winter-start analyses (testing the time-of-start effect

between summer-start and winter-start blocks at 26 and 30 weeks) were conducted (see Chapter 4).

Periphyton composition – To get a broad overview of the periphytic response to macrophyte shape and pygmy perch predation, the periphyton components were lumped into the broad categories of detritus, green algae and blue-green algae because the 13 components still resulted in too many zero values to meet the assumptions of parametric MANOVA (Tabachnick and Fidell 2001). These categories were the dependent variables analysed by two-factorial randomised-block repeated-measures MANOVA (where the factors were macrophyte shape and predator treatment) for the summer-start analysis, and by three-factorial randomised-block repeated-measures MANOVA (where the factors were macrophyte shape, predator treatment and time-of-start) for the winter-start analysis. These categories comprised over 99% of the periphyton, varied proportionally with one another, and met the assumptions of a multivariate analysis of variance which assert that the number of dependent variables should not exceed the number of observations in a cell (Tabachnick and Fidell 2001, Quinn and Keough 2002) – in this case there were three observations per cell. Red algae, moss and fungal hyphae were excluded as they rarely occurred, contributing less than 0.4 ± 0.2 % to the periphyton. Pillai's Trace was used to determine significance (Tabachnick and Fidell 2001) and simple effects tests were performed to interpret significant interactions. These analyses were conducted using SAS Version 6.12 and SYSTAT Version 9.

Changes in the 13 individual components of the periphyton composition were assessed by performing two-factorial non-parametric MANOVA on the data from 2, 26 and 30 weeks (this model does not permit missing cells, so the periphyton composition at 6 and 10 weeks was not analysed). The data were arcsine transformed, and post-hoc comparisons were conducted for significant effects and interactions. These analyses were conducted using NP-MANOVA (Anderson 1999). Non-metric multidimensional scaling (MDS) was performed on the data from all sampling events to provide a visual representation of the separation of treatments on the basis of periphyton composition differences. I could not use IndVal Version 2.0 on proportional data so comparisons were made visually to estimate which components characterised periphyton differences between treatments.

Periphyton biomass – The changes in periphyton biomass throughout time were assessed with univariate analyses, by conducting two-factorial randomised-block repeated-measures ANOVA on the AFDW data from the summer-start blocks. Missing values from the winter-start blocks at 26 weeks (due the accidental loss of 8 samples) necessitated the exclusion of these data, hence a simple three-factorial randomised-block ANOVA was conducted on the 30 week data to assess time-of-start effects on periphyton biomass. Data were fourth-root transformed, and planned comparisons were performed on the main effects of macrophyte shape (*Eleocharis* v. *Triglochin*, and *Triglochin* v. *Myriophyllum*) and predator treatment (control v. access, access v. predators enclosed, and predators enclosed v. predators excluded).

Results

The detritus component of the periphyton consisted of 76% amorphous detritus (on average), 17% senescent macrophyte tissue, 5% terrestrial woody detritus, and the remaining 2% of inorganic and invertebrate detritus. Blue-green algae consisted of 75% filamentous and 25% colonial algae, and green algae consisted of 90% filamentous algae, 5% diatoms, and 5% colonial algae.

Periphyton composition

The summer-start repeated-measures parametric MANOVA showed the proportions of green algae and blue-green algae appeared to vary inversely with one another, such that where there was less green algae, there was correspondingly more blue-green algae, but both were constrained by the proportion of detritus (Figure 1). Macrophyte shape significantly influenced the proportion of each periphyton component ($F_{6,40}$ = 4.951, $p<0.001$), as *Triglochin* supported the largest proportion of detritus (at least 25% more than *Eleocharis* and *Myriophyllum*; Figure 1a). *Myriophyllum* supported the least amount of green algae (30% less than *Eleocharis* and *Triglochin*) and correspondingly the most blue-green algae (24% more than *Eleocharis* and *Triglochin*; Figure 1a). The proportion of each periphyton category also significantly varied over time ($F_{12,10} = 4.761$, $p<0.01$; Figure 1b), as the proportion of detritus was relatively stable over the summer sampling events (2,6 and 10 weeks) but increased during the winter months (26 and 30 weeks). Green algae comprised 20-25% of the periphyton at 2,6 and 10 weeks, but only 8% at week 26 during winter (Figure 1b). Correspondingly, the greatest proportion of blue-green algae occurred at 26 weeks

(45%) before decreasing again to 31% at 30 weeks. There were no significant interactions (all $p>0.371$) nor were there any predator treatment effects ($p>0.09$) in the summer-start analysis.

The winter-start analysis showed a strong effect of the time-of-start factor, as there was hardly any blue-green algae, and correspondingly more green algae and detritus, in the winter-start blocks than the summer-start blocks (Figure 2). This lack of blue-green algae in the winter-start blocks gave rise to the time-of-start factor significantly interacting with macrophyte shape, predator treatment, and time. Simple effects tests on the significant interaction between macrophyte shape and time-of-start ($F_{6,86} = 3.253$, $p<0.007$) showed there was 23% less detritus ($F_{2,69} = 4.034$, $p<0.03$) and 23% more blue-green algae ($F_{2,69} = 0.949$, $p<0.01$) on the structurally simple macrophyte, *Eleocharis*, than either of the other two macrophytes in the summer-start blocks (Figure 2a). In the winter-start blocks, there was 22% less detritus ($F_{2,69} = 3.182$, $p<0.05$) but 43% more green algae ($F_{2,69} = 3.922$, $p<0.03$) on *Eleocharis* due to the low proportions of blue-green algae in these blocks (Figure 2a).

Simple effects test of the significant time-of-start interaction with predator treatment ($F_{9,132} = 1.979$, $p<0.05$) showed significance arose in the summer-start blocks only, where the pygmy perch exclosures supported 38% less detritus ($F_{3,68} = 4.752$, $p<0.006$) but 27% more blue-green algae ($F_{3,68} = 4.582$, $p<0.007$) than any other predator treatment (Figure 2b). Despite the appearance to the contrary (Figure 2b), the proportion of green algae did not significantly vary between predator treatments ($F_{3,68} = 1.177$, $p>0.325$), nor was there any significant variation between the periphyton categories in the winter-start blocks ($F_{9,204} = 1.032$, $p>0.416$).

Finally, the significant interaction between time-of-start and the week of sampling ($F_{3,42} = 3.567$, $p<0.03$) showed no significant variation in the periphyton categories in the summer-start blocks ($F_{9,204} = 1.477$, $p>0.229$), but in the winter-start blocks there was a 70% reduction in the proportion of blue-green algae between 26 and 30 weeks ($F_{1,70} = 4.832$, $p<0.04$; Figure 2c). As blue-green algae only comprised 3% of the periphyton at 26 weeks and 0.9% at 30 weeks, this result was not considered to be particularly important. The proportions of green algae and detritus did not significantly vary over time in the winter-start blocks (all $p>0.401$).

Non-parametric MANOVA of all 13 periphyton components at 2 weeks showed macrophyte shape significantly affected periphyton composition ($F_{2,24} = 2.123$, $p<0.04$). The periphyton composition significantly differed between *Eleocharis* and *Myriophyllum* ($t_2 = 1.886$, $p<0.02$) but not between *Eleocharis* and *Triglochin* ($t_2 = 1.463$, $p>0.086$) or *Myriophyllum* and *Triglochin* ($t_2 = 0.892$, $p>0.534$). Macrophyte shape had no significant effects on the periphyton composition at 26 weeks ($F_{2,60} = 1.356$, $p>0.227$). But at 30 weeks, macrophyte shape again significantly influenced the periphyton composition ($F_{2,60} = 2.630$, $p<0.02$); the periphyton on *Eleocharis* significantly differed in composition from that on *Triglochin* ($t_2 = 1.752$, $p<0.025$) and *Myriophyllum* ($t_2 = 1.856$, $p<0.02$). There were no significant effects of pygmy perch predation (all $p>0.548$), nor any significant interactions (all $p>0.530$), on the periphyton composition in any of the non-parametric MANOVA tests.

MDS of the data from all sampling events (including 6 and 10 weeks) resulted in a three-dimensional solution with a stress of 0.125. From the ordination plots (Figure 3) it can be seen there was little difference in the clustering of samples between the macrophytes, and the *Eleocharis* samples were slightly more negative along Dimensions 2 and 3. There was also a tendency for the winter-start samples to separate from the summer-start samples along Dimension 1, however it was not as marked a separation as that seen for the macroinvertebrate samples. Visual analysis of the periphyton composition between macrophytes (Figure 4) indicated the periphyton on *Eleocharis* differed from that on *Triglochin* and *Myriophyllum* by having relatively smaller proportions of amorphous detritus and colonial blue-green algae, and relatively more filamentous green algae. *Eleocharis* was also the only plant on which red filamentous algae was recorded.

Periphyton biomass

Periphyton biomass, as measured by AFDW, increased over time and was consistently greater on *Myriophyllum*, the most structurally complex plant. The summer-start analysis revealed a significant interaction between macrophyte shape and time ($F_{8,80} = 2.930$, $p<0.02$; Figure 5a); the biomass of periphyton was significantly greater on *Myriophyllum* and also showed a greater increase over time on this plant than on *Triglochin* or *Eleocharis*. The winter-start analysis (excluding the 26 week data due to the missing samples as mentioned in the *Methods* section) found both macrophyte

shape ($F_{2,55}$ = 24.785, p<0.001; Figure 5b) and predator treatment ($F_{3,55}$ = 4.679, p<0.01; Figure 5c) significantly affected periphyton biomass. Planned comparisons showed there was 62% more periphyton on *Myriophyllum* than on *Triglochin* ($F_{1,54}$ = 33.420, p<0.001), but only 8% more periphyton on *Triglochin* than on *Eleocharis* ($F_{1,54}$ = 1.407, p>0.241). In the predator treatments, the greatest amount of periphyton occurred in the access cages suggesting a cage effect (Figure 5c). Planned comparisons showed the access cages had 29% more periphyton than the control cages ($F_{1,54}$ = 4.558, p<0.04), and 49% more than the predator enclosures ($F_{1,54}$ = 10.392, p<0.005); and there was only 9% more periphyton in the pygmy perch exclosures than the enclosures (F1,54 = 0.040, p>0.843).

Discussion

I tested the combined effects of macrophyte shape and fish predation to determine if fish predators had indirect effects on periphyton and if these effects were mediated by the habitat in which they occurred. I found macrophyte shape affected both the composition and the biomass of the periphyton, and while I was expecting any indirect effects of fish predators to be more apparent in the structurally simple macrophyte, where pygmy perch would be most likely to affect grazer abundance, these expectations were not met. I will discuss the effects of macrophyte shape and indirect predator effects in turn, followed by temporal and seasonal effects.

Habitat structure

Macrophyte shape influenced both the biomass and the composition of the periphyton community. *Myriophyllum*, the most complex macrophyte, consistently supported the greatest biomass of periphyton. Considering *Myriophyllum* also supported a greater number of grazers, this corroborates the tendency for higher grazer abundances at high periphyton densities (Feminella and Hawkins 1995, Jones et al. 1998). A structurally complex habitat is generally considered to have a greater surface area, providing more space for the attachment of periphyton and thereby explaining greater macroinvertebrate abundances (Heck and Crowder 1991). However, as shown in Chapter 2, *Myriophyllum* and *Triglochin* have equivalent surface areas, yet do not support the equivalent amount of periphyton. Jones et al. (1999) also found differently shaped macrophytes support different periphyton abundances, but in the opposite

direction; there was more periphyton on *Littorella uniflora*, which has a tufted structure similar to *Triglochin*, than on *Elodea nuttallii*, which with more leaves could be considered more structurally complex. Interestingly, when they used artificial imitations of these macrophytes, the patterns of periphyton biomass reversed; there was more periphyton on *Elodea*, corroborating my results and suggesting effects of macrophyte structure alone.

Different macrophytes can create different microenvironments in a river (Gregg and Rose 1982), and *Myriophyllum* may provide more protection against physical disturbance and thereby encourage more periphyton growth. Gregg and Rose (1982) showed the highly-dissected leaf structure of a *Ranunculus* species decreased local current velocities in the water column, thereby reducing shear stress on the periphyton, and supported more periphyton growth. While flows were very slow in the littoral zone of the Macquarie River during this experiment, physical disturbance and damage to the periphyton may also be caused by fish swimming through macrophytes and brushing their surfaces. The arrangement of leaves on *Myriophyllum* is such that half the leaf's surface faces inwards towards the main stem, therefore half the available surface area is protected from this type of disturbance, whereas *Eleocharis* has its entire surface exposed and offers no protection from physical disturbance for periphyton.

Physical disturbance of a periphyton community can also interrupt successional processes and lead to compositional changes in the community (Bronmark 1989), however, the periphyton components that separated the *Eleocharis* community from the other two macrophytes were predominantly filamentous forms of algae (green, blue-green and red filamentous algae were proportionally more abundant on *Eleocharis*) which are less able to withstand shear stresses without damage than diatoms and colonial forms of algae (Biggs and Thomsen 1995). Thus, the preponderance of filamentous forms on *Eleocharis* may be due to other effects of shape rather than protection from physical disturbance.

Eleocharis also supported far less detritus while *Triglochin* had the greatest proportion of detritus. *Triglochin*, with its tuft of leaves, and *Myriophyllum*, with its many dissected leaves around the stems, both have a morphology more likely to passively collect and accumulate detritus as it settles out of the water column compared to the straight-sided reed structure of *Eleocharis*. Less detritus on

Eleocharis therefore results in more space available for the attachment of filamentous growth forms. Thus, the shape of macrophytes can have an influence on the composition of the periphyton (Bronmark 1989).

Predator effects

There was only a slight predator effect on the composition of periphyton, with more blue-green algae found in the pygmy perch exclosures, but only in the summer-start blocks, and only at weeks 26 and 30, during winter. Furthermore, the effects of pygmy perch took over six months to manifest in the composition of the periphyton, and did not vary according to macrophyte shape. The predator exclosures also supported a higher abundance of vulnerable invertebrate herbivores (see Chapter 4), which suggests pygmy perch do have indirect effects on the periphyton composition, albeit weak effects.

An increase in blue-green algae with greater densities of algal grazers appears to be relatively common and is explained by competitive interactions between green and blue-green algae (Cattaneo 1983, Sterner 1989, Heck and Crowder 1991, Rosemond 1996, Polis et al. 2000). Blue-green algae have a tough, indigestible monosaccharide sheath which makes them a poor food for grazers (Jones et al. 1998); therefore, they can become proportionally dominant as green-algae are preferentially consumed by grazers (Bronmark 1989). While there is no corresponding decrease in the proportion of green algae in the predator exclosures, there was proportionally less detritus. If the green algae were resistant to herbivory (Dudley and D'Antonio 1991), the vulnerable invertebrate herbivores (*Atalophlebia australis*, *Austrochiltonia australis* and ostracods), which increased in abundance in response to the exclusion of pygmy perch, may have been consuming detritus instead; the leptophlebiidae mayfly (*Atalophlebia australis*) in particular has been shown to consume large amount of amorphous detritus (Chessman 1986). Thus, in the pygmy perch exclosures, the consumption of detritus by invertebrates may have released space for the increased growth of blue-green algae.

The fact that this pattern is only seen in the summer-start blocks during the winter suggests the preponderance of blue-green algae may also be influenced by the season. Sterner (1989) showed blue-green algae became dominant in lake phytoplankton during autumn because they were less nitrogen-limited than green algae, and the

presence of *Daphnia* grazing on green algae exacerbated this pattern. The likelihood of nitrogen limitation in the Macquarie River is very small; firstly because it collects agricultural runoff from upstream and around Fosterville, and secondly, the winter-start blocks showed no such dominance of blue-green algae. Nevertheless, the point made by Sterner (1989) is the same; given the initial establishment of blue-green algae, grazers can further increase its dominance by removing a competitor.

While there were differences between predator treatments on periphyton biomass, with biomass being greater in the access cages, I suspect this is a cage artifact rather than any effect of pygmy perch. Despite monthly scrubbing of the cage walls (to prevent them becoming clogged with periphyton), the access cages also appeared to have more peripyhton around the open panels. The open cages may have allowed more water circulation and therefore more opportunity for periphyton establishment, or they may have permitted more light than the walls of the closed cages and therefore better growth conditions. However, given there were no differences between the enclosures and exclosures, I was confident the cage effect did not influence potential pygmy perch effects.

There were no pygmy perch effects on the biomass of periphyton in this study, indicating that the greater abundance of vulnerable invertebrate herbivores in the fish exclosures did not influence periphyton biomass. Despite strong indirect effects of fish predators on periphyton biomass observed in some studies (Power 1990, Martin et al. 1992, Bronmark 1994), there are two possibilities why periphyton biomass did not respond to the predator treatments in this study. Firstly, the AFDW of periphyton reflects both the losses and gains in biomass such that the autotrophic production of the periphyton may have been at a great enough rate to compensate for losses due to grazer consumption (Gresens 1995). Lovgren and Persson (2002) used this mechanism to explain the lack of indirect effects by juvenile perch (*Perca fluviatilis*) on the periphyton biomass of artificial macrophytes, suggesting that the nutrient regeneration provided by filtering cladocerans compensated for the negative effects of grazing cladocerans.

Secondly, and more likely, the increase in vulnerable herbivores in the pygmy perch exclosures represented only a subset of the entire herbivore community. The patterns in the periphyton biomass probably reflected pressure from all herbivores, many of which were not affected by pygmy perch predation. The highly speciose

macroinvertebrate community in the Macquarie River does not have a simple trophic structure with sharply delineated trophic levels, and is therefore an impediment to strong indirect effects of fish on primary resources, particularly trophic cascades (Power 1992, Strong 1992, Polis et al. 2000).

Temporal and seasonal effects

Analyses of the broad categories of periphyton showed a very strong time-of-start effect; the proportion of blue-green algae was remarkably lower in the winter-start blocks than the summer-start blocks, which, besides giving rise to significant interactions with the main treatment effects, suggested a strong effect of season on the outcomes of periphyton successional processes.

The summer-start blocks showed an increase in blue-green algae into winter as the proportion of green algae decreased in winter. Cattaneo (1983) found almost the complete opposite, with the blue-green algal component of a periphyton community on artificial macrophytes becoming more dominant over summer, coinciding with increased grazing pressure by oligochaetes and chironomids. Blue-green algae has also been found to become dominant in phytoplankton communities in late summer as they can tolerate high grazer pressure as well as low nutrients levels (Rosemond 1996). However, the macroinvertebrate abundance (and hence grazing pressure) in the littoral zone of the Macquarie River also increased into winter (see Chapter 4), which suggests the observed seasonal effects on the periphyton composition may be partly due to concurrent effects on the macroinvertebrate community.

In the Macquarie River blue-green and green algae tended to vary inversely with each other over time which suggests space may be a limiting resource and there are competitive interactions between them (Rosemond 1996, Jones et al. 1998). The strong time-of-start effect supports the notion of competition between the two algae. The fact that there were lower proportions of blue-green algae in the winter-start blocks indicates they were unable to establish in the colder months, when water temperatures can drop to 5°C (Humphries 1995), and could only become competitively dominant after establishing during the warmer months of summer. Thus, the season may have had an effect on successional processes in the periphyton. In marine subtidal systems where space is a limiting factor for sessile communities, season has been shown to alter competitive interactions during the recruitment and

settlement of sessile organisms, and hence the successional outcomes of the community assemblage (Underwood and Anderson 1994, Nandakumar 1996). Given water temperatures and the macroinvertebrate assemblage vary over time in the Macquarie River (Chapter 4, Humphries 1995), the effects of season on periphyton successional processes warrant further investigation.

Periphyton biomass only showed temporal effects, steadily increasing over time, particularly on *Myriophyllum*. This corroborates my personal observations of greater periphyton biomass over winter and spring in the Macquarie River which prompted the staggered-start design employed in this experiment. Water discharge in the Macquarie River is at its highest at this time of year (Chris MacGeorge, Bureau of Meteorology, Tasmania, unpubl. flow data, Humphries 1995), so this increase in periphyton abundance probably reflects organic enrichment due to agricultural runoff from upstream of, and around, Fosterville (Davies and Humphries 1996). This may also indicate why there were no seasonal effects on periphyton biomass; the organic enrichment during winter may have encouraged enough periphyton growth in the winter-start cages to match that in the summer-start cages. Nevertheless, this lack of a seasonal effect on periphyton biomass highlights the importance of considering the composition of periphyton as well as the biomass. Many studies investigating indirect effects of fish on periphyton resources have measured effect size with periphyton biomass (e.g. Bronmark 1994, Bernot and Turner 2001, Lovgren and Persson 2002). In this experiment, subtle effects of predator treatment and seasonal variation were picked up by analysis of the composition, which would have been missed had I focussed simply on the biomass.

Conclusions

Macrophyte shape had a strong, consistent effect on the biomass and composition of the periphyton. The morphology of each plant probably creates different microenvironments which is reflected in the periphyton composition, and the complex structure of *Myriophyllum* may provide a surface protected from physical disturbance to allow more periphyton growth. There were only weak indirect effects of pygmy perch on the periphyton composition, and these did not vary with plant shape, indicating the effects of macrophyte structural complexity on pygmy perch foraging are not transferred to the rest of the community. It appears pygmy perch do not have

strong effects, probably because the macroinvertebrate community of the Macquarie River is diverse and can channel effects of pygmy perch down different paths such that the resultant effect on the periphyton community is weak. This is further explored in Chapter 6.

Table 1: The periphyton components which comprised the periphyton, and their corresponding descriptions (see Appendix B for identification keys used). The presence of each component was scored to give the relative proportions of each, and the broad categories of detritus, green and blue-green algae, consisting of their respective components, were used for MANOVA.

Broad categories used for MANOVA	Periphyton components	example
detritus	amorphous	shapeless organic material
	inorganic	silt, sand grains
	macrophyte	senescing macrophyte leaves
	woody	twigs, terrestrial leaves
	invertebrate	caddis cases, exuvia
green algae	filamentous	*Oedogonium, Zygogonium*
	colonial	*Chaetophora*
	diatoms	*Navicula, Amphora, Gomphonema*
blue-green algae	filamentous	*Tolypothrix*
	colonial	*Rivularia*
	red algae	*Batrachospermum*
	fungal hyphae	
	moss	

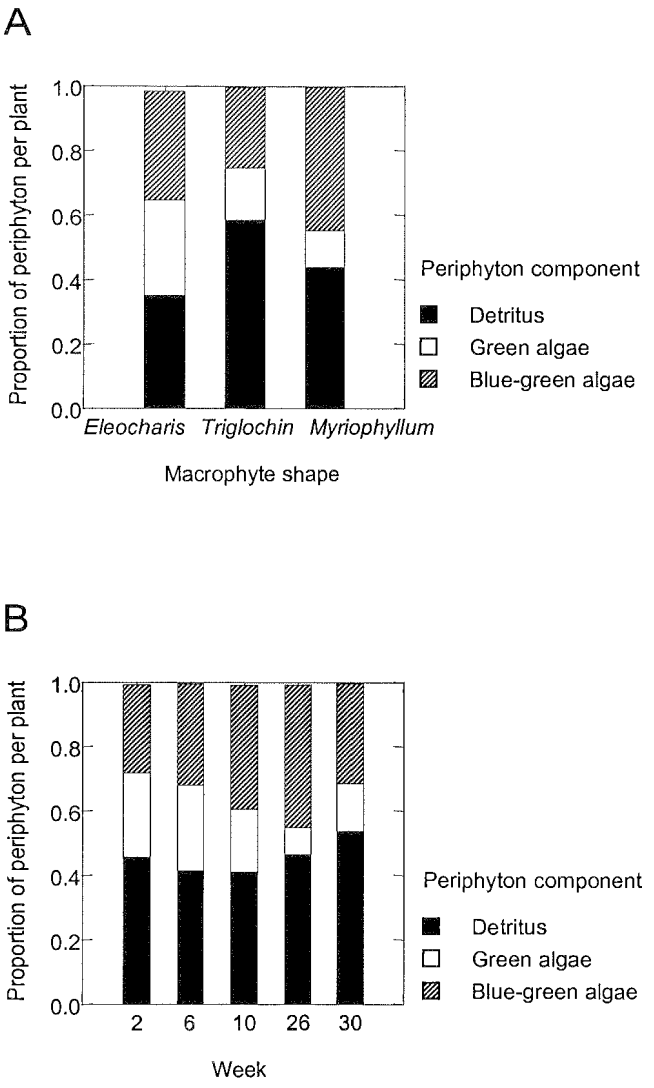

Figure 1: The proportion of detritus, green algae, and blue-green algae comprising the periphyton per plant, in the summer-start analysis, on each macrophyte shape (A), and at each sampling event (B). The three broad components do not necessarily add to 100% due to the exclusion of moss, fungi and red algae from the analyses.

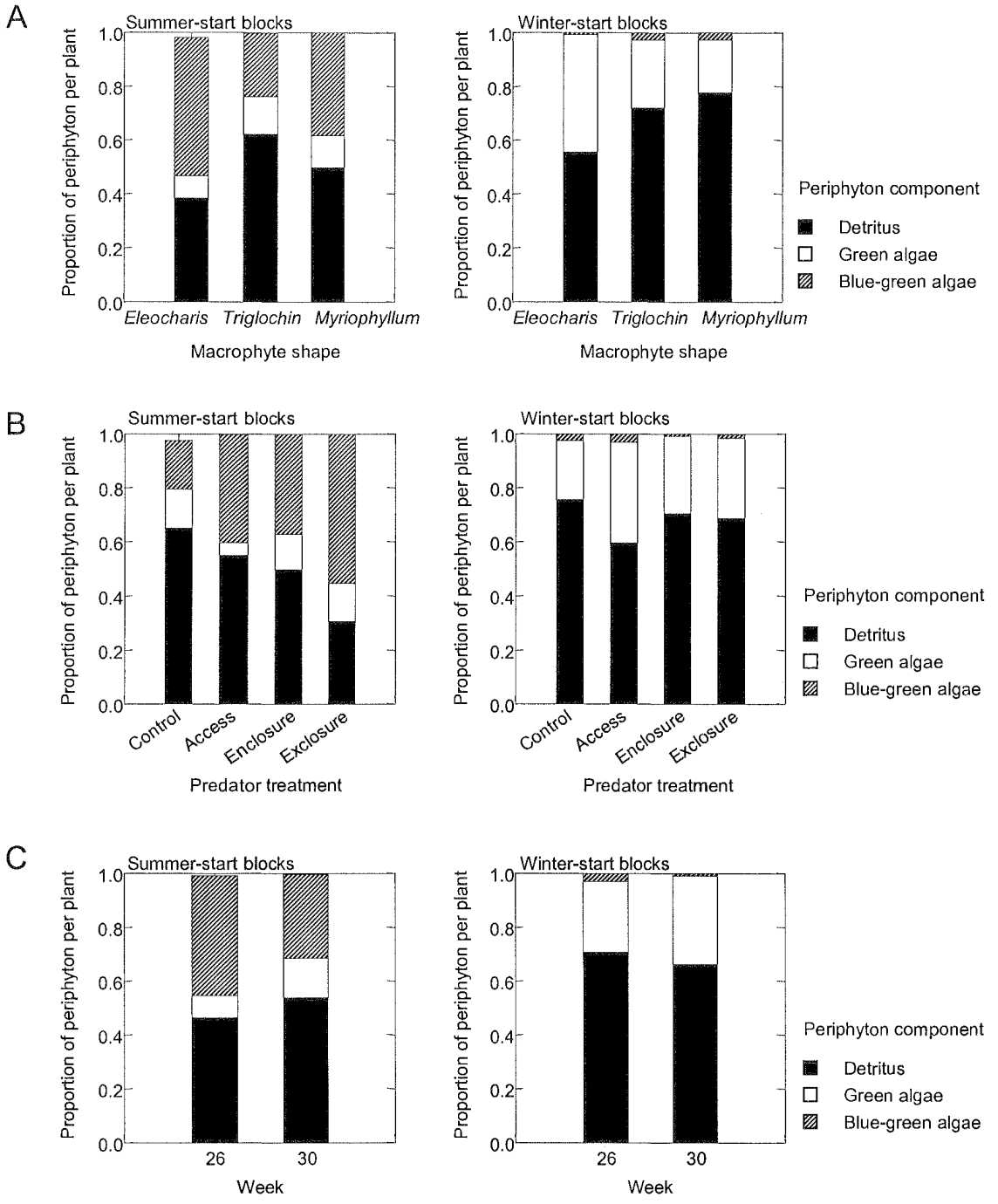

Figure 2: The proportion of detritus, green algae and blue-green algae comprising the periphyton per plant, in the summer-start and winter-start blocks of the winter-start analysis, on each macrophyte (A), in each predator treatment (B), and at 26 and 30 weeks (C).

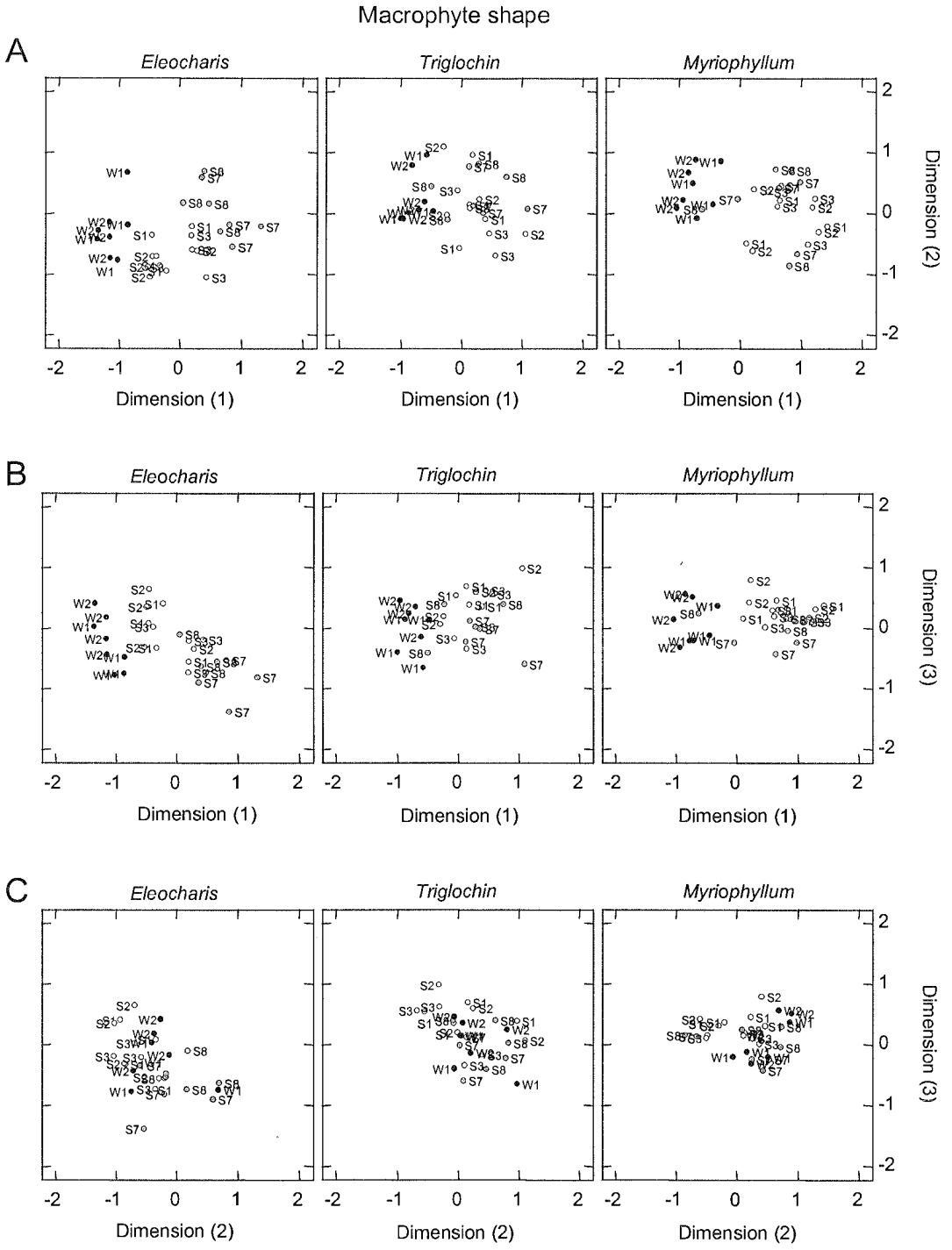

Figure 3: MDS plots showing the separation of periphyton communities over dimensions 1 and 2 (A), dimensions 1 and 3 (B), and dimensions 2 and 3 (C). Samples are grouped according to macrophyte, and labelled by time - the letter denotes the block (Summer-start or Winter-start) and the number denotes the month of sampling after the block was initiated (so S7 is a summer-start block sampled at 26 weeks, and W1 is a winter-start block sampled after one month, which coincides with 26 weeks).

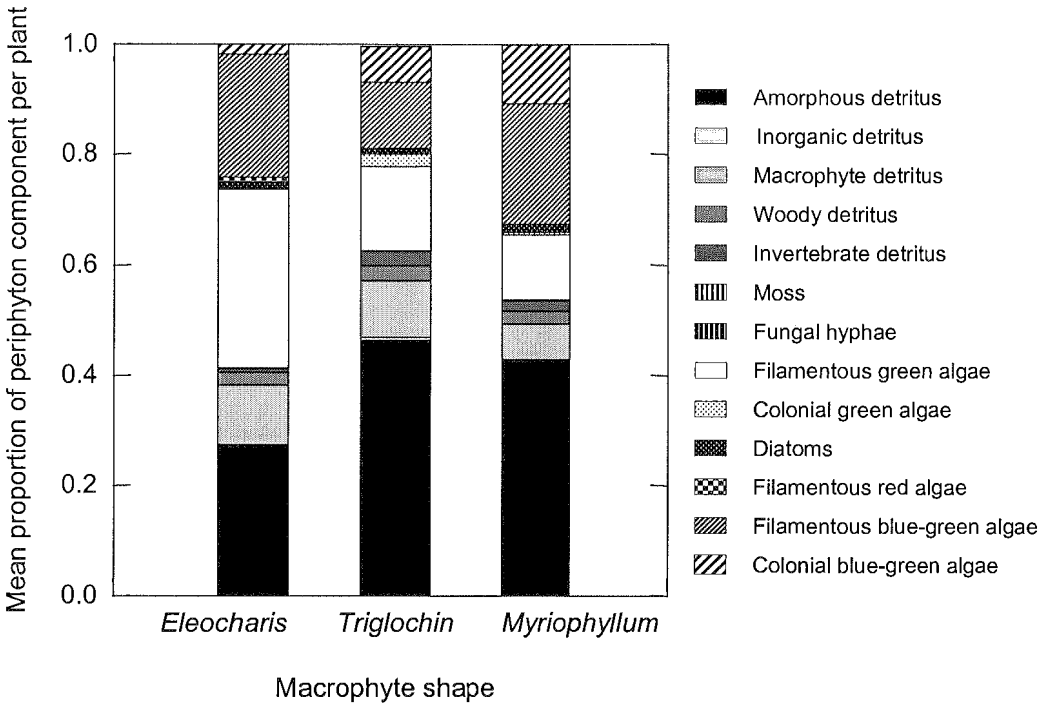

Figure 4: The mean proportions of each periphyton component contributing to the periphyton on each macrophyte shape (pooled over all sampling times).

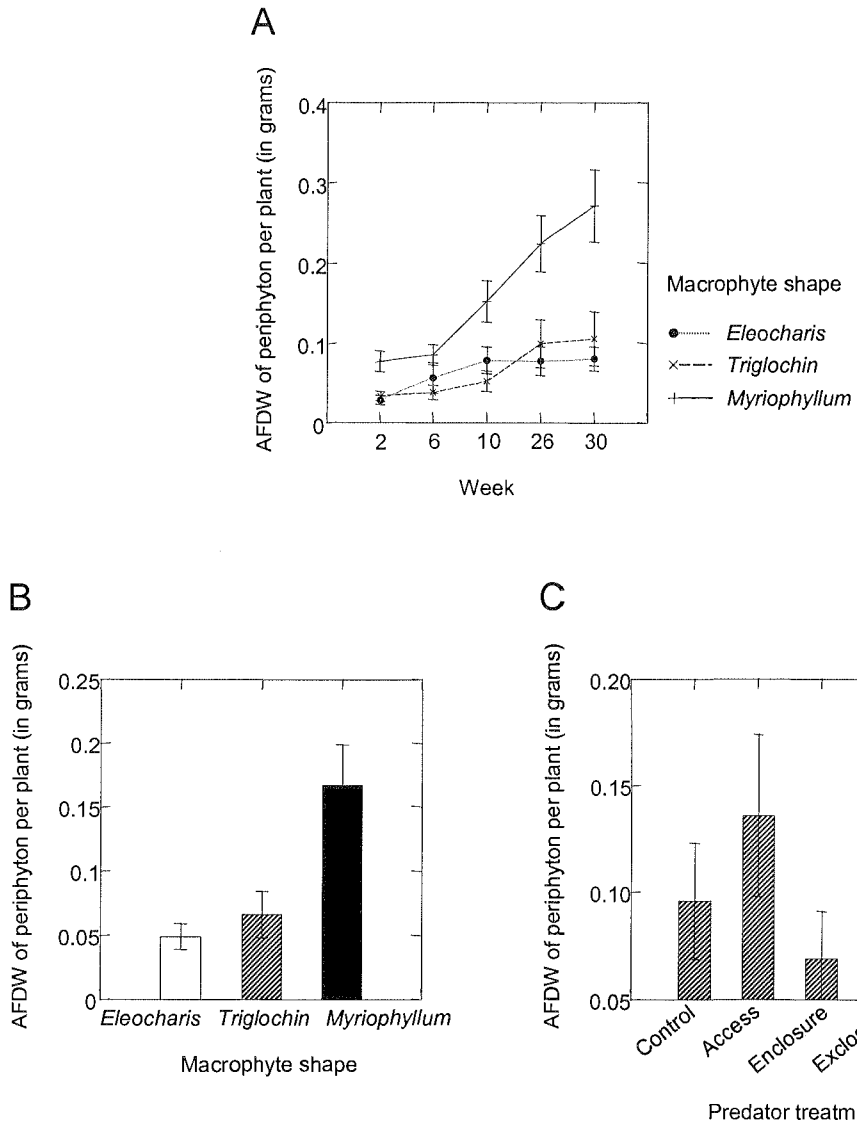

Figure 5: The AFDW (in grams) of periphyton per plant, on each macrophyte in the summer-start analysis, on each macrophyte in the winter-start analysis at 30 weeks only (B), and in each predator treatment in the winter-start analysis at 30 weeks only (C).

Chapter 6: General Discussion

This research addressed the role of habitat structure in the trophic interactions of a freshwater community. Generally, more structurally complex macrophytes support a greater abundance and diversity of macroinvertebrates and epifauna (Crowder et al. 1998). The most often-cited mechanism for this pattern is that more structurally complex macrophytes provide more refuge from fish predation (Heck and Crowder 1991). I tested this hypothesis by 1) quantifying the shape and refuge space of three differently shaped macrophytes, 2) quantifying the prey-capture success of two predators while varying macrophyte density and shape, and 3) quantifying the direct and indirect effects of fish predation in the same three macrophytes. I will summarise each of these in turn, then provide a synthesis, and finish by addressing the implications in the context of the community ecology of food webs.

Summary of findings

While some studies have attempted to measure macrophyte structural complexity, it has usually involved the use of a habitat-specific measure which does not allow a comparison of the role of habitat structure between systems. In order to find a broadly applicable index of macrophyte structural complexity, I used nine different indices of structural complexity to determine which best quantified macrophyte shape and best related to the distribution of macroinvertebrates. Despite finding my *a priori* classification of each macrophyte was corroborated by each index, I found these indices were highly intercorrelated and no single index met the requirements of an index of habitat structure. Rather, combinations of indices provided better descriptions of macrophyte shape and macroinvertebrate distribution. For example, *Myriophyllum* with its highly dissected leaf structure was best described by the indices measuring the fractal dimension at coarser scales and the refuge space associated with the structure, whereas *Eleocharis* was best described by indices measuring "whole plant" attributes (surface area and plant volume) and the fractal dimension at finer scales. Yet there were two structural indices which measured macrophyte shape, but did not describe one particular macrophyte better than another: the interstitial space size / predator size index and the fractal dimension at Scale 2 (5 × magnification). These two indices were also the most highly related to

macroinvertebrate abundance and taxon richness, which were the highest on the most structurally complex plant, *Myriophyllum*. Therefore, I concluded that macroinvertebrates were responding to a combination of refuge from predation (measured by Sp/Pr) and the surface rugosity at 5 × magnification (measured by D_2), both of which described the macrophyte shape, and both of which should be included in the development of a broadly applicable index of macrophyte structure.

If macroinvertebrates are responding to the refuge function of macrophytes, then it follows that predators should be less effective, capturing fewer prey, in more structurally complex plants and at higher plant densities. Moreover, if habitat structure affects the prey-capture success of a single predator, then it may also influence the effectiveness of two predators combined, particularly if it mediates interactions between the predators. I conducted a laboratory experiment to determine the influence of both macrophyte density and shape on the prey capture success of a predatory damselfly and the southern pygmy perch. Contrary to expectations, macrophyte density had no effect on the prey capture success of either predator, but both predators were significantly less effective in *Myriophyllum* compared to *Eleocharis* and *Triglochin*. Furthermore, the structurally complex *Myriophyllum* actually amplified the impact of the negative interaction between the predators on prey numbers; in their response to the presence of pygmy perch, damselflies were less able to capture prey in *Myriophyllum*, thus less prey than expected were consumed in this plant. While these results would be expected to differ depending on prey identity, they further support the mechanism of increased prey refuge in more structurally complex macrophytes as an explanation for macroinvertebrate distributions on macrophytes.

If the prey-capture success of predators is lower in a more structurally complex habitat, then their direct and indirect effects, and therefore their functional significance, in a food web may also be of a lesser magnitude in a more structurally complex habitat. To assess this hypothesis, and the relevance of my laboratory results, I conducted a field caging experiment testing the effects of macrophyte shape and pygmy perch structure on the macroinvertebrate and periphyton communities in the macrophyte beds of the Macquarie River. I found macrophyte shape strongly influenced the abundance and composition of both the macroinvertebrate and periphyton communities. Furthermore, the effects of macrophyte shape were consistent through time and between seasons. While pygmy perch had relatively weak

effects on the macroinvertebrate community, only influencing the abundance of vulnerable herbivores, there was also some indication of indirect effects on the periphyton composition, although they took over six months to manifest and did not vary according to macrophyte shape. The unexpected result from this field experiment was that pygmy perch had their greatest impact on vulnerable invertebrate herbivores in *Myriophyllum*, the most structurally complex macrophyte.

Synthesis of findings

Despite my research indicating that the more structurally complex *Myriophyllum* influences the distribution of macroinvertebrates by providing more surface rugosity and more refuge space, and that pygmy perch are less effective at capturing prey in this plant, it appears that in the field, vulnerable macroinvertebrates (amphipods, ostracods and red spinner mayflies) were at a greater risk of predation on this plant. There are numerous potential explanations as to why this occurred.

Effect measurement

The fact that I measured the effects of predators differently in each experiment, may have contributed to these different, apparently contradictory, results. Firstly, I found the distribution of the entire macroinvertebrate community, rather than a subset of the community, was best described by the amount of refuge space and the surface rugosity at 5 × magnification. Vulnerable invertebrate herbivores were consistently more abundant on *Myriophyllum*, which had measurably more refuge space yet was the plant in which pygmy perch had their strongest effects. Hacker and Steneck (1990) showed the abundance of marine amphipods was greatest on highly dissected forms of algae where there was enough space to occupy their body volume, but still enough structure on which to cling. Thus it is possible the herbivores were responding to the shape of the plant (as described by the fractal dimension) rather than the refuge space, but the surface rugosity was also greater on *Myriophyllum*. It would be necessary to separately manipulate rugosity and prey refuge in order to determine which aspect of plant structure invertebrate herbivores were responding to.

Secondly, in the laboratory experiment, predator effect size was measured by the number of mosquito larvae consumed rather than changes in vulnerable herbivore abundance. While mosquito larvae may have used the macrophyte structure differently than invertebrate herbivores, they were deliberately chosen to represent a

large, mobile and exposed prey item, which were the same criteria used for choosing the taxa in the vulnerable herbivore group. However, pygmy perch may have preferentially selected these prey in *Myriophyllum* compared to the prey in *Eleocharis* and *Triglochin*. Anderson (1984) showed that largemouth bass consumed guppies in low densities of plastic *Elodea* plants, but shifted to foraging on damselflies as density increased and they had more difficulty detecting guppies. It is possible that pygmy perch actively selected vulnerable invertebrate herbivores in *Myriophyllum* compared to the other two plants, but I have observed them to target mobile swimming invertebrates, regardless of their species, in holding tanks and in the field (D.M.Warfe, *pers.obs.*). Furthermore, analyses of their gut contents show their diets reflect the abundances of macroinvertebrates in the macrophyte beds of the Macquarie River (Humphries 1995), which indicates they feed opportunistically and are unlikely to show dietary selectivity.

Therefore, differences in the measurement of pygmy perch effects probably did not contribute to their unexpectedly greater impacts in *Myriophyllum*, which suggests this result may be due to the behaviour of both pygmy perch and their prey.

Prey behaviour

Myriophyllum supported twice as many vulnerable invertebrate herbivores than either *Eleocharis* and *Triglochin*, so the encounter rate between pygmy perch and prey may simply be greater on this plant. But as I mentioned in Chapter 4, I would expect to find more corroborative evidence in the literature, rather than the common pattern of fish predators consuming less prey as habitat structure increases. Thus, the invertebrate herbivores may be altering their behaviour in *Myriophyllum*, either as a result of other interactions or as result of the perception of refuge, such that they increase their exposure and thereby their risk of predation in this plant.

Invertebrate predators, predominantly damselfly larvae, were also more abundant in *Myriophyllum*, thus there was more opportunity for damselfly-herbivore interactions. In much the same manner that the escape response of mayflies to dragonfly predators increased their risk of predation by bluegill sunfish (Swisher et al. 1998), the escape response of the invertebrate herbivores may have increased their risk of predation to pygmy perch. The herbivores included amphipods and mayfly larvae, both of which tend to swim away at contact with the coenagrionid damselfly (D.M.Warfe, *pers.obs*).

This would then have increased their exposure to, and their risk of predation from, pygmy perch.

Similarly, competitive interactions between the vulnerable herbivores and other herbivores, such as caddisflies, may have also resulted in a swimming response of amphipods and ostracods, increasing their risk of predation. Coen et al. (1981) found that the refuge space provided by seagrasses was a limited resource for caridean shrimps, and the competitive loser suffered a greater risk of predation by pinfish. Despite there being measurably more refuge space on *Myriophyllum*, there was also a much greater abundance and diversity of animals on this plant, which may have limited the amount of available space and increased the number of potential competitive interactions.

Another reason why vulnerable invertebrate herbivores may have increased their mobility and exposure in *Myriophyllum* is they may have been able to "perceive" the reduced risk of predation provided by this plant. Mayfly larvae have been shown to assess their risk of predation by fish and alter their behaviour accordingly (McIntosh and Townsend 1996, Tikkanen et al. 1996). For example, McIntosh and Townsend (1996) showed that mayflies spent more time grazing on the tops of stones, where they are more at risk of predation by benthivorous fish, when common river galaxias are absent than when they are present. Likewise, Uiblein et al. (1996) found the ostracod *Cyridopsis vidua* could assess and alter its risk of predation from cyprinid fish by increasing its swimming activity and moving into more dense macrophyte cover. However, these studies imply that prey must be able to perceive the presence of a predator in order to exhibit changes in behaviour. In streams, macroinvertebrates have been shown to detect predators by the changes in hydrodynamic pressure waves and sound caused by their movement (Dodson et al. 1994). Given that macrophytes with a highly dissected leaf structure can decrease local current velocities in the water column (Gregg and Rose 1982), the structure of *Myriophyllum* may have similarly dampened the mechanical cues of pygmy perch so the herbivore prey were unable to detect their presence and therefore did not alter their behaviour, and reduce their exposure, accordingly.

Predator behaviour

The mechanisms invoking an increased risk of predation to prey as they increase their exposure in *Myriophyllum* depend on pygmy perch being able to take advantage of their increased exposure. As discussed in detail in Chapters 3 and 4, pygmy perch can shift their mode of foraging to a search strategy as macrophyte density and structural complexity increase, because they are small enough not to be hampered by macrophyte structure, and thereby annulling the perceived refuge role of high plant density (Chapter 3) and the structurally complex shape of *Myriophyllum* (Chapter 4).

Crowder and Cooper (1982) found bluegill sunfish consumed the most prey at intermediate macrophyte densities; as prey numbers increased with plant density, bluegills consumed more prey, but at higher densities the plants impeded bluegill mobility so they consumed less prey. The pygmy perch used in this experiment were about half the size of the bluegills used by Crowder and Cooper (1982), therefore they are less likely to impeded by macrophyte structure and thus can take advantage of the greater prey abundances on *Myriophyllum*.

Pygmy perch are a small fish at risk from predation by brown trout and redfin perch in the Macquarie River, thus it is possible that the more complex structure of *Myriophyllum* also afforded a predation refuge for pygmy perch. Small or juvenile fish, such as bluegill sunfish, have been shown to alter their habitat use, shifting to vegetated habitats, in the presence of larger piscivorous fish (Werner et al. 1983, Bean and Winfield 1995). Savino and Stein (1982, 1989) have shown that largemouth bass are less effective at greater macrophyte densities, and one of the reasons for this was that their bluegill sunfish prey changed their behaviour from schooling, at low plant densities, to dispersing amongst plant stems at higher densities. As brown trout and redfin perch are much larger than pygmy perch, they are more likely to be physically hampered by macrophyte structure, and pygmy perch may therefore be able to perceive the greater refuge provided by increased density and are small enough to disperse more amongst the stems without being impeded. Bluegill sunfish also have barred colour patterns which have been suggested to reduce their risk of detection at high plant densities (Savino et al. 1992). Similarly, the mottled colouration of pygmy perch (Figure 1, Chapter 3) may have blended with the finely dissected leaves of *Myriophyllum*, reducing their risk of detection by trout and redfin in this macrophyte in particular. A fruitful avenue of research to address the question of refuge for pygmy

perch would be to compare the effects of macrophyte structure and piscivore predation on pygmy perch.

The unexpected result of pygmy perch not being affected by macrophyte density and having their strongest effects in the most structurally complex macrophyte may be due to an increased encounter rate between pygmy perch and their prey. This increased encounter rate is most likely to arise from a combination of vulnerable herbivores being more numerous and exposed, with the increased mobility of pygmy perch in this plant. Vulnerable herbivores may be more exposed to predation due to their perception of a greater refuge in *Myriophyllum*, due to responses to negative interactions with other members of the community, or both. Pygmy perch may be mobile in *Myriophyllum* because they can perceive the greater refuge from piscivore predation afforded by this plant.

But despite the greater effects of pygmy perch in *Myriophyllum*, these effects did not cascade down to periphyton. There were indirect effects of pygmy perch on the composition (but not the biomass) of the periphyton community, but they did not vary with macrophyte shape. Furthermore, these effects were very weak, only appearing in the summer-start cages after six months. Had the experiment continued for longer, their indirect effects may have been stronger, particularly as, after summer, the vulnerable invertebrate herbivores increased in abundance in every predator treatment except the pygmy perch enclosures. As it stands, however, I found no evidence of strong indirect effects of pygmy perch, and I suggest this is a direct result of the strong influence of macrophyte structure on the macroinvertebrate and periphyton communities.

Implications of findings

A classic example of strong predator effects is a trophic cascade, where a predator's effects on their immediate prey have cascading effects on lower trophic levels (Carpenter et al. 1987). Implicit in the concept of trophic cascades is the phenomenon of "runaway consumption" (sensu Strong 1992) in that trophic cascades rely on strong interactions for their presence (Pace et al. 1999, Polis et al. 2000). However, community trophic cascades, where the biomass of the primary resource is substantially altered, have only been demonstrated in relatively simple systems – systems with either little habitat structure (if any), and/or systems with a simple and

strongly defined trophic structure (Polis et al. 2000, Schmitz and Suttle 2001). Indeed, Strong (1992) and Polis (1999) have further suggested that consumers generally do not have strong impacts, and trophic cascades are the exception rather than the rule. As a result, there has been much debate about how often they genuinely occur, although even advocates of the generality of trophic cascades concede "it is possible that [they] are less likely under conditions of high diversity or extensive omnivory" (Pace et al. 1999). The macrophytes in the Macquarie River support both these conditions.

Macroinvertebrates were very abundant and diverse, particularly on *Myriophyllum*, which had measurably more space and surface rugosity and is therefore more likely to support a more diverse range of microhabitats for macroinvertebrates. High diversity communities are able to channel the effects of trophic interactions down many different pathways, and are therefore more able to buffer and absorb these effects than simple, low diversity communities (Strong 1992, Polis et al. 2000). Strong (1992) clearly illustrates this concept with the analogy of a torrent of water flowing over a barren glacial landscape compared to a web of dissected creeks in a vegetated watershed; both conduct great volumes of water, but in the latter it is finely differentiated and thus well buffered.

As a result of this diverse community supported by structurally complex macrophytes, pygmy perch consume prey on the basis of their abundance, mobility and exposure, and body size rather than their trophic status (Chapter 4, Humphries 1995) and thus are omnivorous like some other fish predators (Morin 1984, Diehl 1988). According to some food web theory, omnivory should be a destabilising influence on food web structure, and therefore rare (May 1973, Pimm 1982). However, more recent research indicates that it is common in a range of food webs and may actually help stabilise webs which have a loose structure without sharply defined and delineated trophic levels (McCann et al. 1998, Polis 1998). McCann et al. (1998) used nonlinear modelling to assess the influence of weak trophic interactions, such as omnivory, on food web dynamics. They found that weak trophic links dampen oscillations between consumers and resources so that having many prey reduces the chances of any particular prey being driven to extinction, thereby promoting community persistence and stability. While this runs counter to previous theory, it better explains the complexity often observed in real communities and the absence of true trophic

cascades in many systems (Polis 1998). Considering negative evidence of strong trophic interactions (and their indirect effects) is seldom published (Polis et al. 2000), systems with a simple trophic structure providing the conditions for a community-level trophic cascade may be much rarer than initially anticipated (Polis and Strong 1996, McCann et al. 1998).

Conclusions

Macrophytes provide an important habitat for macroinvertebrates in freshwater systems and it has been hypothesised that this is because of their refuge function. In this system however, pygmy perch did not have strong effects on the macroinvertebrate prey community and, despite having lower prey-capture success in *Myriophyllum*, their strongest effects were in the macrophyte theoretically providing the most refuge for prey. However, macroinvertebrates did not respond to just the amount of refuge from pygmy perch predation; they also responded to the actual shape of the plants, occurring in greater abundance on the macrophyte with a more structurally complex shape (as measured by the fractal dimension). *Myriophyllum* provided a greater abundance and diversity of habitats which in turn supported a greater abundance and diversity of macroinvertebrates and a greater abundance of periphyton. As a result of the greater abundance and diversity of prey supported by *Myriophyllum*, the effects of pygmy perch predation were well buffered. In this system, macrophyte structure strongly affected the structure of both the macroinvertebrate and periphyton communities, primarily through effects of its shape, and secondarily through its effects on pygmy perch predation. Clearly, when investigating the effects of trophic interactions such as predation on community structure, it is crucial to consider the habitat in which they are occurring.

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Appendix A

Gut contents of Ischnura heterosticta tasmanica *from the Macquarie River, Tasmania.*

Forty-three individuals of *Ischnura heterosticta tasmanica* larvae were collected from a variety of macrophyte beds (*Myriophyllum variifolium*, *Eleocharis sphacelata*, *Triglochin procera*, *Scirpus fluitans*, and *Vallisneria gigantea*) in the Macquarie River in the summer of 1997. Each larva was measured and their gut contents were mounted and examined under a compound microscope. I counted the head capsules of macroinvertebrate prey items and assumed they represented entire invertebrates that had been wholly consumed. Prey were identified to order and family where possible. See over for table of results.

	PREY ITEM	Ch.	Or.	Ta.	Am.	Chy.	Co.	Eph.	Ple.	TOTAL
Larval body size	Larval head width									
13.5	3.0		8							8
9.5	2.5		2							2
6.5	1.5		1	2						3
15.5	3.5		2						1	3
9.5	2.0	1	5							6
13.5	3.0	1	2							3
10.5	2.5	1	2							3
13.0	2.5		5							5
10.0	2.5		2							2
15.0	3.5		8							8
11.0	2.5		3							3
13.5	3.0		9	1	1					11
13.0	3.0		5	2						7
10.0	2.5		4							4
10.0	2.5				1					1
9.0	2.0		1		1					2
11.0	3.0		4		1					5
12.5	3.0		2			1				3
6.5	1.5		2			1				3
12.0	2.5		1		1					2
13.5	3.0		5							5
10.5	2.5		2					1		3
13.0	3.0							2		2
10.0	2.5							1		1
16.0	3.5		2							2
7.0	2.0		3							3
8.0	1.5		1							1
11.0	3.0	1	1	1						3
11.0	3.0		2							2
14.0	3.0	1	4		1			1		7
7.0	2.0	1	1							2
13.0	3.0							2		2
13.0	3.0		1		1	5	1	1		9
13.0	2.5		1					1		2
13.0	3.0							5		5
12.0	3.0	1	2			2	5			10
7.0	2.5						2			2
9.0	2.0		3			11				14
10.0	2.5					3				3
5.5	0.8	1	2							3
9.0	1.0	3	10							13
10.0	3.5	4	11							15
TOTAL		**15**	**119**	**6**	**7**	**23**	**8**	**14**	**1**	

Column key: Ch. = Chironominae, Or. = Orthocladiinae, Ta. = Tanypodinae, Am. = Amphipoda, Chy. = Chydoridae, Co. = Copepoda, Eph. -= Ephemeroptera, and Ple. = Plecoptera.

Appendix B:

Identification keys used for macroinvertebrates and periphyton

Macroinvertebrates:

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Personal communications:

Peter McQuillan (Lepidoptera), School of Geography, University of Tasmania.

Alastair Richardson (Amphipoda), School of Zoology, University of Tasmania.

Tom Sloane (Coleoptera), School of Zoology, University of Tasmania.

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Appendix C:

Macroinvertebrate taxon list

The macroinvertebrate taxa sampled over the duration of the field experiment, identified to genus where possible. Each row represents a taxon that was enumerated.

Cnidaria				*Hydra* sp.
Oligochaeta		Naididae		
Nematoda				
Nemertea				
Hirudinea		Glossiphonidae		
Platyhelminthes	Tricladida			
Crustacea	Cladocera	Daphniidae		
		Chydoridae		
	Copepoda			
	Ostracoda			
	Amphipoda	Ceinidae		*Austrochiltonia australis*
	Decapoda	Atyidae		*Paratya australiensis*
Mollusca	Bivalvia	Sphaeriidae		*Sphaerium* sp.
	Gastropoda	Planorbidae		*Physastra gibbosa*
				Gyraulus tasmanicus
		Hydrobiidae		*Beddomeia* complex
		Ancylidae		*Ferrissia tasmanica*
Insecta	Diptera	Chironomidae	Tanypodinae	
			Chironominae	Tanytarsiini
				Chironomini
			Orthocladiinae	
		Ceratopogonidae		
		Stratiomyidae		*Odontomyia* sp.
		Empididae		
		Culicidae	Anophelinae	
		Simuliidae		
	Neuroptera	Sisyridae		
	Trichoptera	Leptoceridae		*Notalina* sp.
				Leptocerus sp.1
				Leptocerus sp.2
				Oecetis sp.
				Triplectides sp.
		Hydroptilidae		*Hellyethira* sp.
				Oxyethira mienica
				Orthotrichia sp.
		Ecnomidae		*Ecnomus* sp.
		Conoesucidae		*Conoesucus* sp.
		Hydropsychidae		*Cheumatopsyche* sp.
		Polycentropodidae		*Plectrocnemia* sp.
		Calamoceratidae		*Anisocentropus* sp.
		Hydrobiosidae		unidentified 1st instars
	Plecoptera	Gripopterygidae		*Dinotoperla marmorata*
				Leptoperla beroe
	Ephemeroptera	Baetidae		*Centroptilum* sp.
		Caenidae		*Tasmanocoenis* sp.
		Leptophlebiidae		*Atalophlebia australis*
				Nousia sp.
	Lepidoptera	Pyralidae		*Nymphula nitens*

	Odonata	Hemicorduliidae		*Hemicordulia* sp.
		Lestidae		*Austrolestes* sp.
		Coenagrionidae		*Ischnura heterosticta tasmanica*
		Telephlebiidae		*Aeshna* sp.
				Austroaeshna sp.
		Gomphidae		*Austrogomphus* sp.
	Hemiptera	Corixidae		*Sigara* sp.
				Micronecta sp.
				Diaprepocoris sp.
		Naucoridae		*Naucoris congrex*
	Coleoptera	Dytiscidae	Hydroporinae	unidentified larvae
				unidentified adults
		Gyrinidae		*Gyrinus* sp.
		Hydrophilidae		unidentified larvae
				Hydrochus sp.
				Helochares australis
		Elmidae		*Austrolimnius* sp.
				Coxelmis sp.
Arachnida	Hydracarina	unidentified mites		
		Limnocharidae		
		Pezidae		
		Oxidae		
		Unionicolidae		
		Arrenuridae		
		Hydromidae		
		Halacaroidae		
		Limnesiidae		
		Orobatida		
		Astigmata		

Appendix D:

Vulnerability scores for macroinvertebrate taxa sampled during the field experiment.

Macroinvertebrate taxa sampled in the field experiment and classified according to their vulnerability to predation by pygmy perch. For each trait (see Table 1, Chapter 4), taxa were given a score 0,1,2 or 4, where the higher score indicates a greater risk of predation. The three highlighted taxa are those with the highest total scores, *Austrochiltonia australis*, *Atalophlebia australis* and ostracods, and they comprised the group "vulnerable invertebrate herbivores".

TAXON	abundance	mobility/ exposure	body size	occurrence in guts	TOTAL	RANK
Hydra	4	0	0	0	4	8
Naididae	4	1	0	0	5	7
Nematoda	4	1	0	0	5	7
Nemertea	4	1	0	0	5	7
Glossiphonidae	0	0	0	0	0	12
Tricladida	4	2	0	0	6	6
daphniid	4	2	0	0	6	6
chydorid	4	1	0	0	5	7
copepod	4	2	0	0	6	6
ostracoda	**4**	**4**	**0**	**2**	**10**	**2**
Austrochiltonia	**4**	**4**	**1**	**4**	**13**	**1**
Paratya	0	4	1	0	5	7
Sphaerium	0	0	0	0	0	12
Physastra	4	0	0	1	5	7
Gyraulus	0	0	0	0	0	12
Beddomeia	0	0	0	0	0	12
Ferrissia	0	0	0	0	0	12
Tanypodinae	4	2	0	1	7	5
Chironomini	4	1	0	1	6	6
Tanytarsiini	4	1	0	1	6	6
Orthocladiinae	4	2	0	1	7	5
Ceratopogonidae	4	2	0	0	6	6
Odontomyia	0	2	0	0	2	10
Anophelinae	0	4	0	0	4	8
Empididae	0	2	0	0	2	10
Simuliidae	0	0	0	0	0	12
Sisyridae	0	2	0	0	2	10
Notalina	1	4	1	0	6	6
Leptocerus sp.1	0	2	0	0	2	10
Leptocerus sp.2	0	2	0	0	2	10
Oecetis	0	2	0	0	2	10

Triplectides	0	2	1	0	3	9
Hellyethira	4	1	0	0	5	7
Oxyethira	0	1	0	0	1	11
Orthotrichia	0	1	0	0	1	11
Ecnomus	4	2	1	0	7	5
Conoesucus	0	2	0	0	2	10
Cheumatopsyche	0	1	1	0	2	10
Plectrocnemia	2	2	0	0	4	8
Anisocentropus	0	2	1	0	3	9
Hydrobiosidae	0	2	1	0	3	9
Dinotoperla	0	1	0	0	1	11
Leptoperla	0	2	1	0	3	9
Centroptilum	0	4	1	0	5	7
Tasmanocoenis	2	2	1	1	6	6
Atalophlebia	**2**	**4**	**1**	**2**	**9**	**3**
Nousia	0	4	1	1	6	6
Nymphula	0	2	1	0	3	9
Hemicordulia	0	2	1	0	3	9
Austrolestes	0	4	1	0	5	7
Ischnura	4	2	1	1	8	4
Aeshna	0	2	1	0	3	9
Austroaeshna	0	2	1	0	3	9
Austrogomphus	0	2	1	0	3	9
Sigara	0	2	0	0	2	10
Micronecta	0	4	0	0	4	8
Diaprepocoris	0	4	0	0	4	8
Naucoris	0	4	1	0	5	7
Hydroporinae larvae	0	4	1	0	5	7
Hydroporinae adults	0	4	1	0	5	7
Gyrinus	0	2	1	0	3	9
Hydrophilid larvae	0	2	0	0	2	10
Hydrochus	0	2	0	0	2	10
Helochares	0	2	0	0	2	10
Austrolimnius	0	2	0	0	2	10
Coxelmis	0	2	0	0	2	10
unidentified mites	0	2	0	0	2	10
Limnocharidae	0	2	0	0	2	10
Pezidae	0	2	0	0	2	10
Oxidae	0	2	0	0	2	10
Unionicol	0	2	0	0	2	10
Arrenurid	0	2	0	0	2	10
Hydromidae	0	4	0	0	4	8
Halacaroidae	0	2	0	0	2	10
Limnesiidae	0	2	0	0	2	10
Orobatida	2	2	0	0	4	8
Astigmata	0	1	0	0	1	11