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LAKE LITTORAL MACROINVERTEBRATE BIOMASS AND COMMUNITY STRUCTURE ACROSS ALKALINITY AND NUTRIENT GRADIENTS

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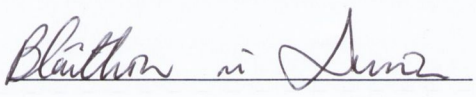

SUMMARY

The aim of this study was to investigate the effects of alkalinity and nutrients on the biomass of lake littoral macroinvertebrate communities. An initial comparative study of nine lakes determined the effect of alkalinity and nutrients on length-mass relationships of eight common aquatic macroinvertebrates among lakes representing an alkalinity and nutrient gradient. The influence of alkalinity and nutrient enrichment on total biomass, mean biomass, and on the slope and intercept of littoral macroinvertebrate size spectra with nutrients, alkalinity and substrate was examined in a comparative study of forty eight lakes, representing an alkalinity and nutrient gradient. The effects of alkalinity and nutrient enrichment on community structure (based on the collective biomass of taxa) and on the proportional biomass of functional feeding groups (FFGs) of lake littoral macroinvertebrates from stony substrates in these forty eight lakes was also investigated.

In an initial study of nine lakes, representing a gradient of phosphorus and alkalinity concentrations, littoral macroinvertebrates from both hard and soft substrates and water chemical variables were measured. Invertebrate samples were frozen, and eight common taxa selected, thawed, measured, dried and weighed to establish the relationship between body length and dry mass, and how this relationship varied among the lakes. The relationship between Ln dry mass and Ln body length, in models of all taxa, was very strong, differing little among lakes with remarkably constant slopes. Alkalinity had a marginal effect on the dry mass of six taxa, and on the slope of the length-mass regression of three taxa; TP had a marginal effect on the dry mass of five taxa and on the slope of the length-mass regression of one taxa. Where alkalinity or TP were found to alter the length mass regressions of taxa, slopes were close to zero within the confidence limits of the length-mass regression, adding little predictive value to the regression. Taxa identified to family or order level did not demonstrate greater residual variability than those identified to species level, although this may be dependent on the range of body shapes within the order/family under examination.

In a broader study, size relationships in the littoral zone were explored for the first time, between seasons, substrates and among 48 lakes with both nutrient and alkalinity gradients. Water chemical variables were measured at each lake, and macroinvertebrates were collected from hard and soft substrates in spring and summer, identified and measured. Size relationships within and among lakes were analysed by means of linear mixed effects models. Stronger within-lake variation (between substrates) than among-lake variation was observed, for models of mean and total biomass; models of size spectra also differed between substrates, with greater mean and total macroinvertebrate biomass found on soft substrates. Strong seasonal effects were observed in models of size structure, biomass-based community structure and proportions of FFGs, within lakes, examined for stony substrates only. More seasonal variation was detected in the biomass of lake littoral macroinvertebrates on hard substrates compared with soft substrates, and a relative decrease in the collective biomass of large invertebrates and a corresponding increase in small invertebrates in summer in both substrates observed. Despite significant within-lake variance between substrates and seasons, consistent patterns in different aspects of lake littoral

macroinvertebrate biomass with alkalinity and nutrients were observed, suggesting macroinvertebrate biomass as an emergent property of lake ecosystems.

Alkalinity had significant marginal effects, on mean and total biomass, on the size structure of littoral macroinvertebrates, and on the proportional biomass of some FFGs ('large' grazers-scrapers, shredders and filter feeders). There was a marginal shift towards larger organisms and significant changes in the distribution and proportional biomass of several taxonomic groups with increasing alkalinity. Models of mean and total biomass, size structure, biomass-based taxonomic composition (in spring) and proportional biomass of predators consistently showed that TN explained variation in the data better than TP. In addition TN effects were frequently curvilinear, suggesting that the standing macroinvertebrate biomass appears to be limited by TN, up to an optimal TN concentration. These results indicate a decline in macroinvertebrate biomass in almost all size classes at TN concentrations $\geq$ 2 mg L^{-1}, and when coupled with studies on macrophytes and algal biomass concentrations which showed optimal growth at similar TN concentrations, it appears unwise for the continued use by the Irish EPA of quality classes, established under the EU Nitrates Directive.

Do mo thuismitheoirí, do mo chlann uilig,

agus do Rory,

le grá mór i gcónaí

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CHAPTER 1

INTRODUCTION

1.1 Thesis Structure

This research addresses some of the knowledge gaps highlighted above. The overall aim is to understand the effects of alkalinity and nutrients on the biomass of lake littoral macroinvertebrate communities. The remaining thesis is laid out as follows:

- ***Chapter 2*** investigates the effect of alkalinity and nutrients on length-mass relationships of eight common aquatic macroinvertebrates among nine lakes;
- ***Chapter 3*** examines changes in the total biomass, mean biomass and slope and intercept of littoral macroinvertebrate size spectra with nutrients, alkalinity and substrate among forty eight lakes;
- ***Chapter 4*** explores the influence of alkalinity and nutrient enrichment on community structure (based on the collective biomass of taxa) and on the proportional biomass of functional feeding groups (FFGs) of lake littoral macroinvertebrates from stony substrates in these forty eight lakes;
- ***Chapter 5*** discusses the overall findings of this study, highlights knowledge gaps and areas for future research, and presents the main conclusions of this body of research.

1.2 Research Aims

The aim of this study is to investigate the effects of alkalinity and nutrients on the biomass of lake littoral macroinvertebrate communities. Ecological communities are a complex array of interacting species, yet underlying internal structures reflected by attributes of body size may relate to many ecological characteristics, such as resource use among communities (Schmid and Schmid-Araya, 2007). Owing to the many physiological correlates of body size (ingestion, excretion, growth and reproduction) as well as correlations with lifespan and home range (Peters, 1983; Cyr, 2000; White et al., 2007) the distribution of body sizes can reveal underlying processes affecting community assembly and persistence, reflecting basic ecosystem processes (Schmidt-Nielsen, 1984; Manly, 1996; Allen et al, 2006). Body size provides a means of integrating approaches based on biomass and energy flux with those based on abundances and populations (Woodward and Warren, 2007). Community body size relationships have shown interesting consistent patterns in the open water zone of lakes,

but research on these relationships has been neglected in the littoral zone. We know little of the effects of natural physical and chemical gradients, or of anthropogenic disturbance, on these relationships. In this study, the effects of nutrients and alkalinity on size relationships among macroinvertebrates in the littoral zone, between seasons and substrates, are explored across a large number of lakes, for the first time.

1.3 The lake littoral zone and benthic macroinvertebrates

The littoral region of the lake is an interface zone between the land of the drainage basin (the 'catchment') and the open water of the lake. The latter includes the benthos (originating from the Greek *benthos* for bottom and including the assemblage of organisms associated with the bottom) and overlying water (Moss, 1998; Wetzel, 2001). The littoral zone extends from the shoreline region between the highest and lowest seasonal water levels, to a depth where sufficient light reaches the benthos for significant photosynthesis, and can thus, support emergent, submerged and rooted floating vegetation and epiphytic algae (Moss, 1998; Wetzel, 2001; Solimini *et al.*, 2006). The littoral zones of shallow lakes (with proportionally large littoral regions) can be extremely important in the energy budgets of lakes, with a large standing biomass of associated submerged macrophytes and epiphytes, significantly contributing to total lake primary production, particularly in clear water lakes (Harrison and Hildrew, 1998; Liboriussen and Jepessen, 2003; Blindow *et al.*, 2006; Nõges *et al.*, 2010).

Benthic macroinvertebrates are defined as organisms that inhabit the bottom substrates of freshwater habitats (e.g. sediments, debris, logs, macrophytes, filamentous algae), for at least part of their life cycle (Rosenberg and Resh, 1993). There is a high diversity among benthic macroinvertebrates found in the littoral zone of lakes (lake littoral macroinvertebrates) owing to the spatial, temporal and three-dimensional heterogeneity of substrates, offering a range of different microhabitats and generally good light, food and oxygen conditions (Rasmussen, 1988; Brodersen *et al.*, 1998). Lake size and lake basin morphometry are significant factors in determining the relative importance of littoral habitats and the potential for littoral invertebrates to contribute to whole-lake secondary production (Vander Zanden and Vadeboncoeur, 2002).

Macroinvertebrates can be useful indicators of water and ecological quality for several reasons: (i) they are ubiquitous and can therefore be affected by environmental perturbations in many different types of aquatic systems and various habitats within those systems; (ii) the large number of species offers a spectra of responses to environmental stresses and their mainly sedentary nature allows effective spatial analyses of pollutant or disturbance effects; and (iii) they have long life cycles compared with other groups, allowing examination of temporal changes caused by disturbances (Lenat *et al.*, 1980; Rosenberg and Resh, 1993). Macroinvertebrates can also be responsive to short term environmental changes which may not be detected by routine chemical monitoring (Reice and Wohlenberg, 1993).

1.4 Benthic macroinvertebrate biomass, size structure and functional feeding groups

Littoral macroinvertebrate communities potentially control the biomass of periphytic algae (James *et al.*, 2000), and recycle detrital material from aquatic (Kornijów *et al.*, 1995) and terrestrial (France, 1995) sources. They also act as a crucial link in the transfer of energy from primary producers and detritus to fish and waterfowl (Hanson, 1990). Moreover, macroinvertebrate biomass and production may be correlated with fish biomass and production in lakes (Hanson and Legget, 1982); with fish growth dependent on the availability of prey of suitable size (Kerr, 1971).

The body size of an organism is correlated with many life-history traits (Calder, 1984), and fundamental biological properties, such as metabolic rates (Peters, 1983; Strayer, 1991; Brown *et al.*, 2004) and influences virtually all aspects of its energy and material flow (Peters, 1983). The size distribution of a community of organisms is, thus, related to nutrient competition, resource availability and use, and predator-prey relations (Peters, 1983) and may be a practical and useful description of the functioning of that community (Peters, 1983; Strayer, 1986). The biomass and size structure of communities in aquatic ecosystems represents a level of organisation closely related to energy transfer, physical habitat characteristics and perturbations, providing an alternative and complementary perspective to a taxonomic analysis of community structure (Hanson, 1990; Rasmussen, 1993; Solimini *et al.*, 2005. Nevertheless, there is

still relatively little known about the size structure of benthic invertebrate communities (Strayer, 1991; Pagola-Carte, 2004). Understanding the factors that affect the abundance and biomass of macroinvertebrates and the size-structure of their communities will improve our understanding of the trophic dynamics of lakes (Hanson, 1990).

The fundamental relationship between linear body measurements and the body mass of an organism, allowing the estimation of body mass from measurements of body length, is a very useful tool in ecological research (Smock, 1980; Miyasaka *et al.*, 2008). However, little is known of how body length-mass relationships change with water chemistry for individual species. One of the objectives of this study is to examine changes in length-mass relationships with alkalinity and nutrients.

Aquatic macroinvertebrate communities are classified into functional feeding groups (FFGs) based on observed patterns of food consumption, morphology of feeding structures (mouthparts and legs) and feeding behaviour (Moog, 1995; Wallace and Webster, 1996; Merrit and Cummins, 1996; Johnson *et al.*, 2004). This is an alternative to classifying these communities by trophic level, because based on analyses of food ingested, most aquatic insects are essentially omnivorous (Merrit and Cummins, 1996). An existing FFG classification scheme (Cummins, 1974; Cummins and Klug, 1979) was modified by Moog (1995), and further developed with the AQEM database of species autoecological traits (Asterics® Version 3.01; http://www.aqem.de) to include ten classes of functional feeding types (shredders, grazer-scrapers, active and passive filter feeders, gathering-collectors, miners, xylophagous, predators, parasites, and other unclassified feeders). Examination of FFGs can be useful in understanding the degree to which the invertebrate biota of a given aquatic system is dependent upon a particular food resource, linking food resources and invertebrate morphological-behavioural adaptations (Cummins, 1974; Merrit and Cummins, 1996). The ratios of different FFGs can change with the varying availability of food resources, and can be used in the ecological assessment of water bodies (Merrit and Cummins, 1996; Johnson *et al.*, 2004; Solimini *et al.*, 2006).

1.5 Nutrient enrichment of freshwaters

Nutrient enrichment (eutrophication) is one of the primary anthropogenic pressures affecting freshwaters globally (Schindler, 2006; Smith *et al.*, 2006). The most recent broad-scale examination of water quality in Ireland (2007 to 2008) identifies nutrient enrichment as the principal pressure on lakes (Environmental Protection Agency, 2009). Diffuse and point source inputs of nutrients from agricultural and urban activities are a major source of phosphorus and nitrogen to surface waters (Carpenter *et al.*, 1998). Inputs of nutrients can alter the structure and function of lake ecosystems (Tolonen, 2004; Brauns *et al.*, 2007; Baldy *et al.*, 2007). Eutrophication of lakes can result in a shift from dominance of benthic to pelagic algal primary production (Vadeboncoeur *et al.*, 2003; Chandra *et al.*, 2005; Liboriussen and Jepessen, 2006). This change in primary production transfers up the food web to littoral invertebrate primary consumers and predators (Vadeboncoeur *et al.*, 2003). Increased nutrients coupled with increased pelagic primary production and algal blooms may also inhibit biomass and reduce species richness of submerged and floating macrophyte growth (James *et al.*, 2005; Gonzaléz *et al.*, 2005; Özkan *et al.*, 2010). However, phytoplankton biomass may reach an asymptote in hypereutrophic lakes as light will eventually begin to limit even mixed-layer phytoplankton production (McCauley *et al.*, 1989).

Research examining the effects of nutrient enrichment on lakes has, in the main, concentrated on the response of phytoplankton (Dillon and Rigler, 1974; Jones and Bachman, 1976; Canfield and Bachman, 1981; Jeppesen *et al.*, 2005) and profundal invertebrate communities (Saether, 1979; Lang, 1997, 1999; Dinsmore *et al.*, 1999). In contrast, the response of lake littoral macroinvertebrate communities to nutrient enrichment has been examined in much fewer studies (Brodersen *et al.*, 1998; Tolonen *et al.*, 2001; White and Irvine, 2003; Brauns *et al.*, 2007; Donohue *et al.*, 2009), probably owing to a view that the structural heterogeneity of the lake littoral zone prevents meaningful use of littoral macroinvertebrate communities for ecological assessment (Rasmussen, 1988; Harrison and Hildrew, 1998; Moss *et al.*, 2003). Nevertheless, a number of studies have significantly related changing nutrient concentrations to littoral macroinvertebrate community structure (Blumenshine *et al.*, 1997; Brodersen *et al.*, 1998; White and Irvine, 2003; Tolonen *et al.*, 2001; Brauns *et al.*, 2007; Donohue, 2008; Donohue *et al.*, 2009), although responses are often nested

within specific habitats (Tolonen *et al.*, 2001; White and Irvine, 2003; Brauns *et al.*, 2007; Donohue, 2008; Donohue *et al.*, 2009).

Changes in benthic macroinvertebrate size relationships with nutrient enrichment have not been extensively investigated for the littoral zones of lakes. Some studies examining lentic littoral macroinvertebrate community size distributions have recorded a significant increase in the abundance of smaller organisms with nutrient enrichment (Hayward and Margraf, 1987; Rasmussen, 1993). The total biomass of littoral macroinvertebrate communities in both streams and lakes appears to increase with nutrient enrichment (Rasmussen, 1988; Baca and Threkeld, 2000; Morin *et al.* 2001). However, the influence of nutrients on littoral macroinvertebrate communities has been found to vary depending on habitat or substrate sampled (Brauns *et al.*, 2007; Donohue *et al.*, 2009; Free *et al.*, 2009), as macrophytes and benthic algae affect nutrient and light availability by sequestering nutrients (Hanson 1990), stabilizing sediments, and reducing phosphorus mobility (Carlton and Wetzel, 1988). There is a wide gap in our understanding of nutrient effects on size relationships among lake littoral macroinvertebrates that this study aims to address.

1.6 The European Water Framework Directive

The European Directive 2000/60/EC, commonly known as the Water Framework Directive (WFD; CEC, 2000), was introduced in recognition of increasing anthropogenic pressures on aquatic environments. It requires the prevention of deterioration of ecological status and pollution of surface waters, with a return to "good water status" by 2015 (or for heavily modified and artificial bodies, "good ecological potential" and "good chemical status"), which is defined as minimal impact from "reference" state. "Reference" state relates to baseline hydromorphological, physicochemical and biological reference conditions of the water body, which has to be decided for each individual water body. To meet this objective, member states are obliged to assess the ecological quality of all standing waters within the European Union (EU), by establishing extensive monitoring programmes of surface and ground waters within the River Basins of their territories. This will, first, establish the quality status of surface waters and second, evaluate whether the status is improving or degrading with time.

The legal requirement of monitoring biological as well as chemical variables provides for a more holistic assessment of the functioning and structure of aquatic ecosystems by EU member states, demonstrating a shift in mindset from viewing water resources as a product to be monitored for chemistry, to one that regards water as a heritage (Solimini *et al.*, 2006). The biological elements to be monitored, referred to in Annex V of the Directive, include phytoplankton, macrophytes and phytobenthos, benthic macroinvertebrates and fish. Annex V specifies that measures of "taxonomic composition and abundance", the "ratio of disturbance sensitive taxa to insensitive taxa" and the "level of diversity", be incorporated into the ecological assessment procedures. The WFD also requires that all water bodies, including lakes, are placed in similar types (termed "ecotype" in the Directive) based on physical criteria such as climate, altitude, size, depth, morphology, geographic location and geology and reference conditions (or "high status"). This allows assessment of ecological status based on comparison with type-specific reference conditions (Moss *et al.*, 2003).

The WFD has generated much research on littoral macroinvertebrates in lakes and their response to anthropogenic pressures (White and Irvine, 2003; Tolonen *et al.*, 2001; Tolonen, 2004; Brauns *et al.*, 2007; Donohue, 2008; Donohue *et al.*, 2009). Nevertheless, our understanding of spatial and temporal patterns among lake littoral macroinvertebrate communities and of their ecological response to pressures, from which monitoring and classification is guided, remains limited (Moss *et al.,* 2003; Donohue *et al.*, 2009). Water chemistry, sediment type, vegetation, habitat structure, lake depth and season have significant effects on littoral macroinvertebrate communities, and interactions among these variables may be mediated by trophic dynamics (Solimini *et al.*, 2006). The distribution of body size, and the use of functional feeding groups have been proposed as possible alternatives for the assessment of lake trophic state using littoral macroinvertebrates (Basset *et al.*, 2004; Solimini *et al.*, 2006) as they have potential as descriptors for comparison across quality elements, across regional areas and other spatial scales (Basset *et al.*, 2004). The objective of this study is to increase our understanding of these descriptors.

CHAPTER 2

A COMPARISON OF LENGTH-MASS REGRESSIONS OF LAKE LITTORAL MACROINVERTEBRATES AMONG LAKES

2.1 Introduction

An organism's size can influence all aspects of its energy and material flow, because physiological processes such as excretion, ingestion, growth and reproduction are scaled to the body size of the individual (Peters, 1983). Therefore, estimating the biomass of aquatic macroinvertebrates is important for understanding the seasonal patterns, life histories, relationships between functional feeding groups, secondary production and trophic energy budgets of food webs (Rigler and Downing, 1984; Meyer 1989; Burgherr and Meyer, 1997; Sabo *et al.*, 2002; Baumgärtner and Rotthaupt, 2003). The fundamental relationship that exists between linear body measurements and the body mass of an organism is a useful tool in ecological research, allowing us to calculate body mass from measuring a linear body dimension (Smock, 1980; Miyasaka *et al.*, 2008).

The biomass of macroinvertebrates can be measured either (i) directly (wet mass, dry mass, ash-free dry mass) or (ii) indirectly, by either estimating the biovolume (e.g. via image analysis) or using body length-dry mass regressions, where an organism's body dimension is measured and the body mass is predicted using a regression previously established from the body length-dry mass relationship (henceforth referred to as length-mass regressions [Smock, 1980; Meyer, 1989; Burgherr and Meyer, 1997; Benke *et al.*, 1999; Baumgärtner and Rotthaupt, 2003]). However, many methods of biomass measurement are considered unsuitable and unreliable. For example, direct measurement of wet mass depends upon factors prone to bias and can be difficult to reproduce, such as the water content of the organism, or the techniques used to remove excess water (Burgherr and Meyer, 1997; Baumgärtner and Rotthaupt, 2003). Weighing the dry mass of preserved animals can be biased by taxon specific mass loss, dependant on the preservative used and the preservation time (Leuven *et al.*, 1985; Meyer, 1989; Burgherr and Meyer, 1997; Benke *et al.*, 1999; Gonzalez *et al.*, 2002; Stoffels *et al.*, 2003). Measurement of dry mass of fresh animals is laborious, specimens are destroyed and accurate measurement of biomass requires access to precision electronic balances (particularly for small specimens). Therefore, although this method is the most accurate, it is often inconvenient or unviable (Rogers *et al.*, 1976; Sample *et al.*, 1993; Burgherr and Meyer 1997; Gonzalez *et al.*, 2002; Sabo *et al.*, 2002). Indirect measurement of biomass through biovolume estimation can be

performed by image analysis, or using volume formulae for cylinders, cubes or spheres (Burgherr and Meyer, 1997; Alcaraz *et al.*, 2003). However, specialised equipment is required for image analysis and biovolume formulae (e.g. formulae for cubes or spheres) may significantly underestimate the mass of larger animals (Morin and Nadon, 1991; Burgherr and Meyer, 1997).

Length-mass regressions are widely accepted as a fast, simple, accurate and non-destructive means of estimating the body mass of aquatic macroinvertebrates (Rogers *et al.*, 1976; Meyer, 1989; Sample *et al.*, 1993; Burgherr and Meyer, 1997; Benke *et al.*, 1999; Gonzalez *et al.*, 2002; Sabo *et al.*, 2002; Baumgärtner and Rotthaupt, 2003; Stoffels *et al.*, 2003). In most instances, the dry mass of aquatic macroinvertebrates is best derived from body length, rather than from head capsule width, as errors in the measurements will have a greater impact when the size range is smaller (Wenzel *et al.*, 1990; Baumgärtner and Rotthaupt, 2003).

Dry mass is most frequently modeled as a power function, or parabolic of body length, in the form of:

$$W = aL^b \qquad [1]$$

where the independent variable (L) is a measure of the body length of the organism (or another linear body dimension), the dependant variable (W) represents the dry mass of the organism and a and b are constants (Smock, 1980; Meyer, 1989; Burgherr and Meyer, 1997; Benke *et al.*, 1999; Johnston and Cunjak, 1999; Miyasaka *et al.*, 2008). This is often presented log transformed as a linear equation:

$$\ln W = \ln a + b \ln L \qquad [2]$$

Although linear, exponential, quadratic and polynomial models have been used in the literature, the power function normally provides a better fit to the data than most other mathematical formulations and has gained the widest acceptance (Mason, 1977; Smock, 1980; Meyer, 1989; Burgherr and Meyer, 1997; Benke *et al.*, 1999; Johnston and Cunjak, 1999).

The length-mass relationships of many stream invertebrates have been compiled over the last number of years from a variety of distinct stream types in locations including North America, Central Europe, New Zealand and Japan (Smock, 1980; Meyer, 1989; Towers *et al.*, 1994; Burgherr and Meyer, 1997; Benke *et al.*, 1999; Johnston and Cunjak, 1999; Sabo *et al.*, 2002; Baumgärtner and Rotthaupt, 2003; Stoffels *et al.*, 2003; Miyasaka *et al.*, 2008). However, few studies have compiled regressions for lentic macroinvertebrates (Baumgärtner and Rotthaupt, 2003; Stoffels *et al.*, 2003).

A number of important factors must be considered when examining the length-mass relationships of aquatic macroinvertebrates. Firstly, intraspecific variation in the length-mass relationships of aquatic macroinvertebrates has been reported by several authors (Schröder, 1987; Johnson *et al.*, 1993; Benke *et al.*, 1999; Johnston and Cunjak, 1999; Gonzalez *et al.*, 2002). For example, Schröder (1987) found differences among the length–mass regressions of the same species of blackfly larvae collected from the inflow and outlet of the same lake. Furthermore, Wenzel *et al.* (1990) found differences in biometric relations for larvae of the *Ecdyonurus venosus* group from softwater and hardwater streams. Secondly, the taxonomic resolution of the regression may also influence the length-mass relationship. Baumgärtner and Rotthaupt (2003) found that equations presented at family level (Chironominae and Orthocladiinae) resulted in poor R^2 values for the regressions. Accordingly, the authors recommended using equations at the lowest possible taxonomic level, to species or genus level if possible (i.e. at a high level of resolution). Models introduced at lower taxonomic levels (e.g. genus or species level) were in many cases found to explain a higher proportion of dry mass (or body length) variation than those at order level (Smock, 1980; Meyer, 1989; Burgherr and Meyer, 1997). Many authors conclude that predictive equations for the lowest possible taxon should be chosen (Smock, 1980; Meyer, 1989; Burgherr and Meyer, 1997; Baumgärtner and Rotthaupt, 2003). Thirdly, it is likely that the length-mass regressions of taxa may differ between streams and lakes (Baumgärtner and Rotthaupt, 2003) and even among water bodies. The body shape of macroinvertebrates may be influenced by varying environmental constraints that influence the slope of the length-mass regression. For example, the unidirectional water flow in streams may result in longer and more flattened stream invertebrates. In contrast, lake littoral macroinvertebrates are subject to multidirectional sheer stress arising from wave action in the littoral zone, resulting in a more robust shape and ultimately, a steeper length–mass regression slope

(Baumgärtner and Rotthaupt, 2003). In addition, strong multidirectional hydraulic stress, coupled with moving substrates in the littoral, may cause the invertebrates to develop protection through a more heavily sclerotised exoskeleton, resulting in a lower regression slope for the lentic macroinvertebrates (Baumgärtner and Rotthaupt, 2003).

Frequently, authors do not develop their own length-mass regression for all taxa and size ranges when comparing the biomass of large numbers of aquatic macroinvertebrates among many samples from different locations, owing to the labour and time involved. This is particularly true for rare species, which may occur at many sites in low abundance, or few sites in low or high abundance (Gauch, 1982). Accordingly, many authors apply a published regression of a species, or related taxa, from geographic locations where the climate, habitats, water chemistry and flow regimes differ (Strayer, 1986; Blumenshine *et al.*, 1997; Stead *et al.*, 2003). Johnston and Cunjak (1999) compared published length-mass relationships amongst studies and found that predicted mass at length was highly variable among models developed in different studies for the same family group (coefficient of variation, ≥25%). They found that the relationships reported in different studies may yield very different predictions, even for closely related taxa. They speculated that this variability resulted from both variations in methods used to construct the models, and from natural spatiotemporal and taxonomic variation in mass at length. However, it has been postulated that differences of up to 20% can be expected between the actual and theoretical biomass derived from length-mass regressions (Wenzel *et al.*, 1990). It is a widely held view that, when it is necessary to use generalised published regressions, those used should be selected from environments that are similar to the communities being studied in terms of location, climatic zone and vegetation characteristics (Gowing and Recher, 1985; Meyer, 1989; Wenzel *et al.*, 1990; Baumgärtner and Rotthaupt, 2003).

Although studies have compared mass predictions in the published literature (Johnson *et al.*, 1993; Baumgärtner and Rotthaupt, 2003), little work has focused on testing differences in mass predictions among water bodies with different water chemistry. To address this knowledge gap, the current study examined the separate effects of total nitrogen (TN), total phosphorus (TP) and alkalinity on mass per unit length of eight common aquatic macroinvertebrates. Length-mass relationships for a set of common

animals were examined to compare these relationships among lakes representing TP and alkalinity gradients.

2.2 Methods and Materials

2.2.1 Study area

Nine lakes were selected for sampling, representing a gradient of phosphorus concentrations (16 to 168 μg L^{-1}) and an alkalinity gradient ranging from 6.3 to 182.4 mg $CaCO_3$ L^{-1}. Lakes were selected from a collection of lakes previously sampled under the North South Shared Aquatic Resources project (NS-SHARE; http://www.nsshare.com). Species distribution and occurrence lists, along with lake water chemistry data, were collated over the course of the NS-SHARE project. This information was used to select lakes based on the frequency of occurrence of target taxa within each alkalinity range and among lakes with a nutrient gradient. Six lakes were sampled in April 2006 (spring) and nine lakes were sampled in July 2006 (summer; Table 3.2). Additional adult *Gammarus* sp. (*G. duebeni*, *G. pulex* and *G. lacustris*) specimens were collected at two higher alkalinity lakes, namely Derrybrick Lough, and Nalughoghe Lough, in April 2006.

2.2.2 Field methods

Macroinvertebrate samples were collected from hard and soft littoral habitats by kick sampling in a depth of approximately 0.5-0.75 m, with a standard 1 mm^2 mesh-size Freshwater Biological Association net. Samples were transferred into plastic containers and covered with lake water. The bottles were immediately frozen in dry ice. Water samples were collected at each lake using a weighted 5 L plastic bottle, with three replicate 0.5 L samples filtered on site. The grid reference location was recorded using a hand-held Global Positioning Satellite (GPS). A photograph of each site was also taken.

2.2.3 Laboratory methods

2.2.3.1 Water chemistry

Water samples were refrigerated on return to the laboratory, and subsequently analysed for total TP, TN and alkalinity. The alkalinity of each filtered sample was analysed by Gran titration with 0.01M H_2SO_4 and the volume of H_2SO_4 at the endpoint was recorded by one of two titration methods. Samples from sites where the alkalinity was judged to be less than 20 mg $CaCO_3$ L^{-1} were measured using 100 ml water samples, and titrated in less than 2 minutes to three endpoints between pH 4.4 and pH 3.7, as per Mackereth

et al. (1989). Samples from sites where alkalinity was estimated to be greater than 20 mg $CaCO_3$ L^{-1} were measured using 50 ml water samples, and titrated to an endpoint of pH 4.5 in less than 2 minutes following Clesceri *et al.* (1989). A magnetic stirrer was used throughout titrations to ensure that sufficient mixing occurred. Gran titration of each pH point was subsequently calculated and plotted against the titrant volume from which the volume in mg $CaCO_3$ L^{-1} was calculated.

The unfiltered water samples were analysed in triplicate for TP by the method of Eisenrech *et al.* (1975). This method involves the conversion of organically bound phosphorus to phosphate, through oxidation by acid persulphate ($K_2S_2O_8$) digestion at 120°C. Triplicate standards of 0, 0.005, 0.025, 0.075, 0.15 and 0.3 mg L^{-1} of TP were used. A sample volume of 25 ml (in triplicate) and standards were pipetted into autoclaveable bottles and 5 ml of digestion reagent was added ($K_2S_2O_8$ dissolved in double distilled water [DDW] containing H_2SO_4). All samples were then autoclaved at 15 psi for 30 minutes. After cooling, 5 ml of each sample or standard was pipetted into a test tube, 1 ml mixed reagent was added (containing 13.5 ml of 3.6N H_2SO_4, 25 ml antimony stock, 25 ml molybdate stock and 0.2 g ascorbic acid), and the test tubes were vortexed. After 10 minutes, absorbance was measured colorimetrically at a wavelength of 882 nm using a Hach DR2000 Spectrometer. A standard curve was created and TP was calculated from the average absorbance of the triplicate samples.

The filtered water samples were analysed in triplicate for TN by the method of Grasshoff *et al.* (1999). This method involved the conversion of organically bound nitrogen and ammonia to nitrate, through oxidation by alkaline persulphate digestion at 120°C. Triplicate standards of 0, 0.1, 0.5, 1.0, and 2.5 mg L^{-1} of TN were prepared. A volume of 50 ml of samples (in triplicate) and standards were pipetted into polypropylene bottles and 5 ml of oxidizing reagent was added (containing NaOH, $K_2S_2O_8$ and boric acid dissolved in DDW). Samples were autoclaved at 15 psi for 30 minutes, and nitrate content was measured using a continuous flow auto-analyser (AutoAnalyser 3, Bran and Luebbe® AACE 5.40).

As a means of quality control, samples of known concentration (QCs) were examined along with lake water samples for each analysis of TP and TN. QC solutions were prepared from different stock reagents to those used for preparation of standards.

2.2.3.2 Macroinvertebrate samples

On return to the laboratory, macroinvertebrate samples were transferred immediately from dry ice to a -12°C freezer for storage. Eight common macroinvertebrate taxa were chosen for the comparison of body-length-mass relationships among lakes (>10 animals per lake). Animals were identified to the most convenient taxonomic level, either order (Diptera [pupae], Acari), family (Chironomidae, Sphaeriidae) or species (*Potamopyrgus jenkinsi*, *Asellus aquaticus*, *Gammarus* sp. [*G. duebeni*, *G. lacustris* and *G. pulex*], *Caenis* sp. [*C. horaria* and *C. luctuosa*]). Once thawed, samples were sieved through a 225 μm mesh sieve and placed into a white tray with fresh water. Target animals in good condition were selected, identified, and the body length measured to the nearest 0.1 mm using a dissecting 40x magnification stereomicroscope with eye graticule and Olympus® KL1500 LCD fibre-optic light source; except molluscs, where the largest dimension of shells was determined. Identifications of macroinvertebrates were made using the keys of Macan (1977), Fitter and Manuel (1986), Elliott *et al.* (1988) and Gledhill *et al.* (1993). Each individual animal was carefully placed into a pre-weighed aluminium dish and oven-dried at 60°C for a minimum of 24 hours. Samples were placed in a desiccator with copper sulphate crystals for approx. 1 hour, before being weighed to the nearest μg on a microbalance (Sartorius ISO 9001). Cased caddis were removed from their cases prior to oven drying. Mollusc regressions included shell mass. Length-mass regressions were developed for genera and families where eight or more individuals were measured.

2.2.4 Statistical analysis

For ease of discussing the results, the regression of Ln body length against Ln dry mass are henceforth referred to as the length-mass regression, and individually referred to as Ln BL and Ln DM, respectively. ANCOVA showed no effect of season (*P* value <0.05) on the slope or intercept of the length-mass regressions. Effect of season (spring or summer) was not considered in further analyses. The effects of alkalinity and nutrients (TN and TP) on the relationship between the body length and dry mass of target taxa were analysed by general linear model analyses (R Development Core Team, 2009). Step-wise model selection was performed, based on Akaike's Information Criterion (AIC [Akaike, 1973]) corrected for small sample size biases (AIC_C) as described by Burnham and Anderson (2004). Akaike's information criterion is an approach to model selection based on information theory which aims to find an

approximate model rather than a true model by estimating the Kullback-Leibler distance between the model at hand and the (unknown) theoretical underlying mechanism generating the data (Burnhan and Anderson, 2004; Weakliem, 2004). The AIC can be written as:

$$-2 \log L + 2p \qquad [3]$$

where L is the maximized log likelihood, and p is the number of free parameters in the model. The AIC value acts as a measure of goodness of fit for an estimated model, offering a measure of the information lost when a given model is used to describe reality (Burnham and Anderson, 2004; Weakliem, 2004). The log likelihood can be thought of as a measure of the information in the model, with an adjustment or "penalty" for each parameter used (Burnham and Anderson, 2004; Weakliem, 2004). Therefore the most parsimonius models with the greatest log-likelihood will have lowest AIC values and thus, the best fit. Given a data set, several competing models may be ranked according to their AIC values, and the model with the lowest AIC score is that to which fits the data best (Weakliem, 2004). However, the value of the AIC is arbitrary, and it is not possible to say if a given difference among models is large or small in an absolute sense; the criteria may show that a smaller model has a lower AIC than that of a larger model (i.e. a model with more terms included), which signifies that the evidence supports the smaller model, and that this will provide better predictions when using the parameter estimates from the sample (Weakliem, 2004). The AIC does not rely on arbitrarily chosen significance levels (Weakliem, 2004).

Forward and backward selection was used to identify the minimal adequate model, which is the most parsimonius model at the lowest value of AIC_C. Models were created using the GLM Procedure and selected with the aid of the step wise procedure (based on AIC values) using the R statistical package (R Development Core Team, 2009). Where two models are compared and found to have a difference in AIC_C values <2 (among models), then both are considered to have substantial evidence to support them (Burnham and Anderson, 2004). Where only one minimal adequate model was produced, the AIC_C values are not discussed further.

Prior to analysis, the relationship between body length and dry mass of each target animal was linearised by transforming body length and dry mass to natural logs (Fig. 2.3). To reduce the level of multicollinearity among parameters, alkalinity and nutrients (both TP and TN) were centered by subtracting the mean from each case, so that the new mean was equal to zero, before further analyses. The nutrients TP and TN were treated in separate analyses, owing to the high correlation between them (Table 2.1). Dummy variables were included (as explanatory variables) to differentiate among species of the same genera (*Caenis horaria* [1] and *C. luctuosa* [0]; *Gammarus duebeni* [1], *G. pulex* [0] and *G. lacustris* [1] *and Gammarus* sp. juveniles [0]). Variables represented by '0' (i.e. *C. luctuosa* and *G.pulex*) were used as reference categories for the other dummy variables for their respective maximal models. Individual interactions between Ln BL and all other variables were included in each of the analyses. Interaction terms between Ln BL and the other terms allows changes in the dynamics of the relationship between Ln BL and Ln DM (or the length-mass regression) in the presence of another variable to be analysed.

2.3 Results

Alkalinity, TP and TN were shown to correlate significantly (Table 2.1). TP and TN were highly correlated; alkalinity was significantly correlated with both nutrients (Table 2.1). The eleven lakes sampled were shown to differ strongly in nutrient concentration and alkalinity (Table 2.2).

Table 2.1: Pearson Correlation matrix showing the correlations among nutrients and alkalinity among all lakes.

	Alkalinity	**TP**	**TN**
Alkalinity	1	0.65***	0.57***
TP		1	0.91***
TN			1

*** denotes *P* value <0.001

Target taxa were not found in all lakes (Table 2.2). Dipteran pupae were found in only three lakes, *Caenis* sp. and *Gammarus* sp. in four lakes, and all other target taxa were found in six lakes or more (Table 2.2). The presence of taxa was not always consistent among seasons (Table 2.2). For example, dipteran pupae occurred in much lower numbers at Lough Shivnagh and Lough Sand, and did not occur at all in Lough Roughan in the summer, presumably owing to emergence as flying adults. With the exception of *Asellus aquaticus*, which were not found in lakes with lower alkalinity concentrations (minimum alkalinity found: 33.4 mg $CaCO_3$ L^{-1}), all taxa were found under a broad range of alkalinity conditions, ranging from 6.3 to 182.4 mg $CaCO_3$ L^{-1} (Table 2.2).

Comparing the predicted mass from length among all regressions, differences among body shapes and the geometry of different taxa are clear (Figs. 2.1 and 2.2). Gastropods (Sphaeriidae and *Potamopyrgus jenkinsi*) are denser animals with heavier mass per unit length than arthropods (Fig. 2.1). Comparing among arthropods, the mites are the heaviest per unit length (owing to their round shape), and chironomids are the lightest per unit length (owing to their cylinder shape). The two crustacean taxa (*Asellus aquaticus* and *Gammarus* sp.) are heavier per unit length than the insects. The insects

Caenis sp., Diptera (pupae) and Chironomidae are longer and more slender in shape, and therefore are the lightest per unit length (Fig. 2.2). A strong linear relationship was observed between the natural log of body length and the natural log of dry mass for each of the target taxa (Figs. 2.3a to 2.3h).

Slopes and intercepts varied among taxa. The intercepts of the arthropod taxa were lower than those of the gastropods. Slopes varied from 2.36 (*P. jenkinsi*) to 3.12 (*Gammarus* sp.), but the slopes of the length-mass regressions of most of the arthropods were close to 3, common to that of many invertebrates and vertebrates including insects, birds, reptiles and mammals (Peters, 1983). The exception was Hydracarina, which has a spherical body shape, and thus a lower slope (mass per unit length is higher). The gastropods had lower slopes and higher intercepts owing to their different body shapes and outer shell. The spherically shaped Sphaeriidae specimens displayed lower intercepts and greater slopes than the cone shaped *Potamopyrgus jenkinsi*, which displayed the highest intercept and lowest slope of all measured taxa.

The regression coefficients of each parameter, produced from the general linear analyses, varied among taxa (Table 2.3). Two maximal models (including either TP or TN) were created for each taxa. As the maximal model for each taxa contained either TP or TN, the corresponding minimal adequate model was referred to as the 'TP model' or the 'TN model' for that taxa, regardless of whether the nutrient (TP or TN) had been eliminated from it. The maximal models contained either TP or TN and their interaction with the natural log of body length as a variable, alongside Ln BL, alkalinity and the interaction of both. Thus, two minimal adequate models were produced for most taxa (one for each nutrient; Table 2.3). However, a single (identical) minimal adequate model was produced for two taxa (the molluscs *P. jenkinsi* and Sphaeriidae) from both maximal models, as TP and TN were eliminated from their respective maximal models (Table .3).

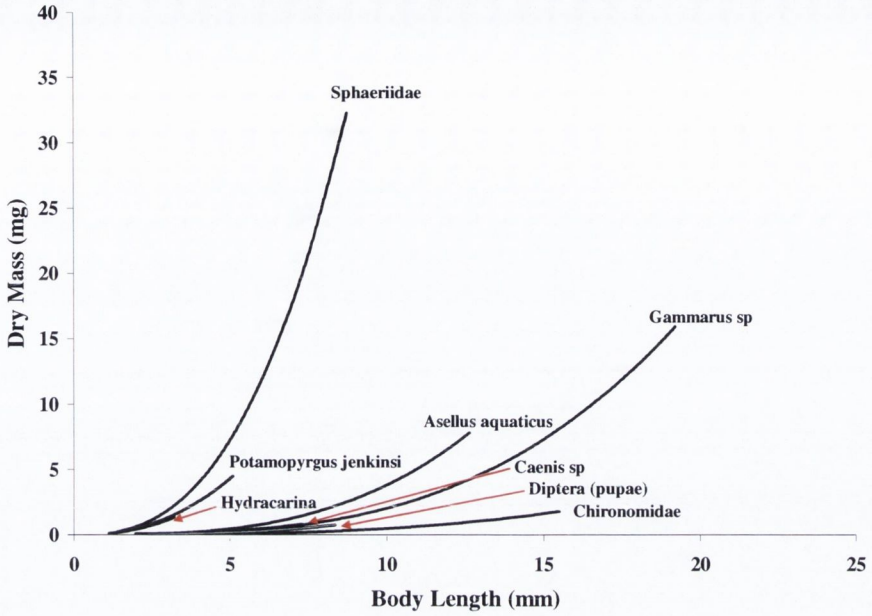


Figure 2.1: Comparison of predicted length-mass relationships for all measured taxa.

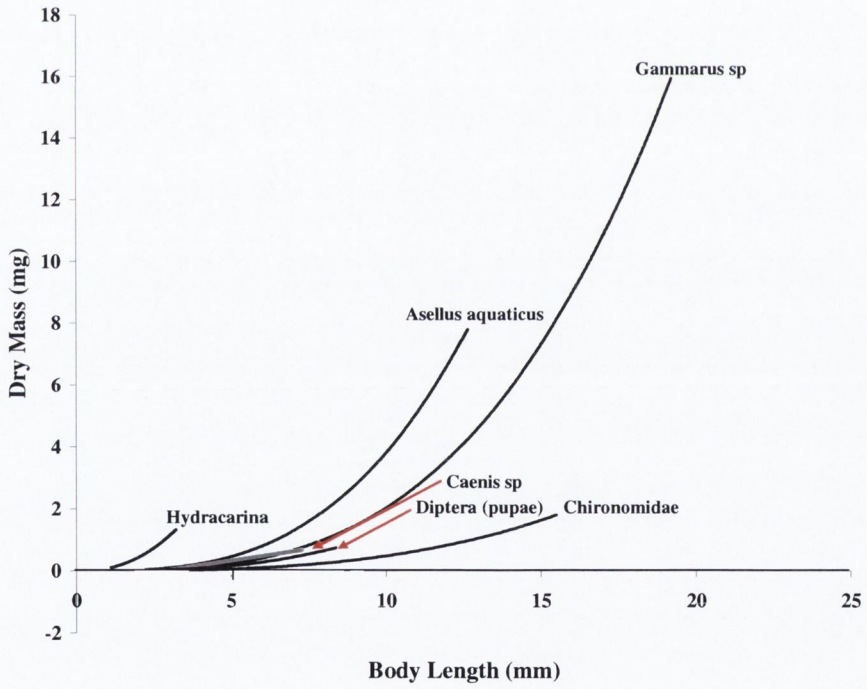


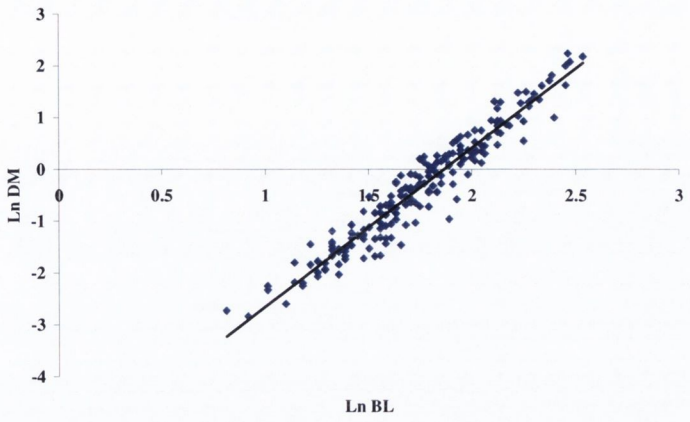
Figure 2.2: Comparison of predicted length-mass relationship for arthropods.

Table 2.2: Summary of the abundance of macroinvertebrate taxa and water chemistry parameters for the examined lakes.

Lake	Season	Order		Family		Species				Water Chemistry		
		Diptera (pupae)	Hydra-carina	Chironomidae	Sphaeriidae	*Gammarus sp.*	*Caenis sp.*	*Asellus aquaticus*	*Potamopyrgus jenkinsi*	TP[1]	TN[2]	Alkalinity[3]
Aillbrack	Spring	-	-	31	19	-	1	-	-	16	0.45	6.3
	Summer	-	-	40	22	-	40	-	-	42	0.71	10.6
Golagh	Spring	-	-	15	6	-	2	-	10	16	0.45	6.4
	Summer	-	-	6	24	-	10	-	20	21	0.47	7.8
Shivnagh	Spring	22	25	49	19	-	40	-	29	32	0.56	11.1
	Summer	7	19	45	3	-	33	-	25	26	0.56	20.5
Melvin	Summer	-	33	40	-	18		20	19	18	0.47	57.4
Fern	Summer	-	32	35	-	-	-	20	-	17	0.37	33.4
Ballydoolagh	Summer	-	13	12	10	44		25	20	41	0.59	62.8
Corglass	Spring	-	4	10	9	-	-	20	-	25	0.72	139.2
	Summer	-	-	39	13	-	-	20	20	50	0.62	136.0
Roughan	Spring	12	5	40	11	-	-	21	-	68	0.78	182.4
	Summer	-	8	37	-	-	-	25	-	36	0.53	162.2
Sand	Spring	17	1	39	3	-	21	20	9	123	1.30	161.0
	Summer	1	9	11	9	-	1	20	17	168	0.99	166.5
Derrybrick	Spring	-	-	-	-	7	-	-	-	21	0.42	153.9
Nalughoghe	Spring	-	-	-	-	12	-	-	-	37	0.53	111.5
Total		**59**	**149**	**449**	**148**	**81**	**148**	**191**	**169**			

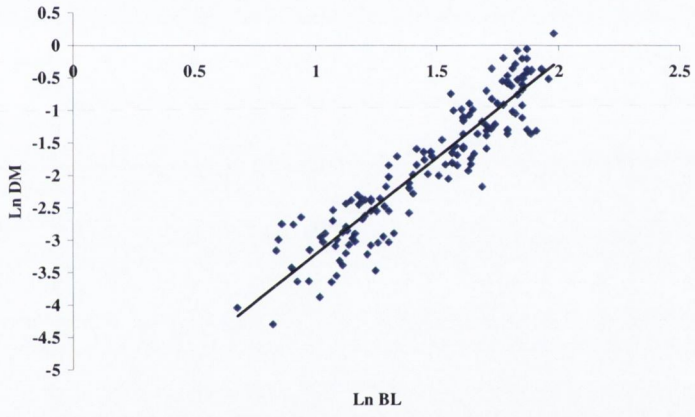
[1]Total Phosphorus (µg L^{-1}); [2]Total Nitrogen (mg L^{-1}); [3]mg $CaCO_3$ L^{-1}; '-' represents not detectable in sufficient quantities.

(a) *Asellus aquaticus*



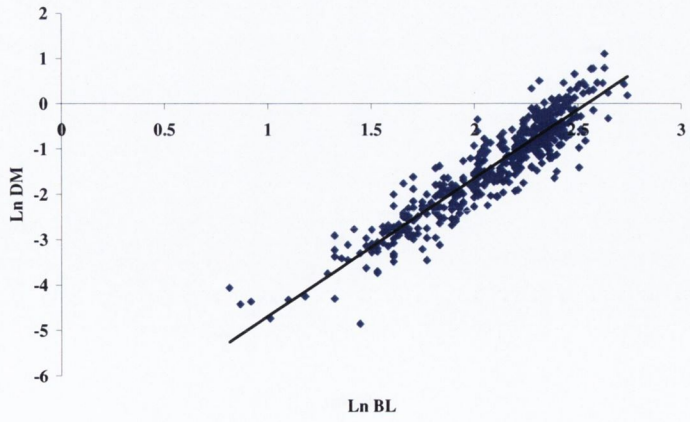
Slope = 3.06

(b) *Caenis* sp.



Slope = 2.97

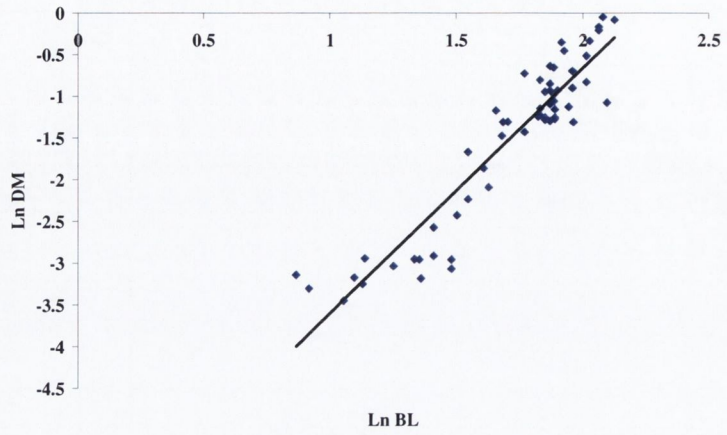
(c) Chironomidae



Slope = 3.03

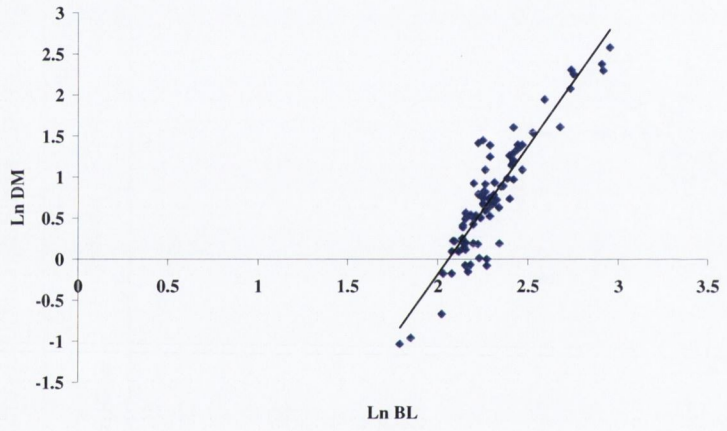
Figure 2.3: (a) to (c) - The natural log of body length (Ln BL) plotted against the natural log of dry mass (Ln DM) for each of the target taxa.

(d) Dipteran pupae



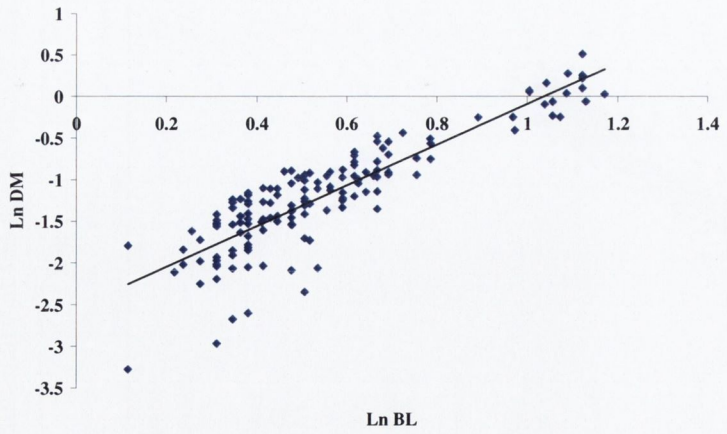
Slope = 2.94

(e) *Gammarus* sp.



Slope = 3.12

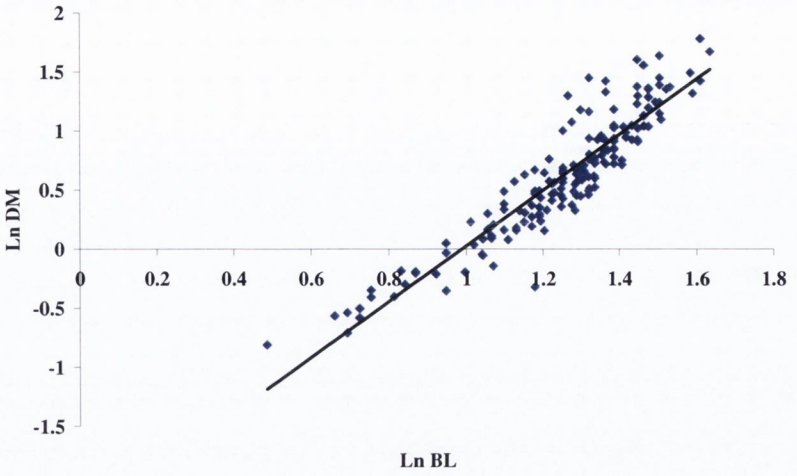
(f) Hydracarina



Slope = 2.44

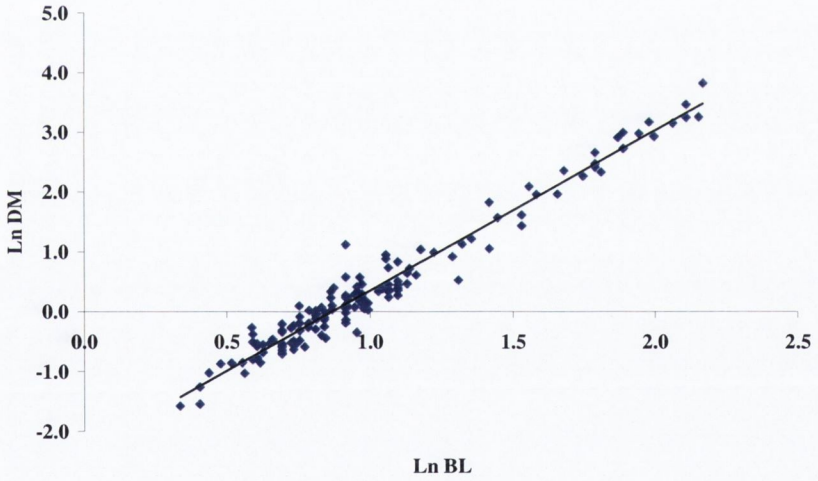
Figure 2.3: (d) to (f) - The natural log of body length (Ln BL) plotted against the natural log of dry mass (Ln DM) for each of the target taxa.

(g) *Potamopyrgus jenkinsi*



Slope = 2.36

(h) Sphaeriidae



Slope = 2.68

Figure 2.3: (g) to (h) - The natural log of body length (Ln BL) plotted against the natural log of dry mass (Ln DM) for each of the target taxa.

Table 2.3: General linear models with the natural log of dry mass of each macroinvertebrate taxa as the dependent variable.

Taxa	Coefficients & Confidence Intervals	Intercept	Ln BL[1]	Alkalinity	TP	TN	Ln BL x Alkalinity	Ln BL x TP	Ln BL x TN	n	Null deviance	Residual deviance	df	AIC_C
Asellus aquaticus	**B**	-5.47	2.92	0.00	-0.00	X	-	-	X					
	2.5%, 97.5% C.I.	(-5.69, -5.25)	(2.80, 3.05)	(0.00, 0.00)	(-0.00, -0.00)	X	-	-	X	191	225.1	12.9	187	37.9
	B	-5.69	3.04	-0.00	X	-0.36	0.00	X	-					
	2.5%, 97.5% C.I.	(-5.92, -5.46)	(2.91, 3.16)	(-0.01, -0.00)	X	(-0.56, -0.16)	(0.00, 0.01)	X	-	191	225.1	14.7	186	64.6
Caenis sp.	**B**	-6.12	2.93	-0.01	0.01	X	-	-	X					
	2.5%, 97.5% C.I.	(-6.41, -5.82)	(2.73, 3.13)	(-0.01, -0.00)	(0.00, 0.02)	X	-	-	X	148	157.9	21.3	144	143.9
	B	-6.07	2.90	-0.00	X	0.97	-	X	-					
	2.5%, 97.5% C.I.	(-6.38, -5.76)	(2.69, 3.11)	(-0.01, -0.00)	X	(0.21, 1.74)	-	X	-	148	157.9	21.7	144	146.5
Chironomidae	**B**	-7.64	3.00	0.00	-0.00	X	0.00	-	X					
	2.5%, 97.5% C.I.	(-7.87, -7.37)	(2.87, 3.11)	(-0.01, 0.00)	(-0.00, -0.00)	X	(0.00, 0.00)	-	X	451	541.7	81.6	446	515.8
	B	-7.64	3.00	0.00	X	-	0.00	X	-					
	2.5%, 97.5% C.I.	(-7.89, -7.39)	(2.88, 3.12)	(-0.01, 0.00)	X	-	(0.00, 0.00)	X	-	451	541.7	81.6	447	519.1
Dipteran pupae	**B**	-6.51	2.93	-	-0.00	X	-	-	X					
	2.5%, 97.5% C.I.	(-6.98, -6.04)	(2.65, 3.20)	-	(-0.01, -0.00)	X	-	-	X	59	56.0	6.1	56	42.4
	B	-6.55	2.96	-	X	-2.12	-	X	1.06					
	2.5%, 97.5% C.I.	(-7.03, -6.08)	(2.68, 3.23)	-	X	(-3.62, -0.63)	-	X	(0.16, 1.95)	59	56.0	5.8	55	42.0

[1] Body Length; 'X' denotes where a covariate was not included in the maximal model; '-' denotes where a covariate was eliminated from a model. TN and TP were analysed in separate maximal models for each of the target taxa. Maximal models for *Caenis* sp. and *Gammarus* sp. also included dummy variables representing each of the species present, and an interaction term of each species present with the natural log of body length. However, the main effects representing species, and their interaction terms, were eliminated from the minimal adequate model for both taxa, and are not presented. Results of the minimal adequate models based on AIC_C values are presented. Alkalnity, TP and TN were centered prior to analysis.

Table 2.3 continued:

Taxa	Coefficients & add. variables	Intercept	Ln BL	Alkalinity	TP	TN	Ln BL x Alkalinity	Ln BL x TP	Ln BL x TN	*G. lacustris*	n	Null deviance	Residual Deviance	df	AICc
Gammarus sp.	B	-6.32	3.09	-	-	-	-	-	-	-0.40					
	2.5%, 97.5% C.I.	(-6.99, -5.64)	(2.80, 3.39)	-	-	-	-	-	-	(-0.58,-0.21)					
											81	38.7	6.0	78	27.1
Hydracarina	B	-2.54	2.47	0.00	-0.01	X	-0.01	0.02	X						
	2.5%, 97.5% C.I.	(-2.66, -2.43)	(2.28, 2.66)	(0.00, 0.01)	(-0.02, -0.01)	X	(-0.01,-0.00)	(0.01, 0.02)	X		149	64.7	10.4	143	40.6
	B	-2.58	2.51	-0.00	X	-1.43	-	X	1.84						
	2.5%, 97.5% C.I.	(-2.70, -2.45)	(2.30, 2.72)	(-0.00, -0.00)	X	(-2.11, -0.74)	-	X	(0.68, 3.00)		149	64.7	12.3	144	63.2
Potamopyrgus jenkinsi	B	-2.34	2.34	-0.01	-	-	0.01	-	-						
	2.5%, 97.5% C.I.	(-2.55, -2.13)	(2.17, 2.50)	(-0.01, -0.00)	-	-	(0.00, 0.01)	-	-		169	46.6	6.2	164	-65.9
Sphaeriidae	B	-2.18	2.53	0.00	-	-	-	-	-						
	2.5%, 97.5% C.I.)	(-2.27, -2.08)	(2.44, 2.62)	(0.00, 0.00)	-	-	-	-	-		148	185.7	5.7	145	-54.8

'X' denotes where a covariate was not included in the maximal model; '-' denotes where a covariate was eliminated from a model.

2.3.1 *Asellus aquaticus*

The minimal adequate TP model for *Asellus aquaticus* included only the main effects (Ln BL, alkalinity and TP), with interaction terms eliminated from the model (Table 2.3). The minimal adequate TN model included the main effects (Ln BL, alkalinity and TN) and the interaction term between Ln BL and alkalinity. The slopes of the relationship between Ln BL and Ln DM varied little among models, and were similar to the slope of the general length-mass relationship (Table 2.3; Fig. 2.3a). The slope of the relationship between alkalinity and Ln DM was small in both models (i.e. approaching zero; positive in the TP model and negative in the TN model) indicating that the effect of alkalinity on Ln DM was small. The slope of the relationship between TP and Ln DM in the first model was negative and close to zero, contributing little to the overall equation. The slope of the relationship between TN and Ln DM in the second model was negative, indicating a decrease in Ln DM with an increase in TN. The slope of the relationship between the interaction term (Ln BL and alkalinity) and Ln DM in the TN model was positive and near to zero, suggesting that alkalinity had only a small, positive effect on the length-mass regression. The AIC_C value of the TP model was slightly over half of that of the TN model (37.9 and 64.6, respectively; i.e. differing by >2), indicating that the minimal adequate TP model best explained the data (Burnham and Anderson, 2004).

2.3.2 *Caenis* sp.

The effect of species (i.e. *C. horaria* or *C. luctuosa*), and the interaction terms between species and Ln BL, were eliminated from both the minimal adequate models (TP and TN) produced for the genus *Caenis*. This indicates that there were no detectable differences in the sizes and length-mass regressions of the two species (Table 2.3). All interaction terms (with Ln BL) were also eliminated from both models, leaving only the main effects remaining in the models (Table 2.3). The slopes of the relationship between Ln BL and Ln DM were similar among both models and to that of the general length-mass relationship (Table 2.3; Fig. 2.3b). The slope of the relationship between alkalinity and Ln DM was negative and approached zero in both models, indicating that alkalinity had a small, negative effect on Ln DM (Table 2.3). The slope of the relationship between TP and Ln DM in the first model was negative, and also approached zero (Table 2.3). The slope of the relationship between TN and Ln DM was

positive, thus indicating an increase in DM with increasing TN. The AIC_C value of the TP model was less than that of the TN model by more than 2 (143.9 and 146.5, respectively), thereby supporting the minimal adequate TP model (Table 2.3).

2.3.3 Chironomidae

Both minimal adequate models produced for Chironomidae included main effects and the interaction between alkalinity and Ln BL (with nutrient interaction terms eliminated; Table 2.3). However, TN was eliminated from the TN model, but TP was not eliminated from the TP model. The slopes of the relationship between Ln BL and Ln DM were similar among both models and to that of the general length-mass relationship (Table 2.3; Fig. 2.3c). The slope of the relationship between alkalinity and Ln DM was zero in both models (the confidence intervals of this relationship included zero in both models), suggesting no change in Ln DM with alkalinity (Table 2.3). The slope of the relationship between TP and Ln DM in the first model was negative and approached zero, demonstrating a small negative effect of TP on Ln DM (Table 2.3). The slope of the relationship between the interaction term (Ln BL and alkalinity) and Ln DM was small and negative in both models, signifying that alkalinity had a small, positive effect on the length-mass regression in both models (Table 2.3). The AIC_C value of the TP model was less than that of the TN model by more than 2 (515.8 and 519.1, respectively) providing further evidence in support of the minimal adequate TP model (Table 2.3).

2.3.4 Dipteran pupae

The TP minimal adequate model constructed for dipteran pupae included only main effects, Ln BL and TP, and excluded alkalinity (Table 2.3). The TN minimal adequate model included the main effects Ln BL and TN, and also included the interaction term between Ln BL and TN. The slopes of the relationship between Ln BL and Ln DM were similar among both models, and were similar to that of the general length-mass relationship (Table 2.3; Fig. 2.3d). The slope of the relationship between TP and Ln DM in the first (TP) model was negative and was close to zero, indicating a small negative effect of TP on Ln DM (Table 2.3). The slope of the relationship between TN and Ln DM in the second model was negative, with very broad confidence limits, indicative of poor goodness of fit (Table 2.3). The slope of the relationship between the

interaction term (Ln BL and TN) and Ln DM in the second model was positive, and also had wide confidence limits, suggesting a broad increase in the slope of the length-mass regression with increasing TN (Table 2.3). The AIC_C values of both models differed by less than 2, and thus, there is evidence to support the use of both the TP and the TN minimal adequate models. However the statistical principle of parsimony leads us to favour the TP model, as it includes fewer variables (Burnham and Anderson, 2004).

2.3.5 *Gammarus* sp.

Identical minimal adequate models were produced by both the TN and TP maximal models, owing to the elimination of both nutrients and the interaction terms in their respective minimal adequate models. This suggested that nutrients did not affect the size (Ln DM) or the length-mass regression of *Gammarus* sp. (Table 2.3). Alkalinity, and its interaction term with Ln BL, were also eliminated from the final model, indicating that alkalinity also did not affect the sizes or length-mass regressions of *Gammarus* sp. The final minimal adequate model included only Ln BL and *G. lacustris* (main effects). The elimination of *G. duebeni* from the model indicated that there were no significant differences in the size of (i) *G. duebeni* and *G. pulex* and (ii) the length-mass regressions of both species. The removal of the interaction between Ln BL and *G. lacustris* indicated that there was no difference in the length-mass regressions of any of the species. However, the inclusion of the main effects of *G. lacustris* indicated a difference in the size (Ln DM) of *G. lacustris* to that of the two other species. The slope of the relationship between Ln BL and Ln DM was similar to that of the general length-mass relationship (Table 2.3; Fig. 2.3e). The slope of the relationship between *G. lacustris* and Ln DM was negative. As all species were represented by dummy variables, this signified that *G. lacustris* specimens were smaller (i.e. had less dry mass) than those of the other species (Table 2.3).

2.3.6 Hydracarina

The minimal adequate TP model was equivalent to the maximal model, as no terms were deleted. The minimal adequate TN model included all main effects, but the interaction of alkanity with Ln BL was excluded. The slope of the relationship between Ln BL and Ln DM was similar between both models, but slightly higher than that of the general length-mass regression (Table 2.3; Fig. 2.3f). In the TP model, the slope of the

relationship between alkalinity and Ln DM was positive but close to zero (Table 2.3). The slope of the relationship between the interaction term (Ln BL and alkalinity) was negative in the TP model, but approaching zero, indicating a decrease in the length-mass regression slope with increasing alkalinity (Table 2.3). The slope of the relationship between TP and Ln DM was negative, but very small; the slope of the relationship between the interaction term (Ln BL and TP) and Ln DM was positive, but close to zero, demonstrating a small increase in the length-mass regression slope with increasing TP. In the TN model, the slope of the relationship between alkalinity and Ln DM was negative, but close to zero (in contrast to the TP model, where this relationship was positive but close to zero; Table 2.3). The slope of the relationship between TN and Ln DM was negative, with relatively broad confidence limits (Table 2.3). The slope of the relationship between the interaction term (Ln BL and TN) and Ln DM in the TN model was positive, with broad confidence limits. The AIC_C value of the TP model was markedly lower than that of the TN model (>2 difference; 40.6 and 63.2, respectively) providing ample evidence in support of the minimal adequate TP model.

2.3.7 *Potamopyrgus jenkinsi*

Identical minimal adequate models were produced by both the TN and TP maximal models, owing to the elimination of both nutrients, and their interaction terms in their respective minimal adequate models. The final model included Ln BL, alkalinity and the interaction term. The slope of the relationship between Ln BL and Ln DM in this model was very similar to that of the general length-mass regression equation (Table 2.3; Fig. 2.3g). The slope of the relationship between alkalinity and Ln DM was negative and approached zero, indicating a small decrease in Ln DM in more alkaline lakes (Table 2.3). The slope of the relationship between the interaction term (Ln BL and alkalinity) and Ln DM in the model was positive, but was close to zero, suggesting a small increase in the slope of the length-mass regression with alkalinity (Table 2.3).

2.3.8 Sphaeriidae

Identical minimal adequate models were produced by both the TN and TP maximal models, owing to the elimination of both nutrients and their interaction terms, and the interaction of Ln BL with alkalinity in their respective minimal adequate models. The final model comprised only two predictors, Ln BL and alkalinity. The slope of the

relationship between Ln BL and Ln DM in this model was lower than that of the general length-mass regression equation (Table 2.3; Fig. 2.3h). The slope of the relationship between alkalinity and Ln DM was positive, but close to zero (Table 2.3).

2.4 Discussion

2.4.1 General comments on published length-mass regressions

Length-mass regressions are a useful tool in ecological research (Johnston and Cunjak, 1999). However, a cautious approach is needed when applying published regressions to study organisms for a given taxa (Smock, 1980; Burgherr and Meyer, 1997; Johnston and Cunjak, 1999; Baumgärtner and Rotthaupt, 2003). A comparison of length-mass regressions among studies found that reported regressions yield different predictions, even for closely related taxa (Johnston and Cunjak, 1999). Some studies were found to consistently predict higher mass at length across all taxa, relative to other studies (Johnston and Cunjak, 1999). For example, regressions presented by Smock (1980) yielded consistently higher predictions of mass at length than those of Burgherr & Meyer (1997) for several families (Johnston and Cunjak, 1999).

There are a number of factors contributing to the discrepancies observed in the literature. First, the size range of organisms used to build the regressions can dramatically affect model predictions; model parameters estimated from a narrow size-range of data may differ markedly from those estimated over a wider size-range (Johnston and Cunjak, 1999). Growth, appearing geometric over a wide length range, may appear linear over a shorter length-range (Johnston and Cunjak, 1999). Second, model-fitting procedures used to build length-mass relationships may also affect model predictions (Johnston and Cunjak, 1999). Third, differences in taxonomic resolution, in methods employed to develop the regressions, as well as in study environments (e.g. catchment, location, climate, water body types and habitats) may also lead to differences among regressions for a given taxon (Gowing and Recher, 1985; Meyer, 1989; Wenzel *et al.*, 1990; Johnston and Cunjak, 1999; Baumgärtner and Rotthaupt, 2003). For example, lake littoral invertebrates were found to exhibit lower length-mass regression slopes when compared to related stream invertebrates (Baumgärtner and Rotthaupt, 2003). Therefore, serious consideration needs to be given to the predictive model chosen (Johnston and Cunjak, 1999).

An unexplored possibility is that sample handling may influence mass at length relationships. Stretching of an animal during measurement may significantly affect the length-mass relationships. This is particularly true for animals such as cased caddis,

where care is required not to stretch the animal as it is being removed from its case. Animals such as Gammaridae or Chironomidae, that are naturally bent, may also be affected by the level to which the worker stretches them to measure them, adding or removing a dimension to the measured body length, and thus altering the produced length-mass relationship.

Applying length-mass regressions at the highest taxonomic is advised for more accurate results (Smock, 1980; Meyer, 1989; Burgherr and Meyer, 1997; Baumgärtner and Rotthaupt, 2003). However, caution is advised when selecting taxa-specific predictive models from the published literature, as the aforementioned differences among studies (e.g. methods, water chemistry, environmental conditions, location and flow) may lead to differences in the predicted mass at length (Johnston and Cunjak, 1999). For example, using a regression from one study to estimate the biomass of species A, and using a regression from a different study for species B, can potentially lead to overestimation of the biomass species A, and underestimation of species B. This is particularly relevant when applying different regressions at species level from various authors to a community of organisms, as it may have significant implications when interpreting the functional importance of taxa. In this context, it is important to consider the comparability of regressions from different sources before applying them to various components of a community of organisms. Furthermore, when using published regressions from different sources, it is advisable to use fewer regressions and if possible, at a higher taxonomic resolution (i.e. lower taxonomic level). This should decrease the potential error and variability that may arise from differences among regressions. Regressions of taxonomically similar organisms should be selected from environments similar to that being studied (e.g. location, climate, limnology, habitats and vegetation characteristics [Gowing and Recher, 1985; Meyer, 1989; Wenzel *et al.*, 1990; Johnston and Cunjak, 1999; Baumgärtner and Rotthaupt, 2003]). However, length mass regressions should ideally be developed for the study habitat and taxon from fresh organisms, covering the size range for which predictions will be made (Johnston and Cunjak, 1999).

2.4.2 The effects of alkalinity and nutrients on length-mass regressions

The results presented in this study showed that alkalinity and nutrients had no effect on the slope of the length-mass regression of five of the eight taxa examined (*Asellus*

aquaticus, *Caenis* sp., Dipteran pupae, *Gammarus* sp. and Sphaeriidae). Alkalinity had a small, limited effect (slope range: -0.01 to 0.01) on the length-mass regression of three of the target taxa examined (Chironomidae, Hydracarina and *P. jenkinsi*) when compared with the slope of the Ln BL and Ln DM relationship. TP had a small, limited effect (slope: 0.02) on the length-mass regression of only one of the target taxa, Hydracarina, when compared the slope of the Ln BL and Ln DM relationship. The dry mass of individuals was found to differ among lakes in response to changes in alkalinity for six target taxa (excluding *Gammarus* sp. and Dipteran pupae) and TP for five taxa (excluding *Gammarus* sp., *P. jenkinsi* and Sphaeriidae). The minimal adequate models of three taxa eliminated all terms associated with nutrients (Sphaeriidae, *P. jenkinsi* and *Gammarus* sp.). Where nutrients remained in models, the minimal adequate TP models best described the data for the target taxa in each case (lower AIC_C values than the TN models).

The slope of the relationship between Ln BL and Ln DM remained remarkably constant when the simple linear model was compared with that of the general linear model, in the case of each of the target taxa, demonstrating the strength of this relationship compared with the marginal effects of the water chemical variables (Table 2.3; Figs. 2.3a to h). Where alkalinity did affect the slope of the length mass regressions, the effects were small, and varied. For the non-biting midge Chironomidae, the Ln DM and Ln BL slope marginally increased with increasing alkalinity levels (Table 2.3). A similar effect was observed for the gastropod *P. jenkinsi.* The Ln DM and Ln BL relationship was moderated by alkalinity by a positive slope of 0.01, but the slope of the alkalinity main effects term was negative (slope: -0.01), indicating that individuals in more alkaline lakes were smaller (i.e. less dry mass) but that their mass per unit length was higher than their counterparts in lower alkalinity lakes. In contrast, alkalinity moderated the Ln DM and Ln BL relationship by a negative slope (-0.01) for the Hydracarina water mites, indicating a limited decrease in the slope of the length-mass regression with alkalinity (Table 3.2). However, the slope of the alkalinity main effects term for Hydracarina was positive, although near zero. This signified that individuals at more alkaline lakes were larger (i.e. more dry mass), but that their mass per unit length was lower than their counterparts in higher alkalinity lakes. Therefore, alkalinity had no clear single effect for all taxa. Similarly, the reported effects of alkalinity on length-mass relationships have varied. Wenzel *et al.* (1990) observed significant differences in

the length-mass relationships of *Ecdyonorus venosus* (Ephemeroptera) group larvae from two softwater Black Forest streams with those from a small hardwater stream in the Lake Constance region. Furthermore, a study on the secondary production of *Paracapnia angulata* (Plecoptera) found highest growth rates in neutral streams, but higher abundance and biomass in acid streams (Griffith *et al.*, 1993).

Crustaceans and molluscs are known to have a preference for calcium-rich water, and may be vulnerable to low calcium levels (Økland, 1980, as cited by Schartau *et al.*, 2008; Killeen *et al.*, 2004). The two crustacean taxa, *Asellus aquaticus* and *Gammarus* sp., occurred at lakes with moderate to high alkalinity concentrations and were not found in low alkalinity lakes (Table 2.2). Nevertheless, there was a marginal increase in the dry mass of *A. aquaticus* with alkalinity, but not in the dry mass of individuals of the crustacean shrimp *Gammarus* sp. (Table 2.3). In contrast, the two molluscs Sphaeriidae, and *P. jenkinsi* occurred across a wide range of alkalinity concentrations, including with similar abundance under very low alkalinity concentrations (<10 mg $CaCO_3$ L^{-1}), and thus appeared to be tolerant of low calcium levels (Table 2.2). The dry mass of Sphaeriidae increased and the dry mass of *P. jenkinsi* decreased with alkalinity, although *P. jenkinsi* individuals had greater mass per unit length at more alkaline lakes.

TP had a small, limited effect (slope: 0.02) on the length-mass regression of only one of the target taxa, the water mite Hydracarina, when compared with the slope and confidence limits of the Ln BL and Ln DM relationship (Table 2.3). The slope of the TP main effects term was negative (slope: -0.01), indicating that individuals at lakes with higher TP concentrations were smaller (less dry mass) but that their mass per unit length was greater than their counterparts in lakes with lower TP concentrations.

Alkalinity levels correlated with TP levels among all lakes (Table 2.1). However, two lakes where mites were sampled, Lough Melvin and Lough Fern, had moderate levels of alkalinity (33.4 / 57.4 mg $CaCO_3$ L^{-1} respectively), but lower TP levels (17 / 18 µg L^{-1}) than Lough Shivnagh, a low alkalinity lake (alkalinity: 11.1 / 20.5 mg $CaCO_3$ L^{-1}, TP: 26 / 32 µg L^{-1}). As such, TP levels did not correlate as strongly with alkalinity levels where Hydracarina were collected (Table 2.2). Therefore, the separate relationships of TP and alkalinity to the length-mass regression of Hydracarina are difficult to discern (i.e. increase in the slope of the length mass regression with alkalinity, but a decrease in

this slope with TP). Individuals at more alkaline lakes were larger, with more dry mass compared with smaller individuals at more TP rich lakes. Differences in the taxonomic composition of lower alkalinity waters, relative to higher alkalinity waters, have been frequently observed (Harriman and Morrison, 1982; Stoner *et al.*, 1984; Johnson *et al.*, 1993; Tierney *et al.*, 1998). Thus, the lower alkalinity lake (Shivnagh) may have had a different taxonomic composition of Hydracarina species to that of the higher alkalinity lakes leading to a difference in slope among lakes with different alkalinity concentrations (Table 2.2).

It has been hypothesized that greater food availability may influence length-mass regressions by (i) greater amounts of food in the guts of animals and (ii) well fed individuals may exhibit higher condition (i.e. body mass at length independent of gut contents [Johnston and Cunjak, 1999]). It is well documented that increases in the nutrient concentrations in lakes lead to an increase in primary productivity, and thus, food availability, with higher concentrations of nutrients contributing to increasing disturbance and loss of species diversity. Both an increase in condition, and a decrease in diversity, may have contributed to the change in the length-mass regression, and the dry mass of Hydracarina individuals with TP (smaller animals with greater mass per unit length at more TP enriched lakes).

The goodness of fit of a length-mass regression model is dependent on the distribution of the measured organisms over the range of the linear body dimensions and the range itself, as well as the nutritional state of the organism; thus, differences of up to 20% between the calculated and actual biomass must be taken into consideration (Wenzel *et al.*, 1990; Burgherr and Meyer, 1997). Therefore, an exact biomass prediction for a single individual is not possible, but solely an estimate of total biomass, given the body length of the individual (Burgherr and Meyer, 1997). In this context, the addition of significant alkalinity and TP main effects and interaction terms, with very small (slope: <0.02) coefficients compared with the confidence limits of the LnBL and Ln DM relationships for each taxon, would not appear to contribute accuracy to a model that is already prone to much greater variability in its principal term (i.e. the length-mass relationship). When considering the added complication of including the additional term, or terms, and applying different regression equations to a great many samples (e.g. there are 175 samples to be examined in the subsequent chapters, each with different

alkalinity and TP levels), with the little additional predictive value that these terms add, there appears to be little merit in complicating the procedure by including these terms.

2.4.3 Taxonomic resolution

The confidence intervals of the slopes of each of the length mass regressions give an indication as to the level of variation of individual points around the regression slope (Table 2.3; Figs. 2.3a to h). Sphaeriidae had the narrowest confidence intervals of all examined taxa (± 0.09), followed by Chironomidae and *Asellus aquaticus* (both ± 0.12), *Potamopyrgus jenkinsi* (± 0.16), Hydracarina (± 0.19), *Caenis* sp. (± 0.20), Dipteran pupae (± 0.27) and *Gammarus* sp. (± 0.29; confidence intervals shown as the amount added or subtracted to the slope; Table 2.3). Accordingly, taxa identified to family or order level did not demonstrate greater residual variability than those identified to species or genus level.

Intra-specific differences in the length-mass regressions among lakes of different nutrient and alkalinity concentrations were examined for two of the target taxa, *Asellus aquaticus* and *Potamopyrgus jenkinsi*. No difference in the slope of the length-mass regression of *Asellus aquaticus* was observed among lakes of different alkalinity and nutrient levels. However, individuals in more alkaline lakes were observed to have marginally greater dry mass (i.e. larger size) than those in less alkaline lakes. In contrast, *A. aquaticus* individuals in lakes with higher TP levels were found to be marginally smaller than those in TP poor lakes. The slope of the *Potamopyrgus jenkinsi* length-mass regression increased marginally (slope: 0.01) with increasing alkalinity concentrations. Individuals of *P. jenkinsi* were found to have slightly less dry mass (slope: -0.01) in more alkaline lakes, although their mass per unit length was higher in these lakes. Intra-specific variation in length-mass relationships among habitats has been observed (Schröder, 1987; Short *et al.*, 1987; Griffith *et al.*, 1993). However, other studies have observed little difference in length mass regressions of benthic invertebrates when compared among habitats (Kovalak, 1978; Smock, 1980; Eggert and Burton, 1994).

Inter-specific differences in the length-mass regressions among species of the same genus were examined for two of the target taxa, *Caenis* and *Gammarus* sp.. *Caenis horaria* and *C. luctuosa* were shown not to differ in dry mass or in length-mass

regression, but the size (i.e. dry mass) of both species was smaller in more alkaline lakes (slope: -0.01) and larger in more TP rich lakes (slope: 0.01). *G. duebeni* and *G. pulex* were found to differ in dry mass from *G. lacustris*; *G. lacustris* specimens were found to be smaller, with lighter dry mass (slope: -0.40) than the other species. However, no marked difference in their body-length mass relationship was observed.

Two taxa were identified to family level (Chironomidae and Sphaeriidae) and two taxa were identified to order level (Hydracarina and Dipteran pupae). The length mass regressions of Dipteran pupae and Sphaeriidae did not differ among lakes except, Sphaeriidae specimens were marginally larger in more alkaline lakes, and Dipteran pupae were marginally smaller in more TP rich lakes (Table 2.3). The length mass regression of Chironomidae was modified by alkalinity (Table 2.3). Chironomidae larvae were marginally smaller in more TP rich lakes and mass per unit length marginally increased in more alkaline lakes. Previous studies of Chironomidae found highly variable length-mass regressions (Balushkina, 1982; Johnston and Cunjak, 1999; Baumgärtner and Rotthaupt, 2003) in contrast to the narrow confidence limits observed in the present study. The poor predictive power was thought to indicate taxonomic variation, owing to the large and diverse constituent species within the Chironomidae family (Johnston and Cunjak, 1999; Baumgärtner and Rotthaupt, 2003). However, in the current study, the predictive power of the Chironomidae length-mass regression was among the best of all taxa, indicated by the narrow confidence limits of the slope of the regression. The observed change in the slope of the length-mass regression with alkalinity could be attributed to differences in the taxonomic composition of lower alkalinity lakes to those with higher alkalinity concentrations (Harriman and Morrison, 1982; Stoner *et al.*, 1984; Johnson *et al.*, 1993; Tierney *et al.*, 1998).

The possibility exists that the effects of alkalinity and TP on the length-mass regression may be a result of differences in the taxonomic composition of lakes with different water chemistry, with different species/genus having slightly different length-mass relationships. Past comparative studies, where regression statistics of invertebrates were employed to identify animals to different levels of taxonomic resolution, have demonstrated that models at lower taxonomic levels (i.e. at higher resolution) had higher coefficents of determination (R^2; Meyer, 1989; Johnston and Cunjak, 1999; Baumgärtner and Rotthaupt, 2003). However the decrease in predictive power observed

with lower taxonomic resolution was not apparent in our results, as the slopes of the length-mass regressions of taxa identified to family or order level did not demonstrate greater confidence intervals than those identified to species level.

Variation in the length-mass regressions at different levels of taxonomic resolution may be dependent on the range of body shapes within the order/family being examined. For example, freshwater larvae of order Ephemeroptera have a wider variation in body shapes among families than freshwater larvae of the order Hydracarina. The low resolution taxa examined in this study (Chironomidae, Sphaeriidae, Hydracarina and Dipteran pupae) have relatively consistent body shapes at all taxonomic levels (i.e. the principle structures, such as head, thorax, abdomen, legs and tails did not differ vastly among constituent species for each of these taxa), perhaps explaining the consistency of variability among all taxa, regardless of taxonomic resolution.

In conclusion, application of length-mass regressions derived from a set of organisms from one lake, to a separate set of organisms from a lake differing in TP and alkalinity, appears to be robust, as differences in the mass per unit length were found to be marginal among lakes differing in both these variables, well within the confidence limits of the overall regression.

2.5 Conclusions

The following points encapsulate the key findings of this study:

- The relationship between Ln dry mass and Ln body length was the strongest relationship in models of all taxa, and slopes (2.34 to 3.09) remained remarkably constant when the simple linear model was compared with that of the general linear model;
- The length-mass regressions of five of the eight taxa examined (*Asellus aquaticus*, *Caenis* sp., Dipteran pupae, *Gammarus* sp., *Potamopyrgus jenkinsi* and Sphaeriidae) were not modified by alkalinity or nutrient changes;
- Alkalinity had a marginal effect on the dry mass of six taxa (excluding *Gammarus* sp. and Dipteran pupae), and on the slope of the length-mass regression of three taxa (Chironomidae, Hydracarina and *P. jenkinsi*);
- Minimal adequate TP models described the data better than TN models for the target taxa in each case (lower AIC_C values), where nutrients were retained in models;
- TP had a marginal effect on the dry mass of five taxa (excluding *Gammarus* sp., *P. jenkinsi* and Sphaeriidae) and on the slope of the length-mass regression of one taxa (Hydracarina);
- Where alkalinity or TP were found to alter the length mass regressions of taxa (Chironomidae, Hydracarina and *P. jenkinsi*), slopes were close to zero (ranging from -0.01 to 0.02) and were well within the confidence limits of the length-mass regression, adding little predictive value to the general length-mass regression;
- Taxa identified to family or order level did not demonstrate greater residual variability than those identified to species level; however, variation in the length mass regressions at different levels of taxonomic resolution may be dependent on the range of body shapes within the order/family under examination; and
- Application of length-mass regressions derived from one set of organisms, to another set of organisms, from lakes differing in TP and alkalinity, appears to be robust, as differences in the mass per unit length were found to be marginal among lakes, well within the confidence limits of the overall regression.

CHAPTER 3

CHANGES IN THE BIOMASS SPECTRUM OF LAKE LITTORAL MACROINVERTEBRATES WITH NUTRIENT ENRICHMENT

3.1 Introduction

The littoral macroinvertebrate community within lakes is an essential link in the transfer of energy from primary producers and detritus to fish (Hanson, 1990). Macroinvertebrate biomass and production correlates with fish biomass and production, and fish growth appears dependant on the availability of prey of suitable size (Kerr, 1971; Hanson and Legget, 1982; Hanson, 1990). The size structure of macroinvertebrates may therefore influence food-chain efficiency, with important implications for ecosystem function (Strayer, 1991; Zimmer, 2001). Understanding the factors that affect macroinvertebrate size-structure and abundance will further our understanding of the trophic dynamics of lakes (Hanson, 1990; Solimini *et al.*, 2005).

3.1.1 Body size

An organism's size influences virtually all aspects of its energy and material flow (Peters, 1983a), is correlated with many life-history traits (Calder, 1984) and influences biotic and abiotic interactions with the environment (Peters, 1983a; Calder, 1984; Strayer, 1991). Allometric theory predicts that physiological processes such as ingestion, excretion, growth and reproduction are scaled exponentially to individual body size (Peters, 1983a; Cyr, 2000). Body size also relates to *inter alia* lifespan and home range (White *et al.*, 2007). Thus, the distribution of body sizes within a community links with community production, metabolism and nutrient turnover (Banse and Mosher, 1980; Peters, 1983a; Strayer, 1986; Rasmussen, 1993; Blumenshine *et al.*, 2000; Stead *et al.*, 2005). Community body size distributions also scale with species density and abundance (Damuth, 1987; Blumenshine *et al.*, 2000), playing an important role in nutrient competition, resource use and predator-prey relations (Peters, 1983a).

As a result of these important population, community and ecosystem level body size based scaling relationships, the distribution of body sizes can reveal underlying processes affecting community assembly and persistence (Allen *et al*, 2006), reflecting basic ecosystem processes (Manly, 1996). These emergent properties of ecosystems have been related to body size distributions across a variety of habitats within and among ecosystems in different biogeographic regions (Brown and Maurer, 1986; Sprules and Munawar, 1986; Cyr *et al.*, 1997; Blumenshine *et al.*, 2000; Sprules and Munawar, 1986; Strayer, 1986; Poff *et al.*, 1993; Solimini *et al.*, 2005).

3.1.2 Introduction to the size spectrum

The density-body mass relationship (reflected in both abundance and collective biomass per unit area of different sized organisms, often categorized into logarithmically increasing size classes) among individuals or among species is a widely studied macro-ecological pattern (Damuth 1981; Dickie *et al.*, 1987; Brown and West, 2000; Gaston and Blackburn, 2000; Kerr and Dickie, 2001; Cohen *et al.*, 2003; Jennings and Mackinson, 2003; White *et al.*, 2007; Blanchard *et al.*, 2009). This relationship exhibits remarkable regularity among systems differing greatly in primary production, temperature and depth (Sheldon *et al.*, 1972; Sprules *et al.*, 1983; Sprules and Munawar, 1986; Boudreau and Dickie, 1992) and is typically described by the slope of a linear relationships between log numerical (N) or biomass (B) density and log body mass (W), or by a power function where untransformed (Jennings *et al.*, 2007).

The density-body mass relationship has been examined from two perspectives: (i) the "cross-species" relationship between log mean abundance or biomass density per species and log mean body size of the species, where data is often from a taxonomically or functionally defined groups of species, such as plants, birds or mammals (Blackburn and Gaston, 1997); or (ii) the relationships for 'all individuals' in a food web or specified size range, where log abundance or total biomass density is summarised for all individuals within a body mass class, and the density-body mass relationship is examined among body mass classes (Dickie, *et al.*, 1987; Jennings *et al.*, 2007). However, although the slopes of the density-body mass relationship can be estimated from either "cross species" relationships, or from "all individuals", the same energetic explanations have been advanced to account for observed slopes (Damuth, 1981; Cyr, 2000; Brown and Gillooly, 2003; Jennings *et al.*, 2007). "Cross species" relationships have been explored more extensively in terrestrial communities, whereas "all individual" relationships have been examined more frequently in aquatic communities, where they are often referred to as "size spectra" (Jennings *et al.*, 2007). Local "cross-species" patterns and "all individual" patterns are linked, as the total number of individuals within a size class (i_s) is equal to the number of species in that size class (s_s) multiplied by the average population density of species in that size class (p_s):

$$i_s = s_s * p_s \qquad [1]$$

This relationship indicates that one pattern may be generated by the other (White *et al.*, 2007). Density-body mass relationships have been examined over many different temporal and spatial scales, from local studies spanning one order of magnitude from a single sample of a small community, to those spanning several orders of magnitude on a regional or global scale, encompassing data from many different studies (Damuth, 1981; Rasmussen, 1993; Blackburn and Gaston, 1997; Jennings *et al.*, 2007).

The "all individual" density-body mass relationship, or size spectrum, follows the model proposed by Sheldon *et al.* (1972). This was originally used to describe patterns of biomass distribution and energy transfer in marine pelagic ecosystems (Sheldon *et al.*, 1972, 1977) and lake plankton communities (Peters 1983), where the collective biomass of organisms ordered into logarithmically increasing size classes was observed to be approximately constant. Constant biomass among log size classes is equivalent to a linear decrease in the log abundance with log size class relationship, with a slope of -1. Theoretically, the size spectrum has been interpreted to reflect the flow of energy from small to large organisms (Kerr, 1974). The size spectrum approach has been used to compare assemblages within and among populations, communities, biogeographic regions or ecosystems (Sprules and Munawar, 1986; Hanson *et al.*, 1989; Strayer, 1991; Poff *et al.*, 1993; Solimini *et al.*, 2001).

The density-body mass relationship reflects many life-history and ecological properties scaled to body size (White *et al.*, 2007). For example, smaller animals tend to grow and reproduce faster but suffer higher natural mortality than larger, longer living animals (Damuth, 1981; Jennings *et al.*, 2002). Thus, annual production to biomass ratios (P/B) are negatively related to body size (Banse and Mosher, 1980; Plante and Downing, 1989; Morin and Dumont, 1994; Huryn and Benke, 2007). However, owing to the greater energy flux among smaller animals, resulting from higher population turnover rates, the standing biomass of smaller animals is considerably less at a given moment than their annual production when compared with larger animals, (Damuth, 1981).

Body size is one of the primary determinants of metabolism, which is predicted to scale to body mass$^{0.75}$ (Gillooly *et al.*, 2001; Rundle *et al.*, 2007). Therefore, body size is predicted to scale to resource use, and the density-body mass relationship is predicted to reveal how resources are partitioned in ecological systems (White *et al.*, 2007). In plant

(Westoby, 1984) and intertidal communities (Hughes and Griffiths, 1988), self-thinning has been observed such that, as organisms grow, there is space for less individuals, leading to a negative relationship between mean organism size and total abundance (White *et al.*, 2007).

The slope of the density-body mass relationship describes the relative abundance of smaller and larger individuals in a community, and the intercept relates to community productivity (Kerr and Dickie, 2001; Shin *et al.*, 2005). Trophic level of feeding often increases with body size, particularly in many "size-structured" aquatic environments, as most non-parasitic predators are larger than their prey (Cohen *et al.*, 1993, 2003). Theoretically this has been interpreted to mean that size structure reflects the flow of energy from small to large organisms (Kerr, 1974). Where size structure represents energy flow through the system, a steeper more negative slope indicates less efficient energy transfer among trophic levels, and an increase in smaller relative to larger organisms (Borgman, 1982). This suggests that reduced energy travels up the food web from prey to predators. Shallower slopes indicate more efficient energy transfer through the web. Although the mean body mass of a species may be weakly correlated with their trophic level, the latter increases continuously with body mass throughout the whole food web (Fry and Quinones, 1994; France *et al.*, 1998; Jennings *et al.*, 2001, 2002a). Thus, assigning all individuals of a species to a single trophic level is inappropriate. Size rather than species-based analyses of food web structure provides a better approach (Jennings and Mackinson, 2003).

3.1.3 Size spectrum patterns at different levels of resolution

The size spectrum has been examined for organisms spanning several orders of magnitude of size (Cohen *et al.*, 2003) and for functionally or taxonomically defined subsets of food webs such as lake plankton (Peters, 1983b; Sprules and Munawar, 1986) or benthos (Strayer, 1986; Rasmussen, 1993).

Ecosystem level size spectra (biomass or abundance based) are typically characterised by subgroups, portrayed by a series of domes, corresponding to major ecological functional groups. In lakes these have been observed to correspond to bacteria, phytoplankton, zooplankton, benthic organisms and fish (Kerr and Dickie, 2001). Therefore, measuring a partial spectrum of a food web may not reflect spatial and

temporal stability observed at an ecosystem level; this may be partially owing to the fact that community level interactions occur at a different timescale than those at an ecosystem level (Kerr and Dickie, 2001). Subordinate size spectra may not adequately describe the relationship between energy availability and scaling, as individuals within subsets may be competing with individuals outside the subsets. Energy available to individuals in different body size classes may not be known (Maxwell and Jennings, 2006). For example, the size spectra of lake littoral benthic macroinvertebrates may be strongly affected by size selective predation by fish, although fish abundance or biomass may not be included in the study. Thus, patterns of partial spectra need to be interpreted with care, while it is also important to examine different scales of resolution, or size ranges of organisms, to gain understanding of the internal structures of size spectra (Kerr and Dickie, 2001).

When the size spectrum of a strongly size-structured food web is examined at low level resolution over several orders of magnitude, the predator-prey mass ratios (PPMR) can help explain the slope and pattern of the spectrum (Jennings and Mackinson, 2003). Many size structured aquatic communities incorporating several trophic levels have demonstrated a slope of approximately -1.0 (Kerr and Dickie, 2001; Jennings and Mackinson, 2003; Blanchard *et al.*, 2009). This is consistent with an energy transfer efficiency between adjacent trophic levels of 10%, and mean ratio of predator to prey mass of four orders of magnitude (Borgman, 1987; Brown and Gillooly, 2003). However, when the size spectrum of a system is examined at a high level of resolution, such as when a single trophic level within a food web is examined, and consumers share a common energy source (e.g. plants sharing sunlight [Belgrano *et al.*, 2002]), "energetic equivalence" has been proposed among individual consumers of differing body size (Damuth, 1981; Jennings and Mackinson, 2003). This theory originated from a study of mammalian herbivore populations, where the observed size spectrum slope was -0.75, so that density (D) scaled with mass (M) $D \alpha M^{-0.75}$ (Damuth, 1981). An individual's metabolic rate (R) (or energy use per individual) has also been observed to scale with body mass: $R \alpha M^{-0.75}$, implying that larger animals are more energy efficient per unit mass than smaller ones (Damuth, 1981; Brown *et al.*, 2004). Therefore the rate of energy use per species per unit area scales as M^0 (Brown and Gillooly, 2003). Damuth (1981) proposed that energy use was independent of M for all organisms

sharing a common energy resource. Such "energy equivalence" (Nee *et al.*, 1991) means that body size does not provide any population with an energetic advantage.

Since this hypothesis was proposed for mammalian herbivore populations, there have been many attempts to test whether Damuth's (1981) hypothesis applied to other groups sharing a common energy source (Blackburn and Gaston, 1997; Belgrano *et al.*, 2002; Li, 2002; Maxwell and Jennings, 2006; Jennings *et al.*, 2007). This has proved difficult, as many studies examine density-body mass relationships in taxon-based subsets of food webs (Gaston and Blackburn, 2000). In addition, many studies lack accurate information on the energy available to animals of different sizes, and whether they feed from a common energy source (Carbone and Gittleman, 2002; Brown and Gillooly, 2003; Jennings and Mackinson, 2003). Energetic equivalence has, however, been shown for the scaling of density and body mass in phytoplankton and plant communities (Belgrano *et al.*, 2002; Li, 2002), although other tests of the theory may have been unreliable, as individuals were not sharing energy, and/or only a proportion of individuals sharing energy were considered (Maxwell and Jennings, 2006; Jennings *et al.*, 2007).

In summary, the size spectrum slope (log density plotted against log body mass class) is approximately -1 in most size-structured aquatic communities which have been investigated across several trophic levels (Kerr and Dickie, 2001; Jennings and Mackinson, 2003; Blanchard *et al.*, 2009). This is consistent with energetic considerations about the efficiency of transfer of resources from one trophic level to another, and the mean ratio of predator to prey body size (Borgmann, 1987; Brown and Gillooly, 2003). Where organisms share a resource (i.e. within a trophic level for communities such as autotrophs, herbivores and detritivores), a shallower slope of approximately -0.75 has been suggested (Damuth, 1981; Brown and Gillooly, 2003; Blanchard *et al.*, 2009).

3.1.4 The normalised size spectrum

Platt and Denman (1977, cited in Kerr and Dickie, 2001) introduced the "normalised size spectrum" (NSS), adapted from the biomass density-body mass spectrum used by Sheldon *et al.* (1972), where the total biomass observed in each size class is scaled by the size of the respective size class, creating a "normalised" spectrum (Kerr and Dickie,

2001). This makes the slope and intercept of the normalised spectrum independent of size classifications, allowing comparison among communities. While removing much of the variation in the data, the method retains most of the information of the size spectra (Baca and Threkeld, 2000; Kerr and Dickie, 2001). This converted Sheldon's hypothesised horizontal spectrum of equal biomass among size classes with a slope near zero, to a normalised spectrum with a slope approximately -1, with the added advantage of a generalised representation of the spectrum (Kerr and Dickie, 2001). When the biomass spectrum is not constant, but demonstrates a size trend, the slope of the NSS can be used to assess the degree of departure from the uniform biomass or "steady state" distribution (Sprules and Munawar, 1986). The normalised biomass of a size class (NB_{sc}) is determined by calculating the log of the total biomass of a size class (Tb_{sc}) divided by the width of the size class (w_{sc}; equation [2]):

$$NB_{sc} = \text{Log}\,[\,Tb_{sc} / Min_{sc}]$$
$$\text{Therefore, } NB_{sc} = \text{Log}\,Tb_{sc} - \text{Log}\,w_{sc}$$
$$\Rightarrow \text{Log}\,Tb_{sc} = NB_{sc} + \text{Log}\,w_{sc} \qquad [2]$$
$$\text{Where width of size class } w_{sc} = Max_{sc} - Min_{sc}$$

The width of a size class is identical to the lower limit of the size class, owing to the logarithmic nature of the size classes. Although not discussed in the literature, the normalised biomass within logarithmically increasing size classes is an approximation of the log abundance-size class relationship within the same size class, where log abundance (Log Ae_{sc}) is estimated from the total biomass within each size class (Tb_{sc}) divided by the mean biomass per individual within each size class (Ab_{sc}; equation [3]).

$$\text{Log}\,Ae_{sc} = \text{Log}\,[\,Tb_{sc} /.\, Ab_{sc}\,]$$
$$\Rightarrow \text{Log}\,Ae_{sc} = \text{Log}\,Tb_{sc} - \text{Log}\,Ab_{sc}$$

Substituting equation [2]:

$$\text{Log}\,Ae_{sc} = NB_{sc} + \text{Log}\,w_{sc} - \text{Log}\,Ab_{sc}$$
$$\text{Or Log}\,Ae_{sc} = NB_{sc} + \text{Log}\,(w_{sc} / Ab_{sc}) \qquad [3]$$

Hence, given a dataset that had calculated total biomass within logarithmically increasing size classes, the slope of normalised biomass among size classes is identical to that of log abundance (calculated indirectly from the total biomass/mean biomass per size class, rather than measured directly), but the intercepts of the different equations

vary (Appendix, Figs. I and II). Thus, the normalised biomass scales with log abundance, and is generally reflective of log abundance patterns.

The NSS is widely used by many authors to describe and compare community size distributions in aquatic communities (Platt and Denman, 1978; Sprules and Munawar, 1986; Borgmann, 1987; Hanson *et al.*, 1989; Hanson, 1990; Ahrens and Peters, 1991; Gaedke, 1992; Sprules and Goyke, 1994; Rodriguez and Magnan, 1993; Rasmussen, 1993; Thiebaux and Dickie, 1993; Morin *et al.*, 1995; Vidondo *et al.*, 1997; Zimmer *et al.*, 2001; Blumenshine *et al.*, 2000; Pagola-Carte, 2004; Zhou, 2006; de Eyto and Irvine, 2007; Sprules, 2008). Autocorrelation of the residuals has been noted, as a result of the tendency of adjacent size classes to vary together (Rasmussen, 1993; Morin *et al.*, 1995; Zimmer., 2001). Nevertheless, acknowledging this, both Rasmussen (1993) and Zimmer (2001) still used NSS analyses, owing to its extreme simplicity and widespread use.

3.1.5 Freshwater size spectra

Studies of freshwater size spectra are generally conducted on subsets of the food-owing to the lower temporal and financial costs involved, so that partial size spectra, focussed on particular communities, are examined. As a result, studies focus on one or two communities of organisms (phytoplankton, zooplankton, macroinvertebrates, fish) within a mesohabitat (macrophyte beds, gravel bed, open water) in a macrohabitat (benthic, pelagic) within an ecosystem (lakes, ponds, streams and rivers). Lake benthic and pelagic habitats are traditionally treated as discrete food webs, with separate producers, decomposers and consumers, rather than strongly linked components of one system (Vadeboncoeur *et al*, 2002). Models of lake pelagic food webs are often simplified to three or four trophic levels (phytoplankton, zooplankton, planktivore fish and piscivores [Vander Zanden *et al.*, 2005]). In this context, empirical evidence from pelagic zones shows a strong relationship between body size and trophic position (Kerr, 1974; Sprules *et al.*, 1983; Sprules and Munawar, 1986; Borgmann, 1987). Relatively constant predator-prey size ratios characterise approximately constant biomass among logarithmically increasing size classes (Kerr, 1974; Sprules *et al.*, 1983; Sprules and Munawar, 1986; Borgmann 1987). The size spectra of the pelagic zone has thus been interpreted to reflect energy flow through the system, demonstrating a remarkably stable pattern (Kerr, 1974; Borgmann, 1987).

In contrast, lake benthic macroinvertebrate size spectra are not typically uniform (Strayer, 1986; Hanson *et al.*, 1989; Hanson, 1990; Morin and Nadon, 1991; Rasmussen, 1993). This is attributed to (i) the presence of large microphagous organisms (filter feeders and deposit feeders [Rasmussen, 1993]) and (ii) the complexity of benthic habitats, leading to greater species richness and enormous differences in behavior, morphology, physiology and other biological characters not clearly related to body size (Strayer, 1991). The strong presence of large benthic microphagous feeders and smaller percentage of macrophagous predators may lead to a weaker correlation with trophic position and less consistent predator-prey size ratios than that of the pelagic zone (Rasmussen, 1993) and less size-structure than that of the pelagic zone. Rasmussen (1993) argued that weaker trophic coupling in the zoobenthos compared with the pelagic community provided a less integrated size spectrum both functionally and statistically. The higher complexity of benthic communities means that energy flow from producer to top predator (i.e. fish) has an extra number of intermediate steps not observed in the pelagic web; consequently, the efficiency of energy transfer to top predators is likely to be lower (Strayer, 1991).

Benthic macroinvertebrate abundance, biomass and size structure has been demonstrated to change with the physical and biological structure of the habitat (Mittelbach, 1981; Hanson, 1990; Rasmussen, 1993; Wellborn *et al.*, 1996; Tolonen *et al.*, 2003; McAbendroth *et al.*, 2005). Macrophyte structure can assist in regulating predator-prey interactions and size structure of littoral invertebrates (Jeppesen *et al.*, 1998; Tolonen *et al.*, 2003). However, observations and experiments examining how differences among habitats affect macroinvertebrate size structure have yielded conflicting results. Hanson (1990) compared weed-beds dominated by macroalgae to those dominated by rooted plants, and found a significant difference in total biomass, taxonomic composition and size structure of the macroinvertebrate communities, where the biomass of large organisms was ten times greater in macroalgae beds (*Chara* sp.) than in rooted plant beds. This led the author to hypothesise that littoral zoobenthic size spectra could be as variable within as among lakes. Hanson (1990) speculated that the abundance of large bodied invertebrates was higher in beds of *Chara* sp. because of the protection offered from fish predation owing to their structural complexity. Although Rasmussen's (1993) analysis of the size structure of lake littoral macroinvertebrates among 11 lakes in Quebec, provided some support for the findings of Hanson (1990),

that plant community structure has importance in determining size spectra variation but also showed that macrophyte biomass and other limnological site variables, such as wave action, provided stronger regression models than macrophyte species composition.

Both Mittelbach (1981) and Rasmussen (1993) found that plant-dwelling invertebrates were on average much smaller than invertebrates living in the littoral sediments. Rasmussen (1993) proposed that this was an indirect result of invertebrates on plant stems and leaves being more exposed to size selective predation by small fishes than those hidden within the substrate. However, Rodriguez and Magnan (1993), who studied the littoral zone of three lakes differing in substratum type, and Morin *et al.* (1995), who examined benthic invertebrates in streams with substrates ranging from sand to boulders, found that size spectra were insensitive to changes in substrata.

A demonstrated increase in the abundance and total biomass of benthic macroinvertebrate with increasing macrophyte cover (Rasmussen, 1988; Paukert and Willis, 2003; Tolonen *et al.*, 2003; Michaletz *et al.*, 2005) has been attributed to increasing structural complexity, with more inhabitable space for smaller animals (McAbendroth *et al.*, 2005) and increased food resources and high heterogeneity within more complex habitats (Diehl, 1992). The structure of macrophyte and macroalgal beds may also provide a refuge against fish predation (Gilinsky, 1984; Hershey, 1985; Winfield, 1986; Diehl, 1988, 1992; Zimmer, 2001; Tolonen *et al.*, 2003). Increases have also been observed in the body size and activity of both predatory and non-predatory invertebrates with increasing macrophyte and macroalgal cover (Zimmer *et al.*, 2001; Tolonen *et al.*, 2003).

Hanson and Legget (1982) demonstrated a correlation of fish biomass with macroinvertebrate biomass and production. Many studies have recorded a decrease in the size of benthic invertebrates with increasing fish predation pressure (Crowder and Cooper, 1982; Morin, 1984; Mittelbach, 1988; Diehl, 1992; Huryn, 1998; Blumenshine *et al.*, 2000). In fishless lakes large invertebrates can replace fish as top predators (Dermott, 1988). Other studies showed that although benthic invertebrate abundance and biomass decreased in the presence of fish, the size structure or mean size did not vary (Zimmer *et al.*, 2001; Michaletz *et al.*, 2005). Size selective predation by fish may reduce the mean body size of the macroinvertebrate community with removal of large

taxa (Townsend and Thompson, 2007). Such effects, however, have been found to be less pronounced in complex habitats such as macrophyte stands, than in open water (Crowder and Cooper, 1982; Diehl, 1988; Diehl, 1992).

Many limnological studies have viewed lake pelagic processes as the crucial element in explaining lake ecosystem functioning, relegating the benthic communities to merely a source or sink of pelagic nutrients or energy (Cyr and Peters, 1996; Vadeboncoeur *et al.*, 2002). Recent evidence has shown that benthic and pelagic production pathways in shallow lakes are intricately linked by mobile consumers such as fish, which have demonstrated an energetic dependency on periphyton and zoobenthos as well as phytoplankton (Blumenshine *et al.*, 1997, Vadeboncoeur *et al.*, 2002; Vander Zanden and Vadeboncoeur, 2002). Allocthonous organic carbon also contributes to benthic-pelagic coupling (Cole *et al.*, 2006). Benthic primary production can be substantial under low nutrient and productivity conditions and may even dominate whole-lake primary production (Wetzel, 1990; Vadeboncoeur *et al.*, 2002, 2003). Benthic production may be particularly important in shallow lakes where benthic and planktonic food webs are more closely linked (Cyr and Peters, 1996; de Eyto and Irvine, 2007). Likewise pelagic production can be important to benthic communities, where sinking planktonic algae provide food to benthic invertebrates (Cyr and Peters, 1996). Benthic invertebrates may feed on both benthic and pelagic algae and invertebrates, which in turn are consumed by fish (Cyr and Peters, 1996).

Few biomass spectra have included both planktonic and benthic organisms. Where benthic and pelagic organisms have been examined together, many authors separate the benthic and pelagic fractions into separate biomass spectra (Cyr and Peters, 1996; Baca and Threkeld, 2000; Blanchard *et al.*, 2009), although pelagic and benthic communities have also been merged to produce a single biomass spectrum with constant slopes (Boudreau and Dickie, 1992; Cyr and Peters, 1997). When trophic coupling between marine benthic and pelagic communities was examined, the disaggregation of whole community data was advocated to account for size structured pelagic processes (predators feeding according to body size) and resource sharing benthic communities (common resource such as detritus or phytoplankton shared [Blanchard *et al.*, 2009]). When datasets were accordingly separated, steeper slopes were observed for the pelagic compared with the benthic community (Blanchard *et al.*, 2009).

3.1.6 Freshwater size spectra and nutrient enrichment

The main anthropogenic pressure affecting lakes in Ireland is nutrient enrichment (eutrophication) associated principally with increases in phosphorus entering surface waters from diffuse run-off from agriculture (Irvine *et al.*, 2001). Nutrient enrichment can distinctly alter energy flow through lake food webs causing shifts from benthic production (such as periphyton and rooted macrophytes) to a dominance of pelagic primary production (Vadeboncoeur *et al.*, 2003). In addition, the process may cause environmental and ecological changes such as light limitation, increased turbidity, decreased biodiversity and ultimately, oxygen limitation.

Many lake pelagic studies document a shallower size spectra slope (Sprules and Munawar, 1986; Ahrens and Peters, 1991) and an increase in larger phytoplankton taxa with nutrient enrichment (Kalff and Knoechel, 1978; Watson and Kalff, 1981; Cottingham, 1999; de Eyto and Irvine, 2007). In contrast, other studies found no significant change in the slope of the size spectra (Cyr and Peters, 1996; Tittel *et al.*, 1998) or a steeper slope was observed (Cyr *et al.* 1997; Baca and Threlkeld, 2000). Steeper size spectrum slopes with nutrient enrichment, suggest that energy accumulates at lower trophic levels. The pelagic size spectrum intercept has been observed to consistently increase with nutrient enrichment (Peters, 1983b; Ahrens and Peters, 1991; Cyr and Peters, 1996; Baca and Threlkeld, 2000; de Eyto and Irvine, 2007), reflecting the increase in primary productivity. However, changes in the shape of the size spectrum are rarely used to describe the size structure of aquatic macroinvertebrate communities (Hanson, 1990; Rodriguez and Magnan, 1993) as there is still relatively little information available on the factors that affect the size structure of benthic macroinvertebrate communities (Strayer, 1991; Pagola-Carte, 2004).

Among lotic littoral macroinvertebrate communities, size spectra appears variable with nutrient enrichment (Rasmussen, 1993; Cyr and Peters, 1996; Michaletz *et al.*, 2005). Morin *et al.* (1995) described a shift towards larger organisms with increasing phosphorus concentrations, while Townsend and Thompson (2007) observed smaller body sizes in more productive environments with coarser substrata. In contrast, Solimini *et al.* (2001) found that the size spectra varied little with changes in stream trophic state and substrate. Stream size spectra generally display little temporal

variation (Morin *et al.*, 1995) and limited variation with substrate size and trophy (Morin and Nadon, 1991; Bourassa and Morin, 1995).

Among lentic littoral macroinvertebrate communities a significant increase in the abundance of smaller organisms with nutrient enrichment has been observed, which reflects a steeper overall size spectrum slope (Hayward and Margraf, 1987; Rasmussen, 1993). However, no significant change in the size structure of macroinvertebrates with nutrient enrichment was observed by either Michaletz *et al.* (2005) or Cyr and Peters (1996). It is difficult to distinguish the effects of productivity and nutrient enrichment from the effects of habitat structure, substrate, fish predation and local disturbance, with often high variability observed among benthic community studies (Strayer, 1991; Rodriguez and Magnan, 1993; Pagola-Carte, 2004). The general patterns of energy flow, such as those described for plankton size spectra (Sheldon *et al*, 1972; Sprules *et al.*, 1983; Sprules and Munawar, 1986) may, therefore, be elusive in lacustrine zoobenthos (Strayer, 1991; Rodriguez and Magnan, 1993).

Alkalinity affects the composition of benthic invertebrate communities, as faunal communities of acidified waters are often reported to be impoverished (Økland and Økland, 1986; Sutcliffe and Hildrew, 1989; Jeffries and Mills, 1990; Johnson *et al.*, 1993; Allott *et al.*, 1997; Tierney *et al.*, 1998), but links with size structure are lacking. Holmgren (2001) found that the body size distributions of macroinvertebrates sampled from limed lakes appeared more similar to circumneutral than to that of two acidic lakes, although results were inconclusive. Nevertheless, limed lakes are not comparable to naturally alkaline systems and little is known of the effects of alkalinity on macroinvertebrate size structure. Another important environmental factor observed to affect macroinvertebrate community structure is the area of a water body (Heino, 2000). Whether this may lead to a change in macroinvertebrate biomass and size distributions has not been examined to date, to the best of my knowledge. Further research into factors affecting benthic invertebrate size spectra has been recommended (Strayer, 1991; Pagola-Carte, 2004; Solimini *et al.*, 2005).

In summary, benthic macroinvertebrate body size and size spectra reflect many autoecological traits, as well as community rates of production, metabolism, nutrient turnover, and can aid in understanding lake trophic dynamics. Nutrient enrichment, the

principal threat to lake water quality in Ireland, has wide ranging potential effects on lake littoral macroinvertebrate community structure and biomass. Lentic macroinvertebrate size spectra were found to reflect an increase in smaller individuals, as well as in the total biomass of invertebrates (and thus the intercepts of size spectra) and to display steeper size spectra slopes with nutrient enrichment. Benthic macroinvertebrate community structure was found to differ among substrates, and with alkalinity, particularly under low alkalinity concentrations. The effects of a broad gradient of alkalinity and nutrient concentrations on the size structure of benthic macroinvertebrates from different substrates, has not been examined in a comparative study among a large number of lakes to date, and the aim of this study is to address this knowledge gap. In this study, the effects of nutrient enrichment and alkalinity on the size distribution of lake littoral macroinvertebrates found in both hard substrates (pebbles, gravel or bedrock) and soft substrates (fine or organic sediments) of forty eight lakes was examined. Lakes were selected to represent a gradient of total phosphorus (TP) concentrations (*in situ* measured range of 2.2 to 160.4 $\mu g\ l^{-1}$) and of alkalinity concentrations (*in situ* measured range of 1.0 to 264.4 mg $CaCO_3\ L^{-1}$).

The following main hypotheses are proposed:

(a) the size spectrum slope will become steeper, with a decrease in mean individual body size and an increase in small animals with nutrient enrichment;

(b) the size spectrum intercept and the total biomass of benthic invertebrates will increase, reflecting greater productivity with nutrient enrichment;

(c) the size spectra differ among substrates, with an increase in larger invertebrates in soft substrates, owing to a higher presence of macrophytes and macroalgae;

(d) the size spectrum intercept will decrease with decreasing alkalinity, arising from an decrease in production, potentially owing to a more impoverished invertebrate community in low alkalinity environments; and

(e) the collective biomass of small invertebrate will increase with benthivorous fish biomass.

Linear mixed effects models were used to analyse the effect of lakebed sustrate and lake water chemistry on mean biomass, total biomass, size diversity, total abundance and size spectra (i.e. normalised biomass across size classes). This model design incorporates random effects (in this context, lake identity, thus specifying the difference

in the variance structure within and among lakes) and fixed effects (both categories [substrates] and gradients [water chemistry; Bolker *et al.*, 2008]). Specification of lake identity as a random effect allows control of the variance-covariance structure of the model (Crawley, 2007). Alternative linear models such as ANCOVA, or multiple and step-wise regression do not have such flexibility. ANCOVA is not appropriate for the data for several reasons such as the assumption of homogeneity of slopes, the preference for equal numbers of samples, the limitation on the number of continuous explanatory variables, and that the order in which explanatory variables (or independent variables) are entered affects the model output. Traditional multiple regression, although more flexible than ANCOVA, does not allow for the inclusion of random and fixed effects, and thus random variability among and within lakes cannot be defined within the model. Additionally, step-wise regression, a model selection process used in multiple regression (where the model is re-fit and terms with the smallest non-significant F statistic excluded; those with the highest significant F statistics included, until a single "best-fitting" model is produced with only significant terms) has several problems associated with it such as bias in parameter estimation, inconsistencies in model selection algorithms and a reliance on a single best model (Whittingham *et al.*, 2006).

Linear mixed effects models were based on information theoretic model selection procedures, allowing models representing different hypotheses to be compared in their entirety (rather than through automated selection procedures) and uncertainty among models is acknowledged, so that several alternative "best fitting" models may be produced and compared (Whittingham *et al.*, 2006). Further detail on model selection based on information theory and Akaike's information criterion (AIC) is given in Chapter 2 (Section 2.2.4).

3.2 Methods and Materials

3.2.1 Study area

Forty eight lakes (Fig. 3.1) were selected for this study, from sixty nine lakes sampled as part of the North South Shared Aquatic Resources (NS-SHARE) project which aimed to develop ecological classification tools (Donohue *et al.*, 2009) to aid in the implementation of the European Water Framework Directive 2000/60/EC (CEC, 2000). Thus, lakes were principally located in Northern Ireland and the border regions of the Republic of Ireland for contractual purposes. Lakes were selected to represent gradients of TP and alkalinity (Table 3.1). The early stages of the NS-SHARE project involved the compilation of baseline information on biotic and abiotic conditions as well as catchment level data on geology and land-use to aid in the selection of suitable study sites. Pilot studies were completed prior to sampling to select and map suitable sites at each lake and verify TP and alkalinity concentrations at each lake. Where present, two principal substrates were sampled at each lake: hard substrates (pebbles, gravel or bedrock) and soft substrates (fine or organic sediments, with or without macrophytes present). Hard and soft substrates were present at, respectively, forty seven and forty one, of the forty eight lakes sampled (Table 3.1). Each of the lakes was sampled for both macroinvertebrates and water chemistry in spring (April or May) and summer (August), at the start and end of growing season, respectively, to account for possible changes in productivity and predation pressure throughout the season. Thirty six of the lakes were sampled in 2005, and the remaining twelve lakes were sampled in 2006.

3.2.2 Field methods

3.2.2.1 Water chemistry

Water temperature was measured and water samples were collected at each lake (using a weighted 5 L bottle with attached rope, which was thrown approximately 10 m from the shore). From this, three replicate 0.5 L samples were filtered, on site, through Whatman® GF/C filter papers, using a hand pumped vacuum filter. Filter papers were stored in the dark in polypropylene centrifuge tubes at 4 °C in 10 ml of methanol prior to chlorophyll *a* analysis. Filtered water was retained in separate light resistant glass bottles.

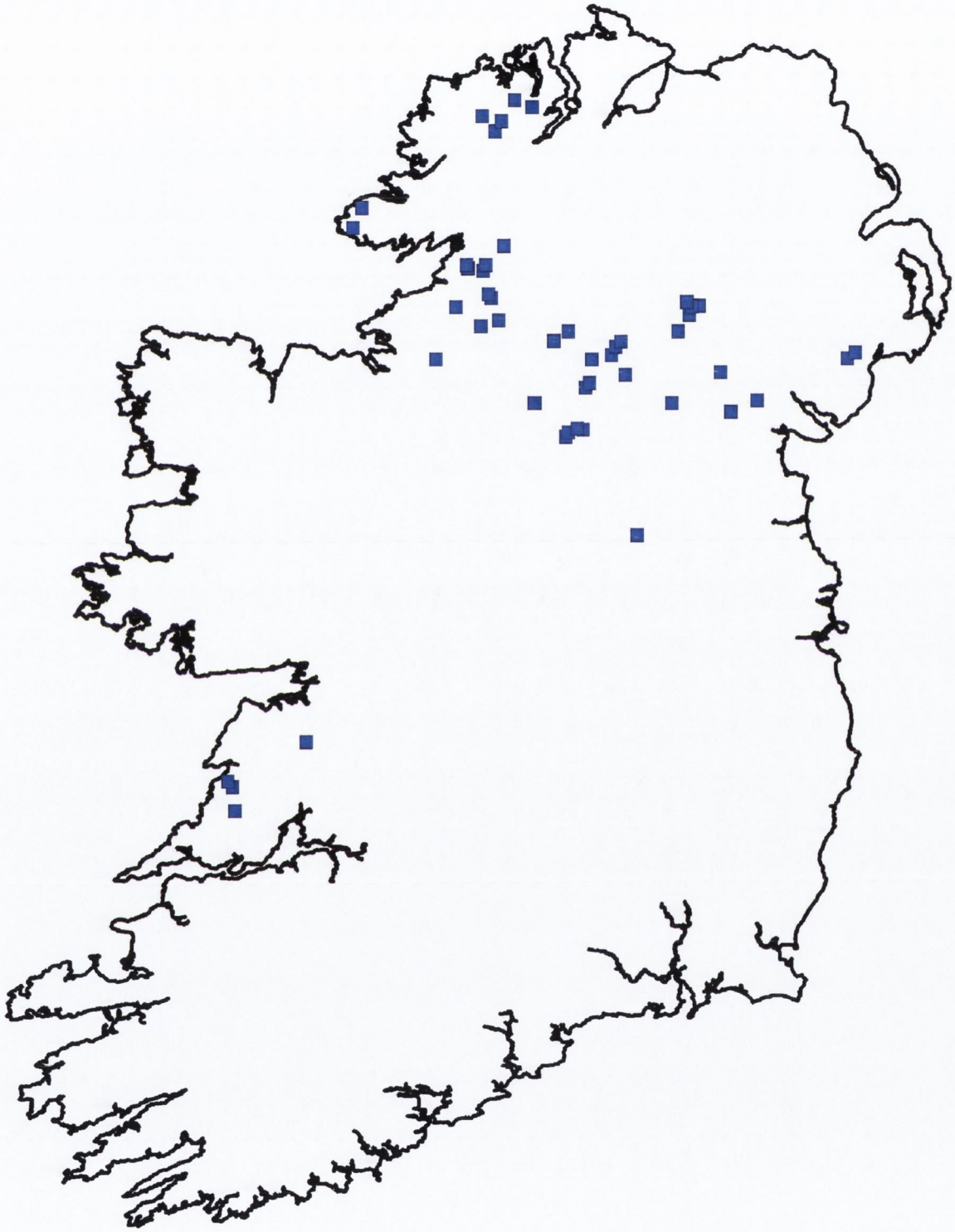


Figure 3.1: Location of lakes sampled in study.

Table 3.1: Measured alkalinity, TP concentrations and available substrates and GPS co-ordinates of each of the lakes sampled in the study.

Low Alkalinity (<20 mg CaCO3 l^{-1})					Moderate Alkalinity (20-100 mg CaCO3 l^{-1})					High Alkalinity (>100 mg CaCO3 l^{-1})				
Lake	Substrates Present	TP ($\mu g\ l^{-1}$)	Easting	Northing	Lake	Substrates Present	TP ($\mu g\ l^{-1}$)	Easting	Northing	Lake	Substrates Present	TP ($\mu g\ l^{-1}$)	Easting	Northing
Beagh	Hard	1	201195	420302	Salt	Hard	4	212441	426229	Bane	Hard, Soft	3	254709	271329
Gartan	Hard, Soft	2	205180	416036	Kiltooris	Hard, Soft	7	167183	396339	Bunny	Hard, Soft	4	137410	196785
Vearty	Hard, Soft	5	199400	365800	Carrigeencor	Hard, Soft	8	183098	333817	Nalughaghe	Hard, Soft	14	236400	324300
Achully	Hard, Soft	9	195503	367077	Cashel Upper	Hard, Soft	9	296800	319600	Corglass	Hard, Soft	17	234766	309005
Derg	Hard, Soft	9	207739	374154	Melvin	Hard, Soft	10	190502	353030	Brantry	Hard, Soft	21	274900	353900
Madral	Hard, Soft	10	195199	367462	Akibbon	Hard, Soft	11	206895	418532	Creeve	Hard	25	273800	351200
Auva	Hard, Soft	12	153857	381171	Lattone	Hard, Soft	17	199879	345447	Carrick	Hard, Soft	34	272400	355200
Formal	Hard, Soft	13	204700	347400	Skale	Hard, Soft	22	230900	344100	Deraith	Hard, Soft	38	233783	309571
Doo	Hard, Soft	17	112024	172065	Castlewellan	Hard	27	332500	336800	Legane	Hard, Soft	47	273700	353800
Aillbrack	Hard, Soft	24	109798	183001	Fern	Hard, Soft	30	217925	423554	Bawn	Hard, Soft	58	230192	307155
Nadarra	Hard, Soft	24	248500	339400	Narye	Hard, Soft	31	239800	333800	Gortnawinny	Hard, Soft	91	251229	328523
Killtyfanad	Hard, Soft	25	157786	388183	Bunerky	Hard, Soft	35	218652	318490	Sand	Hard, Soft	184	237800	326400
Keagh	Hard	35	110078	181132	Emy	Hard, Soft	41	269195	344079					
Shivnagh	Hard, Soft	42	200408	367384	Town	Hard, Soft	61	230997	308042					
Corry	Hard, Soft	52	246500	336400	Tullynawood	Hard, Soft	68	286000	329500					
Meenameen	Hard, Soft	67	202900	355900	Altnadua	Hard, Soft	71	331300	334900					
Glencreawan	Hard, Soft	142	202500	356500	White	Hard, Soft	104	267123	318738					
Natroory	Hard	182	250000	340300	Ross	Hard, Soft	107	288500	315500					

3.2.2.2 Macroinvertebrate samples

Macroinvertebrate samples were collected from the littoral zone of each lake. Three representative sites of each substrate, where present, were selected. If, on visual inspection, sufficient area of either hard or soft substrate was not available to conduct three one minute kick samples, that substrate was not sampled. Large reed beds or sites near inflows or noticeable discharges were not sampled. Macroinvertebrate sampling at each lake was done by kick sampling for 1 min with a standard Freshwater Biological Association kick net of 1 mm^2 mesh size, at each of the three replicates, for each of the substrates present. Therefore, a total of three 1 min kick samples were taken for each substrate present on each sampling occasion. Kick sampling techniques varied slightly between substrates. Hard substrates were sampled by shuffling backwards, thoroughly disturbing the substrate with each foot in turn whilst the hand-net was weaved in front of the active foot on each occasions, ensuring that the disturbed macroinvertebrates were captured in the net. Soft substrates were disturbed using the hand-net itself, knocking it gently against the substrate, to collect macroinvertebrates without associated large quantities of sediments.

The three replicate 1 min samples of each substrate were combined, to capture variation at sites, but not sample variance. Samples were roughly sorted on site, whereupon any cobbles and large macrophytes were carefully examined, ensuring that attached macroinvertebrates were included in the sample, before being discarded. The samples were then preserved on site in >90% industrial methylated spirits (IMS). A field sheet with details of the sample site was completed, the GPS position recorded and a photograph of the site was taken. Additional samples were taken from eleven lakes for the development of length mass regressions for twenty two taxa. The sampling protocol is outlined in Section 2.2.2 of Chapter 2.

3.2.3 Laboratory Methods

3.2.3.1 Water Chemistry

Water samples were analysed for alkalinity, dissolved total organic carbon, colour, pH, conductivity and chlorophyll *a* following standard methods (Standing Committee of Analysts, 1983). Filtered water samples were analysed for alkalinity, dissolved total organic carbon (DTOC) and colour, and unfiltered samples were analysed for pH, conductivity, TP and total nitrogen (TN). Chlorophyll *a* was analysed by heating

previously filtered Whatman® GF/C filter papers, stored in methanol filled centrifuge tubes, in a water bath at 65 °C to further extract pigment from the filter paper into the methanol. Samples were centrifuged and analysed for chlorophyll *a* in a Shimadzu® UV-1601 UV-visible spectrophotometer by measuring absorbance against a methanol blank at both 665 nm (chlorophyll peak) and 750 nm (turbidity correction [Standing Committee of Analysts, 1983]).

One of two methods was used to analyse the alkalinity of each filtered sample. Samples from sites where alkalinity was estimated to be greater than 20 mg $CaCO_3$ l^{-1}, were analysed following Clesceri *et al.* (1989), where 50 ml of filtered water was titrated with 0.01M H_2SO_4 in less than 2 min, until a pH of 4.5 was reached and the volume of H_2SO_4 at the endpoint was recorded. Samples from sites where the alkalinity was judged to be less than 20 mg $CaCO_3$ l^{-1} were analysed following Mackereth *et al.* (1989), where 100 ml of filtered water was titrated with 0.01M H_2SO_4 under 2 min to three endpoints between pH 4.4 and pH 3.7, and the volume of H_2SO_4 at each endpoint was recorded. The Gran titration of each pH point was subsequently calculated and plotted against the titrant volume, from which the volume in mg $CaCO_3$ l^{-1} was calculated. DTOC was measured by thermocatalytic combustion/non-dispersive infrared gas analysis using a Shimadzu® TOC-5000A by acidifying approximately 10 ml of filtered water with drops of 2M HCL added, followed by a high purity air purge to eliminate inorganic carbon prior to measurement of total carbon. The colour of filtered water samples were measured using a Hach® DR2000 spectrophotometer.

pH was measured on 50 ml of water using a Jenway 3030 pH meter. Conductivity was measured on approximately 100 ml of water using a WTW Microprocessor conductivity Meter LF196 and recorded as µS cm^{-1}. TP and TN were analysed following the methods of Eisenreich *et al.* (1975) and Grasshoff *et al.* (1999), respectively, as outlined in Section 2.2.3.1 of Chapter 2. As a means of quality control, known concentrations (QCs) were run along with lake water samples for each analysis of TP and TN. The stock reagents used to prepare QCs was different to that used for preparing concentration standards. The QC concentration used for TP and TN analyses were 25 µg TP l^{-1} and 1 mg NO_3-N l^{-1}, respectively.

3.2.3.2 Macroinvertebrate sample splitting procedure

Owing to a high abundance of macroinvertebrates in many of the lakes sampled, some samples were split, repeatedly where required, before sorting. This was achieved using a specially constructed splitting device (Fig. 3.2), through which the mixed samples were poured, and collected underneath in two large plastic beakers. The proportion of sample split was determined by weighing each half of the sample within a sieve and from this the fraction of sample contained within each portion was calculated. One portion of the sample was then stored in IMS. A minimum ratio of 40:60 was maintained between splits. Where this ratio was greater, the splitting procedure was repeated after pooling the splits. Where samples were very large, the splitting procedure was continued until more manageable numbers were present, to a minimum of 500 animals.

Before sample processing commenced, Chi Squared Tests were performed to assess the sample splitting procedure and determine whether the calculated fraction of split sample was representative of the proportion of animals in each part of the split sample. Eight samples were split into two parts (*i* and *j*), and the sample weight of each part was recorded, allowing calculation of the estimated fraction of the total that each part represented. Samples were then sorted, and the total number of animals in each part was recorded. For each sample, the number of animals "expected" in a randomly chosen split (E_i) was calculated from the estimated proportion of that split based on sample weight (P_i) multiplied by the observed animals found in the other split (O_j) divided by the calculated proportion of the other split (P_j):

$$E_i = P_i * (O_j / P_j) \qquad [4]$$

The observed (O_i) and expected values (E_i) of the same split (split *i*) were compared by means of a Chi Squared test to examine if there was a statistically significant difference between them at each lake (Table 3.2).

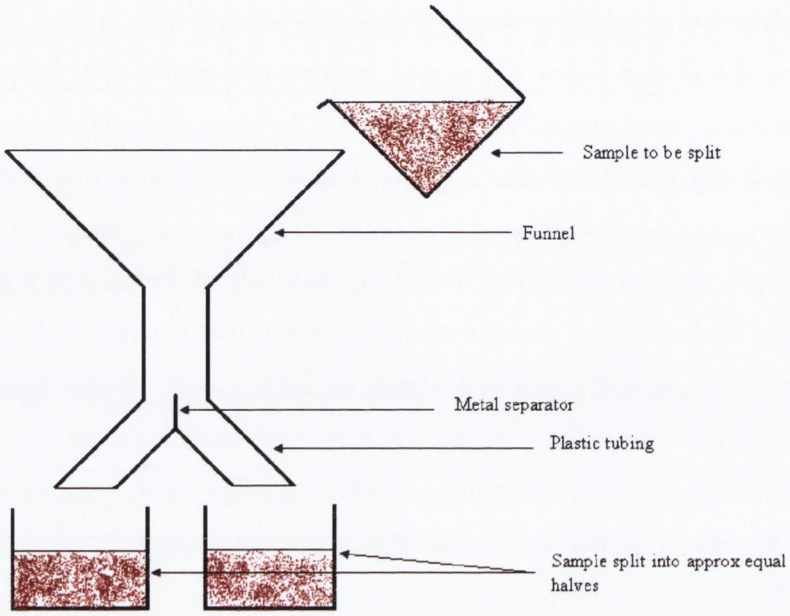


Figure 3.2: Diagram of splitting device for macroinvertebrate samples.

All lakes were compared by adding all Chi Squared values. For seven of the eight samples tested, the observed number of animals was not significantly different to the expected numbers (at $P < 0.05$). Only the Cashel Upper sample had significantly different number of animals than was expected from the splitting procedure. Comparing all lakes, proportions predicted by the splitting procedure were not significantly different from the observed proportions of animals, confirming reliability of the splitting procedure (Table 3.2).

Table 3.2: Chi Squared Test comparing observed (O_i) with expected ($E_i = P_i * (O_j / P_j)$ for eight samples (for individual lakes P value <0.05 when $X^2 > 3.84$ [df = 1] and for all lakes combined $X^2 > 25.00$ [df = 15]).

Lake	Df	X^2
Deraik	1	0.01
Tullynawood	1	2.85
Castlewellan	1	1.27
Muckno	1	2.69
Cashel Upper	1	11.98
Roughan	1	3.72
Bawn	1	0.05
Derrybrick	1	0.55
All Lakes	15	23.12

3.2.3.3 Sorting, identification and measurement

Samples were poured through a 0.5 mm^2 mesh sieve; macroinvertebrates were sorted on white trays into broad taxonomic groups, and placed into small labeled glass bottles, containing >90% IMS for later identification. Macroinvertebrates were identified and the body/shell length measured to the nearest 0.1 mm, using a dissecting 40x magnification stereomicroscope with eye graticule and Olympus® KL1500 LCD fibre-optic light source. Most macroinvertebrate taxa were identified to species level using the keys of Edington and Hildrew (1995), Elliot and Mann (1979), Elliot *et al.* (1988), Fitter and Manuel (1986), Friday (1988), Gledhill *et al.* (1993), Holland (1972), Hynes (1977), Macan (1977), Miller (1996), Reynoldson and Young (2000), Richoux (1982), Savage (1989), Savage (1999) and Wallace *et al.* (2003). However, dipteran larvae, odonatan larvae and Collembola were identified to family level, whereas nematodes, oligochaetes and Hydrachnidia were not identified further.

The body length (tip of head to tip of last segment; ignoring cerci, antennae and mouthparts) of all invertebrates with the exception of oligochaetes, gastropods and Hydrachnidia, was measured. Oligochaetes were categorized into size classes based on their body width and body length to increase the speed of measurement. The largest dimension of Gastropoda, Unioniidae bivalves (including shell) and Hydracarina was measured. Where a large number (>25) of one species or taxa were present, all individuals were identified, but a random subsample was measured: where animals were identified to a higher resolution (species/genus) a 25 animal subsample was taken; when animals were identified to a lower resolution (family/class/order) a subsample of 50 animals was taken, owing to a potential for higher variability in animal sizes present where several species may be pooled. The subsample was taken randomly by scattering the total number of animals on a wide gridded Petri dish after mixing the sample well within the Petri dish, randomly selecting a grid square and measuring all individuals within that square. This procedure was repeated until a sufficient number of animals (25 or 50) had been measured. Where animals had been partially destroyed and body length measurements were not possible to take, head width measurements were made. Random samples were identified by an independent expert individual to ensure quality assurance of identification. Where samples were split, the proportion identified and measured was extrapolated to give an estimate of the total abundance and biomass in the sample.

3.2.3.4 Body length-mass regressions

For procedures followed for identifying, measuring, drying and weighing taxa for the development of length mass regressions see Section 2.2.3.2 of Chapter 2. The following additional taxa were identified, measured and dried to produce length-mass regressions: Coleoptera (larvae and adults), *Bithynia tentaculata*, Corixidae (larvae and adults), Heptageniidae, Hirudinea, Hydracarina, *Leptophlebia* sp., *Planorbis albus*, *P. contortus, P. carinatus,* and Trichoptera (cased and uncased). *Bithynia tentaculata,* Coleoptera (adults only), Corixidae (larvae and adults), Heptageniidae, Hirudinea, *Planorbis albus*, *P. contortus, P. carinatus* and all Trichoptera were identified to species level, *Leptophlebia* sp. to genus level, coleopteran larvae to family level, and Hydracarina to order level, and all were measured, dried and weighed. Some of these taxa were pooled into broader taxonomic groups to estimate a more generally applicable regression.

Oligochaetes disintegrated very rapidly in frozen samples and could not be used to create length mass regressions. Thus, previously collected preserved specimens from Lough Creeve, Lough White and Lough Doo were used to estimate the regressions. Preserved oligochaete specimens from lakes Aillbrack, Creeve, Vearty, White and Doo, were also used to establish the mean mass of size classes, detailed in section 3.2.4.1.

3.2.4 Statistical analysis

3.2.4.1 Body length-mass regressions

Linear regressions of the relationship between the natural log of the dependent variable, dry mass, and the natural log of the independent variable, body length (or other linear body dimensions) were modelled for twenty two taxa. As discussed in Section 2.1 of Chapter 2, the power function normally provides a better fit to the allometric relationship between mass and length, which becomes linear after log transformation (Mason, 1977; Smock, 1980; Meyer, 1989; Burgherr and Meyer, 1997; Benke *et al.*, 1999; Johnston and Cunjak, 1999). Summary statistics of these relationships are shown in Table 3.3.

The regressions were then applied to all measured individuals of these taxa. Two regression equations were developed for oligochaetes (Table 3.4). The first was to describe the relationship between oligochaete body length and dry mass. This was only

applied to a small number of samples, where only the body length of individuals was recorded, at the very beginning of sample processing. The second regression describes the relationship between oligochaete body length and body width with dry mass of individuals. For taxa where it was not possible to develop regressions in the study (owing to insufficient numbers of animals), published regressions were applied.

Table 3.3: Summary statistics of the length-mass regression for each of twenty two taxa. The regression equation was expressed as Ln DM = Ln a + b*Ln BL; where DM = dry mass, Ln a = intercept; b = slope; BL = body length/measured body dimension.

Taxon	Ln a	±SE	b	±SE	R^2	n	Size Range (mm)
Asellus aquaticus	-5.71	0.11	3.06	0.06	0.93	189	2.3 - 12.6
Bithynia tentaculata	-2.33	0.20	2.69	0.09	0.96	34	4.0 - 10.9
Caenis sp.	-6.18	0.15	2.97	0.10	0.85	146	2.0 - 7.3
Chironomidae	-7.72	0.12	3.03	0.06	0.86	449	2.3 - 15.5
Coleoptera (A)	-4.31	0.18	3.30	0.14	0.91	56	2.3 - 5.7
Coleoptera (L)	-6.42	0.55	2.99	0.36	0.69	34	2.9 - 11.5
Corixidae (A)	-5.40	0.36	3.23	0.17	0.91	35	5.7 - 14.1
Corixidae (L)	-4.30	0.25	2.57	0.16	0.90	30	3.4 - 6.2
Dipteran pupae	-6.54	0.26	2.94	0.15	0.87	59	2.4 - 8.4
Gammarus sp.	-6.69	0.16	3.23	0.08	0.91	173	3.3 - 19.2
Heptageniidae	-6.55	0.91	3.04	0.41	0.87	10	4.5 - 11.3
Hirudinea	-8.14	2.14	3.67	1.04	0.67	8	6.6 - 11.6
Hydracarina	-2.54	0.07	2.44	0.11	0.76	145	1.1 - 3.2
Leptophlebia sp.	-6.68	0.70	2.85	0.35	0.85	14	5.1 - 10.7
Planorbiidae	-1.80	0.06	2.07	0.06	0.94	99	1.4 - 8.7
Planorbis albus	-1.79	0.07	1.96	0.06	0.96	38	1.7 - 4.5
Planorbis carinatus	-2.56	0.17	2.55	0.11	0.98	11	2.4 - 8.7
Planorbis contortus	-1.72	0.08	2.09	0.08	0.93	50	1.4 - 4.7
Potamopyrgus jenkinsi	-2.33	0.10	2.36	0.08	0.85	168	1.6 - 5.1
Sphaeriidae	-2.33	0.05	2.68	0.04	0.96	148	1.4 - 8.8
Trichoptera (C)[1]	-6.48	0.36	2.73	0.15	0.81	84	5.4 - 25.6
Trichoptera (U)[2]	-3.63	0.69	1.51	0.27	0.41	47	9.1 - 19.4

[1] (C) refers to cased caddis, and [2] (U) refers to uncased caddis.

Published regressions used for taxa not measured directly were chosen based on several criteria, including latitude and geography of water body, size range, slope and comparability with those developed for similarly shaped animals for the study. Regressions applied to taxa from the orders Turbellaria, Gastropoda (*Ancylus* sp. and *Acroloxus* sp.) and Plecoptera, and for the Ceratapogoniidae family of Diptera were published by Meyer (1989). Regressions applied to the orders Odonata, Diptera (excluding Chironomidae and Ceratopogoniidae), Ephemeroptera (*Ephemera danica*

only), Gastropoda (*Anadonta* sp. only), Sialiidae and Pyraliidae were published by Benke *et al.* (1999). Regressions applied to the several Gastropoda species (*Lymnaea* sp., *Myxas glutinosa*, *Succinea* sp. and *Physa* sp.) were published by Baumgartner and Rothhaupt (2003). A regression published by Smock (1980) was applied to the hemipteran *Velia* sp.. An appropriate aquatic regression was not found for *Agryoneta aquaticus*, and therefore, a terrestrial Arachnida regression was applied (Sabo, 2002). Where head widths were measured instead of body length, the regressions of Meyer (1989) and Benke *et al.* (1999) were applied.

Table 3.4: Summary statistics of the length-mass regression for oligochaetes. The multiple linear regression equation was expressed as Ln DM = Ln a + b_1*Ln BL+ b_2*Ln BW; The bivariate linear regression was expressed as Ln DM = Ln a + b*Ln BL; where DM = dry mass, Ln a = intercept; b = slope; BL = body length; BW = body width.

Regression Type	Ln a	±SE	b_1 (BL)	±SE	b_2 (BW)	±SE	R^2	N	Size Range BL (mm)	Size Range BW (mm)
Bivariate Linear Regression (DM~BL)	-4.41	0.18	1.16	0.09	n.a.	n.a.	0.52	154	2.5-18.1	n.a.
Multiple Linear Regression (DM~BL+BW)	-3.53	0.28	1.10	0.09	0.94	0.24	0.57	154	2.5-18.1	0.3-0.6

n.a. - not applicable

3.2.4.2 Oligochaete size classes

Oligochaetes were categorised into size classes for many samples to allow faster sample processing. The size categories were developed based on the frequencies of lengths of body length and body widths of animals found in lakes Glencreawan, Nadarra, Meenameen, Melvin, Bunerky, Akibbon, Lattone and Kiltooris. These lakes incorporated a wide range of alkalinity (6.6 to 59.7 mg $CaCO_3$ l^{-1}) to allow for taxonomic differences that may have occurred owing to differences in alkalinity among lakes. This was achieved by ordering all animals regardless of origin according to body length and body width. Size categories were then created based on similar numbers of animals in each of four categories increasing in size by 3.1 mm among categories (Small, Medium, Long, Very Long) and of body widths based on three categories, increasing in size by 0.2 mm among categories (Thin, Medium and Fat; Table 3.5). The observed mean dry mass of animals was calculated for each category from the preserved animals measured (by category) dried and weighed (Table 3.6). The mean mass was then applied to all animals that were categorized into size classes. Therefore, when the data preparation was complete, all measurements of identified animals were converted

from body length/body dimension/size class to body mass on the basis of the aforementioned regressions (both published and developed for the study), or on the basis of the mean mass of the corresponding size category.

Table 3.5: Size categories developed for ease of measurement of oligochaetes, detailing category name, size range within the category, and number of animals documented in each category, developed from pooled data collected at lakes Glencreawan, Nadarra, Meenameen, Melvin, Bunerky, Akibbon, Lattone and Kiltooris.

BL Category	BL (mms)	n	BW Category	BW (mms)	n
Small (S)	<3.1	106	Thin (T)	<0.4	148
Medium (M)	3.1-6.2	154	Medium (M)	0.4-0.6	157
Long (L)	6.2-9.4	84	Fat (F)	>0.6	135
Very Long (V)	>9.4	96			

Table 3.6: Mean dry mass of each size category of oligochaete, number of animals (n) collected to produce the mean, standard error of the mean (SE), and the range of sizes of body length (BL) and body width (BW) of animals in each category, produced from animals collected at lakes Aillbrack, Creeve, Vearty, White and Doo.

Category (BL-BW)	DM Mean	n	±SE	BL (mm)	BW (mm)
S-T	0.03	18	0.00	<3.1	<0.4
S-M	0.04	20	0.00	<3.1	0.4-0.6
S-F	0.03	1		<3.1	>0.6
M-T	0.05	26	0.01	3.1-6.2	<0.4
M-M	0.09	139	0.01	3.1-6.2	>0.6
M-F	0.13	27	0.02	3.1-6.2	0.4-0.6
L-T	0.12	31	0.01	6.2-9.4	<0.4
L-M	0.15	78	0.01	6.2-9.4	0.4-0.6
L-F	0.44	32	0.05	6.2-9.4	>0.6
V-T	0.16	16	0.02	>9.4	<0.4
V-M	0.28	49	0.02	>9.4	0.4-0.6
V-F	1.01	53	0.12	>9.4	>0.6

3.2.4.3 Fish taxa abundance and biomass

Additional data on fish taxa abundance (catch per unit effort [cpue]) and biomass (biomass per unit effort [bpue]) was provided by the Central Fisheries Board of Ireland (CFB) for fifteen of the lakes examined in this study (Table 3.7). Sampling was conducted using a combination of gill nets (benthic and floating) and fyke nets on dates between July and October 2005 and June and October 2006. All fifteen lakes were, therefore, sampled within several weeks of macroinvertebrate summer sampling.

Table 3.7: Lakes sampled for fish by the CFB for the NS-Share project including lake name, sampling dates and fish cpue and bpue.

Lake	Date Sampled	Total cpue	Total bpue
Auva	10/08/2005	0.2	7.4
Bawn	27/09/2005	3.0	131.9
Corglass	04/07/2005	1.1	106.9
Deralk	04/10/2005	2.5	122.6
Fern	19/09/2005	0.5	71.9
Gartan	05/09/2005	0.6	33.8
Kiltyfannad	09/08/2005	0.6	38.4
Madral	24/08/2005	0.2	19.3
Salt	20/09/2005	0.4	17.8
Shivnagh	25/08/2005	0.3	26.6
Town	26/09/2005	1.4	78.1
Veagh	15/08/2005	0.3	35.5
Vearty	01/09/2005	0.3	41.7
Corry	16/10/2006	0.2	65.8
Nadarra	20/06/2006	0.1	17.1

3.2.4.4 Biomass spectra

Biomass spectra were constructed by ordering each individual's biomass (dry mass) within a sample into 22 logarithmically increasing size classes (log_2 based; Table 3.8). From this, the following calculations were made for individual samples: (i) the sum of biomass within each size class; (ii) the abundance of animals within each size class; (iii) the log (log_2 based) of the abundance within each size class; (iv) the proportion (%) of total biomass within each size class; (v) the proportion (%) of total abundance within each size class; and (vi) the normalised biomass [log_2 (total biomass in each size class)/(weight range in size class or "size class width"[1])] within each size class. Normalised size spectra were determined for each lake by regressing log_2 [(total biomass in each size class)/(weight range in size class or "size class width")] against the midpoint of each size class. The range of size classes used was based on the range of observed biomass (between 0.001 mg and 789.52 mg dry mass; Table 3.8). Where measurements of specific species had been subsampled, the biomass of each of the individuals from the proportion subsampled (e.g. 25 or 50 animals out of total of n_{sp} animals) was extrapolated up to an estimate E_{sb} to represent the unmeasured individuals within each size class. E_{sb} is calculated by multiplying the biomass or abundance of each individual measured in the subsample, I_i, by the proportion of subsample that it

[1]For example, the size class width of size class midpoint -3 would be 0.09 mg (0.18 mg - 0.09 mg).

represented P_{sb}. P_{sb} is calculated by dividing the total number of animals in the sample, n_{sp}, by the number of animals measured in the subsample M_{sb} (e.g. 25 or 50).

So, where $(n_{sb} / M_{sb}) = P_{sb}$ [5]

Then $I_i \times P_{sb} = E_{sb}$ [6]

When the total abundance or total biomass within each size class was calculated the value E_{sb} was incorporated into the size class biomass rather than the value I_i.

Where samples were split, the biomass or abundance of each individual I_j within the sample was extrapolated up to an estimate E_t by dividing I_j by the proportion of sample used Pr_s; thus the total biomass and abundance in each size class is extrapolated to represent a full sample. Therefore where total biomass or total abundance within each size class was calculated the value E_t was incorporated rather than the value I_j.

Thus, $[I_j / Pr_s = E_t]$ [7]

Where subsamples were found within split samples the extrapolated estimate E_x is derived by dividing the subsample estimate E_{sb} by the proportion of sample used Pr_s.

$$E_x = E_{sb} / Pr_s \quad [8]$$

Table 3.8: Midpoint of each size class on the Log_2 scale and its equivalent range in milligrams.

Size Class Midpoint (Log_2 Scale)	Biomass Range (mg)		
-11	0.0003	-	0.0007
-10	0.0007	-	0.0014
-9	0.0014	-	0.003
-8	0.003	-	0.006
-7	0.006	-	0.01
-6	0.01	-	0.02
-5	0.02	-	0.04
-4	0.04	-	0.09
-3	0.09	-	0.18
-2	0.18	-	0.35
-1	0.35	-	0.71
0	0.71	-	1.4
1	1.4	-	2.8
2	2.8	-	5.7
3	5.7	-	11
4	11	-	23
5	23	-	45
6	45	-	91
7	91	-	181
8	181	-	362
9	362	-	724
10	724	-	1448

3.2.4.5 Normalised size spectra

For the purposes of comparison with other studies, a normalised size spectra was constructed. Normalised biomass (*nb*) is derived following Platt and Denman (1975):

$$nb = \text{Log}_2 \text{ (total biomass in each size class / weight range in size class)} \quad [9]$$

However, there was a possible loss of some small animals through the net mesh, particularly the more elongated cylindrical taxa, owing to the net mesh size (1 mm^2). Morin *et al.* (2004) reported a comprehensive study on the retention of organisms within sieves based on mesh size of the sieve. They found that >90% of benthic invertebrate biomass was retained by a 1 mm^2 mesh sieve but that only ~33% of invertebrate density was retained compared with that retained by a 0.063 mm^2 mesh sieve. They documented that sieves retained all organisms with body lengths ten times larger than the mesh opening. However, they also found that the probability of more cylindrical animals (e.g. oligochaetes) being retained by larger meshes was lower than that of animals of the same body length but with more appendages (e.g. Ephemeroptera) or with a more spherical shape (e.g. Gastropoda; Fig. 3.3).

The sieving procedure carried out in this study was not performed according to Morin *et al.* (2004); animals were collected using a 1 mm^2 mesh net and sieved through a 0.5 mm^2 mesh, rather than passed through a geometric series of sieves. My study was, thus, more likely to retain more small animals in the 1 mm^2 mesh, owing to (i) the single event of kick sample with this net mesh, and thus subsequent clogging, and (ii) more conservative sieving with 0.5 mm^2 mesh thereafter. Nevertheless, to be conservative, a parallel analysis was performed, where the size distributions produced were treated as equivalent to that of the 1 mm^2 mesh produced by Morin *et al.* (2004). Accordingly, two parallel linear mixed effects model analyses were carried out. One accounted for possible loss of animals through the mesh by incorporating a biomass cut-off point (henceforth called the truncated dataset) and one incorporating all the data regardless of size (henceforth called the quadratic dataset).

The biomass cut-off point for the truncated dataset was based on results of Morin *et al.* (2004). Examining the retention probability functions for taxa of different body shapes in a 1 mm^2 mesh sieve (Fig. 3.3), it was considered likely that spherical animals of

length 2.5 mm (Araneae, Hydrachnidia, adult Coleoptera, and Gastropoda), oval animals of length 5 mm (Amphipoda, Anisoptera [Odonata], Isopoda, Hirudinea, Turbellaria and Corixidae), cylindrical animals with long appendages of length 7.5 mm (Ephemeroptera, Zygoptera [Odonata], Megaloptera, larval Coleoptera and Plecoptera) and long cylindrical animals of length 10 mm (Chironomidae, Ceratopogonidae, Trichoptera, and Dipteran pupae) would all be retained. When length-mass regressions were applied to these organisms at these lengths, the dry mass of all animals was <1.25 mg, corresponding to animals occupying all size classes less than or equal to size class 0 (size class midpoint at Log_2) which had a maximum biomass (dry mass) of 1.4 mg. Animals in size classes smaller than size class 1 were, therefore, excluded from the truncated dataset.

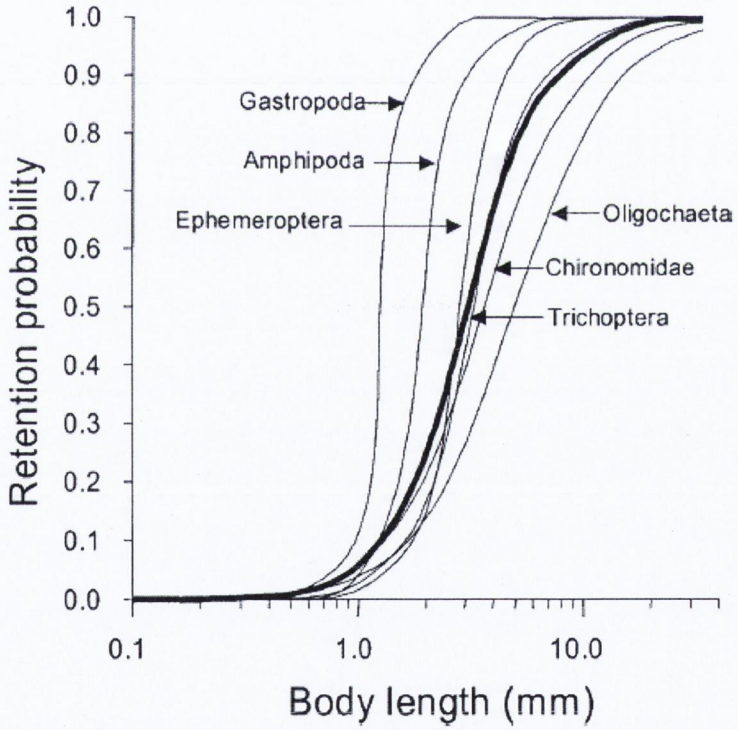


Figure 3.3: Fitted retention probability functions of the numerically dominant taxa retained by a 1 mm sieve. The thick line represents the retention probability for all taxa, including rare ones (reproduced from Morin *et al.* [2004]).

Some samples had no animals in many of the larger size classes, and as each sample had only one data point (of normalised biomass) per occupied size class, it was necessary to also exclude samples that had fewer than four occupied size classes $\leq$ size class 1, to allow for a meaningful comparison among samples. This eliminated 77 samples,

leaving 98 samples remaining (Table 3.9). However, the remaining samples had a sufficiently large TP gradient within each alkalinity range to test the effects of nutrient enrichment on the normalised size spectrum.

Table 3.9: Details of samples included and excluded from the truncated dataset. Those included had animals in at least 4 size classes ≤ size class 1.

Typology/ Substrate	No. samples excluded	No. samples included	TP range (µg L^{-1}) (samples included)	Total no. samples
Hard	50	45	4 - 184	95
Soft	27	53	2 - 184	80
TOTAL	**77**	**98**		**175**

3.2.4.6 Size diversity

The measure of size diversity was calculated for each of the samples following Quintana *et al.* (2008). Size diversity is estimated from a Shannon size diversity expression based on the Shannon-Wiener index, which is adapted for continuous rather than discrete variables. It is computed by means of non-parametric kernel estimation, after data standardization (by division of sample geometric mean). It takes the form of an index $\mu_2(X)$ corresponding to the probability density function $p_x(x)$ of the size of the individuals, and takes the following integral form:

$$\mu_2(X) = -\int_0^{+\infty} p_X(x) \log_2 p_X(x)\, dx \qquad [10]$$

Size diversity values were then included as response variables in LME models, to establish the effect of change in water chemical variables on the size diversity index (detailed in Section 3.2.4.3).

3.2.4.7 Glossary of abbreviated statistical terms

A number of statistical terms are abbreviated in the text:

AIC: Akaike's information criterion, is a parameter used in model selection based on information theory which aims at finding an approximate rather than a true model (Akaike, 1973).

AIC_C : Akaike's information criterion corrected for small sample size bias (Burnham and Anderson, 2004).

LME: Linear mixed effects model, incorporating random and mixed effects.

ML: Maximum likelihood, a statistical framework that finds the parameters of a model that maximizes the probability of the observed data (the likelihood; Bolker *et al.*, 2008).

REML: Restricted maximum likelihood, an alternative to ML that estimates the random-effect parameters (i.e. standard deviations) averaged over the values of the fixed-effect parameters (Bolker *et al.*, 2008).

VIF: Variance inflation factor, is an index used to assess collinearity among explanatory variables, measuring the change in variance of each coefficient with collinearity among explanatory variables (Montgomery and Peck, 1992).

3.2.5 Data exploration

Both correlation matrices (Pearson product-moment) and variance inflation factors (VIF) were used to assess collinearity among explanatory variables to be included in mixed effects models (Table 3.10; Table 3.11). As correlation coefficients are based on pairwise comparisons, VIF were also used to aid in the assessment of collinearity among explanatory variables and removal of some variables before analysis, following Zuur *et al.* (2009). The VIF is an index that measures how much the variance of a coefficient increases through collinearity among explanatory variables. One of the explanatory variables is set as the response variable with others remaining as explanatory variables within a linear regression (Montgomery and Peck, 1992). The VIF for the variable selected as the response variable (B_1) is then given by:

$$\text{VIF}\ (B_1) = 1 / (1 - R^2) \qquad [11]$$

where R^2 is the coefficient of determination of the regression equation outlined above. A different explanatory variable is set as response variable in a series of regressions (if there are k explanatory variables, then this process is carried out k times (Zuur *et al.*, 2007). The underlying rationale is that if, for example, three explanatory variables, X_1, X_2 and X_3 are highly correlated, and one of them is set as the response variable, then the R^2 of the linear regression model will be high and the VIF value from the model will be large (Zuur *et al.*, 2007). A VIF value >5 indicates a high level of collinearity, as it means that the variation in the response variable is explained well by the other variables (Montgomery and Peck, 1992). Zuur *et al.* (2009) used a more conservative VIF cut-off

value of 3 to define unacceptable levels of collinearity among variables, which was adopted as the cut-off value in this study. This process is automated in the statistical programme R by using the "corvif" function, which provides a summary table of the VIF value of each explanatory variable included together (R development core team, 2009; Zuur *et al.*, 2009). However, the biological importance of a variable was also considered before elimination on statistical grounds. Based on all the aforementioned factors, one variable was removed at a time, and the VIF values were recalculated until the most parsimonius and logical set of explanatory variables remained (Table 3.10).

There were high levels of correlation among conductivity, alkalinity and pH (Table 3.11). Conductivity also had the highest VIF value among explanatory variables and was excluded from further analysis (Table 3.10). Colour was the next variable to be removed owing to its high correlation with alkalinity, pH and DOC (Table 3.11) and high VIF value (Table 3.10), and eliminated from further analysis to simplify model interpretation.

Table 3.10: Table displaying the change in VIF values among explanatory variables with the inclusion and exclusion of correlated variables. '-' appears where a variable was excluded.

	VIF	VIF	VIF	VIF	VIF	VIF	VIF
Area (ha)	1.05	1.04	1.04	1.03	1.02	1.02	1.02
pH	2.39	2.38	**2.37**	-	-	-	1.16
Alkalinity (mg $CaCO_3$ l^{-1})	9.96	3.04	2.50	1.23	1.18	1.14	-
Conductivity (µS cm^{-1})	**10.90**	-	-	-	-	-	-
TP (µg l^{-1})	2.69	2.59	2.59	2.46	1.46	-	-
Chlorophyll *a* (µg l^{-1})	1.98	1.97	1.94	1.86	-	1.19	-
TN (mg l^{-1})	2.89	2.14	2.13	2.12	-	-	1.20
DOC (mg l^{-1})	3.63	3.62	1.31	1.30	1.28	1.04	1.05
Colour (PtCo)	3.85	**3.76**	-	-	-	-	-

While the remaining variables now had VIF values <3, correlation among some variables was still very strong (pH and alkalinity; TP, TN, chlorophyll *a*; Table 3.10; Table 3.11). Although the VIF value of alkalinity was higher than that of pH, alkalinity is more biologically relevant for the analysis being conducted; the presence of calcium carbonate in waterbodies has been shown to be particularly important for chitinous invertebrates and molluscs and crustaceans. Macroinvertebrate community structure in acid, low alkalinity waterbodies (<20 mg $CaCO_3$ l^{-1}) differs from that in waterbodies

with higher alkalinity (Stoner *et al.*, 1984; Harriman and Morrison, 1982; Johnson *et al.*, 1993, Tierney *et al.*, 1998). Additionally, Irvine *et al.* (2002) identified that increases in pH among lakes were buffered at concentrations of alkalinity >10 mg $CaCO_3$ l^{-1}. Therefore alkalinity was retained for further analysis and pH was eliminated.

Table 3.11: Pearson product-moment correlation matrix displaying the relationships among explanatory variables.

	Area	pH	Alkalinity	Conductivity	TP	Chlorophyll *a*	TN	DOC	Colour
Area (ha)	1								
pH	0.031	1							
Alkalinity (mg $CaCO_3$ l^{-1})	-0.071	0.722	1						
Conductivity (µS cm^{-1})	-0.091	0.690	0.924	1					
TP (µg l^{-1})	-0.140	0.104	0.342	0.376	1				
Chlorophyll *a* (µg l^{-1})	-0.114	0.233	0.356	0.524	0.660	1			
TN (mg l^{-1})	-0.141	0.288	0.344	0.437	0.598	0.625	1		
DOC (mg l^{-1})	-0.078	-0.157	-0.022	-0.042	0.429	0.184	0.169	1	
Colour (PtCo)	0.024	-0.364	-0.396	-0.427	0.132	-0.082	-0.098	0.758	1

As the nutrients TP and TN are primary agents of nutrient enrichment, it is not surprising that there is a strong correlation among TN, TP and chlorophyll *a*. However, all three variables (henceforth referred to as "nutrient enrichment" terms) are strongly biologically relevant to the analysis, and therefore, were analysed in separate models. The remaining three models showed no sign of collinearity among variables according to their VIF values (Table 3.10). Each of the response and fixed variables were graphed, and many of the response variables when plotted against nutrient enrichment terms exhibited a curvilinear relationship. Consequently, a quadratic enrichment term was added into each maximal model as well as a linear term (e.g. TP and TP^2). An interaction term between substrate and the quadratic term was also included in each maximal model.

For the final LME analysis, which examined the effect of substrate, alkalinity, DOC, nutrient enrichment and the biomass per unit effort of several fish taxa on the total biomass of two size classes, TP and TN were analysed in separate models. A Pearson correlation matrix was constructed to examine correlations among all variables (Table 3.12). There was a high level of correlation among all fish taxa (Table 3.12). Pike bpue and cyprinid bpue were also both strongly correlated with alkalinity (Table 3.12). Upon examination of the VIF values, each fish taxon was examined in separate analyses and

alkalinity was excluded from analyses where cyprinid bpue and pike bpue were examined (Table 3.13). Thus eight final models were run for each size class, four including TP and four including TN.

Table 3.12: Pearson product-moment correlation matrix displaying the relationships among explanatory variables for the final analysis, which examined 15 lakes and included data on fish.

	Alkalinity	TP	TN	DOC	Perch bpue	Pike bpue	Cyprinid bpue	Salmonid bpue
Alkalinity (mg $CaCO_3$ l^{-1})	1							
TP (µg l^{-1})	0.515	1						
TN (mg l^{-1})	0.592	0.873	1					
DOC (mg l^{-1})	0.174	0.476	0.371	1				
Perch bpue	0.591	0.510	0.595	0.389	1			
Pike bpue	0.793	0.246	0.305	0.298	0.616	1		
Cyprinid bpue	0.900	0.536	0.594	0.271	0.586	0.776	1	
Salmonid bpue	-0.376	-0.126	-0.250	-0.119	-0.697	-0.482	-0.487	1

Table 3.13: Table displaying the change in VIF values among explanatory variables for the final analysis. '-' appears where a variable was excluded.

	VIF	VIF	VIF	VIF	VIF	VIF	VIF
Alkalinity (mg $CaCO_3$ l^{-1})	8.62	4.74	1.74	1.73	1.60	-	-
TP (µg l^{-1})	2.97	2.61	1.88	1.67	1.64	1.23	1.58
DOC (mg l^{-1})	1.78	1.77	1.53	1.42	1.39	1.40	1.37
Perch bpue	4.31	3.67	3.49	1.92	-	-	-
Pike bpue	4.68	**4.40**	-	-	-	1.14	-
Cyprinid bpue	**8.17**	-	-	-	-	-	1.43
Salmonid bpue	4.29	3.34	**3.33**	-	1.83	-	-

3.2.6 Data analysis

In the first set of analyses, the effects of substrate, lake area, alkalinity, DOC and the nutrient enrichment terms on a series of response variables (size diversity per sample [p.s.], mean individual biomass p.s., total biomass p.s. and total abundance p.s.) were analysed in linear mixed effects models (LME; R development core team, 2009). Owing to strong correlations, nutrient enrichment terms (TP, TN and chlorophyll *a*) were analysed separately in three different models for each of the response variables. An additional quadratic enrichment term was also added to examine if a curvilinear

relationship described the relationship between enrichment terms and the response variable. Individual interactions between substrate and all other variables were included in the maximal model of each of the analyses. These interaction terms allowed changes in the dynamics of the relationship between substrate and the response variable, in the presence of another variable, to be analysed. The homogeneity of variance within the residuals of the maximal model for each of the response variables was analysed, and mean biomass per individual, total biomass and total abundance were log transformed ($\log_{10} Y$) to achieve a normal distribution of these response variables.

The random structure of each maximal model was selected by comparison of the AIC_C values (explained in Section 2.2.4 of Chapter 2) among maximal models with different random structures, based on restricted maximum likelihood (REML) following Crawley (2007) and Zuur *et al.* (2009). Maximum likelihood (ML) is a statistical framework that estimates the standard deviations of the random effects and maximises the probability of the observed data (Bolker *et al.*, 2009). REML is an alternative to ML that estimates the random-effect standard deviations averaged over the values of the fixed-effects parameters, and is thus less biased than the corresponding ML estimates as the variance components are estimated without being affected by the fixed effects (they are invariant to the values of the fixed effects [Crawley, 2007; Bolker *et al.*, 2009]). The likelihood ratio (LR) test determines the contribution of a single (random or fixed) variable by comparing the fit for nested models (models with and without the factor) and thus can aid in assessing the significance of particular factors, and choosing the better of a pair of nested models (Bolker *et al.*, 2009).

A maximal model with lake identity as the only random effect was compared with that of a maximal model with season (spring and summer) nested within lake identity (based on REML estimations). The model with lake identity as the only random effect had a lower AIC_C in each instance, and was thus adopted as the random structure for each of the models. Backward selection, based on maximum likelihood (ML) was used to establish optimal fixed effects and identify the minimal adequate model which was the most parsimonius model at the lowest value of AIC, following Crawley (2007) and Zuur *et al.* (2009). The ML function, in contrast with the REML function, does not change with re-parameterization of the fixed effects (Pinheiro and Bates, 2000). Changing the matrices of fixed effects in a REML based models results in a

corresponding change in the restricted likelihoods of the fixed effects; LME models fit using REML, with different fixed effects structures, cannot be compared on the basis of their restricted likelihoods (Pinheiro and Bates, 2000). In particular, LR tests are not valid under these circumstances (Pinheiro and Bates, 2000) and, therefore, ML must be used when comparing fixed effects among models using LR tests (Pinheiro and Bates, 2000; Bolker *et al.*, 2009).

The model simplification process of elimination of fixed terms, was based initially on *p*-values produced from the t-statistic; those with the highest non-significant *p*-values were eliminated from the succeeding model. The original and succeeding models were then compared based on an LR test and a comparison with AIC values, to justify the elimination of the non-significant term, following Crawley (2007) and Zuur *et al.* (2009). Where two minimal adequate models are compared and found to have a difference in AIC values <2, then both are considered to have substantial evidence to support them (Burnham and Anderson, 2004). The residuals of all models were checked for homogeneity of variance and normality.

In the second set of analyses, the effects of substrate, alkalinity, DOC and nutrient enrichment (with separate analyses for TP, TN and chlorophyll *a* in turn) on the normalised biomass among size classes, were analysed in LME models. This was done in two separate analyses. One, the quadratic dataset, included all data collected, and the other (truncated dataset) accounted for possible loss of animals through the mesh (see Section 3.2.4.5). The distribution of normalised biomass among size classes was truncated, so that only the range of size classes containing biomass was included for each of the samples. This was done to avoid multiple zero values skewing results. However, where zero values occurred within a distribution of normalised biomass among size classes, this value was included, as this shows that animals within some size classes were rare, and is part of the pattern of the size distribution. There was a large difference in the shape of the distribution of normalised biomass among size among the quadratic and truncated analyses (Figs. 3.4 and 3.6). The distributions of the quadratic analyses were dome shaped – and thus a quadratic size class term was incorporated into the analysis, which was effectively an interaction term of size class with itself (Quinn and Keough, 2002). Owing to the addition of this term (and its subsequent interaction

terms), and in order to reduce the size of the maximal model, soft and hard substrates were treated in separate models.

As in the previous analyses, a maximal model with lake identity as the only random effect was compared with that of a maximal model with season (spring and summer) nested within lake identity (based on REML estimations). The model with season nested within lake identity had a lower AIC for both the quadratic and truncated analyses, and was thus adopted as the random structure for each of the models. The effect of substrate, lake area, alkalinity, DOC and nutrient enrichment terms, as well as an additional quadratic enrichment term (to see if a curvilinear relationship described the relationship between the enrichment term and the response variable) on the relationship of normalised biomass and size class (or the distribution of normalised biomass among size classes) was modelled by incorporating each of the aforementioned explanatory variables (fixed effects) both as main effects and as interaction terms with the size class term. In the analyses of the quadratic dataset, an additional interaction term was included, between the explanatory variables and the quadratic size class term. Backward selection, based on ML estimation, was used to establish the fixed effects structure and identify the minimal adequate model.

The effect of fish on invertebrate biomass was included in a final model, using data from the 15 lakes where fish data was available. The size spectrum was divided into two size classes, representing the total biomass of 'small' and 'large' animals, and treated as separate response variables. The effects of substrate, alkalinity, DOC, nutrient enrichment and the biomass per unit effort of several fish taxa on the total biomass of small and large animals was analysed in LME models (Table 3.7). This allowed examination of potential size selective predation by fish taxa. Lake area was eliminated from these analyses to reduce the number of variables in the maximal model, owing to its deletion from all previous models for the overall dataset. The total biomass of the smaller size class was calculated by adding the biomass of all individuals <1.41 mg (Table 3.14). Likewise, the total biomass of the larger size class was calculated by adding the biomass of all individuals ≥ 1.41 mg (Table 3.14). The total biomass of each size class was then treated as a response variable in separate models (eight models were created for each response variable; see Section 3.2.4.2). The random effects structure was established by comparing a maximal model with lake identity as the only random

effect with that of a maximal model with season (spring and summer) nested within lake identity, based on REML estimations. The model with season nested within lake identity as the random structure had a lower AIC_C and was adopted as the random structure for each of the models. The AIC_C value was used for these analyses rather than the AIC value (used for the previous analyses) in selecting both the random structure and the minimal adequate model, as the sample size was small compared with previous analyses with respect to the number of estimated parameters (Burnham and Anderson, 2002). Backward selection, based on ML estimation was used to establish the fixed effects structure and identify the minimal adequate model. The homogeneity of variance within the residuals of the maximal model for each of the response variables was analysed. The total biomass 'small' response variable was log transformed ($\log_{10}$ Y), and the total biomass 'large' was square root transformed to achieve a normal distribution with homogenous variance.

Table 3.14: Biomass and pooled size classes of two size classes for samples from the 15 lakes with fish data available.

Size class name	Size classes pooled:	Biomass (mg)
Total biomass "small"	Size Class -12 to 0	<1.41
Total biomass "large"	Size Class 1 to 10	≥1.41

3.3 Results

3.3.1 General patterns in the size spectrum

The normalised biomass among size classes produced from data pooled from all samples displayed a dome shape, suggesting a quadratic relationship between normalised biomass and size class midpoint (and thus termed 'the quadratic dataset'; Fig. 3.4). There was a peak in the normalised biomass of samples from hard substrates in size class -1, and in that of soft substrates in size class 1. Samples from soft substrates displayed higher normalised biomass than those from hard substrates in the larger size classes ≥-1. In the smaller size the distribution of normalised biomass appeared similar among substrates.

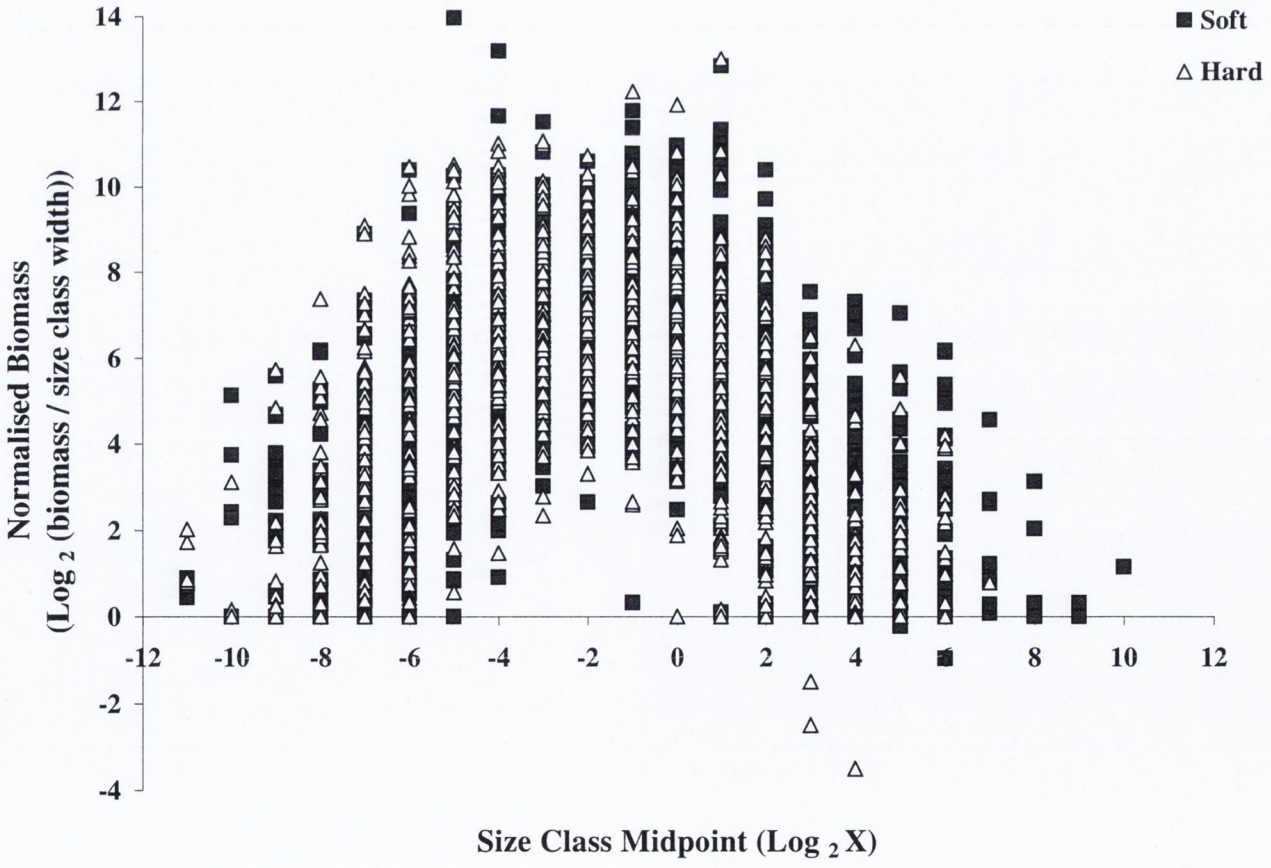
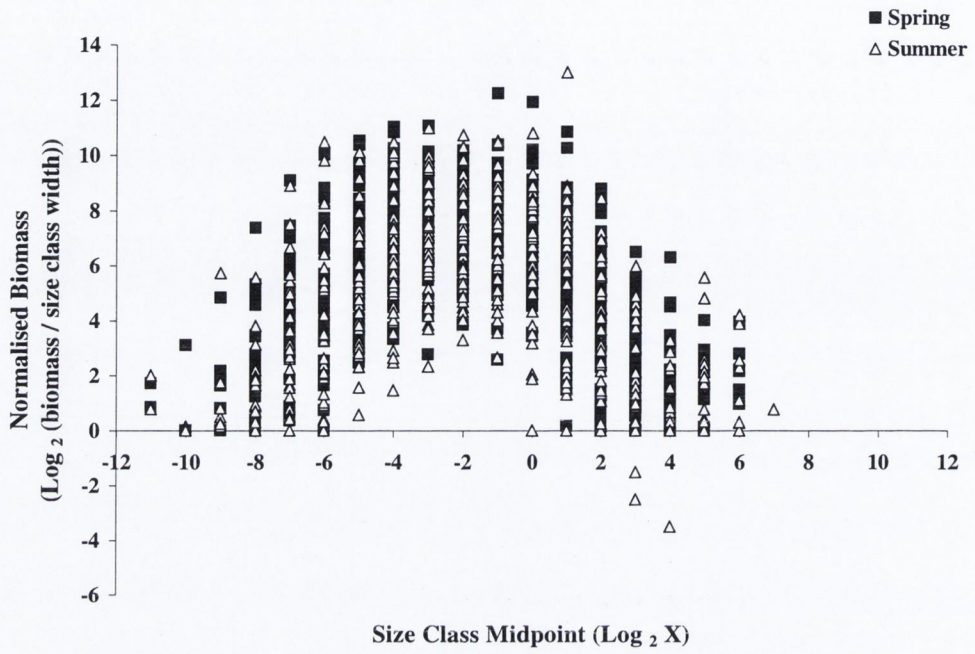


Figure 3.4: Distribution of normalised biomass among size classes for pooled data from all samples (from different seasons, n = 2, and both substrates, n = 2, for all lakes, n = 48) used in the analysis (n = 175) of the quadratic dataset.

(a)



(b)

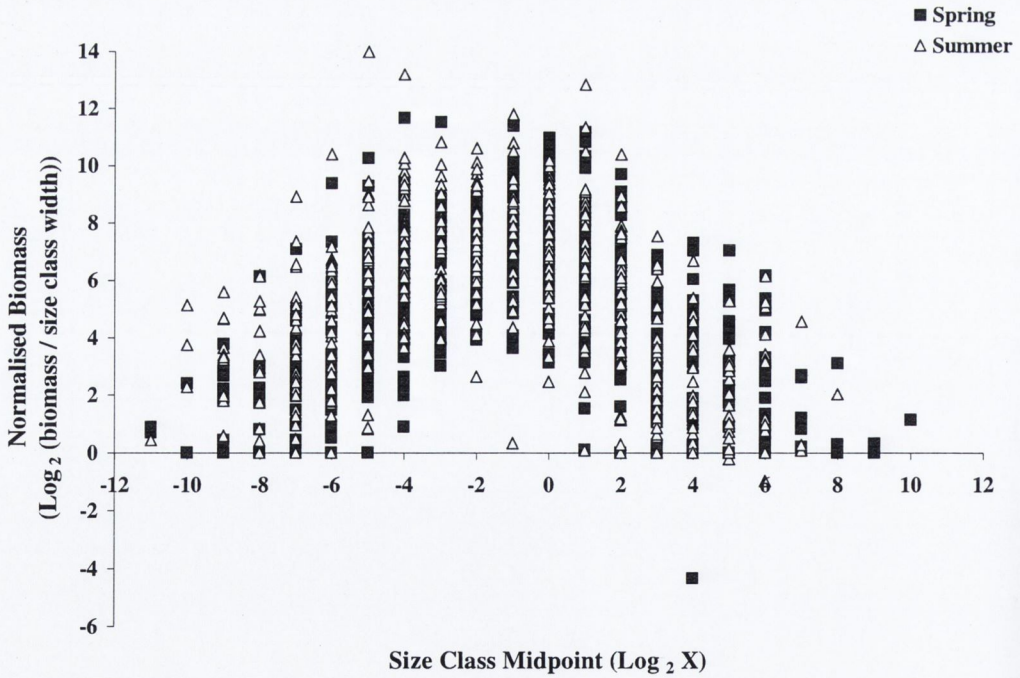


Figure 3.5: Distribution of normalised biomass among size classes of pooled data used in the analyses of the quadratic dataset for (a) hard and (b) soft substrates, in spring and summer.

The distribution of normalised biomass among size classes differed more among substrates than among seasons (Fig. 3.5). There appeared minimal seasonal change in normalised biomass among size classes in hard substrates (Fig. 3.5a), but greater biomass among the smaller size classes of the soft substrates in summer, and less in the larger size than in spring (Fig. 3.5b). The distribution of normalised biomass among size classes, from pooled data using the truncated dataset, depicted a strong linear relationship (Fig. 3.6), with narrow confidence intervals around the slope (Fig. 3.6). The negative slope is approximately -1, indicating equal biomass among size classes, supporting Sheldon's hypothesis (Sheldon, 1972). Examining the slopes of individual samples (from the analysis of truncated data), showed these were broadly consistent among samples, appearing similar to the pooled slope (Figs. 3.6 and 3.7). The intercepts, however, varied among samples, suggesting differences in the normalised biomass of all individuals in size class 0 (Fig. 3.7).

3.3.2 Models of single metrics

3.3.2.1 Size diversity

There were similarities among all models for the first set of minimal adequate LME models (where size diversity, $\log_{10}$ mean individual biomass per sample, $\log_{10}$ total biomass per sample and $\log_{10}$ total abundance per sample were the response variables) such as the elimination of lake area, DOC, and all substrate interaction terms from all minimal adequate models (Table 3.15). All three minimal adequate size diversity models were identical, owing to the elimination of the interaction terms and main effects of TP, TN and chlorophyll *a* (Table 3.15). It is noteworthy that all other explanatory variables (such as substrate, area, DOC and alkalinity) were also eliminated from the final minimal adequate model, leaving the model empty of fixed effects (Table 3.15). The variance components of the model reveal that lake identity as a random effect accounted for almost 20% of variance. However, owing to the elimination of all fixed effects terms, variance in size diversity among lakes could not be explained by any of the fixed effects examined, and the intercept corresponded to the mean value of size diversity, with a low standard error (Table 3.15).

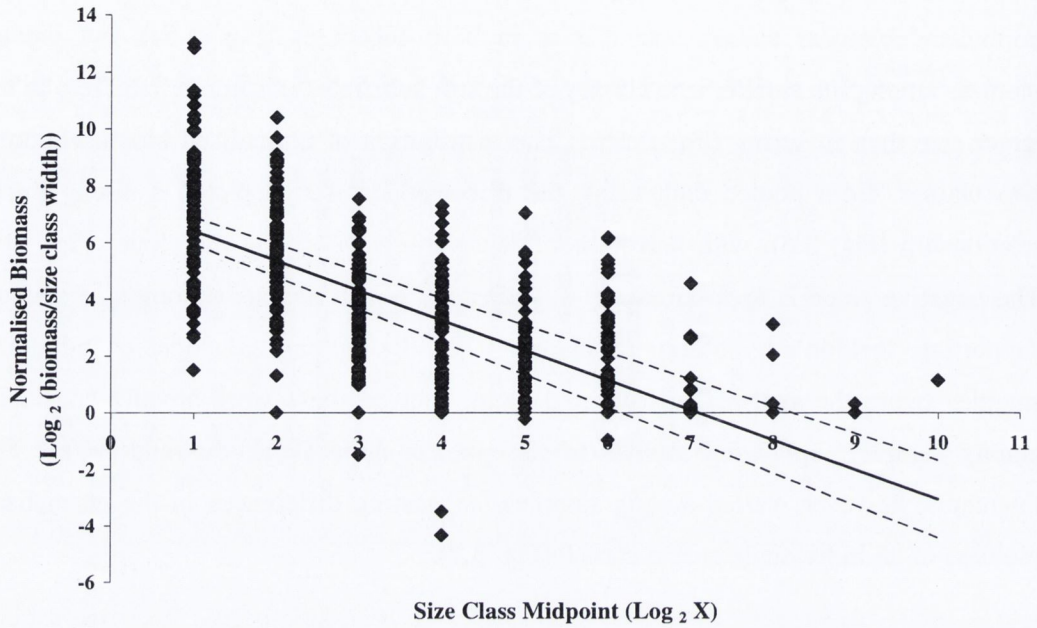


Figure 3.6: Change in normalised biomass among size classes for pooled data used in the analysis of the truncated dataset (normalised biomass from all size classes <1 and samples with fewer than four occupied size classes removed). Slope = -1.05 $\pm$ 0.05.

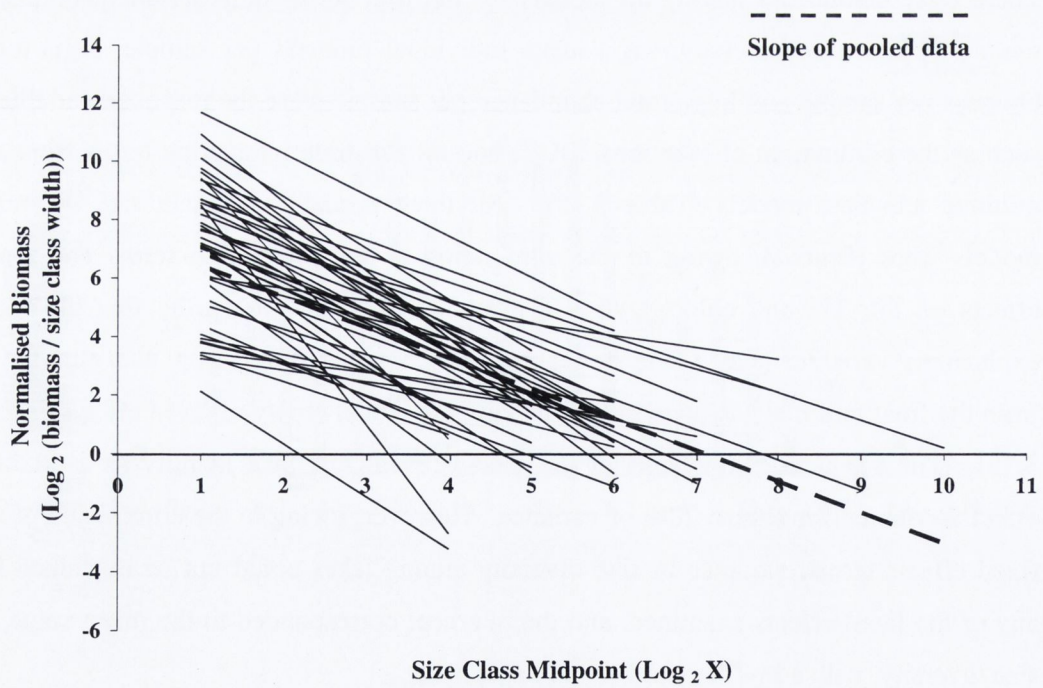


Figure 3.7: Slope trend-lines showing change in normalised biomass among size classes of each individual sample using the truncated dataset (where all size classes <1 were eliminated).

Table 3.15: Minimal adequate mixed effects models for the first set of LME models, for the response variables: size diversity, log_{10} mean individual size per sample (Log_{10} MIS), log_{10} total biomass per sample and log_{10} total abundance per sample. Enrichment term (E) refers to linear (E) and quadratic (E^2) terms describing effects of TP, TN and chlorophyll *a* (chl. *a*), analysed in separate models. All models had the same random structure (lake identity as random effect). Soft was the reference category for substrate. Interactions between substrate and each explanatory variable were included in maximal models and eliminated from minimal adequate models of each response variable.

			Fixed Effects							Variance components (Random Effects)				
Response Variable	**Enrichment Term (E)**	**Coeffs.[e]**	**Intercept**	**Substrate**	**Area**	**A[f]**	**DOC[g]**	**E**	**E^2**	**Intercept**	**Residual**	**n[h]**	**df[i]**	**AIC[j]**
Size Diversity	TP[b]- TN[c] - chl. *a*[d]	B	2.28	-	-	-	-	*	*					
	(µg l^{-1} / mg l^{-1}/µg l^{-1})	SE	0.03	-	-	-	-	*	*	19.40	80.60	175	127	96.5
Log_{10} MIS[a]	TP[b]	B	-0.32	0.26	-	0.00	-	-	-					
	(µg l^{-1})	SE	0.04	0.04	-	0.00	-	-	-	23.66	76.34	175	125	89.4
Log_{10} MIS[a]	TN[c]	B	-0.38	0.25	-	0.00	-	0.11	-					
	(mg l^{-1})	SE	0.06	0.04	-	0.00	-	0.05	-	25.46	74.54	175	124	91.4
Log_{10} MIS[a]	chl. *a*[d]	B	-0.26	0.25	-	0.00	-	0.01	-0.00					
	(µg l^{-1})	SE	0.05	0.04	-	0.00	-	0.00	0.00	24.39	75.61	175	123	115.4
Log_{10} Total Biomass	TP[b]	B	2.44	0.23	-	0.00	-	-	-					
	(µg l^{-1})	SE	0.09	0.06	-	0.00	-	-	-	38.80	61.20	175	125	271.7
Log_{10} Total Biomass	TN[c]	B	2.57	0.22	-	0.00	-	0.48	-0.30					
	(mg l^{-1})	SE	0.10	0.06	-	0.00	-	0.17	0.15	35.61	64.39	175	123	272.7
Log_{10} Total Biomass	chl. *a*[d]	B	2.57	0.22	-	0.00	-	0.01	-0.00					
	(µg l^{-1})	SE	0.09	0.06	-	0.00	-	0.00	0.00	33.71	66.29	175	123	292.5
Log_{10} Total Abundance	TP[b]	B	2.85	-	-	0.00	-	0.01	-0.00					
	(µg l^{-1})	SE	0.07	-	-	0.00	-	0.00	0.00	21.94	78.06	175	124	253.7
Log_{10} Total Abundance	TN[c]- chl. *a*[d]	B	2.74	-	-	0.00	-	**	**					
	(mg l^{-1})	SE	0.06	-	-	0.00	-	**	**	29.06	70.94	175	126	225.4

[a]Mean Individual Size; [b]Total Phosphorus; [c]Total Nitrogen; [d]Chlorophyll *a*; [e]Coefficients; [f]Alkalinity; [g]Dissolved Organic Carbon; [h]No. of samples; [i]Degrees of freedom; [j]Akaike's information criterion;'-' denotes where a variable was eliminated; '*' represents where minimal adequate models were identical for TP, TN and chlorophyll *a* models; '**' represents where minimal adequate models were identical for TN and chlorophyll *a* models.

3.3.2.2 Log mean individual size

Three minimal adequate models were produced for the response variable $\log_{10}$ mean individual size per sample (log MIS), one for each of the nutrient enrichment terms examined. Both TP linear and quadratic main effects were eliminated from the TP minimal adequate model, with substrate and alkalinity remaining as fixed effects in the final model. The slope of the relationship between substrate and log MIS was positive, with low SE, indicating an increase in the log MIS in soft substrates (Table 3.15). The slope of the alkalinity-log MIS relationship was positive but close to zero (Table 3.15). It was apparent that substrate had a greater influence on the log MIS than alkalinity. The variance components indicated that random variance among lakes accounted for approximately 24% of overall variance in log MIS.

Although similar to that of the TP model, the minimal adequate TN model of the log MIS response variable (keeping alkalinity and substrate terms in the model) differed with the retention of TN (Table 3.15). The coefficient of the substrate main effect indicated, as before, greater log individual size in soft substrates (Table 3.15). Similarly, the alkalinity main effect coefficient was comparable with that of the TP model, with a positive value close to zero, but the nutrient enrichment term, TN, was retained, indicating an increase in log MIS with increasing TN concentrations (Table 3.15). The variance components of both models were similar, with a slight increase in variance explained by lake identity in the TN model (Table 3.15). The chlorophyll *a* minimal adequate model of the log MIS response variable had similar values for the substrate and alkalinity terms as those of the previous two models, but retained both linear and quadratic enrichment terms, indicating a curvilinear change in the log MIS with chlorophyll *a*, although heteroscedasticity is pronounced (Fig. 3.8).

The AIC values were compared among models of log MIS, to establish which model fit the data best. As the difference between the AIC value of the chlorophyll *a* log MIS model and those of the TN and TP models was >>2, the chlorophyll *a* model was not considered the best fit to the data (Table 3.15). Likewise, the AIC value of the TN model was marginally higher (>2) than that of the TP model, indicating the TP model (which had originally included the enrichment term TP, and subsequently eliminated it), fitted the data best (Table 3.15). However, during the model selection process, the TN model was compared with the TP model using an ANOVA function based on ML, and

the inclusion of the TN term was found to significantly improve the maximum likelihood and fit of the model (P value <0.05).

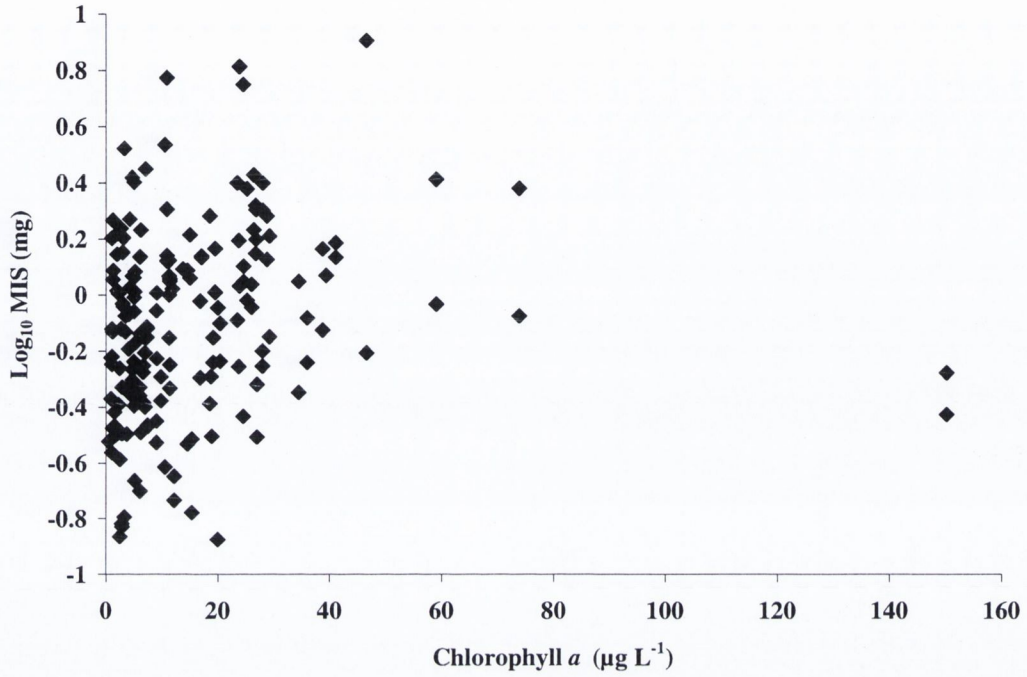


Figure 3.8: Relationship between $\log_{10}$ mean individual size (log MIS) and chlorophyll *a* ($\mu g\ l^{-1}$) among samples.

All the final presented models are REML derived, although the model selection is conducted using ML estimation (for further information on comparison among models see section 3.2.4.3). Based on REML estimations, the TP model provided a better fit to the data from the information given. However, based on ML estimation, the TN model provided a better fit to the data, and removal of the TN term significantly decreased the fit of the model. Accordingly, both models were considered to explain the data equally well.

3.3.2.3 Log total biomass

The minimal adequate models of the $\log_{10}$ total biomass per sample response variable (log total biomass) were similar to those of the log MIS models (Table 3.15). The TP log total biomass model retained only alkalinity and substrate, eliminating lake area, DOC and both linear and quadratic enrichment terms (TP and TP^2). Like the log MIS TP model, the slope of the substrate term was positive, indicating a greater increase in log total biomass in soft substrates. The slope of the alkalinity term was positive but

close to zero (Table 3.15). The TN log total biomass model had similar alkalinity and substrate coefficients. This model retained both linear and quadratic TN terms, indicating a curvilinear relationship between log total biomass and TN (Table 3.15). The positive coefficient of the linear TN term indicates that log total biomass increases with increasing TN, but the negative quadratic term indicates a downward force on this relationship (Table 3.15). The relationship between log total biomass and TN was convex, with log total biomass displaying an initial increase with TN, followed by a possible decrease at TN concentrations >1.4 mg l^{-1} (Fig. 3.9).

The chlorophyll *a* total biomass model retained substrate and alkalinity in the minimal adequate model (Table 3.15). The coefficients were very similar in value to those of the previous log total biomass models, where log total biomass displayed a small increase in value within soft substrates, and increased marginally where alkalinity increased (Table 3.15). Linear and quadratic enrichment terms were retained in the model, both with values close to zero, indicating a marginal curvilinear relationship between log total biomass and chlorophyll *a* (Fig. 3.10).

The coefficient of the linear chlorophyll *a* term was positive and close to zero, indicating a marginal increase of log total biomass with increasing chlorophyll *a* (Table 3.15). However, the quadratic term was negative, although close to zero, suggesting a very small downward force on the linear log total biomass-chlorophyll *a* relationship, apparent when examined graphically (Fig. 3.10). The variance of the random effects of all three models was similar, accounting for more than a third of the total variance in all of the models (Table 3.15). AIC values of the TP and TN models were similar (with a difference of less than 2) among minimal adequate log total biomass models, indicating that both models fitted the data equally. The AIC value of the chlorophyll *a* model was much greater than for these models (>2 difference) signifying that this model did not fit the data as well (Table 3.15).

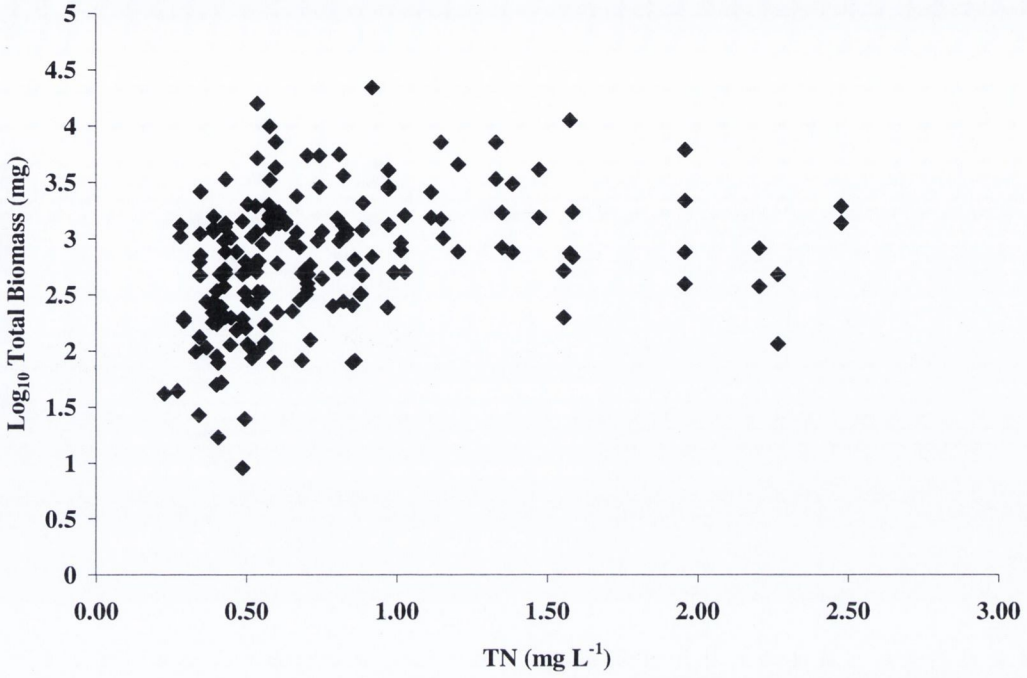


Figure 3.9: Relationship between $\log_{10}$ total biomass and TN (mg l^{-1}) among samples.

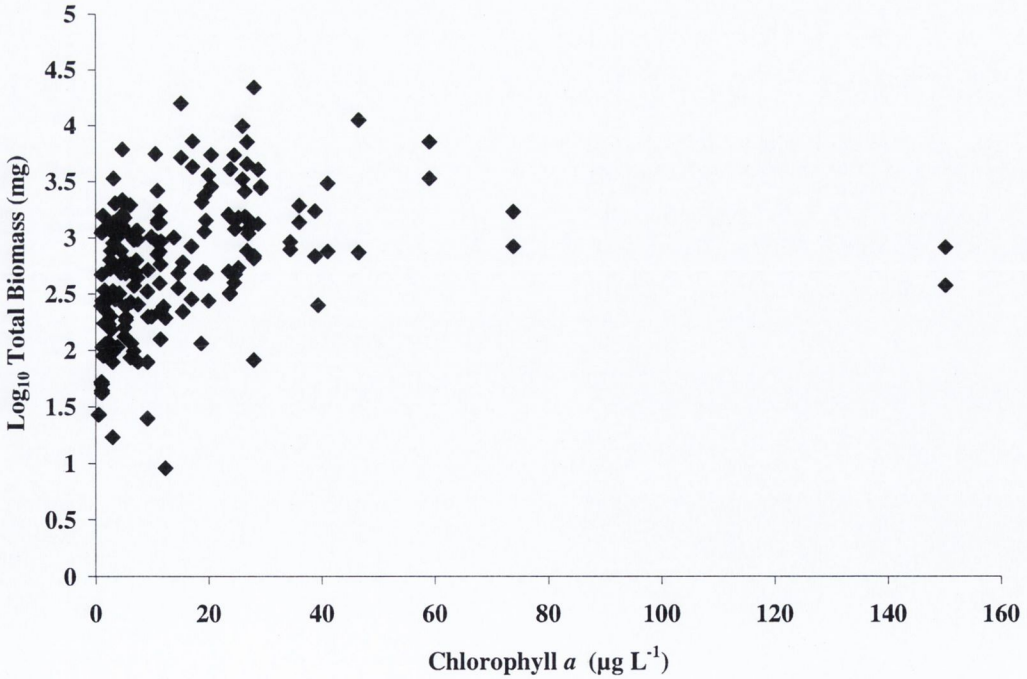


Figure 3.10: Relationship between $\log_{10}$ total biomass of each sample and chlorophyll *a* (µg l^{-1}) among samples.

3.3.2.4 Log total abundance

The three minimal adequate models of the $\log_{10}$ total abundance per sample response variable (log total abundance) were structurally similar (Table 3.15). This may be, in part, owing to the elimination of substrate and the retention of alkalinity, which had an equally low marginal value in all minimal adequate models, indicating a very small increase in log total abundance with alkalinity. The TP log total abundance model retained both linear and quadratic enrichment terms, indicating a curvilinear relationship between log total abundance and TP among samples (Fig. 3.11). The value of the linear coefficient was very close to zero with a positive value, and the quadratic term had an even smaller negative coefficient, indicating a slight downward force on the increasing linear term. However, maximum log total abundance occurred at approximately 20 µg l^{-1}, with similar values of log total abundance found at approximately 30 and 50 µg l^{-1} (Fig. 3.11). Random variance among lakes accounted for approximately 21% of variance in the model.

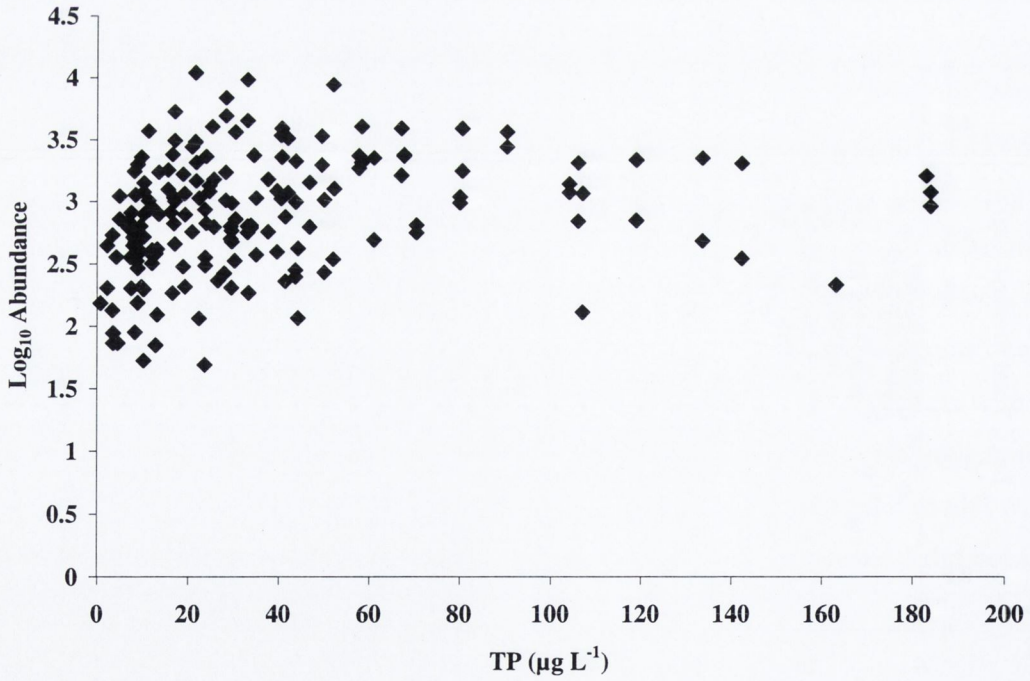


Figure 3.11: Relationship between $\log_{10}$ total abundance and TP (µg l^{-1}) among samples.

The TN and chlorophyll *a* minimal adequate models were identical, owing to the elimination of these enrichment terms from both models (Table 3.15). This TN-chlorophyll *a* model included only one fixed effect, alkalinity, which had a small positive coefficient, similar to those in previous models. Random variance among lakes accounted for approximately 29% of variance in the model. The AIC value of the TP model was much greater (>2 difference) than that of the TN-chlorophyll *a* model, indicating that the latter model was a better fit to the data.

3.3.3 Models of size spectra based on the quadratic dataset

Size spectrum models of the quadratic dataset were divided into two parts: (i) models created using data from hard substrates; and (ii) those created from soft substrates (Table 3.16). All of the models of hard substrates had similar intercept values, and retained both linear and quadratic size class terms, indicating a curvilinear relationship between normalised biomass among size classes in hard substrates. The coefficients of both these terms were negative with low standard errors. This curvilinear shape resulted from the additional downward force of the quadratic term on the equation. For models of normalised biomass among size classes, where SC^2 and E^2 interaction terms were retained (Tables 3.16 and 3.17), additional analysis was conducted, to help visualize changes in the normalised biomass-size class relationship with enrichment terms (both linear and quadratic). This was achieved by solving the linear equation associated with the LME model for different values of the enrichment term within different size classes, based on equations following Jaccard *et al.* (1990) and creating a graph of the resulting values for normalised biomass among size classes. As an example, the minimum adequate TN model for hard substrates (Table 3.16) can be summarised by:

$$\mathbf{Y} = b_0 + b_1\mathbf{SC} + b_2\mathbf{SC}^2 + b_3\mathbf{DOC} + b_4\mathbf{DOC}*\mathbf{SC} + b_5\mathbf{TN} + b_6\mathbf{TN}^2 + b_7\mathbf{TN}*\mathbf{SC} + b_8\mathbf{TN}*\mathbf{SC}^2 + b_9\mathbf{TN}^2*\mathbf{SC} + u + e \quad [12]$$

Where Y = normalised biomass, b_0 = intercept, SC = size class, A = alkalinity; u = random effects; *e* = error.

To calculate the effect of TN on normalised biomass for different fixed size classes (SCs), the value of -10 (representing size class -10) was substituted, as an example, for

each SC in the equation, and substitute each coefficients value into the equation (Table 3.16):

$$\mathbf{Y} = 6.51 + -0.46*(-10) + -0.13*(-10)^2 + 0.02*\mathbf{DOC} + 1.97*\mathbf{TN} - 1.63*\mathbf{TN}^2 - 0.01*\mathbf{DOC}*(-10) + 0.30*\mathbf{TN}(-10) - 0.26*\mathbf{TN}^2(-10) + u + e$$

Yielding:

$$\mathbf{Y} = -2.35 + 0.13*\mathbf{DOC} - 1.07*\mathbf{TN} + 0.98*\mathbf{TN}^2 \quad [13]$$

If DOC is replaced by the average DOC value among samples (9.3 mg l^{-1}), the calculation can be continued for different values of SC at a number of concentrations of TN (or likewise for any of the enrichment terms) for each of the models with quadratic interaction effects. This enables graphical visualisation of the change in the NB-SC relationship for different concentrations of the enrichment term (Figs. 3.12 to 3.17). Similarly, this approach was adopted to graph the normalised biomass- size-class relationship (NB-SC) relationship for the truncated dataset, where quadratic enrichment terms were retained in the models (Figs. 3.18 to 3.22). The variance of the random effects for all three hard substrate models indicated that lake identity accounted for very little variance (between ~0% and 3% of variance). However, seasonal variance within lakes accounted for a much greater amount of variance (~40%) of normalised biomass among samples in each of the hard substrate models.

3.3.3.1 Quadratic models of size spectra from hard substrates

The TP model of normalised biomass among hard samples was structurally different from that of the TN and chlorophyll *a* models, as alkalinity and the linear alkalinity-size class interaction term were retained, and all DOC terms were eliminated (Table 3.16). The quadratic alkalinity-size class interaction term was eliminated from this model. Alkalinity had a very low positive coefficient value within this model, where a marginal increase in the normalised biomass, and in the intercept of the NB-SC relationship occurred with increasing alkalinity (Table 3.16). The size class-alkalinity interaction term was also retained in this model, but had an even lower coefficient value, demonstrating a very small positive effect on the slope of the NB-SC relationship (Table 3.16). This signifies a slightly greater alkalinity effect on larger size classes. The linear and quadratic enrichment terms were retained in the model, as well as a linear

enrichment-size class term, all with values close to zero. The positive coefficient of the linear TP main effect, coupled with the negative quadratic TP main effect, denotes that there was a curvilinear relationship between normalised biomass and TP (Fig. 3.12). This indicates an initial increase in the intercept of the NB-SC relationship, followed by a subsequent decrease in this intercept. TP also mediated a marginal linear increase in the slope of the NB-SC relationship, as evinced by the positive coefficient of the SC-TP interaction term (Table 3.16).

Normalised biomass was relatively unchanging in all size classes, where TP concentrations were ≤ 40 µg L^{-1} (Fig. 3.12). However, normalised biomass in almost all size classes, appeared to decrease with increasing TP where concentrations exceeded 40 µg L^{-1}, with a greater effect on the normalised biomass of smaller size classes, and a marginal effect on large size classes (Fig. 3.12).

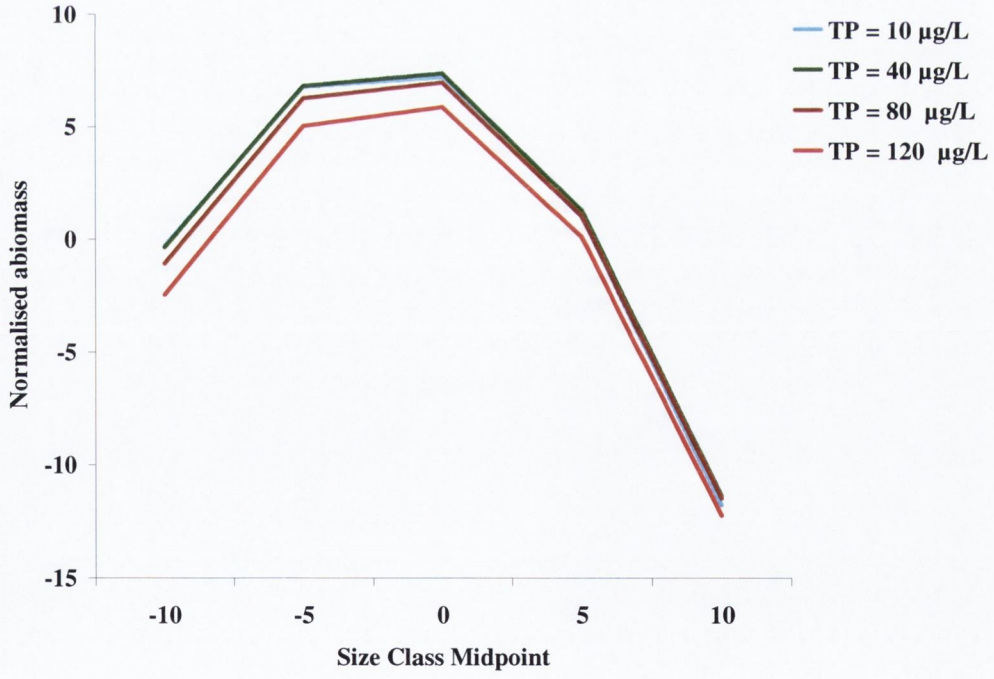


Figure 3.12: The distribution of normalised biomass (from all samples, quadratic analysis) from hard substrates among size classes modeled at different concentrations of TP, based on the minimal adequate TP model.

Table 3.16: Minimal adequate mixed effects models of the quadratic dataset, with normalised biomass (NB) as the response variable and where hard and soft substrates were analysed in separate LME models. Enrichment term (E) refers to both linear (E) and quadratic (E^2) terms to describe the effects of TP, TN and chlorophyll *a* (chl. *a*), analysed in separate models. All models had the same random structure, where season was nested within lake identity. Interaction terms between (i) size class (SC) and each of the explanatory variables and (ii) the size class quadratic term (SC^2) and each of the explanatory variables were included in the maximal model. Two of these interaction terms were eliminated from all minimal adequate models and are not presented (Alkalinity (Alk) x SC^2 and TP x SC^2).

			Fixed effects															Variance components					
Response variable	Enrichment Term (E)	Coeffs.	c[1]	SC	SC^2	Alk[b].	DOC[c]	E	E^2	Alk x SC	Alk x SC^2	DOC x SC	DOC x SC^2	E x SC	E^2 x SC	E x SC^2	E^2 x SC^2		Lake	Season / Lake	Residual	n[d]	df[e]	AIC[f]
NB	TP	B	6.37	-0.65	-0.13	0.01	-	0.02	-0.00	0.00	-	-	-	0.00	-	-	-	Intercept	0.00	37.04	62.96			
(Hard)	($\mu g\ l^{-1}$)	SE	0.26	0.02	0.00	0.00	-	0.01	0.00	0.00	-	-	-	0.00	-	-	-	SD	0.00	1.29	1.69	95	11	4698
NB	TN	B	6.51	-0.46	-0.13	-	0.02	1.97	-1.63	-	-	-0.01	-	0.30	-0.26	-	-	Intercept	2.71	37.03	60.26			
(Hard)	($mg\ l^{-1}$)	SE	0.48	0.04	0.00	-	0.04	0.56	0.54	-	-	0.00	-	0.05	0.05	-	-	SD	0.36	1.31	1.68	95	12	4651
NB	chl. *a*	B	6.33	-0.48	-0.14	-	0.03	0.06	-0.00	-	-	-0.01	-	0.01	-0.00	-	-	Intercept	0.00	39.92	60.08			
(Hard)	($\mu g\ l^{-1}$)	SE	0.43	0.04	0.00	-	0.04	0.01	0.00	-	-	0.00	-	0.00	0.00	-	-	SD	0.00	1.35	1.66	95	12	4673
NB	TP	B	6.27	-0.54	-0.12	0.01	-0.01	0.02	-0.00	0.00	-	0.01	0.00	-	-	-	-	Intercept	0.00	22.31	77.69			
(Soft)	($\mu g\ l^{-1}$)	SE	0.55	0.05	0.01	0.00	0.05	0.01	0.00	0.00	-	0.00	0.00	-	-	-	-	SD	0.00	1.02	1.90	80	13	4553
NB	TN	B	6.01	-0.26	-0.09	0.01	-	0.63	-0.31	-0.00	-0.00	-	-	0.35	-0.30	0.04	-0.04	Intercept	3.70	21.43	74.87			
(Soft)	($mg\ l^{-1}$)	SE	0.31	0.04	0.01	0.00	-	0.60	0.56	0.00	0.00	-	-	0.06	0.06	0.01	0.01	SD	1.01	6.01	0.31	80	15	4534
NB	chl. *a*	B	5.84	-0.46	-0.11	0.01	0.04	0.03	-0.00	0.00	-	0.01	-	0.00	-0.00	-	-	Intercept	2.78	21.41	75.80			
(Soft)	($\mu g\ l^{-1}$)	SE	0.49	0.05	0.00	0.00	0.04	0.01	0.00	0.00	-	0.00	-	0.00	0.00	-	-	SD	1.01	5.84	0.49	80	14	4567

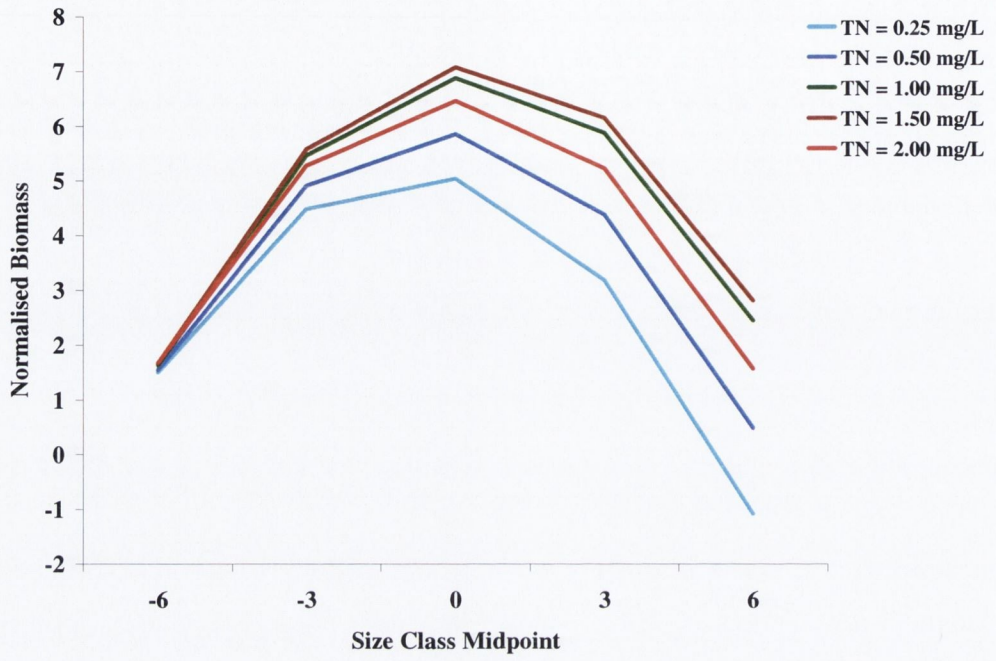
[a]Intercept; [b]Alkalinity; [c]Dissolved Organic Carbon; [d]No. of samples; [e]Degrees of freedom; [f]Akaike's information criterion; '-' denotes where a variable was eliminated; '*' represents where minimal adequate models were identical for TP and chlorophyll *a* models.

The minimal adequate TN and chlorophyll *a* models were structurally similar, as both retained DOC main effects and DOC-SC interaction terms, as well as linear and quadratic enrichment terms, and linear and quadratic enrichment-size class interaction terms (Table 3.16). The DOC main effect term retained in the TN model for normalised biomass in hard substrates had a SE value that was much greater than the coefficient itself; demonstrating no direct relationship between normalised biomass and DOC.

The low negative coefficient value of the DOC-SC interaction effect had a small SE value relative to its own value, indicating that DOC mediated a marginal decrease in the slope of the NB-SC relationship (Table 3.16; Fig. 3.13b). With increasing DOC concentration, there was, therefore, a marginally greater decrease in normalised biomass in larger size classes. The linear and quadratic TN main effects were both retained in the model; the linear term with a positive coefficient value, and the quadratic term with a slightly lower negative value, both with lower SEs, indicating a curvilinear relationship, where the quadratic term exerted a downward pressure on the positive linear relationship between normalised biomass and TN. The positive linear (TN-SC) and negative quadratic (TN^2-SC) size-class-enrichment interaction effects were also retained in the model, indicating a curvilinear change in the NB-SC relationship with TN (Fig. 3.13a). Graphically, this demonstrated an initial increase in normalised biomass among increasing size classes (with the greatest increase in the largest size classes, and a slight decrease in the smallest size classes) with increasing TN concentrations. However, this is a curvilinear response in the NB-SC relationship to TN, so that the increase was slightly depressed at a TN concentration of 1.5 mg l^{-1}, subsequently decreasing at 2.0 mg l^{-1} (Fig. 3.13a).

Similar to the TN model, the coefficient of the DOC main effect in the chlorophyll *a* model had a SE value greater than the coefficient value, indicating that DOC did not directly affect normalised biomass among samples (Table 3.16). However, the DOC-SC interaction term was also retained, with a low negative coefficient value and a small SE value relative to its own value. This signifies that DOC mediated a marginal decrease in the slope of the NB-SC relationship, suggestive of a marginally greater decrease in normalised biomass in larger size classes with increasing DOC.

(a)



(b)

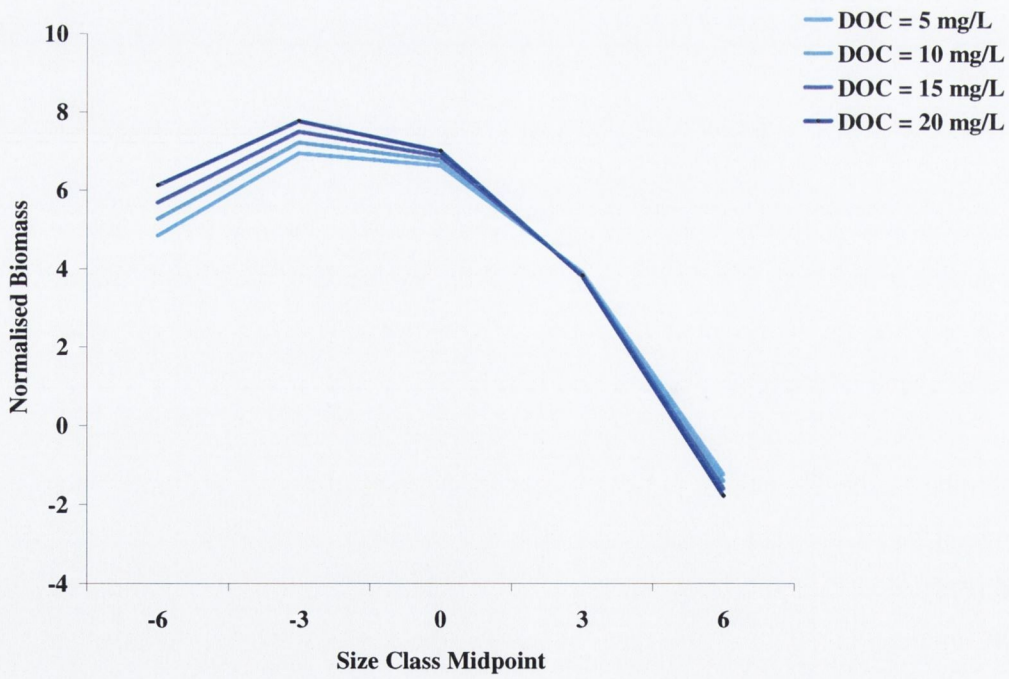


Figure 3.13: The distribution of normalised biomass (from all samples, quadratic analysis) from hard substrates among size classes modeled at different concentrations of TN (a) and different concentrations of DOC (b), based on the minimal adequate TN model.

The linear and quadratic chlorophyll *a* main effects were both retained in the model. The linear term demonstrated a positive coefficient value, but with a much lower value than that of the enrichment term in the TN model, whilst the quadratic term showed a marginally negative value, indicating a marginally curvilinear relationship between normalised biomass and chlorophyll *a* (Table 3.16; Fig. 3.14). The positive linear (chlorophyll *a*-SC) and negative quadratic (chlorophyll a^2-SC) size-class-enrichment interaction effects were also retained in the model, both with values close to zero, indicating a marginal curvilinear change in the NB-SC relationship with chlorophyll *a* (Fig. 3.14). This was shown to indicate an initial increase in normalised biomass among increasing size classes (with the greatest increase in the largest size classes, and a slight decrease in the smallest size classes) with increasing chlorophyll *a* concentrations (Fig. 3.14). This increase was gradually depressed in magnitude, with highest normalised biomass in the larger size classes at a chlorophyll *a* concentration of 75 $\mu g\ l^{-1}$, subsequently decreasing at a chlorophyll *a* concentration of 90 $\mu g\ l^{-1}$ (Fig. 3.14). The AIC values of the TP and chlorophyll *a* models of normalised biomass in hard substrates were much greater than that of the TN value (>2 difference), indicating that the TN model described the data best.

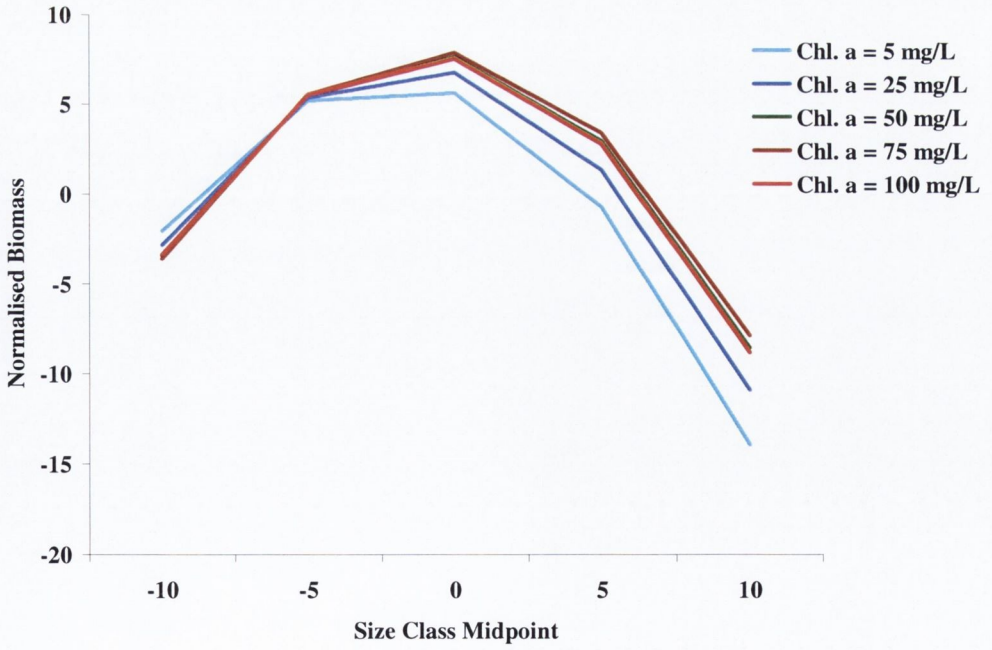


Figure 3.14: The distribution of normalised biomass (from all samples, quadratic analysis) from hard substrates among size classes modeled at different concentrations of chlorophyll *a*, based on the minimal adequate chlorophyll *a* model.

3.3.3.2 Quadratic models of size spectra from soft substrates

The three models of normalised biomass among soft samples retained most of the main effects included in the maximal model (Table 3.16). All of the soft substrate models had similar intercept values which were slightly lower than those of the models of hard substrates, and retained both linear and quadratic size class terms (Table 3.16). The coefficients of both terms were negative with low standard error in all models (Table 3.16). The models based on data from soft substrates differed from those based on hard substrates in several ways. Although both soft models had similar, but lower intercepts, with higher SE values, both the linear and quadratic size class terms were greater (less negative), suggesting that the NB-SC relationship had a shallower curve than the dome depicted in the hard substrates models. The variance components of the random effects for all three soft substrate models indicated that lake identity accounted for little variance (between approximately 0% to 3% of variance), but that seasonal differences within lakes accounted for approximately 21 to 22% of variance in normalised biomass among samples. Therefore, season accounted for less variance in soft substrate models than in hard substrate models, where variance explained by season was approximately 40%.

The TP model of normalised biomass among soft samples retained all main effects in the minimal adequate model (Table 3.16). The alkalinity main effect and the alkalinity-SC interaction term were retained, both with positive coefficients, but with values close to zero. Larger size classes had marginally greater normalised biomass with increasing alkalinity. The DOC main effect, DOC-SC and DOC-SC^2 interaction terms were also retained in the minimal adequate TP model. The DOC main effect had an SE value that was much greater than the coefficient itself, indicating that there was no direct relationship between normalised biomass and DOC. However, the low positive coefficient values of both DOC-SC interaction effects indicated that DOC mediated a positive curvilinear change in the NB-SC relationship, with greater normalised biomass in larger size classes with increasing concentrations of DOC (Table 3.16). The linear and quadratic TP main effects were both retained in the model, but higher order TP interaction effects were eliminated. The linear TP term had a positive coefficient value close to zero, and the quadratic term had a very small negative value. Therefore, although TP did not affect the slope of the NB-SC relationship, the intercept within each of the size classes initially increased, with increasing TP, but owing to the curvilinear

nature of the TP-normalised biomass relationship, the intercept decreased at greater values of TP (Fig. 3.16).

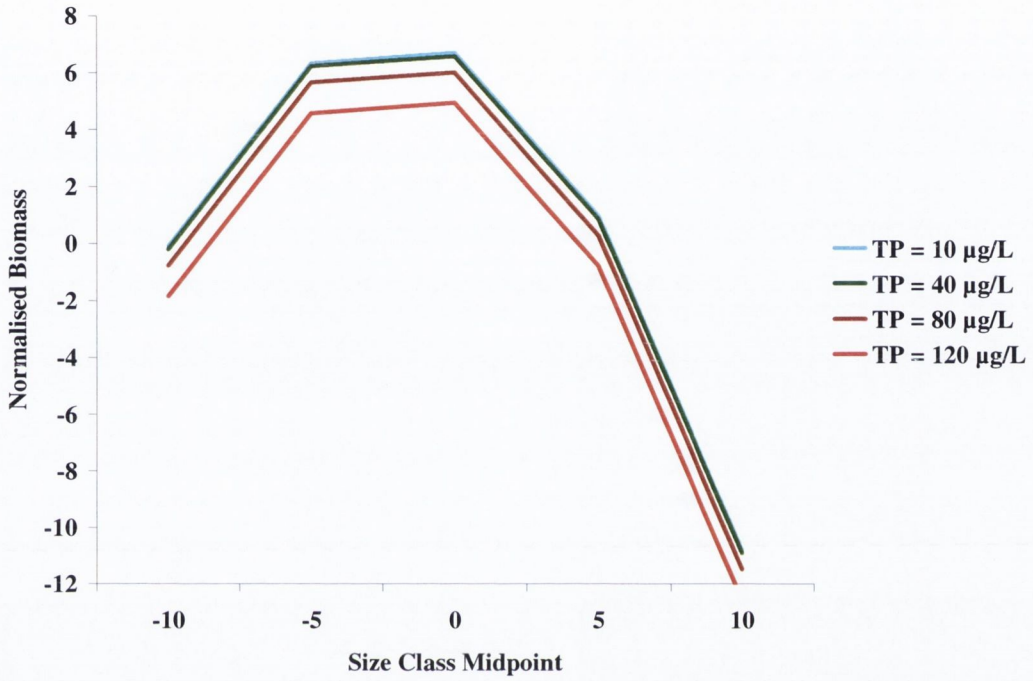


Figure 3.15: The distribution of normalised biomass (from all samples, quadratic analysis) from soft substrates among size classes modeled at different concentrations of TP, based on the minimal adequate TP model.

The minimal adequate TN model eliminated all DOC terms, but retained the alkalinity main effect and the linear and quadratic alkalinity-SC interaction effects, as well as both linear and quadratic enrichment terms and all enrichment-SC linear and quadratic interaction effects. The small positive coefficient of the alkalinity main effect indicated that normalised biomass in soft substrates increased with increasing alkalinity concentrations (indicating an increase in the intercept of the NB-SC relationship; Table 3.16). The coefficient of the linear alkalinity-SC interaction term was negative with an SE value greater than that of the coefficient, indicating that alkalinity did not mediate a linear change in the NB-SC relationship (Table 3.16). The coefficient of the quadratic alkalinity-SC^2 relationship was negative, but close to zero with a low SE value (Table 3.16). This implies that with increasing alkalinity, normalised biomass in smaller size classes increased more than those in larger size classes. Both the linear and quadratic TN main effects had large SE values similar to the coefficient values, indicating no

direct relationship between the normalised biomass in soft substrates and TN (Table 3.16). However, the four TN-SC interaction effects had much greater coefficient values, suggesting that TN mediated a change in the slope of the NB-SC relationship.

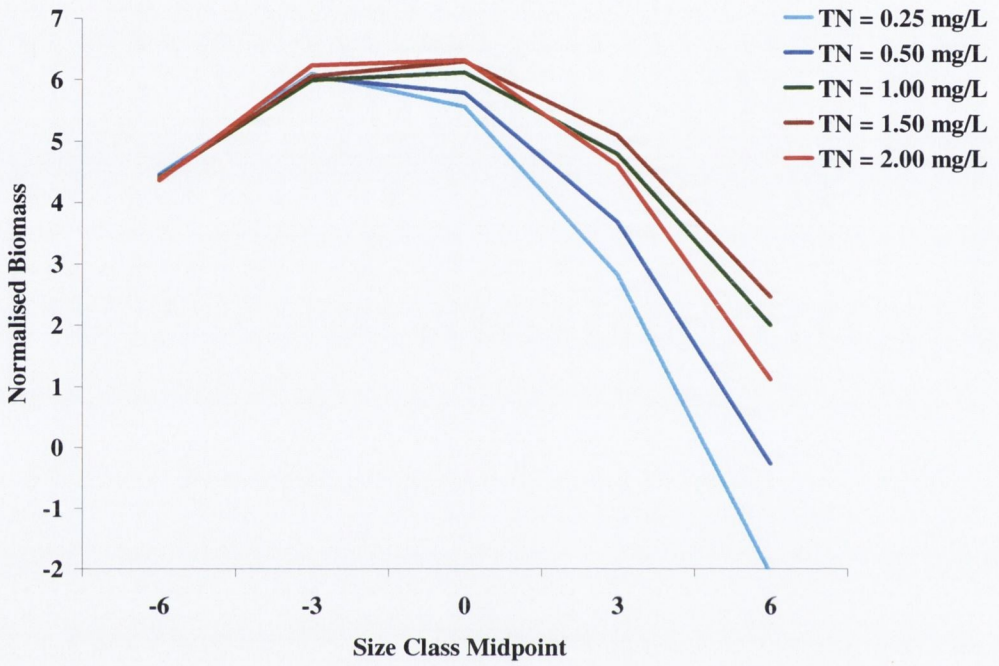


Figure 3.16: The distribution of normalised biomass (from all samples, quadratic analysis) from soft substrates among size classes modeled at different concentrations of TN, based on the minimal adequate TN model.

TN was associated with a greater change in the normalised biomass of larger size classes (Fig. 3.16). Initially (< 1.5 mg TN L^{-1}), the normalised biomass among size classes increased, but at a decreasing rate with increasing TN concentrations. At a concentration of 2 mg l^{-1} of TN, normalised biomass among large size classes decreased sharply, and slightly decreased in the smallest size class, whereas the normalised biomass of intermediate sized organisms (size classes -5 and 0) continued to increase (Fig. 3.16).

The minimal adequate chlorophyll *a* model of normalised biomass in soft samples retained all main effects. All quadratic size class interaction terms were eliminated, but linear size class interaction terms were retained. The coefficient of the alkalinity main effect was small and positive with a lower SE, indicating an increase in normalised

biomass in soft substrates with increasing alkalinity concentrations, and an increase in the intercept of the NB-SC relationship; as also observed in the two other minimal adequate models of normalised biomass among size classes in soft substrates (Table 3.16). The linear alkalinity-SC interaction term had a small negative coefficient, close to zero, with low SE (Table 3.16). The DOC main effect had a small coefficient, with an SE of equal value, indicating that there was no direct relationship between normalised biomass in soft substrates and DOC (Table 3.16). However, the positive small coefficient of the DOC-SC interaction term suggested that increasing DOC concentrations mediated an increase in the slope of the NB-SC relationship, with the greatest increase in the normalised biomass of large size classes.

Both the linear and quadratic chlorophyll *a* main effects had small positive coefficient values, demonstrating a curvilinear relationship between normalised biomass and chlorophyll *a* in soft substrates (Table 3.16; Fig. 3.17). Initially, the downward force of the negative quadratic term created a dome shaped relationship with both terms increasing, then, declining with further increasing chlorophyll *a* (Fig. 3.17). The interaction effects between the linear and quadratic chlorophyll *a* terms and size class were also retained in the model, both with small coefficients; the linear term positive with a value almost zero, and the quadratic term was negative with an even smaller value close to zero (Table 3.16). The normalised biomass in all size classes appeared to increase initially, up to an optimal concentration of approximately 0.75 $\mu g\ l^{-1}$. Thereafter, the normalised biomass in the smallest size classes continued to increase, but declined in the two largest size classes (Fig. 3.17). Comparing the AIC values among soft substrate models, it is apparent that the TN model is a better fit to the data (AIC value >2 lower) than both the TP and the chlorophyll *a* model (Table 3.16). In summary, both the hard substrate TN model and the soft substrate TN model appear to fit the data better than the other minimal adequate models, indicating that TN may play an important role in explaining changes in the NB-SC relationship.

3.3.4 Models of size spectra based on the truncated dataset

All models, of both hard and soft substrates, retained the size class main effect, reflecting the strong negative linear relationship between normalised biomass and size class midpoint described previously (Figs. 3.6 and 3.7; Table 3.17). Alkalinity was also

retained in all minimal adequate models. However, the retention of other fixed effects differed among models.

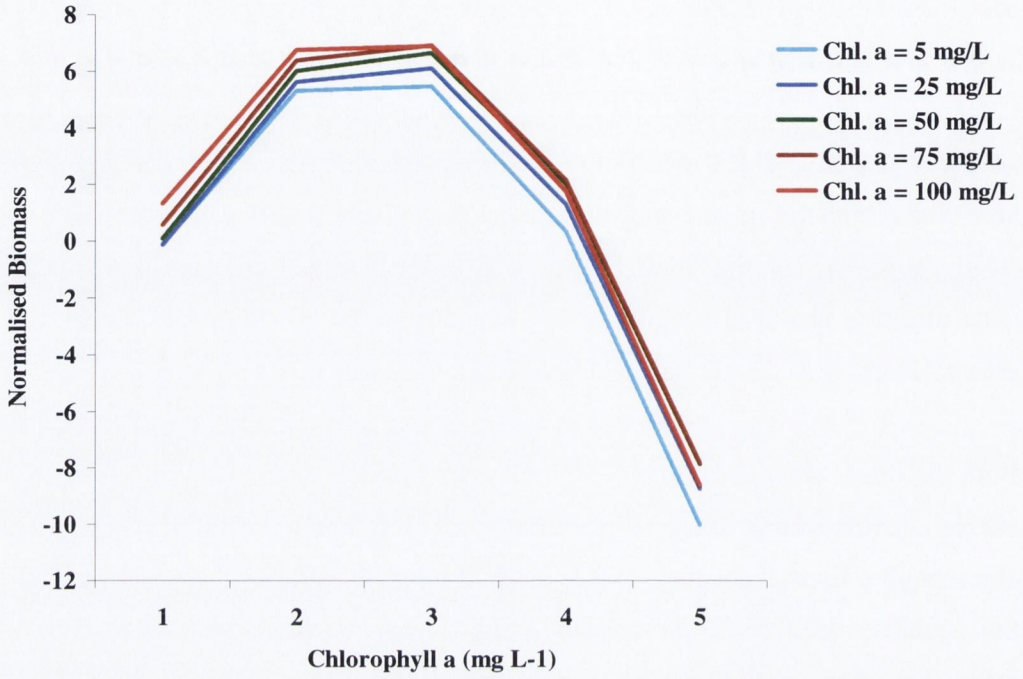


Figure 3.17: Quadratic analysis of the distribution of normalised biomass from soft substrates among size classes modeled at different concentrations of chlorophyll *a*, based on the minimal adequate chlorophyll *a* model.

3.3.4.1 Truncated models of size spectra from hard substrates

Two minimal adequate models were produced for the normalised biomass from hard substrates (using truncated samples with sufficient biomass in size classes greater than size class zero; Table 3.17). The minimal adequate TP model of normalised biomass from hard substrates eliminated the DOC main effect and interaction term with SC, but retained the alkalinity and linear and quadratic enrichment main effects, as well as interaction terms between each of the latter and size class midpoint. Initially, the coefficient of the alkalinity main effect was small with a value close to zero and a low SE, demonstrating a linear increase in normalised biomass with increasing alkalinity concentrations. The coefficient of the alkalinity–size class interaction term was also close to zero with a negative value, suggesting a decrease in the slope of the NB-SC relationship with increasing alkalinity concentrations (Table 3.17). Both the linear and quadratic TP main effect terms had SE values greater than that of the coefficient,

indicating no direct relationship between normalised biomass and TP (Table 3.17). However, the linear and quadratic interaction terms both had coefficients greater than their SE values, where the linear TP-SC interaction term had a positive coefficient with a value approaching zero, and the quadratic TP^2-SC interaction term with a negative coefficient, suggests a downward force on the positive relationship of the TP-SC interaction (Table 3.17; Fig. 3.18). The slope of the NB-SC appeared to increase with increasing TP concentrations, but with a declining intercept. Therefore, although the normalised biomass of the smallest animals decreased slightly, the largest animals increased considerably with increasing TP concentrations. Variance among lakes accounted for approximately 9% of total random variation, with variance among seasons within lakes accounting for approximately 21% of variation (Table 3.17).

The minimal adequate TN and chlorophyll *a* models were identical, following the elimination of all enrichment main effects and interaction terms from each of their maximal models (Table 3.17). The alkalinity and DOC main effects and interaction terms with substrate were all retained. The small positive coefficient of the alkalinity main effect indicated that there was a marginal increase in normalised biomass with increasing concentrations of alkalinity (and an increase in the NB-SC intercept with increasing alkalinity; Table 3.17), but with a marginal decrease in the slope of the NB-SC relationship (Fig. 3.19a). The graphical representation of the effect of the alkalinity main effect and interaction term on the NB-SC slope demonstrated an overall increase in the normalised biomass among size classes with alkalinity, with the greatest increase occurring in the smallest size classes, and a smaller change in normalised biomass in larger size classes, with alkalinity (Fig. 3.19a).

Table 3.17: Minimal adequate mixed effects models of the truncated dataset, with normalised biomass (NB) as the response variable in a series of LME models. Enrichment term (E) refers to both linear (E) and quadratic (E^2) terms describing effects of TP, TN and chlorophyll *a* (chl. *a*), analysed in separate models. All models had the same random structure, where season was nested within lake identity. Soft substrate was the reference category for the substrate term. Interaction terms between (i) substrate and each of the explanatory variables and (ii) size class (SC) and each of the explanatory variables were included in the maximal model. All interaction terms except for those with DOC were eliminated from all minimal adequate models and are not presented. An identical minimal adequate model was produced for the separate TN and chlorophyll *a* models of normalised biomass on hard substrates owing to the elimination of these variables from their respective models.

			Fixed effects											Variance components					
Response variable	**Enrichment Term (E)**	**Coeffs.**	**c[a]**	**SC**	**Alk[b]**	**DOC[c]**	**E**	**E^2**	**DOC x SC**	**Alk x SC**	**E x SC**	**E^2 x SC**		**Lake**	**Season / Lake**	**Residual**	**n[d]**	**df[e]**	**AIC[f]**
NB	TP	B	6.20	-0.91	0.02	-	-0.01	0.00	-	-0.00	0.01	-0.00	Intercept	18.86	21.17	59.98			
(Hard)	($\mu g\ l^{-1}$)	SE	0.58	0.14	0.01	-	0.01	0.00	-	0.00	0.00	0.00	SD	0.84	0.89	1.50	44	11	969.5
NB	TN - chl. *a*	B	7.49	-1.49	0.01	-0.10	*	*	0.04	-0.00	-	-	Intercept	27.26	14.45	58.28			
(Hard)	($mg\ l^{-1}$/ $\mu g\ l^{-1}$)	SE	0.90	0.21	0.01	0.08	*	*	0.02	0.00	-	-	SD	1.02	0.75	1.50	44	9	923.2
NB	TP	B	8.58	-1.48	0.01	-0.07	0.02	-0.00	0.03	-	-	-	Intercept	9.30	26.53	64.17			
(Soft)	($\mu g\ l^{-1}$)	SE	0.86	0.12	0.00	0.07	0.01	0.00	0.01	-	-	-	SD	0.58	0.99	1.53	53	10	1278
NB	TN	B	9.26	-1.49	0.00	-0.10	2.40	-2.26	0.03	-	-	-	Intercept	17.49	18.65	63.86			
(Soft)	($mg\ l^{-1}$)	SE	0.88	0.12	0.00	0.07	0.71	0.83	0.01	-	-	-	SD	0.80	0.83	1.53	53	10	1248
NB	chl. *a*	B	8.52	-1.48	0.01	-0.06	0.05	-0.00	0.03	-	-	-	Intercept	14.27	21.23	64.50			
(Soft)	($\mu g\ l^{-1}$)	SE	0.78	0.12	0.00	0.06	0.02	0.00	0.01	-	-	-	SD	0.72	0.88	1.53	53	10	1274

[a]Intercept; [b] Alkalinity; [c] Dissolved Organic Carbon; [d] no. of samples; [e] degrees of freedom; [f] Akaike's information criterion; '-' denotes where a variable was eliminated; '*' represents where minimal adequate models were identical for TN and chlorophyll *a* models;

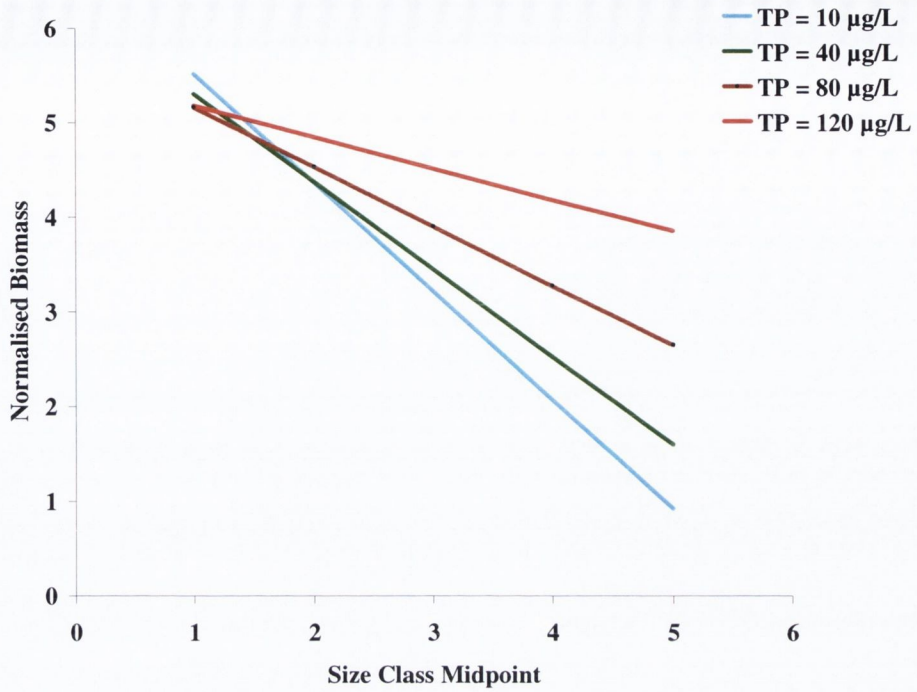
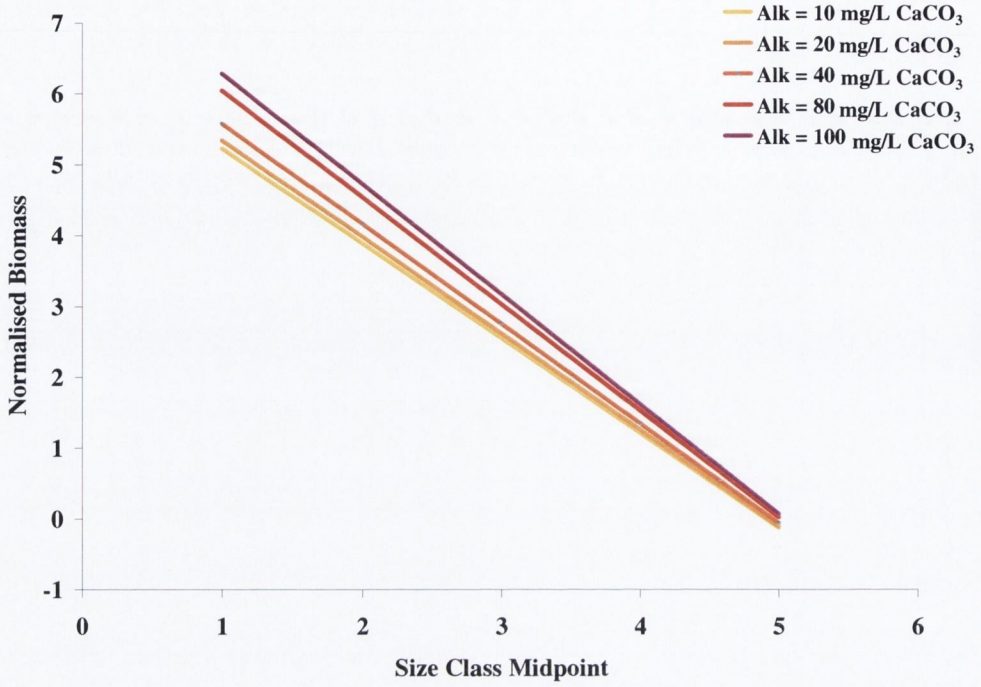


Figure 3.18: The distribution of normalised biomass from hard substrates among size classes (from the truncated dataset), modeled at different concentrations of TP, based on the minimal adequate TP model.

The DOC main effect had a negative coefficient, with a high relative SE value that approached that of the coefficient (Table 3.17), demonstrating a weak relationship between DOC and normalised biomass. However, the small positive coefficient of the DOC-SC interaction term indicated increasing DOC concentrations mediated an increase in the slope of the NB-SC relationship (Fig. 3.19b). Graphically, the increasing slope of the NB-SC relationship increased with increasing DOC concentrations; as the normalised biomass of small size classes were observed to decrease, the normalised biomass of intermediate size classes did not change and the normalised biomass of large size classes increased (Fig. 3.19b). Variation among lakes accounted for 27% of random variance among samples, whereas variation between seasons within each lake accounted for 14% of variance in the model. Accordingly, variance among lakes accounted for more variance in the TN-chlorophyll *a* model, whereas variance among seasons within lakes accounted for more variance in the TP model. The AIC value of the TN-chlorophyll *a* model was much less than that of the TP model, by a difference >2, indicating that the TN-chlorophyll *a* model fit the data best (Table 3.17).

(a)



(b)

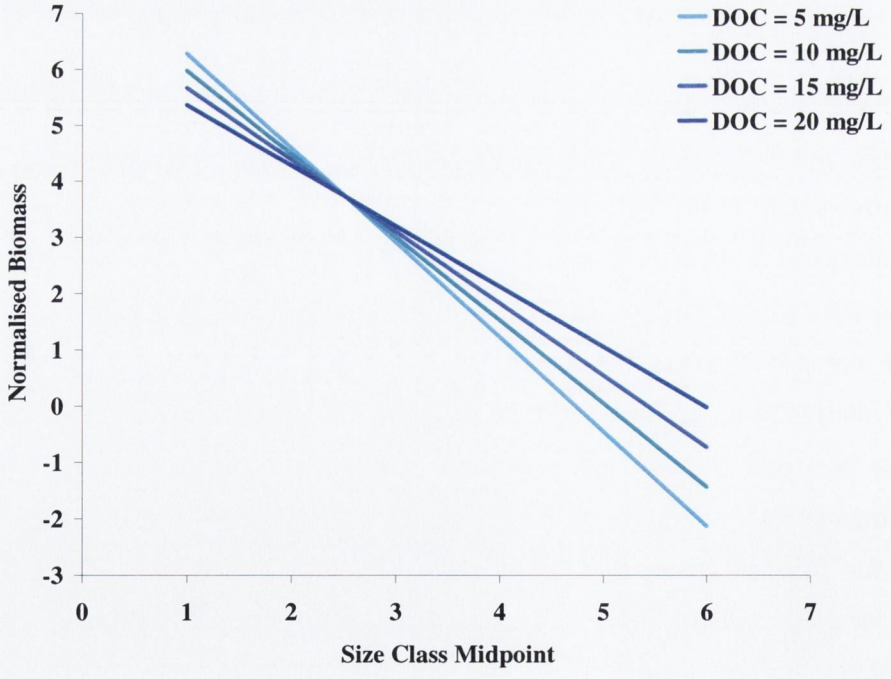


Figure 3.19: The distribution of normalised biomass from hard substrates among size classes, based on the truncated dataset, modeled at different concentrations of (a) alkalinity and (b) DOC based on the minimal adequate TN-chlorophyll *a* model.

3.3.4.2 Truncated models of size spectra from soft substrates

The three minimal adequate models of normalised biomass in soft substrates (from the truncated analysis) were structurally identical, having all retained the SC, alkalinity, DOC and linear and quadratic enrichment main effects, as well as the DOC-SC interaction term (Table 3.17). The coefficient of the SC main effect was almost identical among models, with a negative value approaching -1.5, indicating a consistent NB-SC relationship among models (Table 3.17). Likewise, the coefficient of the alkalinity main effect had a similar positive value close to zero in all three models, demonstrating a linear increase in the normalised biomass of soft substrates with increasing alkalinity concentrations. Thereafter, the models differ slightly in the coefficients of their fixed effects (Table 3.17).

The coefficient of the DOC main effect in the minimal adequate TP model had a negative value, with a SE value slightly less than that of the coefficient itself, indicating a very weak negative relationship between DOC and normalised biomass. The coefficient of the DOC-SC interaction term was small and positive, indicating a DOC mediated increase in the slope of the NB-SC relationship (Table 3.17). The linear and quadratic TP terms were small and positive and smaller and negative, respectively, indicating a curvilinear relationship between normalised biomass and TP. Although this relationship did not affect the slope of the NB-SC relationship, it did affect the intercept. With increasing TP concentrations, normalised biomass among all size classes initially increased, to an optima at a TP concentration of approximately 80 $\mu g\ l^{-1}$, after which it decreased (Fig. 3.20). The variance explained by random variation among lakes (~9%) was approximately a third of that explained by seasonal differences within lakes (~27%).

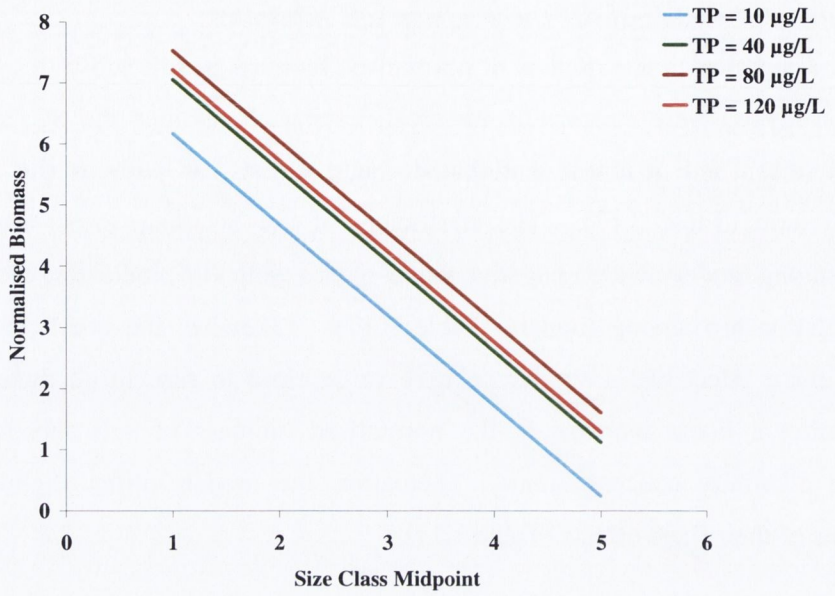


Figure 3.20: The distribution of normalised biomass from soft substrates among size classes (from the truncated dataset) modeled at different concentrations of TP based on the minimal adequate TP model.

The coefficient of the DOC main effect in the TN minimal adequate model of normalised biomass among soft substrates had a small negative value, with a smaller yet similar SE value, demonstrating a weak negative relationship between DOC and normalised biomass (Fig. 3.20). The coefficient of the DOC-SC interaction term had a similar value to that of the DOC-SC coefficient in the TP model, with a small positive value close to zero, signifying an increase in the slope of the NB-SC relationship with increasing DOC concentrations (Table 3.17). The coefficients of the linear and quadratic TN main effects had a much greater negative and positive value, respectively, than those seen for the enrichment terms in the TP model. Together, these terms indicate a strong curvilinear relationship between normalised biomass among soft substrates with TN. Illustrated graphically, this curvilinear relationship resulted in an increase in the intercept of the NB-SC relationship initially with increasing TN up until a concentration of approximately 1.5 mg l^{-1} after which the normalised biomass among all size classes decreased (Fig. 3.21a). Random variation between seasons within lakes was similar to variation among lakes (17-19%).

The coefficient of the DOC main effect in the chlorophyll *a* minimal adequate model had a SE value greater than that of the coefficient, indicating that there was no direct

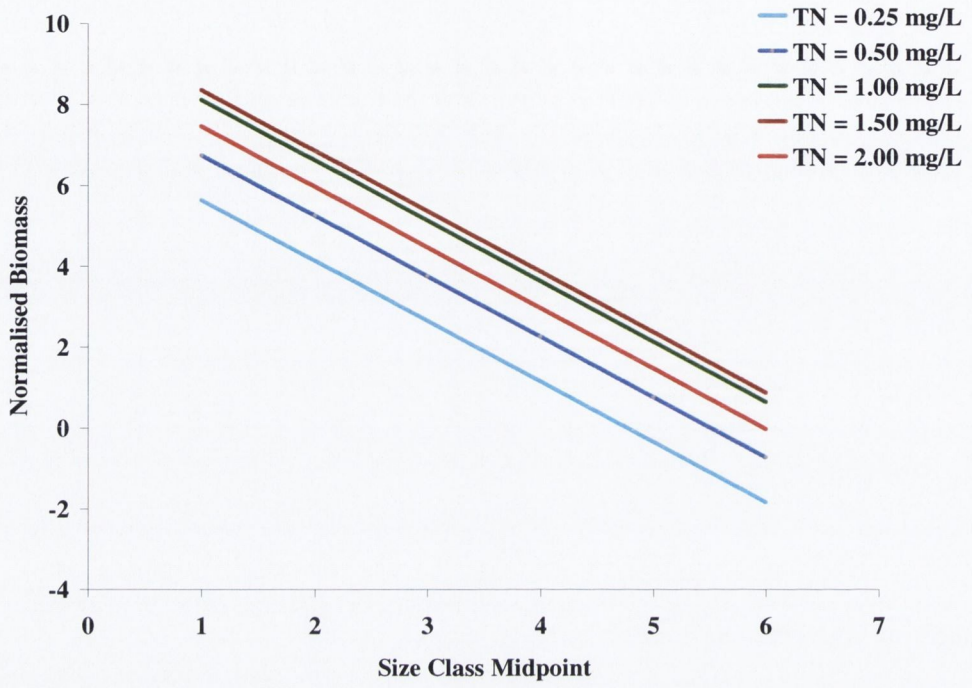
relationship between DOC and normalised biomass (Table 3.17). However, the DOC-SC interaction term had a positive coefficient with a value close to zero, indicating that increasing DOC concentrations resulted in a small increase in the slope of the NB-SC relationship. The coefficients of the linear and quadratic chlorophyll *a* terms were much lower than that observed for the enrichment terms in the TN model. The positive coefficient of the linear chlorophyll *a* term, coupled with a small negative coefficient for the quadratic chlorophyll *a* term, indicated a curvilinear relationship between normalised biomass and chlorophyll *a*. This was demonstrated by an initial increase in the value of the intercept with increasing chlorophyll *a* concentrations (up to approximately 75 $\mu g\ l^{-1}$), followed by a smaller decrease in the intercept at higher concentrations (Fig. 3.22). Random variance among lakes accounted for ~14% of all variance, whereas seasonal variation within lakes accounted for approximately 21% of total variance. The AIC values of the TP and chlorophyll *a* were similar in value, and much greater than that of the TN model, indicating that the TN model fit the data best from the information given.

3.3.5 Models of fish and macroinvertebrate biomass

Total biomass of macroinvertebrates was examined from two size classes (designated "small" and "large" [log transformed and square root transformed, respectively]) from 15 lakes where data on the biomass of several fish taxa was available (which were included as fixed effects in separate models). This generated eight minimal adequate models, four for each of the two size classes examined (Table 3.18). The nutrient enrichment terms (TP and TN) were eliminated from all models. In terms of the models for the log total biomass of "small" individuals (log TB "small"), the four minimal adequate models retained few variables in their minimum adequate models (Table 3.18). The perch model retained the most variables, including substrate, DOC, perch, and the DOC-substrate interaction term (Table 3.18)

Log TB "small" increased with increasing DOC, substrate and perch biomass (Table 3.18). However, the substrate-DOC interaction term displayed a marginally negative coefficient; revealing a much larger increase in log TB "small" with increasing DOC in hard substrates than in soft substrates (Fig. 3.23).

(a)



(b)

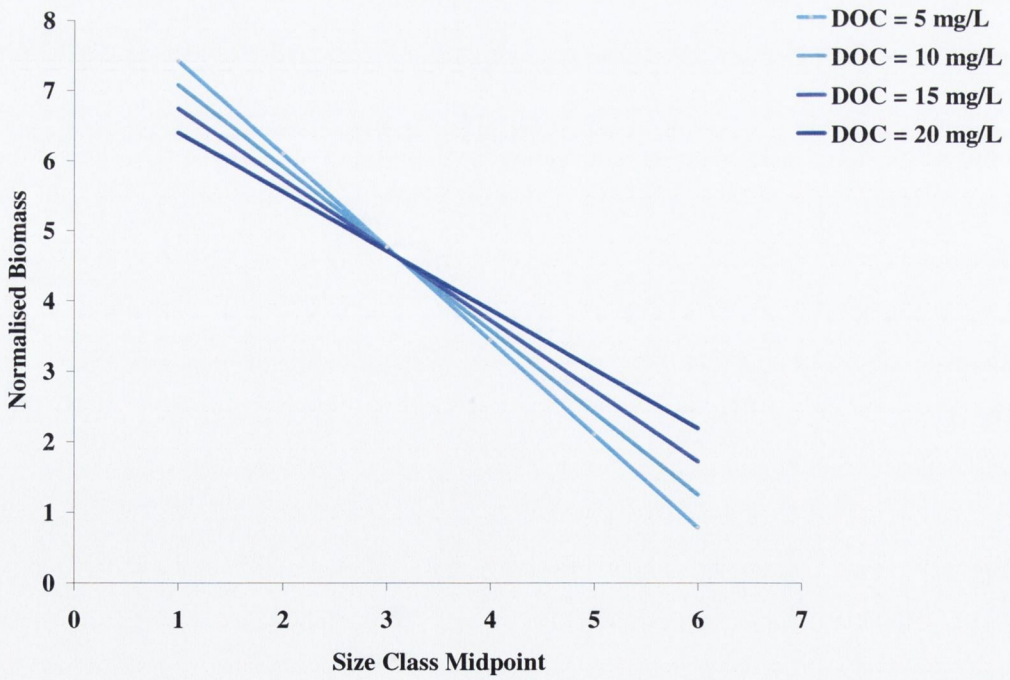


Figure 3.21: The distribution of normalised biomass (from the analysis of the truncated dataset) from soft substrates among size classes modeled at different concentrations of (a) DOC and (b) TN, based on the minimal adequate TN model.

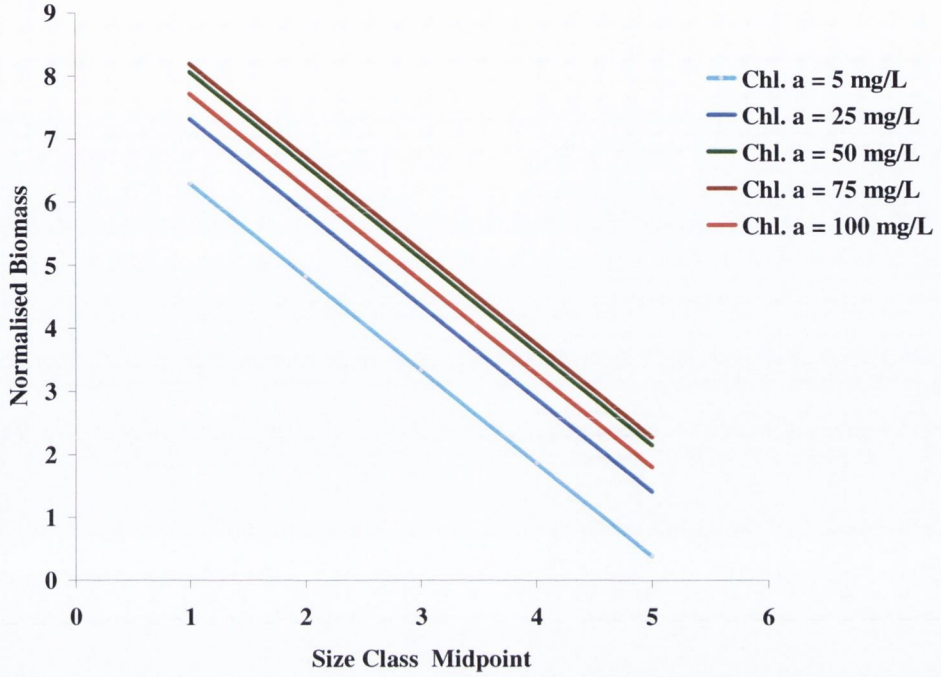


Figure 3.22: The distribution of normalised biomass (from the analysis of the truncated dataset) from soft substrates among size classes modeled at different concentrations of chlorophyll *a* based on the minimal adequate chlorophyll *a* model.

The salmonid model eliminated all terms except that of alkalinity, which exhibited a marginal positive relationship with log TB "small" (Table 3.18). The AIC_C value of this model was lower than that of the perch model (by >2), signifying a better fit to the data (Table 3.18). The third model, which examined changes in the log TB "small" with pike biomass per unit effort, eliminated all variables from the minimal adequate model (Table 3.18). It displayed the lowest AIC_C value among the four minimal adequate models, indicating that none of the variables adequately described the data. The final cyprinid model of log TB "small" retained only cyprinid biomass per unit effort (bpue) in the minimal adequate model, indicating an increase in the log total biomass of small individuals with a marginal increase in cyprinid biomass. However, the AIC_C value of this model, as well as the perch and salmonid models, were much greater than that of the empty pike model, and thus none of the variables presented best described the log total biomass of "small" individuals.

All models of the square root of total biomass "large" (sr TB "large") eliminated DOC from their minimal adequate models, but retained substrate (Table 3.18). The perch model retained alkalinity, substrate, perch and the perch-substrate interaction term (Table 3.18). The alkalinity main effect was small and positive, indicating a small increase in the sr TB "large" with increasing alkalinity. The coefficient of the substrate term was large, with a large SE. This is indicative of a general increase in the sr TB "large" in soft substrates. The coefficient of the perch main effect was small with a very large SE, indicating that perch did not have a direct effect on the log total biomass large. However the perch-substrate interaction term had a positive coefficient. This relationship was further investigated graphically, revealing a greater increase in the slope of the perch bpue- sr TB "large" relationship in soft substrates to that described in hard substrates (Fig. 3.24). Nevertheless, the sr TB of "large" individuals increased with increasing perch bpue in both habitats (Fig. 3.24).

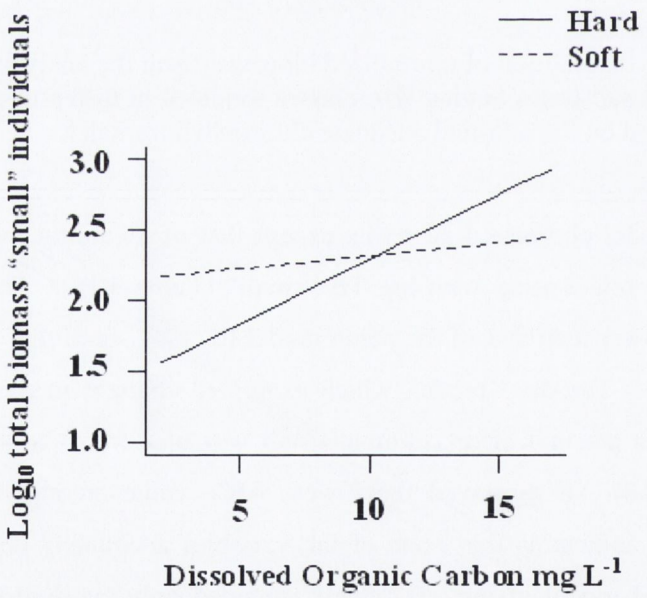


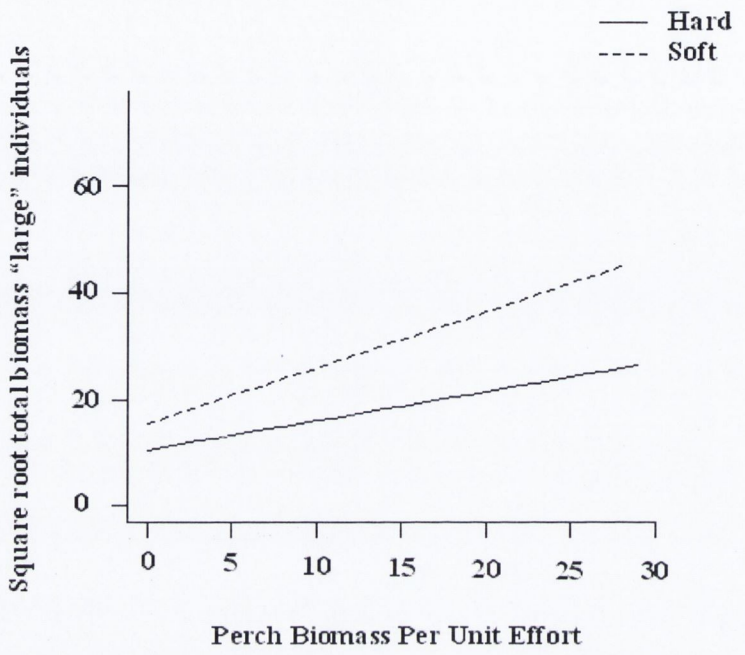
Figure 3.23: The relationship between dissolved organic carbon and the $\log_{10}$ total biomass of "small" macroinvertebrate individuals in different substrates.

Table 3.18: Minimal adequate mixed effects models for the analysis of 15 lakes that had corresponding fish data, with the total biomass (T.B.) of two size (TB small and TB large) classes analysed as separate response variables. Eight maxminal models were created for each of the response variables, with each fish taxa analysed separately with either TP or TN. All models had the same random structure, where season was nested within lake identity. Soft substrate was the reference category for the substrate term. Interaction terms between substrate and each of the explanatory variables and were included in the maximal model. However several interaction terms were eliminated from all minimal adequate models and are not presented.

			Fixed effects												Variance components				
Model	Response variable	Coeffs.	c[a]	Alk.[b]	DOC[c]	TP	TN	Substrate	Perch bpue	Salmonid bpue	Pike bpue	Cyprinid bpue	DOC x Substrate	Perch x Substrate	Lake	Season / Lake	Residual	df[d]	AIC_C[e]
1	Log_{10} TB Small	B	1.48	-	0.06	*	*	0.66	0.02	X	X	X	-0.06	X	0.00	X	100.00	7	103.71
		SE	0.26	-	0.03	*	*	0.44	0.01	X	X	X	0.04	X					
2	Log_{10} TB Small	B	2.05	0.00	-	*	*	-	X	-	X	X	-	-	36.25	X	63.75	4	93.45
		SE	0.12	0.00	-	*	*	-	X	-	X	X	-	-					
3	Log_{10} TB Small	B	2.22	X	-	*	*	-	X	X	-	X	-	X	43.65	X	56.35	3	85.12
		SE	0.11	X	-	*	*	-	X	X	-	X	-	X					
4	Log_{10} TB Small	B	2.06	X	-	*	*	-	X	X	X	0.01	-	X	32.69	X	67.31	4	91.52
		SE	0.11	X	-	*	*	-	X	X	X	0.00	-	X					
5	SR TB Large	B	7.91	0.18	-	*	*	4.49	0.05	X	X	X	-	0.54	0.00	50.81	49.19	8	428.90
		SE	3.00	0.05	-	*	*	3.26	0.24	X	X	X	-	0.21					
6	SR TB Large	B	6.63	0.22	-	*	*	10.67	X	-	X	X	-	X	0.00	41.08	58.92	6	428.52
		SE	2.76	0.04	-	*	*	2.64	X	-	X	X	-	X					
7	SR TB Large	B	9.94	X	-	*	*	10.45	X	X	2.00	X	-	X	21.86	31.86	46.28	6	431.03
		SE	3.21	X	-	*	*	2.67	X	X	0.53	X	-	X					
8	SR TB Large	B	7.94	X	-	*	*	10.48	X	X	X	0.45	-	X	9.02	35.40	55.58	6	429.29
		SE	2.89	X	-	*	*	2.66	X	X	X	0.09	-	X					

[a]Intercept; [b]Alkalinity; [c]Dissolved Organic Carbon; [d]degrees of freedom; [e]Akaike's information criterion corrected for small sample size bias; "X" appears where a variable was not included in the maximal model; '-' denotes where a variable was eliminated; '*' represents where minimal adequate models were identical for TP and TN models;

(a)



(b)

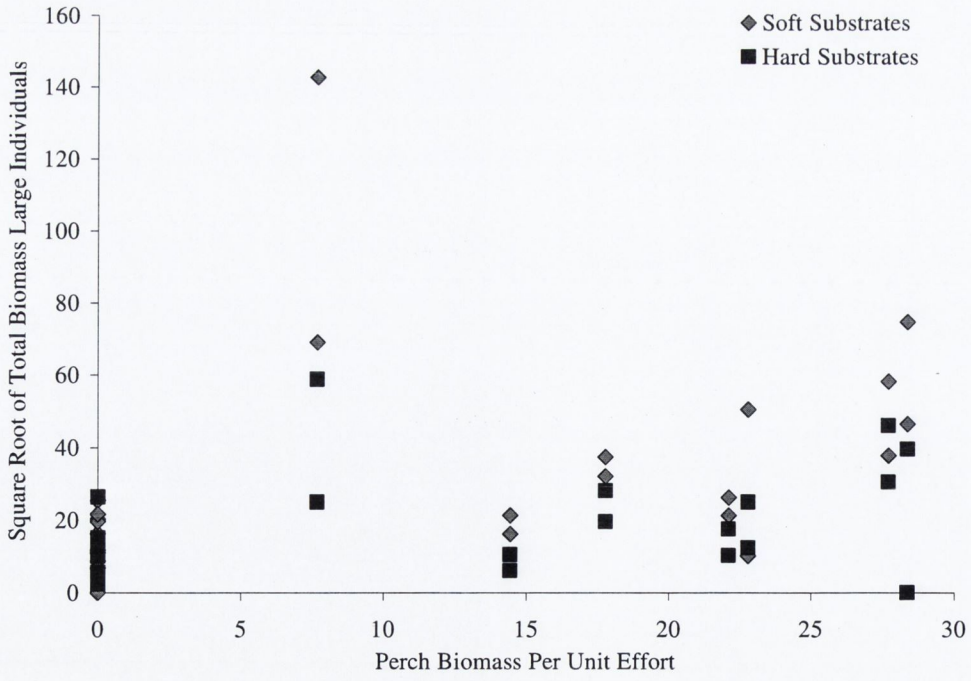


Figure 3.24: The relationship between the square root of the total biomass of large individuals and perch biomass per unit effort in both substrates predicted by the model (a) and from the observed raw data (b).

The salmonid model eliminated salmonid bpue from the minimal adequate model, retaining alkalinity and substrate only (Table 3.18). Alkalinity displayed a positive coefficient with a low SE, indicating an increase in the square root of TB "large" with increasing alkalinity. Substrate displayed a very high coefficient with relatively low SE, with a higher value than that of the intercept, indicating that the sr TB "large" was much greater in soft substrates. The pike model retained pike bpue and substrate in the minimal adequate model (Table 3.18). Substrate displayed a high positive coefficient similar to that in the salmonid model, signifying a large increase in the sr TB "large" in soft substrates. The positive coefficient of the pike bpue main effect also indicated that sr TB "large" increased with increasing biomass of pike (Table 3.18). The cyprinid model retained substrate and cyprinid bpue in its minimal adequate model. The coefficient corresponding to substrate was similar in value and SE to that of the pike and the cyprinid models, depicting a large increase in the square root of total biomass of large individuals in soft substrates. The coefficient of the cyprinid bpue main effect displayed a small positive value, indicating a small increase in sr TB "large" with increasing cyprinid biomass (Fig. 3.25).

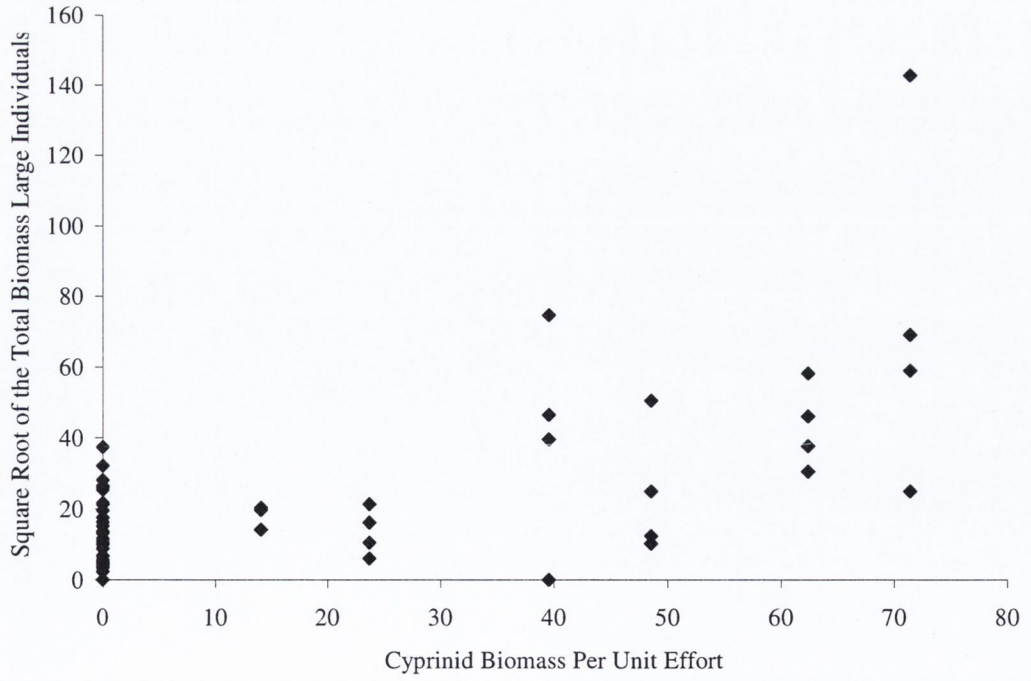


Figure 3.25: The relationship between the square root of the total biomass of large individuals and cyprinid biomass per unit effort plotted based on raw data.

The lowest AIC_C value among the four minimal adequate models was displayed by the salmonid model, which included only alkalinity and substrate (Table 3.18). However, it did not differ by >2 than the perch or cyprinid model, and thus, these three models explain the data equally well. The AIC_C value of the pike model was greater than that of the remaining three models by >2, indicating that the data does not to fit the model as well as the other three models (Table 3.18). Of these three models, the principle of parsimony favours the salmonid model, which had the fewest variables included (Burnham and Anderson, 2004). However, both the perch and cyprinid models can only be compared with the minimal adequate salmonid model, and not to each another, as each has different terms included. The remaining terms in the salmonid model (alkalinity and substrate) are found within each of these other models. Both the perch and the cyprinid minimal adequate models include terms which upon exclusion increase the AIC value by >2 (the perch model retained perch bpue and the perch-substrate interaction, and the cyprinid model retained cyprinid bpue). Thus, the terms decrease the fit of the data to the model, and after having been tested in a ML estimated log-likelihood ANOVA test, the included terms significantly improved the fit of the model. However, the AIC_C value, derived based on REML estimation, indicates little difference among these models, suggesting the most parsimonious model (i.e., the salmonid model) is the best fit. Owing to these differences in the results from the ML and REML based estimations, no model emerges as the best fit to describe the data. Thus, all three models are considered equal alternatives.

3.4 Discussion

Among models used to investigate the influences on biomass attributes of littoral invertebrates, the effect of substrate was shown to be of prime importance, where greater mean individual size and total biomass, and more organisms in the large size classes were found on soft substrates. TN explained slightly less variability than substrate, but consistently displayed a better fit to the data than either TP or chlorophyll *a*, mediating a curvilinear change in almost all models of biomass and size spectra. Alkalinity consistently explained a marginal amount of variation among samples in all biomass based models and almost all size spectra models. DOC explained variation in almost all size spectrum models but not in mean or total biomass. Total biomass, mean size and size spectra displayed remarkable consistency among lakes, suggesting biomass as an important emergent property of lake ecosystems.

It has been hypothesized that littoral macroinvertebrate size spectra are as variable within as among lakes (Hanson, 1990). The results presented in this Chapter suggest that the size structure, mean individual and total biomass of littoral macroinvertebrate communities were more variable within lakes (among substrates) than they were among lakes varying widely in water chemical conditions. Each of the best fitting minimal adequate models are discussed in turn; all other models are not discussed.

Based on REML estimated AIC values, seasonal effects did not explain any additional random variation in single metrics, such as size diversity, mean individual size, total biomass and total abundance, and thus was excluded as a random effect from the models. Seasonal differences were found, however, to explain random variation in all models of size structure (Tables 3.16 and 3.17). In the best fitting quadratic size spectra models, seasonal differences explained almost 40% of variation in hard substrates, and approximately 20% of variation in soft substrates. In the best fitting truncated models, seasonal differences explained less variation; 14% in hard substrates, and 19% in soft substrates. Seasonal changes may be more pronounced for smaller than larger invertebrates, as greater seasonal variation were found on hard substrates in the quadratic dataset compared with soft substrates, where there was a greater abundance of smaller organisms present. Likewise, there was more seasonal variation in the quadratic compared with the truncated dataset. The greatest seasonal variation was found in the

quadratic dataset, on hard substrates, where more large invertebrates were present in spring and more small animals in summer (Fig. 3.5a). The emergence of most univoltine insects in summer (Solimini *et al.*, 2006) may explain the decrease in large animals from summer samples. Previous studies found substantial seasonal variation in the distribution of littoral macroinvertebrates size attributed to emergence of late instars, appearance of new generations, selective predation by fish and breeding mortality of some taxa (Mittelbach, 1981; Hanson *et al.*, 1989). However, Rodríguez & Magnan (1993) found that the biomass spectrum of benthic invertebrates maintained a constant shape across time across three lakes, although invertebrate abundance differed significantly among seasons. In my study of Irish lakes, quadratic size spectra were very consistent among lakes (with low random variance) but not between seasons (high seasonal random variance). Truncated size spectra did not exhibit such a strong seasonal influence, with less consistency among lakes (higher random variance). This may have resulted from the elimination of the smallest individuals from the dataset.

3.4.1 Comparison of quadratic and truncated size spectra

The potential underestimation of small sized animals (i.e., those in size classes 0 or less) as a result of the $1mm^2$ mesh size, is likely to have occurred on a sliding scale, with undersampling of smallest organisms most likely. The underestimation should have been consistent among samples, as all were sampled for the same duration, by the same workers, using the same mesh size. The exclusion of potentially underestimated small organisms from truncated size spectra models could explain the loss of the TN mediated pattern observed in hard substrates in the quadratic dataset (see Section 3.4.5). The full, quadratic, dataset revealed that the size structure of the sampled lakes was best described by a dome, displaying a quadratic relationship in the normalised biomass among size classes (Fig. 3.4). When the dataset was truncated to remove a possible sampling bias, a linear relationship between normalised biomass and size class was evident. The collective slope of all samples (truncated dataset) was very close to -1, indicating an overall trend of equal biomass among size classes, and suggesting the presence of several trophic levels, as hypothesized by Brown and Gilooly (2003).

Benthic invertebrate communities size spectra have most often been characterised by a domed shape, where intermediate sized organisms have the highest density (Morin and Nadon, 1991; Rasmussen, 1993; Rodriguez and Magnan, 1993; Bourassa and Morin,

1995; Morin *et al.*, 1995; Leaper and Rafaelli, 1999; Schmid *et al.*, 2000; Baca and Threkeld, 2000; Solimini *et al.*, 2001; Basset *et al.*, 2004; Solimini *et al.*, 2005). This is similar to the size distribution found in many planktonic communities (Sprules and Munawar, 1986; Strayer, 1986; De Eyto and Irvine, 2007). A characteristic biomass deficiency in the lower size classes has been observed in studies of benthic macroinvertebrates (Hanson *et al.*, 1989; Morin and Nadon, 1991; Rasmussen, 1993). It has been suggested that periphyton may make up a large portion of the biomass in this range, and that the size spectrum would exhibit a better regression if periphyton were included in the analysis (Morin and Nadon, 1991; Rasmussen, 1993). Nevertheless, a study on sieve retention probabilities suggest that smaller individuals may be underestimated (Morin *et al.*, 2004).

Whether the size structure of the benthic macroinvertebrate community is best described by a dome or a linear relationship remains to be established. Several authors argue that subsets of the spectrum such as the benthic and planktonic communities of lakes are more accurately described by quadratic terms rather then linear ones (Baca and Threkeld, 2000; Maxwell and Jennings 2006; De Eyto and Irvine, 2007). The strong curvilinear relationship between size spectra and TN was lost in hard substrates when the dataset was truncated. However, the small DOC mediated change in the size spectrum seen in soft substrates in the truncated analysis, was not observed in the quadratic dataset. The quadratic datasets also included a much larger number of samples, with more detailed size distribution for each sample, and so are arguably more representative of size spectra across lakes; because the sampling method was the same at all sites, any underestimation owing to un-retained animals, is likely similar among all sites. Consistent patterns in the size structure with different water chemical variables also emerged in spite of potential sampling error, and truncation of the data led to changes in some relationships.

3.4.2 The effect of substrate on best fitting models

Increases in mean body-size, total biomass and normalised biomass of littoral macroinvertebrates on soft compared with hard substrates were evident (Tables 3.15 - 3.18, and Figs. 3.4 and 3.5). These result concur with hypothesis (c) that size spectra differ among substrates, with an increase in larger invertebrates in soft substrates, owing to a higher presence of macrophytes and macroalgae. Models of the size diversity and

log abundance of benthic macroinvertebrates were the only models where no differences among substrates were observed. The hard substrates examined were structurally simpler than soft substrates, encompassing gravel, pebbles, cobbles or bedrock, often with attached algae but rarely with macrophytes. This is in comparison with the more complex structures frequently associated with soft sediments, such as rooted macrophytes and macroalgae. A rise in the abundance and biomass of benthic macroinvertberates with increasing macrophyte or macroalgal cover has been well documented (Gerking, 1957; Gerrish and Bristow, 1979; Wiley *et al.*, 1984; Rasmussen, 1988; Paukert and Willis, 2003; Tolonen *et al.*, 2003; Michaletz *et al.*, 2005). It has been hypothesized that this results from increased food resources and reduced fish predation, coupled with increases in large mobile invertebrates and invertebrate predators, high heterogeneity and more perceived space with the greater structural complexity of the habitat (Diehl, 1992; Zimmer *et al.*, 2001; Tolonen *et al.*, 2003; McAbendroth *et al.*, 2005). Such an explanation concurs with the greater mean size and total biomass of macroinvertebrates found in soft compared with hard substrates (Table 3.15).

In some instances, size spectra were found not to vary among substrates in lakes (Rodriguez and Magnan, 1993) and streams (Bourassa and Morin, 1995; Solimini *et al.*, 2001). In contrast, Hanson (1990) found large differences in the size distributions of two contrasting macrophyte communities in a small deep Canadian lake. This finding was supported by examination of size spectra from 53 sites in 11 lakes in Quebec, where plant community structure was found to be important in determining size spectra variation (Rasmussen, 1993). Size structure varied among sites with macrophyte communities differing in density and species in a calcareous Irish lake (McGoff, 2009). The size spectra of fishless wetlands were found to differ from wetlands containing fish, with an increase in the intercept (and in the normalised biomass of almost all size classes) but no significant change in the slope of size spectra (Zimmer *et al.*, 2001). In the quadratic dataset, the reduced normalised biomass in the large size classes of invertebrates found in hard substrates, indicating fewer large individuals in hard substrates than in soft substrates (as normalised biomass is approximately equal to log abundance; equation [ii]), but similar intermediate and small sized organisms in hard and soft substrate (Table 3.16), reflects: (i) decrease in mean size; (ii) decrease in total biomass; and (iii) change in shape of the quadratic size spectra observed in hard

substrates, compared with soft ones (Tables 3.15 and 3.16; Figs. 3.4 and 3.5). However, the identical slope observed for the best fitting models of hard and soft substrates for the linear truncated dataset indicate that there was no change in the size structure of the truncated dataset among substrates. Rather, the increase in the intercept for the soft substrates indicated that all size classes had more biomass in soft substrates.

Water chemistry appeared to mediate changes in macroinvertebrate size across both substrates. That coefficient values for substrate were the highest among fixed effects of models of total and mean biomass, indicates greater within-lake (between hard and soft substrates) than among-lake variance. Nevertheless, differences were evident in the responses of littoral macroinvertebrate normalised biomass to water chemical changes among lakes for each substrate. Zimmer *et al.* (2001) attributed increased abundance of large bodied invertebrates in proximity to macrophytes partially to the structural complexity of macrophyte beds. Within macrophyte beds, Tolonen *et al.* (2003) also found an increase in large mobile taxa sensitive to fish predation; taxa which were virtually absent from the open water zone. Hanson (1990) found that weed-beds dominated by macroalgae such as *Chara* sp. contained ten times more large organisms to those dominated by structurally less complex, rooted, plants. Each of these studies linked structural complexity to refuge from fish predation; a hypothesis that has been proposed many times (Gilinsky, 1984; Hershey, 1985; Winfield, 1986; Diehl, 1988; 1992; Zimmer, 2001; Tolonen *et al.*, 2003). Fish, as predators, usually prefer large and mobile prey, which they search for actively (Wellborn *et al.*, 1996). Conversely, many invertebrate predators prefer small, sessile or sluggish prey (Allan *et al.*, 1987). This leads to habitats subject to high fish predation, such as the open water zone, favouring invertebrate prey species of low activity and small body size; in contrast with habitats subject to high invertebrate predation, such as dense macrophyte beds, favouring high activity and large body size (Crowder and Cooper, 1982; Mittelbach, 1988; Wellborn *et al.*, 1996; Tolonen *et al.*, 2003). Similar mechanisms allowing for a greater abundance of larger animals may have been operating in the Irish lakes.

An additional potential explanation for the patterns observed was proposed by Strayer (1991; based on a model suggested by Lodge *et al.*, 1988), that small sized fauna are more effective in exploiting small and sparsely distributed patches of high-quality food amidst high volumes of sediment which may lead to a greater specialisation of diet in

smaller non-predatory benthic animals. Conversely, large body size could be favourable to benthic invertebrates in dense vegetation beds with ample high quality food in the form of decaying macrophytes, root exudates and bacteria associated with plant roots, periphytic algae and retained allochtonous detritus, leading to greater omnivory among larger non-predatory benthic invertebrates (Strayer, 1991). However, it has also been reported that the size spectra of benthic invertebrates in the littoral zones of lakes (Rodriguez and Magnan, 1993) and streams (Morin *et al.*, 1995) did not change among substrates. My results demonstrated that, comparing macroinvertebrate communities on hard and soft substrates (i) the community were on average larger, with a greater collective biomass on soft substrates, and (ii) changes in size spectrum with water chemistry differed among substrates, supporting the view that the presence of macrophytes and macroalgae on soft substrates affects macroinvertebrate size spectra.

3.4.3 The effect of alkalinity on best fitting models

The retention of alkalinity in almost all models of macroinvertebrate abundance and biomass, coupled with the consistent small positive value of the main effect in all models where it was retained, indicates that alkalinity plays a consistent, albeit marginal, role in explaining differences in the body size, biomass and abundance of macroinvertebrate communities across the study lakes. Alkalinity was only excluded from the model of normalised biomass among size classes from hard substrates based on the quadratic dataset and the model of size diversity (which retained no fixed effects). Macroinvertebrate communities in acidic waterbodies are reported to have a lower abundance and taxa richness than those found in circumneutral or alkaline water bodies (Økland and Økland, 1986; Sutcliffe and Hildrew, 1989; Jeffries and Mills, 1990; Johnson *et al.*, 1993; Allott *et al.*, 1997; Tierney *et al.*, 1998; Dangles *et al.*, 2004). Dangles *et al.* (2004) demonstrated that taxonomic richness of benthic invertebrates in three naturally acid streams were not significantly different from three circumneutral control streams in northern Sweden, contrasting with a data-set of French streams representing a gradient from circumneutral to acidified streams, which showed decreasing richness to be typical of acidified streams. Nevertheless, the species composition differed among streams of different pH in both data-sets (Dangles *et al.*, 2004).

Macroinvertebrate size structure differed between substrates and datasets in relation to alkalinity (Tables 3.16 and 3.17). However, the coefficients of the alkalinity terms, where retained, had similar values of <0.01, indicating a marginal change in the size structure with alkalinity. Alkalinity mediated a change in normalised biomass in the truncated model of hard substrates, and in both quadratic and truncated models of soft substrates, where the intercept of normalised biomass among size classes increased marginally with alkalinity, and mediated a change in the slope of the NB-SC relationship in the truncated size structure of hard substrates and the quadratic size structure of soft substrates (Tables 3.16 and 3.17). The interaction term was approximately equal for both models where the slope of the NB-SC relationship was changed, and overall the data suggest that high alkalinity was associated with enhanced normalised biomass of large organisms on both hard and soft substrates. In hard substrates, the deletion of the alkalinity term from the quadratic model of size structure, contrasts with the increase in intercept and change in slope of the NB-SC relationship with alkalinity in the truncated model. This suggests alkalinity mediated a change in the normalised biomass among larger size classes (and not among small size classes) in hard substrates. Thus, the results are broadly in agreement with the hypothesis (d), that the size spectrum intercept would decrease with decreasing alkalinity, although the changes with alkalinity were more pronounced in larger size classes.

The small coefficients of all alkalinity terms, indicating very small increases in the body size, collective biomass and in the slope of the size structure with increasing alkalinity, may also reflect differences in the taxonomic composition of acidic waterbodies. For example, some coleopterans (e.g., Elmidae) and many ephemeroptera species disappear with increased acidity (Harriman and Morrison, 1982; Stoner *et al.*, 1984; Tierney *et al.*, 1998), and crustaceans and molluscs are vulnerable to low calcium levels (Økland, 1980, as cited by Schartau *et al.*, 2008). Low alkalinity waters can often have rich acid-tolerant invertebrate faunas (such as species of *Leuctra* stoneflies (Hynes, 1970). Johnson *et al.* (1993) proposed that benthic abundance and biomass are unreliable indicators of acid stress, because acid-tolerant taxa replace acid-sensitive ones. Holmgren (2001) found, nevertheless, that the body size distributions of macroinvertebrates sampled from six limed lakes were more similar to four circumneutral lakes than to that of two acidic lakes. Furthermore, Strayer (1991)

suggested that the size spectra of the zoobenthos may vary among lakes as a function of water hardness.

The results from the Irish lakes presented here indicate that abundance, biomass and size structure of macroinvertebrates from the low alkalinity lakes differed only slightly from that of higher alkalinity lakes, with (i) a slight increase in the mean size, total biomass and total abundance of macroinvertebrates and (ii) a marginally greater increase in the biomass and abundance of large individuals within the macroinvertebrate size structure with increasing alkalinity.

3.4.4 The effect of dissolved organic carbon on best fitting models

The elimination of DOC from all models of single metrics indicate that there was no direct relationship between DOC and mean size, total biomass, total abundance and size diversity. However, DOC was shown to mediate a change in the NB-SC relationship in both the quadratic and truncated models of hard substrates, and in truncated models of soft substrates. DOC within lakes may be produced by (i) autochthonous processes (e.g., macrophytes, phytoplankton, animals' inefficient grazing, excretion, defecation and death) or (ii) allochthonous processes (i.e., influx from the surrounding catchment area of the lake; Williamson *et al.*, 1999; Biddanda and Cotner, 2002). It is hypothesised that lakes are principally autotrophic (and thus, in near metabolic balance) when DOC concentrations are <5 mg l^{-1} and principally heterotrophic at higher concentrations (Prairie *et al.*, 2002; Hanson *et al.*, 2003). Only seven of the forty eight lakes had concentrations of DOC below this concentration, indicating most lakes had allocthonous organic carbon contributing to the lake carbon budget. Allochthonous DOC has been suggested to play a pivotal role in lake respiration and metabolic processes (Wetzel 1992; Dillon and Molot 1997; Prairie *et al.*, 2002; Hanson *et al.*, 2003), fueling a large part of bacterial metabolism (Wetzel, 1992; Tranvik, 1992) and associated with high bacterial production (Hessen, 1992). This may explain the increase in small individuals on hard substrates with increased DOC shown in the quadratic TN model, as increased bacterial production with rising DOC concentrations may have caused an increase in primary consumers. The absence of the smallest benthic animals in the truncated models of size structure may account for the differences in the patterns among quadratic and truncated models of hard substrates. Models of both hard and soft substrates of the truncated dataset showed an almost

identical response to DOC, where the smallest animals decreased with increasing DOC, suggesting increased energy efficiency through the food web with DOC. However, as the scale of the spectrum excludes the smallest organisms, these "small" animals are the "intermediate" sized animals of the quadratic spectrum and may not represent microbial consumers (Figs. 3.13b, 3.19b and 3.21b). The change in the size structure seen in the quadratic model of hard substrates, in contrast, indicates a biomass accumulation in the smallest animals with DOC (and thus decreased efficiency of energy transfer with DOC) and a comparatively marginal change among the larger animals in the size range examined in the truncated dataset. Thus, the results from the truncated models merit cautious interpretation.

3.4.5 The effect of nutrient enrichment on best fitting models

TN consistently explained variation in mean and total biomass, as well as size spectra of littoral macroinvertebrates among lakes. In all models where enrichment terms were retained, TN provided a better fitting model than models including TP or chlorophyll *a*. The curvilinear response in the total and normalised biomass of benthic invertebrates suggests an association with TN optima for benthic invertebrates standing biomass, although differing between hard and soft substrates. A strong correlation between TP and chlorophyll *a* concentrations in lakes has been well documented (Dillon and Rigler, 1974; Jones and Bachman, 1976; Canfield and Bachman, 1981; Jeppesen *et al.*, 2005), and it is frequently assumed that lake primary production is phosphorus limited. However, Smith (1982) demonstrated that the TP:TN ratio also influenced chlorophyll *a* concentrations among 228 lakes. Likewise, Phillips *et al.* (2008) found that although TP was a stronger predictor of chlorophyll *a* concentrations, TN concentrations were also significantly related to chlorophyll *a*. Increased nitrogen concentrations were demonstrated to have a negative effect on the species richness of floating and submerged macrophytes (James *et al.*, 2005) and combined TP and TN treatments were shown to significantly reduce biomass of submerged macrophyte species, less than those in single nutrient treatments in 24 experimental field exposures in a shallow lake (Gonzaléz *et al.*, 2005). Donohue (2008) found planktonic algae were nitrogen limited in four of nine Irish study lakes, and benthic algae were nitrogen limited at seven of the nine lakes, where the isotopic ratios of both algal forms were examined. The nine lakes examined by Donohue (2008; four of which were also examined in my study) exhibited a nutrient gradient similar to that of my lakes. Therefore there is some evidence to

suggest that below an optimal TN concentration, littoral macroinvertebrate biomass in lakes may be affected directly or indirectly by nitrogen limitation.

3.4.5.1 Models of single metrics

In one of the two best fitting models, mean individual size displayed a linear increase with TN (Table 3.15). Although the enrichment term was excluded from the second best fitting model of mean size, ML estimated AIC indicated that the former model (i.e., including TN) explained the data best (Table 3.15). This is contrary to that of hypothesis (a) that a decrease in mean individual body size would occur with nutrient enrichment. However, these findings are in agreement with observational studies that examined the change in benthic invertebrate body size in response to nutrient enrichment (Morin *et al,*. 1995; Bourassa and Morin, 1995). Results from gradually enriched experimental mesocosms also suggested a greater mean body size of sediment-associated invertebrates in fertilized treatments than in controls (Blumenshine *et al.*, 1997). In contrast, however, several authors have reported a significant increase in small invertebrates with enrichment (Hayward and Margraf, 1987; Rasmussen, 1993; Bourassa and Morin, 1995). Other studies in lakes (Cyr and Peters, 1996) and impoundments (Michaletz *et al.*, 2005) found no change in the mean size of macroinvertebrates with nutrient enrichment. Although it appears from the literature that there is large variability in the response of mean invertebrate size to nutrient enrichment, many of these studies attributed changes observed to effects on taxonomic composition with eutrophication (Hayward and Margraf, 1987; Rasmussen, 1993; Blumenshine *et al.*, 1997; Michaletz *et al.*, 2005). Such changes have been well documented (White, 2001; Tolonen *et al.*, 2001; Romanuk *et al.*, 2006; Donohue *et al.*, 2009), although it is far from clear what mechanisms drive such changes.

Macroinvertebrate total biomass was observed to have a curvilinear relationship with TN in one of two best fitting models of log total biomass (Table 3.15). Although the enrichment term was excluded from the second best fitting models of total biomass, ML estimated AIC indicated that the former model explained the data best (Table 3.15). This finding is only partially in keeping with hypothesis (a) which predicted that the total biomass would increase with nutrient enrichment, as although total biomass did initially increase with TN, above an optimal TN concentration total biomass declined with TN. Boudreau and Dickie (1992) demonstrated that the intercepts of biomass size

spectra appeared to increase with productivity. This has been found in many pelagic studies (Peters, 1983b; Ahrens and Peters, 1991; Cyr and Peters, 1996; Baca and Threkeld, 2000; De Eyto and Irvine, 2007). Rasmussen (1988) found a significant increase in total zoobenthic biomass with increasing chlorophyll *a* concentrations in a study of 71 sites on 12 lakes. Similarly, nutrient enrichment within streams (Morin *et al.* 2001), as well as within experimental mesocosms (Baca and Threkeld, 2000), has resulted in increases in the total biomass of benthic invertebrates.

In terms of the extent of the nutrient gradient and quantity of lakes examined, this study represents, to the best of my knowledge, the largest study of lake littoral benthic community biomass to date. Consequently, it is possible that some previous studies did not have sufficient data from lakes above an optimal nutrient concentration to reveal the curvilinear change in total invertebrate biomass with enrichment, thus revealing only a linear increase. There is a clear need for further investigation into this curvilinear change, based on large datasets with a broad TN or nutrient gradient.

Nutrient enrichment did not appear to alter the total abundance of animals among samples, as all nutrient terms were removed (owing to redundancy) from all models of log total abundance, leaving a single minimal adequate model remaining (Table 3.15). Although macroinvertebrate abundance has been shown to be positively correlated with lake productivity and nutrient enrichment (Bourassa and Morin, 1995; Blumenshine *et al.*, 1997; Donohue *et al.*, 2009), other studies have shown no significant change in abundance with nutrient enrichment (Dinsmore *et al.*, 1999; Paujkert and Willis 2003; Michaletz *et al.*, 2005).

3.4.5.2 Size spectra models

The best fitting quadratic models of macroinvertebrate size structure for both substrates displayed linear and quadratic changes in the size spectra slope with TN (Table 3.16; Figs. 3.13 and 3.16). The size spectra intercept also significantly increased with TN in hard, but not soft, substrates (Table 3.16). The greatest changes in normalised biomass with changing TN concentrations, were observed in the large size classes for both models with only a minimal change in smaller size classes. With increasing TN, normalised biomass increased with increasing TN in all size classes in both quadratic size spectra models, up to a nutrient optima, after which the nature of the curvilinear

change differed between substrates (Figs. 3.13a and 3.16). Above this optimal concentration, normalised biomass of all size classes $\geq$ -5 decreased in hard substrates, in contrast with the normalised biomass on soft substrates, which increased in intermediate size classes and decreased in the smallest and largest size classes, suggesting that most invertebrates in hard substrates were negatively affected by high concentrations of TN, but only the largest (and, to a very small extent, the smallest invertebrates) in soft substrates were negatively affected by TN.

In contrast, truncated size spectra models showed no change in size spectrum slope with TN in either substrate, although the size spectrum intercept in soft substrates displayed a curvilinear response to TN. The best fitting truncated model of the size structure of hard substrates excluded all TN terms, and variance among samples for this dataset was explained by alkalinity and DOC changes (Table 3.17; Fig. 3.19b). Similar to the intercept of the quadratic size spectra model of hard substrates, the intercept of the truncated size spectra of soft substrates increased with TN until a optima was reached, which then declined, at the highest TN concentrations (Table 3.17; Fig. 3.21a). Contrary to hypothesis (a) that size spectra slopes would become steeper with nutrient enrichment reflecting increases in smaller organisms relative to larger organisms, the biomass of small organisms appeared to vary little and the biomass of larger organisms displayed a much greater curvilinear change with nutrient enrichment (with the exception of the quadratic model of hard substrates). Hypothesis (b) which predicted that the intercept would increase with nutrient enrichment, was observed initially, until an optimal TN concentration, but differed from the prediction above this TN concentration where the intercept declined with TN.

The observed effect of nutrients on size spectra present a number of interesting ecological questions:

(1) Why did the quadratic models of hard and soft substrates differ?

(2) Why did quadratic and truncated size spectra models differ in their response to TN?

(3) Why did larger animals, and not smaller animals, show the greatest fluctuation in biomass with TN?

(4) What caused the curvilinear response to TN?

Why did the quadratic models of hard and soft substrates differ?

Size spectra in some studies were found to vary with changes in vegetation density, biomass and community structure in lakes (Hanson, 1990; Rasmussen, 1993; McGoff, 2009). However, size spectra in other studies were invariant among substrates in lakes (Rodriguez and Magnan, 1993) and streams (Bourassa and Morin, 1995; Solimini *et al.*, 2001). Nevertheless, the influence of nutrients on littoral macroinvertebrate communities has been found to vary depending on habitat or substrate sampled (Brauns *et al.*, 2007; Donohue *et al.*, 2009; Free *et al.*, 2009). Macrophytes and benthic algae affect nutrient and light availability by sequestering nutrients (Hanson 1990), stabilizing sediments, and reducing phosphorus mobility (Carlton and Wetzel, 1988). Donohue *et al.* (2009) found the abundance and taxonomic composition of benthic invertebrates on hard substrates was better related to TP and chlorophyll *a* concentrations than on soft substrates. Brauns *et al.*, (2007) found that community composition on sandy substrates significantly correlated with TP concentration, but was less important than wind exposure in explaining changes in community structure on reed beds and stony habitats. The slightly different significant relationships between TN and size structure in different substrates found in my work may, therefore, reflect differences in the ability of the associated habitat to buffer nutrient effects (e.g. soft substrates with macrophytes and stony substrates with no cover). It may also reflect changes in the taxonomic composition of the different substrates, found in an abundance based study which included these samples as a subset of a larger dataset (Donohue, 2008).

Why did quadratic and truncated size spectra models differ in their response to TN?

In hard substrates, the significant change in the slope and intercept of the quadratic size spectra was not reflected in the truncated size spectra, where all TN terms were eliminated from the model. However, 50 samples were excluded from the quadratic (whole) dataset, owing to an insufficient number of data-points in size classes ≥ 1 in these samples (see Section 3.2.4.1.5 for more detail), leaving 45 hard samples remaining in the truncated dataset (Table 3.9). Furthermore, although the TP and TN gradient within the truncated dataset were similar to that found in the full quadratic dataset, only three samples of hard substrates had TN concentrations greater than the approximate optimal concentration of 1.5 mg l^{-1}, compared with eight samples of both hard and soft substrates in the quadratic dataset. Thus, it is likely that the exclusion of more than half

of the hard samples from the truncated dataset may have obscured the pattern detected in the quadratic models of hard substrates (Tables 3.13 and 3.15, Fig. 3.13).

In soft substrates, TN mediated a curvilinear change in both the truncated and quadratic best fitting size spectra models; however, this change occurred in the intercept of the truncated model, and in the slope of the quadratic model (Tables 3.16 and 3.17; Figs. 3.16a and 3.21). Although the biomass of all size classes present in both models (≥ 1) increased with increasing TN concentrations (up to the hypothesized optimal TN concentrations) in a similar pattern in both models, at high TN concentrations (above the optima) the change in slope seen in the quadratic model was not reflected in the truncated model (Figs. 3.16 and 3.21). Twenty seven soft samples were excluded (from the whole dataset), leaving 53 samples for the truncated dataset. Only six of the eight samples with TN concentrations higher than the optima remained in this truncated dataset, and the exclusion of these two samples may explain the loss of this pattern from the truncated dataset at high TN concentrations. In summary, the exclusion of data-points and elimination of 77 samples (correction because of the use of the 1 mm^2 mesh size), may have obscured the size-structure-nutrient relationship.

Why did larger animals, and not smaller animals, show the greatest fluctuation in biomass with TN?

In quadratic size spectra models of both substrates, the greatest response to increased TN concentrations was observed in the largest size classes, although the effect was muted in hard substrates (Figs. 3.13 and 3.16). Several possible explanations are examined:

(a) that "snapshot" sampling does not sufficiently describe variation in smaller macroinvertebrates among lakes, owing to greater energy fluctuation, and gives a less accurate representation of annual production than larger animals;

(b) that larger organisms having a higher sensitivity to nutrient change;

(c) that a change in the competitive ability of non-size structured large primary consumers occurs; or

(d) that a greater energy transfer efficiency occurs in the size structured component of the benthic food web, represented by predator-prey interactions, to the largest predators.

As smaller animals often have a higher associated energy flux, owing to a tendency for higher population turnover rates, standing biomass of smaller animals may be considerably less at a given moment than their annual production, compared with larger animals (Damuth, 1981) and, thus, a "snapshot" study such as this one reflects changes in the production of larger animals better than changes in smaller ones. Nevertheless, population biomass has been shown to correlate with annual production in animals of different sizes (Waters, 1977; Banse and Mosher, 1980; Stephenson and Morin, 2009). Predictions from quadratic models of both substrates show greater variability in the collective biomass of larger size classes, compared with relative stability in the smaller size classes with TN (contrasting with the hypothesized greater energy fluctuation among smaller animals; Figs. 3.13a and 3.16), rather than the expected high variability in smaller animals owing to high turn-over, and thus, "snapshot" sampling does not appear to explain the greater change in the biomass of larger animals with TN.

Another possible explanation relates to a higher sensitivity of larger organisms to nutrient enrichment. Large rare species, are usually the first lost with system disturbance (Petchey *et al.*, 1999; Raffaelli, 2004; Woodward, 2009). However, in our study individuals of all sizes increased with increasing TN until the optima, (larger individuals by a greater proportion than smaller ones) above which larger individuals suffered the largest decreases in collective biomass. Owing to the presence of many large microphagous organisms (i.e. filter feeders and deposit feeders) occurring within benthic invertebrate communities, a direct "bottom-up" effect (i.e. increased primary producers with TN in turn leading to increased primary consumers), may not result in a direct increase in the collective biomass of the smallest size classes, as seen in pelagic communities (Rasmussen, 1993). These large primary consumers and detritus feeders distort the size structure and energy flow relationship of predator-prey interactions observed in pelagic communities (Rasmussen, 1993), and may result in increases of animals of all sizes with greater primary production. However, at high nutrient levels with potential loss of food resources (e.g. through increased turbidity), it is likely that the larger, less abundant, and thus more vulnerable individuals were lost first as predicted by Petchey *et al.* (1999) Raffaelli (2004) and Woodward (2009).

Bourassa *et al.* (1995) also found that the abundance of the largest benthic invertebrates in streams increased more, with increasing phosphorus concentrations, than smaller

animals. The authors hypothesised that this may reflect the ability of larger invertebrates to sequester food and/or space from their smaller competitors in eutrophic conditions, or simply, that only the large invertebrates are food limited, which may also explain the initial patterns of greater increase in larger invertebrates in my study.

It is also possible that the size structured component of the community had increased energy transfer efficiency under increasing TN concentrations below a TN optima. The shift from benthic to pelagic primary production observed with eutrophication is predicted to distinctly alter energy flow through lake food webs (Vadeboncoeur *et al.*, 2003). In size structured communities, where size structure represents energy flow through the system, a steeper more negative slope indicates less efficient energy transfer among trophic levels, and an increase in smaller organisms relative to larger organisms (Borgman, 1982). Accordingly, the increase in large organisms with increasing TN concentrations to an optima may indicate that, for the size structured component of the benthic invertebrate community (i.e., that represented by predator-prey interactions), energy flows through the food web to the largest predators more efficiently.

What caused the curvilinear response to TN?

The response of both stream and lake macroinvertebrate communities' size spectra to nutrient enrichment has varied among studies (Rasmussen, 1993; Cyr and Peters, 1996; Michaletz *et al.*, 2005). Although studies of stream benthic invertebrate size spectra have shown a shift towards larger organisms with increasing nutrient concentrations (Morin *et al.*, 1995; Bourassa *et al.*, 1995), none have demonstrated a curvilinear change in this relationship at high nutrient concentrations. Several reports have found little change in benthic invertebrate size structure with increasing enrichment of various water bodies (Solimini *et al.*, 2001 [single stream]; Cyr and Peters, 1996 [24 lakes]; Michaletz *et al.*, 2005 [30 impoundments]). Other studies have reported an increase in the biomass of smaller organisms with nutrient enrichment (Hayward and Margraf, 1987; Rasmussen, 1993). In an experimental study where nutrients were added to mesocosms, and a shift towards smaller benthic and pelagic organisms was recorded, the authors conceded that the temporal scale may not have been sufficiently long to show increases in larger consumers and that, owing to the time of year when the study was conducted, low consumption may have reduced trophic transfer of energy (Baca and Threkeld, 2000).

Changes in the taxonomic composition of the community with nutrient enrichment are well documented (White, 2001; Tolonen *et al.*, 2001; Romanuk *et al.*, 2006; Brauns *et al.*, 2007). In such cases, species sensitive to changes in environmental quality may be replaced by more tolerant species (Hellawell, 1986), possibly changing predator-prey interactions affecting energy transfer. A decrease in larger predators under high nutrient conditions may explain the increase in intermediate size organisms, with the latter relieved from predation pressure and a decrease in large organisms above the TN optima in the soft samples. A decrease in all size classes above a putative TN optima was documented in all but the smallest size classes on hard substrates, suggesting a decrease in food resources for all invertebrates on hard substrates under high nutrient conditions. Jeppesen *et al.* (2003) documented increasing zooplankton biomass with increasing TP concentrations and an increase in grazing pressure was recorded with increasing lake trophic status until mesotrophic nutrient concentrations were reached. The authors also observed a change in grazing pressure and in zooplankton body size in eutrophic lakes, potentially resulting from a change in primary production with increasing filamentous cyanobacteria. Jeppesen *et al.* (2003) predicted increases in benthic production (benthic invertebrates, algae or detritus) under eutrophic conditions owing to low zooplankton grazing and high sedimentation of phytoplankton. Putative changes in predation and competition may have resulted from changes in the parts of the food web which were beyond the scope of this study with TN, such as food sources (benthic and planktonic algae, detritus), pelagic competitors (zooplankton; small benthivorous fish) or fish predators.

Comparing the results of many different studies is difficult owing to the differences not only spatial and temporal scale, but also in the range of nutrients examined (Table 3.19). For example, comparing studies that documented a shift towards larger organisms with increasing nutrients, Morin *et al.* (1995) documented TP ranges less than 60 $\mu g\ l^{-1}$ and although Bourassa and Morin, (1995) studied streams with higher TP concentrations; of nine study streams only two had TP concentrations greater than 70 $\mu g\ l^{-1}$. It is possible that a curvilinear change in size structure with nutrient enrichment, if present, would not have been detected in these cases as the gradient of enrichment was not large enough. However, many hydrological, hydromorphological, abiotic and biotic differences separate the abundance and biomass of benthic invertebrate stream communities from

those of lake communities, and thus, the results from stream studies are not always comparable with lake benthic invertebrate studies.

Two studies documented no change in size structure with enrichment. Solimini *et al.* (2001) recorded only the available nutrients, where soluble reactive phosphate and NO_3-N had a range of between 190 – 680 $\mu g\ l^{-1}$ and 0.63 – 1.44 $mg\ l^{-1}$, respectively, indicating that the TP and TN concentrations were even higher (Solimini *et al.*, 2001). Therefore, the minimum nutrient concentrations for Solimini *et al.* (2001) were high in comparison to my study, and the benthic invertebrate size structure may have changed little, owing to a high baseline of enrichment. Alternatively, the differences among sites may have been explained by a curvilinear change in size structure with enrichment, which was not statistically examined in their study. Likewise, the TP concentrations of impoundments studied by Michaletz *et al.* (2005) were very high, outside the range examined here, and benthic invertebrate communities may stabilise at such high levels of enrichment.

Finally, Baca and Threkeld (2000) observed an increase in smaller animals in response to nutrient enrichment during the enrichment of mesocosms over a nine-week period. The authors recognise that the experimental period may not have allowed sufficient time for the energy to transmit through the food web to allow a change in the biomass of larger organisms (Baca and Threkeld, 2000). However, relatively little is known about the factors that affect the size structure of benthic macroinvertebrate communities, and further research is warranted (Strayer, 1991; Zimmer *et al.*, 2001; Pagola-Carte, 2004).

Both quadratic size spectrum models (both hard and soft substrates) and the truncated model of soft substrates indicated a similar TN optima (Figs. 3.13, 3.16 and 3.21). Models of total and mean biomass also displayed a curvilinear relationship with TN (Table 3.15). TN consistently explained models of benthic invertebrate biomass better than either TP or chlorophyll *a*, suggesting that the benthic macroinvertebrate growth in these lakes is TN limited at low concentrations, and may reflect deteriorating lake condition better than TP or chlorophyll *a* at high TN concentrations.

Table 3.19: Compilation of principal studies examining changes in freshwater benthic invertebrate size structure with nutrient enrichment.

Authors	Waterbody	TP[a] range (µg/L)	TN[b] range (mg/L)	Chl. *a*[c] range (µg/L)	Shift in spectrum (with enrichment)
Bourassa and Morin (1995)	Streams	2-54	0.24- 0.76		↑ larger individuals
Morin *et al.* (1995)	Streams	10-129		0.6 - 7.9	↑ larger individuals
Solimini *et al.* (2001)	Stream	SRP[4] given	NO_3-N given	3.2 - 33.0	No change
Michaletz *et al.* (2005)	Impoundments	11000-18800	326-2120		No change
Baca and Threkeld (2000)	Mesocosms	0-3500	0-1.7		↑ smaller individuals
Rasmussen (1993)	Lakes	"Oligotrophic" - "Strongly Eutrophic"			↑ smaller individuals

[a]Total Phosphorus; [b]Total Nitrogen; [c]chlorophyll *a*;

3.4.6 Changes in benthic invertebrate biomass with changing fish biomass

Fish predation has been demonstrated to affect the size structure of benthic macroinvertebrate communities (Crowder and Cooper, 1982; Diehl, 1992; Zimmer *et al.*, 2001; Blumenshine *et al.*, 2000). Many fish are energetically dependant on periphyton and zoobenthos as well as phytoplankton for much of their growth and production (Hanson and Legett, 1982; Blumenshine *et al.*, 1997; Blumenshine *et al.*, 2000; Vadeboncoeur *et al.*, 2002; Vander Zanden and Vadeboncoeur, 2002). However, the effects of vertebrate predation on size structure may be weaker in lacustrine zoobenthos than in zooplankton owing to the availability of physical refuges and the widespread occurrence of morphological and behavioural defences in benthic invertebrates (Strayer, 1991). The best fitting minimal adequate model of the biomass of small organisms was empty of all fixed terms and variance was not explained by changes in substrate or water chemistry, nor was it explained by changes in the biomass of the four fish species examined in separate models for the 15 study lakes where data on fish biomass was available (Table 3.18).

In contrast, three minimal adequate models of the biomass of the large organisms fit the data equally well (Table 3.18). The principle of parsimony should lead to the models with the least terms (models 6 and 8) being favoured. Model 6 indicates that a greater abundance (or collective biomass) of large animals was seen in soft substrates, and total biomass of large animals increased with increasing alkalinity (Table 3.18). This reflects the general relationships described in this study, where the mean biomass, total biomass and normalised biomass of large size classes of benthic invertebrates was significantly greater on soft substrates, and marginally greater under more alkaline conditions.

Model 5 included the same two terms found in model 6, with two additional terms (perch bpue and perch bpue-substrate interaction). Although these models originally contained different terms, model 6 is a reduced version of model 5. During the reduction of model 5, an ANOVA test based on ML estimations indicated that the elimination of the perch terms would worsen the fit of the model, with an increase in the AIC_C value once the perch (*Perca fluviatilis*) interaction term was removed. Thus, according to ML estimates, model 5 explained the data better than model 6. Nevertheless the standard error of the perch main effect was 5 times that of the coefficient, indicating no linear relationship between the total biomass of large

invertebrates and perch biomass. Furthermore, the positive coefficient of the perch-substrate interaction term indicates that although the total biomass of large invertebrates generally increases with increasing perch biomass in soft substrates, the relationship was not significant in hard substrates (Fig. 3.24b). The standard error of this term is half the value of the coefficient, indicating high variability in this relationship. Given the low number of sample sites where perch was recorded, it is difficult to assess how robust this relationship is. Model 8 depicts an increase in the biomass of large invertebrates on soft substrates, and with increases in cyprinid biomass (Table 3.18; Fig. 3.25). The increase in the total biomass of large individuals with cyprinid biomass is much clearer than that seen for perch. However, as there are very few sites where Cyprinidae were present, these results should be interpreted with care. There is no provision in this analysis for the inclusion of two fish terms in the same model owing to the effects of multicollinearity among fish species. Alkalinity was not included in the cyprinid model for the same reason. Therefore, it is possible that all of these variables contribute to explaining the variation in the total biomass of the large invertebrates.

All three best fitting models of the total biomass of large invertebrates retained the substrate main effect (indicating greater biomass in soft substrates), which consistently explained the greatest amount of variation in large individual invertebrate biomass among samples compared with fish biomass; with nutrients and DOC being eliminated from all models. Thus, within-lake-variation (substrate, fish biomass) was greater than among lake-variation (water chemistry). Substrate also explained most variation in littoral macroinvertebrate biomass in models of all 48 lakes, and equally, biomass was greater on soft substrates for these lakes. Increased food resources, high heterogeneity and more perceived space with the greater structural complexity of the macrophyte stands associated with soft substrates, as well as protection from fish predation among the stands may also contribute to the increase in large bodied invertebrates on soft substrates (see Sections 3.1.5 and 3.4.2 for references). Thus, contrary to hypothesis (e) that collective biomass of small invertebrate would increase with benthivorous fish biomass, the collective biomass of large individuals appeared to increase with fish (cyprinid and perch) biomass.

Fish have been shown to search actively for prey, preferring large and mobile species, in contrast with many invertebrate predators which prefer small, sessile or sluggish

prey, that are easier to capture; this leads to open water habitats being subject to high fish predation, favouring more inactive small invertebrate species, in contrast with dense macrophyte beds subject to high invertebrate predation, favouring highly active large species (see Sections 3.1.5 and 3.4.2 for references). In the current study, the results from model 5 indicated that the total biomass of large individuals increased in soft substrates with increasing perch biomass, but no change occurred in hard substrates with increasing perch biomass (Fig. 3.24b). Likewise, model 8 indicated that the total biomass of large individuals increased on both substrates with increasing cyprinid biomass. These results are contrary to the commonly held view on benthivorous fish effects, which predict that size selective predation by fish reduces mean body size in a community by reducing the mean size of prey species that occur there or by driving larger taxa extinct (Blumenshine *et al.*, 2000; Tolonen *et al.*, 2003; Hall *et al.*, 2007; Townsend and Thompson, 2007).

Both experimental and manipulative studies have indicated that benthivorous fish reduce the number of large-sized active taxa, and result in increases in small cryptic invertebrate species (Crowder and Cooper, 1982; Morin, 1984; Mittelbach, 1988; Diehl, 1992; Huryn 1998; Blumenshine *et al.*, 2000). Blumenshine *et al.* (2000) found that fish predation shifted benthos size structure towards smaller organisms in three lakes where fish were experimentally manipulated. A study examining lakes with and without fish found that large invertebrates have replaced fish as top predators (Dermott, 1988,). The effects of fish predation on the abundance, body size and community structure of macroinvertebrates has been found to be lower in vegetation than in open water (Crowder and Cooper, 1982; Diehl, 1988; Diehl, 1992; Diehl & Eklöv, 1995; Diehl & Kornijów, 1998). The ability of fish to perceive attack or capture prey within complex habitats is limited (Diehl 1988). Previous studies have suggested that adult perch and cyprinids avoid stands of macrophytes because of the predation risk by pike (*Esox lucius*), remaining instead in open water (Eklöv & Hamrin, 1989; Laffaille *et al.*, 2001). None of the reviewed literature has documented an increase in larger organisms with increasing fish biomass, as was the case in both substrates in model 8, and in soft substrates in model 5. As this pattern cannot be explained by patterns observed in previous studies, it needs to be treated with caution, particularly owing to the absence of some fish species from over half of the lakes, and to the small sample size. However,

patterns relating to substrates are clear for these 15 lakes, and consistent with models of the larger dataset as well as patterns observed in the literature.

3.4.7 Differences among metrics

With the exception of size diversity, there were notable similarities among the best fitting models of biomass based metrics e.g., mean individual size, total biomass and size structure (truncated and quadratic based). In each case, substrate explained the most variability among samples, followed by a curvilinear change in almost all biomass based models with TN, followed by marginal changes with alkalinity. DOC explained variation in almost all size spectrum models but not in mean or total biomass.

Size diversity provides a unique value for each sampled size distribution and has been used to describe the shape of size spectra in several studies (Quintana *et al.*, 2002; Brucet *et al.*, 2006; Quintana *et al.*, 2006; Gascón *et al.*, 2009). However, the minimal adequate size diversity model produced here eliminated all terms, meaning that none of the fixed terms adequately described the data. In addition, the size diversity value was largely invariant among lakes. Previous studies have documented size diversity to co-vary with both biotic and abiotic variables. For example, the size diversity of zooplankton communities in a Mediterranean salt marsh were negatively correlated with species diversity (Brucet *et al.*, 2006). Gascón *et al.* (2009) examined patterns in the size diversity of benthic macroinvertebrates in Mediterranean salt marshes and found that size diversity varied with dissolved inorganic nitrogen and with predatory pressure among the six basins examined. However, aside from the inclusion of a substrate term, no biotic variables were included in this analysis. Although size diversity has been applied as a surrogate for size spectra, most of the variability among size spectra described in this study was among substrates, with size diversity values invariant among substrates.

The best fitting model of log abundance indicated that only changes in alkalinity concentrations caused a change (although minimal, given the low coefficient) in the abundance of invertebrates among lakes among all variables examined. Abundance has yielded conflicting results in previous studies such that a positive correlation was observed with nutrient enrichment in some reports (Bourassa and Morin, 1995; Blumenshine *et al.*, 1997; Donohue *et al.*, 2009) but not in others (Dinsmore *et al.*,

1999; Paujkert & willis 2003; Michaletz *et al.*, 2005). Lake area has also been observed to affect macroinvertebrate community structure (Heino, 2000). However, in my study, lake area was eliminated as a fixed effect from models of size diversity, mean individual size, total biomass, and log abundance and thus was not perceived to affect changes in macroinvertebrate biomass among lakes.

3.4.8 Potential factors affecting benthic invertebrate size distributions

Relationships within the benthos and changes in benthic macroinvertebrate size spectra may be more difficult to interpret than those in the pelagic owing to:

(i) greater habitat complexity in the benthos from macrophytes, and differences among substrates;

(ii) predation by fish, which can cause a shift in benthos size structure towards smaller organisms whereas predation by invertebrates can increase abundance of larger invertebrates;

(iii) trophic interactions within benthic invertebrate communities are not entirely driven by body size (e.g., filter feeders, scavengers) and some invertebrates do not change food source as they grow (and thus trophic level is only weakly correlated with body size);

(iv) benthic macroinvertebrates occupying a subset of the lake foodwebs; representing partial size spectra, and the influence of predators or competitors outside the recorded spectra;

(v) benthic macroinvertebrate communities not discretely separate from the pelagic community.

There is limited information available on the size structure of benthic animal communities and on the factors that affect the size structure of benthic invertebrate communities (Strayer, 1991; Pagola-Carte, 2004; Solimini *et al.*, 2005) and conspicuous variability observed among lakes and among studies (Strayer, 1991; Rodriguez and Magnan, 1993). However, the findings from this study, which examined benthic macroinvertbrate biomass and size structure among lakes on a larger spatial scale with larger nutrient and alkalinity gradients than previously investigated, indicate that the size spectrum follows a curvilinear trend in response to increasing TN concentrations, and a linear increase in response to alkalinity increase for the study lakes examined. Several authors have predicted that "size trends" in the size spectra in response to

anthropogenic pressure have potential for use in assessment of ecosystem health, complementary to taxonomic analysis (Rasmussen, 1993; Holmgren, 2001; Basset *et al.*, 2004; De Eyto and Irvine, 2007). The observed curvilinear response to nutrient status change warrants further study, and has potential for use in monitoring.

3.4.9 Use of linear mixed effects models

The use of LME models, and the ensuing model selection process, although time consuming, allowed much greater flexibility and insight into changes in macroinvertebrate size spectra among lakes, substrates, seasons and size classes than multiple regression or generalised linear models owing to the inclusion of random factors, manual model reduction, and the basis in information theory. Unlike other statistical methods, where final results are automatically generated through a statistical program, the information theory based LME models, when computed through the R statistical program, were manually reduced, so that the process of model reduction is transparent, and the researcher has complete control at each stage of the process. For data such as size spectra where changes among two variables are to be analysed, and the complex nature of multiple continuous variables need to be retained, as well as a nested random structure, LME models are the most reliable and flexible analytical approach, available.

3.5 Conclusions

The following points encapsulate the key findings of this study:

- Substrate explained the most variation in the mean and total biomass and in the size structure of lake littoral macroinvertebrate communities from 48 lakes, with greater mean and total biomass and an increase in the normalised biomass of larger size classes of macroinvertebrate communities found on soft substrates than those found on hard substrates;
- Increasing alkalinity concentrations resulted in marginal increases in the abundance, mean biomass, total biomass and in a shift towards larger organisms in the size structure of macroinvertebrate communities on both substrates;
- Increasing DOC concentrations mediated changes in the slope of the size spectra of macroinvertebrates from hard and soft substrates;
- TN consistently explained changes in the mean and total biomass and the size structure of lake littoral macroinvertebrate communities in both substrates better than TP or chlorophyll *a*. It is possible that TN may provide a more useful metric for assessment of nutrient effects within the benthos or indeed the whole lake than previously thought;
- A curvilinear change in the total biomass and size structure of lake littoral macroinvertebrate in both habitats was observed with increasing TN concentrations, with suggestions of an optimal concentration; and
- Models of the total biomass of large individuals from 15 lakes where fish data were examined yielded conflicting results with the literature on the effect of fish predation on invertebrate size. Substrate was observed to explain the most variation in the biomass of large individuals among lakes. However sample sizes were small and results need to be interpreted with caution.

CHAPTER 4

BIOMASS-BASED CHANGES IN LAKE LITTORAL MACROINVERTEBRATE COMMUNITY STRUCTURE AND FUNCTIONAL FEEDING GROUPS WITH NUTRIENT ENRICHMENT

4.1 Introduction

The heterogeneity of substrates within the littoral zone of lakes leads to a high species diversity within lake littoral macroinvertebrate communities (Brodersen *et al.*, 1998; White and Irvine, 2003). However, the resulting mosaic of habitat patches can create difficulties in standardising sampling (Brodersen *et al.*, 1998; White and Irvine, 2003). Changes in the community structure of lake littoral macroinvertebrates with lake trophic state have been examined in several studies (Macan and Maudsley, 1969; Kornijow, 1988; Kansanen *et al.*, 1990; Petridis, 1993; Brodersen *et al.*, 1998; White and Irvine, 2003; Brauns *et al.*, 2007; Donohue, 2008). The effects of trophic state on macroinvertebrate community structure are often nested within habitat types (Tolonen *et al.*, 2001; White and Irvine, 2003; Brauns *et al.*, 2007; Donohue, 2008; Donohue *et al.*, 2009). Thus, stratification of sampling within lakes (according to habitats) is an important consideration.

Brodersen *et al.* (1998) minimised the effects of substrate heterogeneity by confining sampling to well-defined littoral stony substrates. Samples from stony substrates in lakes are well suited to comparative studies among lakes, as they are more likely to be constant over seasons, with less variation among communities over time compared with soft substrates (fine or organic sediments). Soft substrates may vary significantly owing to seasonal variation in the growth of associated rooted macrophytes and macroalgae (Donohue *et al.*, 2009). Changes in the community structure of littoral macroinvertebrates from stony substrates in lakes with nutrient enrichment have been examined in relatively few studies (Macan and Maudsley, 1969; Brodersen *et al.*, 1998; Brauns *et al.*, 2007; Donohue, 2008). Significant changes in the community structure of stony substrates were recorded in some studies (Brodersen *et al.*, 1998; Donohue, 2008) and not in others (Brauns *et al.*, 2007). In a study of changes in macroinvertebrate community structure (based on proportional abundance) among 66 lakes, which included all 48 lakes examined here, conducted over the same time period, Donohue (2008) found that differences in community structure among lakes were significantly related to TN and alkalinity. Local-scale variables such as habitat, riparian and ecosystem variables) have been shown to explain a greater proportion of variance in macroinvertebrate communities among stony sites than regional scale variables (such as variables relating to the catchment, ecoregion and geographic location) in a study of 538

lakes and 694 streams (Johnson *et al.*, 2004). However, the latter study did not include anthropogenically affected sites. In contrast, Townsend *et al.* (2003) found catchment-scale variables, such as percentage pasture within the catchment, accounted for more variance in stream communities than other levels of spatial scale (reach, bedform, geographic position) but included anthropogenically affected streams in the study. Johnson *et al.* (2004) suggested that nutrient concentrations in water bodies are influenced by regional scale processes which may have caused the difference in findings between the studies.

Based on food ingested, most aquatic insects are essentially omnivorous (Merrit and Cummins, 1996). For example, insects that chew leaf litter in streams ('shredders') ingest not only leaf tissue and associated microbiota, but also diatoms and other attached algae as well as very small invertebrates (Merrit and Cummins, 1996). Classifying macroinvertebrate communities by trophic level is thus difficult. Accordingly, an alternative classification system was developed, whereupon invertebrates are categorized into functional feeding groups (FFGs) based on the previously observed patterns of food consumption, morphology of feeding structures (mouthparts and legs) and feeding behaviour, rather than on the food actually ingested (e.g. as determined from gut content analysis [Moog, 1995; Wallace and Webster, 1996; Merrit and Cummins, 1996; Johnson *et al.*, 2004]).

An existing FFG classification scheme (Cummins, 1974; Cummins and Klug, 1979) was further modified by Moog (1995) to include 10 classes of functional feeding types. The principal groups are 'shredders' (feeding on fallen leaves, plant tissue and coarse particulate organic matter [POM]), 'grazer-scrapers' (feeding on epilithic algae, biofilm, POM, tissues of living plants), 'filter feeders' (feeding on suspended fine POM and coarse POM, whirled micro prey), 'gathering-collectors' (feeding on sedimented fine POM), 'miners' (feeding on leaves of aquatic plants), 'xylophagous' (feeding on woody debris), 'predators' (feeding on invertebrate prey), 'parasites' (feeding on host invertebrate/fish), and 'other' (unclassified feeders). Although some invertebrates may have specialised mouthparts for ingesting a particular food type, they may utilise other resources and act as generalist feeders (Mihuc and Minshall, 1995; Mihuc, 1997). Likewise, because of life history changes, an animal may use different resources at different life stages (Johnson *et al.*, 2004). Therefore, animals may be classified into

more than one feeding group. The system developed by Moog (1995) involves the allotment of 10 points divided among the FFGs to account for different resource use by a species, and involves use of the AQEM database of species autoecological traits (Asterics® Version 3.01; http://www.aqem.de). Much of the research on freshwater invertebrate diet, and that used to create the AQEM database, is derived from stream data, and information on differences in the diet and consumption patterns of many lake macroinvertebrate species is limited. Johnson *et al.* (2004) proposed that differences between lake and stream macroinvertebrate community structure reflect differences in the uptake and use of coarse particulate organic matter (CPOM), owing to longer retention times of CPOM in lake littoral regions than in streams, but that the quality of organic matter was probably similar between the two systems, based on their large-scale study of Swedish lakes and streams.

In lotic systems, functional traits of macroinvertebrates, such as FFGs, have played a key role in the assessment of anthropogenic degradation (Lamouroux *et al.*, 2004; Finn and Poff, 2005; Compin and Céréghino, 2007; Heino, 2007). However, use of macroinvertebrate FFGs in examination and characterization of lake littoral macroinvertebrate communities has been limited (Johnson and Goedkoop 2002; Johnson *et al.*, 2004; Tolonen *et al.*, 2003; Heino, 2008). With nutrient enrichment, generalist feeders (such as gathering-collectors and filter feeders) that have a broad range of available food, and thus, are more tolerant of pollution, would be expected to increase, contrasting with specialist feeders, such as grazer-scrapers and shredders that rely on live plant and algal material, which may be sensitive to enrichment with changes in available food resources (Barbour *et al.*, 1996). Increases in gathering-collectors tolerant to nutrient enrichment have been observed among streams (Barbour *et al.*, 1996; Cross *et al.*, 2006) and among lakes (Heino, 2000). Increased microbial activity and nutrient quality of the detritus with nutrient enrichment explained this significant increase in gathering-collectors, when a detritus-based stream was experimentally enriched and was compared with an adjacent reference detritus-based stream (Cross *et al.*, 2006).

In the current study, a biomass-based approach to structural and functional feeding changes in littoral macroinvertebrates was adopted by examining the mean, collective and proportional biomass of each taxa, rather than examining the more conventional

abundance of taxa. The body size of an individual is correlated with many life-history traits (Calder, 1984) and is predicted to scale exponentially with physiological processes such as excretion, ingestion, growth and reproduction (Peters, 1983; Cyr, 2000). Individual body size and population biomass has been shown to correlate with cohort and annual production rates (Waters, 1977; Banse and Mosher, 1980; Plante and Downing, 1989; Stephenson and Morin, 2009). Therefore, although no direct functional measurement of littoral macroinvertebrate communities were made, a biomass-based (rather than abundance-based) examination of the taxonomic composition and functional feeding of these communities implies functional changes that may be occurring, and assists us in gaining a better understanding of change in communities among lakes with nutrient enrichment.

4.1.1 Aims

Interesting trends in the emergent property of body size and in size spectra in response to nutrient enrichment were reported in Chapter 3. The principal aim of this chapter is to uncover change in community and functional feeding structures underlying the changes observed in Chapter 3. All analyses are based on either collective biomass (community structure) or proportional biomass of taxa or FFGs; data on abundance was not examined. As the biomass of large invertebrates fluctuated most in response to increasing nutrient pressure (Chapter 3), the data was partitioned to examine structural and functional change in 'large' macroinvertebrates separately from changes in the 'whole' dataset, which included all invertebrates.

Donohue's abundance-based study of lake littoral macroinvertebrates from a larger collection of lakes which included all lakes examined here, conducted over the same time period, found that changing nutrient concentrations were significantly related to changes in the community structure and FFGs based on proportional abundance (Donohue, 2008). These findings, coupled with the curvilinear change in size structure and in total and mean biomass observed in response to changes in nutrient state reported in Chapter 3, are hypothesised to reflect a corresponding change in the functional feeding group proportional biomass and in the biomass-based community structure with increasing trophic state. Given that changes in total nitrogen (TN) concentrations best explained changes in size structure and total and mean biomass, it is hypothesised that changes in TN concentrations will also explain biomass-based structural changes among

lake macroinvertebrates significantly better than changes in total phosphorus (TP) concentrations.

Owing to the observed marginal changes in total and mean biomass and size structure with alkalinity, with a greater change among larger organisms, it is hypothesized that changes in community structure (based on collective biomass) with alkalinity will reflect that of abundance based patterns observed by Donohue (2008), but that these changes will be more pronounced among large individuals. In addition, an increase in the proportional biomass of gathering-collectors with increasing nutrient concentrations is expected, as observed in previous studies of FFG change with nutrient enrichment (Barbour *et al.*, 1996; Cross *et al.*, 2006; Heino, 2000), reflecting shifts in the functional processing of matter.

In summary, it is hypothesised that:

(a) Community structure will significantly alter with alkalinity (as reported by Donohue [2008]) and will be more pronounced among 'large' invertebrates (reflecting the greater change in biomass observed with these variables in Chapter 3);

(b) TN concentrations will explain more change in community structure than TP (reflecting patterns in the total biomass, mean biomass and size structure of the communities);

(c) Significant shifts will be observed in (i) collective biomass of community structure and (ii) proportional biomass of FFG of lake littoral macroinvertebrates with TN (mirroring those seen in Chapter 3, and recorded by Donohue [2008]);

(d) Increases in the proportional biomass of gathering-collectors will be observed with enrichment (reflecting change in functional processing of matter).

4.2 Methods and Materials

4.2.1 Study area

Forty eight lakes were selected, principally located in Northern Ireland and the border regions of the Republic of Ireland (Fig. 3.1). Lakes were chosen to represent a gradient of TP (2.2 to 160.4 $\mu g\ L^{-1}$) and alkalinity concentrations (1.0 to 264.4 mg $CaCO_3\ L^{-1}$; Table 3.1, Chapter 3). Details on these lakes are described in Chapter 3 (Section 3.2.1). Hard substrates (i.e. pebbles, gravel or bedrock) were sampled in spring (April or May) and summer (August) for both macroinvertebrates and water chemistry. Thirty six of the lakes were sampled in 2005, and the remaining twelve lakes were sampled in 2006. Ninety five samples were collected from hard substrates in total (48 lakes in 2 seasons, with the exception of Lough Corry in summer, where no hard substrate remained).

4.2.2 Water samples

Water samples were collected from each lake using a weighted 5 L bottle with attached rope, which was thrown approximately 10 m from the shore. Water samples were analysed for pH, alkalinity, conductivity, colour, dissolved organic carbon (DOC) and chlorophyll *a* following standard methods (Standing Committee of Analysts, 1983). TN was analysed following Grasshoff *et al.* (1999) and TP was analysed according to Eisenreich *et al.* (1975). Details on methods employed in the collection and analysis of the water samples are described in Chapter 3 (Sections 3.2.2.1 and 3.2.3.1).

4.2.3 Macroinvertebrate samples

Macroinvertebrate samples were collected from the littoral zone of each lake. Three representative sites of hard substrate were selected in each lake. Macroinvertebrate sampling was conducted by kick sampling for 1 min at each of the three replicate sites, using a standard Freshwater Biological Association kick net of 1 mm^2 mesh size. The replicate 1 min samples were combined, sieved and preserved on site in >90% industrial methylated spirits (IMS). Further detail on the collection of macroinvertebate samples is provided in Chapter 3 (Section 3.2.2.2). Owing to a high abundance of macroinvertebrates in many of the lakes sampled, samples were split where required, to give a minimum of 500 animals. Samples were sorted and macroinvertebrates identified and measured. Most macroinvertebrate taxa were identified to genus or species, with the exception of dipteran larvae, odonatan larvae and Collembola, which

were identified to family level. Nematodes, oligochaetes and Hydracarina were not further identified. Measurements generally involved the measurement of the body length of the invertebrate. However, oligochaetes were categorized into size classes based on their body width and body length, and the largest dimension of Gastropoda, Unioniidae bivalves (including shell) and Hydracarina was measured. All individuals were identified, but a random subsample was taken where a large number of one taxon was present (i.e. >25). Where animals were identified to species/genus, a 25 animal subsample was taken. When animals were identified to a lower resolution, a subsample of 50 animals was taken; thus providing a more accurate sample of size distribution where invertebrates grouped as one taxon were potentially a mixture of species. Further detail on the identification and processing of macroinvertebrate samples is described in Chapter 3 (Section 3.2.3.2).

Length mass regressions were used to estimate the body mass of each measured organism from the body length, or body dimension measured. Length mass regressions were developed for most taxa, described in detail in Chapters 2 (Section 2.2.3.2) and 3 (Sections 3.2.3.4 and 3.2.4.1). For taxa where it was not possible to develop regressions in the study (owing to insufficient numbers of animals), published regressions from Smock (1980), Meyer (1989), Benke *et al.* (1999), Sabo (2002) and Baumgartner and Rotthaupt (2003) were applied. Details on the selection of published length-mass regressions, and on the taxa to which published regressions were applied, are provided in Chapter 3 (Section 3.2.4.1).

4.2.4 Statistical analysis

4.2.4.1 Data preparation

Data on the total biomass of each species within each sample was divided into two parts: (i) 'large' invertebrates species (mean dry mass $\geq$1.41 mg).and (ii) 'small' invertebrate species (mean dry mass <1.41 mg). These parts were compared with the complete 'whole' dataset on the biomass of each species within each sample. As the summer samples from Lough Bawn and Lough Kiltyfannad included no 'large' species, these samples were not included in the 'large' dataset, leaving 93 samples remaining. The cut-off point of 1.41 mg was the same as that used in Chapter 3 for separation of data for the 'truncated' and 'quadratic' size spectra, to account for loss of small animals

through the net mesh, and was used to separate 'small' and 'large' animals for LME models that included data on fish (Chapter 3, Sections 3.2.4.5 and 3.2.6). Although 'small' animals may have been subject to some losses through the mesh (as described in detail in Chapter 3, Section 3.2.4.5), this group, nevertheless, constitutes greater than half of the total biomass within all samples (when pooled) and includes the median of the pooled size distribution (Chapter 3, Fig. 3.3). Use of the same cut-off point as that used in Chapter 3 allows for ease of comparison among data from both chapters.

4.2.4.2 Data analysis

The PRIMER statistical program was used for multivariate analyses, non-metric Multi Dimensional Scaling (MDS), cluster analysis, PERMANOVA, BEST and global BEST analyses. Instead of using data based on the abundance of each species/taxa present to examine structural changes in community structure with environmental variables, data based on the collective biomass of each species/taxa was used to uncover changes in the taxonomic composition. The collective biomass of small, numerically abundant taxa may, therefore, be similar to that of large rarer taxa. Each dataset of lake littoral macroinvertebrate taxa biomass was transformed by fourth root and a Bray-Curtis similarity matrix was constructed. From this, an MDS ordination plot was constructed based on an underlying triangular similarity matrix, with similarities calculated between every pair of samples, and samples ordinated on a 'map' according to the ranks of the similarities (Clarke and Warwick, 2001).

Hierarchical agglomerative cluster analysis was used to organize samples into groups, and groups into clusters, based on the similarity of the biomass-based species composition of samples. PERMANOVA is a non-parametric routine for testing the simultaneous response of one or more factors (e.g. season) in an analysis of variance (ANOVA) design. This approach is based on resemblance measures (e.g. similarity matrix such as Bray-Curtis constructed from species biomass data), using permutation methods (Anderson *et al.*, 2008). The method partitions the total sum of squares according to the experimental design specified, calculating an appropriate distance-based *pseudo* F statistic for each term in the model, based on the expectations of means squares, comparable to the calculation of the F statistic in ANOVA models, but without a known distribution under the true null hypothesis. *P*-values are calculated using appropriate permutations for each term (Anderson *et al.*, 2008).

BEST analysis searched for high rank correlations between a secondary fixed sample similarity matrix (e.g. species biomass data) and resemblance matrices generated from different subsets of a primary matrix (e.g. normalised environmental variables) by conducting a full search of all possible combinations of variables from the primary datasheet (Clarke and Gorley, 2006). *Rho* lies in the range (-1 to +1) with the extremes of *Rho* = -1 and +1 corresponding to the cases where the two sets of ranks are in complete opposition or complete agreement. Values of *Rho* close to zero correspond to the absence of any match between the two patterns, but typically *Rho* is positive (Clarke and Warwick, 2001). The effects of TN, TP and chlorophyll *a* were analysed in separate BEST analyses owing to a high level of correlation among the three variables (see Chapter 3 [Section 3.2.5] for further detail). Global BEST, a non-parametric form of the Mantel test, was used to test the statistical significance of the BEST results. This is a permutation test which tests the null hypothesis of "no agreement in the multivariate pattern" corresponding to the rank correlation coefficient *Rho* = 0; *Rho* is measured by comparing two independently derived resemblance matrices, by random permutation of one set of labels relative to the other. *Rho* is recalculated for successive permutations, and if less than 5% of these values are greater than the real *Rho*, the null hypothesis can be rejected at *P* value <0.05. The model with a *Rho* value closest to 1 or -1 (e.g. including TN, TP or chlorophyll *a*), and with the lowest significance value was presented in the results. Bubble plots of the MDS ordinations were constructed for environmental variables found to significantly explain the species composition of the samples, based on the BEST and global BEST analyses. BEST analyses were also used to highlight taxa which were important for structuring the overall community composition among all samples, where the taxonomic matrix is permuted against a copy of itself, and variable sets (species) are selected from the second matrix such that their sample ordination matches "near-perfect" to that of the first matrix (full species set), deemed to be when *Rho* exceeds 0.95 (Clarke and Warwick, 2001).

Relationships between environmental variables (i.e. lake area, alkalinity, DOC, TP, TN and chlorophyll *a*) and the proportional biomass of Araneae, Bivalvia, Coleoptera, Crustacea, Diptera, Ephemeroptera, Gastropoda, Heteroptera, Hirudinea, Hydracarina, Lepidoptera, Megaloptera, Odonata, Oligoghaeta, Plecoptera, Trichoptera and Turbellari, were examined using Spearman rank correlation.

The effects of season, lake area, alkalinity, DOC and TN on a series of response variables based on FFG data (total biomass of grazer-scrapers, shredders, gathering-collectors, filter feeders and predators) were analysed in linear mixed effects models using 'whole' and 'large' datasets (LME; R development core team, 2009). Summer was the reference category for the fixed effect of season. Lake identity was included as a random effect in each of the models.

To account for differences in total biomass among lakes, a correction factor was included in the model. The correctional factor was derived from dividing a proportional FFG biomass response variable into its two constituent parts: total FFG biomass (tb_i) divided by total biomass of the sample (TB_i) and log transforming the response variable (tb_i / TB_i). The linear equation was restructured so that the log of total FFG biomass is the new response variable, with a correction for total biomass (summarised in equations [1] to [3]).

$$\textbf{Log } (\textbf{tb}_i \textbf{ / TB}_i) = b_0 + b_1X_1 + b_2X_2 + ...+ b_nX_n + u + e \quad [1]$$

$$\Rightarrow \textbf{ Log tb}_i \textbf{ – Log TB}_i = b_0 + b_1X_1 + b_2X_2 + ...+ b_nX_n + u + e \quad [2]$$

$$\Rightarrow \textbf{ Log tb}_i = b_0 + b_1X_1 + b_2X_2 + ...+ b_nX_n + b_{n+1}\textbf{ Log TB}_i + u + e \quad [3]$$

Where b_0 = intercept; u = random effects; e = error.

A quadratic enrichment term was added to examine whether a curvilinear relationship described the relationship between TN and the response variable. Interaction terms between alkalinity and TN, and season and TN, were also included in each maximal model. Backward selection based on maximum likelihood (ML) was used to identify the minimal adequate model (the most parsimonious model at the lowest value of AIC) following Crawley (2007) and Zuur *et al.* (2009). The ML function must be used when comparing fixed effects among models using LR tests (Pinheiro and Bates, 2000; Bolker *et al.*, 2009). Model simplification was based initially on *p*-values produced from the *t*-statistic; those with the highest non-significant *p*-values were eliminated from the succeeding model. The original and succeeding models were then compared based on a likelihood ratio (LR) test (which determines the contribution of a single variable by comparing the fit for nested models; Bolker *et al.*, 2009) and comparison with AIC values, to justify the elimination of the non-significant term (Crawley, 2007; Zuur *et al.*,

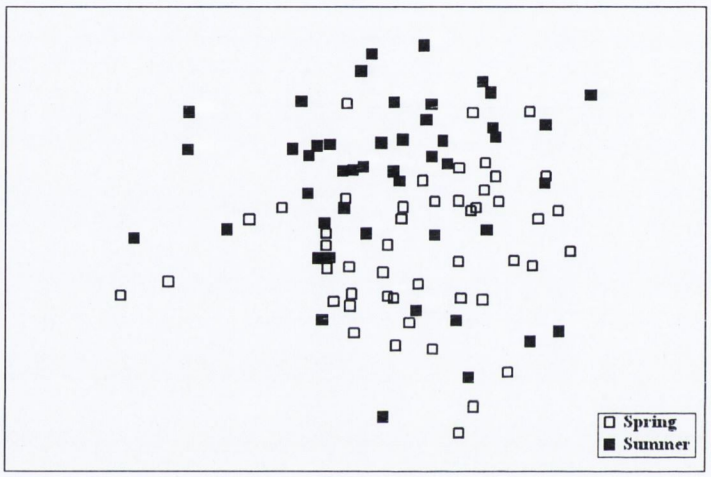
2009). Where two minimal adequate models are compared and found to have a difference in AIC values <2, then both are considered to describe the data equally well (Burnham and Anderson, 2004). The residuals of all models were checked for homogeneity of variance and normality. Model selection is explained in greater detail in Chapters 2 [Section 2.2.4] and 3 [Section 3.2.6].

4.3 Results

4.3.1 Analysis of biomass-based taxonomic composition of 'whole' dataset

To assess seasonal differences in the whole macroinvertebrate community across lakes, MDS plots were generated using fourth root transformed biomass data. Community composition biomass appeared to differ among seasons (Fig. 4.1).

(a)



(b)

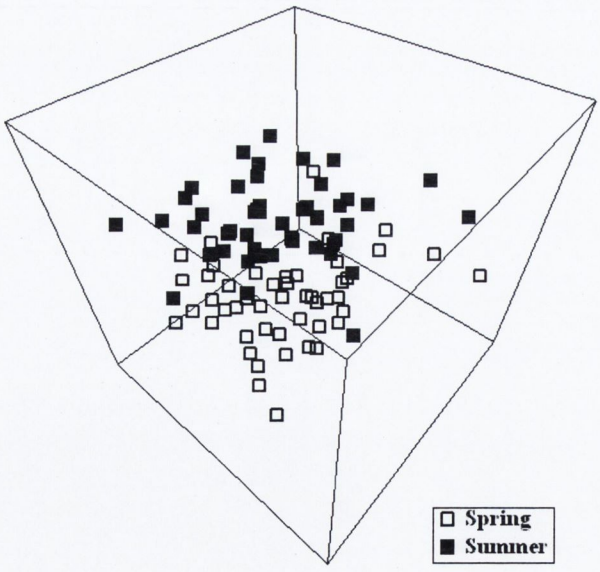


Figure 4.1: MDS of Bray-Curtis similarities from fourth root transformed data on lake littoral macroinvertebrate species biomass of the 'whole' dataset in (a) two dimensions (stress = 0.28) and (b) in three dimensions (stress = 0.2); n = 95.

The stress values of both two dimensional and three dimensional MDS plots are high at 0.28 and 0.2, respectively (Fig. 4.1). The stress value indicates the extent of disagreement between the rank order of the original matrix and the rank order of the distances on the ordination plot, and is distorted by compressing multi-dimensional data into two dimensional plots, decreasing in three dimensions, and again with increasing numbers of dimensions. A stress value of zero would indicate that the ordination was completely successful in representing the original data. Stress values between 0.2 and 0.3 should be treated with caution, particularly those in the upper half of this range, as they are close to being arbitrarily placed (Clarke and Warwick, 2001). The three dimensional plot with the lower stress value represents the data better, and depicts a clear division in the community structure between seasons (Fig. 4.1b).

PERMANOVA was used to estimate statistically whether biomass-based macroinvertebrate community structure differed between seasons, by analysing the entire community biomass matrix against the factor 'season' (Table 4.1). Biomass-based community structure was found to significantly differ between seasons ($P \leq 0.05$).

Table 4.1: PERMANOVA table detailing seasonal effects on lake littoral macroinvertebrate species biomass from the 'whole' dataset (n = 95).

Dataset	Factor	df	SS	MS	*Pseudo* F	*P* value (perm)
'whole'	Season	1	14574	14574	5.77	**0.0001**
'whole'	Residual	93	234930	2526	n.a.	n.a.

n.a. - not applicable.

Owing to differences in the macroinvertebrate community structure between seasons, BEST analysis was conducted on samples from each season separately for the 'whole' dataset. BEST and Global BEST analysis of 'whole' spring samples indicated TN significantly explained changes in community structure (Table 4.2). In separate analyses, neither TP nor chlorophyll *a* combined with the other environmental variables, were significantly related to community structure (Appendix, Table II). BEST analysis of 'whole' summer samples, however, indicated lake area may be of importance in structuring macroinvertebrate communities, but Global BEST showed that this variable was not significant in explaining changes.

Table 4.2: Water chemical variables best describing lake littoral macroinvertebrate species biomass from the 'whole' dataset in spring and summer, as determined by the BEST analysis.

Dataset	n	BEST Values	*P* value[1]	BEST variables
'whole' Spring	48	*Rho* = 0.234	**0.008**[1]	- TN
'whole' Summer	47	*Rho* = 0.116	0.304[1]	- Lake area

[1]Significance values produced from global BEST analysis.

Bubble plots were used to visualise differences in each of the variables chosen by BEST to significantly explain changes in community structure among spring samples (Fig. 4.2). Cluster analyses did not reveal any further grouping of samples and thus, cluster graphs were not overlaid. Samples with higher TN concentrations were oriented loosely towards the lower half of the graph, indicating more similar community structure to one another than those with lower TN, which were loosely orientated towards the upper half of the graph (Fig. 4.2). The high stress level associated with the MDS indicates that the ordination is not an excellent representation of the data.

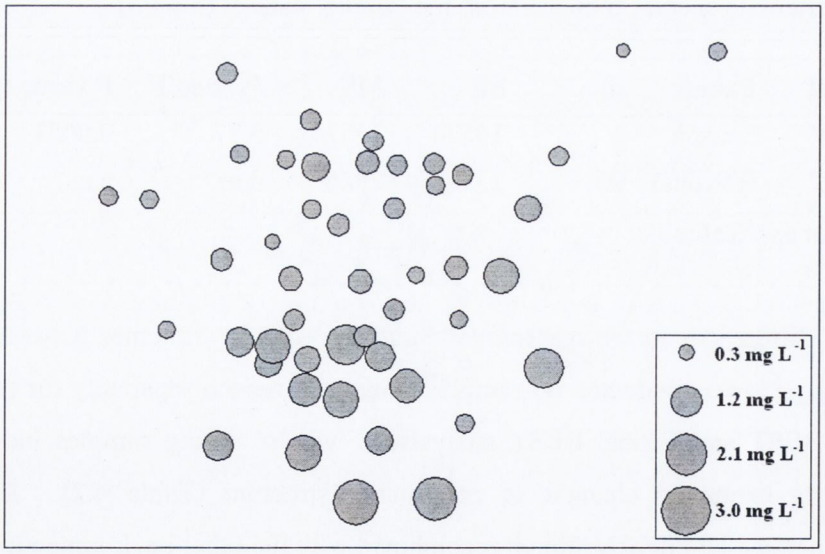


Figure 4.2: MDS of Bray-Curtis similarities from fourth root transformed data on lake littoral macroinvertebrate species biomass in spring from the 'whole' dataset indicating changing TN concentrations (mg L^{-1}; stress = 0.24); n = 48.

BEST analysis of biomass-based taxonomic structure of macroinvertebrate communities from the 'whole' dataset found that 41 taxa best described community composition of

spring samples and 32 taxa best described summer samples. Twenty-three of the taxa that best described community composition were common to spring and summer samples (Table 4.3).

Table 4.3: Species significantly describing community structure among samples of the 'whole' dataset chosen using the BEST analysis.

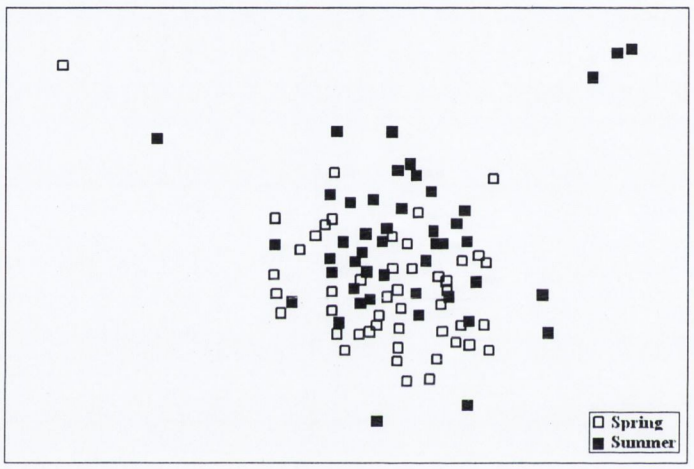
Spring	Summer
Anabolia nervosa	*Asellus aquaticus**
Argyroneta aquatica	*Bithynia leachii*
*Asellus aquaticus**	*Bithynia tentaculata*
*Caenis horaria**	*Caenis horaria**
Caenis luctuosa	Chironomidae*
Centroptilum luteolum	*Cloeon simile**
Ceratopogonidae	Coenagrionidae*
Chironomidae*	Diptera *
*Cloeon simile**	*Dryops* sp. (larvae)
Coenagrionidae*	*Erpobdella octoculata**
Cymatia bonsdorffii	*Gammarus* sp (juveniles)*
Diptera *	*Gammarus duebeni*
Dugesia sp.	*Gammarus pulex**
Enallagma cyathigerum	*Haliplus* sp. (larvae)*
*Erpobdella octoculata**	*Helobdella stagnalis*
Gammarus sp. (juveniles)*	Hydracarina*
*Gammarus pulex**	*Limnius volckmari* (larvae)
Glossiphonia complanata	*Nebrioporus assimilis* (adult)*
Haliplus sp. (adult)	*Nebrioporus depressus* (adult)
Haliplus sp. (larvae)*	Oligochaeta*
Hydracarina*	*Oulimnius* sp. (adult)*
Hygrotus quinquelineatus (adult)	*Polycelis nigra/tenuis**
Leptophlebia sp.	*Polycentropus* sp.*
Leptophlebia vespertina	*Potamopyrgus antipodarum**
Limnephilus lunatus	*Radix balthica**
Limnephilus marmoratus	*Sialis lutaria**
Nebrioporus assimilis (adult)*	*Sigara distincta**
Oligochaeta*	*Sigara falleni**
Oulimnius sp. (adult)*	*Sigara* sp.
Physa fontinalis	Sphaeriidae*
Planorbis carinatus	Tabanidae
*Polycelis nigra/tenuis**	*Tinodes waeneri**
Polycentropus sp.*	
*Potamopyrgus antipodarum**	
*Radix balthica**	
*Sialis lutaria**	
*Sigara distincta**	
*Sigara falleni**	
Siphonoperla torrentium	
Sphaeriidae*	
*Tinodes waeneri**	

* appears where taxa occur on both spring and summer BEST derived taxa lists

4.3.2 Analysis of biomass-based taxonomic composition of 'large' dataset

MDS plots, generated using fourth root transformed biomass data, displayed a loose division of the community structure of the 'large' dataset between seasons (Fig. 4.3). Although there was a less defined separation of seasons than that seen in the 'whole' dataset (Fig. 4.1), the stress values of both two dimensional (0.18) and three dimensional (0.14) MDS plots were lower, and the greater number of dimensions in the latter ordination provided a better representation of the data (Fig. 4.3).

(a)



(b)

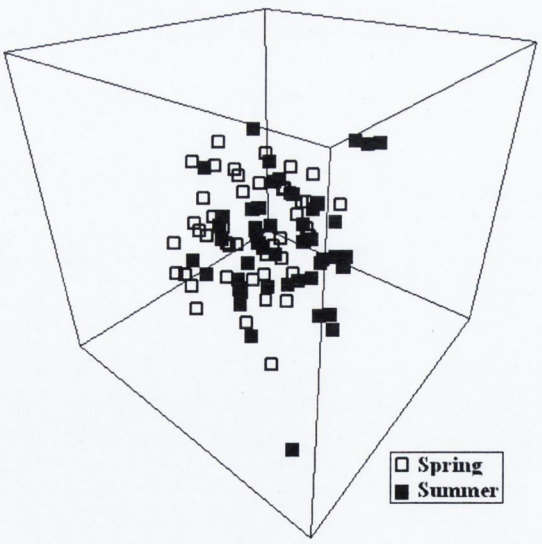


Figure 4.3: MDS of Bray-Curtis similarities from fourth root transformed data on 'large' lake littoral macroinvertebrate species biomass in hard substrates in (a) two dimensions (stress = 0.18) and (b) three dimensions (stress = 0.14); n = 93.

PERMANOVA analysis revealed that biomass-based macroinvertebrate community structure of the 'large' macroinvertebrate dataset differed significantly between seasons, with a lower *pseudo* F ratio than that found for the 'whole' dataset, likely in response to the less defined difference among seasons seen in the MDS ordinations (Fig. 4.3; Table 4.4).

Table 4.4: PERMANOVA table, examining seasonal effects on lake littoral macroinvertebrate species biomass of 'large' dataset (n = 93).

Dataset	Factor	df	SS	MS	*Pseudo* F	*P* value (perm)
'large'	Season	1	11439	11439	2.86	**0.0002**
'large'	Residual	91	364020	4000	n.a.	n.a.

n.a. - not applicable.

BEST and Global BEST analysis conducted on spring samples of the 'large' dataset, indicated that changes in alkalinity and TN concentrations may be of importance in explaining changes in community structure; separate analyses where TP or chlorophyll *a* were included had lower *Rho* values (Appendix, Table II). However, results from Global BEST analysis indicated that none of these variables were statistically significant (Table 4.5). Analysis of 'large' summer samples indicated that chlorophyll *a* may be of importance in explaining changes in community structure, but the *Rho* value was almost zero, indicating no match between the community composition similarity matrix and chlorophyll *a* concentrations (Table 4.5). Results from Global BEST analysis displayed a *p* value approaching 1, also indicating that this variable explained almost no change in composition (Table 4.5).

Table 4.5. Water chemical variables best describing lake littoral macroinvertebrate species biomass of spring samples of the 'large' dataset as determined by the BEST analysis.

Dataset	n	BEST Values	*P* value[1]	BEST variables
'large' Spring	48	*Rho* = 0.138	0.143	Alkalinity; TN
'large' Summer	45	*Rho* = 0.025	0.886	Chlorophyll *a*

[1]Significance values produced from global BEST analysis.

To further investigate the relationship between 'large' spring samples and alkalinity and TN, owing to the low (although non significant) *P* value produced from the BEST analysis, an initial MDS ordination was examined, before production of bubble plots (Fig. 4.4). This ordination revealed that a single spring sample, from Tullynawood Lake was very dissimilar to the other samples (Figure 4.4). Raw data showed that a single family (Diptera: Tipulidae) was the only recorded taxa present within the sample. As a result of the distance of this sample from all other samples in the MDS plot, and to ensure that the BEST analysis was not distorted by its inclusion, the BEST analysis was re-run excluding this sample from the "large" spring dataset (Table 4.6).

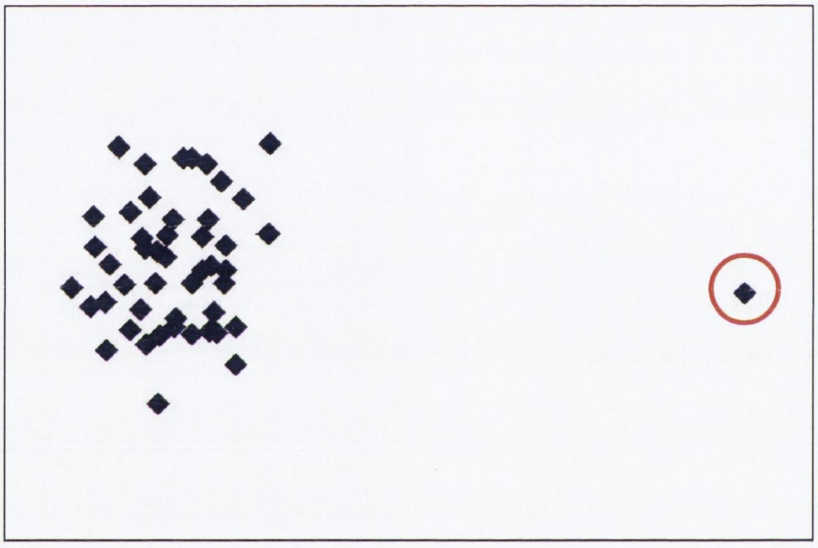


Figure 4.4: MDS of Bray-Curtis similarities from fourth root transformed data on 'large' lake littoral macroinvertebrate species from spring samples (stress = 0.18); n = 48. Tullynawood Lake sample is circled in red.

Results from the re-run BEST and Global BEST analysis, where the Tullynawood Lake sample was excluded, suggested that differences in alkalinity and DOC help describe changes in the community structure of spring samples, differing from initial analyses of the dataset (Table 4.6). This indicated that the Tullynawood sample had a large influence on the previous result, particularly on the choice of TN as an environmental variable important in explaining community change (Table 4.6). The *Rho* value was close to zero, and a high *p* value was produced, indicating little match between the community composition similarity matrix and alkalinity or DOC concentrations (Table 4.6).

Table 4.6: Water chemical variables best describing lake littoral macroinvertebrate species biomass of spring samples of the 'large' dataset, with sample from Tullynawood removed as determined by the BEST analysis.

Dataset	n	BEST Values	*P* value[1]	BEST variables
'Large' Spring	47	*Rho* = 0.089	0.341	- Alkalinity
(Tullynawood excluded)				- DOC

[1]Significance values produced from global BEST analysis.

BEST analysis of macroinvertebrate community structure biomass from the 'large' dataset found that 17 taxa best described community composition of spring samples and that 12 taxa best described summer samples; 9 of which were common to both spring and summer samples (Table 4.7).

Table 4.7: Species significantly describing community structure among samples of the 'large' dataset, chosen using the BEST analysis.

Spring	Summer
Anabolia nervosa	*Bithynia tentaculata*
Ancylus fluviatilis	*Enallagma cyathigerum**
Asellus aquaticus	*Erpobdella octoculata*
*Enallagma cyathigerum**	*Gammarus duebeni**
Ephemera danica	*Gammarus pulex**
*Gammarus duebeni**	*Nebrioporus assimilis* (adult)*
*Gammarus pulex**	*Nebrioporus depressus* (adult)*
Limnephilus lunatus	*Potamopyrgus antipodarum**
Limnephilus marmoratus	*Radix balthica**
Nebrioporus assimilis (adult)*	*Sigara distincta**
Nebrioporus depressus (adult)*	Sphaeriidae*
*Potamopyrgus antipodarum**	Tabanidae
*Radix balthica**	
Sialis lutaria	
*Sigara distincta**	
Sigara falleni	
Sphaeriidae*	

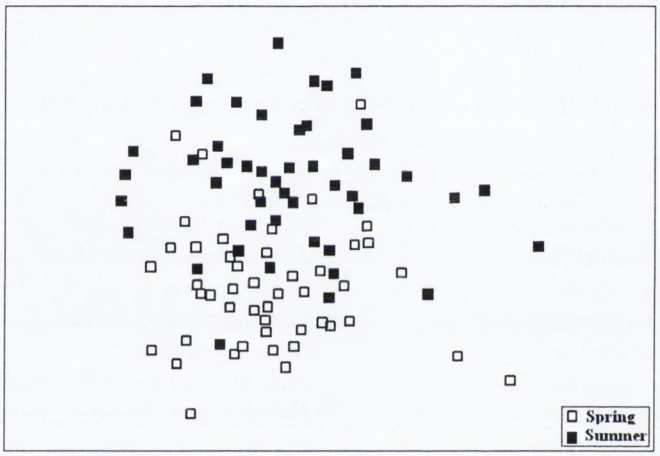
* appears where taxa occur on both spring and summer BEST derived taxa lists

4.3.3 *Post hoc* analysis of 'small' dataset

Results from the BEST analyses showed that changes in community structure were significantly related to changing TN concentrations in the 'whole' dataset, but no environmental variables were significantly related to community structure in the 'large' dataset. Therefore, data that described the biomass-based taxonomic structure of 'small' invertebrates (i.e. mean body mass <1.41 mg within each sample) was analysed in the same way as both 'whole' and 'large' datasets. This determined if a

corresponding shift in community structure with environmental variables was observed among the small invertebrates. MDS plots were generated using fourth root transformed biomass data to examine seasonal differences in the 'small' macroinvertebrate community dataset across lakes (Fig. 4.5). Community composition biomass appeared to differ between seasons (Fig. 4.5). Similar to the 'whole' dataset, the stress values of both two dimensional and three dimensional MDS plots were moderately high at 0.28 and 0.21, respectively. The three dimensional plot had a lower stress value, indicating a better representation of the data, and depicting a clear division in the community structure of 'small' invertebrates between seasons (Fig. 4.5b).

(a)



(b)

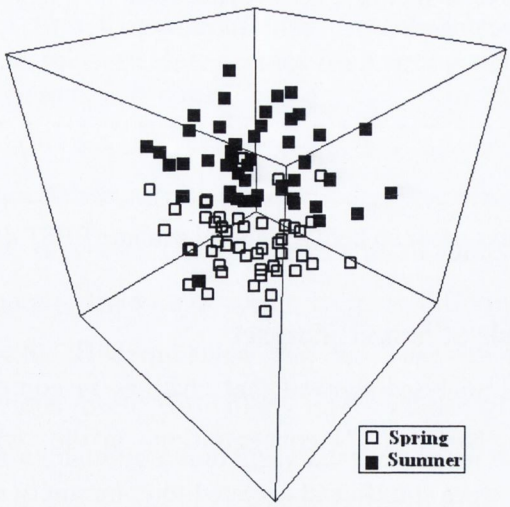


Figure 4.5: MDS of Bray-Curtis similarities from fourth root transformed data on lake littoral macroinvertebrate species biomass of the 'small' dataset in (a) two dimensions (stress = 0.28) and (b) three dimensions (stress = 0.21); n = 95.

PERMANOVA analysis revealed that the biomass-based macroinvertebrate community structure of the 'small' macroinvertebrate dataset differed significantly between seasons, and had a greater *pseudo* F ratio than that of the 'large' or 'whole' datasets. This indicated a greater divide in the community structure, reflected in the more defined separation of seasons in the MDS ordinations, compared with the other datasets (Fig. 4.5; Table 4.8).

Table 4.8: PERMANOVA analysis examining seasonal effects on lake littoral macroinvertebrate species biomass of 'small' dataset (n = 95).

Dataset	Factor	df	SS	MS	*Pseudo* F	*P* value (perm)
'small'	Season	1	16675	16675	7.04	**0.0001**
'small'	Residual	93	220340	2369	n.a.	n.a.

n.a. - not applicable.

BEST and Global BEST analyses of the 'small' dataset was conducted on samples from spring and summer in separate analyses. Results indicated that changes in the community structure of 'small' spring samples was significantly explained by TN and lake area (Table 4.9); TP and chlorophyll *a* were not related to community structure, (Appendix, Table II). BEST analysis of 'small' summer samples indicated that alkalinity, DOC may be of importance in structuring macroinvertebrate communities, but Global BEST showed that this variable was not significant in explaining changes.

Table 4.9: Water chemical variables best describing lake littoral macroinvertebrate species biomass of 'small' dataset as determined by the BEST analysis.

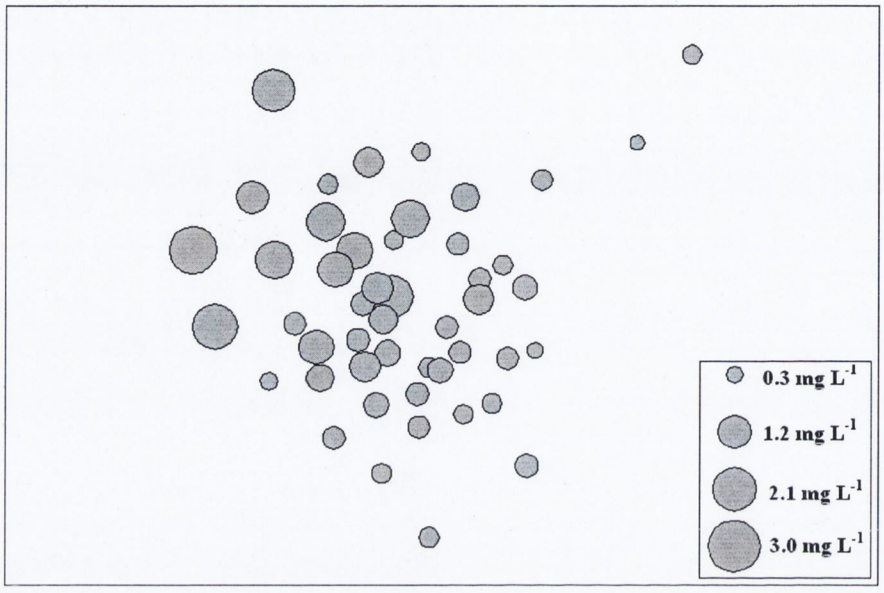
Dataset	n	BEST Values	*P* value[1]	BEST variables
'Small' Spring	48	*Rho* = 0.222	0.041	Lake area; TN
'Small' Summer	47	*Rho* = 0.139	0.21	Lake area

[1]Significance values produced from global BEST analysis.

Bubble plots, depicting variables chosen by BEST to significantly explain changes in community structure among 'small' spring samples did not reveal strong grouping of samples according to either TN or lake area, nor did cluster analyses and thus, cluster graphs were not overlaid (Fig. 4.6). Samples with higher TN concentrations were

loosely oriented towards the top left and centre of the graph (Fig. 4.6a). No pattern was evident in the community structure with lake area (Fig. 4.6b).

(a)



(b)

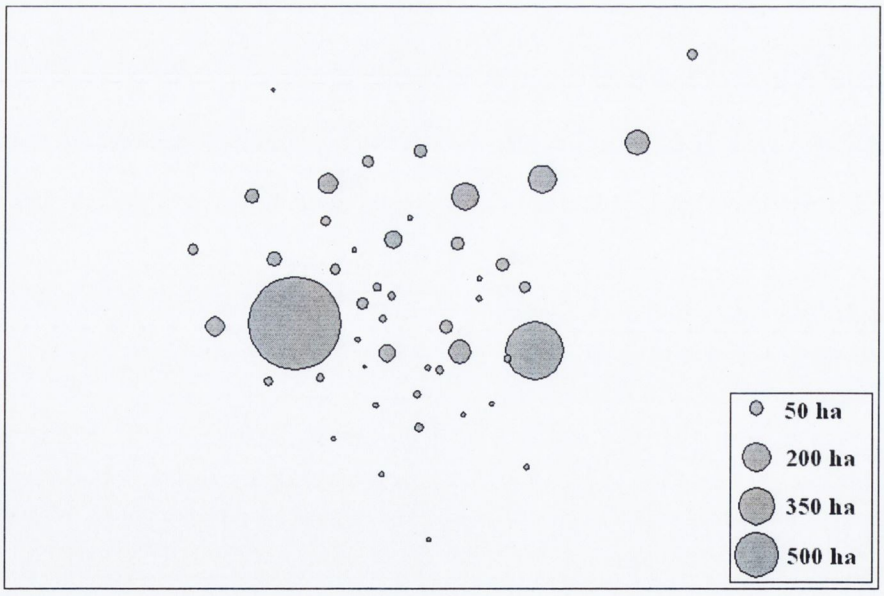


Figure 4.6: MDS of Bray-Curtis similarities from fourth root transformed data on lake littoral macroinvertebrate species biomass in spring from the 'small' dataset indicating (a) changing TN concentrations (mg L^{-1}) and (b) changing lake area; stress = 0.28; ha – hectares; n = 48.

BEST analysis of macroinvertebrate community structure biomass from the 'small' dataset found that 28 taxa best described community composition of spring samples and that 28 taxa best described summer samples, 18 of which were common to both spring and summer samples (Table 4.10).

Table 4.10: Species significantly describing community structure among samples of the 'small' dataset, chosen using the BEST analysis.

Spring	Summer
*Asellus aquaticus**	*Asellus aquaticus**
*Caenis horaria**	*Bithynia leachii*
*Caenis luctuosa**	*Caenis horaria**
Centroptilum luteolum	*Caenis luctuosa**
Ceratopogonidae	Chironomidae*
Chironomidae*	*Cloeon simile**
*Cloeon simile**	Diptera*
Diptera*	*Dryops* sp. (larvae)
Dugesia sp.	*Gammarus duebeni*
Ephemera danica	*Gammarus lacustris*
Gammarus sp. (juveniles)*	*Gammarus* sp. (juveniles)*
Haliplus sp. (adult)*	*Haliplus* sp. (adult)*
Haliplus sp. (larvae)*	*Haliplus* sp. (larvae)*
Heptageniidae	*Helobdella stagnalis*
Hydracarina*	Hydracarina*
Leptophlebia sp.	*Limnius volckmari* (adult)*
Leptophlebia vespertina	*Limnius volckmari* (larvae)
Limnephilus lunatus	Oligochaeta*
Limnius volckmari (adult)*	*Oulimnius* sp. (adult)*
Oligochaeta*	*Oulimnius* sp. (larvae)
Oulimnius sp. (adult)	*Polycelis nigra/tenuis*
Polycelis sp.*	*Polycelis* sp.*
Polycentropus sp. *	*Polycentropus* sp. *
*Potamopyrgus antipodarum**	*Potamopyrgus antipodarum**
Sigara scotti	*Sigara dorsalis/striata*
Siphonoperla torrentium	*Sigara* sp.
Sphaeriidae*	Sphaeriidae*
*Tinodes waeneri**	*Tinodes waeneri**

* appears where taxa occur on both spring and summer BEST derived taxa lists

4.3.4 Trends in collective and mean biomass of BEST identified taxa

Of the 252 taxa found among all samples, 23 were found to best describe community structure both in spring and in summer, and were varied in mean collective biomass (for the 'whole' dataset; Table 4.3; Fig. 4.7). Several species categorised principally as a mixture of grazer-scrapers, shredders and gathering-collectors (G-S-G) had higher collective biomass than those of other FFGs (i.e. *Asellus aquaticus*, *Gammarus pulex* and *Potamopyrgus antipodarum*; Fig. 4.7). *A. aquaticus* and *G. pulex* displayed moderate variability associated with the mean collective biomass; however, *P. antipodarum* had high associated error (Fig. 4.7). The filter feeding bivalve Sphaeriidae had moderate mean collective biomass, varying considerably among samples, but nevertheless contributing considerable biomass per sample (Fig. 4.7). Four taxa were classified as gathering-collectors (other taxa were categorised partially detritivorous, according to Moog [1995]; Fig. 4.7). Oligochaetes had higher mean collective biomass per sample than the two corixid species, *Sigara distincta* and *S. falleni*, and had approximately five times the collective biomass per sample of the small mayfly *Caenis horaria* (Fig. 4.7). Predatory taxa had the lowest mean collective biomass among all taxa (Fig. 4.7). Among predators, the leech species, *Erpobdella octoculata*, and flatworm species *Polycelis nigra/tenuis*, had the largest collective biomass, whereas the damselfly Coenagrioniidae and the alder fly *Sialis lutaria* had the smallest collective biomass (Fig. 4.7).

There were 111 taxa present among all samples of 'large' invertebrates, of which 9 taxa were found by BEST analysis to best describe community structure of the dataset in both spring and summer (Table 4.7; Fig. 4.8). Species principally categorized as G-S-G (*P. antipodarum*, *Gammarus pulex* and *G. duebeni*) and the filter feeding Sphaeridae had the highest collective biomass of all the species selected by BEST, followed by the detritivorous corixid *S. distincta*, and the principally G-S-G gastropod *Radix balthica* (Fig. 4.8). As was observed with the 'whole' dataset, *P. antipodarum* had very high variability associated with the mean value. Sphaeriidae also had high relative error, compared with the much lower variability of *G. pulex* and *G. duebeni*. Predatory species (*Enallagma cyathigerum* and the adult beetles *Nebrioporus assimilis* and *N. depressus*) had the smallest collective biomass of all species (Fig. 4.8).

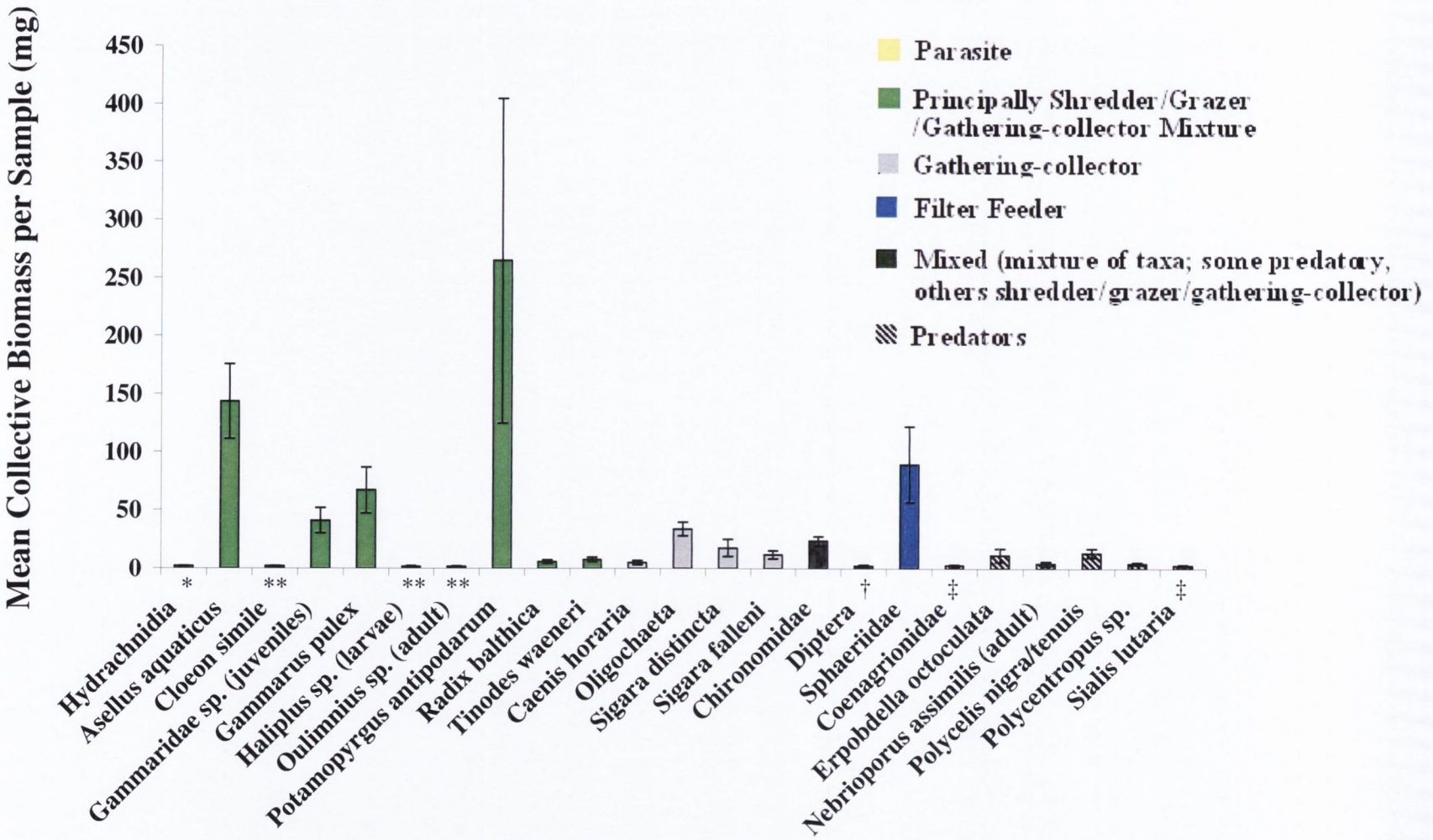


Figure 4.7: Mean collective biomass per sample (where present) of species shown by BEST analysis to describe the community structure of the 'whole' dataset. Error bars represent the standard error of the mean; n = 95. Symbols are entered below bars that are too small to distinguish pattern: * denotes parasite; ** denotes principally shredder / grazer / gathering-collector; † denotes mixed; ‡ denotes predator.

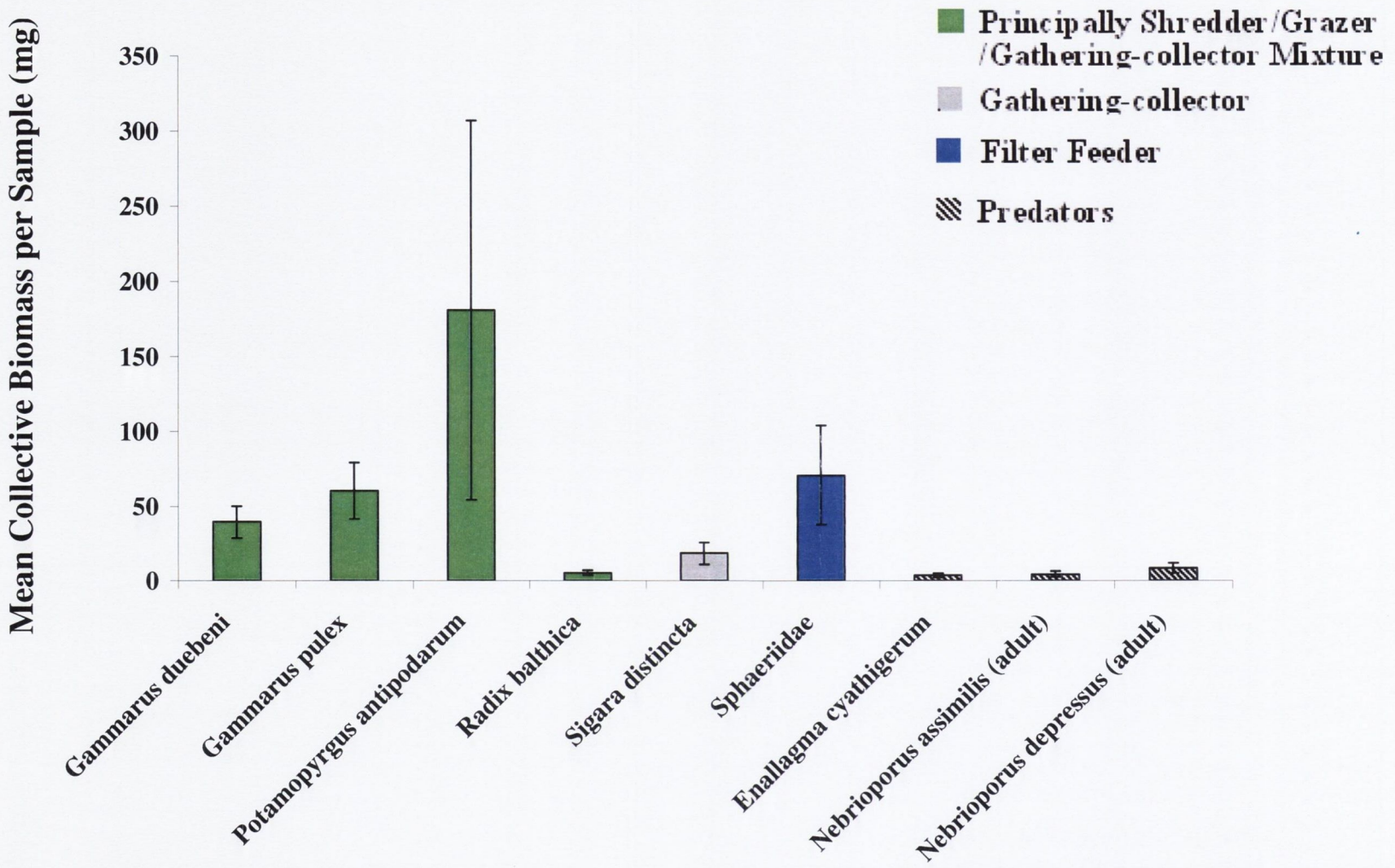


Figure 4.8: Mean collective biomass per sample of species (where present) shown by BEST analysis to describe the community structure of the 'large' invertebrates. Error bars represent the standard error of the mean; n = 93.

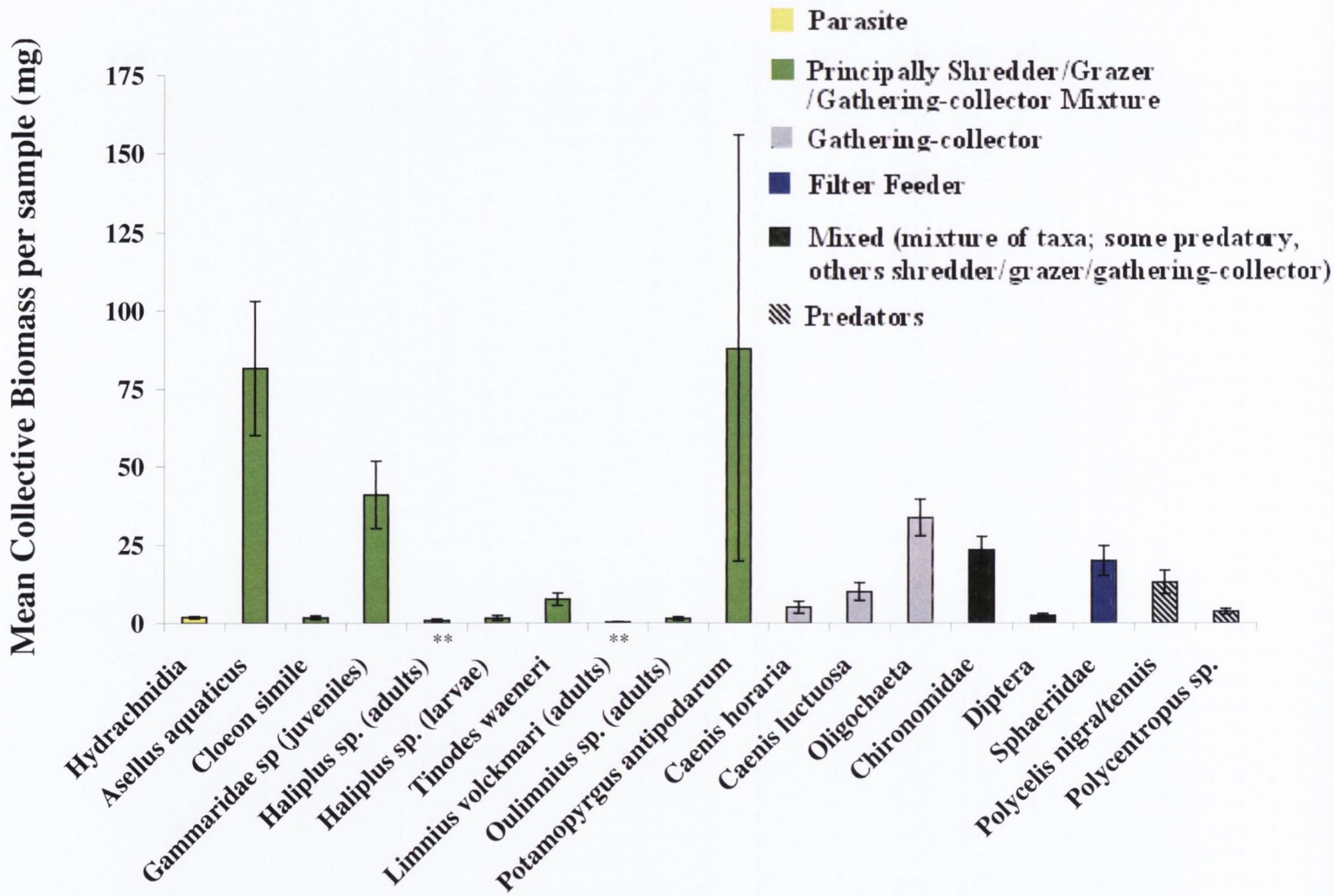


Figure 4.9: Mean collective biomass per sample of species (where present) shown by BEST analysis to describe the community structure of the 'small' invertebrates. Error bars represent the standard error of the mean; n = 95. **denotes principally shredder / grazer / gathering-collector;

Of the 207 taxa of 'small' invertebrates among samples, 18 taxa were selected by BEST analysis to best describe community structure in spring and summer (Table 4.10; Fig. 4.9). As observed in the 'whole' and 'large' datasets, *P. antipodarum* had the greatest collective biomass of all species, but also had high associated error (Fig. 4.9). *Asellus aquaticus* and Gammaridae (juveniles) also had high collective biomass per sample, with small associated error (Fig. 4.9). The six other species classified as a mixture of G-S-G (*Cloeon simile*, *Haliplus* sp., *Tinodes waeneri,* adults of *Limnius volckmari* and *Oulimnius* sp.) had low mean collective biomass (Fig. 4.9). Of the three gathering-collector taxa, Oligochaeta had relatively high mean collective biomass with low error associated, compared with the two mayflies *Caenis luctuosa* and *C. horaria*, which had relatively low collective biomass per sample (Fig. 4.9). The functionally mixed Chironomidae had moderately high collective biomass, with low associated error, compared with Diptera which had much lower collective biomass (Fig. 4.9). Sphaeriidae, the only filter feeding taxon selected, also had moderately high collective biomass and the two predatory taxa *Polycelis nigra/tenuis* and *Polycentropus* sp. had slightly lower collective biomass (Fig. 4.9). In all, the mixed G-S-G primary consumers had the greatest collective biomass within samples, and although there were a greater number of core species that best described the community structure in the 'small' dataset than observed among 'large' invertebrates, the collective biomass of those species was less than that of the fewer 'large' invertebrates (Fig. 4.9).

The mean biomass of each of the taxa identified by BEST to be important in describing community structure of the 'whole' community varied considerably among taxa (Fig. 4.10). The largest taxa were a mixture of functional groups: *Radix balthica* (G-S-G mixture), *Erpobdella octoculata* (predator), *Sigara distincta* and *S. falleni* (gathering-collectors; Fig. 4.10). Some of these larger species had highly variable mean biomass (*R. balthica* and *E. octoculata*) contrasting with the stability of the mean biomass of the two corixid gathering-collectors (*S. distincta* and *S. falleni*). Intermediate sized taxa represented a mixture of FFGs, with three mixed G-S-G species (*Asellus aquaticus*, *Gammarus pulex* and *Potamopyrgus antipodarum*), the filter feeding Sphaeridae, and three predator taxa (*Nebrioporus assimilis* adults, *Polycentropus* sp. and *Sialis lutaria*; Fig. 4.10), all with relatively low associated variance. There was a notable contrast in the largely invariate mean biomass of *P. antipodarum* and the highly variable collective biomass among samples seen in 'whole', 'large' and 'small' datasets (Figs. 4.7 to 4.9).

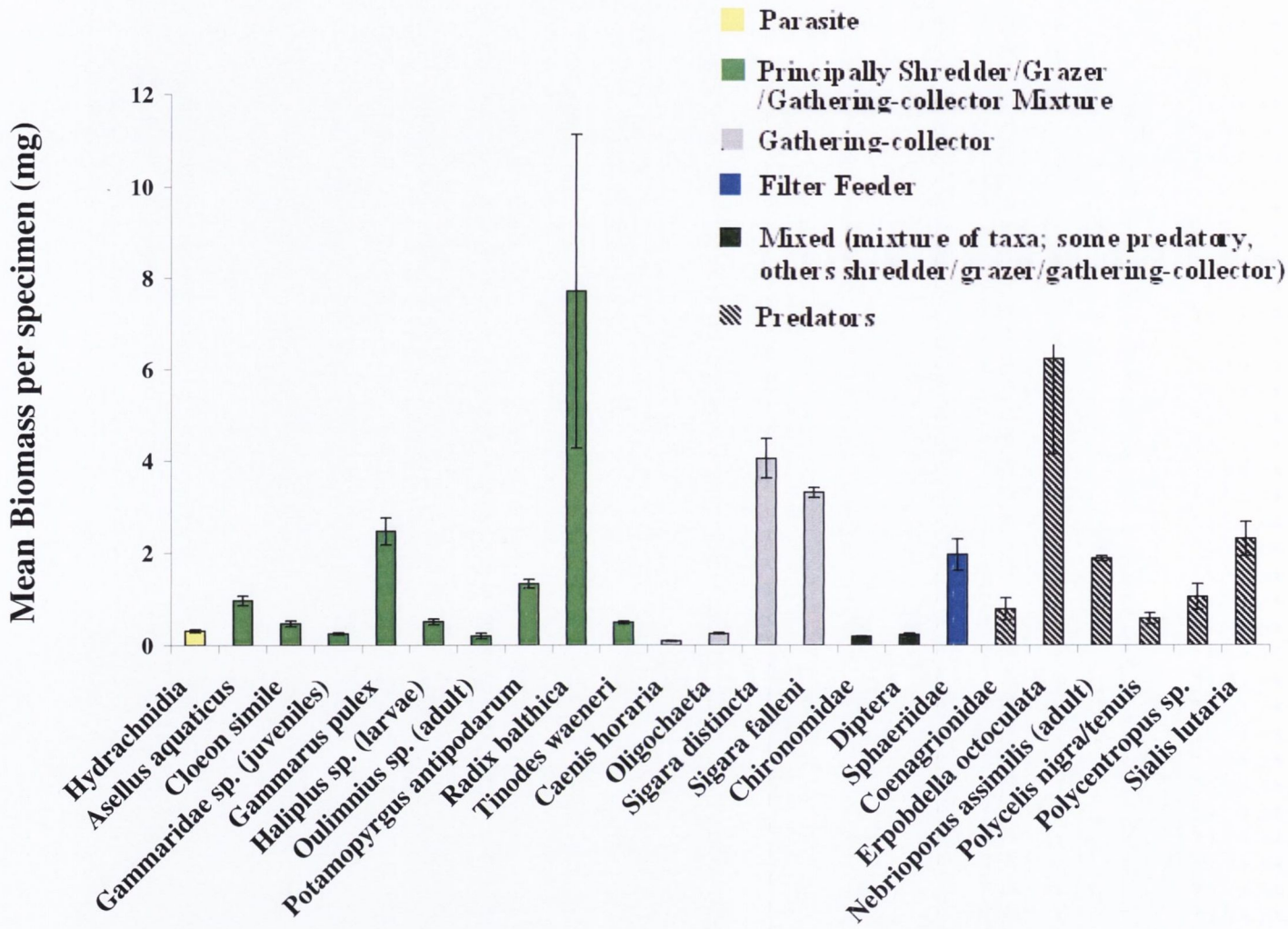


Figure 4.10: Mean biomass per specimen of species shown by BEST analysis to describe the community structure of the 'whole' dataset. Error bars represent the standard error of the mean; n = 95.

The remaining 12 smallest taxa included taxa from all FFGs, most with relatively low variability in their mean biomass (Hydracarina, *Cloeon simile,* Gammaridae sp. [juveniles], *Haliplus* sp. [larvae], Oligochaeta, Chironomidae, *Tinodes waeneri*, Diptera and *Polycelis nigra/tenuis*; Fig. 4.10).

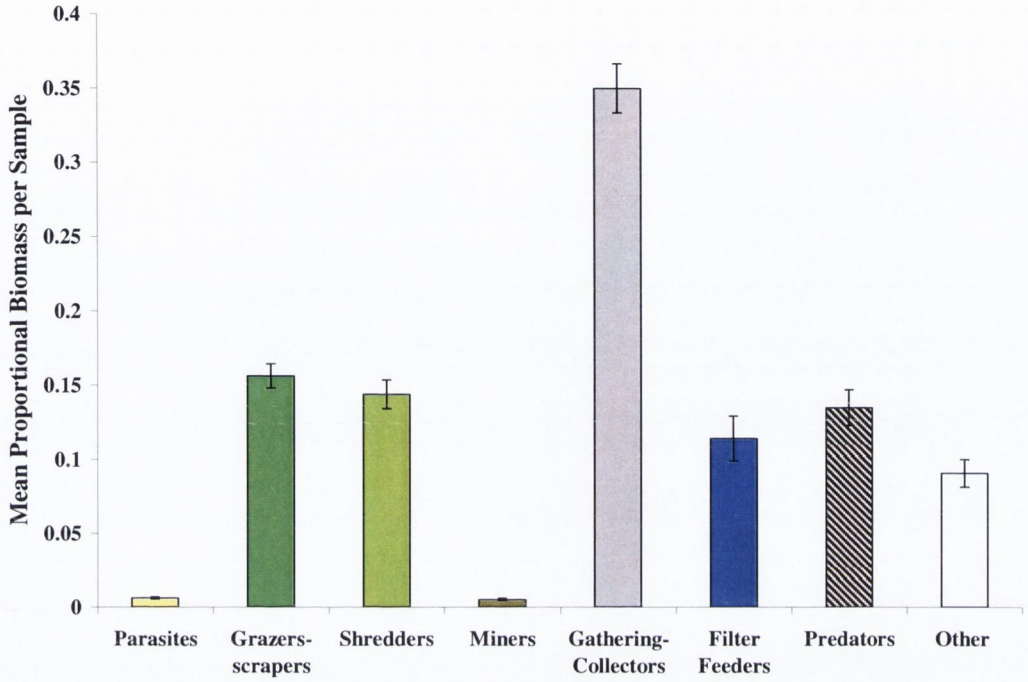
4.3.5 Trends in the proportional biomass of each functional feeding group

Before commencing the LME analysis of the proportional biomass of each FFG for the 'large' and 'whole' datasets, histograms were constructed to visualise the mean proportion of each FFG (average across lakes; Fig. 4.11) and to show changes in mean percentage of each FFG among lakes in relation to TN (Fig. 4.12). The proportion of each FFG per sample had relatively small standard error, and varied little among samples in the 'whole' dataset (Fig. 4.11a). Gathering-collectors had the highest proportions within samples, representing approximately one third of the biomass within each sample; whereas parasites and miners had the lowest (< 1% of each sample; Fig. 4.11a). The remaining principal FFGs (grazer-scrapers, shredders, predators and filter feeders) each corresponded to ~10 to 16% of each sample (Fig. 4.11a).

The proportional biomass of each FFG in the 'large' dataset had slightly higher associated variation than in the 'whole' dataset. Gathering-collectors were similarly represented among the 'large' invertebrates, corresponding to ~30% of biomass in each sample (Fig. 4.11b). However, predators contributed more to the total biomass of 'large' invertebrates, corresponding to approximately one quarter of the total biomass of each sample (Fig. 4.11b). Grazer-scrapers, filter feeders and shredders were similarly distributed within the 'large' samples to that of the 'whole' samples, although there was a slight increase in the proportion of shredders in the 'large' samples (Fig. 4.11b).

No consistent change in the proportion of each FFG with increasing TN concentrations was apparent for either 'whole' or 'large' datasets (Fig. 4.12). Variation in the proportion of each FFG was lower in the 'whole' dataset than in the 'large' dataset (Fig. 4.12). Predators were, however, present in higher proportions at low TN concentrations in the 'large' dataset (Fig. 4.12).

(a)



(b)

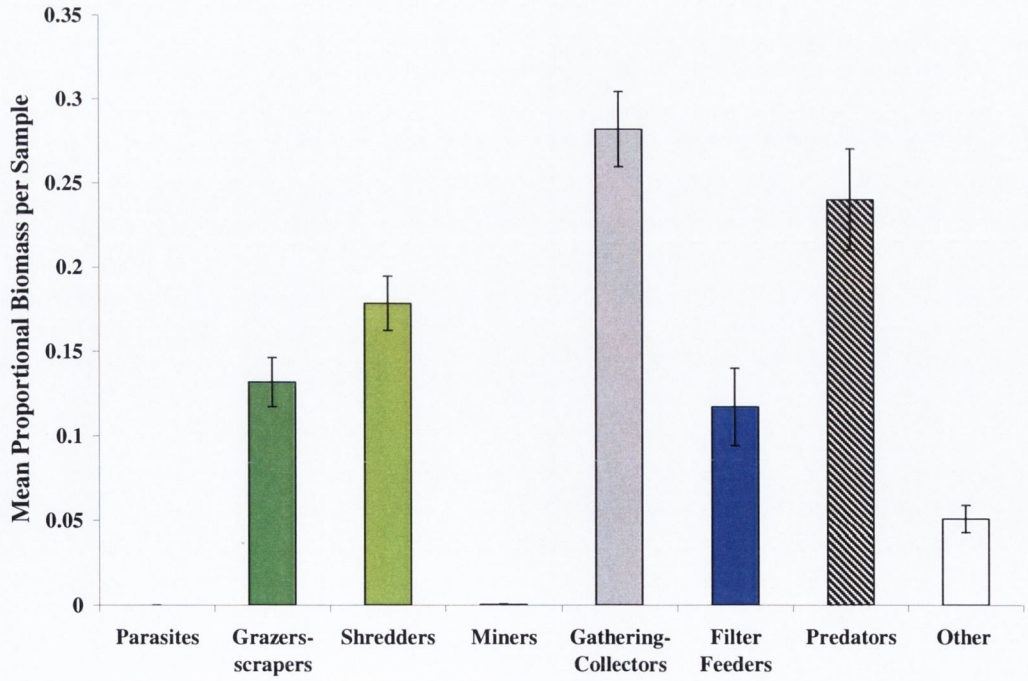
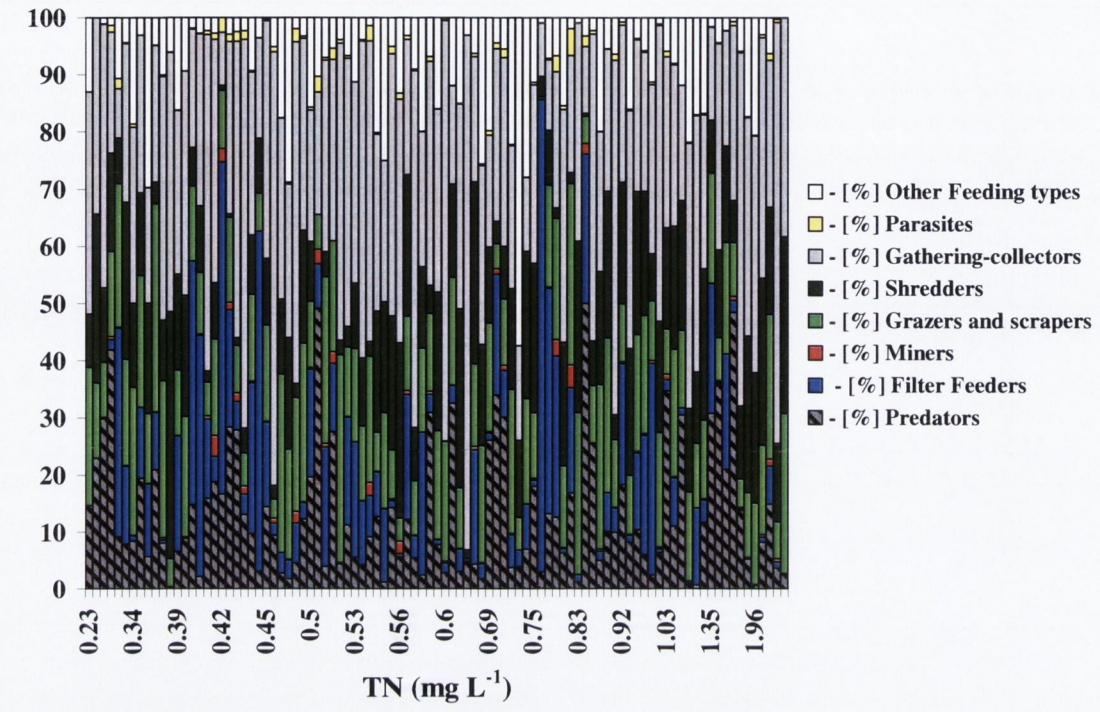


Figure 4.11: Histograms showing mean proportional biomass of each FFG per sample for the (a) 'whole' dataset (n = 95) and (b) 'large' dataset (n = 93). Error bars represent the standard error of the mean.

(a)



(b)

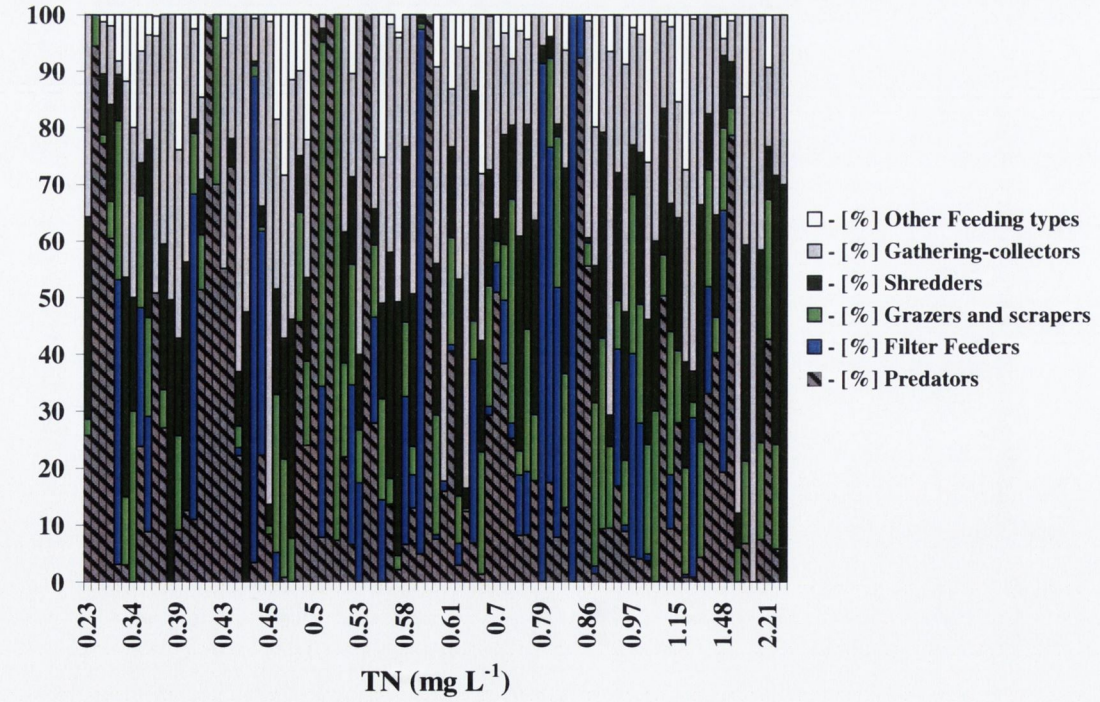


Figure 4.12: Histogram depicting percentage of each FFG per sample, ordered according to sample TN concentration (mg L^{-1}) for the (a) 'whole' dataset and (b) 'large' dataset.

4.3.6 Relationships between taxa and environmental variables

Spearman rank correlations used to examine relationships between taxa groups and environmental variables indicated that Araneae, Gastropoda, Lepidoptera and Trichoptera were not significantly related to environmental variables (data not presented). The proportional biomass of Coleoptera, Crustacea and Plecoptera increased, and of Bivalvia, Diptera, Megaloptera and Odonata decreased with lake area. The proportional biomass of Crustacea, Hirudinea, and Turbellaria increased and of Ephemeroptera and Plecoptera decreased with alkalinity (Table 4.11). DOC concentrations were not significantly related to the proportional biomass of any of the taxa groups (Table 4.11). Coleoptera, Ephemeroptera and Plecoptera significantly decreased with increasing nutrients (TP and TN) and chlorophyll *a* concentrations (Table 4.11). Heteroptera increased with increasing TN concentrations, and Hydrachniidia and Oligochaeta significantly decreased with increasing chlorophyll *a* concentrations (Table 4.11). In summary, Ephemeroptera and Plecoptera were the most sensitive to change in water chemistry and in lake area, followed by Coleoptera.

Table 4.11: Spearman rank correlation matrix of proportional biomass of taxa groups significantly correlated with environmental variables (mean of samples from two seasons for 48 lakes). Significant relationships are in bold font.

	Lake area	Alkalinity	DOC	TP	TN	Chlorophyll *a*
Bivalvia	**-0.354***	-0.061	0.136	0.054	-0.164	-0.041
Coleoptera	**0.437****	-0.268	-0.074	**-0.354***	**-0.363***	**-0.326***
Crustacea	**0.406****	**0.603*****	-0.202	0.136	0.274	0.224
Diptera	**-0.348***	-0.170	0.310	-0.002	-0.057	-0.079
Ephemeroptera	0.087	**-0.546*****	-0.097	**-0.576*****	**-0.752*****	**-0.723*****
Heteroptera	-0.100	0.274	0.104	0.223	**0.335***	0.246
Hirudinea	0.283	**0.343***	0.074	0.175	0.252	0.209
Hydracarina	0.202	-0.206	-0.058	-0.239	-0.191	**-0.320***
Megaloptera	**-0.297***	0.118	0.134	0.067	0.117	0.183
Odonata	**-0.490*****	0.032	-0.073	-0.117	-0.056	-0.027
Oligochaeta	0.046	-0.218	0.147	-0.186	-0.143	**-0.323***
Plecoptera	**0.366***	**-0.505*****	-0.125	**-0.362***	**-0.426****	**-0.461****
Turbellaria	0.056	**0.424****	0.021	0.275	0.213	0.256

Significance levels: * *P* value <0.05; ** *P* value <0.01; *** *P* value <0.001.

Four taxa groups (Araneaea, Gastropoda, Lepidoptera and Trichoptera) were not significantly related to any of the variables examined (and thus not presented in Table 4.11). Lepidoptera and Araneaea were rare among lakes, and likely occurred too infrequently for detection of relationships with lake area and water chemistry. In contrast Gastropoda and Trichoptera were common among lakes, and these taxa groups appear to be tolerant to change in water chemistry and lake area.

4.3.7 Analysis of functional feeding group biomass of 'whole' dataset

Owing to the inclusion of the log total biomass (TB) correction factor in all models, each of the FFG biomass response variables represented effectively the proportional biomass rather than total biomass of the FFG, for both 'whole' and 'large' datasets. The fixed effects of lake area, DOC, and all TN interaction terms were eliminated from minimal adequate models of all response variables of FFG biomass ('whole' dataset; Table 4.12).

The minimal adequate model of the response variable log TB predators retained season, and linear and quadratic TN terms as fixed effects (Table 4.12). The slope of the relationship between season and log TB predators was negative, with a low standard error (SE), indicating a decrease in predator biomass relative to total biomass in summer (Table 4.12). The retention of both linear and quadratic TN terms indicated a curvilinear relationship between log TB predators and TN (Table 4.12). The positive coefficient of the linear TN term indicates that log TB predators increases with increasing TN relative to log TB, but the negative quadratic term indicates a downward force on this relationship (Table 4.12). Owing to the higher value of the linear and quadratic TN coefficients, it was apparent that TN had a stronger relationship with log TB predators than season. The variance components of the model reveal that lake identity as a random effect accounted for approximately 53% of variance in the model.

The minimal adequate model produced for the response variable log TB filter feeders retained only season as a fixed effect (Table 4.12). The slope of the relationship between season and log TB filter feeders was positive with a low SE, indicating an increase in filter feeders in summer (Table 4.12). Random variance among lakes accounted for approximately 61% of variance in the model.

Table 4.12: Minimal adequate mixed effects models for the response variables log total biomass (TB) predators, log TB filter feeders, log TB gathering-collectors, log TB shredders, and log TB grazer-scrapers, from the 'whole' dataset*.

		Fixed effects								Variance components (Random effects)				
Response variable	**Coeffs.**	**Intercept**	**Log TB Correction**	**Season**	**Area**	**Alk.**	**DOC**	**TN**	**TN^2**	**Intercept**	**Residual**	**n**	**df**	**AIC**
Log TB Predators	B	-0.29	0.65	-0.19	-	-	-	0.67	-0.30	52.67	47.33	95	7	110.9
	SE	0.25	0.08	0.06	-	-	-	0.35	0.14					
Log TB Filter Feeders	B	-1.05	0.85	0.32	-	-	-	-	-	61.16	38.84	95	5	176.2
	SE	0.34	0.12	0.08	-	-	-	-	-					
Log TB Gathering-Collectors	B	-0.37	0.98	-0.14	-	-	-	-	-	22.25	77.75	95	5	-26.0
	SE	0.11	0.04	0.04	-	-	-	-	-					
Log TB Shredders	B	-1.47	1.15	-	-	0.00	-	-	-	41.52	58.48	94	5	101.0
	SE	0.20	0.08	-	-	0.00	-	-	-					
Log TB Grazer-Scrapers	B	-0.76	0.97	-	-	-	-	-	-	8.27	91.73	93	4	6.2
	SE	0.12	0.05	-	-	-	-	-	-					

*A correction factor of log TB was included as a fixed effect in each model (see Section 4.2.4.2 for details). Fixed effects included in the maximal models were season, alkalinity (Alk.), dissolved organic carbon (DOC), total nitrogen (TN), a quadratic TN term and interaction effects between TN and season, and TN and alkalinity. All models had the same random structure (lake identity as random effect). Interaction effects were eliminated from minimal adequate models of each response variable.

Season was the only fixed effect retained in the minimal adequate model of log TB gathering-collectors (Table 4.12). However, the negative coefficient of the season term with low SE indicated that gathering-collectors decreased in summer (Table 4.12). Lake identity as a random effect accounted for approximately 22% of variance in the model.

The minimal adequate model of log TB shredders included alkalinity as the only fixed effect retained in the model. The slope of the alkalinity-log TB shredders relationship was positive but close to zero, indicating marginal increases in the total biomass of shredders under increasingly alkaline conditions. Random variation among lakes accounted for almost 42% of variance in the model.

All fixed effects were eliminated from the minimal adequate model of log TB grazer-scrapers (with the exception of the log TB correction factor; Table 4.12) indicating that none of the explanatory variables significantly explained variation in log TB grazer-scrapers among lakes. The variance components of the model reveal that lake identity as a random effect accounted for approximately 8% of variance.

4.3.8 Analysis of functional feeding group biomass of 'large' dataset

Similar to the analysis of the 'whole' dataset, lake area and DOC were eliminated from all models of FFG biomass for the 'large' dataset. The quadratic TN term, and the season-TN interaction term were also eliminated from all minimal adequate models (Table 4.14). The minimal adequate model of log TB predators retained only season as a fixed effect (Table 4.14). The slope of the season-log TB predator relationship was negative, with an SE half of that of the coefficient, indicating that predators decreased in summer, but that this relationship was variable (Table 4.14). Random variance among lakes accounted for approximately 38% of variance.

The minimal adequate model of log TB filter feeders retained several fixed effects, which were season, alkalinity, TN and the alkalinity-TN interaction effect (Table 4.14). The positive coefficient of the season term with a low SE indicated that log TB filter feeders increased in summer (Table 4.14). The alkalinity main effect had a positive value close to zero with a low SE, indicating a small increase in log TB filter feeders with increasing alkalinity. The TN main effect had a positive value, with an SE half of

that of the coefficient, indicating a weak relationship between log TB filter feeders and TN with high associated variability. The interaction term between alkalinity and TN had a small negative coefficient close to zero with a small relative SE, demonstrating that TN mediated a decrease in the slope of the alkalinity-log TB filter feeders relationship (Fig. 4.13a).

More than half of all samples contained no 'large' filter feeders (50 of the 93 samples; Table 4.13; Fig. 4.13). 'Large' filter feeders were absent from more than 70% of samples with low or very high TN concentrations, but were present in more than 60% of samples with moderate-high TN concentrations (i.e. 0.50 to 1.49 mg L^{-1}; Table 4.13). Likewise, 'large' filter feeders were absent from almost 70% of low alkalinity lakes, increasing in occurrence with alkalinity (Table 4.13). Lakes with moderate to high TN concentrations (0.5 to 1.25 mg L^{-1}) displayed a slightly curvilinear relationship between log "proportional filter feeders" and alkalinity, with highest proportions of filter feeders in lakes with less than 110 mg $CaCO_3$ L^{-1} (Fig. 4.13). The log proportion of filter feeders at moderate concentrations of TN (i.e. 0.50 to 0.74 mg L^{-1}) was relatively constant, principally representing moderate alkalinity lakes; the majority of filter feeders at higher concentrations of TN (>0.75 mg L^{-1}) were in high alkalinity lakes with slightly lower proportional biomass. Thus, the proportion of filter feeders within moderate and high alkalinity lakes decreased with increasing TN (Fig. 4.13b). However, as high alkalinity lakes with filter feeders frequently had high concentrations of TN, it is difficult to distinguish alkalinity from nutrient effects (Fig. 4.13). Random variation among lakes accounted for almost 47% of variance in the model.

Table 4.13: Details of the presence of filter feeders (FFs) within samples divided into four ranges of TN concentration, and three ranges of alkalinity concentration.

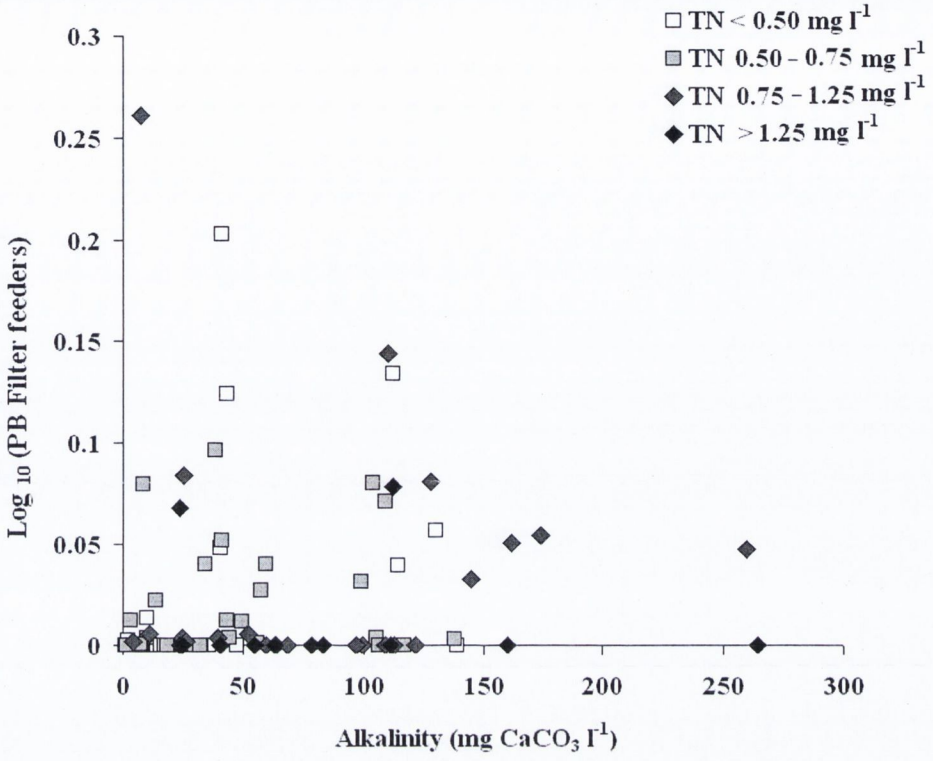
TN range (mg L^{-1})	Total no. samples	No. samples FFs absent	Percentage samples FFs absent
0.23 to 0.49	28	20	71%
0.50 to 0.74	31	12	39%
0.75 to 1.25	22	8	36%
1.25 to 2.50	12	10	83%
Alkalinity range (mg $CaCO_3$ L^{-1})			
Low (0 to 20)	32	22	69%
Moderate (20 to 100)	38	19	50%
High (>100)	23	9	39%

Table 4.14: Minimal adequate mixed effects models for the response variables log total biomass (TB) predators, log TB filter feeders, log TB gathering-collectors, log TB shredders, and log TB grazer-scrapers, from the 'large' dataset*.

		Fixed effects									Variance component (Random Effects)s				
Response variable	**Coeffs.**	**Intercept**	**Log TB Correction**	**Season**	**Area**	**Alk.**	**DOC**	**TN**	**TN^2**	**Alk x TN**	**Intercept**	**Residual**	**n**	**df**	**AIC**
Log TB Predators	B	0.21	0.53	-0.26	-	-	-	-	-	-	36.38	63.62	93	5	188.8
	SE	0.24	0.09	0.11	-	-	-	-	-	-					
Log TB Filter Feeders	B	-1.28	0.59	0.34	-	0.01		0.58		-0.01	46.76	53.24	93	8	250.2
	SE	0.37	0.12	0.13	-	0.00		0.29		0.00					
Log TB Gathering-collectors	B	-0.67	1.00	-	-	-	-	-	-	-	13.93	86.07	93	4	141.5
	SE	0.17	0.07	-	-	-	-	-	-	-					
Log TB Shredders	B	-0.87	0.94	-	-	0.00	-	-	-	-	0.00	100.00	93	5	154.3
	SE	0.16	0.08	-	-	0.00	-	-	-	-					
Log TB Grazer-scrapers	B	-1.01	0.95	-	-	0.00	-	-	-	-	0.00	100.00	93	5	153.2
	SE	0.16	0.07	-	-	0.00	-	-	-	-					

*A correction factor of log TB was included as a fixed effect in each model (see Section 4.2.4.2 for details). Fixed effects included in the maximal models were season, alkalinity (Alk), dissolved organic carbon (DOC), total nitrogen (TN), a quadratic TN term, and interaction effects between TN and season, and TN and alkalinity. All models had the same random structure (lake identity as random effect). Interaction effects between season and TN were eliminated from the minimal adequate models of each response variable.

(a)



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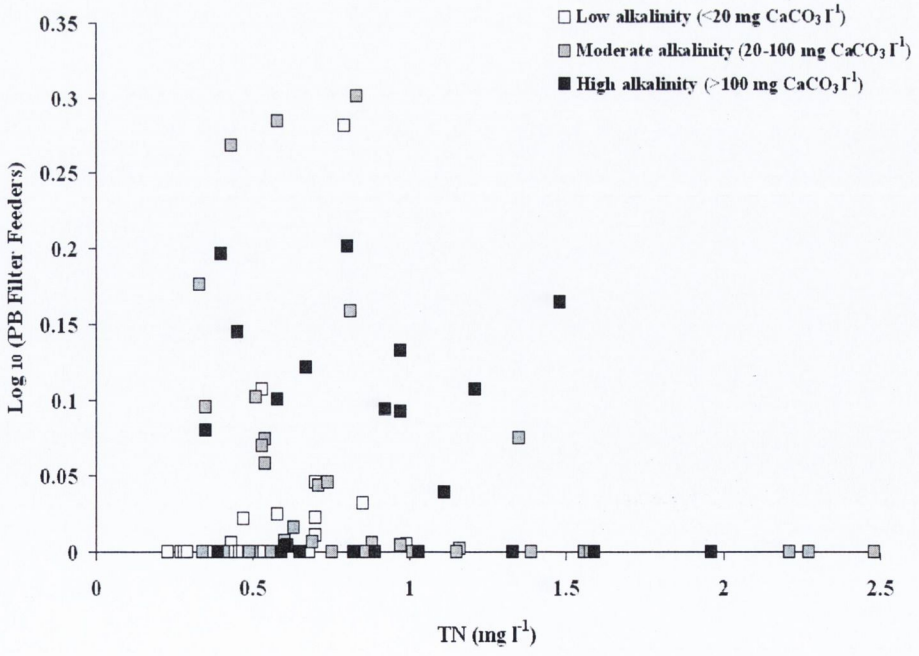


Figure 4.13: Relationship between log TB filter feeders and (a) alkalinity at different TN concentrations and (b) TN at different alkalinity concentrations; plotted from the raw data.

The minimal adequate model of log TB gathering-collectors was empty of fixed effects (with the exception of the log TB correction factor) indicating that none of the explanatory variables significantly explained variation in log TB gathering-collectors among lakes (Table 4.14). Random variation among lakes accounted for almost 14% of variance in the model.

Alkalinity was the only fixed effect retained in the minimal adequate model of log TB shredders (Table 4.14). The coefficient was positive but very close to zero, with an SE value slightly less than half of the coefficient value, indicating a marginal increase of log TB shredders with alkalinity, but with a high variability associated with the term (Table 4.14). The variance components of the model indicated that lake identity accounted for almost no random variation.

The minimal adequate model of log TB grazer-scrapers also retained alkalinity as the only fixed effect (Table 4.14). The positive coefficient had a value close to zero with a SE slightly less than half of its value (similar to the relationship seen in the minimal adequate model of log TB shredders), thereby indicating a marginal increase, but with high variability with increasing alkalinity concentrations (Table 4.14). Random variance among lakes accounted for almost no variance in the model (Table 4.14).

4.3.9 Analysis of change in predatory and filter feeding taxa with TN

4.3.9.1 Predators

Minimal adequate models of FFG biomass indicated that predators had a positive curvilinear relationship with increasing TN concentrations for the 'whole' dataset. Following Moog (1995), predatory taxa shown by BEST analysis as important drivers of the observed patterns (Table 4.3) were used to exemplify patterns in the data (Fig. 4.14). As curvilinear relationships between proportional biomass of taxa and TN were visually examined, and each taxa were absent from a large number of samples across the TN spectra, regression statistics are not presented in the graphs (Fig. 4.14).

Coenagrioniidae proportional biomass was highly variable across TN concentrations, with no apparent difference between seasons (Fig. 4.14a). Only 22 of the 95 samples from the 'whole' dataset contained Coenagrioniidae, occurring across a wide range of TN concentrations, with no clear pattern with TN (Fig. 4.14a). Although only 18 of the

95 samples contained *Erpobdella octoculata,* the proportion of *E. octoculata* was greatest in summer samples at moderate TN concentrations (~0.50 to 0.75 mg L^{-1}), decreasing with high TN, and with a left skewed distribution. No specimens were found in samples with high TN concentrations in summer, and there was a negative linear relationship with TN in the spring samples (Fig. 4.14b). *Nebrioporus assimilis*, present in only 19 of 95 samples, displayed a relatively low proportional biomass within all samples, with the exception of a single summer sample, and changed little with season and with increasing TN concentrations (Fig. 4.14c). In contrast, the proportion of *Polycelis* sp. (present in 54 of 95 samples) was highly variable, displaying a loosely curvilinear change in spring with increasing TN, but a more variable relationship with TN in summer, but with overall greater proportions in spring (Fig. 4.14d).

The proportional biomass of *Polycentropus* sp. (present in 36 of 95 samples), appeared consistently greater at moderate TN concentrations in summer, displaying a left skewed modal relationship with TN in both spring and summer samples (Fig. 4.14e). *Sialis lutaria* (present in 27 of the 95 samples), had a relatively linear decrease in proportional biomass with increasing TN concentrations, and present more frequently in spring samples, in higher proportions (Fig. 4.14f). In summary, proportional biomass of all predatory species was lowest at high TN concentrations (>1.5 mg L^{-1}) and highest at moderate TN concentrations (~0.5 to 0.75 mg L^{-1}) and differed between seasons for many of the taxa examined (Fig. 4.14).

Coenagrioniidae had highly variable mean biomass among samples, with greater mean biomass in spring than summer samples, with a loosely but non significant (P = 0.44) negative relationship with TN concentrations, (Fig. 4.14g). The mean biomass of *E. octoculata* was highly variable between seasons, and had a variable and non significant relationship with TN (P value = 0.16); only three samples with TN concentrations >1 mg L^{-1} contained the species (Fig. 4.14h). However, the mean biomass of *Nebrioporus assimilis* adults was consistent among samples and differed little between seasons, displaying a marginal decrease in size with increasing TN concentrations, and with a P value close to statistical significance (P value = 0.075; Fig. 4.14i).

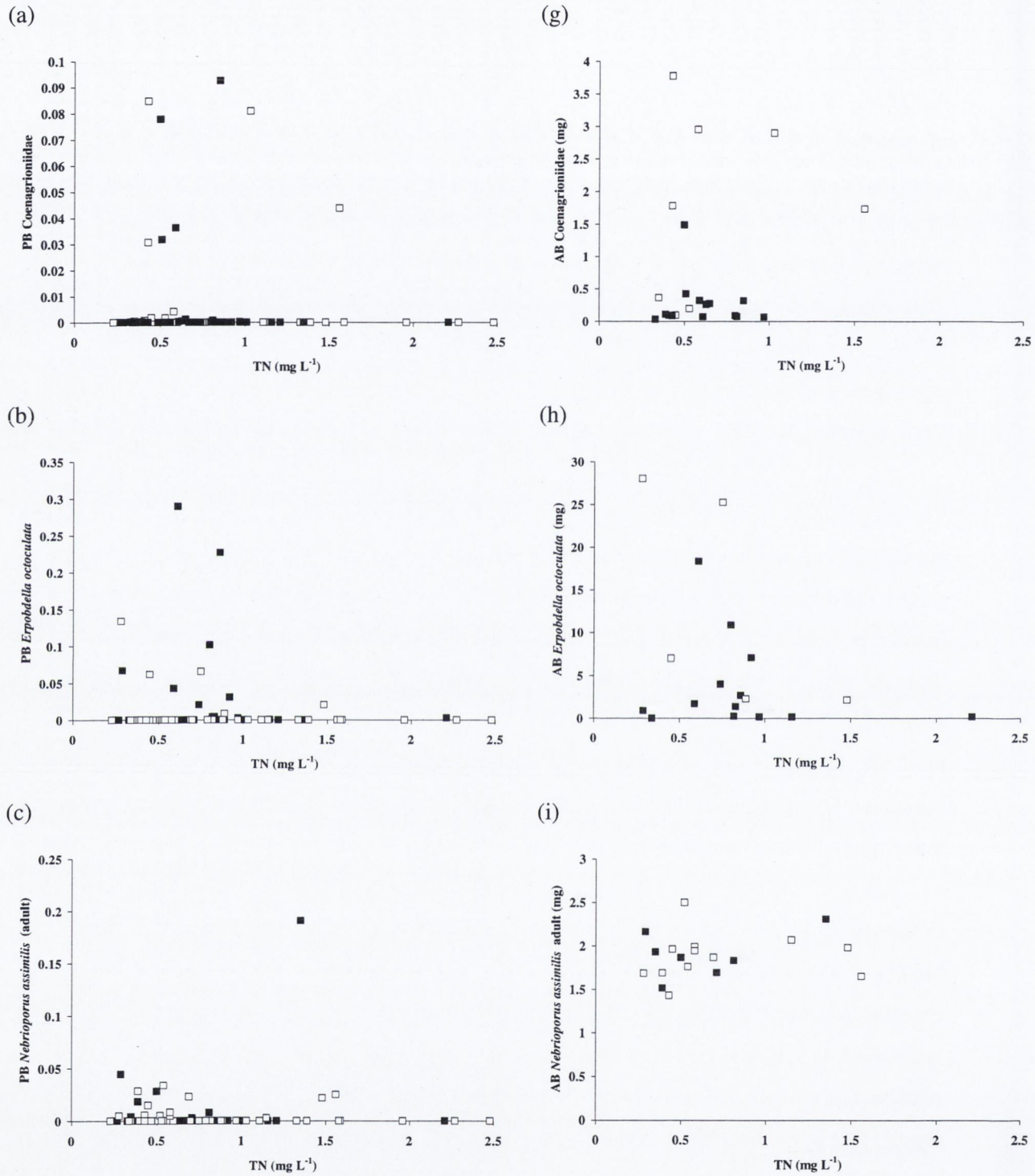


Figure 4.14 Graphs (a) to (f) show relationships between the proportional biomass [PB] of key taxa [(a) Coenagrioniidae (b) *Erpobdella octoculata* (c) *Nebrioporus assimilis* {adult} (d) *Polycelis* sp. (e) *Polycentropus* sp. (f) *Sialis lutaria*] and TN (mg L^{-1}); Graphs (g) to (l) show relationships between the mean biomass [AB; mg] of key taxa [(g) Coenagrioniidae ($R^2 = 0.03$; $P = 0.43$) (h) *Erpobdella octoculata* ($R^2 = 0.12$; $P = 0.16$) (i) *Nebrioporus assimilis* (adult; $R^2 = 0.17$; $P = 0.075$) (j) *Polycelis* sp. ($R^2 = 0.00$; $P = 0.86$) (k) *Polycentropus* sp. ($R^2 = 0.00$; $P = 0.88$) (l) *Sialis lutaria* ($R^2 = 0.03$; $P = 0.39$)] and TN (mg L^{-1}); □ = Spring ■ = Summer.

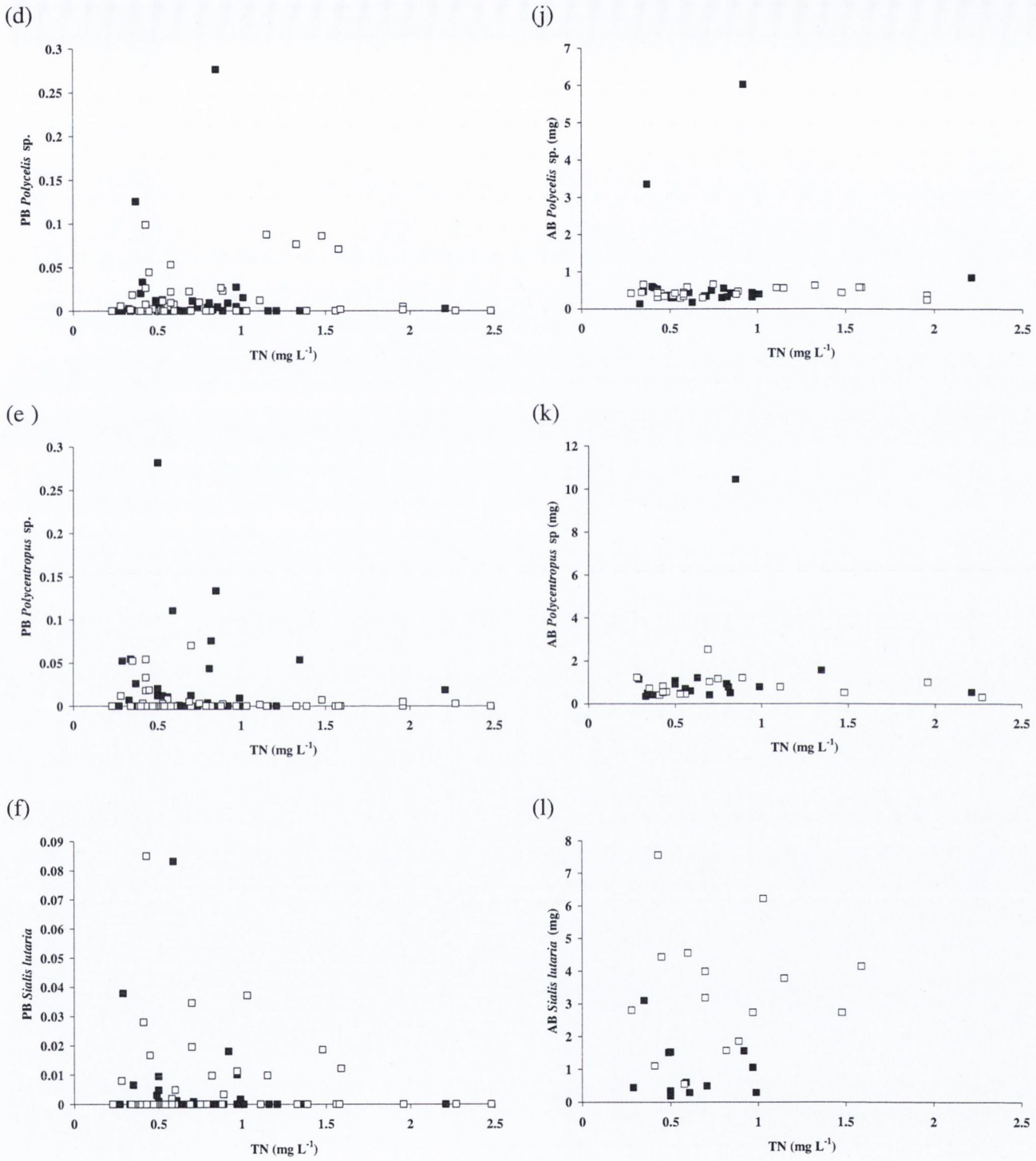


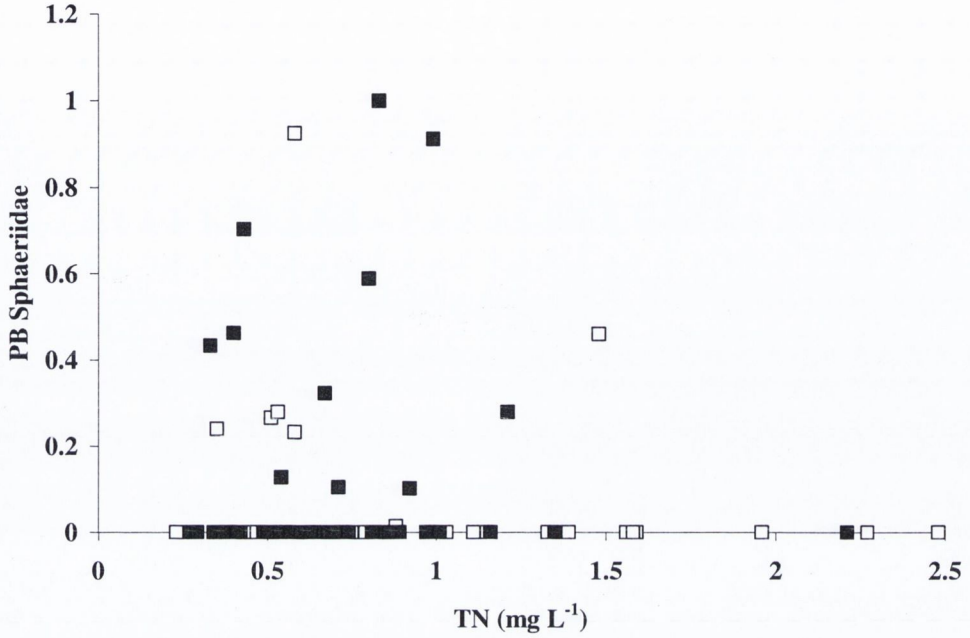
Figure 4.14 Graphs (a) to (f) show relationships between the proportional biomass [PB] of key taxa [(a) Coenagrioniidae (b) *Erpobdella octoculata* (c) *Nebrioporus assimilis* {adult} (d) *Polycelis* sp. (e) *Polycentropus* sp. (f) *Sialis lutaria*] and TN (mg L^{-1}); Graphs (g) to (l) show relationships between the mean biomass [AB; mg] of key taxa [(g) Coenagrioniidae ($R^2 = 0.03$; $P = 0.43$) (h) *Erpobdella octoculata* ($R^2 = 0.12$; $P = 0.16$) (i) *Nebrioporus assimilis* (adult; $R^2 = 0.17$; $P = 0.075$) (j) *Polycelis* sp. ($R^2 = 0.00$; $P = 0.86$) (k) *Polycentropus* sp. ($R^2 = 0.00$; $P = 0.88$) (l) *Sialis lutaria* ($R^2 = 0.03$; $P = 0.39$)] and TN (mg L^{-1}); □ = Spring ■ = Summer.

The mean biomass of *Polycelis* sp. and *Polycentropus* sp. varied little with increasing TN concentrations, and were not significantly related to TN (Fig. 4.14j and k). Although *Polycelis* sp. had exceptionally large mean biomass in two summer samples, in general there was little difference in mean biomass between seasons (Fig. 4.14j). Likewise, *Polycentropus* sp. differed little in mean biomass between seasons with the exception of a single summer sample, where mean biomass was much greater (Fig. 4.14k). Finally, the mean biomass of *S. lutaria* had a high level of variation among samples, and did not display a significant relationship with TN (Fig. 4.14l).

4.3.9.2 Filter feeders

Minimal adequate models of FFG biomass for the 'large' dataset indicated that filter feeders had a positive linear relationship with TN, alkalinity and with the interaction effect between these variables. Following Moog (1995), only one filter feeding taxa, Sphaeriidae, was shown by the BEST analysis (Table 4.7) as an important descriptor of the observed patterns. The proportional biomass of Sphaeriidae in the 'large' dataset was highly variable among samples, with the highest proportions recorded at moderate TN concentrations in summer (Fig. 4.15a). Proportional biomass was lower in spring samples. The mean biomass of Sphaeriidae was variable between seasons, with the greatest mean biomass recorded at low and moderate TN concentrations (<0.75 mg L^{-1}), and was not significantly related to TN ($P = 0.88$; Fig. 4.15b).

(a)



(b)

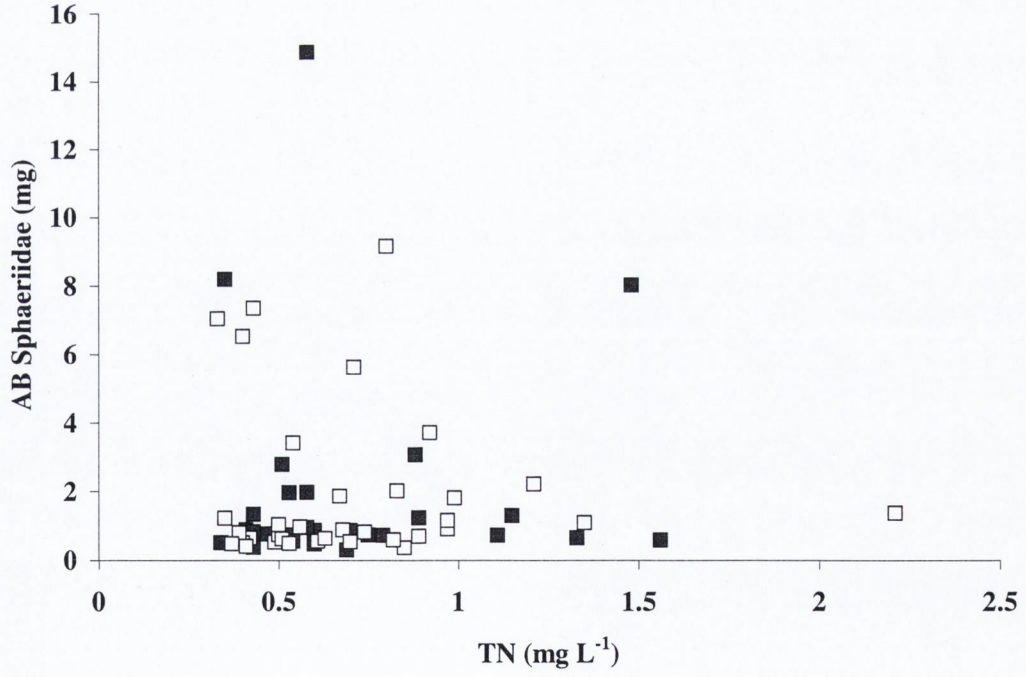


Figure 4.15: Relationship between (a) the proportional biomass (PB) of Sphaeriidae and TN (mg L^{-1}) and (b) the mean biomass (AB) of Sphaeriidae and TN (mg L^{-1}; R^2 = 0.00; P = 0.88); □ = Spring ■ = Summer.

4.4 Discussion

For clarity, all analyses conducted were based on either collective biomass (community structure and BEST analyses) or proportional biomass of taxa (Spearman rank correlations of taxa groups) or FFGs (LMEs); abundance of taxa or FFGs was not examined. Thus, when changes in taxonomic composition or FFGs are discussed below, this refers to patterns in either collective or proportional biomass.

4.4.1 Overall patterns in biomass-based community structure and functional feeding groups

Significant seasonal differences in the biomass of taxonomic and FFG littoral invertebrate groups were found among samples (Figs. 4.1, 4.3, 4.5; Tables 4.1, 4.4, 4.8). For both the 'whole' dataset and the subset of 'small' invertebrates, changes in the biomass-based taxonomic structure were significantly related to TN in spring, but not in summer. In contrast, the taxonomic structure of 'large' invertebrates was not significantly related to any environmental variable. Analysis of the 'whole' dataset averaged over seasons indicated that Coleoptera, Ephemeroptera and Plecoptera proportional biomass declined significantly with TP, TN and chlorophyll *a*. In addition, Heteroptera increased with TN and Hydracarina and Oligochaeta decreased with chlorophyll *a*.

The proportional biomass of predators among lakes initially increased with TN, but subsequently decreased under high concentrations of TN, depicting a curvilinear relationship between the two variables. However, the proportion of 'large' predators was not significantly related to TN. The proportions of filter feeders, gathering-collectors and predators differed between seasons in the 'whole' dataset. Predator and filter feeder proportional biomass differed between seasons among 'large' invertebrates.

TN mediated a change in the marginal relationship between the proportion of 'large' filter feeders and alkalinity. Alkalinity was associated with a marginal increase in the proportional biomass of shredders in both 'whole' and 'large' datasets, and grazer-scrapers in the 'large' dataset. Crustacea, Hirudinea, and Turbellaria increased and Ephemeroptera and Plecoptera decreased with alkalinity. The proportional biomass of Coleoptera, Crustacea and Plecoptera increased, and of Bivalvia, Diptera, Megaloptera

and Odonata decreased with lake area. Seasonal and enrichment based changes in the biomass-based structure of functional groups of littoral macroinvertebrates were clearer when the 'whole', compared with the 'large', dataset was examined. This contrasts with results from Chapter 3, which indicated that large individuals exhibited the greatest fluctuations in biomass with increasing TN, indicating that changes in invertebrate functional feeding groups with nutrient enrichment are more evident across the entire size spectrum of invertebrates, and not within confined data subsets.

4.4.2 Structural and functional feeding patterns in the biomass of littoral macroinvertebrates

Most taxa that best described community structure among all samples had highly variable collective biomass (all datasets; Figs. 4.7 to 4.9). However, the mean biomass of many taxa had much lower variation, with the exception of the mollusc *Radix balthica* and predatory leech *Erpobdella octoculata* (Fig. 4.10). Both these species had highly variable mean biomass and are subject to continuous growth (as opposed to moulting and metamorphosizing invertebrates).

Upon examination of BEST selected taxa important to community structure, functionally mixed taxa that are partially grazer-scrapers, shredders and gathering-collectors (G-S-G) have the greatest collective biomass within samples (Figs. 4.5 to 4.7). However, when the proportions of each FFG are examined, it is clear that gathering-collectors have the highest proportional biomass among all FFGs, comprising approximately one third of the total biomass of samples (Fig. 4.11a); suggesting the importance of allochthonous detritus for benthic macroinvertebrate production (Mihuc, 1997). BEST reported taxa with notably high collective biomass among samples were: *Potamopyrgus antipodarum* (featuring as important to community structure, according to BEST, in all three datasets), *Asellus aquaticus*, *Gammarus pulex* and Sphaeriidae (also featuring in all BEST derived taxa lists; Figs. 4.7 to 4.9). Both *P. antipodarum* and Sphaeriidae had mean biomass' on the boundary of 'small' and 'large' (1.33 ± 0.18 mg [mean biomass ± SE], and 1.97 ± 0.68, respectively), which explains their importance within 'small' and 'large' datasets.

Among the BEST reported important structuring taxa, predatory species had relatively small collective biomass in the 'whole' and 'large' datasets (Figs. 4.7 and 4.8). Predators accounted for approximately a quarter of total biomass among 'large' invertebrates, and approximately 14% of total biomass of the 'whole' dataset (Fig. 4.10). These seemingly contradictory patterns may be owing to a high proportion of taxa that are partially predatory (Moog, 1995), contrasting with the principally predatory taxa presented, chosen by BEST analysis to best represent community structure. An alternative possibility is that a high proportion of rare predatory taxa among lakes were excluded by BEST analysis.

Many large BEST selected taxa were non-predatory; for example, G-S-G taxa (*G. pulex* and *R. balthica)* and detritivorous taxa (*S. distincta* and *S. falleni*) were larger than most predatory taxa, with the exception of *E. octoculata* (Fig. 4.10). The presence of large microphagous organisms (filter feeders and deposit feeders) among benthic communities contrasts with pelagic communities, owing partially to the availability of physical refuges among macrophytes, pebbles or sand (Strayer, 1991; Rasmussen, 1993; see Chapter 3, Section 3.1 for detailed description).

The proportion of FFGs differed between the 'whole' and 'large' datasets (Fig. 4.10). Although gathering-collectors dominated the biomass of the 'whole' and 'large' datasets, there was a slight decrease in the proportion of gathering-collectors, and a large increase in the proportion of predators when only 'large' invertebrates were examined (Fig. 4.11); with a slight decrease in grazer-scrapers and increase in shredders. The greater proportion of predators detected among 'large' invertebrates reflects an increase in trophic level with body size, commonly found in many ecosystems, as most non-parasitic predators are larger than their prey (Cohen *et al.*, 1993, 2003; Jennings *et al.*, 2002).

4.4.3 Season

There were significant seasonal differences in littoral macroinvertebrate community structure and in proportional FFGs. Community structure differed significantly between seasons for all datasets (i.e. 'small', 'large' and 'whole' datasets; Tables 4.1, 4.4 and 4.8; Figs. 4.1, 4.3 and 4.5). The *pseudo F* ratio produced from the PERMANOVA analysis was highest for the comparison of community structure between seasons within

the 'small' dataset, and lowest for the comparison within the 'large' dataset, indicating that the largest seasonal differences in community structure were among the 'small' invertebrates (Tables 4.1, 4.3 and 4.5).

Results from Chapter 3 indicated that the size structure of littoral macroinvertebrates on hard substrates also differed between seasons, with more pronounced seasonal differences among smaller invertebrates, and a shift from a higher presence of large invertebrates in spring to an increase in smaller animals in summer (Chapter 3, Fig. 3.4). Upon examination of the taxa lists chosen by BEST analysis to represent core taxa important to community structure, it was revealed that many species present on spring lists and absent on summer lists were insect larvae, with potential for summer emergence (10 of 18 taxa from the 'whole' dataset; 5 of 8 taxa from the 'large dataset; and 8 of 11 taxa from the 'small' dataset; Tables 4.3, 4.7 and 4.10). Coupled with a loss of large animals in summer (Chapter 3), the significant taxonomic change between seasons (PERMANOVA analysis; Tables 4.1, 4.3, and 4.5) is likely owing to a loss of univoltine insects from the littoral zone with summer emergence (Solimini *et al.*, 2006).

The decline in large invertebrates together with changes in community structure between spring and summer have been observed in previous studies, and has been attributed to selective predation by fish, emergence of late instars or the appearance of new generations, or all of these (Mittelbach, 1981). An abundance and presence/absence based analysis of the 'whole' dataset, along with an additional 18 lakes sampled in both seasons also found that season had a strong relationship with community structure, with season explaining the highest amount of variance in the presence/absence dataset and the second highest amount of variance in the proportional abundance dataset (Donohue, 2008). Furthermore, Donohue (2008) also concluded that seasonal effects on community structure were likely owing to emergence of most univoltine insects in the summer. However, Rodriguez and Magnan (1993) found only limited seasonal variation in the littoral communities of three small shallow Laurentian Shield lakes, differing in fish species composition and substratum types, where the taxonomic structure based on biomass and the overall shape of the biomass spectrum combination were relatively constant from May until August.

Although differences in the community structure of spring samples of 'whole' and 'small' datasets were explained by changing TN concentrations, variance in the community structure of spring samples from the 'large' dataset was not explained by any of the environmental variables examined. This suggests that change in the taxonomic composition of the 'small' invertebrates with TN was driving change in the community structure of the 'whole' dataset (Tables 4.2, 4.5 and 4.9). However, changes in the community structure of summer samples from all datasets were not explained by any of the environmental variables examined (Tables 4.2, 4.5 and 4.9). The loss of species richness in summer samples, likely caused by the emergence of adult insects, may explain the lack of significant change in community structure of summer samples with TN. This is particularly so if the emerging species were TN sensitive or tolerant, and thus, contributing to the significant relationship between community structure and TN observed for spring samples. The greater taxa richness observed among the 'small' dataset may explain why changes in community structure with TN were identified among 'small' invertebrates, and not among the less diverse 'large' invertebrates.

Changes in the proportional biomass of many FFGs with season were evident for both the 'whole' and 'large' datasets (Tables 4.12 and 4.13). The proportion of predators and gathering-collectors decreased, and the proportion of filter feeders increased in summer in the 'whole' samples (Table 4.12). Likewise, the proportion of predators decreased and filter feeders increased in summer in the 'large' dataset, but the proportion of gathering-collectors did not change, suggesting that the decrease in detritivorous invertebrates in summer occurred among 'small' invertebrates (Table 4.14).

Season also explained a significant amount of variation in the proportional abundance of FFGs in an analysis conducted by Donohue (2008) which examined samples from the 'whole' dataset as well as samples from another 19 lakes. Seasonal variation in the proportion of FFGs have been found in lotic (Hawkins and Sedell, 1981; Canton and Chadwick, 1983; Murphy and Giller, 2000; Callisto *et al.*, 2001) and lentic (Kornijow and Scibior, 1999) benthic macroinvertebrate communities. The assemblages of epiphytic invertebrate feeding groups displayed clear seasonal differences in their relative abundance, dominance structure and densities in a small shallow eutrophic Polish lake (Kornijow and Scibior, 1999). These changes were attributed to seasonal variation in (a) periphytic and planktonic algae presence, (b) the nutrient content of

macrophyte tissue, (c) detritus from decomposing leaves, (d) habitable leaf surface area in summer and (e) interactions between algae-detritus feeders and predatory invertebrates (Kornijow and Scibior, 1999). Hawkins and Sedell (1981) found seasonal shifts in FFGs in four study streams; two streams recorded increasing shredder and decreasing gathering-collectors in summer. In contrast, an increase in gathering-collectors and a decrease in shredders in summer was recorded in a longitudinal study of benthic invertebrate FFGs along a 46 km stretch of an American river, attributed to seasonal changes in the species composition (Canton and Chadwick, 1983). Thus, functional shifts appear to be variable among water bodies, dependant upon the food resources and life-cycles of the invertebrates present, as well on seasonal changes in the habitats present.

4.4.4 Lake area

The taxonomic composition of 'small' invertebrates was significantly related to lake area (Table 4.8). However, the community structure of 'large' and of 'whole' datasets, were not related to lake area. Coleoptera, Crustacea and Plecoptera increased, and Bivalvia, Diptera, Megaloptera and Odonata decreased in proportional biomass with lake area. In Heino's study (2000) of littoral macroinvertebrates in 21 lakes in Finland, the area of the water body was the principal environmental factor explaining changes in the taxonomic structure of the macroinvertebrate communities. This pattern was attributed to differences in the physical habitats present at smaller bog lakes compared with larger lakes with well developed littoral zones and occurring in principally forested catchment and riparian zones (Heino, 2000). However, in my study of Irish lakes, there was a high diversity of catchments and of lake alkalinity, which did not relate to lake area. My study also contrasted with Heino's (2000) study, in that patterns in the taxonomic composition were examined only on stony substrates, to reduce habitat heterogeneity. Nevertheless, it has been suggested that larger lakes may provide more niche opportunities to species, owing to wider habitat variability (Tonn and Magnuson 1982; Heino, 2008).

A comparison of the macroinvertebrate benthos of stony habitats in Swedish lakes and streams, found that for lakes, lake surface area explained significant amounts of the variance in community composition (Johnson *et al.*, 2004). They proposed that lake surface area was probably a proxy for wave action, with communities of large lakes

experiencing greater wave action than those of small lakes (Johnson *et al.*, 2004). This may explain the significant relationships between lake area and some taxa groups. For example, Megaloptera (single species present, *Sialis lutaria*) and Odonata, which have a preference for sheltered waters, were found to decrease with increasing lake area (Elliot, 1996; Hammond, 1997; Table 4.11). Bivalves, found to decrease with lake area, also require a stable substrate in which to bury (Killeen *et al.*, 2004). However, the most common bivalve taxa present in my study, Sphaeriidae, is frequently found in streams as well as lakes (Johnson *et al.*, 2004); and many species of Plecoptera, in particular, have a preference for fast flowing stony streams and rivers (Fitter and Manuel, 1986). These results support the view proposed by Johnson *et al.* (2004), that lake area relates to wave action.

Lake area was excluded from all models of proportional FFGs for both 'whole' and 'large' datasets, indicating no clear functional composition change in lakes with lake size (Tables 4.12 and 4.14). This contrasts directly with the results of a study of 48 Finnish boreal lakes which found that lake surface area was of prime importance in explaining change in the functional feeding structure of littoral macroinvertebrate communities (Heino, 2008). Johnson *et al.* (2004) also found that lake surface area was a strong predictor of the functional feeding composition of macroinvertebrate communities. However, few other studies have examined the effect of lake area on littoral macroinvertebrate structure or function, and further research is recommended.

4.4.5 Alkalinity

Lake littoral macroinvertebrate community structure was not significantly related to alkalinity concentrations in any of the datasets, leading to the rejection of hypothesis (a). This result is surprising as the taxonomic composition of water bodies with low pH is generally different, if not impoverished, compared with those of circumneutral or alkaline water bodies, owing to the loss of acid sensitive species, and taxa vulnerable to low calcium concentrations, which are often replaced with acid-tolerant fauna (Økland and Økland, 1986; Sutcliffe and Hildrew, 1989; Jeffries and Mills, 1990; Johnson *et al.*, 1993; Allott *et al.*, 1997; Tierney *et al.*, 1998; Dangles *et al.*, 2004).

Nevertheless, significant changes in several taxa groups with alkalinity were recorded (Table 4.11). Crustacea, Hirudinea, and Turbellaria significantly increased and

Ephemeroptera, and Plecoptera significantly decreased with alkalinity (Table 4.11). Crustacea are vulnerable to low pH and low calcium levels (Jeffries and Mills, 1990; Johnson *et al.*, 1993). Likewise, species of Hirudinea are most abundant when alkalinity exceeds 60 mg L^{-1} and few species occur at low alkalinity, possibly owing to reduced prey abundance at low alkalinity (Elliott and Mann, 1979). Dolisy and Dohet (2003) found that Ephemeroptera were sensitive to increasing alkalinity. Nevertheless, many ephemeroptera species disappear with increased acidity (Harriman and Morrison, 1982; Stoner *et al.*, 1984; Tierney *et al.*, 1998); while species of Plecoptera, such as *Leuctra* are known to be acid-tolerant (Hynes, 1970). Thus, although the overall taxonomic structure was not significantly related to changes in alkalinity, the proportional biomass of several taxonomic groups did significantly change with increasing alkalinity concentrations, indicating some structural change with alkalinity.

The proportions of 'large' shredders, filter feeders, and grazer-scrapers increased marginally with alkalinity (Table 4.14). However, only the proportion of shredders was shown to increase (marginally) with alkalinity in the 'whole' dataset (Table 4.12). Molluscan scraper species were also found to increase with increasing water hardness in a study of 21 Finnish lakes (Heino, 2000). Heino (2008) found that lake hardness was important in determining the functional structure of macroinvertebrate communities, in a separate examination of 48 boreal Finnish lakes. The latter study also reported that sprawling shredders (those inhabiting the surfaces of floating leaves of vascular hydrophytes or fine sediments) and sprawling grazer-scrapers were significantly and positively related to alkalinity. It is likely that an increase in the size and collective biomass of crustacean and molluscan shredders and grazer-scrapers, known to prefer more alkaline waters, caused the marginal increase in their proportional biomass. It has been suggested that the scraper community may be more sensitive to acidity changes than nutrient enrichment (Resh and Jackson, 1993). That the 'large' grazer-scrapers within this study marginally increased with alkalinity, but did not change with TN supports this hypothesis (Table 4.14). The principal filter feeder is the molluscan bivalve Sphaeriidae, known also to have a strong preference for calcium-rich water (Killeen *et al.*, 2004). Results from Chapter 3 suggest that owing to the marginal positive relationship of size spectra, mean biomass and total biomass with alkalinity, animals are larger on average, and have more collective biomass with increasing alkalinity (Chapter 3, Tables 3.15 to 3.17), explaining why more functional groups

among 'large' individuals displayed a positive relationship with alkalinity than among all individuals within the 'whole' dataset.

There was a significant change in the biomass-based taxonomic composition of several key taxa with alkalinity (Table 4.11). Yet the BEST analysis did not detect any significant relationship between the biomass-based community structure and alkalinity, suggesting that this analysis may not be adequate for examining community shifts with alkalinity (Tables 4.2, 4.5, 4.9). Donohue (2008) found a significant relationship between the abundance based taxonomic structure of an expanded dataset (198 samples from 66 lakes), which included abundance data from all samples in the 'whole' dataset (95 samples from 48 lakes), and pH, alkalinity and conductivity, using canonical correspondence analysis (CCA). There are several possibilities explaining discrepancies between these two results (i) CCA is more sensitive to community shifts with environmental variables, (ii) proportional abundance is a more sensitive measure of taxonomic structure than proportional biomass and (iii) the smaller dataset used in my study was not adequate to detect relationships with alkalinity.

Addressing (i): differences between the two types of analyses (BEST BioEnv and CCA) are difficult to assess. CCA is a direct ordination of biotic data according to their associated environmental variables, constraining the ordination of the biotic data by multiple regression on the environmental data in a separate matrix (ignoring all biotic data unrelated to the measured environmental variables). The BEST BioEnv procedure involves a rank correlation (using the Spearman coefficient) between biotic and abiotic (environmental variables) similarity matrices, where high rank correlations between a biotic similarity matrix with all possible combinations of the abiotic variables is completed (Clarke and Warwick, 2001). Without conducting both analyses on each dataset (both on 'whole' dataset and on Donohue's [2008] dataset), it is difficult to assess the sensitivity of each method. CCA is more frequently used in the analysis of changes in macroinvertebrate community structure with environmental variables.

In reference to (ii), a comparison of biomass and abundance based measures is discussed at length in Section 4.4.8, but in summary: proportional biomass accounts for animal size, and gives an indication as to the functional importance of the species owing to a correlation between biomass and productivity. Abundance based measures result in

a bias against large species that account for, and consume more biomass, but have fewer individuals, and may not be as sensitive in detecting community change among larger rarer individuals with enrichment. It is thus likely that biomass based measures are more responsive to overall community change among all sizes of invertebrates, than abundance based measures.

Finally, addressing (iii): samples from both hard and soft substrates were included within Donohue's (2008) CCA analysis, with samples from 18 additional lakes, so that 198 samples were included in the CCA analysis compared with 95 hard samples found in the 'whole' dataset in the current study, and thus a dataset double the size might uncover clearer structural patterns with environmental change.

4.4.6 Dissolved organic carbon

DOC was found to explain shifts in the size spectra of the 'whole' (quadratic) and 'large' (truncated) datasets (Chapter 3; Tables 3.16 and 3.17). However, DOC was not significantly related to community structure, or to the proportional biomass of any taxonomic group, and was removed from all models of FFG proportional biomass (Table 4.2, 4.5 4.8, 4.11, 4.12 and 4.14). Thus patterns in community size structure with DOC could not be explained by differences in community structure or functional feeding.

4.4.7 Nutrient enrichment

As hypothesised, TN was significantly related to biomass based structural changes in littoral macroinvertebrates among lakes, where TP or chlorophyll *a* concentrations were not, for the spring samples of the 'whole' and 'small' datasets. TP has repeatedly been observed to correlate strongly with chlorophyll *a* concentrations in lakes (Dillon and Rigler, 1974; Jones and Bachman, 1976; Canfield and Bachman, 1981; Jeppesen *et al.*, 2005) leading to the assumption that lake primary production is phosphorus limited. Recent evidence, however, suggests that nitrogen may play a more important role than previously thought (Gonzaléz *et al.*, 2005; James *et al.*, 2005; Jeppesen *et al.*, 2005). Submerged and floating macrophyte species richness was shown to decrease with increasing nitrogen concentrations in 60 shallow lakes in Poland and the UK (James *et al.*, 2005). After combined nutrient treatments (TP and TN) the biomass of submerged

macrophyte species was significantly less than those in single nutrient treatments, in 24 experimental field exposures fixed in, and exposed to, the sediment in a shallow lake (Gonzaléz *et al.*, 2005).

That TN was significantly related to biomass based structural changes in the spring samples of the 'whole' dataset, was in partial agreement with part (i) of hypothesis (c), and concurred with the abundance based analyses of community structure conducted by Donohue (2008) on a larger dataset which included the 'whole' samples. TN, TP and chlorophyll *a* explained significant variation in a proportional abundance based analysis; TN and chlorophyll *a* explained significant variation in a presence/absence based analysis of community structure. In both analyses, TN, as a conditional effect, explained more variance by forward selection in CCA ordination, than the other enrichment terms (Donohue, 2008). An examination of littoral invertebrate communities in the stony substrate of 39 lakes along a nutrient gradient revealed that changes in community structure were explained by chlorophyll *a* and TP, but not TN (Brodersen *et al.*, 1998). In a study of spatial and water chemical variation among 21 lentic water bodies in Finland (ponds and lakes), TN was found to account for more variation in the structure of macroinvertebrate assemblages than TP (Heino, 2000). However, where the community composition of littoral macroinvertebrates in 36 central German lakes (with wide ranging nutrient conditions) was examined, the taxonomic composition of macroinvertebrates from stony habitats was not significantly related to nutrient conditions, but distinct differences in the taxonomic composition of communities on coarse woody debris, roots and sand were found between oligotrophic and hypertrophic lakes (Brauns *et al.*, 2007).

The taxonomic structure (based on biomass) of summer samples of all datasets were not significantly related to any environmental variables, likely owing to the summer emergence of many univoltine adult insects, and resulting loss of species (see Section 4.4.2 and references therein). The community structure of 'large' invertebrates in either season was not related to any environmental variables, suggesting that 'small' invertebrates were driving the observed change in 'whole' community structure with TN (in spring). This finding contrasts somewhat with results from Chapter 3, where biomass of the larger sized individuals fluctuated most with TN (Chapter 3, Tables 3.16 and 3.17). Large, rare species, often at higher trophic positions, are usually the first lost

with system disturbance (Petchey *et al.*, 1999; Raffaelli, 2004; Woodward, 2009) and thus, changes in the community structure of large invertebrates with increasing TN concentrations could be obscured by loss of species. Rasmussen (1993) found two independent size modes (i.e. modes of "small" and "large" size fractions) when size spectra of littoral invertebrates among 11 lakes in Quebec were compared; where the biomass of the small size fraction was related to trophic status (and macrophyte biomass) and that of the large fraction was related to wave exposure.

Coleoptera, Ephemeroptera and Plecoptera significantly decreased with increasing nutrients (TP and TN) and chlorophyll *a* concentrations (Table 4.11). The species richness and density of Ephemeroptera and Plecoptera are often described as sensitive to most types of pollution including nutrient enrichment (Lenat, 1988; Norris and Georges, 1993; Bauernfeind and Moog, 2000; White, 2001; Camargo *et al.*, 2004). Donohue (2008) also found a significant negative correlation between the proportional abundance of Coleoptera, Ephemeroptera and Plecoptera and TP, TN and chlorophyll *a*. Hydrachniidia and Oligochaeta significantly decreased with increasing chlorophyll *a* concentrations (Table 4.11); while increases in oligochaete abundance, and in tubifid taxa in particular, have previously been associated with nutrient enrichment (Wiederholm, 1980; Johnson *et al.*, 1993; Norris and Georges, 1993). Nevertheless, a review by Brinkhurst (1974) found no general relationship between oligochaete abundance and lake trophic state. Fernandez *et al.* (2004) found no change in the proportion of Hydrachniidia mites in experimentally enriched mesocosms in a shallow lake in north-west Spain. Thus, the cause of decrease in proportional biomass of these taxa with chlorophyll *a* is unclear.

The proportional biomass of Heteroptera increased significantly with TN (Table 4.11). Most Heteropteran taxa present were from the family Corixidae, and two species were chosen by BEST analysis as important in describing community structure among all lakes: *Sigara falleni* and *S. distincta*. Donohue (2008) found both *S. falleni* and *Callicorixa praeusta* were highly tolerant of eutrophic water. White (2001) found that *S. falleni* was associated with eutrophic conditions and Biesiadka and Szczepaniak (1987) found both *S. falleni* and *C. praeusta* to be indicative of eutrophic conditions. Thus definite taxonomic shifts were evident with nutrient enrichment in all analyses.

TN was of prime importance in explaining changes in the proportional biomass of predators (Table 4.12). A strong curvilinear relationship between the two variables was found, with the proportion of predators initially increasing and subsequently decreasing with increasing TN concentrations (Table 4.12). Donohue (2008) found that the proportional abundance of predators increased significantly with chlorophyll *a*, although only linear changes with nutrients and chlorophyll *a* were analysed (spearman rank correlation). The proportion of predators consistently increased with several different anthropogenic impacts compared with reference streams in a study of 11 German streams (Rawer-Jost *et al.*, 2000). Likewise, the production of invertebrate predators in an experimentally enriched stream increased over time with nutrient addition (Cross *et al.*, 2006). However, other studies recorded no change in the abundance or biomass based proportion of predators with nutrient enrichment (Kerans and Karr, 1994; Poepperl, 1999; Blocksom *et al.*, 2002). Cross *et al.* (2006) found that the magnitude of the effect of nutrient enrichment on predators was not as great as observed for most primary consumers and invertebrate prey, hypothesising that this reflected a loss of energy with each trophic transfer. Nevertheless, as most non-parasitic predators are larger than their prey (Cohen *et al.*, 1993) it is likely that a greater proportion of large individuals will be predatory. Thus, as curvilinear change in size spectra with TN observed in Chapter 3 were most pronounced in larger size classes, this may have resulted from a greater change among predators with TN.

Patterns within the predators did not mirror that of benthic primary consumers within the 'whole' dataset. As no relationship in the biomass of benthic primary consumer feeding groups with increasing TN concentrations was observed, it is possible that the initial increase in predators with TN may have resulted from enhanced food resources within the pelagic zone (e.g. zooplankton prey), with moderate enrichment, and subsequent depression of all food resources causing a corresponding decrease in predators under high nutrient concentrations. The overall biotic response to nutrient enrichment has been hypothesised to be curvilinear or non-linear, as although nutrient enrichment generally stimulates process rates, associated stressors such as anoxia and siltation may have the opposite effect (Baldy *et al.*, 2007; Compin and Cereghino, 2007; Woodward *et al.*, 2009). Cross *et al.* (2006) suggested that an alternative equilibrium quantity of total benthic organic matter lower than reference conditions would occur with prolonged enrichment in their experimentally enriched stream, such that benthic

matter would have a curvilinear relationship with enrichment over time, initially increasing, but ultimately decreasing with prolonged enrichment.

A weak relationship was displayed between 'large' filter feeders and TN, mediated by an interaction effect with alkalinity (Table 4.13). Although the minimal adequate model indicated that there was an overall increase in the proportion of 'large' filter feeders with TN, the relationship was weak, influenced by samples with no filter feeders; filter feeders were absent most frequently from samples with low alkalinity concentrations, or at highest and lowest TN concentrations (Table, 4.13; Figure 4.13). The proportion of filter feeders within moderate and high alkalinity lakes decreased with increasing TN concentrations (Figure 4.13b). High alkalinity lakes with filter feeders present often had very high concentrations of TN, creating difficulties in distinguishing alkalinity effects from nutrient effects (Fig. 4.13). The relationships between biomass, abundance, diversity and distribution of filter feeders and nutrient enrichment are often difficult to interpret because many factors influencing them are confounded (e.g. food quality and quantity, stable substrates, temperature [Richardson and Mackay, 1991; Whiles and Dodds, 2002]).

Nutrient enrichment has been shown to cause a decrease in the abundance or biomass of filter feeders in streams (Barbour *et al.*, 1996; Poepperl, 1999). Donohue (2008) found a significant decrease in the proportional abundance of filter feeders with increasing TP, TN and chlorophyll *a* concentrations in soft substrates, although no relationship was seen in hard substrates. The overall trend in my study was of a decrease in proportional biomass of filter feeders with nutrient enrichment, with a strongly interacting alkalinity effect (Figure 4.13b). Food (seston) quality and quantity appear to be strongly correlated with the density of filter feeders within water-bodies, such that a decrease in nutritional quality results in decreased abundance and biomass of filter feeders (Morin and Peters, 1988; Richardson and Mackay, 1991). However, no evidence for limitation of filter feeders by seston quality was found in a study of filter feeding taxa from headwaters and rivers within Kansas River drainage system (Whiles and Dodds, 2002). It is hypothesised that filter feeders selectively remove and ingest particles that promote growth (high quality food) even in systems where low quality particles dominate (Richardson and Mackay, 1991; Whiles and Dodds, 2002). Dilution of food resources by additional refractory particles with enrichment, even when the concentration of high-

quality food is maintained or slightly increased, reduces the amount of high-quality food per unit mass of filtrate, and refractory particles may clog filtering structures and hamper feeding (Morin and Peters 1988; Richardson and Mackay, 1991). The proportional biomass of predators was the only measure of FFGs that was significantly related to measures of nutrient enrichment in my study (Table 4.12).

There was no change in the proportional biomass of gathering-collectors with TN in either dataset, contrary to hypothesis (d) proposed in the introduction. In contrast, Donohue (2008) found that the proportion of gathering-collectors decreased with nutrient enrichment. Oligochaetes, of which most taxa are gathering-collectors (Moog, 1995), decreased with increasing chlorophyll *a*. This is in agreement with the findings of Donohue (2008; Table 4.11). However, organically polluted waters are often dominated by tolerant gatherer species (Barbour *et al.*, 1996). The abundance and biomass of shredders and gatherers increased highly significantly with nutrient enrichment, when a detritus-based stream was experimentally enriched (before and after comparison) and when the enriched stream was compared with an adjacent reference detritus-based stream (Cross *et al.*, 2006). In this instance, enhanced detrital quality (through increased microbial activity and nutrient content) despite carbon loss (through increased decomposition and respiration) resulting from nutrient enrichment of the treatment stream, caused an ~226% increase in invertebrate production (Cross *et al.*, 2006). However, a 45% reduction in leaf litter owing to increased microbial and invertebrate consumption was also recorded in this study, leading the authors to hypothesise that longer term enrichment may impact consumers through eventual reductions in the quantity of detritus (Cross *et al.*, 2006). Results from Heino (2008) also recorded an increase in the abundance of gatherer-collector functional groups with TP among 48 lakes, possibly owing to higher nutrient concentrations of fine organic material in more nutrient-rich lakes. As the nutritional value of food resources were not examined in the current study, it is only possible to speculate that there was a high variability in the quantity and in the nutritional value of detritus with nutrient enrichment among lakes, possibly owing to morphological differences among lakes, such as area, depth, slope, differences in available habitats, riparian and catchment characteristics, as well as differences in available food resources.

Primary and secondary consumers are predicted to increase with system productivity and nutrient enrichment (Cross *et al.*, 2006). However, none of the FFGs representing benthic primary consumers were significantly related to nutrient enrichment in any of the models, and thus no overall shift in proportions of FFGs was detected, contrary to part (ii) of hypothesis (c), leading us to reject this part of the hypothesis. The feeding roles of many freshwater macroinvertebrate taxa may be highly flexible and involve omnivory (Mihuc 1997; Dangles 2002). The AQEM database accounts for variation in the feeding habits of species by distributing a 10 point score among FFGs where evidence of mixed feeding is found. For example, the leech *Helobdella stagnalis* is scored ten points as a predator, whereas the common isopod *Asellus aquaticus* is scored as a shredder (three points), grazer-scraper (three points) and gathering-collector (four points). However, some taxa have high feeding plasticity, such that given differences in the proportions of resources present, a change in resource use may occur, and the AQEM point score system cannot represent this change. For example, *Gammarus* sp. can eat fresh aquatic plant material, leaf litter, and other invertebrates as well as juvenile fish, thus acting as grazer-scraper, shredder, gathering-collector and predator (MacNeil *et al.*, 1997). This feeding plasticity is linked to the success of *Gammarus* sp. in persisting in and colonising or invading disturbance-prone ecosystems (MacNeil *et al.*, 1997).

The common species *Gammarus pulex* is scored (Moog, 1995) as shredder (six points), grazer-scraper (one point), gathering-collector (two points) and predator (one point). However a study of *G. pulex* from a variety of habitats in the Isle of Man found a high prevalence of animal material in the gut of the species (Hynes, 1954). Where ecosystems are disturbed, and resource conditions change, many invertebrates switch between foods as they become available (Kostalos and Seymour, 1976; Short and Ward, 1987). Thus, *Gammarus* sp. may act principally as shredders in one season, gathering-collectors in another season within the same habitat, and be mainly predatory under different conditions in a different habitat (MacNeil *et al.*, 1997). However, if food resources change with nutrient enrichment induced disturbance, causing a shift in the diet of many invertebrates, then pre-assigned FFGs, regardless of how they are partitioned, are no longer useful, as the sensitivity in detecting functional or trophic change among habitats or ecosystems is blurred (MacNeil *et al.*, 1997). Nonetheless, some species exhibit little spatial (resource based) and ontogenetic change in feeding;

such as for active filter feeding bivalves (e.g. Sphaeriidae) and some predatory taxa (e.g. Hirudinea, Odonata, some species of Coleoptera). That these two FFGs were both significantly related to nutrient enrichment, yet variance in all other FFGs, often assigned partially to taxa, (i.e. taxa are partially grazer-scrapers, gathering-collectors and shredders) were not, highlights the usefulness of examining taxa with simple feeding habits, and reinforces the need for caution in interpretation of FFG change among omnivorous taxa. Donohue (2008) found that the generalist feeding habits increased in importance with nutrient enrichment in stony habitats. Owing to the broad range of food available to them, generalists are more tolerant to pollution that might alter availability of certain food (Barbour *et al.*, 1996). It is possible, therefore, that a change in benthic primary consumers did occur with nutrient enrichment, but that this was not reflected in the FFGs owing to dietary shifts with changes in food resources. It is also possible that predators increased consumption of pelagic consumers with enrichment. Further study is required to understand the pathway through which predator production might increase.

4.4.8 Comparing biomass and abundance based community measures

Cohort and annual production rates have been shown to correlate with population biomass (Waters, 1977; Banse and Mosher, 1980; Stephenson and Morin, 2009). The use of biomass instead of abundance in multivariate examinations of changes in the taxonomic composition of macroinvertebrate communities, and in models of FFGs, gives a snapshot indication of production, more indicative of the functional contribution of species to the community then abundance. However, as the biomass turnover rate is much faster in small short lived species than in larger, longer lived ones, the snapshot picture of the biomass does not capture the greater flux in energy, and consequently biomass, among the smaller organisms, that is captured when annual production is examined.

Analyses using abundance as the main measure of taxonomic structure may inadvertently provide a biased picture of structural change, and of the functional importance of smaller sized taxa. For example, a high abundance of a Caenidae mayfly and low abundance of a Coenagrioniidae damselfly within a dataset would suggest that the Caenid was more functionally important. However, examination of the collective biomass of these taxa may reveal that Caenidae had a small collective biomass (owing

to their tiny body size) compared with a high collective biomass recorded for the large and less abundant predatory Coenagrioniidae. Thus abundance may not accurately reflect the functional importance of each of these taxa. Nevertheless, patterns from analyses conducted by Donohue (2008) which included abundance-based data from this study, produced similar results in terms of both change in the taxonomic composition, and in the proportion of FFGs to those produced here, indicating that both measures are highly correlated.

4.5 Conclusions

The following points encapsulate the key findings of this study:

- Strong seasonal differences in the biomass-based community structure and in proportions of FFGs were evident; proportional biomass of predators and gathering-collectors decreased and of filter feeders increased, in summer;
- No structural changes with alkalinity were evident from BEST analyses; however, proportional biomass of Crustacea, Hirudinea, and Turbellaria increased and of Ephemeroptera and Plecoptera decreased significantly with alkalinity; the proportional biomass of all shredders ('large' and 'whole' datasets) and of 'large' grazer-scrapers increased with alkalinity;
- The biomass-based taxonomic structure of 'small' invertebrates was significantly related to TN in spring but not in summer, likely owing to emergence of insect species in summer; this pattern appeared to be driving the change in community structure of the 'whole' dataset with TN also evident in spring but not summer; 'large' invertebrates were not related to TN;
- Proportional biomass of Coleoptera, Ephemeroptera and Plecoptera significantly decreased with TP, TN and chlorophyll *a*; Heteroptera increased with TN; Hydracarina and Oligochaeta decreased with chlorophyll *a*;
- The proportional biomass of predators had a significant curvilinear relationship with TN, initially increasing and, subsequently, decreasing with high concentrations of TN; and
- The proportional biomass of 'large' filter feeders had a significant relationship with both TN and alkalinity, with a significant interaction effct, indicating a change in the relationship between filter feeders and alkalinity with increasing TN; the proportional biomass of filter feeders decreased with increasing TN under moderate and high alkalinity concentrations.

CHAPTER 5

DISCUSSION

5.1 Research Aims

The aim of this study was to investigate the effects of alkalinity and nutrient enrichment on the biomass of lake littoral macroinvertebrate communities. A comparative study of nine lakes determined the effect of alkalinity and nutrients on length-mass relationships of eight common aquatic macroinvertebrates among lakes representing an alkalinity and nutrient gradient, reported in Chapter 2. A study of forty eight lakes, representing a broad alkalinity and nutrient gradient, assessed changes in the total biomass, mean biomass and slope and intercept of littoral macroinvertebrate size spectra with nutrients, alkalinity and substrate, in Chapter 3. The influence of alkalinity and nutrient enrichment on the community structure (based on the collective biomass of taxa) and on the proportional biomass of functional feeding groups (FFGs) of lake littoral macroinvertebrates from stony substrates in forty eight lakes was examined in Chapter 4.

5.2 Within lakes: The role of substrate and season in structuring lake littoral macroinvertebrate size spectra, and on biomass-based community structure and FFGs

Within-lake variation (between substrates) was greater than among lake variation for models of mean and total biomass (Chapter 3). Models of size spectra also differed between substrates (Chapter 3). In addition, strong seasonal changes in lake littoral macroinvertebrate size structure and community structure (based on biomass of taxa) were observed within lakes. Nevertheless, in spite of this strong variability within lakes, consistent change with water chemical variables was observed among the lakes. Changes in the proportional and collective biomass of taxa and proportional biomass of FFGs were examined for only hard substrates, in order to reduce habitat heterogeneity and to allow a clearer comparison among lakes (Chapter 4). Thus, differences in the biomass of lake littoral macroinvertebrates between substrates were examined in detail, in Chapter 3.

5.2.1 Substrate

The effect of substrate was shown to be of prime importance in explaining variance in models of mean and total macroinvertebrate biomass, with large differences observed in

the size structure of lake littoral macroinvertebrates between hard and soft substrates. Macroinvertebrates found on soft substrates displayed greater mean and total biomass and an increase in the normalised biomass of larger size classes compared with macroinvertebrate communities on hard substrates. Further evidence in support of this trend was found in models of the total biomass of large individuals from 15 lakes where fish data was included, where the total biomass of 'large' individuals was also greater on soft substrates. More complex structures are frequently associated with soft sediments, such as rooted macrophytes and macroalgae, compared with the structurally simpler hard substrates comprising of gravel, pebbles, cobbles or bedrock. Increases in abundance and biomass of benthic macroinvertberates with increasing macrophyte or macroalgal cover have been well documented, hypothesised to result from increased food resources and reduced fish predation, coupled with increases in large mobile invertebrates and invertebrate predators, high heterogeneity and more perceived space with the greater structural complexity of the habitat (see Chapter 3, Section 3.4.2 and references therein).

Differences in the response of benthic macroinvertebrate size spectra to water chemical change, between substrates, were observed. This finding concurs with results from other studies that found the effects of trophic state on macroinvertebrate community structure were nested within habitat types (Tolonen *et al.*, 2001; White and Irvine, 2003; Brauns *et al.*, 2007; Donohue, 2008; Donohue *et al.*, 2009).

5.2.2 Season

Strong seasonal effects were observed in models of size structure, biomass-based community structure and in models of proportions of FFGs, within lakes. Large seasonal variation was observed in the collective biomass and in the biomass-based community structure of 'small' invertebrates relative to 'larger' ones. Although only the taxonomic composition of invertebrates on hard substrates was assessed in my study, the findings showed more seasonal variation in the biomass of lake littoral macroinvertebrates on hard substrates relative to those on soft substrates. In contrast, greater seasonal variation in the taxonomic composition of lake littoral macroinvertebrates on soft substrates, relative to hard substrates, was found in a study of thirty six North-German lowland lakes (Brauns *et al.*, 2007). A relative decrease in the collective biomass of large invertebrates and a corresponding increase in small

invertebrates in summer was found in both substrates in my study. This could be attributable to several factors, including the emergence of many univoltine insects in summer, the appearance of new generations and possible selective predation by fish, although the exact mechanism is speculative.

5.3 Among lakes: The role of alkalinity and nutrients in structuring lake littoral macroinvertebrate length-mass relationships, size spectra, and on biomass-based community structure and FFGs

The effects of alkalinity and nutrients on different aspects of lake littoral macroinvertebrate biomass (e.g. mass per unit length, total biomass, mean biomass, size structure, community structure based on biomass of taxa, proportional biomass of FFGs) were examined among lakes. There is a likely interaction between nutrients and alkalinity owing to the quality of soils in calcareous and karst catchments and, therefore, a likelihood of agricultural land use, resulting in increased diffuse nutrient loadings. In analyses such as linear mixed effects models and general linear models (LMEs and GLMs respectively), multiple variables are examined together, and the partial effects of each variable is quantified separately, while controlling for the effects of all other relevant variables. Thus, the effect of either alkalinity or nutrients was controlled for, whilst examining the effect of one or the other.

5.3.1 Alkalinity

Throughout this study, alkalinity had a significant, but marginal effect, repeatedly estimated as close to zero, on several aspects of the biomass of lake littoral macroinvertebrates. However, in contrast to these remarkably consistent marginal effects, alkalinity was found to significantly alter the distribution and proportional biomass of several taxonomic groups (Crustacea, Ephemeroptera, Hirudinea, Plecoptera, Turbellaria). Another study, conducted over the same time period, also found significant changes in taxonomic composition based on proportional abundance with alkalinity (Donohue, 2008). Thus, although alkalinity may be important in explaining changes in the taxonomic composition among lakes, it appears to have only a marginal effect on (i) the mass per unit length of some invertebrates (and no effect on the length-mass relationships of others), (ii) predicted dry mass, mean size and total

biomass of macroinvertebrates and (iii) the size structure of littoral macroinvertebrates communities, with a marginal shift towards larger organisms. Further evidence towards a greater response among larger organisms was seen when the proportional biomass of 'large' grazers-scrapers, shredders and filter feeders was shown to increase marginally with alkalinity, a pattern mirrored only by shredders in the 'whole' dataset.

The European Water Framework Directive (WFD; Council of the European Communities Directive 2000/60/EC; CEC, 2000) requires that a typology ("ecotype") be established for different water bodies and reference conditions (or "high status") for each type determined. A European study of shallow lakes (ECOFRAME) devised a core scheme that comprised 48 ecotypes, incorporating gradients of climate, lake area, geology of the catchment and conductivity (Moss *et al.*, 2003). Owing to the correlation between invertebrate biomass and production (Banse and Mosher, 1980; Stephenson and Morin, 2009) my results imply that zoobenthic production is only marginally influenced by alkalinity effects, differing among taxa (Chapters 2 and 3). Taking this marginal effect into consideration, combined with the elimination of lake area from all statistical models of size structure, these results suggest that size structure varies only marginally among lake types. Therefore, although a lake typology approach is needed in terms of the taxonomic composition of organisms in lakes differing in alkalinity, the biomass and size structure of lake littoral macroinvertebrates is relatively comparable among all lakes. A similar conclusion, but on a much smaller dataset, was suggested by the findings of De Eyto and Irvine (2007). The obvious overall conclusion from these results is that alkalinity *per se* has only a small marginal effect on community-level littoral macroinvertebrate size structure.

5.3.2 Nutrient enrichment

There were some significant differences in the relationships of the various aspects of lake littoral macroinvertebrate biomass examined, with the nutrients total nitrogen (TN) and total phosphorus (TP). For example, the dry mass of five taxa (out of eight taxa examined) was found to change marginally with TP, which explained variation in the dry mass of these taxa better than TN (Chapter 2). TP also caused a marginal increase in the mass per unit length of a single taxon, Hydracarina (Chapter 2). In contrast, models of lake littoral macroinvertebrate mean and total biomass, size structure, both of biomass-based taxonomic composition (in spring only) and of proportional biomass of

predators, showed consistently that TN explained the data better than TP or chlorophyll *a*. In addition, TN effects were frequently curvilinear (Chapters 3 and 4). These results suggest that there are differences among species in their response to either TP or TN, but that overall patterns of benthic invertebrate biomass are more strongly governed by changing TN concentrations then by TP; in addition, the standing biomass is limited by TN, likely to an optimal range of TN concentrations, above which the biomass gradually declines. This curvilinear pattern was evident across size classes in both substrates (with similar optimal TN concentrations observed in the size structure in both substrates) and in the total biomass. The greater response of littoral macroinvertebrates to TN than TP suggests a greater role of nitrogen as a limiting nutrient in lakes than is the conventional view. The optimum 'plateau' also suggests subsequent limitation of production at high TN concentration.

A similar curvilinear response to increasing nutrient pressure has been observed among communities of primary producers, suggesting that changes in macroinvertebrate food resources may have initiated the observed change in macroinvertebrate biomass (Weatherhead and James, 2001; Declerck *et al.*, 2005; Free *et al.*, 2009). For example, a unimodal response in the biomass and diversity of macrophyte communities to nutrient increase has been documented (Gonzaléz Sagrario *et al.*, 2005; James *et al.*, 2005). Likewise, periphytic algal biomass (algae found attached to submerged surfaces) was found to have a curvilinear relationship with increasing nutrients, with an optimal biomass at intermediate nutrient concentrations, suggesting a shift from nutrient limitation to light limitation at high nutrient concentrations, triggered by increased phytoplankton biomass (Liboriussen and Jepessen, 2006; Hansson, 1992). However, phytoplankton biomass has been observed to reach an asymptote in hypereutrophic lakes, as light will eventually begin to limit even mixed layer phytoplankton production (McCauley *et al.*, 1989). Thus it is likely that macroinvertebrate food resources in the study lakes changed with increasing nutrient concentrations, and that this had a significant part to play in the unimodal change in macroinvertebrate biomass and size structure.

The transfer of energy to higher trophic levels is less efficient in more productive systems (Downing *et al.* 1990; Sager and Richman 1991; Cyr *et al.*, 1997; Jeppesen *et al.*, 2003). As primary producer biomass was not examined in this study, it is not

possible to deduce whether less efficient energy transfer was responsible for the more significant decrease in the largest size classes under high nutrient concentrations; yet, the unimodal relationship between the proportion of predators and nutrient enrichment supports this view.

Primary production in lakes is often considered TP limited owing to the strong correlation between TP and chlorophyll *a* (Schindler, 1977; Dillon and Rigler, 1974; Jones and Bachman, 1976; Canfield and Bachman, 1981; Jeppesen *et al.*, 2005; Özkan *et al.*, 2010). However, recent experimental and field studies have suggested that TN may play a greater role in the functioning of temperate shallow lakes than previously thought (Heino, 2000; James *et al.*, 2005; Donohue, 2008; Özkan *et al.*, 2010). My results suggest that up to an optimum level, TN may limit the standing biomass, size structure and mean biomass of benthic macroinvertebrates and proportional biomass of predators in the littoral zones of many Irish lakes. In a study of nine Irish lakes (four of which were also examined in the current study) with a similar nutrient gradient to that of my study lakes, Donohue (2008) found planktonic algae were nitrogen limited (with C:N ratios greater than 8.3, as proposed by Healey and Hendzel [1980]) in four of the nine lakes, and benthic algae were nitrogen limited (with C:N ratios >10 and N:P ratios <13, as deduced by Hillebrand and Sommer [1999]) at seven of the nine lakes. These observations support the findings of my study.

The optimal macroinvertebrate overall biomass and biomass among size classes was consistently observed (in both substrates) at TN concentrations of approximately 1.5 mg L^{-1} (optimal concentration was in the range of 1 to 2 mg L^{-1}). González Sagrario *et al.* (2005) found a high probability of losing submerged macrophytes in temperate shallow lakes above TN concentrations of 1.2 to 2 mg L^{-1} in mesocosm experiments. Macroinvertebrate communities are likely to respond to changes in macrophyte density and diversity as macrophytes provide shelter, habitat and refuge from predators, as well as acting as a food source (Weatherhead and James, 2001; Declerck *et al.*, 2005; Rennie and Jackson, 2005; Penning *et al.*, 2008; Free *et al.*, 2009). Based on published data on benthic and planktonic algae, Dodds *et al.* (1998) using a cumulative frequency method, proposed concentrations of 0.7 mg l^{-1} TN for the boundary of oligotrophic-mesotrophic rivers and 1.5 mg l^{-1} TN for the mesotrophic-eutrophic boundary, which corresponded

to the upper third of the distribution (and maximum algal biomass). When the same method was applied to data from Smith (1982) on lake algal biomass in 228 northern temperate lakes, lower boundary concentrations were calculated for lakes (0.5 mg l^{-1} TN for the oligotrophic-mesotrophic boundary and 1.26 mg l^{-1} TN for the mesotrophic-eutrophic boundary; Dodds *et al.*, 1998).

As no WFD standards have, as yet, been established for nitrates in lakes or rivers, the Irish Environmental Protection Agency (EPA), for reporting purposes, included six quality classes, established under the EU Nitrates Directive (91/676/EEC), ranging from 0 to >50 mg NO_3 L^{-1}, with the separation of 'good' from 'moderate' water quality (in relation to nitrates) at nitrate concentrations >10 mg NO_3 L^{-1} (EPA, 2009). The 'good-moderate' boundary is an important threshold for assessment, and for meeting the objectives of the WFD, for a return to 'good water status' by 2015. Converting the NO_3 concentration of 10 mg L^{-1}, established as the threshold for the 'good-moderate' boundary, to NO_3-N, to allow comparison with TN concentrations, the concentration becomes NO_3-N 2.36 mg L^{-1}. Although, nitrate is the common form of inorganic N entering freshwaters from the catchment, dissolved organic nitrogen (DON) often constitutes over half of the total soluble N in freshwaters (Wetzel, 2001). Therefore, an NO_3-N concentration of 2.36 mg L^{-1} represents an even higher TN concentration of natural water. My results indicate a sharp decline in macroinvertebrate biomass in almost all size classes and in the total biomass at TN concentrations of 2 mg L^{-1}. Given the observed decline in macrophytes at similar concentrations from mesocosm experiments (González Sagrario *et al.*, 2005), and the peak in algal biomass concentrations observed among lakes with concentrations of TN > 1.26 mg l^{-1} (Dodds *et al.*, 1998), continued use of the 'Nitrate' Directive derived quality classes by the EPA appears unwise.

The observed relationship between macroinvertebrate biomass and TN has important implications for monitoring, as investigation into macroinvertebrate based lake classification metrics may be founded on TP or chlorophyll *a* gradients and tolerance of macroinvertebrates to TP concentrations (Brodersen *et al.*, 1998; Brauns *et al.*, 2007; Donohue *et al.*, 2009). For the effective implementation of the WFD, there is a strong need to further investigate the relationship between TN and lake littoral macroinvertebrate growth, production and community structure so that functional

change as well as taxonomic change is incorporated into the development of ecological monitoring classification tools.

Although the WFD emphasizes the use of taxonomy in assessing ecological quality, Article 20 provides for the development of alternatives to taxonomic measurements. Size structure has potential as an index of ecosystem function, reflecting patterns in standing biomass and energy flow, and would provide a useful complementary technique to taxonomic measures, particularly in long-term monitoring, as well as in the monitoring of protected sites. The use of size spectra in monitoring planktonic organisms under the WFD has been suggested (De Eyto and Irvine, 2007). Studies of lake littoral invertebrate size spectra are in their infancy, however, and require further investigation for the development of a useful metric.

5.4 Knowledge gaps and future research

The results of my study show consistent patterns in the biomass and size structure of lake littoral macroinvertebrates with nutrient enrichment. Such a result has implications for monitoring of ecological status and needs further investigation. Questions remain, however, on changes in consumer-resource relations, such as patterns of change in predation and in consumption with nutrient enrichment, which would aid in the interpretation of size spectra.

Predator-prey mass ratios (PPMR) can help explain patterns in size spectra (Jennings and Mackinson, 2003). Inefficient energy transfer through food chains restricts net energy supply to larger individuals (predators), and thus, the total supply of energy available to small animals is greater than that available to large (Jennings *et al.*, 2007). Where food webs are size structured, the rate at which available energy decreases with increasing mass will depend on the mean PPMR as well as the trophic transfer efficiency (TE), where TE is the proportion of prey production converted to predator production (Jennings and Mackinson, 2003). Evidence from marine food webs has shown that smaller mean predator–prey body size ratios are characteristic of more stable environments and that food chains are longer when mean predator–prey body size ratios are small (Jennings and Warr, 2003). The transfer of energy through a large number of trophic levels, identified by steeper size spectra slopes, may characterise stable

environments (Jennings and Mackinson, 2003). The identification of change in PPMRs with nutrient enrichment could assist in interpretation of size spectra change with nutrient enrichment. Stable isotope analysis (SIA) measures what is assimilated into invertebrate body tissue, rather than what is merely ingested (James *et al.*, 2000); as such, SIA could assist in this process by identifying food sources and following their pathway up through the food web.

As benthic littoral communities include large microphagous feeders such as large filter feeding invertebrates, they may not be as strongly-size structured as pelagic communities (Rasmussen, 1993). Blanchard *et al.* (2009) disaggregated community data collected in the North sea into predator and detritivore (filter feeders and deposit feeders) components, where one comprised predators feeding on each other according to body size and the other comprised animals sharing food and examined size spectra slopes within the two trophic pathways in separate size spectra analyses. A similar approach to examining lake littoral macroinvertebrate communities may offer insight into patterns of resource allocation and energy use within these communities.

There is a strong need for more species specific information on feeding plasticity among lentic littoral macroinvertebrates with nutrient enrichment. Invariant functional feeders, such as life-long filter feeders and predators, displayed significant relationships with water chemical variables. However, use of FFGs (based on the AQEM database) in the assessment of ecosystem health, based on taxa with variable feeding patterns over their life cycle, and with nutrient enrichment, led to ambiguous results in my study. This was because feeding plasticity could not be accounted for, and thus, the pathway for predator increase with nutrient enrichment (and subsequent decrease) was difficult to identify. Further investigation into this pathway would be beneficial for understanding functional change in the lake littoral zone with nutrient enrichment.

5.5 Conclusions

The key findings of this study were:

- Stronger within-lake variation (between substrates) than among-lake variation was observed, for models of mean and total biomass; models of size spectra also differed between substrates, with greater normalised biomass among larger size classes on soft substrates and greater mean and total macroinvertebrate biomass found on soft substrates compared with hard substrates;

- Strong seasonal effects were observed in models of size structure, biomass-based community structure and proportions of FFGs, within lakes, with more seasonal variation in the biomass of lake littoral macroinvertebrates on hard substrates compared with soft substrates, and a relative decrease in the collective biomass of large invertebrates and a corresponding increase in small invertebrates in summer in both substrates observed.

- Despite significant within-lake variance between substrates and seasons, consistent patterns in different aspects of lake littoral macroinvertebrate biomass with alkalinity and nutrients were observed, suggesting macroinvertebrate biomass as an emergent property of lake ecosystems;

- Alkalinity had significant marginal effects on the mass per unit length of some invertebrate taxa (three of eight taxa examined), on individual biomass, mean and total biomass of macroinvertebrates, and on the proportional biomass of some FFGs and on the size structure of littoral macroinvertebrates communities, with a marginal shift towards larger organisms with increasing alkalinity; the proportional biomass of 'large' grazers-scrapers, shredders and filter feeders, and of shredders in the 'whole' dataset was also shown to marginally increase with alkalinity; changes in alkalinity concentrations had significant effects on the distribution and proportional biomass of several taxonomic groups;

- TP caused a marginal increase in the mass per unit length of a single taxon, Hydracarina, and in the dry mass of five taxa (of eight taxa examined), explaining changes in dry mass better than TN for these taxa; in contrast, models of mean and total biomass, size structure, biomass-based taxonomic composition (in spring) and proportional biomass of predators consistently showed that TN explained variation in the data better than TP; In addition TN effects were frequently curvilinear, suggesting that although there are differences among species in their response to either TP or TN, the standing macroinvertebrate biomass appears to be limited by TN, up to an optimal TN concentration, above which macroinvertebrate biomass gradually declines;

- These results indicate a sharp decline in macroinvertebrate biomass in almost all size classes and in the total biomass at TN concentrations of 2 mg L^{-1}, and when coupled with previous studies where a decline in macrophytes from mesocosm experiments, and a peak in algal biomass concentrations was observed among lakes, at similar TN concentrations, it appears unwise for the continued use by the Irish EPA of quality classes, established under the EU Nitrates Directive, as thresholds for the 'good-moderate' boundary are proposed for significantly higher concentrations. The current study has yielded insight into change in length-mass relationships, in patterns of size structure and biomass, and in biomass-based measures of community structure and proportions of FFG, with nutrient and alkalinity, among communities of lake littoral macroinvertebrates for the first time.

CHAPTER 6

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APPENDIX

Appendix

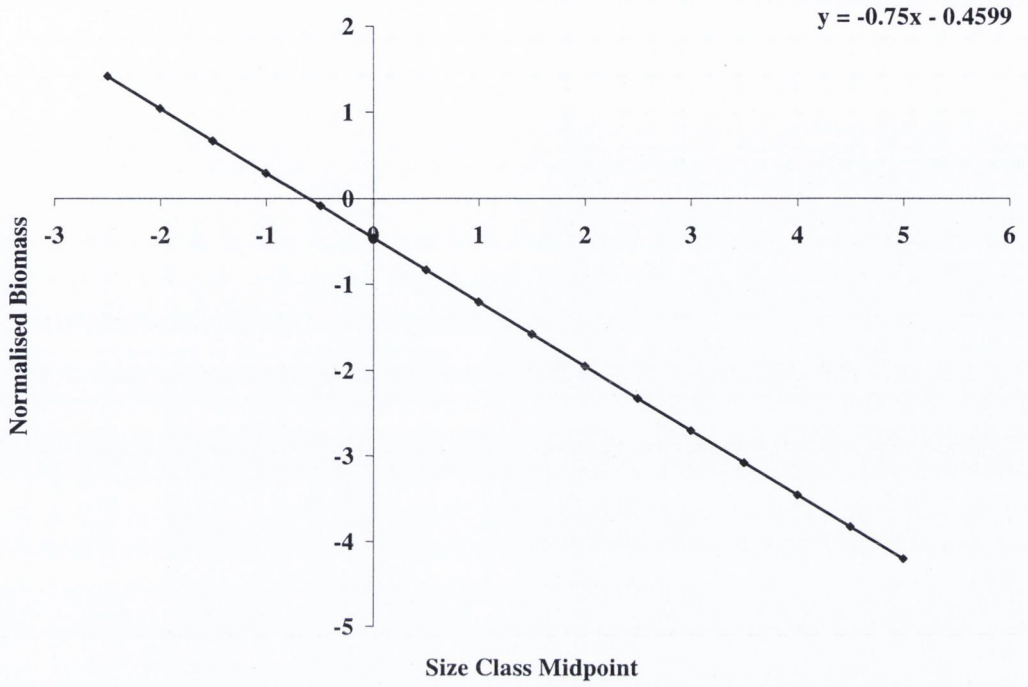

Figure I: Normalised biomass among size classes for Dataset a.

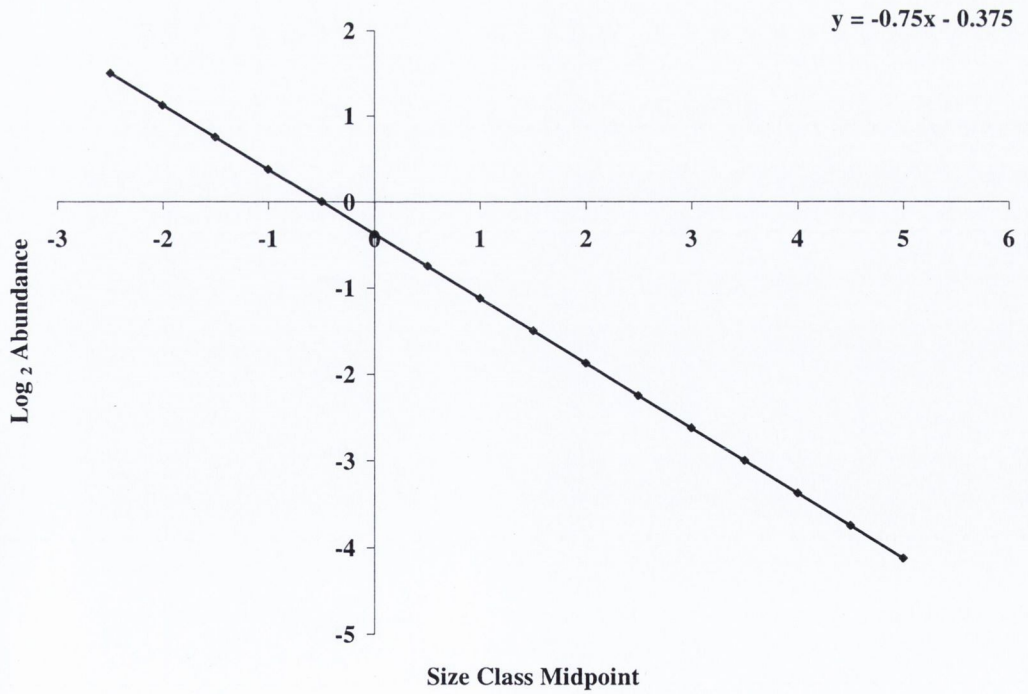

Figure II: Log abundance among siz classes (abundance derived from collective biomass of animal within each size class divided by mean size of individuals within size class) for Dataset a.

Table I: Principle water chemical concentrations measured at each lake

Lake	Season	Year	Lake Area (ha)	Alkalinity (mg CaCO3 l^{-1})	DOC (mg l^{-1})	TP (µg l^{-1})	TN (mg l^{-1})	Chlorophyll *a* (mg l^{-1})
Achully	Spring	2005	5.36	4.6	12.66	8	0.41	5.21
Achully	Summer	2005	5.36	8.4	11.96	13	0.42	0.96
Aillbrack	Spring	2005	4.45	5.6	7.29	24	0.43	6.76
Aillbrack	Summer	2005	4.45	11.0	11.94	42	0.85	7.45
Akibbon	Spring	2006	44.46	34.1	8.51	8	0.51	3.35
Akibbon	Summer	2006	44.46	41.0	6.03	11	0.43	3.11
Altnadua	Spring	2005	2.5	25.0	6.38	70	1.96	11.32
Altnadua	Summer	2005	2.5	36.0	4.95	30	0.49	19.53
Auva	Spring	2005	18.75	1.7	6.12	10	0.54	2.97
Auva	Summer	2005	18.75	2.9	11.87	24	0.49	12.29
Bane	Spring	2005	75.14	139.2	3.39	3	0.39	1.91
Bane	Summer	2005	75.14	114.6	3.37	7	0.35	1.04
Bawn	Spring	2005	17.2	111.3	10.02	58	1.96	4.73
Bawn	Summer	2005	17.2	118.0	9.10	58	0.82	20.00
Beagh	Spring	2005	152.29	1.0	4.33	1	0.23	1.07
Beagh	Summer	2005	152.29	2.2	7.72	5	0.27	1.07
Brantry	Spring	2005	17.5	109.0	7.96	21	0.82	4.26
Brantry	Summer	2005	17.5	105.4	7.62	28	0.61	11.36
Bunerky	Spring	2006	74.57	59.7	10.87	26	0.75	7.32
Bunerky	Summer	2006	74.57	68.7	9.83	35	0.89	24.65
Bunny	Spring	2005	102.53	130.4	4.01	4	0.45	1.65
Bunny	Summer	2005	102.53	112.2	5.25	4	0.40	1.32
Carrick	Spring	2005	3.1	113.0	8.04	34	1.03	23.52
Carrick	Summer	2005	3.1	106.8	8.28	7	0.65	11.51
Carrigeencor	Spring	2005	43.42	40.8	5.68	8	0.35	10.93
Carrigeencor	Summer	2005	43.42	43.5	3.81	33	0.33	6.67
Cashel Upper	Spring	2005	5.678	23.6	7.36	9	1.56	9.17
Cashel Upper	Summer	2005	5.678	32.6	7.83	10	0.50	5.04
Castlewellan	Spring	2005	34	25.0	5.16	27	0.97	11.95
Castlewellan	Summer	2005	34	26.3	4.08	16	1.16	13.83
Corglass	Spring	2005	34.28	138.0	9.48	17	0.60	17.12
Corglass	Summer	2005	34.28	128.4	9.24	52	0.92	28.04
Corry	Spring	2006	6.5	3.1	14.53	40	0.70	14.62
Creeve	Spring	2005	14.75	121.6	6.18	25	0.89	18.81
Creeve	Summer	2005	14.75	116.9	6.44	28	0.59	9.10
Deraik	Spring	2005	14.12	145.2	11.55	38	1.11	26.41
Deraik	Summer	2005	14.12	161.7	11.60	44	0.97	28.76
Derg	Spring	2006	858.92	3.3	7.67	16	0.28	3.47
Derg	Summer	2006	858.92	4.4	6.12	9	0.29	5.28
Doo	Spring	2005	129.91	3.4	10.87	17	0.52	6.08
Doo	Summer	2005	129.91	7.0	11.22	19	0.68	16.90
Emy	Spring	2005	52.38	97.1	10.72	41	1.15	26.78
Emy	Summer	2005	52.38	99.4	10.47	28	0.74	20.44
Fern	Spring	2005	180.29	39.8	8.09	30	0.88	23.76
Fern	Summer	2005	180.29	43.5	7.56	50	0.71	11.47
Formal	Spring	2006	8.8	8.1	16.29	22	0.43	2.54
Formal	Summer	2006	8.8	18.5	13.53	13	0.50	9.10
Gartan	Spring	2005	202.79	9.9	6.29	2	0.47	2.65
Gartan	Summer	2005	202.79	11.7	8.35	10	0.39	2.00
Glencreawan	Spring	2006	21.7	17.0	11.90	22	0.34	5.02
Glencreawan	Summer	2006	21.7	23.6	9.14	142	1.35	73.89

Table I continued

Lake	Season	Year	Lake Area (ha)	Alkalinity (mg CaCO3 l^{-1})	DOC (mg l^{-1})	TP (µg l^{-1})	TN (mg l^{-1})	Chlorophyll *a* (mg l^{-1})
Gortnawinny	Spring	2005	5.06	264.4	11.91	90	1.33	59.05
Gortnawinny	Summer	2005	5.06	259.4	10.23	81	0.97	29.21
Keagh	Spring	2005	6.95	2.0	10.09	35	0.43	4.71
Keagh	Summer	2005	6.95	3.1	14.62	30	0.51	3.76
Killtyfanad	Spring	2005	7.18	7.7	6.69	25	0.53	6.15
Killtyfanad	Summer	2005	7.18	11.6	10.91	17	0.40	1.54
Kiltooris	Spring	2006	43	29.9	7.76	44	0.56	2.24
Kiltooris	Summer	2006	43	32.2	7.10	7	0.56	3.02
Lattone	Spring	2006	30	44.4	11.81	19	0.69	6.99
Lattone	Summer	2006	30	49.4	10.08	17	0.63	10.95
Legane	Spring	2005	5.6	112.3	8.17	47	1.48	23.87
Legane	Summer	2005	5.6	110.1	8.82	33	0.80	25.24
Madral	Spring	2005	13.32	8.2	9.65	12	0.53	7.14
Madral	Summer	2005	13.32	10.2	8.64	11	0.39	1.24
Meenameen	Spring	2006	17.1	13.6	25.54	80	0.70	24.56
Meenameen	Summer	2006	17.1	25.5	21.13	67	0.81	10.60
Melvin	Spring	2006	2197.07	57.3	10.77	19	0.54	2.50
Melvin	Summer	2006	2197.07	59.5	8.58	10	0.53	6.12
Nadarra	Spring	2006	4.2	6.6	10.92	9	0.45	9.86
Nadarra	Summer	2006	4.2	7.8	7.66	24	0.50	6.28
Nalughaghe	Spring	2005	7.5	103.7	7.38	14	0.58	5.10
Nalughaghe	Summer	2005	7.5	109.4	8.26	50	0.67	19.24
Narye	Spring	2005	8	56.0	7.41	31	0.60	15.42
Narye	Summer	2005	8	47.6	7.55	10	0.41	2.89
Natroory	Spring	2005	9.2	4.1	15.82	183	0.99	18.98
Natroory	Summer	2005	9.2	7.3	13.14	163	0.79	39.38
Ross	Spring	2006	90.25	54.7	8.90	44	2.27	18.63
Ross	Summer	2006	90.25	64.0	7.64	107	0.86	27.97
Salt	Spring	2005	28.73	24.6	2.73	4	0.40	1.06
Salt	Summer	2005	28.73	25.2	2.61	8	0.34	0.56
Sand	Spring	2005	23.3	160.4	15.17	184	1.59	38.81
Sand	Summer	2005	23.3	174.1	16.13	134	1.21	26.82
Shivnagh	Spring	2005	14.93	10.2	10.93	42	0.58	4.71
Shivnagh	Summer	2005	14.93	7.3	16.46	29	0.70	4.08
Skale	Spring	2005	4.7	38.5	5.36	22	0.58	26.00
Skale	Summer	2005	4.7	41.1	5.51	33	0.54	15.07
Town	Spring	2005	31.09	63.3	11.69	61	1.39	41.09
Town	Summer	2005	31.09	83.2	10.77	119	2.21	150.16
Tullynawood	Spring	2005	30.4	40.8	9.32	68	2.48	36.03
Tullynawood	Summer	2005	30.4	52.1	9.63	41	0.83	27.06
Vearty	Spring	2005	44.3	5.5	6.53	5	0.43	4.43
Vearty	Summer	2005	44.3	7.0	7.75	12	0.37	1.20
White	Spring	2005	53.58	78.7	9.85	104	1.58	46.57
White	Summer	2005	53.58	99.4	11.18	106	1.01	34.49

Table II: Water chemical variables best describing lake littoral macroinvertebrate species biomass of 'whole', 'large' and 'small' dataset as determined by the BEST analysis, with a different enrichment term included in each analysis.

Dataset	Enrichment term	BEST Values	BEST variables
"Whole" Spring	TP	*Rho* = 0.157 *P* value = 0.141	- Area
"Whole" Spring	TN	*Rho* = 0.234 ***P* value = 0.008**	- TN
"Whole" Spring	Chlorophyll *a*	*Rho* = 0.157 *P* value = 0.123	- Area
"Whole" Summer	TP	*Rho* = 0.116 *P* value = 0.304	- Area
"Whole" Summer	TN	*Rho* = 0.116 *P* value = 0.335	- Area
"Whole" Summer	Chlorophyll *a*	*Rho* = 0.116 P value = 0.324	- Area
"Small" Spring	TP	*Rho* = 0.187 *P* value = 0.09	- Area
"Small" Spring	TN	*Rho* = 0.222 ***P* value = 0.041**	- Area - TN
"Small" Spring	Chlorophyll *a*	*Rho* = 0.187 *P* value = 0.082	- Area
"Small" Summer	TP	*Rho* = 0.139 *P* value = 0.228	- Area
"Small" Summer	TN	*Rho* = 0.139 *P* value = 0.21	- Area
"Small" Summer	Chlorophyll *a*	*Rho* = 0.139 *P* value = 0.255	- Area
"Large" Spring	TP	*Rho* = 0.075 *P* value = 0.46	- Area
"Large" Spring	TN	*Rho* = 0.138 *P* value = 0.143	- Alkalinity - TN
"Large" Spring	Chlorophyll *a*	*Rho* = 0.075 *P* value = 0.48	- Area
"Large" Summer	TP	*Rho* = -0.007 *P* value = 0.965	- Alkalinity
"Large" Summer	TN	*Rho* = -0.007 *P* value = 0.957	- Alkalinity
Large" Summer	Chlorophyll *a*	*Rho* = 0.025 *P* value = 0.886	- Chlorophyll *a*

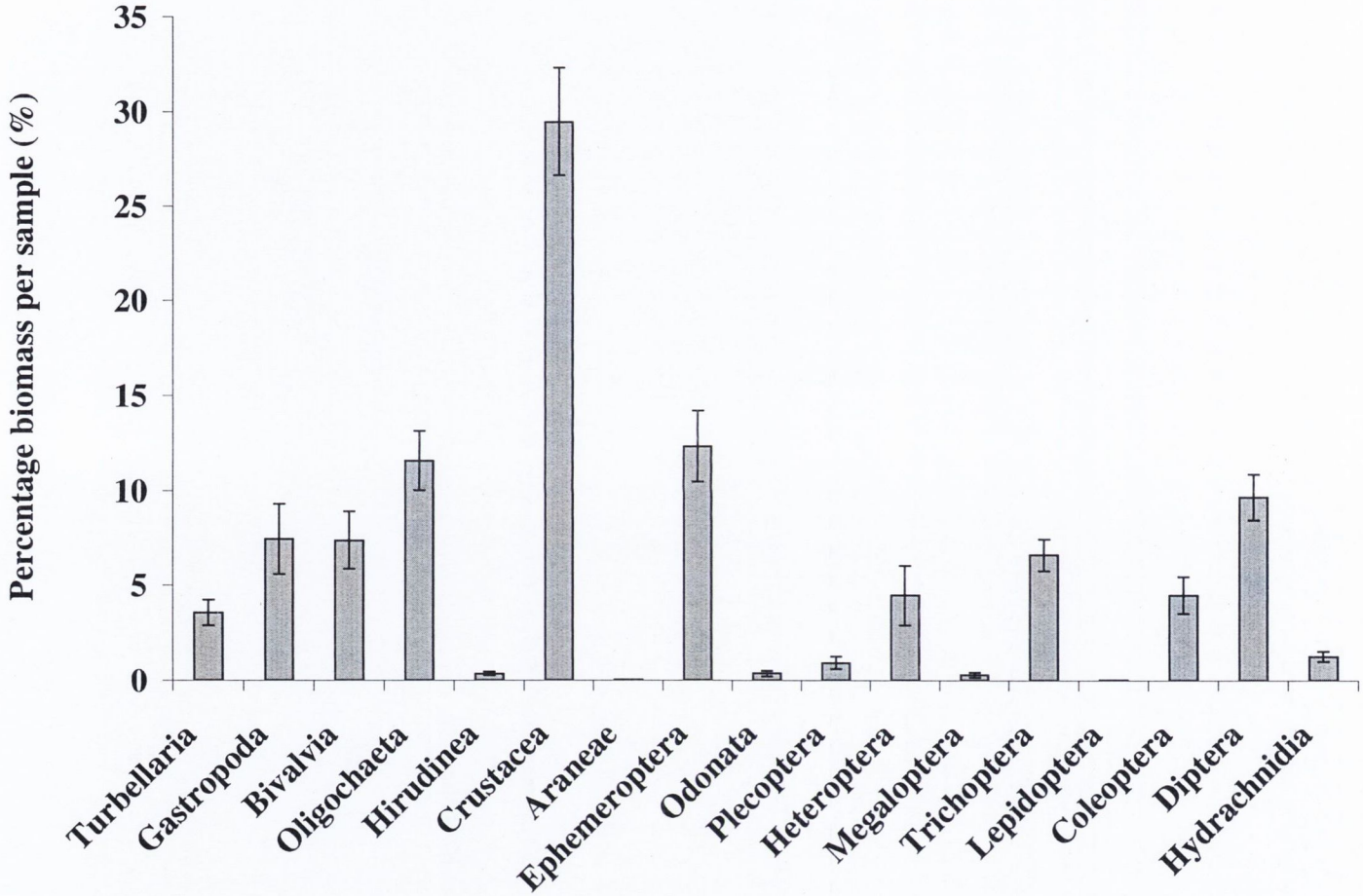

Figure III: Percentage biomass of principal taxonomic groups present within 'small' dataset.

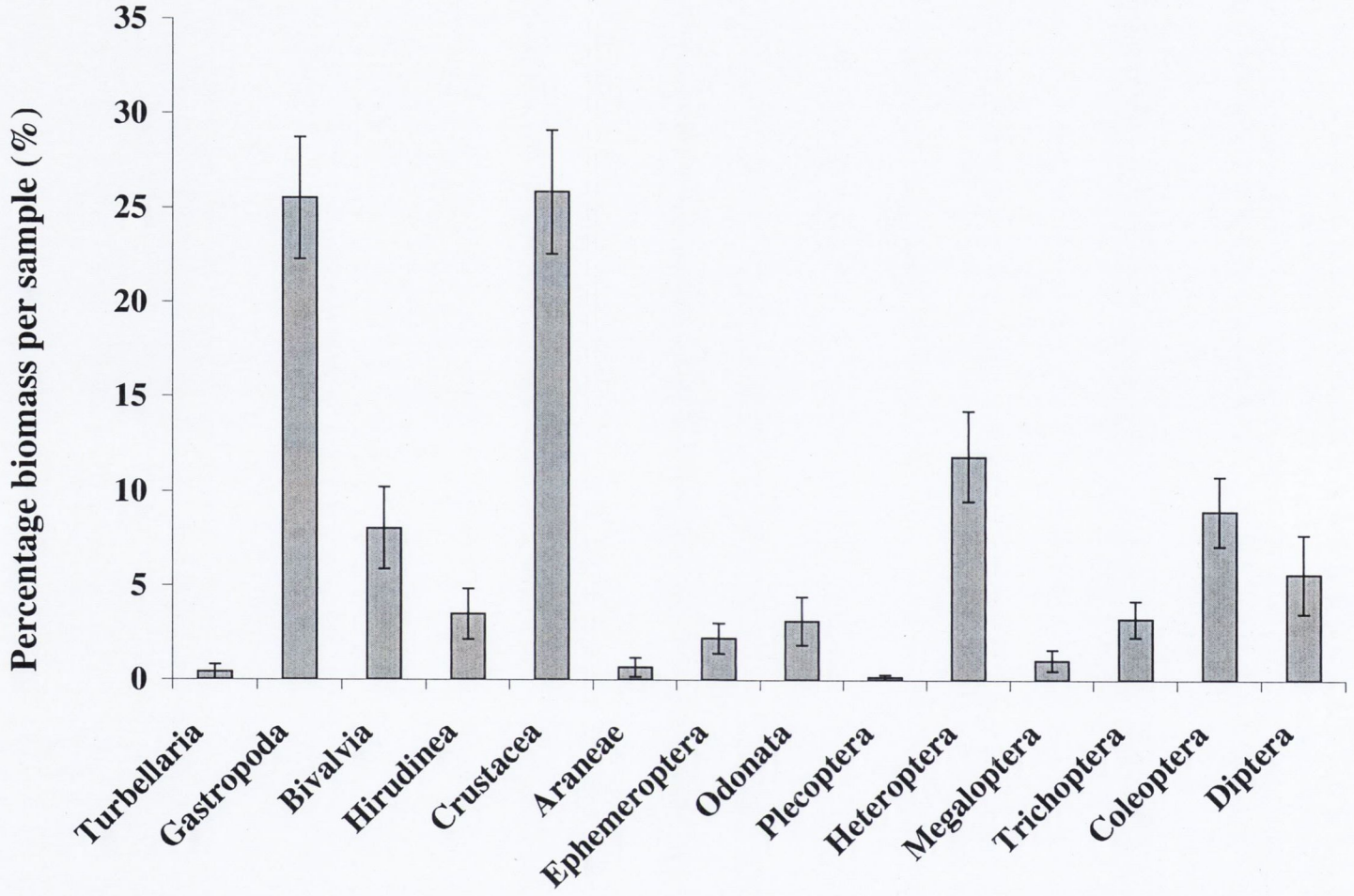

Figure IV: Percentage biomass of principal taxonomic groups present within 'large' dataset.

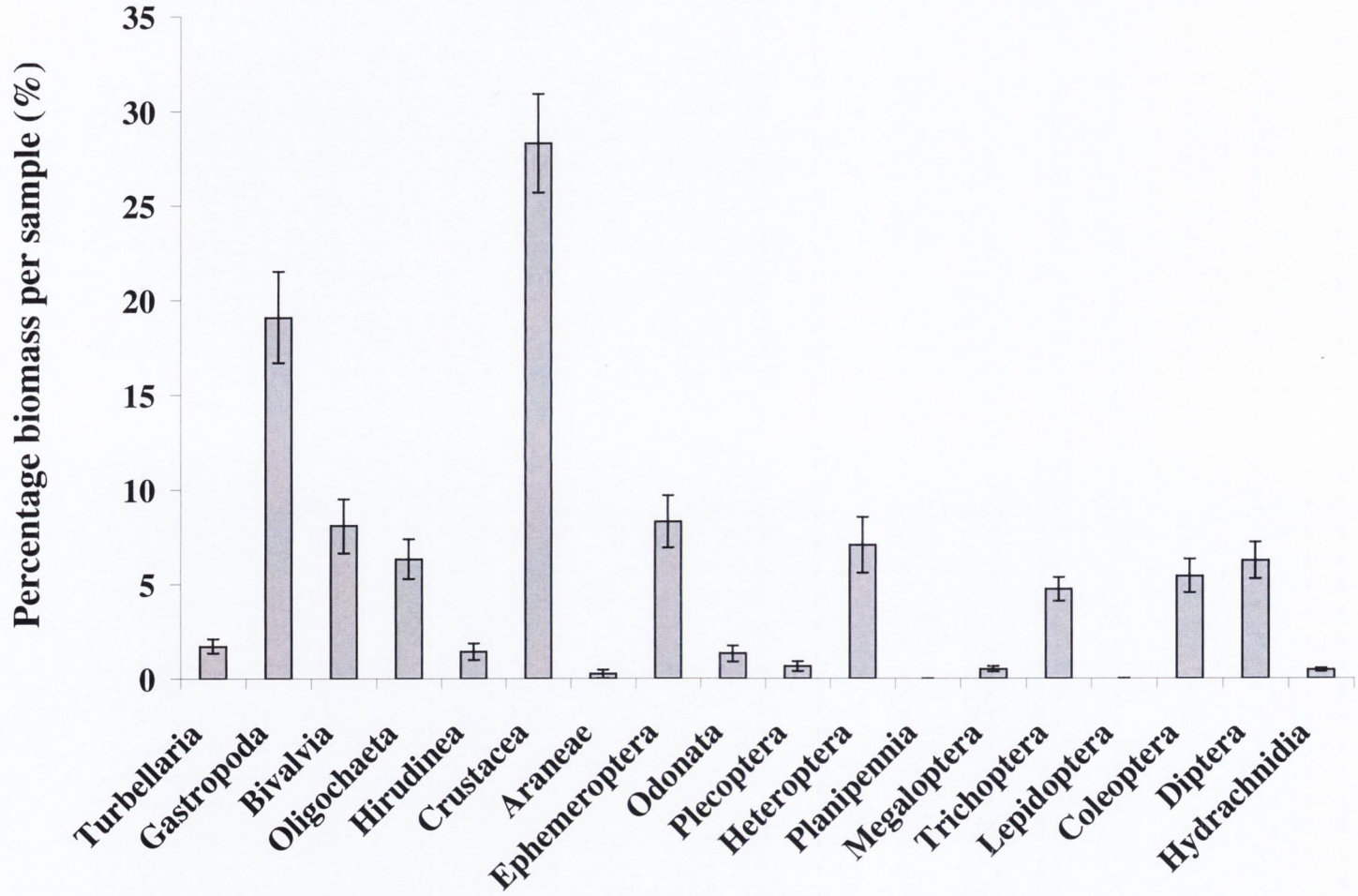

Figure V: Percentage biomass of principal taxonomic groups present within 'whole' dataset.

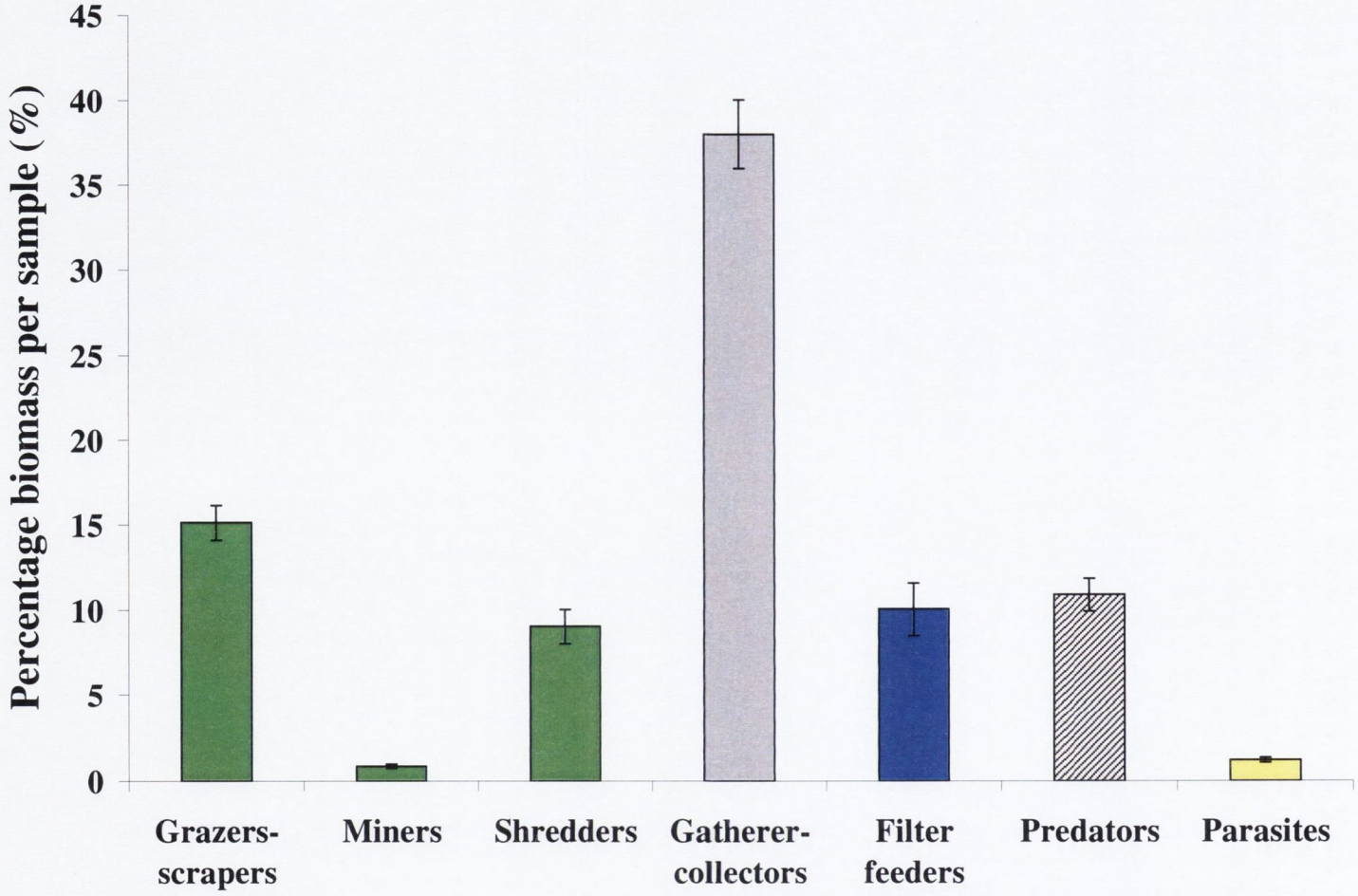

Figure VI: Percentage biomass of principal functional feeding groups present within 'small' dataset.