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# FUNCTIONAL SIGNATURES OF MODEL CRUSTACEANS IN LAKE LITTORAL ZONES IN RESPONSE TO NUTRIENT STATUS AND LOCAL HABITAT

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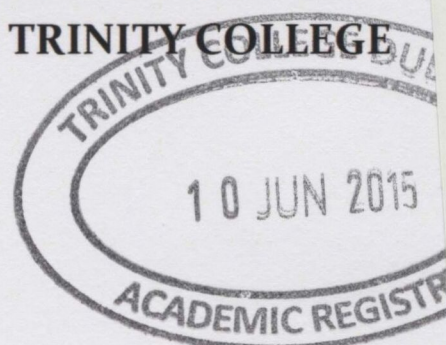
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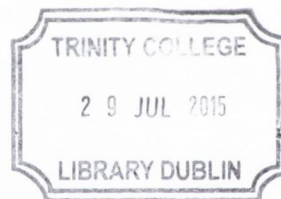


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Saliha Yeşim Tunalı





## SUMMARY

Littoral zones of lakes are critical for linking, physically and energetically, terrestrial and aquatic habitats. Disruption of this link through modification of lake riparian habitats can affect the structure and functioning of littoral communities. Understanding the functional responses of macroinvertebrate communities to alterations in riparian habitat and in-lake nutrient status provides an alternative to more structural assessment of littoral invertebrates that are increasingly used in assessment of lake status. The general aim of this study was to test the use of elemental signatures as functional indicators of the response of four common macrocrustacean species, as model organisms, to alterations to riparian habitat and nutrient status across a series of lakes. Three approaches were used to quantify ecological function: carbon and nitrogen stable isotope ratios, isotopic niche metrics and C:N:P stoichiometry. I used these measures to compare influences of natural woodland with modified habitats, represented by grassland shorelines, on the functioning of these species.

In the first study, I assessed the effects of two distinct types of shoreline habitat (woodland and grassland) on the carbon and nitrogen isotopic variation of *Asellus aquaticus*, *Gammarus duebeni* and *Crangonyx pseudogracilis* across eight Irish lakes along a nutrient enrichment gradient. Linear mixed-effects models were used to analyse the isotopic data. Water colour emerged as an important water chemistry variable for invertebrate isotopic variation, but analyses suggested that there were limitations regarding the use of macroinvertebrate  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures as indicators of nutrient status as species had different responses to nutrient enrichment. There was a significant effect of shoreline habitat type on both the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of *A. aquaticus* and *G. duebeni*, and on the  $\delta^{15}\text{N}$  signatures of *C. pseudogracilis*, with distinct responses across species, suggesting specific dietary needs. The study also highlighted the importance of species-specific traits, such as body size, in explaining species' isotopic variation within lakes.

In the second study, competitive overlap between one native (*G. d. celticus*) and two invasive (*G. pulex* and *C. pseudogracilis*) amphipod species were compared across three lakes with different natural combinations of these species. Using isotopic niche metrics, which included standard deviation of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , standard ellipse area and overlap, relative distance and angle, the study

highlighted the high trophic position of *C. pseudogracilis* compared with *Gammarus* spp. and provided no evidence for the predation of *Gammarus* spp. on *C. pseudogracilis*. This contradicts earlier findings. The narrow ranges of both stable isotopes and C:N:P elemental ratios suggest *C. pseudogracilis* as a species with the most specific feeding. Overlap in isotopic niche between *Gammarus* spp. and other species varied across lakes. Isotopic niche metrics could provide a novel approach for testing changes in species' trophic interactions in spatially distinct habitats.

In the final study of this thesis, I investigated the taxonomic and spatial variation of the C:N:P stoichiometry of littoral macroinvertebrates within and across three lakes by comparing sites adjacent to woodland and grassland shoreline habitats. The results of this study indicated that taxonomy, rather than the spatial differences are more likely to be the primary driver of among and within lake variation of C:N:P stoichiometry. Significant stoichiometric differences between *Gammarus* spp. and *C. pseudogracilis* were demonstrated for the first time.

To all the little beings,  
that were once crawling  
at the bottom of a lake...  
*Gammarus, Crangonyx, Asellus...*

I acknowledge you all.

This piece of work is dedicated to you.

Fascinated by your nature

I set out on this journey

As I wondered, I asked:

What are you eating, algae or dead leaves?

How are you surviving, when we cut all the trees?

Where are you hiding, under the water, in the mud?

I had to tell others, what it was all about...

Hardly anyone knew, may be the little roach

Your babies were kept, hidden in a pouch...

Once you were alive and

a part of an intricate web

Before your lives were taken

to make this research happen.

I would have done it differently

if only I knew another way...

I truly hope one day,

We will learn how to look after our Home

Without any need to detach you from your home.

May all the lives, taken for science, count!

Ho!





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# CHAPTER 1

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## 1. GENERAL INTRODUCTION





## 1.1 General research aims

The general aim of this study was to assess the use of carbon and nitrogen stable isotope signatures, isotopic niche metrics and elemental stoichiometry as indicators of habitat use by four common littoral macrocrustacean species, *Asellus aquaticus*, *Gammarus duebeni celticus*, *G. pulex* and *Crangonyx pseudogracilis*. The application of these model organisms to test differences in ecological function provides a potentially novel approach to lake assessment and contrasts with the more structural metrics being developed for policy compliance (e.g. Hering et al. (2010)) In particular, the study relates to new approaches and metrics for the assessment of lake hydromorphology (McGoff and Irvine 2009, Jurca et al. 2012, Miler et al. 2013, 2014) using communities of lake littoral invertebrates.

In an ecological context, “function” may have different meanings denoting multiple processes, roles of species, ecosystem services or the functioning of whole systems (Jax 2005). In this study, for the “function”, I refer to both “how individuals of different species interact” and “which roles do different species have in littoral ecosystems” (Jax 2005). Moreover, with the application of stoichiometric and isotopic metrics, I aim to contribute to the understanding of “littoral functioning”, which refers to ecological processes occurring in littoral zones (Jax 2005).

The first aim of this study was to examine, using carbon and nitrogen stable isotopes, the effects of two distinct types of shoreline habitat (woodland and grassland) on the functioning of three of the taxa, *A. aquaticus*, *G. d. celticus* and *C. pseudogracilis*, across a nutrient enrichment gradient, illustrated by eight Irish lakes (**Chapter 2**). As a species-specific trait, body size was used as potential factor influencing within-lake variability of species’ isotopic signatures while accounting for environmental variation across lakes.

The second aim was to quantify the competitive overlap between one native (*G. d. celticus*) and two invasive (*G. pulex* and *C. pseudogracilis*) amphipod species, using isotopic niche metrics across three lakes with different combinations of these species (**Chapter 3**). To provide a benchmark for comparison, the isopod species *A. aquaticus* was used as a reference species because of its commonality to all three lakes and functional similarity to amphipods. Isotopic niche metrics, including standard deviation of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , standard ellipse area and overlap, relative

distance and angle were applied to assess species' ecological interactions and functional role in the food web.

The third aim was to further investigate the taxonomic and spatial variation of the C:N:P stoichiometry of littoral macroinvertebrates within and across three lakes by comparing sites adjacent to woodland and grassland shoreline habitats using all four species; *G. d. celticus*, *G. pulex*, *C. pseudogracilis* and *A. aquaticus* as model organisms (**Chapter 4**).

## 1.2 Thesis structure

The thesis is organized so as to build an understanding of the ecology of common macrocrustacean taxa in lake littoral zones and to provide an overview of the application of stable isotopes and stoichiometry as indicators of ecosystem functioning across habitats.

- **Chapter 1** introduces the general aims and the outline of the study, and provides background information on studied littoral macrocrustacean species, littoral and shoreline habitats, elemental stoichiometry, stable isotope analysis and isotopic niche approach.
- **Chapter 2** investigates the effects of environmental gradients, shoreline habitat type, and species' body size on the carbon and nitrogen isotopic variability of *A. aquaticus*, *G. d. celticus* and *C. pseudogracilis* across eight lakes along a nutrient gradient. Understanding the variability of stable isotopes would enhance their use both as indicators of habitat and nutrient as well as tracers of trophic interactions.
- **Chapter 3** assesses the competitive overlap of three amphipod species, *G. duebeni*, *G. pulex* and *C. pseudogracilis*, in reference to isopod *A. aquaticus*, using isotopic niche metrics. This provides insights on the mechanisms of coexistence or dominance among the native and invasive species of amphipods.
- **Chapter 4** explores the spatial and taxonomic variability of C:N:P stoichiometry of four common littoral macrocrustacean species across three Irish lakes. Differences in elemental stoichiometry can reflect nutrient limitations on species in respect to different habitat use.
- **Chapter 5** discusses the overall findings of this study and presents the main conclusions.



### 1.3 Lake littoral zones and shoreline habitat modification

Aquatic systems are connected with their surrounding landscape in various ways (Richardson 2008). Both terrestrial (allochthonous) and lake (autochthonous) sources of carbon support secondary production in lakes (Vadeboncoeur *et al.* 2003). The relative contribution of these carbon sources to the littoral macroinvertebrate production may depend on both in-lake and catchment scale processes (France 1995). Processes that connect aquatic ecosystems and their surrounding landscape can be disrupted directly or indirectly by anthropogenic modifications of the riparian and shoreline habitats (Harrison and Harris 2002), influencing the structural and functional composition of littoral macroinvertebrate communities at the local scale (Brauns 2008, Jurca 2012). Particularly in agricultural landscapes, replacement of structurally complex woodland habitats with more simply structured grasslands is a common type of such disruptions in Ireland (Plate 1.1). Consequently, in this study, I compared natural/semi-natural woodland with putatively modified grassland shoreline habitats, mostly maintained by livestock, normally cattle, as examples of hydromorphological modifications, following an approach used by Rowan *et al.* (2006) and subsequently used to test for effects of lake riparian structure on littoral (McGoff and Irvine 2009, Jurca *et al.* 2012).

Impacts of riparian habitat alterations on macroinvertebrate community structure and functioning in the river and stream ecosystems have been studied widely (Dole *et al.* 2006, Bergfur *et al.* 2009, Mckie and Malmqvist 2009, Young and Collier 2009, Dolédec *et al.* 2011). Despite some recent studies on the impacts of lakeshore development on littoral food webs (Udy *et al.* 2006, Rosenberger *et al.* 2008, Brauns *et al.* 2011), studies linking the functional responses of lake littoral communities to riparian habitat are rather limited, and especially so in relation to grassland dominated shorelines. Vander Zanden *et al.* (2005) found macroinvertebrate nitrogen stable isotopes as useful indicators of urban and agricultural land use, but impacts on specific taxa were not discussed.





Plate 1.1 Woodlands (top) and grasslands (bottom) are the two common and distinct shoreline habitat features in Irish lakes. (Photo by S. Y. Tunali).

Modification of natural shoreline habitats can have consequences for littoral habitats and their accompanying biological communities as a result of, for example, lower buffering to nutrient flow, less allochthonous nutrient input, increased irradiance and less intact habitat structures associated with grassland shoreline habitats (Brauns *et al.* 2011, Jurca *et al.* 2012, Miler *et al.* 2013). Nutrient enrichments, through manure and fertilisers derived from riparian grasslands, can lower water quality at large scales (Vander Zanden *et al.* 2005). Even though nutrient enrichment can alter elemental compositions at the organismal level,



inducing functional shifts in the dietary preferences of the consumer species (Bergfur *et al.* 2009), it is unclear to what extent this can be measured at local scales.

Since littoral habitats are energetically coupled with riparian zones through allochthonous leaf litter and coarse woody debris (CWD) inputs, degradation of woodland sites often result in loss of important food sources for detrital consumers (Christensen *et al.* 1996, Schindler and Scheuerell 2002, Brauns *et al.* 2011). CWD, which alters water movements, also provides physical structure for aquatic organisms and can function as refuges from predation (Christensen *et al.* 1996). However, a study of 16 northern temperate lakes indicated a significant reduction of CWD density in deforested shorelines (Christensen *et al.* 1996). Furthermore, riparian habitat structure has important roles in dispersal, and in the mating and oviposition behaviour of adult aquatic insects (Harrison and Hildrew 2001, Harrison and Harris 2002). Also, owing to a lack of canopy coverage in riparian grassland, higher light attenuation can alter littoral temperature and microclimate as well as influencing the extent and type of primary production (benthic vs. pelagic) (Vadeboncoeur *et al.* 2003).

Finally, modification of shoreline habitats can also alter the physical structure of littoral habitats (Brauns *et al.* 2011). For instance, when livestock grazing on grasslands at lakeshore, have access to the lake littoral zone, they can disturb the bottom sediment, which can cause resuspension of sediment and nutrients as well as restricting the development of macrophyte beds. Structurally rich submerged macrophytes are highly important not only for littoral communities but also for whole lake ecology; processes include nutrient regulation, suppressing phytoplankton growth, providing refuge for zooplankton and macroinvertebrates against fish predation, and reducing physical disturbance from wave action (Vakkilainen 2005).

## 1.4 Study species

This study focused on four macrocrustacean species common to the littoral habitats of lakes in Ireland: the isopod *Asellus aquaticus* and the amphipods *Gammarus duebeni celticus*, *Gammarus pulex* and *Crangonyx pseudogracilis* (Plate 1.2). Amphipod and isopod crustaceans often dominate benthic macroinvertebrate communities across a variety of freshwater and brackish habitats, in terms of both



numbers and/or biomass (Gledhill *et al.* 1993). Common in freshwater bodies throughout the year, they constitute an important food source for many fish (Welton 1979, Macneil *et al.* 1999). Because of their widespread distribution and high abundance throughout the year, they provide good model organisms to study effects of anthropogenic pressures, including shoreline habitat modifications and nutrient enrichment.



Plate 1.2 Four macrocrustacean species investigated in this study: *G. duebeni* (top left), *G. pulex* (top right), *C. pseudogracilis* (left bottom) and *A. aquaticus* (right bottom). Note: Species' actual sizes are not presented in proportion. (Photo credits: Gevelsberg (2013); Cann (2014); Fenwick (2014); Hydra-Institute (2014)).

The genus *Gammarus* has over 100 species, widely spread throughout the northern hemisphere (Karaman and Pinkster 1977). Owing to their potential powerful influence on benthic communities and ecosystem processes, including algal production and decomposition, they are potential keystone taxa (Woodward *et al.* 2008). Because of their ability of effective processing of large amounts of coarse particulate organic matter (CPOM), *Gammarus* spp. have been traditionally regarded mainly as shredders (Cummins 1962). However recent studies have highlighted their versatile functional, including predatory, role (Macneil *et al.* 1997, Kelly *et al.* 2002).



*Gammarus duebeni celticus* Pinkster & Stock (1970) is an endemic freshwater amphipod species to Ireland (along with the less common *Gammarus lacustris* Sars, 1863 and subterranean *Niphargus kochianus irlandicus* Schellenberg, 1932). *Gammarus duebeni duebeni* is a subspecies of *Gammarus duebeni* (Liljeborg, 1854) occurring mainly in coastal saline environments (Pinkster *et al.* 1970). *Gammarus duebeni celticus* (referred as *G. duebeni* in rest of chapter) is also commonly found in Britain, Isle of Man and Western Brittany (Piscart *et al.* 2007, Dick 2008). Its distribution, however, appears to be increasingly restricted in the last century following the arrival of other non-indigenous freshwater amphipods (Costello 1993). Among them, *Gammarus pulex* (Linnaeus, 1758) is the most widely distributed freshwater amphipod in mainland Europe and Britain, where it originates. The gradual replacement of *G. duebeni* by *G. pulex* has been observed following the arrival of the latter species to Ireland in the 1950s (Strange and Glass 1979).

*Crangonyx pseudogracilis* Bousfield, 1958, originally from Northern America, is another non-indigenous amphipod species in Ireland that likely arrived within the last century. Other than a few published records in Northern Ireland, the distribution of *C. pseudogracilis* in the rest of Ireland is not well known, especially compared with *Gammarus* spp. Despite being considered to be more tolerant to pollution, *C. pseudogracilis* was able to survive in good quality waters occupied by *Gammarus* spp. (Macneil *et al.* 2000). In fact, in a recent study, *C. pseudogracilis* was recorded in all of the nine lakes sampled during my study, while *G. duebeni* and *G. pulex* were limited to seven and five of the lakes, respectively (Jurca 2012). Results of recent laboratory experiments suggested heavy predation by both *G. duebeni* and *G. pulex* on the smaller *C. pseudogracilis* (MacNeil *et al.* 1999, 2003). Mainly because of its inferior competitive position against *G. pulex*, *C. pseudogracilis* is not considered as a major threat for the native *G. duebeni* (Dick 2008). Even though it is mainly described as an herbivore (Gledhill *et al.* 1993), the trophic ecology of *C. pseudogracilis* is not well known, and especially when compared with *G. pulex*.

An almost mutually exclusive distribution between *G. duebeni* and *G. pulex* has been considered as a 'competitive exclusion' case in earlier studies (Hynes 1954). Despite the recent emphasis on mutual intraguild predation, that is predation between potentially competing species (MacNeil *et al.* 2003), as the main driver of the exclusion among the native and invading amphipods in Ireland, the

underlying processes of interspecific competition among these species are still largely unknown (Dick 2008). Studies demonstrating the competitive interaction of these common amphipod species in the field are still lacking. When their diverse functional roles in freshwaters are considered, understanding the competitive relationship between these common macrocrustacean species is important.

*Asellus aquaticus* (Linnaeus, 1758), commonly known as the water louse, is a native isopod species to Ireland. It is known as an extreme generalist and adaptable species and is the only species of its genus inhabiting large parts of Europe successfully (Sket 1994). First of all, *A. aquaticus* demonstrates a wide-range distribution in Ireland (Free *et al.* 2006), occurring in various types of freshwater habitats (Sket 1994), which has been associated with its high tolerance to organic pollution, especially when compared with *Gammarus spp.* (Basset 1993, MacNeil *et al.* 2002). Secondly, there is a considerable niche overlap between *A. aquaticus* and *Gammarus spp.* Despite being both traditionally considered as detritivorous, mainly feeding on microbially conditioned autochthonous and allochthonous CPOM (Graca *et al.* 1994a, 1994b, Leberfinger *et al.* 2010), studies using gut content analysis also suggest that algae are a regular part of the diet of *Asellus* and, to a lesser degree, of *Gammarus pulex* (Moore 1975). Therefore, with its robust distribution pattern and similar trophic niche to *Gammarus spp.*, using *A. aquaticus* as reference species provide advantages for comparing lakes with potentially different amphipod communities.

## 1.5 EU Water Framework Directive and functional measures

EU Water Framework Directive (WFD) requires that the ecological quality of surface waters should be quantified as ‘an expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters’ (European Commission 2000, Sandin and Solimini 2009). Even though the ‘structure’ term here refers to both the biological structure of ecosystems, and physical and chemical settings, there is no specific definition for the ‘functioning’, which has been owing to the assumption that measures of biological structures (e.g. biodiversity) relate directly to the functioning properties of ecosystems such as nutrient cycling and decomposition (Sandin and Solimini 2009). However, structural and functional attributes of ecosystems may not necessarily respond to



stress in similar ways. Thus, this study aims to contribute to our understanding of the functioning of aquatic systems and to move towards developing functional measures (or indicators) for the assessment of its health.

In this study, I used three different approaches to assess the functional responses of the macrocrustacean species to degradation of lake shoreline habitats and to explore the trophic relationship among them. The first two are based on utilising stable isotopic data in two different ways, whereas the third is based on ecological stoichiometry of macrocrustacean species. The carbon isotopic ratios ( $\delta^{13}\text{C}$ ) are commonly used for tracing the carbon source of consumers (Vander Zanden and Rasmussen 1999), while  $\delta^{15}\text{N}$  can be used for estimating their trophic level within food webs (Cabana and Rasmussen 1996).

The first approach involved testing the carbon and nitrogen stable isotope signatures of species as indicators of shoreline habitat modification and nutrient enrichment through exploration of within and across-lake variation of isotopic data in linear mixed-effects models (Chapter 2). The second approach consisted of using species' isotopic signatures to estimate a number of isotopic niche metrics (*e.g.* isotopic niche width) for the quantification of the competitive interactions among species (Chapter 3). The final approach I used as a functional measure is the ecological stoichiometry, expressed in this work as the C:N:P elemental ratios of organisms (Chapter 4). Elemental ratios can be indicators of the source and quality of organic matter, thus spatial and taxonomical variation of invertebrate C:N:P ratios can inform us about nutrient limitations and anthropogenic impacts on lakes.





### **2. VARIABILITY OF MACROINVERTEBRATE $\delta^{13}\text{C}$ AND $\delta^{15}\text{N}$ WITHIN AND AMONG LAKES**





## 2.1 Introduction

The stable isotopes of carbon and nitrogen are commonly used to study ecological interactions and energy flows in freshwater systems. Carbon stable isotope ratios ( $\delta^{13}\text{C}$ ) are used for tracing the original sources of dietary carbon, as they tend to vary substantially among primary producers, but change slightly with trophic level (Deniro and Epstein 1978). In lakes,  $\delta^{13}\text{C}$  have been used to differentiate sources of energy between littoral or pelagic zones as  $\delta^{13}\text{C}$  values of periphyton (attached algae) and detritus in the littoral zones tend to be more enriched (less negative  $\delta^{13}\text{C}$ ) in comparison with phytoplankton in the water column (France 1995). On the other hand, nitrogen stable isotope ratios ( $\delta^{15}\text{N}$ ) increase in a predictable fashion with each trophic level (3-4‰), indicating the trophic level of consumers within food webs (Post 2002). One of the main advantages of the stable isotope technique is integration of time and space into an organism's trophic ecology (Layman *et al.* 2012).

Differences in carbon and nitrogen sources have been demonstrated to influence  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of primary producers and their consumers in aquatic systems (Cabana and Rasmussen 1996, Schindler *et al.* 1997, France and Cattaneo 1998, Post 2002, Solomon *et al.* 2008). Even though spatial variation of consumer nitrogen stable isotopes occurs within and across lakes, and thereby introduces some uncertainty to what they convey for interpreting food web interactions (Vander Zanden MJ *et al.* 1999, Post 2002), the extent of inherent variation among organisms is usually masked by larger differences among their source material. For example, sewage and fertilizers have distinct  $\delta^{15}\text{N}$  values (Heaton 1986). Nitrate or ammonium based fertilizers show  $\delta^{15}\text{N}$  values in the range -4 to 4‰ since they are derived by industrial fixation of atmospheric nitrogen (Heaton 1986). On the other hand, animal waste and sewage typically show  $\delta^{15}\text{N}$  values higher than +10‰, because of  $^{15}\text{N}$  enriched ammonium and nitrate (Heaton 1986). Recent studies have found an increase of macroinvertebrate  $\delta^{15}\text{N}$  signatures along nutrient enrichment gradients (Harrington *et al.* 1998, Vander Zanden *et al.* 2005, Bergfur *et al.* 2009), suggesting potential use of  $\delta^{15}\text{N}$  signatures as indicators of anthropogenic nutrient sources.

Similar to  $\delta^{15}\text{N}$ , lake-to-lake  $\delta^{13}\text{C}$  differences of macroinvertebrate consumers feeding on the same food sources in different lakes reflect  $\delta^{13}\text{C}$  baseline variation, with different primary producers carrying distinct  $\delta^{13}\text{C}$  signals (France 2000). For example, phytoplankton shows depleted  $\delta^{13}\text{C}$  signatures as a result of sequestering aquatic  $\text{CO}_2$  more rapidly and discriminating against the heavier  $^{13}\text{C}$  (Finlay 2001). In contrast, periphyton display more  $\delta^{13}\text{C}$ -enriched signatures as a result of more restricted nutrient uptake because of a stronger boundary layer effect, especially in more stagnant waters (Finlay 2001). Additionally, the  $\delta^{13}\text{C}$  signatures of the primary producers are majorly affected by the  $\delta^{13}\text{C}$  of dissolved inorganic carbon (DIC), which can vary depending on its origin. For instance, DIC produced by organic matter decomposition can be  $\delta^{13}\text{C}$ -depleted (France 2000), whereas an increase in lake primary production can lead to enrichment of DIC  $\delta^{13}\text{C}$  and consecutively in the entire lake food web (Schindler *et al.* 1997). An increase of periphyton  $\delta^{13}\text{C}$  has been observed with increased productivity associated with nutrient enrichment (Finlay 2001). Moreover, an increase in  $\delta^{13}\text{C}$  values of littoral invertebrates could be linked to elevated exploitation of phytoplankton following nutrient enrichment, suggesting a shift in primary productivity from benthic to pelagic habitats of lakes (Vadeboncoeur *et al.* 2003).

### **2.1.1 Shoreline habitat effects**

Even though multi-lake studies provide useful information on variation of macroinvertebrate elemental signatures across lakes (Vadeboncoeur *et al.* 2003, Donohue 2008, Borderelle *et al.* 2009, Bergfur *et al.* 2009), little is known about small scale variability of stable isotope values of specific macro-invertebrates. However, a study on small scale spatial variation of littoral macroinvertebrate isotopic signatures of Lake Jyväsjärvi in Finland revealed significant differences among seven sampling sites, related mainly to lake-specific characteristics such as harbours and major inlets (Syvaranta *et al.* 2006).

Littoral macroinvertebrates rely on both allochthonous (*e.g.* leaf litter and woody debris) and autochthonous (*e.g.* benthic and planktonic algae, macrophytes) energy resources with their characteristic carbon isotope signatures, and of potential availability to littoral macroinvertebrates in variable quantities depending on the nature of littoral and shoreline habitats (Schindler and Scheuerell 2002). Modification of shoreline habitats, associated with changes in



land use practices, can influence the littoral food webs by altering the physical structure and diversity of habitats (Brauns *et al.* 2011, Jurca 2012). For example, clear cutting of riparian vegetation can result in a reduction of habitats provided by coarse woody debris and submerged tree roots (Schindler and Scheuerell 2002, Brauns *et al.* 2011). Decline of littoral habitat heterogeneity associated with shoreline modifications can affect the quantity and diversity of food sources available to littoral macroinvertebrates (Schindler and Scheuerell 2002). Cole *et al.* (2006) showed that allochthonous inputs were important food sources for the entire lake food web, particularly in oligotrophic lakes. In contrast, Brauns *et al.* (2011) found a lower contribution of terrestrial coarse particulate organic matter (CPOM) to the trophic basis and littoral food webs in modified “developed” in comparison with undeveloped shorelines in three German lowland lakes. The extent that shoreline modification affects the carbon and nitrogen dynamics of littoral foodwebs likely varies with both the extent of physical modification and lake nutrient state.

In Ireland, two common riparian and shoreline habitat features exist: semi- natural woodlands and managed grasslands. Woodland shoreline habitats are characterised by the presence of trees and shrubs while grass and herbs are the common vegetation cover at grassland shorelines, which are maintained by livestock, normally cattle, grazing. It is expected that differences in riparian habitat will affect the ecology in the adjacent water body, that can be expected to manifest in different energy (carbon) and nutrient (phosphorus and nitrogen) inputs to the littoral zone. Examples of such differences between grassland and woodland dominated shorelines would be a lower buffering of nutrient flow across grass habitats compared with woodland, less allochthonous carbon from grassland than woodland habitats, increased irradiance (Reed *et al.* 1994) in littoral zones adjacent to grassland than woodland, and less complex habitat (*e.g.* from tree roots (Brauns *et al.* 2011)) in sites bordered by grasslands compared with woodland. These factors would likely influence the structure and function of littoral macro-invertebrate communities.

### **2.1.2 Ontogenetic diet shifts**

Ontogenetic dietary shifts can be detected in the stable isotope signals among individuals of the same species, related by, for example; metabolism linked to



body size, availability of food sources linked to mouth size, and speed of movement (Doi *et al.* 2010). Size dependent predation and cannibalism has been documented for various *Gammarus spp.* (Dick *et al.* 1993, Macneil *et al.* 1997). A shift in the species' trophic position and size related changes in  $\delta^{15}\text{N}$  signature has been detected in various crustacean zooplankton species (Matthews and Mazumder 2008). Progressive enrichment of the  $\delta^{15}\text{N}$  of opossum shrimp, *Mysis relicta*, was linked to increasing body size, indicating a shift from a diet similar to the bulk zooplankton to a more carnivorous one (Branstrator *et al.* 2000). Moreover, smaller individuals of a species may prefer different microhabitats to ones occupied by larger individuals in order to avoid inter- or intra-specific predation (McGrath *et al.* 2007). Such size-dependent feeding in different habitats can also lead to variation in the  $\delta^{13}\text{C}$  signature of individuals in the same population, as in the dipteran *Chaoborus spp.* (Persaud and Dillon 2009) and deposit-feeding polychaetes (Hientschel 1998).

### 2.1.3 Chapter objectives

In this study, the effects of the two distinct types of shoreline habitats (woodland and grassland) on the isotopic variation of three common littoral macrocrustacean species, *Asellus aquaticus*, *Gammarus duebeni celticus* and *Crangonyx pseudogracilis*, across eight Irish lakes were examined along a nutrient enrichment gradient. In a recent study, these three species have been recorded together in seven out of eight lakes across Ireland (Jurca 2012). Their competitive niche overlap across three lakes (all three species in Lough Brackley, only *G. d. celticus* and *A. aquaticus* in Loughs Fern and Muckno) is explored in Chapter 3 of this study. *Asellus aquaticus* is a widely distributed freshwater isopod species, native to Ireland. *Gammarus duebeni celticus* (referred as *G. duebeni* in rest of chapter) is an endemic amphipod species in Ireland, whose distribution has declined with the arrival of other amphipod species, primarily *G. pulex*, in the last century (Strange and Glass 1979). *Crangonyx pseudogracilis* is a non-native amphipod species that arrived in Ireland in mid 1950s from northeast America (Costello 1993). Both biotic (*e.g.* predation and parasitism (MacNeil *et al.* 2003)) and abiotic factors (*e.g.* physico-chemical conditions (Holland 1976, Macneil *et al.* 2000, MacNeil *et al.* 2002)) have been suggested to influence the distribution of these species.

The specific objectives of this study were: 1) to explore the general response of species'  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic signatures to nutrient status; 2) to assess differences of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic ratios of species between sites adjacent to woodland and grassland shoreline habitats across lakes with a nutrient enrichment gradient; and 3) to evaluate the effect of species-specific traits of body size on the variation of species'  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures while accounting for the environmental variation across lakes.

I hypothesise that  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of macroinvertebrates: 1) vary along a nutrient enrichment gradient; 2) reflect differences in riparian habitats within lakes; and 3) vary with body-size.

## 2.2 Methods

### 2.2.1 Study lakes

Loughs Akibbon, Carrigeencor, Kiltorris, Fern, Brackley, Muckno, Scur and Sillan (Figure 2.1) were used to test effects of environmental gradients and habitat types on species' carbon and nitrogen stable isotopes.

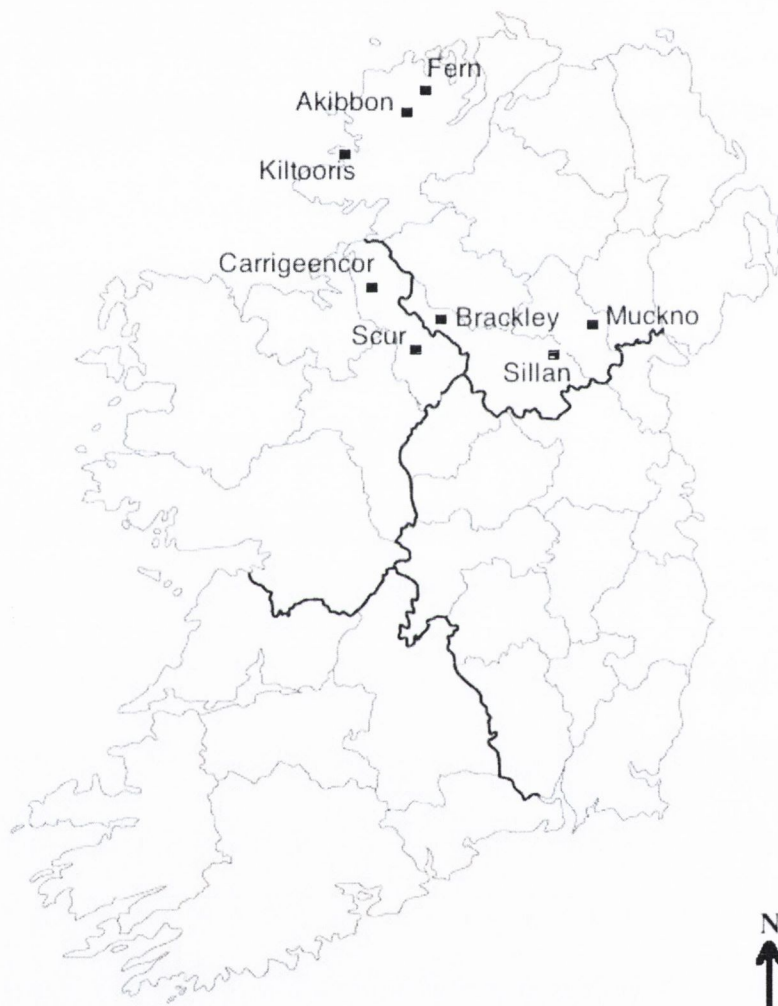


Figure 2.1 Locations of the studied lakes on the map of Ireland. (Map source: Russell (2010)).

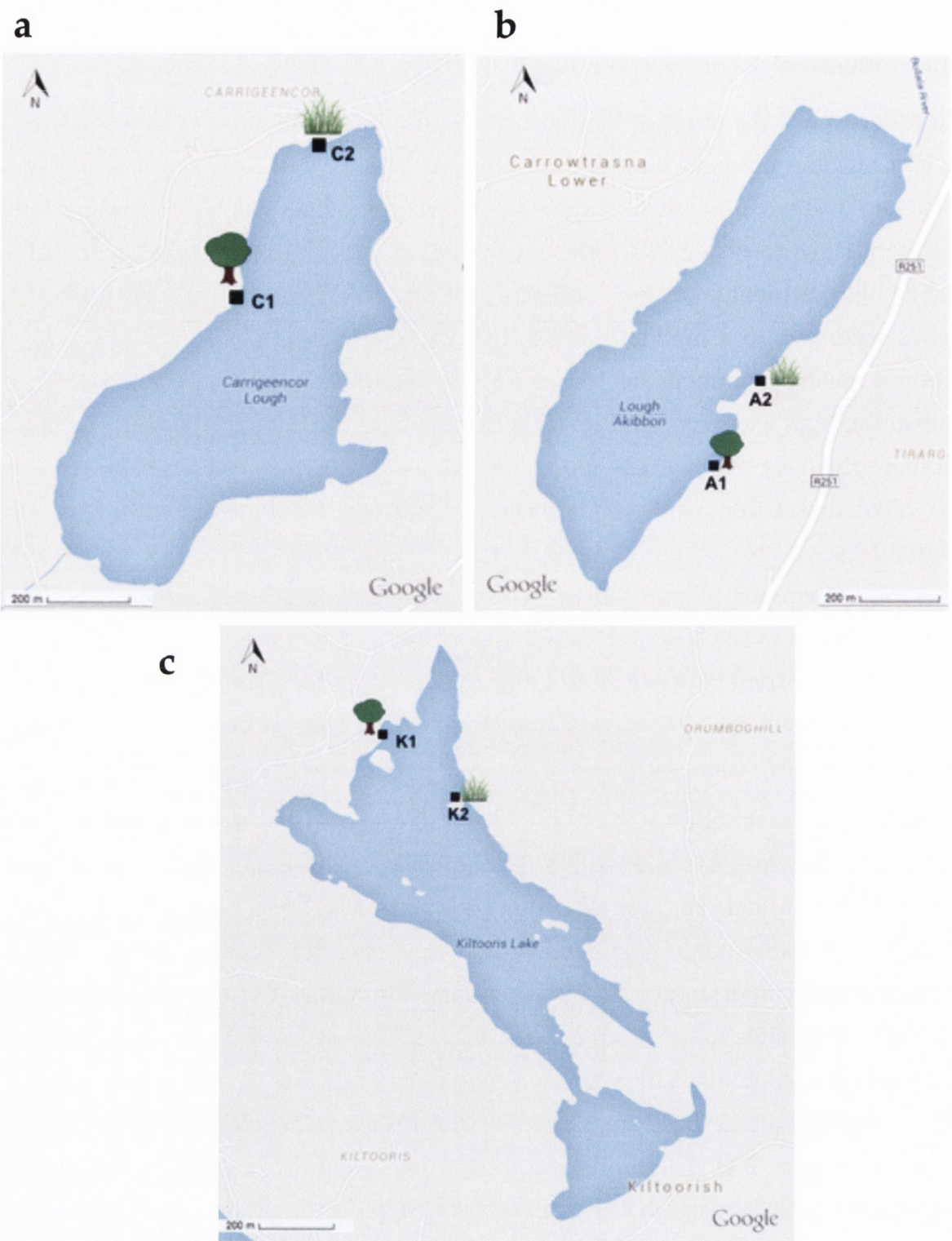
Lake selection took account of historical total phosphorus (TP) data and satellite images of riparian habitats. Since invasive zebra mussel (*Dreissena polymorpha*) can strongly influence the benthic community structure and functioning as well as the carbon and nitrogen isotope values of amphipods (Gergs *et al.* 2011), no lakes with visible *D. polymorpha* colonies were included in the study. Loughs Fern, Brackley



and Muckno, lying within a medium TP range ( $35\text{--}109\ \mu\text{g l}^{-1}$ ) had been sampled more intensively in order to conduct the studies presented in Chapters 3 and 4.

In order to test the effect of habitat types on invertebrate stable isotopes across a wider nutrient gradient range, five additional lakes to Loughs Fern, Brackley and Muckno were chosen from the lower and higher ends of the TP range. Low nutrient lakes consisted of Carrigeencor, Akibbon and Kiltorris (TP:  $23\text{--}36\ \mu\text{g l}^{-1}$ ), while high nutrient lakes included Scur and Sillan (TP:  $165\text{--}329\ \mu\text{g l}^{-1}$ ). All studied lakes were within a moderate alkalinity range ( $48\text{--}61\ \text{mg CaCO}_3\ \text{l}^{-1}$ ) (Table 2.1). Despite being common in Ireland, marl lakes, characterised by calcareous substrata, high alkalinity, and low colour, were not included in the study because of their distinctly negative effect on lake DIC  $\delta^{13}\text{C}$  isotopic signatures (Borderelle *et al.* 2008) and subsequently, on primary producer and macroinvertebrate  $\delta^{13}\text{C}$  signatures. Also, alkaline lakes ( $> 100\ \text{mg CaCO}_3\ \text{l}^{-1}$ ) have distinct macroinvertebrate communities (Donohue 2008). Total nitrogen (TN) concentrations in the lakes that were studied ranged between  $0.4\text{--}1.5\ \text{mg l}^{-1}$ , while water colour varied between  $59\text{--}422\ \text{mg l}^{-1}\ \text{PtCo}$  (Table 2.1). The highest percentage of the catchment land cover of a majority of the lakes consisted of pasture and agricultural land (Table 2.2).

Sampling of invertebrates and associated environmental features took place between late August and early October 2009. Shoreline habitat type and accessibility influenced site selection within each lake. Sampling locations included sites adjacent to woodland or grassland shoreline habitats. Stretches with visible human interference at the woodland shorelines (*e.g.* picnic sites) were avoided. Two sites (one woodland and one grassland) were sampled in Loughs Carrigeencor, Akibbon, Kiltorris, Scur and Sillan (Figures 2.2–2.3) whereas six sites (three woodland and three grassland) were sampled in Loughs Fern, Brackley and Muckno (Figure 2.4). This unbalanced sampling protocol reflected the logistical constraints of the project that this study was funded through, but was considered legitimate as it expanded the number of lakes across the nutrient gradient as described above. As such it provides finer scale information on three of the lakes, and coarser scale information on the spatial variability within and among all 8 lakes. The statistical analysis using linear mixed models (see below) accounted for this unbalanced, and nested-measurements study design.

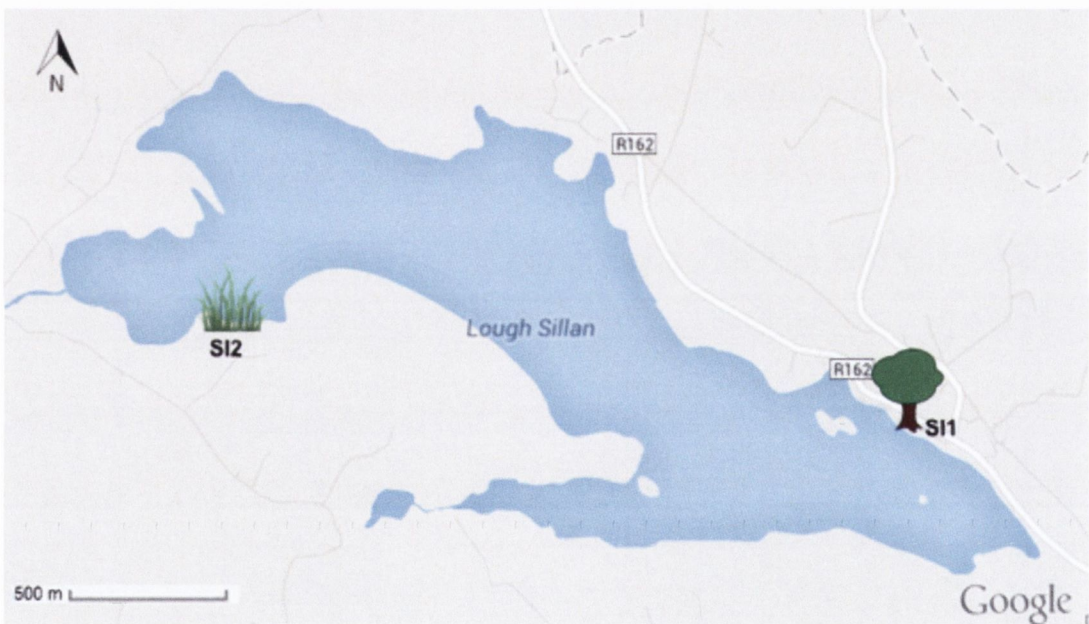




a



b





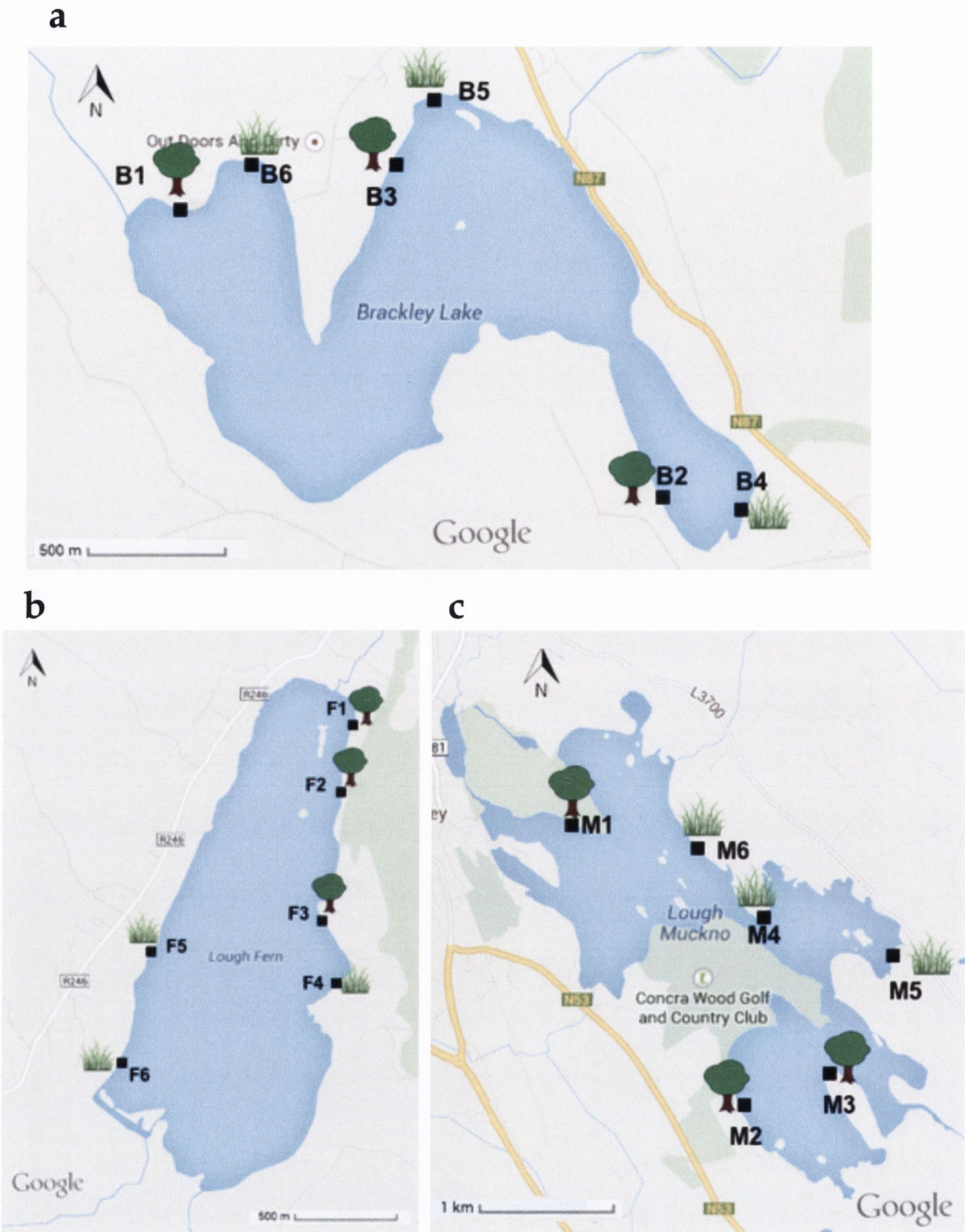


Figure 2.4 Locations of sampling sites in Lough Brackley, Co. Cavan (a), Lough Fern, Co. Donegal (b) and Lough Muckno, Co. Monaghan (c). Symbols of tree and grass represent the woodland and grassland shoreline habitats, respectively. (Map Source: Google 2014).

Table 2.1 Water chemistry variables (mean  $\pm$  SE) of the eight study loughs, measured between late August and early October 2009 and used for the PCA (see section 2.2.5.1). Number of sites (n) per shoreline habitat was 3 for Loughs Fern, Brackley and Muckno, while water chemistry values for the rest of the lakes were based on single site measurements for each shoreline habitat type (Shoreline Habitats = **W**: Woodland and **G**: Grassland).

| Lake         | Shoreline Habitat | Alkalinity<br>(mg $\text{CaCO}_3 \text{ l}^{-1}$ ) | TP<br>( $\mu\text{g l}^{-1}$ ) | TN<br>(mg $\text{l}^{-1}$ ) | Chl- <i>a</i><br>( $\mu\text{g l}^{-1}$ ) | TOC<br>(mg $\text{l}^{-1}$ ) | Colour<br>(mg $\text{l}^{-1}$ PtCo) |
|--------------|-------------------|----------------------------------------------------|--------------------------------|-----------------------------|-------------------------------------------|------------------------------|-------------------------------------|
| Carrigeencor | W                 | 48.9                                               | 19.1                           | 0.37                        | 12.9                                      | 6.4                          | 158                                 |
|              | G                 | 47.9                                               | 26.7                           | 0.45                        | 4.6                                       | 6.8                          | 158                                 |
| Akibbon      | W                 | 37.7                                               | 24.7                           | 0.83                        | 4.2                                       | 10.6                         | 216                                 |
|              | G                 | 39.5                                               | 23.3                           | 0.94                        | 4.0                                       | 9.1                          | 211                                 |
| Kiltooris    | W                 | 30.4                                               | 8.5                            | 0.79                        | 2.0                                       | 8.6                          | 92                                  |
|              | G                 | 29.0                                               | 64.1                           | 0.72                        | 1.1                                       | 9.7                          | 96                                  |
| Fern         | W                 | 28.6 $\pm$ 0.2                                     | 37.7 $\pm$ 5.6                 | 1.03 $\pm$ 0.11             | 4.9 $\pm$ 1.0                             | 11.3 $\pm$ 0.3               | 282 $\pm$ 2                         |
|              | G                 | 30.1 $\pm$ 1.2                                     | 32.7 $\pm$ 2.0                 | 0.94 $\pm$ 0.02             | 3.1 $\pm$ 0.4                             | 10.8 $\pm$ 0.1               | 274 $\pm$ 6                         |
| Brackley     | W                 | 48.5 $\pm$ 1.3                                     | 51.3 $\pm$ 8.2                 | 1.29 $\pm$ 0.09             | 19.1 $\pm$ 1.8                            | 14.2 $\pm$ 0.1               | 228 $\pm$ 6                         |
|              | G                 | 57.9 $\pm$ 10.3                                    | 66.9 $\pm$ 25.2                | 1.34 $\pm$ 0.02             | 13.4 $\pm$ 2.0                            | 14.2 $\pm$ 0.9               | 126 $\pm$ 3                         |
| Muckno       | W                 | 60.8 $\pm$ 6.2                                     | 100.0 $\pm$ 4.1                | 1.38 $\pm$ 0.04             | 20.8 $\pm$ 7.7                            | 14.8 $\pm$ 3.3               | 132 $\pm$ 27                        |
|              | G                 | 58.3 $\pm$ 5.7                                     | 117.8 $\pm$ 14.5               | 1.54 $\pm$ 0.10             | 20.2 $\pm$ 9.1                            | 13.0 $\pm$ 0.6               | 158 $\pm$ 14                        |
| Scur         | W                 | 44.6                                               | 90.3                           | 1.4                         | 12.9                                      | 19.5                         | 454                                 |
|              | G                 | 61.6                                               | 568.0                          | 1.5                         | 11.4                                      | 18.2                         | 391                                 |
| Sillan       | W                 | 60.7                                               | 223.5                          | 1.7                         | 81.9                                      | 8.3                          | 62                                  |
|              | G                 | 60.9                                               | 106.9                          | 1.0                         | 17.5                                      | 8.6                          | 70                                  |

Table 2.2 Physical characteristics of the eight study loughs.

| Lake                              | Carrigeencor | Akibbon | Kiltooris | Fern   | Brackley | Muckno | Scur  | Sillan |
|-----------------------------------|--------------|---------|-----------|--------|----------|--------|-------|--------|
| Altitude (m)                      | 45           | 79      | 7         | 20     | 58       | 86     | 62    | 94     |
| Lake Area (km <sup>2</sup> )      | 0.44         | 0.44    | 0.43      | 1.81   | 1.67     | 3.57   | 1.14  | 1.62   |
| Catchment Area (km <sup>2</sup> ) | 2.63         | 8.06    | 5.55      | 206.17 | 17.43    | 109.68 | 62.87 | 51.26  |
| Forest                            | 0            | 3       | 0         | 2      | 10       | 1      | 6     | 0      |
| Pasture                           | 41           | 0       | 51        | 18     | 49       | 5      | 36    | 17     |
| Land Cover (%) Agriculture        | 1            | 40      | 0         | 23     | 6        | 84     | 17    | 77     |
| Peat                              | 15           | 45      | 0         | 35     | 9        | 2      | 23    | 0      |
| Other                             | 43           | 12      | 49        | 22     | 26       | 8      | 18    | 6      |



### **2.2.2 Water and macroinvertebrate sampling**

Water samples were collected from every site of each lake using a weighted plastic 5 l bottle attached to a 10 m long rope, before invertebrate sampling took place. Filtered water samples for the analysis of total organic carbon (TOC) were transferred into amber glass vials and digested with a few drops of HCl. Also, unfiltered water samples were transferred into amber glass vials and autoclavable glass bottles for the analyses of total nitrogen (TN) and total phosphorus (TP), respectively. Three 0.5 l water samples were filtered through Whatman® GF/C filter paper after collection with a portable vacuum filtration system and stored in polypropylene centrifuge tubes with 10 ml methanol and kept in darkened cool boxes for later chlorophyll-*a* (Chl-*a*) analysis. Site-specific composite water samples (in 5 l bottles) were kept chilled in cool boxes until returned to laboratory and stored in a dark fridge (-4°C) for maximum five days prior to analysis for pH, conductivity, alkalinity, and colour.

Macroinvertebrate samples for stable isotope analysis (SIA) were collected from the littoral zones using kick sampling with a standard hand net (mesh size 0.5). At each site, a maximum of three macroinvertebrate samples were collected when different habitat patches existed. If inspection of the littoral zone indicated a homogeneous habitat, usually one and sometimes two samples (one from near and another away from the shore, minimum 5 m apart) were collected. Invertebrate samples were put into clean plastic bottles, after draining the water using a metal sieve (500 µm). Samples were frozen upon collection dry ice, transferred to a freezer on arrival back to the laboratory and kept frozen at -40°C until processing for SIA, following Donohue (2008).

### **2.2.3 Water chemistry analysis**

Water samples were analysed for alkalinity (Mackereth *et al.* 1989), colour, total phosphorus (TP) (Eisenreich *et al.* 1975), total nitrogen (TN), chlorophyll-*a* (Standing Committee Of Analysts 1983) and non-dissolved total organic carbon (TOC) measures after returned back to the laboratory in the end of the field sampling. Colour was measured with a Hach® DR2000 spectrophotometer after filtration through Whatman® GF/C filter paper. TOC and TN were measured in a Bran & Luebbes continuous flow AutoAnalyzer 3. All water chemistry analyses

took place in the Centre for the Environment, The University of Dublin, Trinity College.

#### **2.2.4 Stable Isotope Analysis (SIA)**

In the laboratory, frozen macroinvertebrates samples were thawed to room temperature before being sorted in a white tray with distilled water. A stereomicroscope (Olympus SZX12) was used for the taxonomical identification of macroinvertebrates. Isopod and amphipod samples were separated into different petri dishes before the identification of taxa, which was based on Gledhill et al. (1993). Body length (from base of first antenna to base of telson) was estimated with a metric ruler at x6.3 magnification in the stereomicroscope. In an earlier study, the eggs and embryos of gammarids were reported to have higher contents of lipid and fatty acid (Sutcliffe 1992), which can be depleted in  $\delta^{13}\text{C}$  when compared with organism's whole body (Peterson and Fry 1987). Therefore, developing embryos and juveniles in the brood pouch of gravid females of all taxa were emptied during microscopic examination.

Minimum adequate mass for the carbon and nitrogen SIA of individual macroinvertebrates, which was determined through test SIA runs, was used to estimate species-specific minimum lengths. Individuals above the minimum lengths were laid into clean petri dishes that were divided into cells with a given number. A random sequencing website (Haahr 2011) was used to select a minimum of six individuals of either sex from each littoral habitat sample for SIA. All animals were dried at 60°C for 24 h in clean petri dishes in a dehydrator. Samples not processed immediately were kept at 4°C for up to 2-3 h. Individual animals were ground into a fine powder using an agate pestle and mortar. Subsamples of 0.6-0.9 mg tissue of individual macroinvertebrate samples were weighted into tin capsules using a microbalance (Sartorius MC-5 with 1 µg readability), folded into tin balls and stored in a desiccator until analysis.

Carbon and nitrogen stable isotope analyses for all samples were performed on Thermo Delta<sup>plus</sup>® CF-IRMS (continuous flow isotopic ratio mass spectrometer) coupled to the CE Instruments 1112 Flash Elemental Analyser by Conflo III Interface, which was operated by ISODAT software and based in the Geochemistry Laboratory at Trinity College Dublin (Dublin, Ireland). Initial carbon and nitrogen isotope ratios were recorded relative to primary standards



that were Pee Dee Belemnite (PDB) and atmospheric air (VAIR), respectively. Isotope values were expressed as relative difference per mil (‰) and in the standard delta ( $\delta$ ) notation:

$$\delta X = \left( (R_{\text{sample}}/R_{\text{standard}}) - 1 \right) \times 100 ; \text{ where } X = {}^{13}\text{C} \text{ or } {}^{15}\text{N}, \text{ and } R = {}^{13}\text{C}/{}^{12}\text{C} \text{ or } {}^{15}\text{N}/{}^{14}\text{N}.$$

Average standard deviations for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  were recorded as  $\pm 0.130\text{‰}$  and  $\pm 0.085\text{‰}$ , respectively (n=523). L-Alanine ( $\delta^{15}\text{N} = -7.767\text{‰}$ ,  $\delta^{13}\text{C} = -25.119\text{‰}$ ) was used as the working standard that was used to calibrate the data to the PDB scale.

### 2.2.5 Statistical analyses

All statistical analysis was performed in R version 3.0.2 (R Development Core Team 2013). Before performing statistical analysis, all lake chemistry variables (TP, TN, alkalinity, colour, Chl-*a* and TOC) were normalised using log transformation in order to meet parametric requirements of the statistical models.

#### 2.2.5.1 Principal Components Analysis (PCA)

As collinearity (correlation between covariates) among lake chemistry variables can affect performance of mixed-effects models (Zuur *et al.* 2010), principal component analysis (PCA) was used to assess the collinearity of the water chemistry variables and characterise the main environmental gradients; transforming correlated variables into a lower number of uncorrelated 'principal component' (PC) axes. Six chemistry variables (alkalinity, colour, TP, TN, TOC and Chl-*a*) were used in the PCA.

Since the chemistry variables were measured on different scale and units, data were scaled to unit variance before performing PCA. Only PC axes with eigenvalues greater than 1 were retained (Dillon and Goldstein 1984), with scores used as fixed explanatory factors in the mixed-effects models. Correlation of the water chemistry variables with each PC axis was determined by examining the loadings of the retained PC axes, used to define the environmental gradients. The cut-point for the PCA loadings was defined as 0.4. Therefore loadings  $> 0.4$  were considered as relatively 'high loadings', while loadings  $< 0.4$  were not interpreted. The R package 'FactoMineR' (Husson *et al.* 2013) was used to perform the PCA



and to visually illustrate the relationship among different chemistry variables and samples collected at each site across the eight lakes.

### 2.2.5.2 Linear mixed-effects models

Linear mixed-effects models to test hypotheses fitted using the “lmer” function based on the ‘lmerTest’ package in R (Kuznetsova *et al.* 2013, R Development Core Team 2013). Mixed effects models account for the spatial grouping of data at lake scale (Bolker *et al.* 2009). Scores of the first and the second principal components (PC1 and PC2) were included as fixed explanatory variables to the mixed models, together with ‘lake’ as a random effect in order to account for among lake variation while testing the effects of habitat types and size of species on within-lake variability of isotope signatures. Akaike’s Information Criterion (AIC) corrected for small sample size (AICc) was used for model selection as recommended by Burnham & Anderson (2004) when  $n/K < 40$ ; where  $n$  and  $K$  represent sample size and number of parameters in a model, respectively. The calculation of AICc was based on the ‘MuMIn’ package in R (Bartoń 2013).

Carbon and nitrogen isotope data of different macroinvertebrate species were analysed in separate models, with lake as the only random effect. Full models of *A. aquaticus* and *G. duebeni* comprised five main explanatory variables as fixed effects and a selection of their interaction terms (Table 2.3). Models of all three species included PC1 and PC2, and invertebrate “body length” (BL) as continuous explanatory variables, and “shoreline habitat type” (SHab; 2 levels: woodland or grassland) as the only categorical explanatory variable. In order to test the shoreline habitat type correctly, a lake was included in the model only if data for at least three individuals of a species were present for both the woodland and grassland shoreline habitats types. Therefore, despite initially being sampled, a ninth lake within the high nutrient range (Lough Rinn), with distinct composition of the three study species between sites adjacent to woodland and grassland shorelines, was excluded in the final study.

Table 2.3 List of all fixed (main and two-way interaction) variables included in the full linear mixed-effects models.

| Main fixed variables     | Two –way interaction variables |
|--------------------------|--------------------------------|
| PC1                      | PC1 x SHab                     |
| PC2                      | PC2 x SHab                     |
| Body Length (BL)         |                                |
| Shoreline habitat (SHab) |                                |

Models were fit with maximum likelihood (ML) estimation that can accommodate models with different fixed-effects structures (Crawley 2007). After the full model was fitted to the data, backward and forward selection was used to identify the minimal adequate model, which is the most parsimonious model with the lowest *AICc* values (Johnson and Omland 2004). Model simplification process included elimination of one fixed explanatory variable at a time, starting from the least significant interaction term (Crawley 2007). The *AICc* values of the original and the reduced models were compared in order to justify the elimination of the non-significant term. An explanatory variable is added back to the model, if the reduced model produced higher *AICc* values than the original model. This was followed until the most parsimonious (final or the minimal adequate) model was reached (Crawley 2007).

Significance of the fixed effects was evaluated using the *p*-values that were generated using the *anova* function in the ‘lmerTest’ R package (Kuznetsova *et al.* 2013), which calculated F statistics of type III hypothesis based on Satterthwaite’s approximation for denominator degrees of freedom (SAS Institute Inc. 1978). Type III hypothesis is sample size independent and suitable for unbalanced data as it allows examination of the significance of the effect of a variable in the model after all other factors have been accounted for (Langsrud 2003). The *summary* function ‘lmerTest’ package (Kuznetsova *et al.* 2013) also provided model summaries, as in the more common ‘lme4’ package (Bates *et al.* 2011), but with *p*-values added for the t-tests on the fixed factors based on Satterthwaite’s approximation (Kuznetsova *et al.* 2013). In the final model, fixed factors with *p*-values < 0.05 were considered significant. Model simplification steps for the carbon and nitrogen



isotope data of *A. aquaticus*, *G. duebeni* and *C. pseudogracilis*, with the corresponding *AICc* values are presented separately in Appendix I.

Distributions of species' isotope values against PC and body size gradients were illustrated using scatterplots and the fitted linear mixed effect models (*lme*) were plotted using linear lines over the original data throughout the chapter (Figure 2.10). Different plotting symbols were used for different lakes. When the shoreline habitat type factor or its interaction term remained in the minimal model, its two levels (woodland and grassland) were plotted in two different colours and separate linear lines with corresponding colours were fitted for each shoreline habitat type.

Sometimes, fitting the *lme* models over the scatter plot of the original data resulted in a visual mismatch, especially when illustrating the PC and shoreline habitat type interactions. This can be mainly attributed to the lake random effect fitted in the *lme* models and to a lesser extent to the distribution of the body size among lakes. In order to explain this further, two distinct approaches to plotting are discussed using the example of the  $\delta^{15}\text{N}$  model of *C. pseudogracilis*.

While Figure 2.5a uses *lme*, which estimates variance contributed by the lake random effect and the residuals, Figure 2.5b displays the same data fitted with a generalised linear model (*glm*). Even though the *glm* model presents a visually better fit, it does not take the correlation among data points within a lake account. This would be a statistical mistake, as the isotopic data points are not independent but are nested within lakes (Figure 2.6) and can lead to estimation of linear effects with entirely the wrong sign. Figure 2.5c demonstrates a third approach to plotting the data, which uses transformed isotopic data, obtained by subtracting the lake mean values from each isotopic value which is an approximation to the process of including a random effect in the generalised linear model framework. This approach allows a better visual fit of the *lme* model results by removing the lake effect in a somewhat ad hoc manner. Although this approach is mathematically naïve, the calculations carried out within the random-effects models are mathematically robust. Transformed plots of all the plots with original data presented throughout the chapter are illustrated in Appendix II.



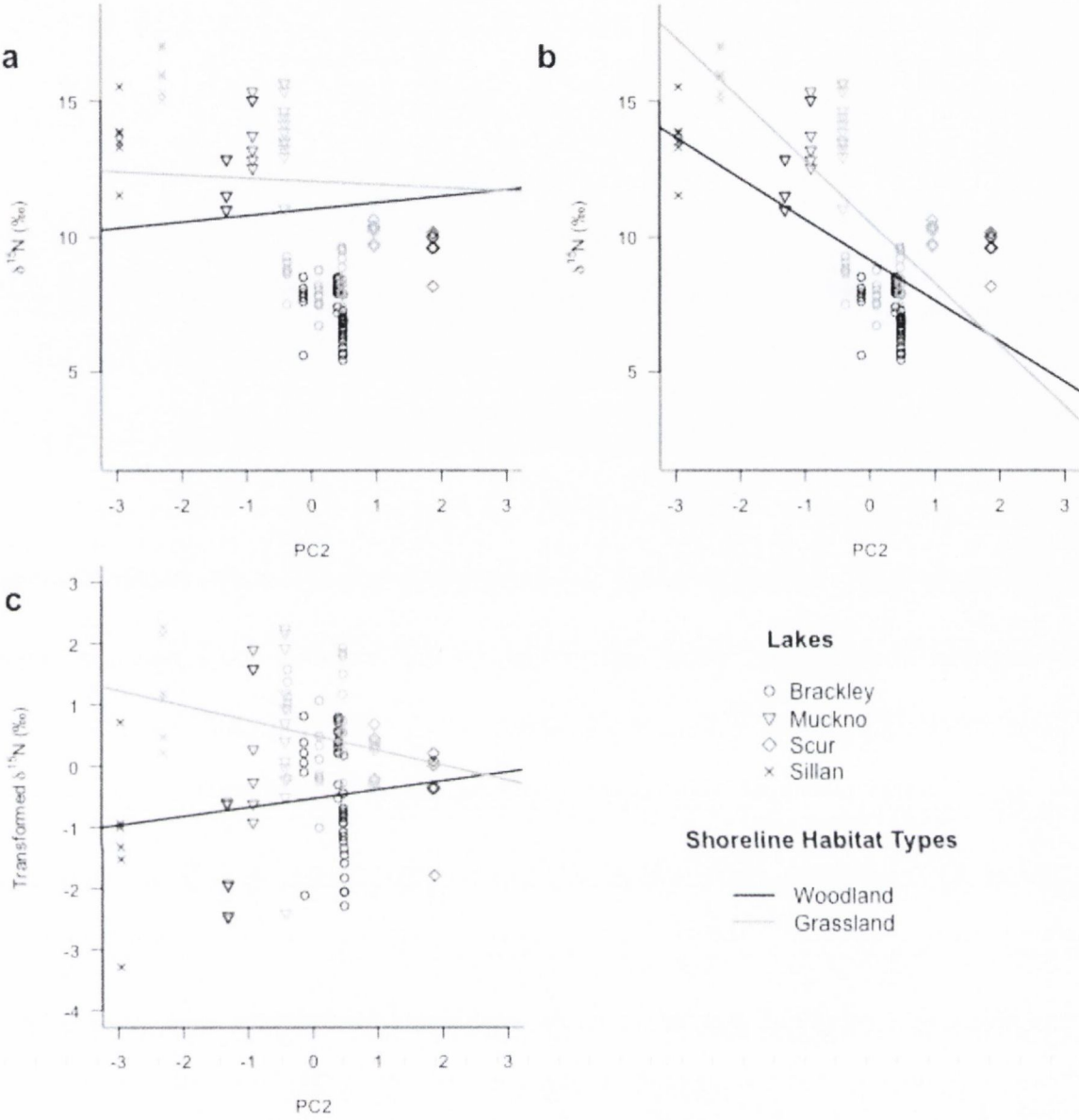


Figure 2.5 Example plots illustrating the distribution of *C. pseudogracilis*  $\delta^{15}\text{N}$  signatures based on **a)** a linear mixed effects model (*lme*) with original data; **b)** a generalised linear model (*glm*) with original data and **c)** *lme* with transformed data, where lake effect is taken into account. Linear lines visualise the model results of different responses of the two shoreline habitat types to the PC2 gradient.

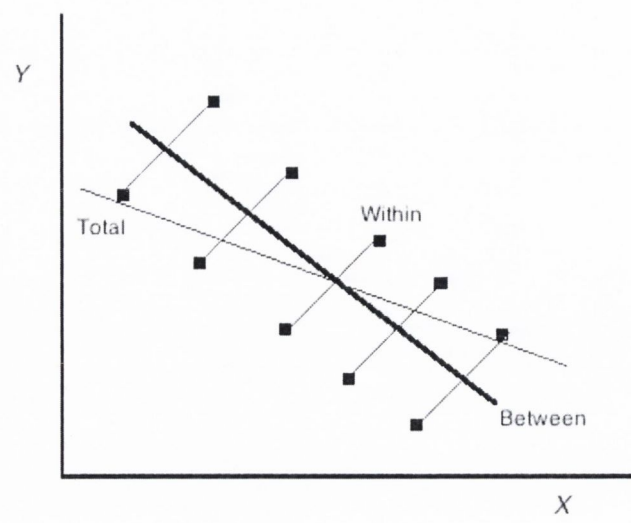


Figure 2.6 Example graph displaying the within, between and total relations among X and Y variables (Snijders and Bosker 2004). The within group effect is actually positive, but we would fail to identify this if we ignored the nesting structure of the data and simply used an ordinary linear model across all the data which would yield a negative slope corresponding to the “total” line. Indeed, such a naïve approach would also underestimate the magnitude of the between-group negative relationship.

As an example, the final  $\delta^{15}\text{N}$  model of *C. pseudogracilis* included the ‘PC2’ and ‘body length’ as the continuous variables, ‘shoreline habitat type’ as the categorical variable with two levels (woodland and grassland, among which the second was set as the reference level) and a significant ‘PC2 x shoreline habitat type’ interaction term (Figure 2.5a). For the simplicity of plotting, when modelling  $\delta^{15}\text{N}$  signatures as a function of PC2 and shoreline habitat type, the intercepts for lines were adjusted using the mean values of the body size, the other significant continuous measure in the minimal model. Based on the estimated coefficients of the final *C. pseudogracilis*  $\delta^{15}\text{N}$  model (Table 2.4), the intercepts and the slopes of the lines for the grassland (G, the reference factor) and the woodland (W) shoreline habitat type (SHab) factors against the PC2 axis were estimated as follows:

|               |                                                  |
|---------------|--------------------------------------------------|
| Intercept (G) | $= A + (D \times \text{mean (Body Length)})$     |
| Intercept (W) | $= A + C + (D \times \text{mean (Body Length)})$ |
| Slope (G)     | $= B$                                            |
| Slope (W)     | $= B + E$                                        |

Table 2.4 Example results of the linear mixed effects model with  $\delta^{15}\text{N}$  isotope values of *C. pseudogracilis* (see Table 2.17 for the full results table) to illustrate the interpretation of the estimated coefficients.

|                   | Estimated Coeff. | Interpretation                                                             |
|-------------------|------------------|----------------------------------------------------------------------------|
| Intercept         | A                | Intercept for Grassland SHab factor                                        |
| PC2               | B                | Slope of $\delta^{15}\text{N}$ with PC2 for the Grassland SHab factor      |
| SHab: W (G)       | C                | Difference between two intercepts of SHab factors (Woodland and Grassland) |
| Body Length       | D                | Slope of $\delta^{15}\text{N}$ with Body Length                            |
| PC2 x SHab: W (G) | E                | Difference between two slopes of the SHab factors                          |



## 2.3 Results

### 2.3.1 PCA results

Two principal component (PC) axes, with eigenvalues greater than one, cumulatively explained 79.2% of the total variation of the six lake chemistry variables collected from each study site (Dillon and Goldstein 1984). The first and the second PC axes (PC1 and PC2) explained 53.8% and 29.0% of the total variation in variables, respectively (Table 2.5).

Table 2.5 Loadings of the variables used in the Principal Components Analysis (PCA) presented together with the eigenvalues, % variance and cumulative % variance explained by the first two principal component (PC) axes which had eigenvalues > 1. Data in bold indicates relatively high loadings (> 0.4) contributing primarily to the corresponding PC axes.

|                                        | PC axis 1   | PC axis 2   |
|----------------------------------------|-------------|-------------|
| Eigenvalue ( $\lambda$ )               | 3.2         | 1.7         |
| % Variance explained                   | 53.8        | 29.0        |
| Cumulative % variance explained        | 53.8        | 82.7        |
| Alkalinity                             | 0.39        | -0.42       |
| Colour                                 | 0.16        | <b>0.69</b> |
| Total Phosphorus (TP)                  | <b>0.47</b> | -0.12       |
| Total Nitrogen (TN)                    | <b>0.49</b> | 0.18        |
| Chlorophyll- <i>a</i> (Chl- <i>a</i> ) | <b>0.43</b> | -0.35       |
| TOC                                    | 0.42        | 0.42        |

Loadings of total phosphorus (TP), total nitrogen (TN) and Chl-*a* were relatively high in PC1, while PC2 was highly positively correlated with colour (Figure 2.7 and Table 2.5). Therefore, nutrient status and colour were identified as the primary drivers of PC1 and PC2, respectively. Because of equally high loadings of total dissolved organic carbon (TOC) in PC1 and PC2, its representation by any single PC axis was not discernible. Distributions of lake sampling sites along these environmental gradients demonstrated that lough Carrigeencor, Akibbon, Kiltorris and Fern, with low PC1 scores ( $< 0$ ), were placed on left half of the PCA bi-plot, confirming relatively low nutrient levels measured in these lakes (TP: 23–36  $\mu\text{g l}^{-1}$ ) (Figure 2.8). On the other hand, a wide PC2 range was observed at both sides of the PC1 axis, indicating a colour driven gradient across lakes (Figure 2.8).

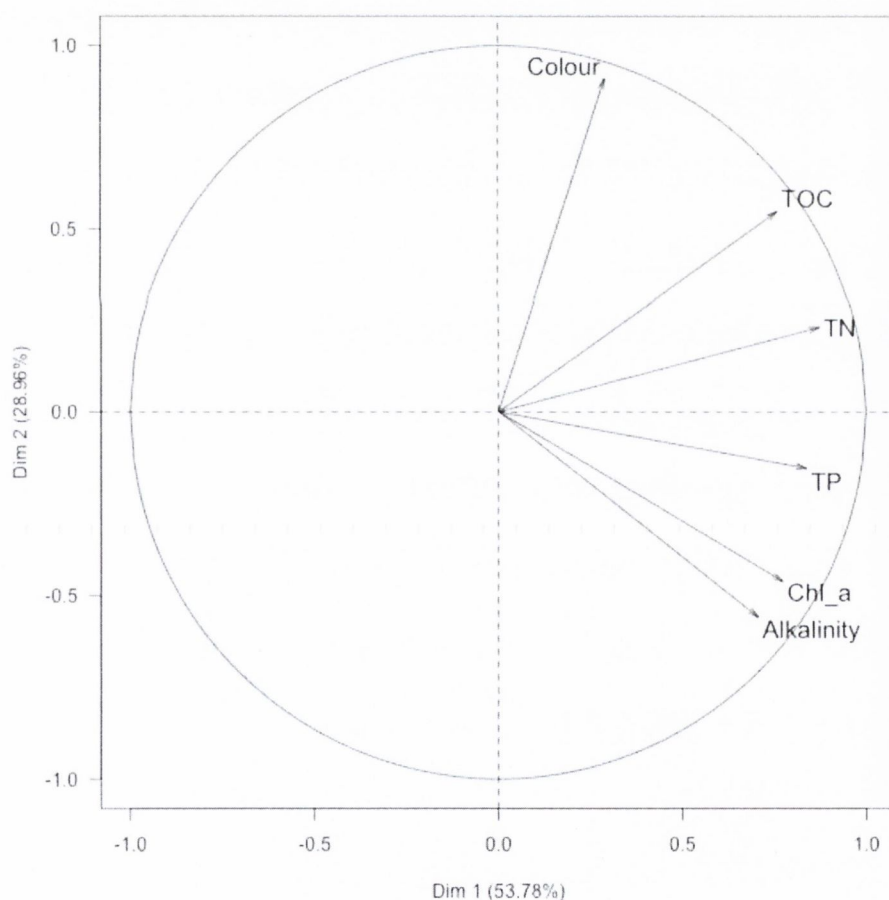


Figure 2.7 The relationship between lake chemistry variables and the principal component analysis (PCA). The first and second PC axes explain 53.7% and 29.0% of the total variation, respectively.

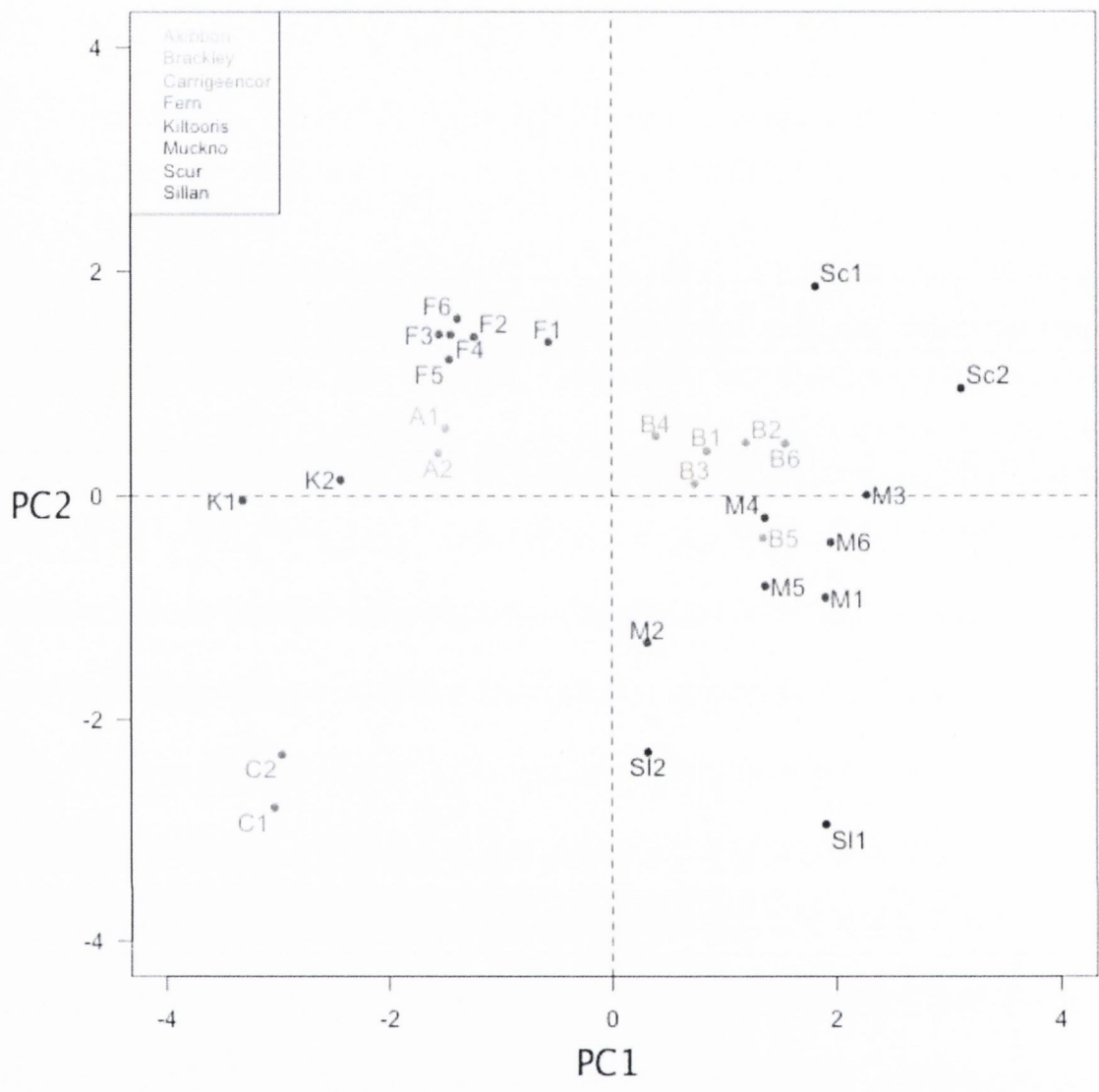


Figure 2.8 Distribution of individual sampling sites on the PCA bi-plot. Nutrient enrichment and colour gradients, primarily represented by PC1 and PC2, respectively, explain 53.7% and 29.0% of the total variation. Letters and numbers indicate different lakes and sites, respectively (A-Akibon, B-Brackley, C-Carrigeencor, F-Fern, K-Kiltorris, M-Muckno, Sc-Scur and Sl- Sillan).



### 2.3.2 *Asellus aquaticus*

*Asellus aquaticus* was found in 7 of the 8 study loughs (Table A 7, Appendix III). In Akibbon, Carrigeencor, Scur and Sillan, samples were taken from two sites (one adjacent to woodland and one to grassland shore), while in Fern, Brackley and Muckno; three sites for each shoreline type were sampled. Summary data for isotopic and body length measures (mean  $\pm$  SE) are presented for each shoreline habitat type for all lakes with *A. aquaticus* in Table 2.6 and for each site for lakes with more than two sampling sites in Table 2.7.

Table 2.6 Comparison of descriptive statistics (mean $\pm$ SE) for the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  stable isotope signatures, and body length (mm) of *A. aquaticus* among the woodland (W) and grassland (G) shoreline habitats across seven loughs where the species was present; where  $n$  = the total number of individual invertebrates used in the analysis for each shoreline type of a lake. \*Site names for lakes with two sampling sites are indicated after the shoreline habitat in parenthesis.

| Lough             | Shoreline Habitat* | n  | $\delta^{13}\text{C} \pm$<br>SE (‰) | $\delta^{15}\text{N} \pm$<br>SE (‰) | Body Length<br>$\pm$ SE (mm) |
|-------------------|--------------------|----|-------------------------------------|-------------------------------------|------------------------------|
| Akibbon           | W (A1)             | 6  | $-26.96 \pm 0.52$                   | $7.86 \pm 0.11$                     | $5.0 \pm 0.4$                |
|                   | G (A2)             | 7  | $-27.09 \pm 0.86$                   | $7.60 \pm 0.08$                     | $6.2 \pm 0.3$                |
| Brackley          | W                  | 24 | $-29.13 \pm 0.50$                   | $4.42 \pm 0.24$                     | $6.5 \pm 0.2$                |
|                   | G                  | 12 | $-29.12 \pm 0.45$                   | $5.23 \pm 0.16$                     | $6.8 \pm 0.4$                |
| Carrigeen-<br>cor | W (C1)             | 6  | $-24.25 \pm 0.29$                   | $4.54 \pm 0.06$                     | $6.7 \pm 0.4$                |
|                   | G (C2)             | 6  | $-24.52 \pm 0.33$                   | $5.22 \pm 0.14$                     | $5.9 \pm 0.2$                |
| Fern              | W                  | 12 | $-26.03 \pm 0.13$                   | $9.09 \pm 0.10$                     | $5.8 \pm 0.2$                |
|                   | G                  | 18 | $-25.95 \pm 0.21$                   | $8.27 \pm 0.31$                     | $6.0 \pm 0.2$                |
| Muckno            | W                  | 12 | $-29.31 \pm 0.27$                   | $10.21 \pm 0.24$                    | $5.8 \pm 0.3$                |
|                   | G                  | 24 | $-30.4 \pm 0.44$                    | $11.48 \pm 0.14$                    | $6.4 \pm 0.3$                |
| Scur              | W (Sc1)            | 12 | $-31.83 \pm 0.46$                   | $7.36 \pm 0.19$                     | $6.1 \pm 0.2$                |
|                   | G (Sc2)            | 12 | $-31.23 \pm 0.84$                   | $7.79 \pm 0.14$                     | $7.3 \pm 0.5$                |
| Sillan            | W (Sl1)            | 6  | $-27.00 \pm 0.41$                   | $11.55 \pm 0.36$                    | $6.6 \pm 0.5$                |
|                   | G (Sl2)            | 5  | $-27.91 \pm 0.38$                   | $13.61 \pm 0.40$                    | $7.9 \pm 0.4$                |

Table 2.7 Comparison of descriptive statistics (mean $\pm$ SE) for the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  stable isotope signatures, and body length (mm) of *A. aquaticus* across the sampling sites of Loughs Fern, Brackley and Muckno (Figure 2.4) where the species were collected from multiple sites of each shoreline habitat type (indicated after site name; W=Woodland and G=Grassland); where  $n$  = the number of total individual invertebrates used in the analysis from each site. Values for sites, where the species was not present ( $n=0$ ), are left blank.

| Lough    | Site   | n  | $\delta^{13}\text{C} \pm$<br>SE (‰) | $\delta^{15}\text{N} \pm$<br>SE (‰) | Body Length<br>$\pm$ SE (mm) |
|----------|--------|----|-------------------------------------|-------------------------------------|------------------------------|
| Brackley | B1 (W) | 12 | $-27.02 \pm 0.28$                   | $5.40 \pm 0.17$                     | $6.6 \pm 0.4$                |
|          | B2 (W) | 12 | $-31.23 \pm 0.41$                   | $3.44 \pm 0.22$                     | $6.4 \pm 0.2$                |
|          | B3 (W) | 0  |                                     |                                     |                              |
|          | B4 (G) | 6  | $-29.86 \pm 0.35$                   | $4.89 \pm 0.05$                     | $7.6 \pm 0.4$                |
|          | B5 (G) | 0  |                                     |                                     |                              |
|          | B6 (G) | 6  | $-28.38 \pm 0.65$                   | $5.87 \pm 0.08$                     | $6.1 \pm 0.5$                |
| Fern     | F1 (W) | 6  | $-26.09 \pm 0.18$                   | $9.02 \pm 0.23$                     | $5.9 \pm 0.4$                |
|          | F2 (W) | 6  | $-25.64 \pm 0.19$                   | $9.03 \pm 0.18$                     | $5.5 \pm 0.2$                |
|          | F3 (W) | 6  | $-26.36 \pm 0.20$                   | $9.22 \pm 0.13$                     | $5.9 \pm 0.3$                |
|          | F4 (G) | 6  | $-26.46 \pm 0.25$                   | $9.27 \pm 0.08$                     | $6.5 \pm 0.3$                |
|          | F5 (G) | 0  |                                     |                                     |                              |
|          | F6 (G) | 6  | $-25.44 \pm 0.19$                   | $7.29 \pm 0.20$                     | $5.5 \pm 0.3$                |
| Muckno   | M1 (W) | 6  | $-29.87 \pm 0.35$                   | $10.86 \pm 0.08$                    | $6.4 \pm 0.5$                |
|          | M2 (W) | 6  | $-28.74 \pm 0.29$                   | $9.57 \pm 0.29$                     | $5.2 \pm 0.3$                |
|          | M3 (W) | 0  |                                     |                                     |                              |
|          | M4 (G) | 6  | $-30.32 \pm 0.30$                   | $11.67 \pm 0.30$                    | $5.6 \pm 0.4$                |
|          | M5 (G) | 6  | $-33.45 \pm 0.70$                   | $10.58 \pm 0.18$                    | $7.3 \pm 0.5$                |
|          | M6 (G) | 12 | $-28.99 \pm 0.50$                   | $11.83 \pm 0.12$                    | $6.3 \pm 0.4$                |

2.3.2.1 *Asellus aquaticus* models with  $\delta^{13}\text{C}$  data

*Asellus aquaticus*  $\delta^{13}\text{C}$  signatures varied between -37.5‰ and -23.3‰ (Figure 2.9). The most parsimonious model for  $\delta^{13}\text{C}$  isotope values of *A. aquaticus* collected across seven lakes incorporated PC1 as the only significant main effect (Table 2.8). Despite their overall insignificant effects, PC2 and shoreline habitat type were retained in the final model owing to their significant interaction. Lake random effect and residuals accounted for 0.61% and 3.17% of the total variance, respectively, signifying relatively low variation associated with lake (Table 2.8).

The final model demonstrated that  $\delta^{13}\text{C}$  of *A. aquaticus* were more negatively affected by the increase in PC1, primarily representing the nutrient gradient. The negative slope of  $\delta^{13}\text{C}$  with the ‘PC2 x shoreline type’ interaction indicated that the effect of the PC2 on  $\delta^{13}\text{C}$  was significantly more negative for *A. aquaticus* associated with woodland in comparison with grassland shorelines (Table 2.8 and Figure 2.9).

Table 2.8 Minimal adequate linear mixed effects model with  $\delta^{13}\text{C}$  isotope values of *A. aquaticus* (n=162) collected from Loughs Akibbon, Carrigeencor, Fern, Brackley, Muckno, Sillan, and Scur. (“SHab” - shoreline habitat type: “W” – woodland or “G” – grassland; (the latter was set as the reference level as shown in parenthesis). Variance explained by ‘Lake’ random effect and ‘Residual’ accounted for 0.61 and 3.17, respectively. AICc= 673.0.

|                   | Estimated Coeff. | SE    | t-value | p        |
|-------------------|------------------|-------|---------|----------|
| PC1               | -0.783           | 0.164 | -4.785  | 0.002 ** |
| PC2               | -0.102           | 0.278 | -0.367  | 0.724    |
| SHab: W (G)       | 0.161            | 0.289 | 0.556   | 0.579    |
| PC2 x SHab: W (G) | -0.447           | 0.223 | -2.006  | 0.047 *  |

Significance codes: \*  $p < 0.05$ .      \*\*  $p < 0.01$ .



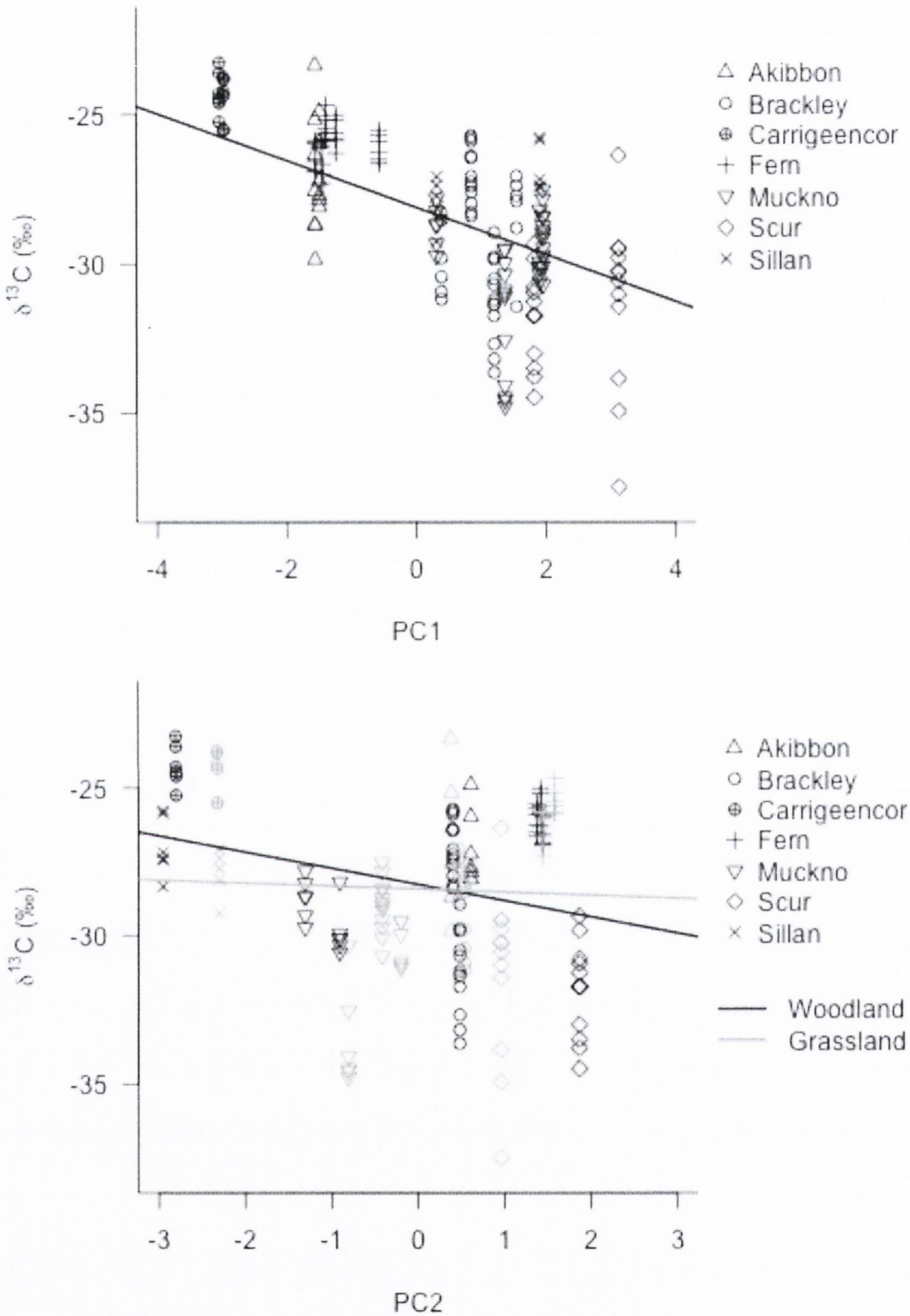


Figure 2.9 Distribution of  $\delta^{13}\text{C}$  signatures of *A. aquaticus* across eight lakes along the PC1 (above) and PC2 (below) axes. Linear lines in the below figure visualise the model illustrating the difference of  $\delta^{15}\text{N}$  signatures to the PC2 gradient at different shoreline habitat types). (See Figure A 1 with the lake effect taken into account).

2.3.2.2 *Asellus aquaticus* models with  $\delta^{15}\text{N}$  data

The  $\delta^{15}\text{N}$  signatures of *A. aquaticus* ranged between 2.3‰ and 14.6‰ across seven lakes (Figure 2.10) The results of the most parsimonious model based on the  $\delta^{15}\text{N}$  isotope signatures of *A. aquaticus* included PC1 and the shoreline habitat type as significant main fixed explanatory variables. Also, the interaction terms between shoreline habitat type and either of the first two PC axes were found to be significant (Table 2.9). Lake random effect and residuals explained 8.69% and 0.55% of the total variance, respectively.

Significant interactions of PC1 axes and shoreline habitat indicate that both PC1 and PC2 main effects on *A. aquaticus*  $\delta^{15}\text{N}$  signatures are significantly different between the two shorelines types. The negative coefficient of “PC1 x SHab” interaction suggested that increased PC1 affected *A. aquaticus*  $\delta^{15}\text{N}$  signatures more negatively in sites associated with woodland shorelines in comparison with grassland shorelines (Table 2.9 and Figure 2.10). In comparison, the positive coefficient value of ‘PC2 x SHab’ interaction effect indicated that there was a significantly greater increase in the  $\delta^{15}\text{N}$  isotopes of *A. aquaticus* in sites associated with woodland shorelines in comparison with grassland shorelines (Table 2.9 and Figure 2.10).

Table 2.9 Minimal adequate linear mixed effects model with  $\delta^{15}\text{N}$  isotope values of *A. aquaticus* (n=162) collected from Loughs Akibbon, Carrigeencor, Fern, Brackley, Muckno, Sillan, and Scur. (“SHab” - shoreline habitat type: “W” – woodland or “G” – grassland (the latter was set as the reference level as shown in parenthesis); “x” indicates interaction between terms). Variance explained by ‘Lake’ random effect and ‘Residual’ accounted for 8.69 and 0.55 respectively. AICc= 421.7.

|                   | Estimated Coeff. | SE    | t-value | p           |
|-------------------|------------------|-------|---------|-------------|
| PC1               | 0.360            | 0.125 | 2.875   | 0.005 **    |
| PC2               | 0.410            | 0.307 | 1.336   | 0.184       |
| SHab: W (G)       | -0.265           | 0.125 | -2.052  | 0.041 *     |
| PC1 x SHab: W (G) | -0.453           | 0.076 | -5.943  | < 0.001 *** |
| PC2 x SHab: W (G) | 0.275            | 0.112 | 2.449   | 0.015 *     |

Significance codes: \*  $p < 0.05$ .      \*\*  $p < 0.01$ .      \*\*\*  $p < 0.001$ .

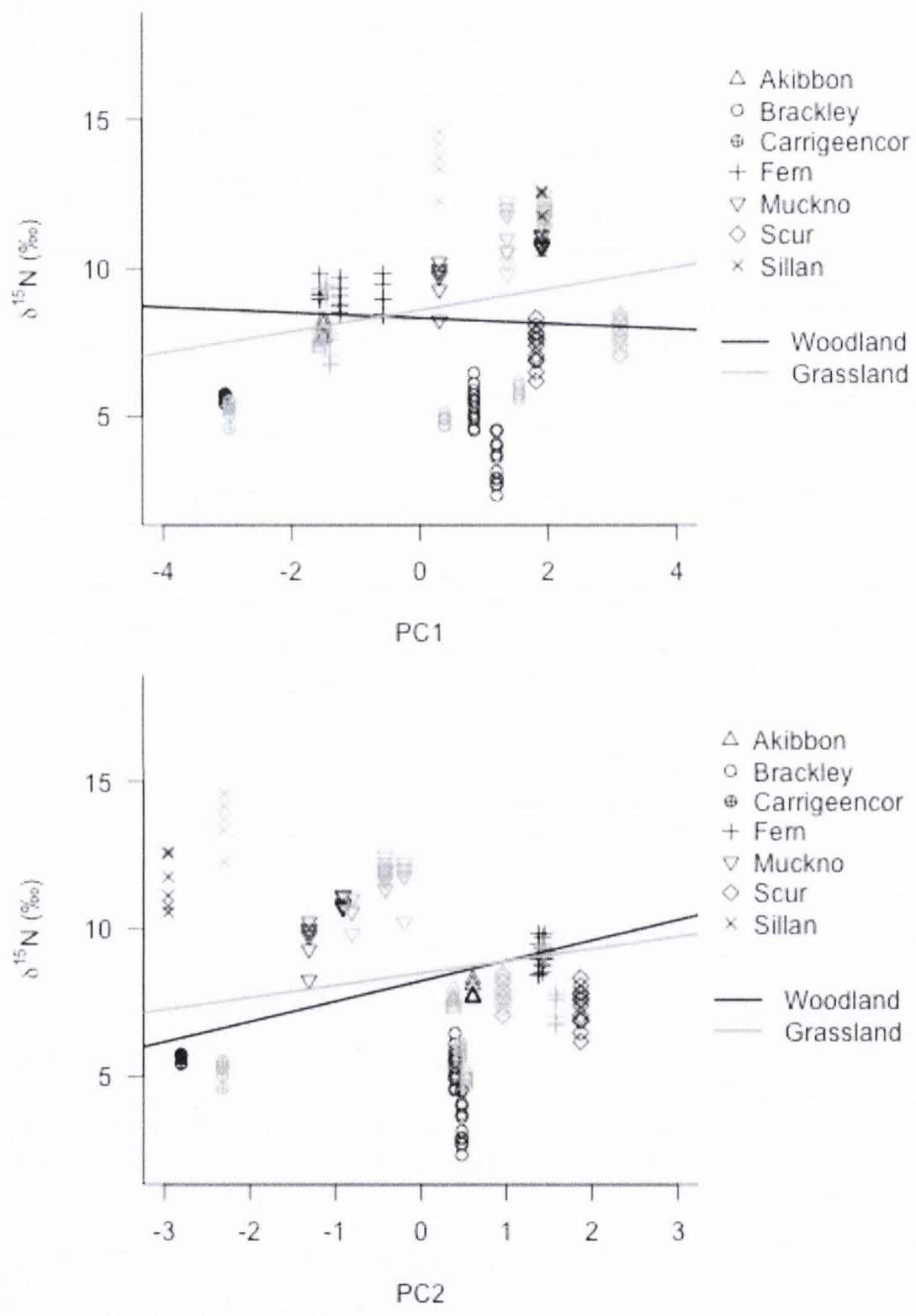


Figure 2.10 Distribution of  $\delta^{15}\text{N}$  signatures of *A. aquaticus* across eight lakes along the PC1 (above) and PC2 (below) axes. Linear lines in the below figure visualise the model illustrating the difference of  $\delta^{15}\text{N}$  signatures to the PC2 gradient at different shoreline habitat types). (See Figure A 2 with the lake effect taken into account).



### 2.3.3 *Gammarus duebeni*

*Gammarus duebeni* was found in 5 of the 8 study loughs (Table A 7, Appendix III). In Akibbon, Kiltorris and Scur, two sites (one adjacent to woodland and one to grassland shore) were sampled. Summary data for *G. duebeni* isotopic and body length measures (mean  $\pm$  SE) are presented for each shoreline habitat type in Table 2.10 and for each site in the more intensively sampled lakes (three sites for each shoreline type), including Loughs Fern and Brackley in Table 2.11.

Table 2.10 Comparison of descriptive statistics (mean $\pm$ SE) for the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  stable isotope signatures, and body length (mm) of *G. duebeni* among the woodland (W) and grassland (G) shoreline habitats across five loughs where the species was present; where  $n$  = the total number of individual invertebrates used in the analysis for each shoreline type of a lake. \* Site names for lakes with two sampling sites are indicated after the shoreline habitat in parenthesis.

| Lough     | Shoreline Habitat * | n  | $\delta^{13}\text{C} \pm$<br>SE (‰) | $\delta^{15}\text{N} \pm$<br>SE (‰) | Body Length<br>$\pm$ SE (mm) |
|-----------|---------------------|----|-------------------------------------|-------------------------------------|------------------------------|
| Akibbon   | W (A1)              | 6  | $-29.24 \pm 0.45$                   | $8.37 \pm 0.17$                     | $12.3 \pm 1.5$               |
|           | G (A2)              | 4  | $-27.52 \pm 0.34$                   | $7.15 \pm 0.26$                     | $10.8 \pm 1.2$               |
| Brackley  | B (W)               | 6  | $-27.67 \pm 0.27$                   | $5.66 \pm 0.32$                     | $10.5 \pm 0.5$               |
|           | B (G)               | 12 | $-30.83 \pm 0.35$                   | $6.00 \pm 0.36$                     | $9.6 \pm 0.4$                |
| Kiltorris | W (K1)              | 6  | $-25.01 \pm 0.18$                   | $5.63 \pm 0.07$                     | $10.4 \pm 0.7$               |
|           | G (K2)              | 6  | $-24.94 \pm 0.26$                   | $6.07 \pm 0.11$                     | $9.9 \pm 0.9$                |
| Fern      | F (W)               | 18 | $-26.92 \pm 0.17$                   | $9.24 \pm 0.15$                     | $8.3 \pm 0.5$                |
|           | F (G)               | 18 | $-27.26 \pm 0.20$                   | $9.34 \pm 0.13$                     | $9.7 \pm 0.5$                |
| Scur      | W (Sc1)             | 3  | $-30.45 \pm 0.52$                   | $7.25 \pm 0.42$                     | $13.4 \pm 1.0$               |
|           | G (Sc2)             | 6  | $-29.74 \pm 0.09$                   | $8.53 \pm 0.12$                     | $13.1 \pm 0.9$               |

Table 2.11 Comparison of descriptive statistics (mean $\pm$ SE) for the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  stable isotope signatures, and body length (mm) of *G. duebeni* across the sampling sites of Loughs Fern and Brackley (Figure 2.4) where the species were collected from multiple sites of each shoreline habitat type (indicated after site names; W=Woodland and G=Grassland); where *n* = the number of total individual invertebrates used in the analysis from each site. Values for sites, where the species was not present (*n*=0), are left blank.

| Lough    | Site   | n  | $\delta^{13}\text{C} \pm$<br>SE (‰) | $\delta^{15}\text{N} \pm$<br>SE (‰) | Body Length<br>$\pm$ SE (mm) |
|----------|--------|----|-------------------------------------|-------------------------------------|------------------------------|
| Brackley | B1 (W) | 0  |                                     |                                     |                              |
|          | B2 (W) | 0  |                                     |                                     |                              |
|          | B3 (W) | 6  | 27.67 $\pm$ 0.27                    | 5.66 $\pm$ 0.32                     | 10.5 $\pm$ 0.5               |
|          | B4 (G) | 6  | -31.27 $\pm$ 0.50                   | 4.84 $\pm$ 0.08                     | 9.4 $\pm$ 0.6                |
|          | B5 (G) | 6  | -30.39 $\pm$ 0.45                   | 7.15 $\pm$ 0.16                     | 9.8 $\pm$ 0.7                |
|          | B6 (G) | 0  |                                     |                                     |                              |
| Fern     | F1 (W) | 0  |                                     |                                     |                              |
|          | F2 (W) | 12 | -26.56 $\pm$ 0.15                   | 9.24 $\pm$ 0.21                     | 8.7 $\pm$ 0.6                |
|          | F3 (W) | 6  | -27.65 $\pm$ 0.22                   | 9.22 $\pm$ 0.21                     | 7.5 $\pm$ 0.5                |
|          | F4 (G) | 6  | -28.06 $\pm$ 0.23                   | 9.80 $\pm$ 0.16                     | 11.1 $\pm$ 0.9               |
|          | F5 (G) | 6  | -27.20 $\pm$ 0.12                   | 9.30 $\pm$ 0.18                     | 8.3 $\pm$ 0.5                |
|          | F6 (G) | 6  | -26.52 $\pm$ 0.34                   | 8.29 $\pm$ 0.23                     | 9.7 $\pm$ 0.9                |

2.3.3.1 *Gammarus duebeni* models with  $\delta^{13}\text{C}$  data

*Gammarus duebeni*  $\delta^{13}\text{C}$  signatures ranged between -32.8‰ and -24.2‰ across five lakes (Figure 2.11). The most parsimonious model for *G. duebeni*  $\delta^{13}\text{C}$  data included PC1, body length, and shoreline habitat type as the significant main fixed explanatory variables. Also, significant interactions were found between the shoreline habitat type and both of the PC axes. Even though PC2 axis main effect was not significant, it remained in the final model owing to its significant interaction with the shoreline habitat type. Lake random effect and residuals explained 28.49% and 0.52% of the total variance (Table 2.12).

Both the main effect of PC1 and its interaction with shoreline habitat type were highly significant. The positive slope between PC1 and *G. duebeni*  $\delta^{13}\text{C}$  relationship suggested a more increasing influence of PC1 on *G. duebeni*  $\delta^{13}\text{C}$  in woodland in comparison with grassland shoreline habitats (Table 2.12 and Figure 2.11). On the other hand, the negative coefficient of ‘PC2 x shoreline habitat’ interaction term

indicated a more negative effect of PC2 on  $\delta^{13}\text{C}$  signature of *G. duebeni* associated with woodland shorelines in comparison with *G. duebeni* associated with grassland shorelines (Table 2.12 and Figure 2.11).  $\delta^{13}\text{C}$  had a positive estimated slope with the 'body length' variable, suggesting a positive effect on *G. duebeni*  $\delta^{13}\text{C}$  (Table 2.12 and Figure 2.15).

Table 2.12 Minimal adequate linear mixed effects model with  $\delta^{13}\text{C}$  isotope values of *G. duebeni* (n=85) collected from Loughs Akibbon, Kiltorris, Fern, Brackley and Scur. ("SHab" - shoreline habitat type: "W" – woodland or "G" – grassland (the latter was set as the reference level as shown in parenthesis), "x" indicates interaction between terms). Variance explained by 'Lake' random effect and 'Residual' accounted for 28.49 and 0.52, respectively. AICc=239.4.

|                   | Estimated Coeff. | SE    | t-value | p          |
|-------------------|------------------|-------|---------|------------|
| PC1               | 1.742            | 0.361 | 4.817   | <0.001 *** |
| PC2               | 0.765            | 0.545 | 1.402   | 0.164      |
| SHab: W (G)       | 3.083            | 0.326 | 9.468   | <0.001 *** |
| Body Length       | 0.970            | 0.403 | 2.406   | 0.018 *    |
| PC1 x SHab: W (G) | 0.443            | 0.122 | 3.617   | <0.001 *** |
| PC2 x SHab: W (G) | -1.548           | 0.269 | -5.747  | <0.001 *** |

Significance codes: \*  $p < 0.05$ . \*\*\*  $p < 0.001$ .



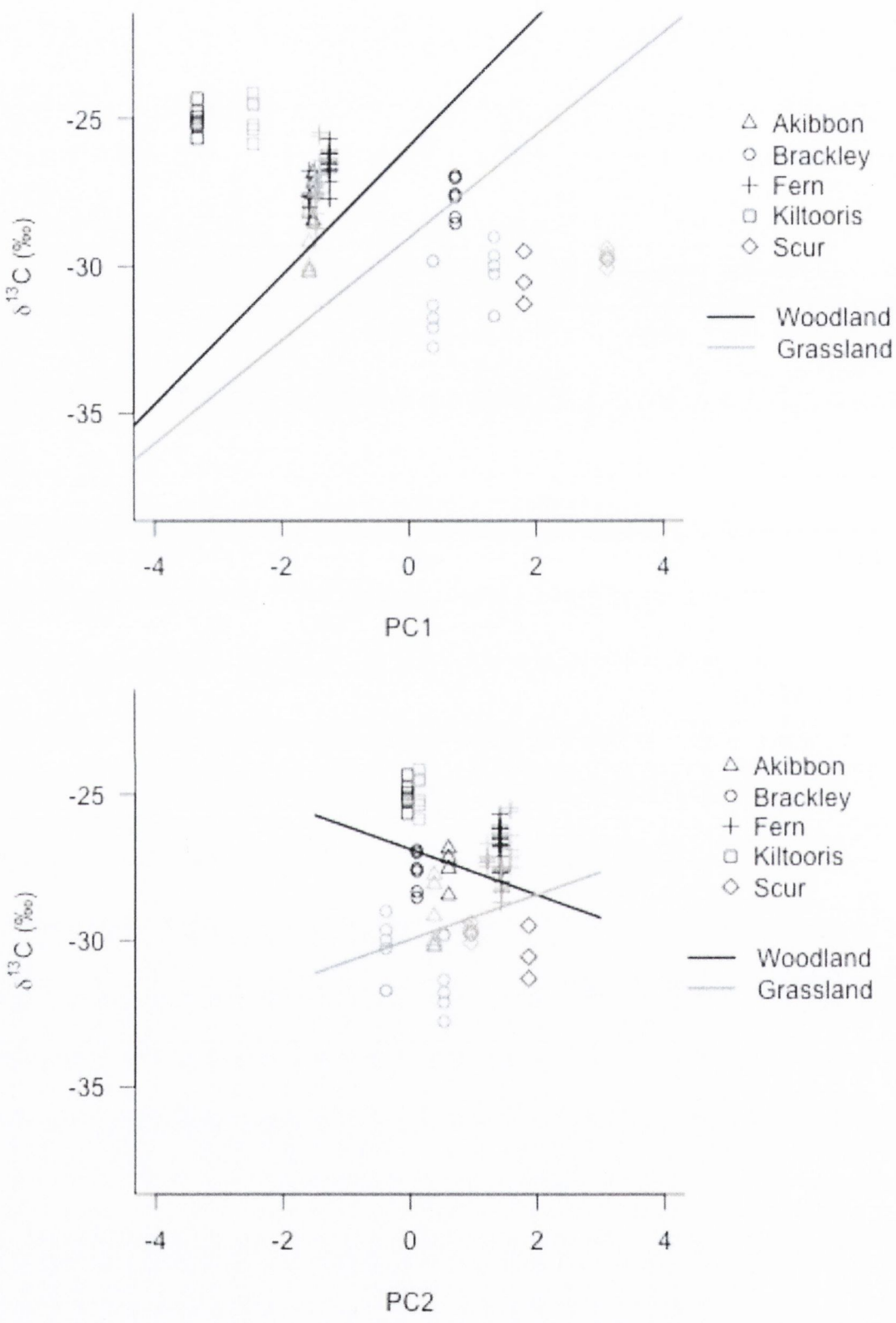
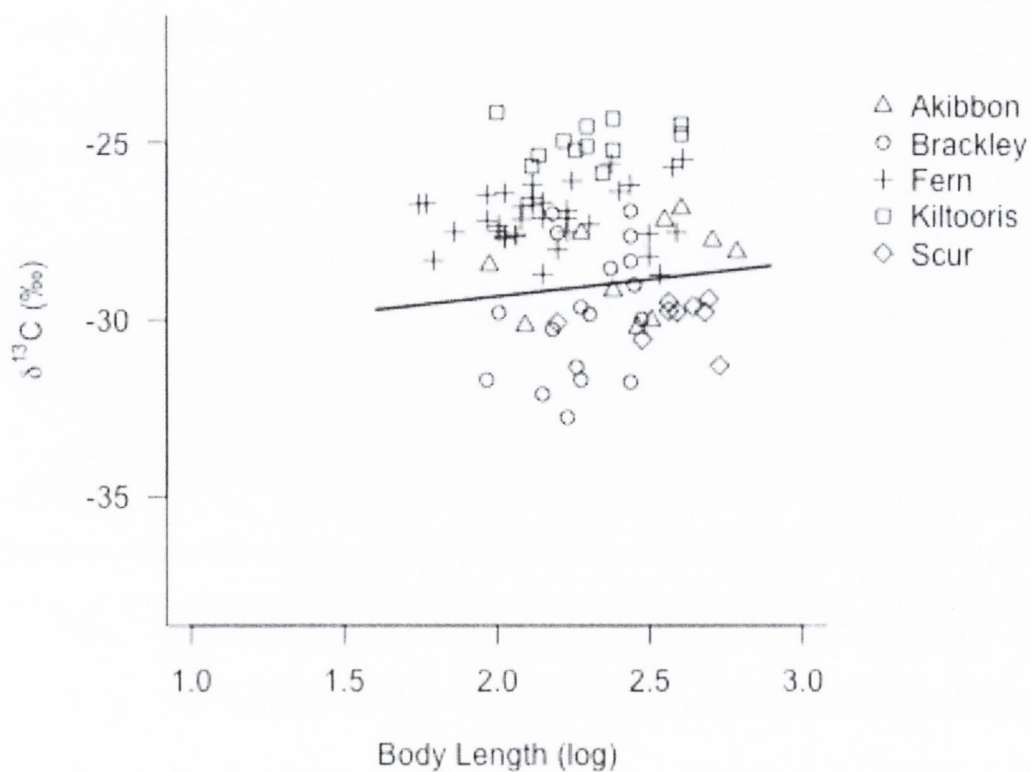


Figure 2.11 Distribution of  $\delta^{13}\text{C}$  signatures of *G. duebeni* across five lakes along the PC1 (above) and PC2 (below) axes. Linear lines visualise the models illustrating the difference of  $\delta^{15}\text{N}$  signatures to the PC1 and PC2 gradients at different shoreline habitat types. (See Figure A 3 with the lake effect taken into account).



### 2.3.3.2 *Gammarus duebeni* models with $\delta^{15}\text{N}$ data

$\delta^{15}\text{N}$  signatures of *G. duebeni* ranged from 4.4‰ to 10.4‰ across five lakes (Figure 2.13). The minimal adequate model of  $\delta^{15}\text{N}$  values of *G. duebeni* included PC2, shoreline habitat type and body length as the main fixed explanatory variables, while PC1 was discarded in the final model, indicating no significant effects on *G. duebeni*  $\delta^{15}\text{N}$  signature. The interaction term between ‘PC2 x shoreline habitat’ was also retained in the final model (Table 2.13). The lake random effect could explain 5.42% of the total variance, while residual variance accounted for 0.24%.

The final model results showed that  $\delta^{15}\text{N}$  signature of *G. duebeni* in woodland shoreline habitats was more positively affected by PC2, primarily representing the colour gradient, in comparison with grassland shoreline habitats. Furthermore, the positive coefficient of body size indicated that increased body length elevated the  $\delta^{15}\text{N}$  signature of *G. duebeni* (Table 2.13).

Table 2.13 Minimal adequate linear mixed effects model with  $\delta^{15}\text{N}$  isotope values of *G. duebeni* (n=85) collected from Loughs Akibbon, Kiltorris, Fern, Brackley and Scur. (“SHab” - shoreline habitat type: “W” – woodland or “G” – grassland; “(G)” (the latter was set as the reference level as shown in parenthesis); “x” indicates interaction between terms). Reference groups of the categorical variables are shown in parenthesis. Variance explained by ‘Lake’ random effect and ‘Residual’ accounted for 5.42 and 0.24, respectively. AICc=165.7.

|                   | Estimated Coeff. | SE    | t-value | p          |
|-------------------|------------------|-------|---------|------------|
| PC2               | -2.097           | 0.243 | -8.634  | <0.001 *** |
| SHab: W (G)       | -0.7141          | 0.181 | -3.940  | <0.001 *** |
| Body Length       | 1.003            | 0.274 | 3.665   | <0.001 *** |
| PC2 x SHab: W (G) | 0.594            | 0.170 | 3.488   | <0.001 *** |

Significance code: \*\*\*  $p < 0.001$ .



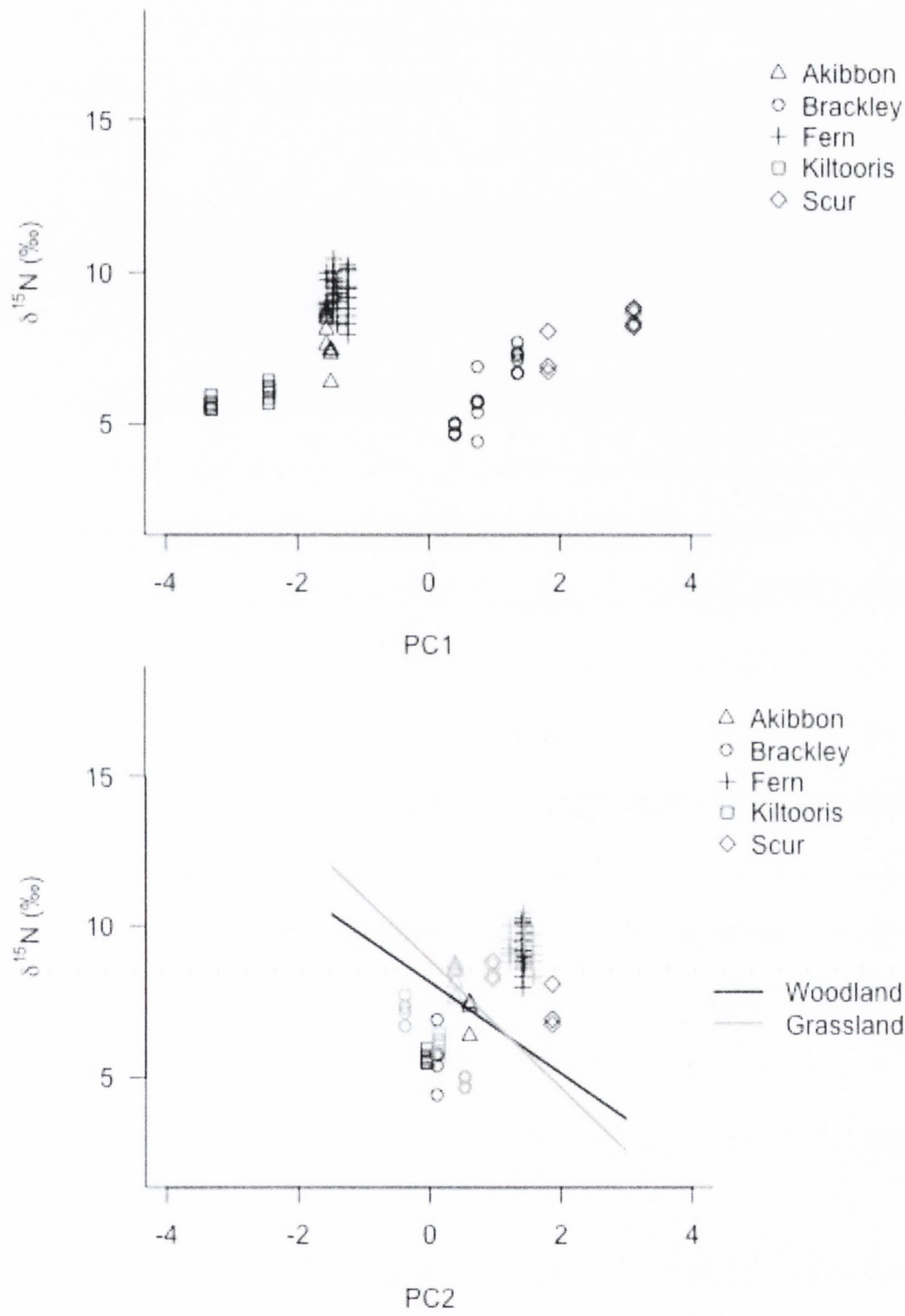


Figure 2.13 Distribution of  $\delta^{15}\text{N}$  signatures of *G. duebeni* across six lakes along the PC1 (above) and PC2 (below) axes. Linear lines in the below figure visualise the model illustrating the difference of  $\delta^{15}\text{N}$  signatures to the PC2 gradient at different shoreline habitat types). (See Figure A 4 with the lake effect taken into account).

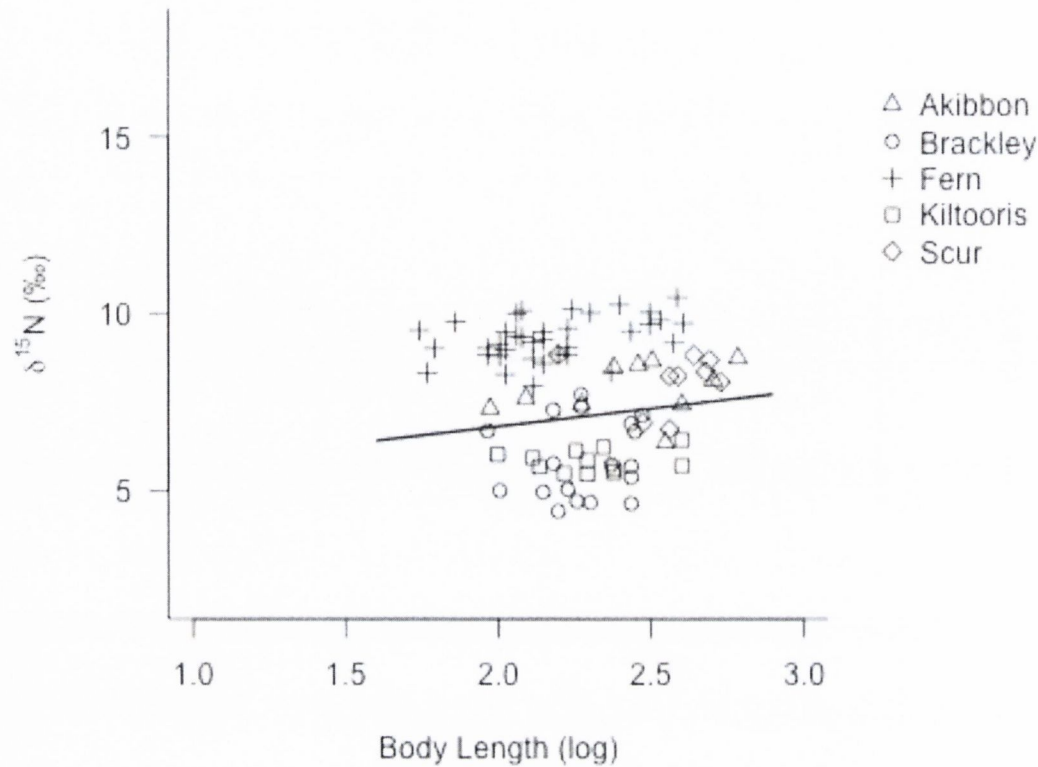


Figure 2.14 Distribution of  $\delta^{15}\text{N}$  signatures of *G. duebeni* across five lakes along a body length (log) gradient. The linear line visualises the model illustrating the response of *G. duebeni*  $\delta^{15}\text{N}$  signatures to body length change. (See Figure A 5 with the lake effect taken into account).

2.3.4 *Crangonyx pseudogracilis*

*Crangonyx pseudogracilis* was found in 4 of the 8 study loughs (Table A 7, Appendix III). Summary data for *C. pseudogracilis* isotopic and body length measures (mean  $\pm$  SE) are displayed for each shoreline habitat type in Table 2.14. Two sites (one adjacent to woodland and one to grassland shore) were sampled in Loughs Scur and Sillan, while samples were collected from more than two sites in Loughs Fern, Brackley and Muckno. Summary data for each site of these loughs are displayed in Table 2.15.

Table 2.14 Comparison of descriptive statistics (mean $\pm$ SE) for the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  stable isotope signatures, and body length (mm) of *C. pseudogracilis* among the woodland (W) and grassland (G) shoreline habitats across four loughs where the species was present; where  $n$  = the total number of individual invertebrates used in the analysis for each shoreline type of a lake. \* Site names for lakes with two sampling sites are indicated after the shoreline habitat in parenthesis.

| Lough    | Shoreline Habitat * | n  | $\delta^{13}\text{C} \pm \text{SE} (\text{‰})$ | $\delta^{15}\text{N} \pm \text{SE} (\text{‰})$ | Body Length $\pm \text{SE} (\text{mm})$ |
|----------|---------------------|----|------------------------------------------------|------------------------------------------------|-----------------------------------------|
| Brackley | W                   | 31 | $-28.97 \pm 0.23$                              | $7.24 \pm 0.15$                                | $6.5 \pm 0.2$                           |
|          | G                   | 31 | $-29.19 \pm 0.25$                              | $8.28 \pm 0.14$                                | $6.1 \pm 0.3$                           |
| Muckno   | W                   | 13 | $-29.01 \pm 0.29$                              | $12.97 \pm 0.42$                               | $7.4 \pm 0.4$                           |
|          | G                   | 12 | $-28.93 \pm 0.38$                              | $14.00 \pm 0.38$                               | $8.4 \pm 0.4$                           |
| Scur     | W (Sc1)             | 7  | $-31.94 \pm 0.44$                              | $9.59 \pm 0.25$                                | $7.3 \pm 0.2$                           |
|          | G (Sc2)             | 12 | $-31.77 \pm 0.53$                              | $10.17 \pm 0.11$                               | $7.7 \pm 0.2$                           |
| Sillan   | W (Sl1)             | 6  | $-26.98 \pm 0.24$                              | $13.60 \pm 0.52$                               | $7.2 \pm 0.4$                           |
|          | G (Sl2)             | 6  | $-27.09 \pm 0.75$                              | $16.05 \pm 0.15$                               | $7.4 \pm 0.3$                           |



Table 2.15 Comparison of descriptive statistics (mean $\pm$ SE) for the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  stable isotope signatures, and body length (mm) of *C. pseudogracilis* across the sampling sites of Loughs Brackley and Muckno (Figure 2.4), where species were collected from multiple sampling sites for each shoreline habitat type (indicated after site names; W=Woodland and G=Grassland); where  $n$  = the number of individual invertebrates used in the analysis from each site. Values for sites, where the species was not present ( $n=0$ ), are left blank.

| Lough    | Site   | n  | $\delta^{13}\text{C} \pm$<br>SE (‰) | $\delta^{15}\text{N} \pm$<br>SE (‰) | Body Length<br>$\pm$ SE (mm) |
|----------|--------|----|-------------------------------------|-------------------------------------|------------------------------|
| Brackley | B1 (W) | 12 | $-28.66 \pm 0.21$                   | $8.06 \pm 0.12$                     | $7.2 \pm 0.1$                |
|          | B2 (W) | 19 | $-29.64 \pm 0.30$                   | $6.61 \pm 0.16$                     | $6.8 \pm 0.1$                |
|          | B3 (W) | 6  | $-27.48 \pm 0.67$                   | $7.59 \pm 0.42$                     | $4.3 \pm 0.2$                |
|          | B4 (G) | 12 | $-29.35 \pm 0.51$                   | $7.75 \pm 0.19$                     | $7.0 \pm 0.2$                |
|          | B5 (G) | 6  | $-28.80 \pm 0.35$                   | $8.68 \pm 0.25$                     | $4.3 \pm 0.5$                |
|          | B6 (G) | 13 | $-29.23 \pm 0.36$                   | $8.60 \pm 0.19$                     | $6.1 \pm 0.4$                |
| Muckno   | M1 (W) | 7  | $-29.60 \pm 0.35$                   | $13.97 \pm 0.44$                    | $6.5 \pm 0.5$                |
|          | M2 (W) | 6  | $-28.32 \pm 0.27$                   | $11.80 \pm 0.35$                    | $8.4 \pm 0.1$                |
|          | M3 (W) | 0  |                                     |                                     |                              |
|          | M4 (G) | 0  |                                     |                                     |                              |
|          | M5 (G) | 0  |                                     |                                     |                              |
|          | M6 (G) | 12 | $-28.93 \pm 0.37$                   | $14.00 \pm 0.20$                    | $6.5 \pm 0.4$                |

2.3.4.1 *Crangonyx pseudogracilis* models with  $\delta^{13}\text{C}$  data

*Crangonyx pseudogracilis*  $\delta^{13}\text{C}$  signatures varied between -35.2‰ and -25.6‰ across four lakes (Figure 2.15). The most parsimonious model for the  $\delta^{13}\text{C}$  isotope signatures of *C. pseudogracilis* comprised PC1, PC2 and the body length main effects with highly significant results. On the other hand, no significant interaction terms were found significant (Table 2.16). Lake random effect and residuals explained 0.00% and 1.53% of the total variance, respectively (Table 2.16).

The model results showed that  $\delta^{13}\text{C}$  isotope values of *C. pseudogracilis* were positively affected by the increase of body length (Table 2.16). There was a negative response of  $\delta^{13}\text{C}$  signatures of *C. pseudogracilis* to either of the PC axes (Table 2.16 and Figure 2.15).

Table 2.16 Minimal adequate linear mixed effects model with  $\delta^{13}\text{C}$  isotope values of *C. pseudogracilis* (n=124) collected from Loughs Brackley, Muckno, Sillan and Scur. Variance explained by ‘Lake’ random effect and ‘Residual’ accounted for 0.00 and 1.53, respectively. AIC=417.1.

|             | Estimated Coeff. | SE    | t-value | p          |
|-------------|------------------|-------|---------|------------|
| PC1         | -0.746           | 0.152 | -4.903  | <0.001 *** |
| PC2         | -0.827           | 0.106 | -7.766  | <0.001 *** |
| Body Length | 1.842            | 0.506 | -3.640  | <0.001 *** |

Significance code: \*\*\*  $p < 0.001$ .

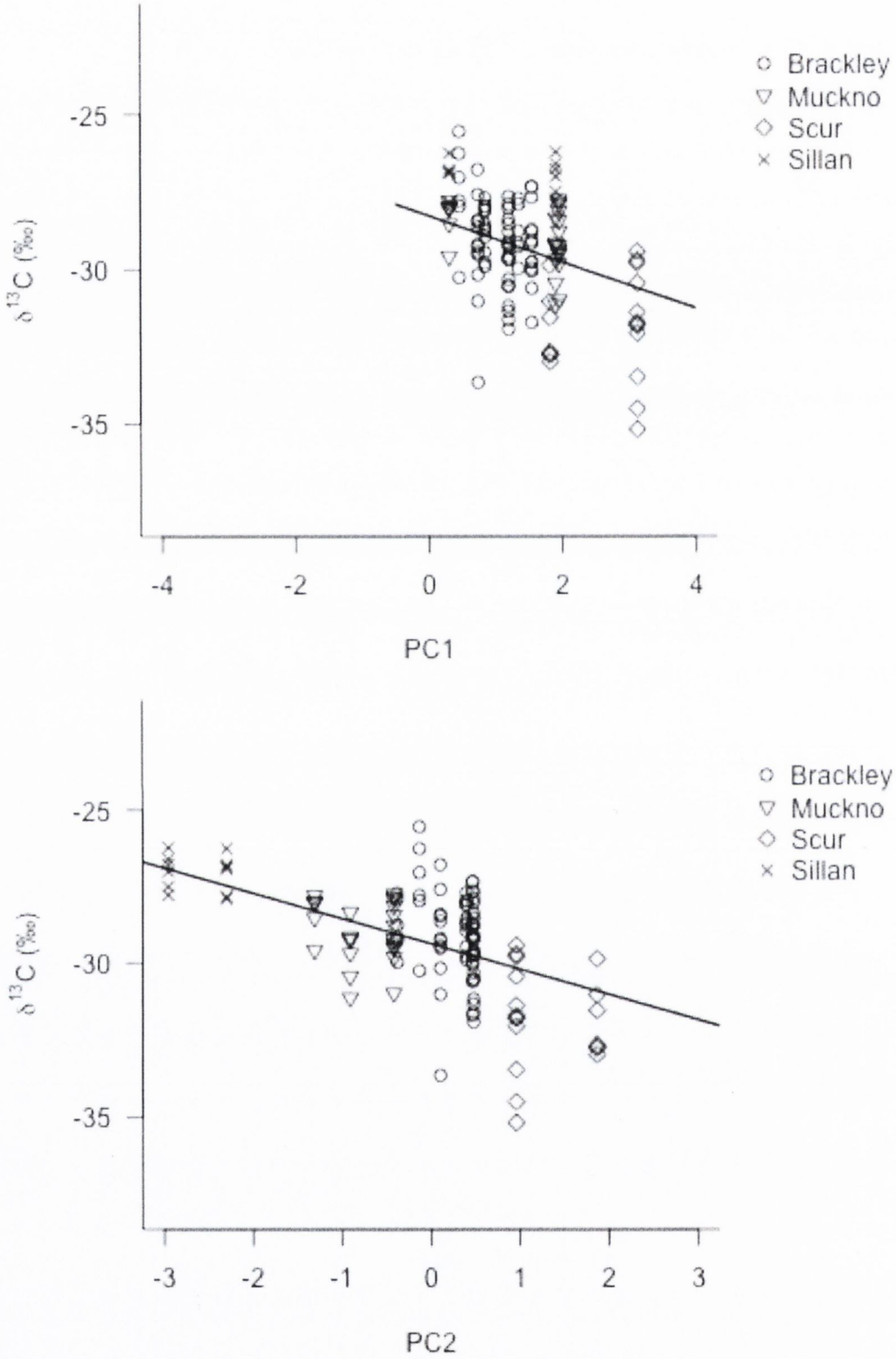


Figure 2.15 Distribution of  $\delta^{13}\text{C}$  signatures of *C. pseudogracilis* across four lakes along the PC1 (above) and PC2 (below) axes. Linear lines visualise the models illustrating the overall response of  $\delta^{15}\text{N}$  signatures to the PC1 and PC2 gradients. (See Figure A 6 with the lake effect taken into account).



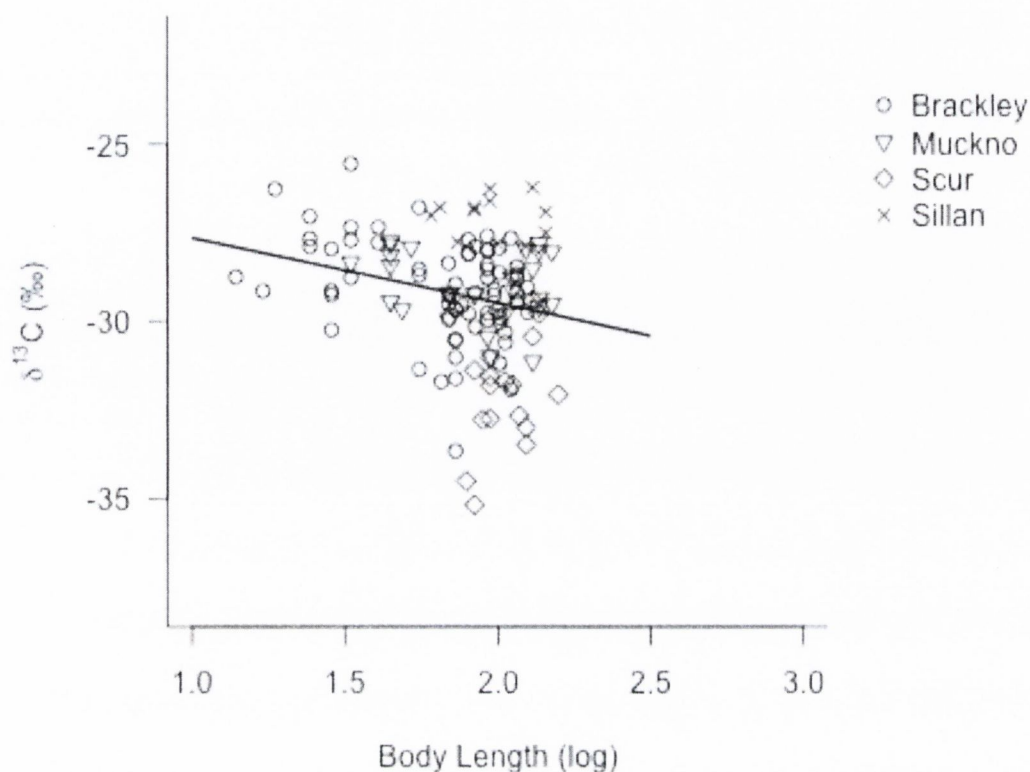


Figure 2.16 Distribution of  $\delta^{13}\text{C}$  signatures of *C. pseudogracilis* across four lakes along the body length (log) gradient. The linear line visualises the model illustrating the response of  $\delta^{15}\text{N}$  signatures to changes in body length. (See Figure A 8 with the lake effect taken into account).

#### 2.3.4.2 *Crangonyx pseudogracilis* models with $\delta^{15}\text{N}$ data

*Crangonyx pseudogracilis*  $\delta^{15}\text{N}$  signatures ranged between 5.4‰ and 17.1‰ across four lakes (Figure 2.17). The significant main fixed explanatory variables in the most parsimonious model for *C. pseudogracilis*  $\delta^{15}\text{N}$  isotope signatures comprised shoreline habitat type, and the body length main effects and the interaction term between 'PC2 x shoreline habitat' (Table 2.17). Similar to the *G. duebeni*, PC1 main effect was not found to be significant and was eliminated in the final model of *C. pseudogracilis* for  $\delta^{15}\text{N}$ . Also, the interaction term between 'PC2 x shoreline habitat' with a low but non-significant *p*-value was left in the final model as its removal resulted in a higher AIC value. Lake random effect and residual variance explained 8.81% and 0.89% of the total variance, respectively.

The final model results revealed significant effects of body length on *C. pseudogracilis*  $\delta^{15}\text{N}$  isotope signatures. The negative estimated coefficient revealed a significant decrease of  $\delta^{15}\text{N}$  signatures of *C. pseudogracilis* with increasing body size (Table 2.17 and Figure 2.18). The  $\delta^{15}\text{N}$  of *C. pseudogracilis* associated with

woodland shoreline habitats were significantly decreased compared with *C. pseudogracilis* associated with grassland shoreline habitats, which is displayed as the difference between the intercepts of the model linear lines plotted for the two shoreline habitat types (Figure 2.17).

Table 2.17 Minimal adequate linear mixed effects model with  $\delta^{15}\text{N}$  isotope values of *C. pseudogracilis* (n=124) collected from Loughs Brackley, Muckno, Sillan and Scur. ("SHab" - shoreline habitat type: "W" – woodland or "G" – grassland (the latter was set as the reference level as shown in parenthesis), "x" indicates interaction between, terms). Variance explained by 'Lake' random effect and 'Residual' accounted for 8.81 and 0.89, respectively. AICc=374.1.

|                   | Estimated Coeff. | SE     | t-value | p          |
|-------------------|------------------|--------|---------|------------|
| PC2               | - 0.116          | 0.412  | -0.280  | 0.779      |
| SHab: W (G)       | -1.030           | 0.1723 | - 5.955 | <0.001 *** |
| Body Length       | - 1.138          | 0.428  | - 2.662 | 0.009 **   |
| PC2 x SHab: W (G) | 0.355            | 0.211  | 1.684   | 0.095      |

Significance codes: \*  $p < 0.05$ .    \*\*  $p < 0.01$ .    \*\*\*  $p < 0.001$ .

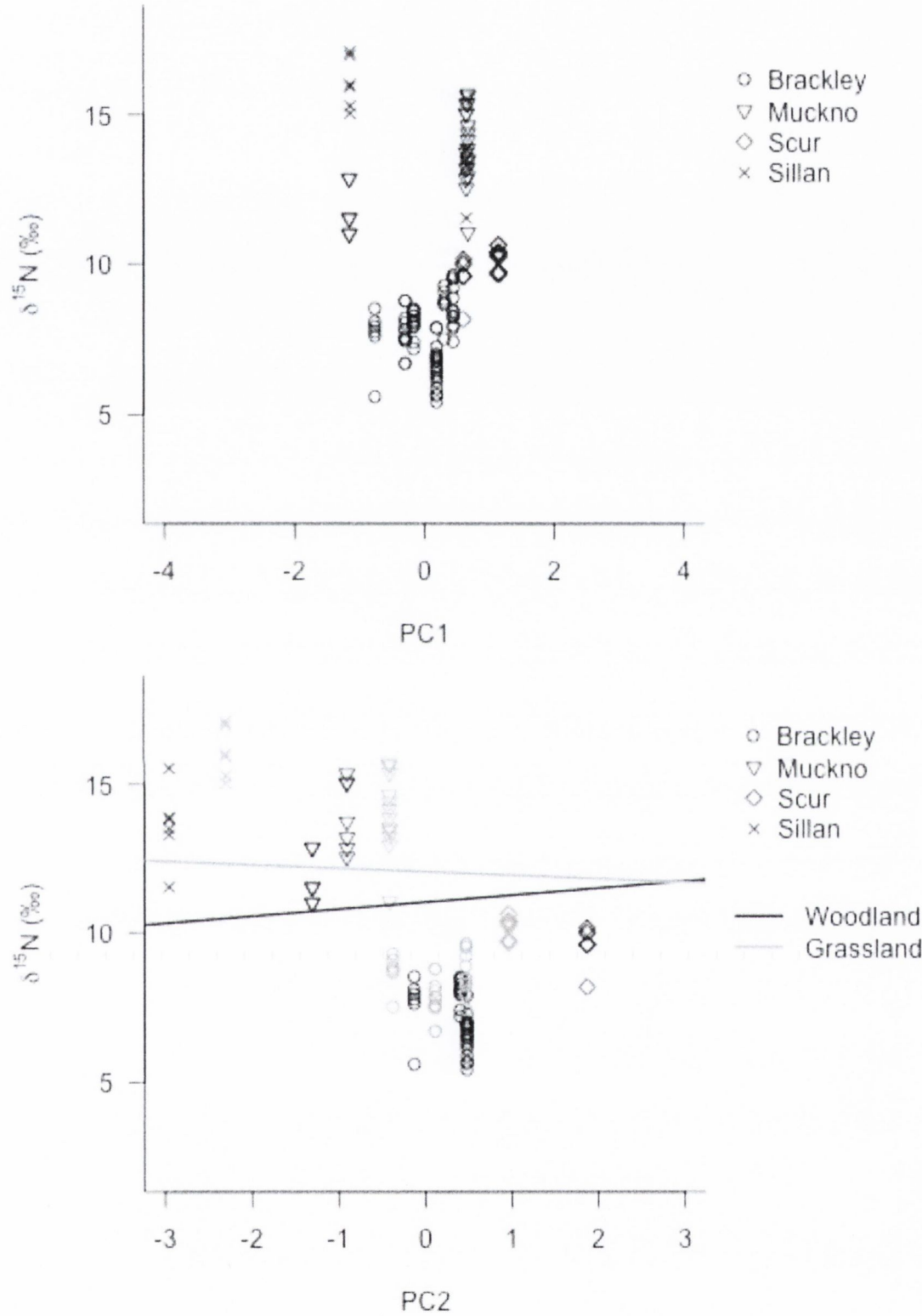
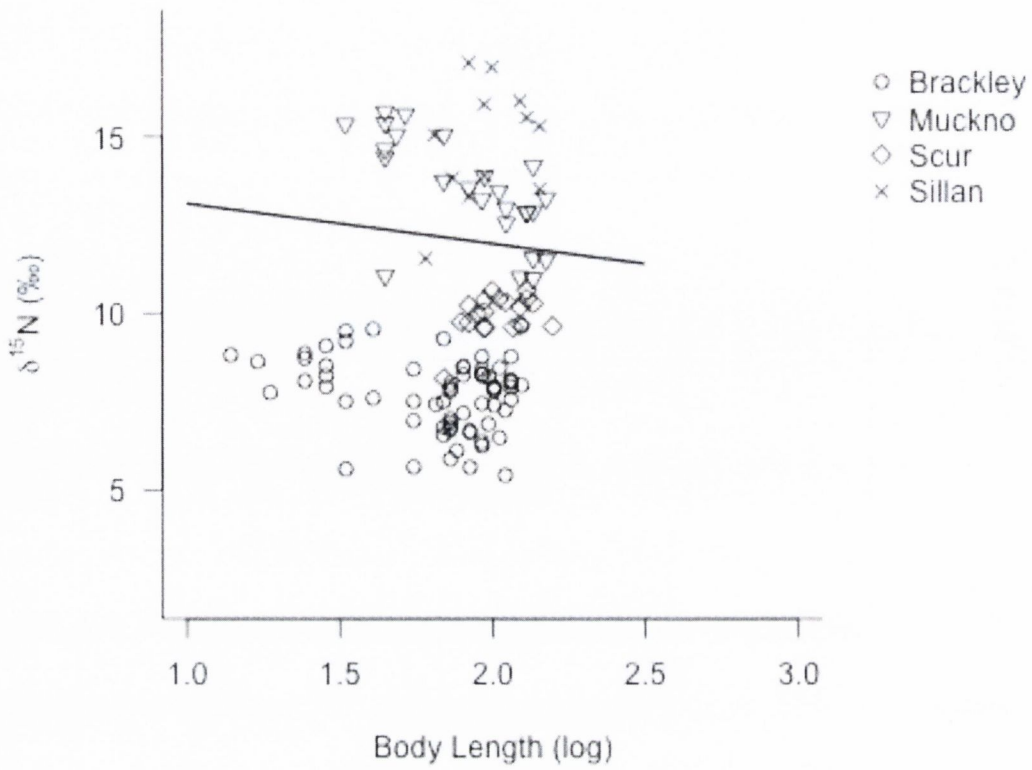


Figure 2.17 Distribution of  $\delta^{15}\text{N}$  signatures of *C. pseudogracilis* across four lakes along the PC1 (above) and PC2 (below) axes. Linear lines in the below figure visualise the model illustrating difference of  $\delta^{15}\text{N}$  signatures to the PC1 and PC2 gradients at different shoreline habitat types. (See Figure A 7 with the lake effect taken into account).





## 2.4 Discussion

### 2.4.1 Random (lake) effect vs. fixed effects

Lake random effect explained 5-9% of total variation of  $\delta^{15}\text{N}$  signatures of *A. aquaticus*, *G. duebeni* and *C. pseudogracilis*. Even though, 28% of total *G. duebeni*  $\delta^{13}\text{C}$  variation could be explained by the lake random effect, no or very low variation could be associated with the lake effect in the *A. aquaticus* and *C. pseudogracilis*  $\delta^{13}\text{C}$  models, after accounting for the fixed effects. In other terms, the variance of  $\delta^{13}\text{C}$  signatures were almost constant under the influence of other significant fixed effects (Crawley 2007). The significant negative PC1 main effect, with no interaction with the shoreline habitat type, for both species, indicates a consistent change of these species'  $\delta^{13}\text{C}$  signatures along the PC1 gradient. The same is true for the negative relation between the PC2 gradient and the  $\delta^{15}\text{N}$  signatures of *C. pseudogracilis*. However, the PC2 effect for the  $\delta^{15}\text{N}$  signatures of *A. aquaticus* differed significantly between the two shoreline habitats. The insignificant lake effect in *C. pseudogracilis* may also reflect its presence only in four lakes (Brackley, Muckno, Scur and Sillan) that were at the higher end of the PC1 ( $> 0$ , Figure 2.8), presenting a much narrower nutrient gradient among lakes when compared with the other two species. Similarly, Bergfur *et al.* (2009) also reported larger variations within species than within sites for both the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of invertebrates across 9 streams in Sweden.

### 2.4.2 Effects of nutrient gradient

#### 2.4.2.1 Species' $\delta^{13}\text{C}$ signature – nutrient enrichment relationship

PC1 main effect, which primarily represents the nutrient gradient (Figure 2.7) was retained in all final mixed-effects models and was significant based on species'  $\delta^{13}\text{C}$  signatures, indicating the importance of nutrient gradient for explaining species'  $\delta^{13}\text{C}$  variation across lakes (Figure 2.9, Figure 2.11 and Figure 2.15). The  $\delta^{13}\text{C}$  signatures of both *A. aquaticus* (Table 2.8) and *C. pseudogracilis* (Table 2.16) decreased along the PC1 gradient. On the other hand, PC1 main effect had an overall positive influence on *G. duebeni*  $\delta^{13}\text{C}$  signatures. The interaction between PC1 and the shoreline habitat type was significant only in the  $\delta^{13}\text{C}$  model of *G. duebeni*, which signified a more increasing effect of PC1 on woodland shoreline



habitats than grassland (Table 2.11). These results support the hypothesis that species'  $\delta^{13}\text{C}$  signatures vary along a nutrient gradient.

In theory, phytoplankton has depleted  $\delta^{13}\text{C}$  signatures as a result of sequestering aquatic  $\text{CO}_2$  more rapidly and discriminating against the heavier  $^{13}\text{C}$  (Finlay 2001). In contrast, periphyton display more  $\delta^{13}\text{C}$ -enriched signatures as a result of more restricted nutrient uptake because of a stronger boundary layer effect, especially in more stagnant waters (Finlay *et al.* 1999). Additionally, the  $\delta^{13}\text{C}$  signatures of the primary producers are much affected by the  $\delta^{13}\text{C}$  of DIC, which can vary depending on its origin. For example, DIC produced by organic matter decomposition can be  $\delta^{13}\text{C}$ -depleted (France 2000), whereas increase in lake primary production can lead to an increase in DIC  $\delta^{13}\text{C}$  and consecutively in the entire lake food web (Schindler *et al.* 1997). For example, an increase of periphyton  $\delta^{13}\text{C}$  has been observed with increased productivity associated with nutrient enrichment (Finlay 2001).

Since eutrophication can result in a decline of periphyton and macrophytes, representing a change in macroinvertebrate food sources, Vadeboncoeur *et al.* (2003) hypothesised that generalist macroinvertebrate species would modify their diets to reflect changes in the availability of food sources changing with increased nutrient concentrations. A study of 27 temporal lakes along an eutrophication gradient indicated a shift in the primary productivity from benthic to pelagic habitats with eutrophication, leading to an increase in the exploitation of phytoplankton by littoral macroinvertebrates in comparison with periphyton (Vadeboncoeur *et al.* 2003).

Following a similar hypothesis to Vadeboncoeur *et al.* (2003), Donohue (2008) tested the changes in the contribution of periphyton and seston to the diets of generalist littoral macroinvertebrates, including *G. duebeni* and *A. aquaticus*, across nine Irish lakes along a nutrient gradient. The results indicated depletion of macroinvertebrate  $\delta^{13}\text{C}$  signatures following an increase on seston reliance in high nutrient lakes (Donohue 2008). Similarly, results of my study suggest negative correlations of *A. aquaticus* and *C. pseudogracilis*  $\delta^{13}\text{C}$  with PC1 (Donohue 2008). In contrast, results of my study yielded conflicting results regarding the influence of nutrient enrichment on *G. duebeni*  $\delta^{13}\text{C}$ . Even though an initial visual inspection of data suggests depletion of *G. duebeni*  $\delta^{13}\text{C}$  along PC1 (Figure 2.11), the results of the minimal mixed effects model indicates an increase of  $\delta^{13}\text{C}$  signatures of *G.*



*duebeni* along the nutrient enrichment gradient (PC1). This contradiction can be partially explained by the high contribution of the lake random effect on the total variance (%28.49) of the minimal *G. duebeni*  $\delta^{13}\text{C}$  model, which was not displayed graphically in Figure 2.11 (but see Figure A 3 for the lake-corrected graph). However, it should be noted that a much smaller sample size (3 samples/species/lake) was used by Donohue (2008) compared with this present study.

Results of my study, however, suggest limitations of using common littoral macroinvertebrate  $\delta^{13}\text{C}$  isotopic signals to predict nutrient enrichment in Irish lakes. The response of macroinvertebrate isotopic composition to the nutrient enrichment gradient (PC1) was not consistent across species, which might reflect specific dietary needs of each species. Without the isotopic values of food sources, it was not possible to predict sources of the observed shifts in species'  $\delta^{13}\text{C}$  signals. However, France (1996) has suggested that separation of allochthonous and autochthonous food sources of consumers was not possible in eutrophic lakes owing to the overlap in the  $\delta^{13}\text{C}$  signatures between terrestrial detritus and those of periphytic algae. For example, no significant relationship was found between the  $\delta^{13}\text{C}$  signatures of various macroinvertebrates and nutrient enrichment across 9 streams in Sweden (Bergfur *et al.* 2009). Moreover,  $\delta^{13}\text{C}$  signatures could not discriminate between allochthonous and autochthonous production and the  $\delta^{13}\text{C}$  of many consumers were found outside the ranges of basal resource  $\delta^{13}\text{C}$  (Bergfur *et al.* 2009).

#### **2.4.2.2 Species' $\delta^{15}\text{N}$ signature – nutrient enrichment relationship**

Results based on the final models with  $\delta^{15}\text{N}$  values indicated a mixed response of species'  $\delta^{15}\text{N}$  signatures to PC1 gradient, primarily representing the nutrient enrichment indices. The models detected a significant effect of PC1 only on *A. aquaticus*  $\delta^{15}\text{N}$ , with significant interaction of PC1 with shoreline habitat type (Table 2.8). On the other hand, model results suggested no influence of nutrient enrichment on the  $\delta^{15}\text{N}$  signatures of neither *G. duebeni* nor *C. pseudogracilis*, as PC1 and its interactions with shoreline habitat type factor were eliminated in the final models (Table 2.12 and Table 2.15). Based on these findings, the initial hypothesis that species'  $\delta^{15}\text{N}$  signatures vary along a nutrient enrichment gradient was supported for *A. aquaticus*, but not for *G. duebeni* or *C. pseudogracilis*.

Generally, an increase of the  $\delta^{15}\text{N}$  of primary producer and their consumers is expected along a nutrient gradient (Vander Zanden *et al.* 2005, Borderelle *et al.* 2009, Bergfur *et al.* 2009). According to a meta-analysis of 36 lakes around the world, seasonal POM  $\delta^{15}\text{N}$  increased from oligotrophic to eutrophic lakes, which could be related to increasing primary productivity driven by phosphorus (Gu 2009). Increase of periphyton  $\delta^{15}\text{N}$  along a nutrient enrichment gradient has also been reflected in invertebrates and fish  $\delta^{15}\text{N}$  across nine boreal streams in Sweden (Bergfur *et al.* 2009). Moreover, increase in fish  $\delta^{15}\text{N}$  across of nine French lakes could be related to nutrient enrichment caused by increased anthropogenic activities in the lake catchments (Borderelle *et al.* 2009). According to a study across 27 Danish lakes spanning a range of trophic states, macroinvertebrate  $\delta^{15}\text{N}$  signatures not only reflected lake trophic status, but also riparian and watershed land use, indicative of nutrient sources to lakes (Vander Zanden *et al.* 2005) (also see 2.4.4).

The inability of the study to detect significant influences of PC1 gradient on the  $\delta^{15}\text{N}$  of *G. duebeni* and *C. pseudogracilis* can be related to the differential contribution of different nitrogen sources with distinct  $\delta^{15}\text{N}$  signatures into lakes. The  $\delta^{15}\text{N}$  signals in certain lakes were unexpectedly low for all species in Loughs Brackley and Scur. For example, in comparison with Muckno or Sillan, the  $\delta^{15}\text{N}$  of *A. aquaticus* and *C. pseudogracilis* were considerably depleted in Loughs Brackley and Scur, respectively, despite similar nutrient enrichment (Figure 2.10 and Figure 2.17). As pasture constitutes the highest percentage of land cover in the catchments of Brackley (49%) and Scur (36%), use of synthetic fertilisers with typically low  $\delta^{15}\text{N}$  signatures (-4 to 4‰, (Heaton 1986)) in the watersheds of these lakes might have contributed to the relatively low signatures in the  $\delta^{15}\text{N}$  baselines. At the same time, we can expect elevated  $\delta^{15}\text{N}$  signatures as a result of manure from farm animals in pastures. Also, Gu & Schelske (2010) found no significant relationships between trophic state indices (TP, TN or Chl-*a*) and particulate organic matter (POM)  $\delta^{15}\text{N}$  across 96 lakes in Florida, USA, which was explained by excessive atmospheric nitrogen fixation in nitrogen limited lakes. Even though nitrogen-fixing cyanobacterial blooms were shown to be the reason of depletion of POM  $\delta^{15}\text{N}$  in highly eutrophic lakes (Gu and Schelske 2010), its overall effect on the  $\delta^{15}\text{N}$  of a lake's higher trophic levels is questionable.



### 2.4.3 Effects of the PC2 gradient

The second principal component axis (PC2), primarily represented by the colour variable, was retained in all the models of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures for all species. Moreover, its interaction with shoreline habitat type was also significant in all models, except the *C. pseudogracilis*  $\delta^{13}\text{C}$  model. Overall results across models suggest a strong explanatory power of colour gradient. While not necessarily causal, these results indicate the importance of colour as a key-water chemical factor influencing macroinvertebrate  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures in Irish lakes. Many Irish lakes have high colour derived from peatland catchments, or breakdown of *in situ* organic matter (Jennings *et al.* 2006). Elevated levels of dissolved organic carbon and water colour in humic freshwaters influence the ecophysiology of autotrophs, reflected in their  $\delta^{13}\text{C}$  (France and Cattaneo 1998, France 1999), however more work is needed to understand their direct impact on macroinvertebrate isotopic signatures.

Water colour can have various direct and indirect effects on macroinvertebrates. Since water colour influences the availability of light that affects the growth of primary producers, increased water colour can affect grazers by inhibiting the growth of periphytic algae (Kullberg *et al.* 1993). Changes in the water colour could affect the behaviour of organisms, especially if they rely on visual cues for feeding or predator avoidance. For example, Wissel, Boeing & Ramcharan (2003) reported lower predation of midge larva, *Chaoborus* and other zooplankton by fish in lakes with high colour. Amphipods have sessile, apposition compound eyes, which form multiple inverted images (Hallberg *et al.* 1980). Also, when its similarities with the morphology of other arthropod eyes are considered, amphipod eyes are assumed to have good resolution and motion detection, and probably a vision of colour (Hallberg *et al.* 1980). Glazier & Deptola (2011) found a positive correlation between the amphipod *G. minus* eye size and the number of fish predators in freshwater springs in USA, suggesting increased eye size in amphipods could be associated with predator avoidance. However, as *G. minus* was mainly considered as a detritivore, the same study did not discuss the possible predatory behaviour of amphipods using visual cues (Glazier and Deptola 2011). Given that amphipods could be susceptible to light and rely on visual cues for predator avoidance and possibly for predation, an influence of water colour on amphipods' isotopic values, as a result of behavioural changes,



can also be expected. For example, invertebrates commonly hide among more complex habitats, such as dense macrophyte beds, in order to avoid fish predation (Macneil *et al.* 1999). However, further investigations are required to determine the importance of water colour on the ecology of amphipods and isopods.

High water colour can be a function of high land-derived organic matter which is typically depleted in  $\delta^{15}\text{N}$  (Peterson and Fry 1987). Peat, covering 16.5% of total land in Ireland, can be an important source of high dissolved organic carbon (DOC) in freshwaters and an indicator of the loading of allochthonous matter (Jennings *et al.* 2006). Among the study lakes, Loughs Scur, Fern and Akibbon, which had relatively high percentages of peat land in their catchments (23%, 35% and 45%, respectively), were also characterised with high water colour (422, 278 and 214  $\text{mg l}^{-1}$  PtCo, respectively) (Table 2.1). Even though colour can also correlate with nutrient enrichment, as a result of increased primary productivity of algae, this would depend on other factors, such as zooplankton grazing (Scheffer 1998). Therefore, high colour detected in Brackley and Muckno could result from a combination of peat in their catchment (9% and 2%, respectively), as well as high primary productivity, revealed by relatively high Chl-*a* measurements (16.3 and 20.5  $\mu\text{g l}^{-1}$ , respectively) (Table 2.1).

#### **2.4.3.1 Species' $\delta^{13}\text{C}$ signature – PC2 relationship**

The results of the mixed-effects models indicated that PC2 main effect was significant only for the *C. pseudogracilis*  $\delta^{13}\text{C}$  model, however its significant interaction with shoreline habitat type in *A. aquaticus* and *G. duebeni* models indicated its important explanatory power for  $\delta^{13}\text{C}$  variability. An overall negative slope PC2 was estimated for both *A. aquaticus* and *C. pseudogracilis*  $\delta^{13}\text{C}$ . On the other hand, a negative relationship of  $\delta^{13}\text{C}$  with PC2 was present for *G. duebeni* associated with only woodland shorelines. However, see Section 2.4.4 for distinct isotopic responses of samples based on shoreline habitats types.

The results regarding the negative relationship between  $\delta^{13}\text{C}$  signatures and colour gradient are generally in concordance with the findings of some earlier studies (France and Cattaneo 1998, France 2000, Grey *et al.* 2000). Grey *et al.* (2000) also found a significant  $\delta^{13}\text{C}$  depletion of particulate organic matter (POM) with increased colour across 26 UK lakes, even though no significant relationship was apparent between POM and zooplankton  $\delta^{13}\text{C}$  (Grey *et al.* 2000). On the other

hand, reduced  $\delta^{13}\text{C}$  signatures were also recorded in leeches in lakes with increased DOC levels (France 2000). Moreover, France & Cattaneo (1998) reported significantly negative relationships of benthic algal  $\delta^{13}\text{C}$  with water colour in a multi-stream study in Canada. Similarly, an inverse relationship was observed between the  $\delta^{13}\text{C}$  values of epilithic algae and dissolved organic carbon (DOC) in lake littoral zones (France 1999). Water colour has been associated with high dissolved organic carbon (DOC) as a result of microbial decomposition of largely terrestrial organic matter (France & Cattaneo 1998). Thus, reduced macroinvertebrates  $\delta^{13}\text{C}$  signatures in high coloured lakes can be related to increased levels of recycled  $\text{CO}_2$  (produced by the microbial breakdown of terrestrial or aquatic organic matter) (France & Cattaneo 1998), which can be carried to higher trophic levels after assimilation by primary producers.

#### **2.4.3.2 Species' $\delta^{15}\text{N}$ signature – PC2 relationship**

Minimal mixed effect models for  $\delta^{15}\text{N}$  indicated contrasting results for different species regarding PC2. The PC2 main effect was found significant only for *G. duebeni*, while its interaction with shoreline habitat type was significant for both *A. aquaticus* and *G. duebeni*. Results showed an overall significant negative effect of PC2 on the  $\delta^{15}\text{N}$  of *G. duebeni* for both shoreline types and an overall positive effect on the  $\delta^{15}\text{N}$  of *A. aquaticus*, with a greater increase associated with woodland shoreline type in comparison with grassland. On the other hand, results suggested a decreasing effect of PC2 on *C. pseudogracilis*  $\delta^{15}\text{N}$  signatures associated with grassland shorelines and an increasing effect in association with woodland shorelines.

In concordance with my findings, Gu & Schelske (2010) reported a negative correlation between water colour and  $\delta^{15}\text{N}$  of particulate organic matter (POM) across 96 lakes in Florida, USA. Moreover, both for *A. aquaticus* and *G. duebeni*, grassland habitats were associated with more depleted  $\delta^{15}\text{N}$  signatures with increasing water colour, in comparison with woodland shorelines. In contrast, *C. pseudogracilis*  $\delta^{15}\text{N}$  demonstrated a positive relationship with the PC2 gradient, while enrichment effect of the colour gradient was significantly different between the  $\delta^{15}\text{N}$  of *C. pseudogracilis* associated with woodland and grassland shorelines.

According to Gu & Schelske (2010), nutrient effect can be more important for the  $\delta^{15}\text{N}$  in low colour lakes, while colour becomes more important in nutrient



enriched lakes as light becomes a limiting factor for phytoplankton. Thus, the substantial difference of 7.1‰ in the mean  $\delta^{15}\text{N}$  signatures of *A. aquaticus* between Loughs Carrigeencor and Sillan, lakes with very close PC2 but dissimilar PC1 scores (Figure 2.10), can be attributed to differences in their trophic state. On the other hand, differences of the mean *A. aquaticus*  $\delta^{15}\text{N}$  signatures among Loughs Scur and Akibbon or Fern, lakes with distinct trophic state and higher colour gradient (high PC2 scores), were much smaller (1.1‰) (Figure 2.10).

#### 2.4.4 Effects of shoreline habitat types

The results of the mixed-effects models revealed that the shoreline habitat type had a significant effect on only the  $\delta^{13}\text{C}$  signatures of *G. duebeni* and on the  $\delta^{15}\text{N}$  signatures of all three species of macroinvertebrates. Additionally, the influence of shoreline habitat type on *A. aquaticus*  $\delta^{13}\text{C}$  signatures was revealed through its significant interaction with PC2. On the other hand, the shoreline habitat type main effect and its interactions with both PC axes were eliminated in the final *C. pseudogracilis* model for  $\delta^{13}\text{C}$ , suggesting no evidence for the influence of shoreline habitats on *C. pseudogracilis*  $\delta^{13}\text{C}$  signatures.

Overall, results of my study suggest the importance of shoreline habitat structures, particularly on the isotopic composition of *G. duebeni* and more on the  $\delta^{15}\text{N}$  isotopic signatures of littoral consumers used in the study than  $\delta^{13}\text{C}$ , which partially supports my second hypothesis. Even though interpreting model results with several significant interaction terms can be complex, their retention in the model selection process indicates their importance in explaining variation in isotope signatures. Furthermore, the estimated slopes and intercepts indicate that responses of different species' isotopic signatures to different shoreline habitat types varied across PC1 and PC2 gradients through interactions.

The current study suggested detectable differences among macroinvertebrate isotopic signatures between woodland and grassland shorelines, despite possible variations in vegetation characteristics or intensity of land use in the shoreline habitats. In addition to water chemistry variables, using more detailed land use data can enhance the predictability of influences of specific anthropogenic activities in shoreline habitats. For example, in a comparative study of macroinvertebrate  $\delta^{15}\text{N}$  signatures across 26 Danish lakes along a nutrient gradient, Vander Zanden *et al.* (2005) found that  $\delta^{15}\text{N}$  could reflect lake trophic



state and total nitrogen loading, however models including riparian zone and watershed land use characteristics had a higher explanatory power to explain the overall variance. In the same study, land use variables in the best performing model included number and density of the farm animals in the watershed, indicating the importance of manure as an important driver of the  $\delta^{15}\text{N}$  enrichment in agricultural landscapes (Vander Zanden *et al.* 2005). In contrast to my study,  $\delta^{15}\text{N}$  signatures could not distinguish the developed and undeveloped sites in a deep oligotrophic lake in USA, mainly because of the mixed signal of fertilizer and human sewage, with low and high  $\delta^{15}\text{N}$  values, respectively (Rosenberger *et al.* 2008).

In particular, the significant effect of 'PC2 x shoreline habitat' interaction has been consistent across all models, except for *C. pseudogracilis*, suggesting different response of isotopic values of *A. aquaticus* and *G. duebeni* to shoreline habitats along the colour gradient. Because of the close relationship between colour and DOC, we can predict different contributions of DOC at sites associated by woodland and grassland habitats.

#### 2.4.5 Effects of body size

The study results indicated significant effects of individual's body length on the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of *G. duebeni* and *C. pseudogracilis*, supporting my third hypothesis. On the other hand, no significant influence of body length main effect was detected on the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of *A. aquaticus*. There was a positive effect of body length on the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of *G. duebeni*. Unexpectedly, a negative relationship was observed between body length and both isotopes of *C. pseudogracilis*. In contrast, Doi *et al.* (2010) could not detect a significant effect of the shell size on the within-species isotopic signal variation of gastropod, *Lemnaea stagnalis*. Also, the  $\delta^{15}\text{N}$  for various ontogenetic stages did not differ significantly among benthic invertebrate species, including *Echinogammarus ischnus*, *Hyalella Azteca* and *Gammarus fasciatus* (Limén *et al.* 2005).

Changes in the isotopic signature of *G. duebeni* suggest a dietary shift and possibly differential habitat use of individuals with varying body lengths. When the predatory nature of *G. duebeni* is considered, it is not surprising to detect body-size related  $\delta^{15}\text{N}$  enrichment, indicating increased trophic status with size. Results

from my study support findings of McGrath *et al.* (2007) on size-related dietary shift of *G. pulex* based on laboratory experiments. The same study highlighted the importance of habitat complexity for avoiding cannibalism of large over small individuals, as the survival of smaller *G. pulex* increased in small sized-substrates, acting as refuges (McGrath *et al.* 2007).

*Crangonyx pseudogracilis* is commonly considered herbivorous (Gledhill *et al.* 1993, MacNeil *et al.* 2013) and body sizes of male *C. pseudogracilis* are known to be smaller when compared with females (Gledhill *et al.* 1993). However, knowledge in the dietary preferences of *C. pseudogracilis* at different life stages is mostly unknown. However, results based on the Chapter 3 of the current study suggesting significantly higher  $\delta^{15}\text{N}$  isotopic values of *C. pseudogracilis* compared with *Gammarus spp.* or *A. aquaticus* may suggest some form of predatory diet. The predatory nature of other *Crangonyx spp.* has been reported by earlier studies (Schwartz 1992, Taylor and Batzer 2010) (also see Section 3.4.2 for a further discussion on species' trophic relationships). Nevertheless, a negative significant relationship between body size and  $\delta^{15}\text{N}$  isotopes is unexpected for a predatory species. These findings suggest a need for further studies on the diet of *C. pseudogracilis* and a possible dietary shift with ontogenetic stages.

## 2.5 Conclusions

The aim of this study was to evaluate the differences of carbon and nitrogen isotope ratios of common littoral invertebrates, *A. aquaticus*, *G. duebeni* and *C. pseudogracilis* between sites adjacent to woodland and grassland dominated shoreline habitats along a nutrient enrichment gradient. Second, assessment of the effect of individual body size on the variation of species' carbon and nitrogen isotope ratios was done, after accounting for the environmental variation across lakes. The following conclusions can be drawn based on the results of this study:

- No or very low variation could be associated with the lake effect in the *A. aquaticus* and *C. pseudogracilis*  $\delta^{13}\text{C}$  models, after accounting for the fixed effects. This signifies a consistent change for the  $\delta^{13}\text{C}$  of *C. pseudogracilis* and *A. aquaticus* along the nutrient gradient.
- Shoreline habitat type had significant effects on both the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of *A. aquaticus* and *G. duebeni*, and on the  $\delta^{15}\text{N}$  signatures of *C.*



*pseudogracilis*. However, the response of isotopic signatures to different shorelines varied among species.

- There was a varied response of species'  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures to nutrient enrichment, suggesting limited ability of littoral macroinvertebrate isotope signatures to act as indicators of nutrient enrichment in the studied lakes. There was a negative effect of nutrient enrichment on *A. aquaticus* and *C. pseudogracilis*  $\delta^{13}\text{C}$  signatures, irrespective of the shoreline type, while there was an overall positive relationship between the nutrient gradient and the *G. duebeni*  $\delta^{13}\text{C}$  values. No influence of nutrient enrichment gradient on *G. duebeni* or *C. pseudogracilis*  $\delta^{15}\text{N}$  signatures could be detected.
- Colour gradient (PC2 axis), either on its own or as an interaction with shoreline habitat were highly important in all models, signifying the importance of colour for littoral consumer  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures in Irish lakes. Generally, there was a negative effect of the colour gradient on species'  $\delta^{13}\text{C}$  signatures. The response of  $\delta^{15}\text{N}$  signatures to the PC2 gradient varied among species, such that they increased for *A. aquaticus* and decreased for *G. duebeni*.
- Body length was significant in explaining species' isotopic variation of *G. duebeni* and *C. pseudogracilis* within lakes, with species-specific responses. While both isotopic signatures increased along a body size gradient of *G. duebeni*, these were negatively associated with body length of *C. pseudogracilis*.





### **3. TROPHIC RELATIONSHIPS REVEALED BY ISOTOPIC NICHE METRICS**





### 3.1 Introduction

Amphipod crustaceans are common in freshwater bodies in Ireland throughout the year, with *Gammarus* spp. (Order Amphipoda) constituting a high percentage of total macroinvertebrate abundance and biomass (Macneil *et al.* 1997, Free *et al.* 2006). *Gammarus duebeni celticus* (referred as *G. duebeni* in rest of chapter) is an endemic freshwater amphipod species in Ireland (Piscart *et al.* 2007), also found in Britain, Isle of Man and Western Brittany (Piscart *et al.* 2007, Dick 2008). *Gammarus pulex* is the most widespread freshwater amphipod in mainland Europe and Britain and most abundant one in Britain. It has major importance as a food for fish (Welton 1979, Mayer *et al.* 2012). Even though native to Europe and Britain, *G. pulex* did not arrive in Ireland until the 1950s, gradually replacing *G. duebeni* in many Irish rivers and lakes (Strange and Glass 1979). *Crangonyx pseudogracilis* is another non-native amphipod species, originally from North America, that arrived in Ireland within the last century (Costello 1993). *Gammarus pulex* and *C. pseudogracilis* are identified as invasive species according to the Irish National Invasive Species Database (NBDC 2010a, 2010b) and they were listed among established high impact invasive species in Ireland ((Minchin 2007). The native and invasive amphipods, including *G. duebeni*, *G. pulex* and *C. pseudogracilis*, have been reported occurring together in the same general locality across rivers and lakes in Northern Ireland (Dick 1996). This strongly implies the interaction of these amphipod species through overlapping habitats, predation and competition (Dick 1996). However, difficulty in distinguishing preserved specimens of *C. pseudogracilis* from juvenile *Gammarus* spp. (Dick *et al.* 1999) might have lead to under-documentation of the former species in Ireland. No recordings of *C. pseudogracilis* was found in some recent nation-wide surveys of lakes in the Republic of Ireland (Free *et al.* 2006, Donohue 2008).

Even though *Gammarus* spp. are mainly described as shredders, they can have other functional roles including collector-gatherers, predators or generalist-detritivores in a variety of freshwater habitats (Macneil *et al.* 1997). Documented by a wide range of studies, the dietary preferences of *G. pulex* includes algal filaments (Agrawal 1965, Moore 1975), decaying as well as fresh leaf material (Welton 1979, Graca *et al.* 1994a, 1994b) and live prey comprising *Asellus aquaticus* (Bollache *et al.* 2008), mayfly nymphs (Kelly *et al.* 2002) and the amphipods *G. duebeni* and *C. pseudogracilis* (Dick 1996, MacNeil *et al.* 2003). Mouthpart

morphology of *G. pulex* supports a view that it is an omnivore (Mayer *et al.* 2012). A lack of evidence for spatial separation of the two gammarid species suggest that the decline of *G. duebeni* in Ireland could result from competition for food and intraguild predation by *G. pulex* (Piscart *et al.* 2011). In an early study by Hynes (1954), the dynamics between the *G. duebeni* and *G. pulex* was described as interspecific competition and the two species as ecological equivalents. Laboratory studies by Dick, Montgomery & Elwood (1993) supported evidence for *G. pulex* predation on *G. duebeni* as a mechanism to explain the invasion success of the former species over the latter. It was demonstrated that especially male *G. duebeni* were more frequent prey of male *G. pulex* predators, while females of both species were subjected to predation by the larger sized males of either species (Dick *et al.* 1993).

*Crangonyx pseudogracilis* has a biotic potential (the number of offspring produced in a year by a typical female) that is 30 times greater than *G. duebeni* and 10 times that of *G. pulex* (Hynes 1955). Unlike other amphipod species, *C. pseudogracilis* also demonstrate active brood care of eggs, which involves females cycling the eggs and ejecting dead embryos within their external brood pouch (Dick *et al.* 1998). Despite these traits, *C. pseudogracilis* does not seem to be as strong a competitor against native *G. duebeni* as *G. pulex*, and its prevalence could relate to greater tolerances of pollution by *C. pseudogracilis*; it appears not to occur in high quality waters where *Gammarus spp.* are abundant (Holland 1976). Nevertheless, a transplant field experiment by Macneil, Dick & Elwood (2000) showed that *C. pseudogracilis* was able to colonise high quality waters as well as *G. pulex* and *G. duebeni*, while *Gammarus spp.* were less capable of surviving in low water quality sites dominated by *C. pseudogracilis*. *Crangonyx pseudogracilis* was also recorded together with other *Gammarus spp.* at certain sites of the same freshwater systems including Lough Neagh in N. Ireland (Dick 1996) and rivers in Isle of Man (MacNeil and Dick 2011). Laboratory experiments testing the predatory interactions among the *Gammarus spp.* and *C. pseudogracilis* revealed high predation of smaller sized *C. pseudogracilis* by *G. pulex*, *G. duebeni* and *G. tigrinus* (Dick 1996). For example, 33% of laboratory populations of *C. pseudogracilis* were eliminated by *Gammarus spp.* within 12 h (MacNeil *et al.* 1999).



### 3.1.1 Isotopic niche approach

While previous studies highlighted the importance of biotic interactions such as predation and parasitism influencing the co-existence and exclusion of the native and invasive amphipod species in Ireland, alongside abiotic factors such as nutrient concentrations and habitat utilisation, detecting interspecific competition between these species remains a challenge (Dick 2008). An alternative way of looking into the competitive interactions of these gammarid species is to compare species isotopic niches using stable isotope analysis (SIA) (Newsome *et al.* 2007). One advantage of stable isotope techniques is that they integrate time and space into an organism's trophic ecology (Layman *et al.* 2007a), whereas the more conventional gut content analysis provides only a snapshot of food items recently ingested (Hyslop 1980). As a practical example, Piscart *et al.* (2011) reported that isotopic signatures were more efficient than gut content analysis in detecting diet shifts of coexisting gammarid species. Gut contents of *G. duebeni* and *G. pulex* in single populations were very similar and consisted mainly of inorganic material, whereas stable isotope signals revealed a wider spectrum of food and diversity of carbon sources assimilated by *G. pulex* than *G. duebeni* (Piscart *et al.* 2011).

Carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) are naturally occurring stable isotopes, commonly used to study the food web structure (Layman *et al.* 2012) and the trophic niche of organisms (Newsome *et al.* 2007). While  $\delta^{13}\text{C}$  is commonly used for tracing spatial and temporal variability in organic matter (basal) sources to freshwater ecosystems (Vander Zanden and Rasmussen 1999),  $\delta^{15}\text{N}$  can be used for estimating the trophic level of consumers within food webs (Cabana and Rasmussen 1996). Owing to differential digestion or fractionation of food during assimilation and metabolism, stable isotope ratios can change between diet of consumers along trophic chains (McCutchan *et al.* 2003). The  $\delta^{13}\text{C}$  isotopic ratio of food sources is passed on to the consumers only with minor enrichment ( $\sim 0.7\text{‰}$ ), whereas there is a higher enrichment in the  $\delta^{15}\text{N}$  ( $\sim 3.5\text{‰}$ ) between consumers and their diet (Peterson and Fry 1987). Different macroinvertebrate food sources are shown to carry distinct  $\delta^{13}\text{C}$  values. Examples include phytoplankton ( $-32$  to  $-23\text{‰}$ ) and terrestrial plants ( $-27$  to  $-13\text{‰}$ ) (Finlay and Kendall 2007). Such variation in the  $\delta^{13}\text{C}$  of the food sources is expected to lead to variation in the  $\delta^{13}\text{C}$  of their macroinvertebrate consumers. On the other hand, variable ratios of  $\delta^{15}\text{N}$  isotopic



ratios of consumers can be attributed to different trophic levels in a food web (Cabana and Rasmussen 1996).

Carbon and nitrogen isotope data is commonly presented in  $\delta^{13}\text{C}$  -  $\delta^{15}\text{N}$  bi-plots. The plotted mean stable isotope signatures have been used to gain quantitative information related to food web structure, including trophic positions, relative contributions of prey items to consumer species, niche shifts, and intra-specific diet variability (Layman *et al.* 2007a). The dimensional space occupied by two or more isotopic signatures is called the  $\delta$ -space (Cummings *et al.* 2012). The isotopic niche of a species or a population, defined as the area in the  $\delta$ -space, is comparable to a subset of the n-dimensional hypervolume suggested by Hutchinson (1957) to define the ecological niche (Newsome *et al.* 2007). Since both diet sources and habitat utilisation are incorporated in the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic values, the isotopic niche is expected to align closely with the ecological niche (Newsome *et al.* 2007). Variance of stable isotope ratios has been used to compare trophic niche width (Bearhop *et al.* 2004). Dispersion in the distribution of points in  $\delta$ -space can estimate total niche width, but is also dependent on the distance between the isotopic values of the available food sources (Newsome *et al.* 2007). Isotopic similarity does not always mean ecological similarity as two individuals with similar isotopic niche may have distinct ecological niches (Layman *et al.* 2012), whereas non-similar isotopic niches between two individuals are more likely to represent non-overlapping ecological niches.

### 3.1.2 Chapter objectives

This study aims to quantify the competitive overlap of three species, one native (*G. duebeni*) and two invasive (*G. pulex* and *C. pseudogracilis*) amphipod species common to Irish freshwater systems, using stable isotopes. Three lakes with different combinations of species were studied (see Table A 8 in Appendix III for species abundances at the meso-habitat scale across sampling sites). In the first lake (Lough Fern), the native *G. duebeni* is the only amphipod species. In the second lake (Brackley Lough), native *G. duebeni* coexists together with the invasive *C. pseudogracilis*, which is widespread throughout the lake and with *G. duebeni*, which is limited to only a few sites. In the third lake, the native *G. duebeni* is presumed extinct, *G. pulex* is the dominant species, while the distribution of *C. pseudogracilis* is restricted to few sites. Owing to its wide distribution and common

presence in Loughs Fern, Brackley and Muckno, as well as to its functional similarities to amphipods, an isopod species, *Asellus aquaticus* was used as a reference species in the comparison of these three lakes.

There are four objectives to the study. The first is to estimate overlap in species' isotopic niches, using the variance of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic ratios and classical analysis of variance (ANOVA). Significant differences in  $\delta^{13}\text{C}$  ratios would indicate species feeding on distinct food sources and/or feeding in different habitats, whereas significantly different  $\delta^{15}\text{N}$  ratios indicates different trophic positions in the food web.

The second objective is to measure species' isotopic ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) dispersion in isotopic space within each lake.  $\delta^{13}\text{C}$  dispersion is used as a function of diversity of the basal food sources, enabling a comparison of species' generalist feeding habits. On the other hand,  $\delta^{15}\text{N}$  dispersion is considered indicative of trophic diversity and omnivory as it represents the vertical structure in a food web.

The third objective is to quantify species' isotopic niche widths to compare competitive niche overlap among species within and across lakes. Moreover, across-lake comparisons provide quantified measure of changes in the niche widths of single species and their overlaps in each lake.

The fourth objective is to measure relative isotopic distances and positions among species. These two newly identified isotopic niche characteristics can be used as additional metrics for separating species' isotopic niches. Even though niche width,  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  dispersion could be the same for any two species, the isotopic space can be used to distinguish ecological niches.

I hypothesise that 1) there will be a contraction in species' isotopic niche width when species occur together and 2) that differences in species' isotopic niches can be revealed through comparison of their relative distance and positioning in isotopic space.



3.2 Methods

3.2.1 Study lakes

Loughs sampled for this study (Lough Fern, Brackley Lough and Lough Muckno) are situated in the northern part of the Republic of Ireland (Figure 3.1). Their total phosphorus (TP) concentrations ranged between 20-45  $\mu\text{g/L}$ . The sampling took place in September 2009. See the Section 2.2.1 for a more detailed description on lake selection criteria and Figure 3.2 for the detailed site locations at each study lough. Shoreline habitat type, habitat integrity and accessibility influenced the site selection within each lake. Sampling locations included sites dominated by three woodland and three grassland shoreline habitats. Site locations of the same shoreline type were spatially separate from each other as much as possible to increase probability of independence of samples.

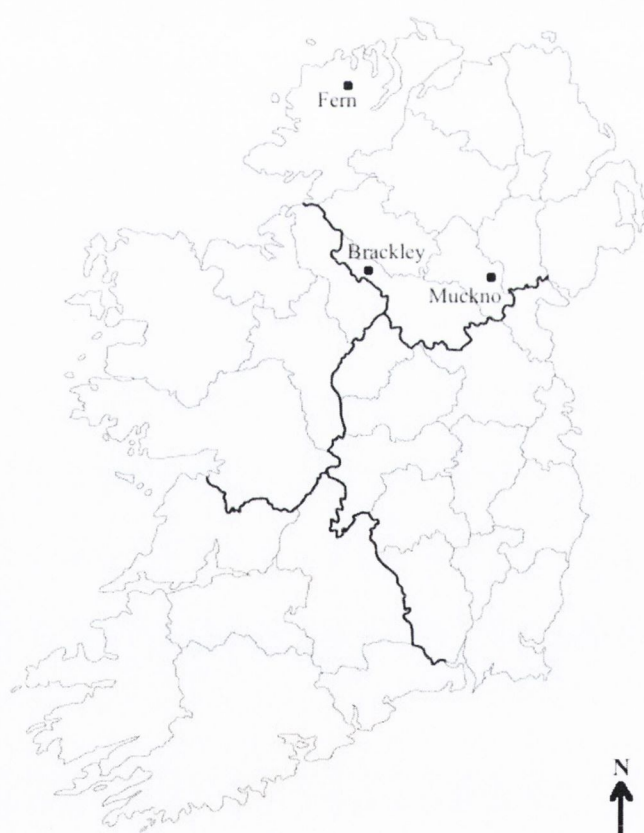


Figure 3.1 Locations of sampling loughs used in this study on the map of Ireland. (Blank map of Ireland by Russell (2010)).





Figure 3.2 Locations of the six sampling sites in Loughs Fern (a), Brackley (b) and Muckno (c). Symbols of tree and grass represent the woodland and grassland shoreline habitats, respectively. See Table A 8 (Appendix III) for a full list of species abundances at each site. (Map source: Google 2014).

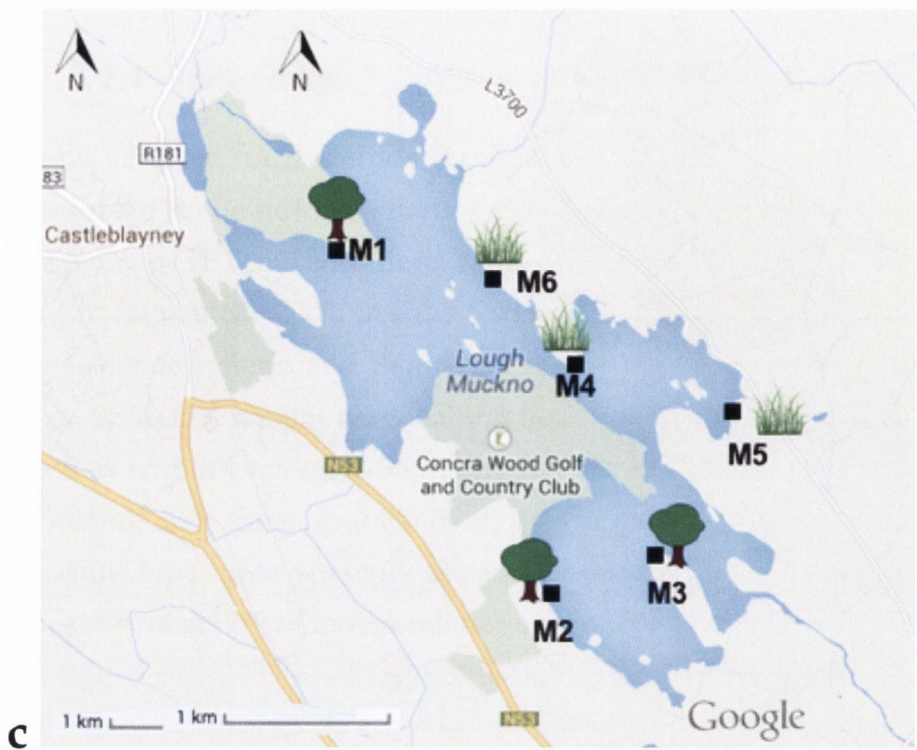


Figure 3.2 (continued) Locations of the six sampling sites in Loughs Fern (a), Brackley (b) and Muckno (c). Symbols of tree and grass represent the woodland and grassland shoreline habitats, respectively. See Table A 8 for a list of species abundances across sites. (Map source: Google 2014).

### 3.2.2 Macroinvertebrate sampling

Macroinvertebrate samples for the stable isotope analysis (SIA) were collected from the littoral zone at six sites adjacent to either woodland or grassland shoreline habitats (but see Section 3.2.4.1), using kick sampling. Common tree species found in the wooded shorelines and the characteristics of littoral habitats were recorded in broad categories, based on substrates and macrophyte availability (Table A 8, Appendix III).

At each site, a maximum of three macroinvertebrate samples were collected when different habitat patches existed. If inspection of the littoral zone indicated a homogeneous habitat, usually one (in most cases in Lough Fern) and sometimes two samples (one from near and another away from the shore) were collected. After draining the water using a metal sieve (500  $\mu\text{m}$ ), invertebrate samples were put into clean plastic bottles and frozen upon collection using dry ice. They were



transferred to a freezer on arrival back to the laboratory and kept frozen at -40°C until processed for SIA, following Donohue (2008).

### 3.2.3 Stable isotope analysis

Frozen macroinvertebrates samples were thawed to room temperature before being sorted in the laboratory. Species were identified using a stereomicroscope, based on (Gledhill *et al.* 1993). Species' abundances, based on samples from individual littoral habitats were recorded. Even though juvenile individuals were counted with adult individuals for the recording of abundances (Table A 8, Appendix III), they were not included in the analysis, because they were below the minimum adequate mass needed for the SIA. Similarly, damaged adult individuals were discarded from the analysis, owing to concerns that processes of decay could affect isotopic signals. Hence, samples where no, or very few, adults, despite high number of juveniles, were not included in the analysis (Table A 8, Appendix III). A random sequencing website (Haahr 2011) was used to select a minimum of six individuals from each littoral habitat for SIA. Prior to SIA, samples were dried at 60°C for 24 h in clean petri dishes in a dehydrator. Individual animals were ground into a fine powder using a marble pastel and mortar. Subsamples of 0.6-0.9 mg tissue of individual animals were weighted into tin capsules using a microbalance (Sartorius MC-5 with 1 µg readability), folded into tin balls and stored in a desiccator until analysis. See Section 2.2.4 for further details of sample preparation for the stable isotope analysis.

Carbon and nitrogen stable isotope analyses for all samples were performed on Thermo Delta<sup>plus</sup>® CF-IRMS (continuous flow isotopic ratio mass spectrometer) coupled to the CE Instruments 1112 Flash Elemental Analyser by Conflo III Interface, which was operated by ISODAT software and based in the Geochemistry Laboratory at Trinity College Dublin (Dublin, Ireland). Initial carbon and nitrogen isotope ratios were recorded relative to primary standards that were Pee Dee Belemnite (PDB) and atmospheric air (VAIR), respectively. Isotope values were expressed as relative difference per mil (‰) and in the standard delta (δ) notation:

$$\delta X = (R_{\text{sample}}/R_{\text{standard}}) - 1) \times 1000; \text{ where } X = {}^{13}\text{C} \text{ or } {}^{15}\text{N}, \text{ and } R = {}^{13}\text{C}/{}^{12}\text{C} \text{ or } {}^{15}\text{N}/{}^{14}\text{N}.$$



Initial  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotope values were transformed before further calculations to account for differences among lakes in isotopic values. First, the centroids in  $\delta^{13}\text{C}$  -  $\delta^{15}\text{N}$  niche space was calculated as the mean of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for all individuals of a species in each lake (Piscart *et al.* 2011). Then, data for each lake were transformed such that the centroid value (mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of *A. aquaticus* in each lake matched with zero, since it is the only species common to all three lakes. Data transformation included subtraction of all invertebrate  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values from the mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (centroid) values of *A. aquaticus* in each lake, respectively. Standard ellipses and isotopic metrics were based on the transformed  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (see Section 3.2.4.2).

### **3.2.4 Data analysis and statistics**

#### **3.2.4.1 Isotopic variation among species**

One-way analysis of variances (ANOVAs) were used to test the variance of transformed  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopes among species within each lake, followed by Tukey's HSD multiple comparison test when significant differences were found. Looking at the variance of isotopic values is a classical approach for identifying major distinction between species' isotopic niches. Significant differences among mean macroinvertebrate  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic values among species indicate a likely separation among their carbon food sources ( $\delta^{13}\text{C}$ ) and trophic positions ( $\delta^{15}\text{N}$ ). Non-overlapping isotope values imply separation of trophic niches between species, but overlapping isotope values not necessarily mean same trophic niches. In order to account for the unbalanced study design, ANOVAs were calculated on the basis of type III Sums of Squares (SS), using the "car" package (Fox and Weisberg 2011) in R 2.14.1 and the Tukey's HSD tests were performed through the "agricolae" package (de Mendiburu 2012), also in the same version of R (R development core team, 2012).

The initial sampling of macroinvertebrates used in the current chapter was designed to compare the sites adjacent to woodland and grassland shoreline habitats, mainly for testing the hypotheses in Chapter 2 (species' isotopic signals would reflect differences in riparian habitats within lakes) and Chapter 4 (elemental stoichiometry species would demonstrate significant differences between the woodland and grassland shoreline habitats within lakes). In order to

quantify the competitive overlap of the study species effectively, as posed in the aims and the hypotheses of this chapter (Section 3.1.2), species sampled from all sites of each lake were aggregated. This facilitated the comparison of species' trophic relationships at the whole-lake level and due to the higher number of samples per species in each lake, it provided for a more reliable analysis (see below for Section 3.2.4.2).

### 3.2.4.2 *Standard ellipse and isotopic niche metrics*

Layman *et al.* (2007b) proposed a set of six quantitative metrics for studying the intraspecific trophic structure using isotope data. Among these metrics the total area of the convex hull (TA), which was calculated using the most extreme data points of a convex hull in the isotope bi-plot, provides a direct measure of population niche width (Layman *et al.* 2007a). Alternative to Layman's convex hull (Layman *et al.* 2007b), Jackson *et al.* (2011) proposed using standard ellipses (SE), which is used for bivariate data and comparable with standard deviation (SD) of univariate data (Batschelet 1981). A standard ellipse covers approximately 40% of the data independent of the sample size (Batschelet 1981) and plotting one involves the calculation of its covariance matrix, which provides the semi-major ( $a$ ) and the semi-minor axes ( $b$ ) and the angle of  $a$  with the x-axis (Jackson *et al.* 2011) (Figure 3.3). The covariance matrix of a standard ellipse, defines its shape and size, while the means of the bivariate data define its location (Jackson *et al.* 2011).

In this study, we adopted the six isotopic niche metrics based on standard ellipses (Jackson *et al.* 2011) (Table 3.1). The first three metrics measure the extent of niche space occupied by single species, such as standard deviations (SD) of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopes, and standard ellipse area (SEA). The last three metrics define the niche space occupied commonly by two species; overlapping SEA, relative distance and relative angle between species. Among these six metrics, SEA has been used in previous studies (Jackson *et al.* 2012, Syväranta *et al.* 2013), while the other five metrics are novel, (e.g. 'Overlapping SEA<sub>C</sub>' for its capacity to assess the trophic interactions between two species even when they occupy spatially distinct habitats). Additional to the SIBER approach (Jackson *et al.* 2011), which suggests an avenue for comparing multiple isotopic niche spaces using standard ellipses,



the ‘relative distance and relative angle’ metrics suggested in this study provides information on the shape and the positioning of one ellipses relative to another.

Table 3.1 List of isotopic niche metrics, their units and what they indicate in the study

|   | Isotopic Niche Metric                         | Unit                       | Indicator                                                                                                                                                                                              |
|---|-----------------------------------------------|----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1 | Standard Deviation (SD) $\delta^{13}\text{C}$ | ‰                          | $\delta^{13}\text{C}$ dispersion as a function of diversity of basal food sources, higher SD $\delta^{13}\text{C}$ indicates generalist feeding behaviour                                              |
| 2 | Standard Deviation (SD) $\delta^{15}\text{N}$ | ‰                          | $\delta^{15}\text{N}$ dispersion as a function of degree of trophic diversity and omnivory, higher SD $\delta^{15}\text{N}$ indicates feeding on multiple trophic levels                               |
| 3 | Standard Ellipse Area ( $\text{SEA}_B$ )      | ‰ <sup>2</sup>             | Species niche width, allows comparison of the relative competitive ability among species and across lakes                                                                                              |
| 4 | Overlapping $\text{SEA}_C$                    | ‰ <sup>2</sup>             | Overlapping niches between species, indicates dietary similarities or distinctions                                                                                                                     |
| 5 | Relative distance                             | ‰                          | Relative spacing between two species (how close two species are to each other). Together with overlapping $\text{SEA}_C$ , indicate niche closeness between species                                    |
| 6 | Relative angle                                | Radians<br>( $-\pi, \pi$ ] | Measures relative positioning of two species, vertical alignment between species indicates feeding on similar basal sources and separate trophic positions (depending also on their relative distance) |

The first two isotopic niche metrics used in this study are the standard deviations (SD) of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopes, which correspond to half the length of the semi major axis  $a$ , and semi minor axis  $b$  of a standard ellipse (Figure 3.3), and can be regarded as functions of horizontal and vertical dispersion of the data points (variability) in the isotopic niche space, respectively (Batschelet 1981). Higher SD  $\delta^{13}\text{C}$  indicates species with more generalist feeding characteristics, feeding on multiple food sources with varying  $\delta^{13}\text{C}$  values, while SD  $\delta^{15}\text{N}$  represents trophic range with higher values suggesting higher trophic diversity for a species. These are therefore comparable with the max/min  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ranges described in Layman *et al.* (2007b).



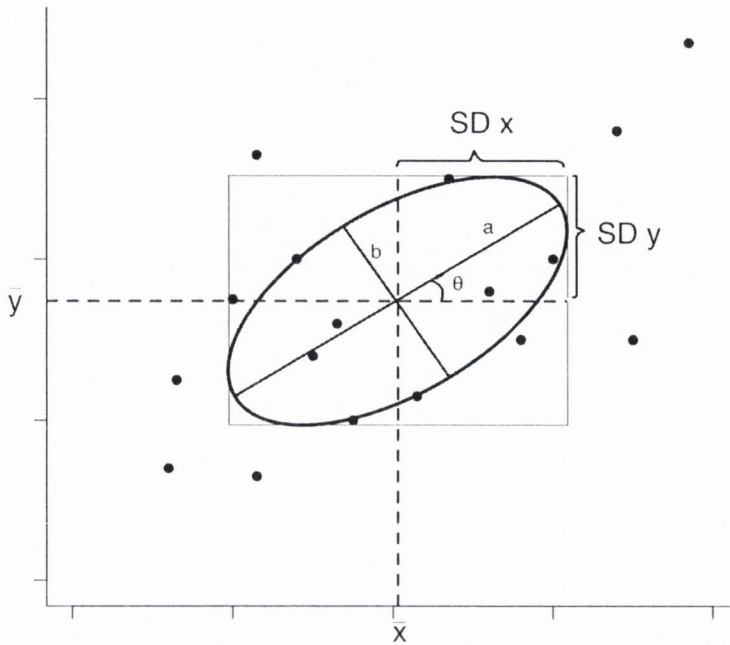


Figure 3.3 Example standard ellipse (SE), demonstrating sample data (filled circles), standard ellipse, semi-major axis  $a$ , semi-minor axis  $b$  and angle between the  $a$  with the horizontal axis ( $\theta$ ). A rectangle with centre at  $(\bar{x}, \bar{y})$  and sides parallel to the  $X$  and  $Y$  axes is drawn around the standard ellipse in order to demonstrate the standard deviation of  $x$  ( $SD\ x$ ) and  $y$  ( $SD\ y$ ), which correspond to half the length of the sides of this rectangle surrounding the SE.

The third metric, Standard Ellipse Area (SEA) was used for estimating species' isotopic niche width since it is more robust to variation in the sample sizes than Layman *et al.*'s TA (Layman *et al.* 2007a, Jackson *et al.* 2011). The SEA, which can be calculated by  $SEA = \pi ab$  (Batschelet 1981), is a point estimate that does not incorporate the uncertainty of the data that allows statistical comparisons (Jackson *et al.* 2011). Bayesian estimate of standard ellipse areas ( $SEA_B$ ) were calculated by simulating ellipse area values for  $10^4$  times using Bayesian approaches in order to reflect the uncertainty in the SEA sizes caused by the sampling process and to compare standard ellipses quantitatively in the isotopic space (Jackson *et al.* 2011).  $SEA_B$  is subject to fluctuations in the estimate of the location of the ellipse and its shape, causing larger uncertainty for smaller sample sizes (Jackson *et al.* 2011). Calculation of probability distributions of  $SEA_B$  enabled statistical comparisons of  $SEA_B$  sizes of species at each lake, which provided an evaluation of species' niche widths (Jackson *et al.* 2011). The pair-wise comparison of species niche widths were expressed by the probability of a species'  $SEA_B$  being smaller than another,

which was expressed as a value between 0 and 1. Probability values closer to 1 represent a higher likelihood of the argument to be true and values over 0.95 demonstrate significant results.

The fourth metric, the overlapping standard ellipse area between species, quantifies dietary similarities and niche overlaps among species. Its estimation was based on the overlapping corrected standard ellipse areas ( $SEA_C$ ) between two species, shown as the shaded area common to both ellipses in Figure 3.4. The correction of the SEA leads to slightly larger ellipse sizes for species with small sample sizes, but with the same geometrical shape (Jackson *et al.* 2011).

The last two isotopic niche metrics, relative distance ( $D_{1,2}$ ) and relative angle ( $\theta_{1,2}$ ) between two species, were derived from  $SEA_B$  to investigate the relationship between two species in the isotopic space (*e.g.* species 1 and 2) (Figure 3.4). Estimation of these metrics required defining the centre of the standard ellipses of both species which were the arithmetic means of the  $\delta^{13}C$  and  $\delta^{15}N$  isotopic values for each species (Batschelet 1981). For example, the central point for species 1 ( $\bar{x}_1, \bar{y}_1$ ) was calculated by:

$$\bar{x}_1 = \frac{1}{n} \sum x_i, \quad \bar{y}_1 = \frac{1}{n} \sum y_i$$

Relative distance between two species (*e.g.*  $D_{1,2}$ ) is a metric for estimating the relative spacing of the two species in the isotopic bivariate space.  $D_{1,2}$  corresponds to the vector between the central points of the standard ellipses of species 1 ( $\bar{x}_1, \bar{y}_1$ ) and species 2 ( $\bar{x}_2, \bar{y}_2$ ) (Figure 3.4) and was calculated by:

$$D_{1,2} = \sqrt{(\bar{x}_1 - \bar{x}_2)^2 + (\bar{y}_1 - \bar{y}_2)^2}$$

Relative angle between two species (*i.e.*  $\theta_{1,2}$ ) is an isotopic niche metric reflecting the relative position between two species in the isotopic space. It was measured as the angle between the x-axis and the vector between the centres of the standard ellipses of species 1 ( $\bar{x}_1, \bar{y}_1$ ) and species 2 ( $\bar{x}_2, \bar{y}_2$ ) (Figure 3.4). The angles were estimated using the *atan2()* function in R (R Development Core Team 2013), which is the arc-tangent function with two arguments:

$$\theta_{1,2} = \text{atan2}((\bar{y}_1 - \bar{y}_2), (\bar{x}_1 - \bar{x}_2));$$

where  $\text{atan2}() = \text{atan}(\bar{y}_1/\bar{x}_1)$ ;

and  $\text{atan}()$  refers to arc-tangent function.

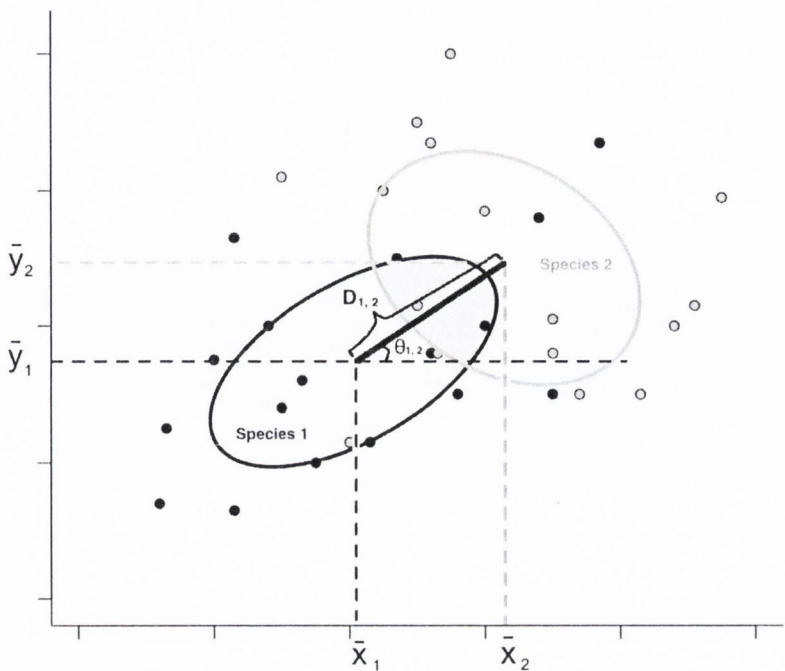


Figure 3.4 Example standard ellipses based on data from two species (Species 1 = black filled circles; species 2 = grey filled circles). The angle from Species 1 to Species 2 ( $\theta_{1,2}$ ) is measured between the horizontal axis and the vector between the centre of the ellipses of Species 1 ( $x_1, y_1$ ) and Species 2 ( $x_2, y_2$ ), which corresponds to the distance between the two species ( $D_{1,2}$ ). The shaded area represents the overlapping standard ellipse area ( $SEA_B$ ) between the two species.

All calculations were based on R statistical package (R Development Core Team 2013). Calculations of isotopic niche metrics were performed using the Stable Isotopes Analysis in R package - SIAR (Parnell *et al.* 2010; R Development Core Team 2011). The summary results for the isotopic distance and angle among species included the mode and the 95% confidence interval (CI). Vectors in the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic space (*e.g.* distance between two species, SD of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) were expressed in parts per thousand or permil (‰), while the areas (*e.g.* species  $SEA_B$ ) were expressed in permil<sup>2</sup> (‰<sup>2</sup>). The angular distances between two species were presented in radians within the range  $(-\pi, \pi]$  (Figure 3.5).



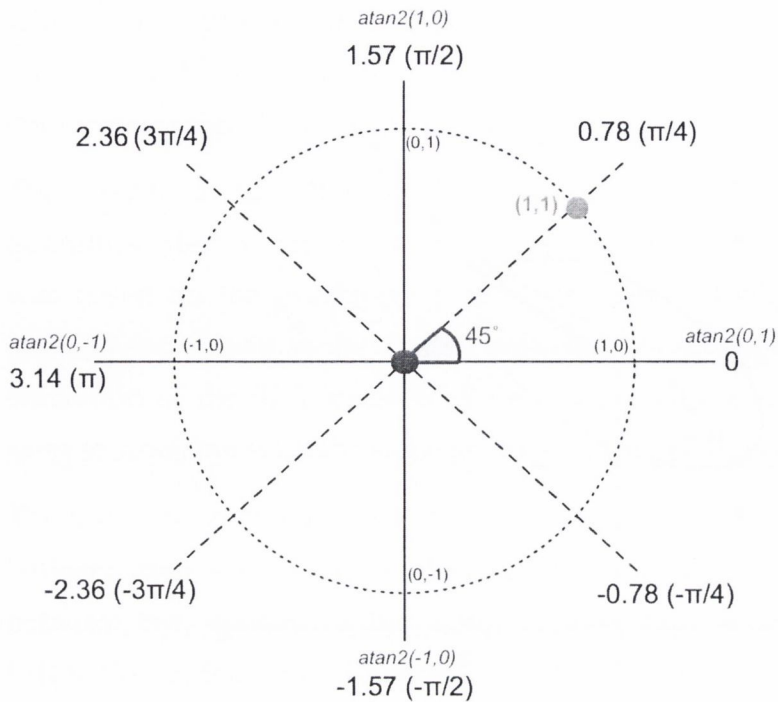


Figure 3.5 Reference diagram for estimating the angle between two species in the isotopic space. The arc-tangent ( $\text{atan2}$ ) function values (in radians, outside the circle) at selected points (inside the circle) are presented on a unit circle. Note that the order of arguments are reversed for the  $\text{atan2}()$  function, hence  $\text{atan2}(y, x)$  corresponds to the point  $(x, y)$ . E.g. the angle from the horizontal x-axis to the vector  $(1,1)$  can be calculated as  $\text{atan2}(1,1) = 45^\circ$  or  $\pi/4 = 0.78$  in radians.

### 3.3 Results

#### 3.3.1 Isotopic variation among species

Separation among species' carbon food sources ( $\delta^{13}\text{C}$ ) and trophic positions ( $\delta^{15}\text{N}$ ) were tested using one-way ANOVAs. The mean  $\delta^{13}\text{C}$  values were significantly different among species in Loughs Fern ( $F_{1,65} = 37.0$ ,  $p < 0.001$ ) and Muckno ( $F_{2,130} = 8.7$ ,  $p < 0.001$ ), but not in Brackley ( $F_{2,119} = 1.2$ ,  $p > 0.05$ ). Here, transformation of isotope values refers to re-centring the isotope data according to the mean isotopic values of *A. aquaticus* in each lake, thus accounting for among-lake differences while absolute differences of isotopic values remained on the same scale (see Section 3.2.3). Summary data for the untransformed carbon and nitrogen isotopic signatures of macroinvertebrates (mean  $\pm$  SE) from each sampling site are displayed in Table 3.2 and Table 3.3, respectively. In Lough Fern,  $\delta^{13}\text{C}$  value of *A. aquaticus* was significantly higher than *G. duebeni* (Tukey's HSD,  $p < 0.05$ ), while in Lough Muckno it was significantly lower than both *G. pulex* and *C. pseudogracilis*, between which the  $\delta^{13}\text{C}$  values did not vary significantly (Tukey's HSD,  $p > 0.05$ ) (Figure 3.6a).

Significant differences were observed among species'  $\delta^{15}\text{N}$  values in all three lakes (Fern:  $F_{1,65} = 8.4$ ,  $p < 0.01$ ; Brackley:  $F_{2,119} = 97.4$ ,  $p < 0.001$  and Muckno:  $F_{2,130} = 23.9$ ,  $p < 0.001$ ). *Gammarus duebeni* had significantly elevated  $\delta^{15}\text{N}$  values compared with *A. aquaticus* both in lough Fern and Brackley, but not in Muckno (Tukey's HSD,  $p < 0.05$ ).  $\delta^{15}\text{N}$  values of *C. pseudogracilis* were also significantly higher than *A. aquaticus* and *Gammarus spp.* in both Brackley and Muckno (Tukey's HSD,  $p < 0.05$ ) (Figure 3.6b).

Table 3.2 Comparison of descriptive statistics (mean±SE) for the  $\delta^{13}\text{C}$  stable isotope signatures of *A. aquaticus*, *G. duebeni* (Fern and Brackley), *G. pulex* (Muckno) and *C. pseudogracilis* across the sampling sites of Loughs Fern, Brackley and Muckno (Figure 3.2). Shoreline habitats associated with each site are indicated after site names (W=Woodland and G=Grassland); where *n* = the number of individual invertebrates used in the analysis from each site. Isotopic values are left blank for sites where the species was not present (n=0).

| Lough    | Site   | <i>Asellus aquaticus</i> |                           |   |      | <i>Gammarus spp.</i> |                           |   |      | <i>Crangonyx pseudogracilis</i> |                           |   |      |
|----------|--------|--------------------------|---------------------------|---|------|----------------------|---------------------------|---|------|---------------------------------|---------------------------|---|------|
|          |        | n                        | $\delta^{13}\text{C}$ (‰) | ± | SE   | n                    | $\delta^{13}\text{C}$ (‰) | ± | SE   | n                               | $\delta^{13}\text{C}$ (‰) | ± | SE   |
| Fern     | F1 (W) | 6                        | -26.09                    | ± | 0.18 | 0                    |                           |   |      |                                 |                           |   |      |
|          | F2 (W) | 6                        | -25.64                    | ± | 0.19 | 12                   | -26.56                    | ± | 0.15 |                                 |                           |   |      |
|          | F3 (W) | 6                        | -26.36                    | ± | 0.20 | 6                    | -27.65                    | ± | 0.22 |                                 |                           |   |      |
|          | F4 (G) | 6                        | -26.46                    | ± | 0.25 | 6                    | -28.06                    | ± | 0.23 |                                 |                           |   |      |
|          | F5 (G) | 0                        |                           |   |      | 6                    | -27.20                    | ± | 0.12 |                                 |                           |   |      |
|          | F6 (G) | 6                        | -25.44                    | ± | 0.19 | 6                    | -26.52                    | ± | 0.34 |                                 |                           |   |      |
| Brackley | B1 (W) | 12                       | -27.02                    | ± | 0.28 | 0                    |                           |   |      | 12                              | -28.66                    | ± | 0.21 |
|          | B2 (W) | 12                       | -31.23                    | ± | 0.41 | 0                    |                           |   |      | 19                              | -29.64                    | ± | 0.30 |
|          | B3 (W) | 0                        |                           |   |      | 6                    | -27.67                    | ± | 0.27 | 6                               | -27.48                    | ± | 0.67 |
|          | B4 (G) | 6                        | -29.86                    | ± | 0.35 | 6                    | -31.27                    | ± | 0.50 | 12                              | -29.35                    | ± | 0.51 |
|          | B5 (G) | 0                        |                           |   |      | 6                    | -30.39                    | ± | 0.45 | 6                               | -28.80                    | ± | 0.35 |
|          | B6 (G) | 6                        | -28.38                    | ± | 0.65 | 0                    |                           |   |      | 13                              | -29.23                    | ± | 0.36 |
| Muckno   | M1 (W) | 6                        | -29.87                    | ± | 0.35 | 18                   | -29.02                    | ± | 0.31 | 7                               | -29.60                    | ± | 0.35 |
|          | M2 (W) | 6                        | -28.74                    | ± | 0.29 | 12                   | -27.57                    | ± | 0.20 | 6                               | -28.32                    | ± | 0.27 |
|          | M3 (W) | 0                        |                           |   |      | 18                   | -28.85                    | ± | 0.20 | 0                               |                           |   |      |
|          | M4 (G) | 6                        | -30.32                    | ± | 0.30 | 12                   | -28.73                    | ± | 0.20 | 0                               |                           |   |      |
|          | M5 (G) | 6                        | -33.45                    | ± | 0.70 | 6                    | -31.59                    | ± | 0.74 | 0                               |                           |   |      |
|          | M6 (G) | 12                       | -28.99                    | ± | 0.50 | 6                    | -27.95                    | ± | 0.27 | 12                              | -28.93                    | ± | 0.37 |



Table 3.3 Comparison of descriptive statistics (mean±SE) for the  $\delta^{15}\text{N}$  stable isotope signatures of *A. aquaticus*, *G. duebeni* (Fern and Brackley), *G. pulex* (Muckno) and *C. pseudogracilis* across the sampling sites of study loughs (Figure 3.2). Shoreline habitats associated with each site are indicated after site names (**W**=Woodland and **G**=Grassland); where *n* = the number of individual invertebrates used in the analysis from each site. Isotopic values are left blank for sites where the species was not present (*n*=0).

| Lough    | Site   | <i>Asellus aquaticus</i> |                           |   |      | <i>Gammarus spp.</i> |                           |   |      | <i>Crangonyx pseudogracilis</i> |                           |   |      |
|----------|--------|--------------------------|---------------------------|---|------|----------------------|---------------------------|---|------|---------------------------------|---------------------------|---|------|
|          |        | n                        | $\delta^{15}\text{N}$ (‰) | ± | SE   | n                    | $\delta^{15}\text{N}$ (‰) | ± | SE   | n                               | $\delta^{15}\text{N}$ (‰) | ± | SE   |
| Fern     | F1 (W) | 6                        | 9.02                      | ± | 0.23 | 0                    |                           |   |      |                                 |                           |   |      |
|          | F2 (W) | 6                        | 9.03                      | ± | 0.18 | 12                   | 9.24                      | ± | 0.21 |                                 |                           |   |      |
|          | F3 (W) | 6                        | 9.22                      | ± | 0.13 | 6                    | 9.22                      | ± | 0.21 |                                 |                           |   |      |
|          | F4 (G) | 6                        | 9.27                      | ± | 0.08 | 6                    | 9.80                      | ± | 0.16 |                                 |                           |   |      |
|          | F5 (G) | 0                        |                           |   |      | 6                    | 9.30                      | ± | 0.18 |                                 |                           |   |      |
|          | F6 (G) | 6                        | 7.29                      | ± | 0.20 | 6                    | 8.92                      | ± | 0.23 |                                 |                           |   |      |
| Brackley | B1 (W) | 12                       | 5.40                      | ± | 0.17 | 0                    |                           |   |      | 12                              | 8.06                      | ± | 0.12 |
|          | B2 (W) | 12                       | 3.44                      | ± | 0.22 | 0                    |                           |   |      | 19                              | 6.61                      | ± | 0.16 |
|          | B3 (W) | 0                        |                           |   |      | 6                    | 5.66                      | ± | 0.32 | 6                               | 7.59                      | ± | 0.42 |
|          | B4 (G) | 6                        | 4.89                      | ± | 0.05 | 6                    | 4.84                      | ± | 0.08 | 12                              | 7.75                      | ± | 0.19 |
|          | B5 (G) | 0                        |                           |   |      | 6                    | 7.15                      | ± | 0.16 | 6                               | 8.68                      | ± | 0.25 |
|          | B6 (G) | 6                        | 5.87                      | ± | 0.08 | 0                    |                           |   |      | 13                              | 8.60                      | ± | 0.19 |
| Muckno   | M1 (W) | 6                        | 10.86                     | ± | 0.08 | 18                   | 11.44                     | ± | 0.24 | 7                               | 13.97                     | ± | 0.44 |
|          | M2 (W) | 6                        | 9.57                      | ± | 0.29 | 12                   | 8.98                      | ± | 0.37 | 6                               | 11.80                     | ± | 0.35 |
|          | M3 (W) | 0                        |                           |   |      | 18                   | 12.61                     | ± | 0.16 | 0                               |                           |   |      |
|          | M4 (G) | 6                        | 11.67                     | ± | 0.30 | 12                   | 12.48                     | ± | 0.14 | 0                               |                           |   |      |
|          | M5 (G) | 6                        | 10.58                     | ± | 0.18 | 6                    | 11.66                     | ± | 0.15 | 0                               |                           |   |      |
|          | M6 (G) | 12                       | 11.83                     | ± | 0.12 | 6                    | 12.68                     | ± | 0.14 | 12                              | 14.00                     | ± | 0.20 |

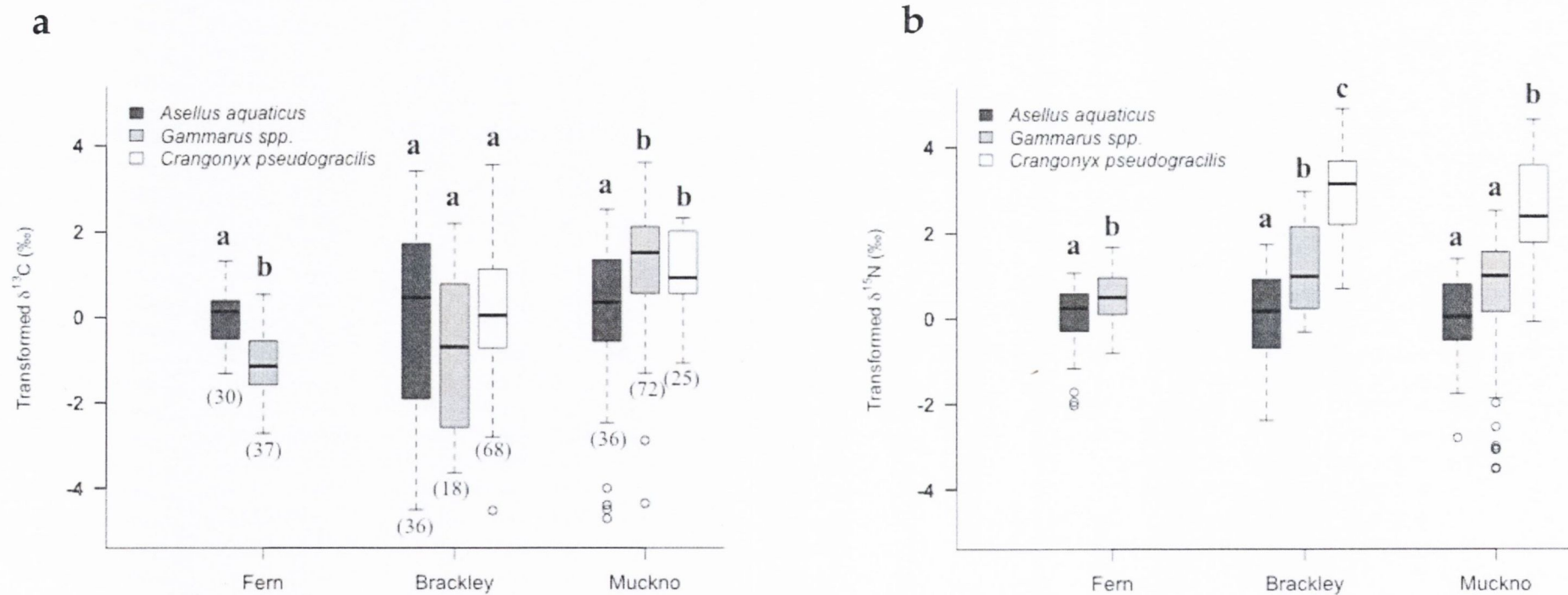


Figure 3.6 Variation among the transformed  $\delta^{13}\text{C}$  (a) and  $\delta^{15}\text{N}$  (b) values of the common macroinvertebrate consumers of Loughs Fern, Brackley and Muckno. Different letters above the boxes indicate significant differences in the isotopic values among species in each lake separately. E.g. For Lough Muckno on the transformed  $\delta^{15}\text{N}$  plot (on the left), letters 'a' above the first two boxes indicate non-significant differences of  $\delta^{15}\text{N}$  values between *A. aquaticus* and *G. duebeni* species, while letter 'b' above the third box indicate significant differences between *C. pseudogracilis* and the other two species. Mean comparisons are based on TUKEY's HSD ( $p < 0.05$ ). The line in the middle of the box represents the median and the lower and upper ends of the box represent the 25% and 75% quartiles, respectively. Sample size is indicated under the boxes (e.g.  $n=36$ ).

### 3.3.1 Comparison of isotopic niche metrics

Standard ellipses for individual species at each lake were used to investigate further the relationships among their isotopic niches (Figure 3.7). The mean isotope value of the isopod *A. aquaticus*, present in all three lakes, was used as a reference during normalisation. In Lough Fern the two native Irish crustacean species, *G. duebeni* and *A. aquaticus* occupied the niche space together. In Lough Brackley, amphipod *C. pseudogracilis* was the most commonly found species in addition to the two native species that were present in Fern. In Lough Muckno, the non-native *G. pulex*, presumed to have replaced *G. duebeni*, was the dominant gammarid species; next to less widely distributed *C. pseudogracilis* (Table A. 8, Appendix III).

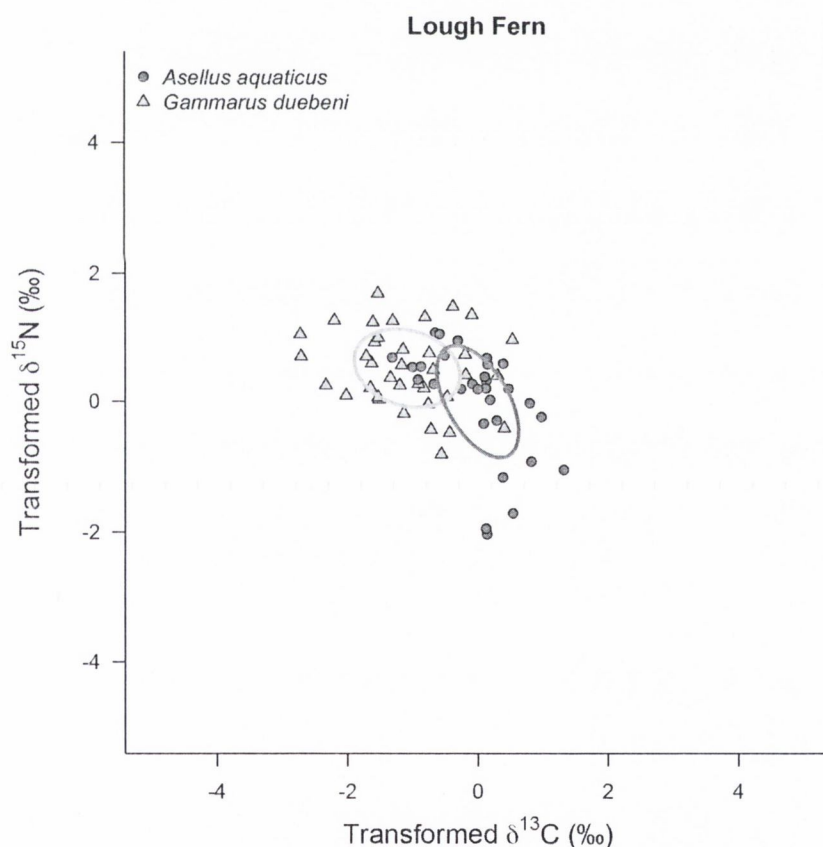


Figure 3.7 Standard ellipses (SEA<sub>B</sub>) estimated for macroinvertebrate species in Loughs Fern, Brackley and Muckno (see next page). Each data point represents an individual macroinvertebrate sample in the transformed  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  bi-plot (see Sections 3.2.3 and 3.2.4 for further explanation).



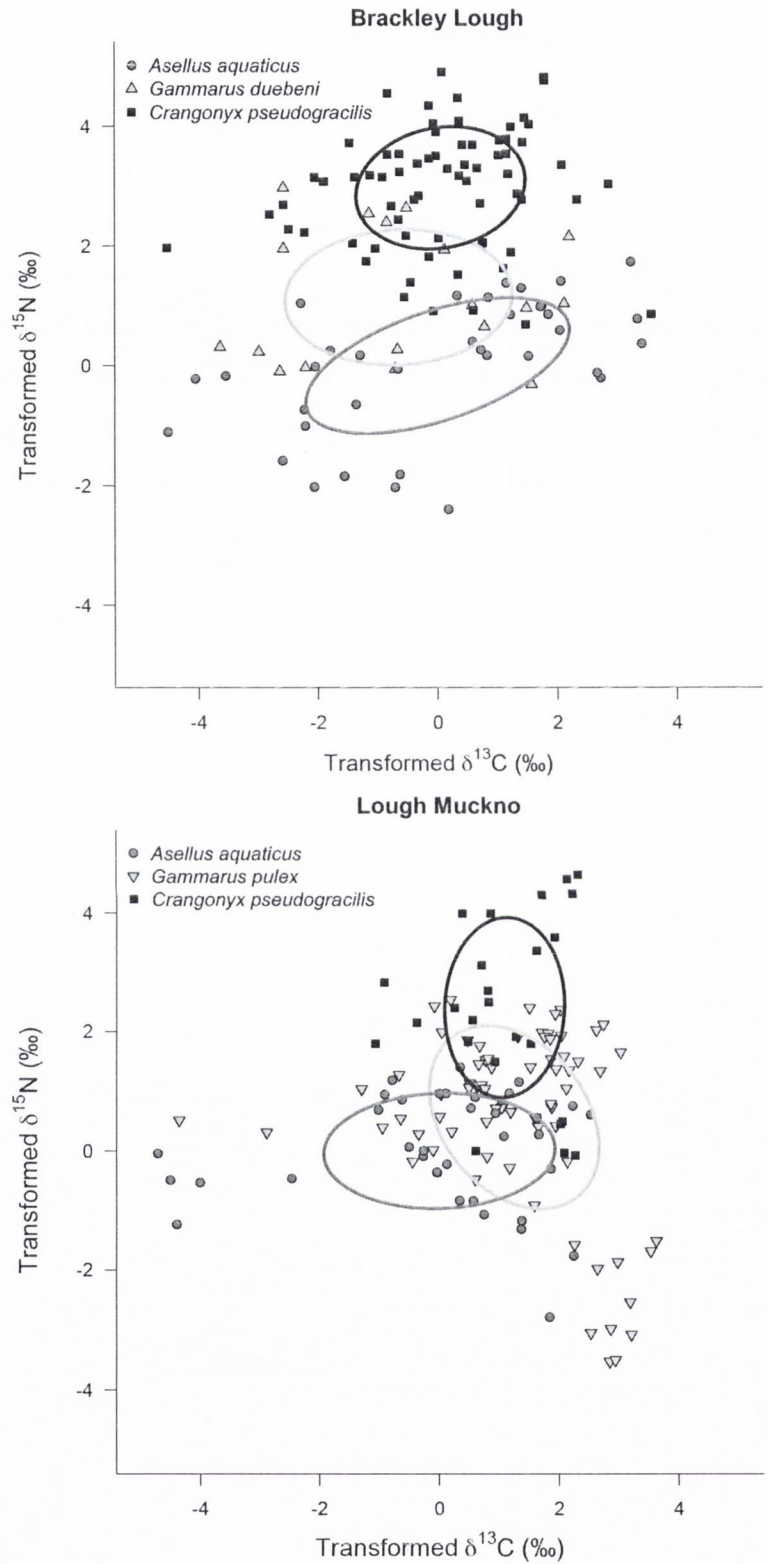


Figure 3.7 (continued) Standard ellipses (SEA<sub>B</sub>) estimated for macroinvertebrate species in Loughs Fern, Brackley and Muckno. Each data point represents an individual macroinvertebrate sample in the transformed  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  bi-plot (see Sections 3.2.3 and 3.2.4 for further explanation).

3.3.1.1  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic dispersions

The SD of  $\delta^{13}\text{C}$  was used as an indication of  $\delta^{13}\text{C}$  dispersion in the isotopic space and as a function of diversity of the basal food sources. It was lower for *A. aquaticus* (0.62‰) than *G. duebeni* (0.80‰) in Lough Fern. In Loughs Brackley and Muckno, *C. pseudogracilis* showed the narrowest  $\delta^{13}\text{C}$  dispersions among all species, while *A. aquaticus* showed wider  $\delta^{13}\text{C}$  dispersions (Brackley: 2.12‰ and Muckno: 1.90‰) in comparison with *G. duebeni* (1.84‰) in Brackley and *G. pulex* (1.41‰) in Muckno (Figure 3.7).

The  $\delta^{15}\text{N}$  dispersion, measured as the SD of  $\delta^{15}\text{N}$  values, was higher for *A. aquaticus* (0.85‰) than *G. duebeni* (0.60‰) in Fern, while they were very similar in Brackley (1.12‰ and 1.10‰ for *A. aquaticus* and *G. duebeni*, respectively). On the other hand,  $\delta^{15}\text{N}$  dispersion of *A. aquaticus* (0.95‰) was narrower than *G. pulex* (1.52‰) in Muckno. *Crangonyx pseudogracilis* (1.01‰) showed slightly narrower  $\delta^{15}\text{N}$  dispersion than the other two species in Lough Brackley. In Muckno, the  $\delta^{15}\text{N}$  of *C. pseudogracilis* had a broader dispersion (1.48‰) than *A. aquaticus* and was very similar to *G. pulex* (Figure 3.7).

Table 3.4 Standard deviation (SD) of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotope values estimated for each macroinvertebrate species in Loughs Fern, Brackley and Muckno; where n = sample size used for plotting the Standard Ellipses. See the Methods Section (3.2.4) for explanation on SD  $\delta^{13}\text{C}$  and SD  $\delta^{15}\text{N}$ .

| Lake     | Species                         | n  | SD $\delta^{13}\text{C}$ | SD $\delta^{15}\text{N}$ |
|----------|---------------------------------|----|--------------------------|--------------------------|
| Fern     | <i>Asellus aquaticus</i>        | 30 | 0.62                     | 0.85                     |
|          | <i>Gammarus duebeni</i>         | 37 | 0.80                     | 0.60                     |
| Brackley | <i>Asellus aquaticus</i>        | 36 | 2.12                     | 1.12                     |
|          | <i>Gammarus duebeni</i>         | 18 | 1.84                     | 1.10                     |
|          | <i>Crangonyx pseudogracilis</i> | 68 | 1.41                     | 1.01                     |
| Muckno   | <i>Asellus aquaticus</i>        | 36 | 1.90                     | 0.95                     |
|          | <i>Gammarus pulex</i>           | 72 | 1.41                     | 1.52                     |
|          | <i>Crangonyx pseudogracilis</i> | 25 | 0.98                     | 1.48                     |

3.3.1.2 Isotopic niche widths

Comparison of niche widths among species within and across lakes were based on the Bayesian Estimated Standard Ellipse Areas ( $SEA_B$ ) as demonstrated in Figure 3.8. Among-lake comparisons showed that *A. aquaticus* and *G. duebeni* both had significantly smaller niche widths in Fern than in Brackley and Muckno (probability: 1). Similar patterns were observed for the niche widths of *Gammarus* spp. and *C. pseudogracilis* between Brackley and Muckno. The probability of *C. pseudogracilis* having a smaller niche width in Brackley than in Muckno was the same for *G. duebeni* in Brackley and *G. pulex* in Muckno (probability: 0.55). On the other hand, there was a lower chance of *A. aquaticus* occupying a smaller niche width in Brackley compared with Muckno (probability: 0.3) (Table 3.5).

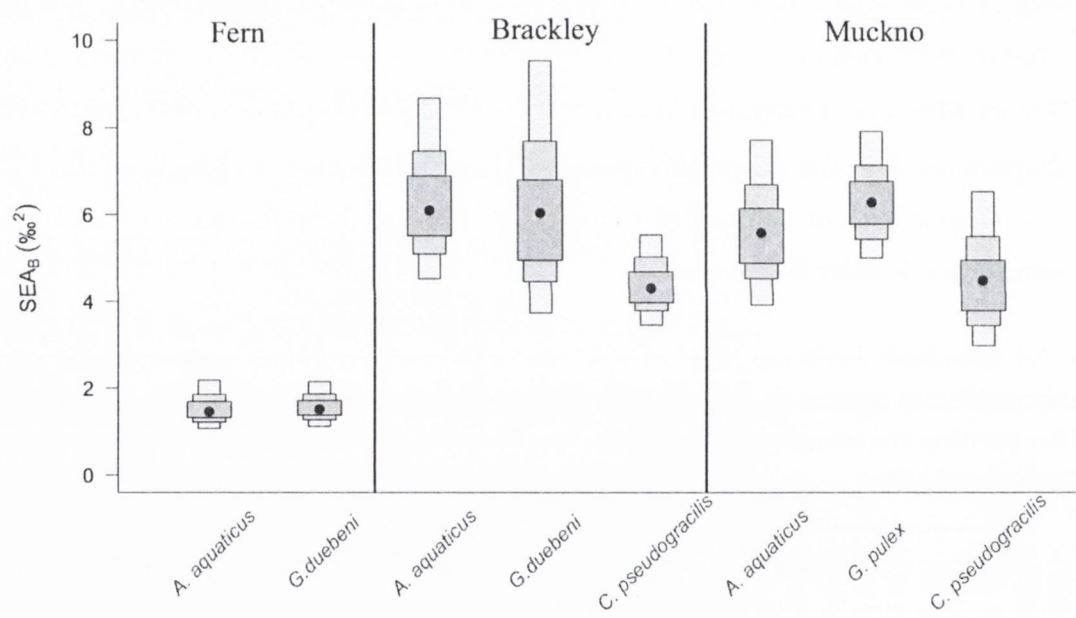


Figure 3.8 Density plot demonstrating the credible intervals for the standard ellipse areas ( $SEA_B$ ) estimated for macroinvertebrate species in Loughs Fern, Brackley and Muckno. Black points correspond to mean  $SEA_B$  values. The three shades of grey areas in the boxes reflect the 95%, 75% and 50% credible intervals.

Pair-wise comparison of  $SEA_B$  between species revealed that *A. aquaticus* had equal probability of having smaller niche widths than *G. duebeni* both in Loughs Fern and Brackley (0.53 and 0.43, respectively). In comparison, the probability of *A. aquaticus* having a smaller niche width than *G. pulex* was relatively higher (0.71) in Muckno. Probabilities of *C. pseudogracilis* having smaller niche widths than *G. duebeni* (in Brackley) or *G. pulex* (in Muckno) were both over 0.9. *Crangonyx pseudogracilis* had a significantly smaller niche width than *A. aquaticus* in Brackley



with a probability of 0.97; whereas the probability of *A. aquaticus* niche width being bigger than *C. pseudogracilis* was 0.81 in Muckno (Table 3.5).

Table 3.5 The pairwise comparison of SEA<sub>B</sub> of species among lakes, which is based on the probability that the SEA<sub>B</sub> of a species in Lake 1 is smaller than in Lake 2. See section 3.2.4 for details on the calculation of SEA<sub>B</sub>.

| Species                  | Lake 1   | Lake 2   | SEA <sub>B</sub> comparison<br>(Probability) |
|--------------------------|----------|----------|----------------------------------------------|
| <i>A. aquaticus</i>      | Fern     | Brackley | 1                                            |
|                          | Fern     | Muckno   | 1                                            |
|                          | Brackley | Muckno   | 0.30                                         |
| <i>Gammarus spp.</i>     | Fern     | Brackley | 1                                            |
|                          | Fern     | Muckno   | 1                                            |
|                          | Brackley | Muckno   | 0.55                                         |
| <i>C. pseudogracilis</i> | Brackley | Muckno   | 0.55                                         |

3.3.1.3 Isotopic niche overlap among species

The overlap between the corrected standard ellipse areas (SEA<sub>C</sub>) of *A. aquaticus* and *G. duebeni* was 0.21‰<sup>2</sup> in Lough Fern, comprising 13.8% and 14.6% of total *G. duebeni* and *A. aquaticus* isotopic niche area, respectively (Table 3.6). The overlap between the SEA<sub>C</sub> of *A. aquaticus* and *G. duebeni* went up to 1.79‰<sup>2</sup> in Brackley, consisting of 26.4% and 27.1% of total niche area, respectively. The SEA<sub>C</sub> overlap between *A. aquaticus* and *G. pulex* in Muckno was 2.46‰<sup>2</sup>, comprising of 37.9% and 41.9% of total *A. aquaticus* and *G. pulex* isotopic niche areas, respectively, and reflecting not only the highest dietary overlap between *A. aquaticus* and a *Gammarus spp.* across lakes but also the highest overlap among all species studied.

The overlap between the SEA<sub>C</sub> of *C. pseudogracilis* and *Gammarus spp.* was higher in Muckno (1.48‰<sup>2</sup>) than in Brackley (0.33‰<sup>2</sup>), indicating a higher dietary overlap between *C. pseudogracilis* and *G. pulex* than *G. duebeni* (Figure 3.7, Table 3.6). The percentages of the overlapping isotopic niche areas of species to their total isotopic niche areas were also higher in Muckno (22.8% and 31.1% for *G. pulex* and *C. pseudogracilis*, respectively) than in Brackley (4.9% and 7.3% for *G. duebeni* and *C. pseudogracilis*, respectively). On the other hand, no dietary overlap was

Table 3.6. Summary results of SIBER metrics for the invertebrate species in Loughs Fern, Brackley and Muckno, including  $SEA_B$  comparisons, total and relative  $SEA_C$  overlaps, distance and angle between species. The pairwise comparisons of  $SEA_B$  are based on the probability that the  $SEA_B$  of Species 1 is smaller than of Species 2. The mode and the 95% confidence interval (CI) are provided for the distances (‰) and angles (radians) between species. Angle between species represents the angle between the x-axis and the vector drawn between species 1 and 2, which equals to the distance between species. See section 3.2.4 for calculation of isotope metrics.

| Lake     | Species 1                | Species 2           | $SEA_B$<br>comparison<br>(Probability) | Total<br>$SEA_C$<br>Overlap<br>(‰ <sup>2</sup> ) | Distance<br>(‰) |         | Angle<br>(radians) |               |
|----------|--------------------------|---------------------|----------------------------------------|--------------------------------------------------|-----------------|---------|--------------------|---------------|
|          |                          |                     |                                        |                                                  | Mode            | 95% CI  | Mode               | 95% CI        |
| Fern     | <i>A. aquaticus</i>      | <i>G. duebeni</i>   | 0.53                                   | 0.21                                             | 1.2             | 0.8-1.6 | 2.68               | 2.43 – 2.98   |
| Brackley | <i>A. aquaticus</i>      | <i>G. duebeni</i>   | 0.46                                   | 1.79                                             | 1.4             | 0.7-2.1 | 2.13               | 1.26 – 2.82   |
|          | <i>C. pseudogracilis</i> | <i>G. duebeni</i>   | 0.91                                   | 0.33                                             | 2.0             | 1.3-2.7 | -1.96              | -2.37 – -1.47 |
|          | <i>C. pseudogracilis</i> | <i>A. aquaticus</i> | <b>0.97</b>                            | 0                                                | 3.0             | 2.5-3.5 | -1.60              | -1.84 – -1.32 |
| Muckno   | <i>A. aquaticus</i>      | <i>G. pulex</i>     | 0.71                                   | 2.46                                             | 1.4             | 0.7-2.0 | 0.40               | 0.02 – 0.88   |
|          | <i>C. pseudogracilis</i> | <i>G. pulex</i>     | 0.93                                   | 1.48                                             | 1.8             | 1.2-2.6 | -1.48              | -1.88 – -1.20 |
|          | <i>C. pseudogracilis</i> | <i>A. aquaticus</i> | 0.81                                   | 0                                                | 2.7             | 2.0-3.4 | -2.00              | -2.27 – -1.71 |



observed between *C. pseudogracilis* and *A. aquaticus* either in Brackley or in Muckno (Figure 3.7, Table 3.6).

### 3.3.1.4 Relative distance between species

The relative spacing among species were revealed as the distances (in units of ‰) between the centroids of the standard ellipses of two species in the isotopic space. The mode of distance between *A. aquaticus* and *G. duebeni* in Fern was the shortest isotopic distance measured among species across lakes (1.2‰, CI: 0.8 – 1.6‰) (Figure 3.7, Table 3.6). Same distances were observed between *A. aquaticus* and *Gammarus* spp. in Brackley and Muckno (mode: 1.4‰, CIs: 0.7 – 2.1‰ and 0.7 – 2.0‰ for *G. duebeni* in Brackley and *G. pulex* in Muckno, respectively). Distances were longer between *C. pseudogracilis* and *A. aquaticus* in Brackley (3.0‰, CI: 2.5–3.5‰) than in Muckno (2.7‰, CI: 2.0 – 3.4‰). The mode of isotopic distance between *C. pseudogracilis* and *Gammarus* spp. in Brackley was 2.0‰ (CI: 1.3–2.7‰), which was slightly longer than it was in Muckno (1.8‰, CI: 1.2 – 2.6‰) (Figure 3.7, Table 3.6).

### 3.3.1.5 Relative angle between species

The relative position of the species to each other was investigated through analysis of the angle between their centroids. Modes of angles from *A. aquaticus* to *G. duebeni* were both within the top left quadrant of the unit circle in Fern and Brackley (2.68 and 2.13 radians; CI: 2.43 – 2.98 and 1.26 – 2.82 radians, respectively), indicating a similar positioning of *A. aquaticus* and *G. duebeni*, regardless of the presence of *C. pseudogracilis* in Brackley (see Figure 3.5 for the reference diagram for angles). However the mode of angle from *A. aquaticus* to *G. pulex* corresponded to the top right quadrant of the unit circle (0.40 radians, CI: 0.02–0.88 radians), reflecting a significant change in the positioning between *A. aquaticus* and the two species of gammarids (Table 3.6). The modes of angles from *C. pseudogracilis* to *G. duebeni* both corresponded to the bottom left quadrant of the unit circle in Loughs Brackley (-1.96 radians, CI: -2.37 – -1.47 radians) and Muckno (-1.48 radians, CI: -1.88 – -1.20 radians), which indicated an almost vertical alignment in Muckno, with *C. pseudogracilis* at a higher position of *C. pseudogracilis* than *G. pulex*. The mode of angle from *C. pseudogracilis* to *A. aquaticus* was -1.60 radians (CI: -1.84 – -1.32 radians) in Brackley and 2.00 radians (CI: -2.27 – -1.71



radians) in Muckno, which corresponded to the bottom left quadrant of the unit circle. However the CIs indicated close alignments with the vertical axis in both lakes, but more in Brackley than in Muckno.

### 3.4 Discussion

#### 3.4.1 Comparisons of species' niche widths and isotopic dispersions among lakes

Differences in the niche widths of species, measured by standard ellipse areas ( $SEA_B$ ), between Lough Fern and the other two lakes corresponded with their carbon and/or nitrogen isotope dispersions. In Lough Fern, both *A. aquaticus* and *G. duebeni* had the smallest niche widths of all lakes, besides the narrowest carbon and nitrogen dispersions. Olsson *et al.* (2009) argued that niche widths at the population level reflect the carbon and nitrogen ranges of the resources available in a lake. Therefore, narrower niche widths and isotopic dispersions of macroinvertebrates, as in Lough Fern, suggests lower diversity of basal food reflecting more limited carbon and nitrogen ranges compared with the other lakes. These results can also be linked to the more homogenous catchment (e.g. with less riparian and littoral habitat structure and complexity) of Lough Fern in comparison with Brackley or Muckno. Broader  $\delta^{13}C$  and  $\delta^{15}N$  dispersions can also be associated with higher dietary generalism (feeding on a diversity of food sources and/or on food with a wide range of  $\delta^{13}C$ ) and omnivory (feeding at more than one trophic level) among species, respectively (Jones and Waldron 2003, Bearhop *et al.* 2004). That the  $\delta^{13}C$  dispersions of all species were broadest in Brackley compared with other lakes, suggests a wider range of basal resources, that are clearly also able to be exploited by the relative generalist feeding behaviour of the studied species. Despite the presence of *C. pseudogracilis* in Brackley and Muckno,  $\delta^{15}N$  dispersions of both *A. aquaticus* and *Gammarus spp.* were broader than in Fern, suggesting increased omnivory in these lakes. Isotopic values of basal food sources may also respond to environmental factors, including lake nutrient levels and productivity (Bergfur *et al.* 2009). There is a likely influence of lake nutrient status as well as water colour on species' isotopic signals as discussed in Chapter 2. For example, increased nutrient levels were shown to have significant effects, particularly on species'  $\delta^{13}C$  signatures, even though the

individual responses of species varied (negative for *A. aquaticus* and *C. pseudogracilis*; and positive for *G. duebni*). Water colour, a major component of the water chemistry acting on species along the secondary axis of the PCA, either on its own or as an interaction with shoreline habitat, were highly significant for explaining species isotopic variation within lakes for all species.

Despite having similar niche widths between Loughs Brackley and Muckno, *C. pseudogracilis* had contrasting dispersions of carbon and nitrogen isotopes in these two lakes. The  $\delta^{13}\text{C}$  dispersion of *C. pseudogracilis* in Muckno was 30% narrower than in Brackley, indicating its use of food sources that are either more diverse and/or within a broader range of  $\delta^{13}\text{C}$  values in Brackley. However, compared with Brackley, its  $\delta^{15}\text{N}$  dispersion was almost 50% larger, representing an extended trophic position in the vertical structure of the food web of Muckno. Decreased  $\delta^{13}\text{C}$  dispersions of both *Gammarus* spp. and *C. pseudogracilis* in Lough Muckno in comparison with Brackley, together with an increase in  $\delta^{15}\text{N}$  dispersions, might also reflect expansion of feeding across trophic groups following a limitation of food sources, and based on herbivorous diets. However, further research is needed to gain a better understanding of species' relative dispersions of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures.

As a result of extreme rainfall over the summer of 2009 (MET Annual Report 2009), high water levels were observed during the sampling period. Even though this resulted in the sampling of littoral habitats with inundated vegetation (particularly at the grassland sites of Loughs Brackley and Muckno), habitats covered by high water levels were still subject to the influences of tree cover (or lack of it). Hence, the presence of allochthonous leaf litter was visibly prevalent in the wooded sites and not the grassland sites. Since the main focus of this chapter is the trophic interactions among species by comparing their isotopic niche widths (in contrast to community composition as used by many monitoring schemes), it represents valid comparisons of isotopic metrics among species and does not undermine the findings, despite differences in the littoral habitat characteristics.

### **3.4.2 Trophic relationships of *Crangonyx pseudogracilis***

The current study showed that *C. pseudogracilis* had the narrowest  $\delta^{13}\text{C}$  dispersions of all species in Brackley and Muckno. Also, the high probability of *C.*



*pseudogracilis* having a smaller niche width than *Gammarus* spp. or *A. aquaticus* (0.9 in average) suggested it had the least generalist feeding tendencies among the species. There was no overlap between the isotopic niches of *C. pseudogracilis* and *A. aquaticus* even though they were often found in the same littoral habitat samples (Table A 8, Appendix III). This indicates distinct ecological niches occupied by the two species, from either feeding on different trophic levels and/or utilizing different microhabitats. My results support the findings of Jones & Waldron (2003) who described trophic separation between *C. pseudogracilis* and *A. aquaticus* in two different lakes. This can potentially represent an advantage for the invasive spread of *C. pseudogracilis* over *G. pulex*, which has been shown to have more overlapping trophic niches with *A. aquaticus* (see Section 3.4.3).

The relative abundance and distribution patterns of *C. pseudogracilis* and *Gammarus* spp. in Brackley and Muckno differed (Table A 8, Appendix III). However, one littoral sample (M6.1) from site M6 was an exception, in which the abundance of *C. pseudogracilis* was 97, in comparison with 3 *G. pulex*. The common presences of *C. pseudogracilis* and *G. pulex* in Muckno was also reflected in their dietary overlap, which was higher than between *C. pseudogracilis* and *G. duebeni*, comprising 31% of *C. pseudogracilis* total isotopic niche width, in comparison with 7% in Brackley. This was also revealed in their isotopic distances, which was closer between *C. pseudogracilis* and *G. pulex* than between *C. pseudogracilis* and *G. duebeni*. These results suggest a higher degree of competitive overlap between *C. pseudogracilis* and *G. pulex* than between *C. pseudogracilis* and *G. duebeni*.

Earlier laboratory studies suggested heavy predation of *C. pseudogracilis* by *Gammarus* spp. (Dick 1996, Macneil *et al.* 1999, MacNeil *et al.* 2003). However, results based on stable isotopes in this study did not provide evidence that *C. pseudogracilis* is a prey of either *G. duebeni* or *G. pulex*. In contrast, *C. pseudogracilis* had significantly higher  $\delta^{15}\text{N}$  values than the other species, suggesting that *C. pseudogracilis* occupied higher trophic positions in the food webs of Loughs Brackley and Muckno. Even though this suggests some form of predatory diet by *C. pseudogracilis*, it would not necessarily indicate its predation on *Gammarus* spp. or *A. aquaticus*.

The predatory nature of *Crangonyx* spp. has been reported by earlier studies including Schwartz (1992), who showed *C. shoemakeri*, a common species of seasonal ponds of North America, capturing and consuming mosquito larvae and



*Daphnia obtusa*. Stable isotope data from a wetland community in south-eastern USA suggested individuals of the Crangonyctidae family feed on midge larvae (Taylor and Batzer 2010). Being described mainly as a herbivorous species (Gledhill *et al.* 1993), knowledge on the diet of *C. pseudogracilis* is rather limited compared with the other *Gammarus* spp. The predatory nature of *Crangonyx* spp. has mostly been disregarded in relation to other amphipod species, mostly because of its much smaller size compared with *Gammarus* spp. (Gledhill *et al.* 1993, Dick 1996). *Crangonyx* demonstrates sexual dimorphism, females being much larger (up to 11 mm) than males (3-4 mm) (Kirkpatrick *et al.* 2006). In comparison, *G. pulex* males can reach up to 2 cm in length, although females are smaller. Stable isotope data from a high nutrient shallow lake in East England suggested predation by *C. pseudogracilis*, which appeared to share the top trophic position together with predatory leeches and the fish, rudd (*Scardinius erythrophthalmus*) (Jones and Waldron 2003).

A possible explanation for the contrasting results on the predation of *Gammarus* spp. on *C. pseudogracilis* can be found in an earlier laboratory study by MacNeil *et al.* (1999) that simulated lake habitats, showing that *C. pseudogracilis* demonstrated more distinct micro-distribution patterns than *G. pulex* or *G. duebeni*. *Gammarus* spp. occurred mainly on or in the sand, in the pondweed or under the provided pond substrate, avoiding open water or tank walls. In contrast, the majority of *C. pseudogracilis* are observed swimming freely in the water column or crawling on the pond walls (MacNeil *et al.* 1999). Even though results presented in this study were based on species' isotope signals at the whole-lake scale, isotope data of *C. pseudogracilis* with *Gammarus* spp. found together in a total of six littoral habitats in Brackley and Muckno supported separation of their isotopic niches also at the meso-habitat scale (Figure A 9, Appendix III). Based on standard ellipses of species from the same littoral habitats, the trophic position of *C. pseudogracilis* was higher than *Gammarus* spp. (and *A. aquaticus*) and no overlaps were observed between their standard ellipses (Figure A 9, Appendix III). Therefore the limited co-existence of *C. pseudogracilis* with *Gammarus* spp. in the same littoral habitats cannot be easily explained by predation of *Gammarus* spp. on *C. pseudogracilis*. Further work on indirect trophic interactions, or competitive bottlenecks between these two species could be a fruitful line of research in understanding the ecology and success of invasions.

### 3.4.3 Trophic relationship between *Gammarus* spp. and *A. aquaticus*

*Asellus aquaticus*, like *Gammarus* spp., is known to be a generalist species, functioning as a detritivore, shredder and grazer (Moog 2002). Particularly in lotic systems, coarse particulate organic matter (CPOM), especially microbially conditioned leaves, constitute an important food source for both *Asellus* and *Gammarus* (Graca *et al.* 1994a). Algae, which is more nutritious than allochthonous leaves, are also an important part of the diet of *A. aquaticus* and, to a lesser degree, *G. pulex* (Moore 1975). Despite considerable niche overlap between the two species (Graca *et al.* 1994a, 1994b), predation of *A. aquaticus* by the *Gammarus* spp. has been documented (Bengtsson 1982, Macneil *et al.* 1997, MacNeil *et al.* 2002, Bollache *et al.* 2008). Moreover, their relative distribution seems to be influenced by water nutrient concentrations, with *Gammarus* likely being more sensitive to organic pollution than *Asellus* (MacNeil *et al.* 2002, Donohue *et al.* 2009).

In Lough Fern, *A. aquaticus* and *G. duebeni* co-existed, with no significant differences between their isotopic niche widths. The fact that their isotopic distance was closest in Fern compared with other two lakes was a result of a relatively narrower niche width, rather than a strong overlap of their isotopic niches. In contrast, their niche overlap was less than 15% of their total isotopic niche areas, the smallest percentage of all lakes. Significant differences in their  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values indicated that, in comparison with *A. aquaticus*, *G. duebeni* had a significantly higher trophic position and was more  $\delta^{13}\text{C}$ -depleted.

In Brackley, where the non-native *C. pseudogracilis* was the dominant amphipod species, *A. aquaticus* was common and widespread while *G. duebeni* had a more limited distribution throughout the lake. The relative abundance of adult *A. aquaticus* versus *G. duebeni* was fewer in two out of three sites where *G. duebeni* was present in Lough Barkley (Table A 8, Appendix III). This can be a result of *A. aquaticus* avoiding habitats with *G. duebeni* because of increased predation risk. Whether this could be related to a higher dietary overlap and increased competition between *G. duebeni* with *C. pseudogracilis* requires further investigation. When the overall isotopic niche widths of *A. aquaticus* and *G. duebeni* were compared at the whole-lake level, no significant differences were found. However, the overlapping niche area between them was greater than it was in



Fern, comprising above 25% of their total isotopic niche areas. The relative positioning between *A. aquaticus* and *G. duebeni* in Brackley were rather similar to Fern; *A. aquaticus* was located at the lower left quadrant of the isotopic space in respect to *G. duebeni* (Figure 3.7). However, their alignment in Brackley was near vertical, which is indicative of their generalist feeding behaviours and *G. duebeni* still occupying higher trophic positions. These were also supported by the ANOVA test results, showing significant differences for  $\delta^{15}\text{N}$  but non-significant differences for  $\delta^{13}\text{C}$  values between species in Brackley.

In Muckno, the native *G. duebeni* is assumed to have become extinct with the arrival of *G. pulex*. As the dominant amphipod species, *G. pulex* co-existed in more habitats with *A. aquaticus*, unlike *G. duebeni* in Brackley. Even though the total niche widths of *G. pulex* in Muckno and *G. duebeni* in Brackley were very similar in size, *A. aquaticus* had a lower chance of having a broader niche width in Muckno than in Brackley (0.3). Therefore, the probability of *A. aquaticus* having smaller niche width than *G. pulex* was higher (0.7) in comparison with *G. duebeni* in Fern or Brackley (average 0.5). Moreover, it was more likely that *G. pulex* showed a higher trophic overlap with *A. aquaticus* (around 40% of species' total niche widths) than *G. duebeni* (25% in Brackley and 15% in Fern). Even though the isotopic distance between *A. aquaticus* and *G. pulex* in Muckno was the same as it was between *A. aquaticus* and *G. duebeni* in Brackley, their relative positioning contrasted. *Gammarus pulex* was significantly more  $\delta^{13}\text{C}$ -enriched than *A. aquaticus* in Muckno, but the  $\delta^{15}\text{N}$  difference between them was not significant, unlike *A. aquaticus* and *G. duebeni* in Fern or Brackley. *Gammarus pulex* was situated only slightly above and to the right of *A. aquaticus* in the isotopic space, constituting almost a vertical symmetry to the positioning of *G. duebeni* and *A. aquaticus* in Fern. Similar trophic positions occupied by *G. pulex* and *A. aquaticus* in Muckno suggests that despite a stronger niche overlap between the two species, they can continue to co-exist in the same habitat which could be a result of reduced predation pressure on *A. aquaticus* by *G. pulex* compared with *G. duebeni* in Brackley and Fern. This supports some earlier studies which showed that predation of *G. duebeni* on juvenile *A. aquaticus* was higher than *G. pulex* (Bollache *et al.* 2008) and predation rates of *G. duebeni* on juvenile *A. aquaticus* was less on adults (MacNeil *et al.* 2002). Moreover, co-existence of *G. pulex* and *A. aquaticus* could have been further facilitated by segregation of their food sources and/or utilisation of different habitats by the two



species (distinct  $\delta^{13}\text{C}$  values) in Muckno. It should be noted that *G. pulex* in Muckno had narrower  $\delta^{13}\text{C}$  dispersions than *G. duebeni* in Brackley, which can either be explained by less generalist feeding tendencies of the former species or differences in the diversity of basal food sources between the two lakes (see Section 3.4.1)

#### 3.4.4 *Gammarus duebeni* vs. *Gammarus pulex*

Because native *G. duebeni* and the invasive *G. pulex* did not co-exist in any of the study lakes, no direct comparisons of their isotopic niches could be made. This supports competitive exclusion of *G. duebeni* by *G. pulex* (Dick *et al.* 1993). Quantification of their isotopic niche width between Brackley and Muckno showed that their isotopic niche widths were almost equal. In spite of that, the  $\delta^{13}\text{C}$  dispersion was highest for *G. duebeni* in Brackley while the  $\delta^{15}\text{N}$  dispersion was highest for *G. pulex* in Muckno. This indicated a broader diversity of carbon sources utilized by *G. duebeni* in comparison with a broader range of trophic position and a higher degree of omnivorous diet by *G. pulex*. According to Piscart *et al.* (2011) who used both gut content and stable isotope analysis to compare single species populations of *G. pulex* and *G. duebeni* from different sections of the same river in N. Ireland, no significant differences were found in the food choices of *G. pulex* and *G. duebeni*. However, the same study reported that a broader spectrum of food sources and a broader diversity of carbon sources was assimilated by *G. pulex* than *G. duebeni* (Piscart *et al.* 2011), which contradicts the results of this study. Also, when both species co-existed, *G. pulex* was significantly  $\delta^{13}\text{C}$ - and  $\delta^{15}\text{N}$ -enriched compared with *G. duebeni* (Piscart *et al.* 2011). Based on the relative positioning of species, this study also showed that *G. pulex* was more  $\delta^{13}\text{C}$ -enriched in Muckno in respect to *A. aquaticus* than *G. duebeni* in Loughs Fern and Brackley. However, relative to *A. aquaticus*, *G. duebeni* was more  $\delta^{15}\text{N}$ -enriched than *G. pulex*.

### 3.5 Conclusions

The results of this chapter can lead to overall conclusions that:

- Niche widths and isotopic dispersions of macroinvertebrates were narrowest in Lough Fern, suggesting less diverse food sources with limited carbon and

nitrogen ranges. Brackley provided food sources with highest diversity and/or widest range of  $\delta^{13}\text{C}$  that supported increased generalist-feeding behaviour for all studied species across lakes. Broader  $\delta^{15}\text{N}$  dispersions of both *A. aquaticus* and *Gammarus spp.* in Brackley and Muckno than in Fern suggest increased chance of omnivory in the former two lakes. Similar to *C. pseudogracilis*, *G. pulex* showed a higher chance of omnivory in Muckno than *G. duebeni* in Brackley.

- With the highest  $\delta^{15}\text{N}$  values, *C. pseudogracilis* occupied the highest trophic positions among studied species both in Brackley and Muckno. This strongly suggests some form of a predatory diet by *C. pseudogracilis*, although not necessarily on *Gammarus spp.* or *A. aquaticus*. These results contrast with earlier arguments for predation of *C. pseudogracilis* by the *Gammarus spp.* in laboratory conditions.
- Niche widths between *G. duebeni* and *A. aquaticus* were not significantly different in Fern or Brackley, while the chance of *G. pulex* having a broader niche width than *A. aquaticus* was higher in comparison with *G. duebeni*. *Crangonyx pseudogracilis* showed the narrowest  $\delta^{13}\text{C}$  dispersions and a very high probability of having a smaller isotopic niche width than *A. aquaticus* or *Gammarus spp.* in Brackley and Muckno, which indicates least generalist feeding tendencies of *C. pseudogracilis* among the studied species.
- The competitive niche overlap among amphipods was higher between *C. pseudogracilis* and *G. pulex* in Muckno compared with *C. pseudogracilis* and *G. duebeni* in Brackley. There was no overlap between the isotopic niches of *C. pseudogracilis* and *A. aquaticus*, indicating distinct trophic positions occupied by the two species. *Gammarus pulex* showed a higher trophic overlap with *A. aquaticus* than *G. duebeni*. More widespread co-existence of *G. pulex* with *A. aquaticus* in comparison with *G. duebeni* in Brackley can be a result of occupying similar trophic positions in Muckno, hence reduced predation risk on *A. aquaticus* by *G. pulex*, as well as segregation of their food sources.
- The two *Gammarus spp.* showed differences in their position relative to *A. aquaticus*; *G. pulex* in Muckno was relatively more  $\delta^{13}\text{C}$ -enriched than *G. duebeni*, which might suggest *G. pulex* consuming more allochthonous food

sources. However, *G. duebeni* was more  $\delta^{15}\text{N}$ -enriched than *G. pulex*, which could suggest a higher predation pressure on *A. aquaticus*.

- Quantification of various features of the species' isotopic niche by adopting the isotopic niche metrics, including standard deviation of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , standard ellipse area ( $\text{SEA}_B$ ) and overlap, relative distance and angle gave a higher potential for providing additional insight about species' ecological interactions and their functional role in the food web, than classical (*e.g.* analysis of variances) approaches.



**4. SPATIAL AND TAXONOMIC  
VARIABILITY OF  
MACROINVERTEBRATE C:N:P  
STOICHIOMETRY**



## 4.1 Introduction

Carbon, nitrogen and phosphorus are the three key nutrients affecting the productivity of aquatic systems. Organisms interacting in food webs have specific elemental requirements. Poor quality food sources (high C:N and C:P) may cause elemental imbalances in consumers, which not only restrict their growth and reproduction, influencing their population dynamics, but have consequences on ecosystem-level nutrient cycling and food web interactions (Sterner and Elser 2002, Hall 2009). Compared with relatively constrained elemental signatures of marine phytoplankton (C:N:P ~ 106:15:1 molar) (Redfield *et al.*, 1958), elemental ratios of primary producers in freshwaters display higher variabilities, mainly driven by large variations in nutrient and light supplies (Hall 2009). Consumers tend to be more stoichiometrically homeostatic than their food sources (Sterner and Elser, 2002). Stoichiometry of organisms is regulated by a series of physiological processes controlling element uptake (*e.g.* selective acquisition of food), incorporation (*e.g.* allocation and storage of excess food) and release (*e.g.* during excretion and respiration) (Frost *et al.* 2005).

The growth rate hypothesis (Elser *et al.* 2000) suggests a dependency of C:P and N:P ratios of organisms' for growth. High P is required for the production of P-rich ribosomal-RNA that is necessary for increased protein synthesis during fast growth (Elser *et al.* 2000, Elser and Hamilton 2007). As growth rates are inversely proportional with organisms' body size (Peters, 1983), relative P contents are expected to be higher in smaller organisms, and high growth rates associated with reduced C:P and N:P ratios (Cross *et al.* 2003).

Rheostasis, which suggests that an organism is able to adjust its body stoichiometry to a changing environment over time (Mrosovsky 1990), has been suggested as an alternative hypothesis to strict homeostasis (Villar-Argaiz *et al.* 2002, Liess and Hillebrand 2005). Villar-Argaiz *et al.* (2002) showed that the body elemental ratios varied in different ontogenetic stages of a copepod species, *Mixodiaptomus laciniatus*, such that adult C:N ratios were significantly lower, while C:P and N:P ratios were significantly higher than in nauplii, therefore suggesting a link between zooplankton body stoichiometry and life history. Findings of seasonal variation in the stoichiometry of benthic invertebrates from a Swedish lake also supported the rheostasis hypothesis (Liess & Hillebrand 2005).



### **4.1.1 Linking littoral benthos stoichiometry and lake shoreline habitats**

According to Forest *et al.* (2003), the P, N, and C contents in the bodies of consumers indicate their needs for these elements and the C:N:P ratio of consumers can be used to estimate nutrient limitations. Despite some stoichiometric studies on stream benthos (Cross *et al.* 2003, Evans-White and Lamberti 2005, Hladysz *et al.* 2009), the application of the stoichiometric framework to littoral benthic food webs is still very limited (but see Frost *et al.* 2003; Liess & Hillebrand 2005; Fink, Peters & Von Elert 2006; Donohue 2008) and the mechanisms that underpin the tolerance of invertebrates for C:N:P ranges is still uncertain.

In comparison with pelagic systems, littoral benthic organisms are closely associated with their inorganic and the organic substrates (Doi 2009). Their nutrient and energy supply depend on the connections from both below (organic substrates, mineral dissolution and groundwater) and above (settling material and water column) for element and energy sources (Fink *et al.* 2006). In addition, littoral zones are characterized by high spatial heterogeneity owing to strong lateral and vertical gradients of environmental conditions and coupled with the riparian zones both spatially (providing habitat and substrate for benthic communities) and energetically (allochthonous inputs of dissolved and coarse particulate organic matter (CPOM)) (Brauns 2008). The proportion of the allochthonous (*e.g.* leaf litter and woody debris) compared with autochthonous (*e.g.* benthic algae, macrophytes) inputs into the benthic system affects the elemental ratios of the particulate organic matter (POM), which in turn, influences the quality of the food sources available to littoral macroinvertebrate consumers (Frost *et al.* 2002). In a  $\delta^{13}\text{C}$  tracer experiment, Cole *et al.* (2006) showed terrestrial carbon constituted 33-70% of total carbon flow to zooplankton, 60-85% to benthic invertebrates and 20-50% to fish, showing the importance of terrestrial food sources to, particularly oligotrophic, lake food webs (Cole *et al.* 2006, Doi 2009). Therefore, alteration in catchment properties, including changes of shoreline habitat vegetation likely affect the connectedness of lake systems to terrestrial subsidies (Doi 2009). Brauns *et al.* (2011) showed that human shoreline development negatively affects the energetic coupling between the littoral and the

riparian zone through disruption of organic matter transfer across the aquatic-terrestrial boundary.

Amphipod and isopod crustaceans are common benthic macroinvertebrates present in freshwater bodies throughout the year, and *Gammarus* spp. (Family Amphipoda) can constitute a high percentage of the total macroinvertebrate abundance and biomass (Macneil *et al.* 1997, Free *et al.* 2006). *Gammarus* spp. are mainly described as shredders, even though they can have other functional roles including collector-gatherers, predators or generalist-detritivores in a variety of freshwater habitats (Macneil *et al.* 1997). They are potential keystone taxa with a potential for powerful effects on structure and ecosystem processes, including algal production and decomposition (Woodward *et al.* 2008, Gerhardt *et al.* 2011, Nelson 2011). Among the amphipods in Irish freshwaters, *Gammarus duebeni celticus* is one of the three native species next to less common *Gammarus lacustris* and subterranean *Niphargus kochianus irlandicus*, whereas *Gammarus pulex*, *Gammarus tigrinus* and *Crangonyx pseudogracilis* are the three non-indigenous freshwater amphipods that arrived in Ireland in the last century (Costello 1993). Since *G. pulex* was introduced to Ireland in 1950s, a gradual replacement of *G. d. celticus* (referred as *G. duebeni* in rest of chapter) has been observed in many freshwater systems (Dick 2008). Earlier studies explained the dynamic of these two *Gammarus* spp. by interspecific competition (Hynes 1954). Recent studies emphasize the importance of predation of *G. pulex* over *G. duebeni* (Dick 2008). Another alien species, *C. pseudogracilis*, with high tolerances of pollution, appear not to occur in high quality waters where *Gammarus* spp. are abundant (MacNeil *et al.* 2002). A possible explanation to this is the predation of both *G. pulex* and *G. duebeni* on the smaller sized *C. pseudogracilis*, especially during moulting (Dick 1996).

Among the two freshwater isopod species in Irish waters, *Asellus aquaticus* is more common (Free *et al.* 2006, Little and Free 2013) than *Proasellus meridianus* (Wolff 1973). *Asellus aquaticus* was found to have the highest total biomass of littoral invertebrates with a key role in detritus processing in three Swedish lakes (Bjelke *et al.* 2005). Allochthonous CPOM was the most important food source to shredders, including *A. aquaticus* at both open and wooded sites of four Swedish streams (Leberfinger *et al.* 2010). Both *Gammarus* spp. and *A. aquaticus* are



suggested to have overlapping niches, feeding on CPOM, particularly microbially conditioned leaves (Graca *et al.* 1994a).

### 4.1.2 Chapter objectives

Characterization of C:N:P stoichiometry of benthic invertebrates provide clues to taxonomic and functional ecology (Frost *et al.* 2002). So far, a few studies have reported the stoichiometry of the isopod *A. aquaticus* (Liess and Hillebrand 2005, Fink *et al.* 2006, Brauns 2008, Donohue 2008); and amphipods *G. duebeni* (Donohue 2008, Hladyz *et al.* 2009) and *G. pulex* (Dorenbosch and Bakker 2011). A few studies have also covered another isopod species, *Asellus militaris* (Evans-White and Lamberti 2005) and other species of gammarids, such as *G. roeseli* (Fink *et al.* 2006, Gücker *et al.* 2011) and *G. pseudolimnaeus* (Evans-White and Lamberti 2005). No studies appear to report the elemental stoichiometry of amphipod *C. pseudogracilis*. Moreover, no studies appear to have linked the stoichiometry of these littoral macroinvertebrates with lake shoreline habitats other than Brauns (2008) who studied the effects of shoreline development on the trophic basis of littoral macroinvertebrate food webs in three German lakes. This study indicated that overall consumer-resource imbalances were highest at natural shorelines compared with the two developed shorelines (retained wall and beach) (Brauns 2008). However, the comparison of macroinvertebrate stoichiometry among shoreline types was based on trophic groups (i.e. scraper and collector) instead of individual species. Comparison of elemental ratios between *G. duebeni* and *A. aquaticus* across 9 Irish lakes along a nutrient gradient by Donohue (2008) showed significant taxonomic differences, particularly of C:N ratios. According to the same study, elemental ratios of these two species displayed much less among-lake variation in comparison with their food sources, while the extend of spatial variation in macroinvertebrate stoichiometry was not discussed.

There are two main objectives of this study. First is to investigate the within- and among-lake spatial variability of littoral macroinvertebrate C:N:P stoichiometry and the influence of shoreline habitat features by comparing sites adjacent to woodland and grassland shoreline habitats. This objective tests the extent of local habitat features on small-scale spatial differences in the elemental stoichiometry, which may be indicative of functional roles of littoral macroinvertebrates.



Second is to compare the taxonomic variability of the C:N:P stoichiometry of littoral macroinvertebrates, using four crustacean species (Amphipoda and Isopoda families) common to Irish freshwaters as model organisms. Taxonomical variability in the elemental stoichiometry can be used to differentiate the functional roles of these ecologically related invertebrate species and compare the degree of homeostasis among them.

I hypothesize that elemental stoichiometry of macroinvertebrate species will demonstrate significant differences: 1) among lakes; 2) between the woodland and grassland shoreline habitats within lakes, following the rheostasis hypothesis; and 3) among species which reflects their functional differences (*e.g.* between *A. aquaticus* and *Gammarus spp.* as reported by Donohue (2008) and between *C. pseudogracilis* and the other species as suggested by the results of Chapter 3).

## **4.2 Methods**

### **4.2.1 Study lakes**

Three lakes, Lough Fern, Brackley Lough and Lough Muckno, within a range of medium nutrient status (TP 35-109  $\mu\text{g/L}$ ) were sampled in September 2009. See the Section 2.2.1 for a more detailed description on lake selection criteria. Shoreline habitat type and accessibility influenced the site selection within each lake. Sampling locations included sites dominated by three woodland and three grassland shoreline habitats (see Figure 3.2 for maps showing the locations of sampling sites at each lake and Table A 8 (Appendix III) for the abundances of species at each site). Site locations of the same shoreline type were judged to be sufficient distance from each other to reduce inter-active effects and in order to cover a whole lake influence. Common tree species in the sites with wooded shorelines were also identified and listed in Table A 8 (Appendix III).

### **4.2.2 Macroinvertebrate sampling**

Macroinvertebrate samples were collected from six sites in the littoral zone, adjacent to three grassland and three woodland shoreline habitats, using kick sampling. At each site, multiple macroinvertebrate samples were collected from different habitat patches (maximum 3) whenever they were present. If inspection of the littoral zone indicated a homogeneous habitat, usually one, sometimes two

samples (one from near and another away from the shore) were collected. Following Donohue (2008), macroinvertebrate samples were frozen upon collection using dry ice until they were transferred to a freezer on arrival back to the laboratory. They were kept frozen at  $-40^{\circ}\text{C}$  until processed for stoichiometry analysis.

### 4.2.3 Stoichiometry analysis

Frozen macroinvertebrate samples were thawed at room temperature before being sorted in distilled water. The isopod and amphipod samples were separated in different petri dishes before species identification and length measurements, using a binocular dissecting stereomicroscope. In order to avoid contamination, all the laboratory material and the surfaces were wiped with 98% ethanol prior to sorting and identification, while equipment (*e.g.* forceps, petri dishes, spoons, etc.) was also wiped with 98% ethanol before handling separate invertebrates. Laboratory latex gloves were worn at all times to minimize sample contamination.

A random sequencing website (Haahr 2011) was used to generate a sequence of random numbers, which were then used to select the necessary total number of macroinvertebrates required for the stable isotope and elemental analysis. During species identification, lengths of individual macroinvertebrates were measured and those above the minimum required length were separated and placed randomly on petri dishes, and divided into cells with a given number. Three to six individuals from each littoral habitat were selected for elemental analysis, depending on the availability of the species in samples. All animals were dried at  $60^{\circ}\text{C}$  for 24 h in clean petri dishes in a dehydrator. Samples not processed immediately were kept at  $4^{\circ}\text{C}$  for up to 2-3 h.

In order to obtain sufficient sample for elemental analysis, generally six individuals of the same macroinvertebrate species were pooled and ground into a fine powder using an agate pestle and mortar. When numbers of individuals were low, a minimum of three individuals were used. For the carbon and nitrogen analysis, subsamples of 0.6-0.9 mg macroinvertebrate tissue were weighted into tin capsules, folded into tin balls and stored in a desiccator until the analysis. All samples were analysed for carbon and nitrogen in a Thermo Delta<sup>plus</sup>® CF-IRMS (continuous flow isotopic ratio mass spectrometer) interfaced with Delta<sup>plus</sup> via a



Conflo III based in Trinity College Dublin Stable Isotope Laboratory (Dublin, Ireland).

To measure total phosphorus contents of invertebrates, subsamples of 0.4-0.5 mg of the remaining material from the each composite sample, used for the C and N analysis, were placed into autoclavable glass bottles and combusted at 300°C in a furnace for 2 h in order to remove organic content. Twenty-five ml of distilled water was added to the cooled glass bottles. Total phosphorus analysis was conducted following the Eisenreich, Bannerman & Armstrong (1975) method, which included potassium peroxide sulphate ( $K_2S_2O_8$ ) digestion of the samples. After the addition of 5 ml of digestion reagent into the bottles, samples were autoclaved at 15 psi for 30 min. After cooling, 5 ml of mixed reagent, which included  $H_2SO_4$ , antimony stock, molybdate stock and ascorbic acid, was added. Two 5 ml subsamples of the mixture were transferred into clean test tubes. After colour development (app. 30 min), sample absorbance was measured using a spectrophotometer (Shimadzu UV-VIS Spectrophotometer UV-1700) at 882 nm.

#### **4.2.4 Data analysis and statistics**

##### **4.2.4.1 Triangular diagrams**

Triangular diagrams were used to illustrate the relative molar carbon (C), nitrogen (N) and phosphorus (P) concentrations of macroinvertebrates. As the proportions of the variables should sum to a constant (unity in this case), the elemental composition data was normalised by dividing the molar C, N and P values with the sum of the three before plotting the triangular diagrams.

Figure 4.1 demonstrates a guideline for reading the triangular diagrams for a given data point. The main axes have standardised scales from 0 to 1 and gridlines divide each main axis at 0.1 units. Each data point within or on the triangle represents relative C, N and P concentrations, which sum to 1. Figure 4.1a displays the estimation of the relative carbon concentration using the dashed gridlines drawn parallel to the P main axis (left). Hence, following the thick gridline from the data point to the C main axis, the value on the carbon scale can be read as 0.4. Also, a data point anywhere along the P main axis, would have 0 carbon concentration, while a data point at the bottom left corner the triangle represents a C concentration of 1, hence 0 concentrations of N and P. The triangular diagrams



run in counter-clockwise directions as indicated by the arrows in Figure 4.1b. When estimating the corresponding data values, the imaginary grids should be drawn parallel to the main axes that are to the left of the variable of interest. Based on this, the relative concentrations of C, N and P for the given data point can be read as 0.4, 0.4 and 0.2, respectively (Figure 4.1b).

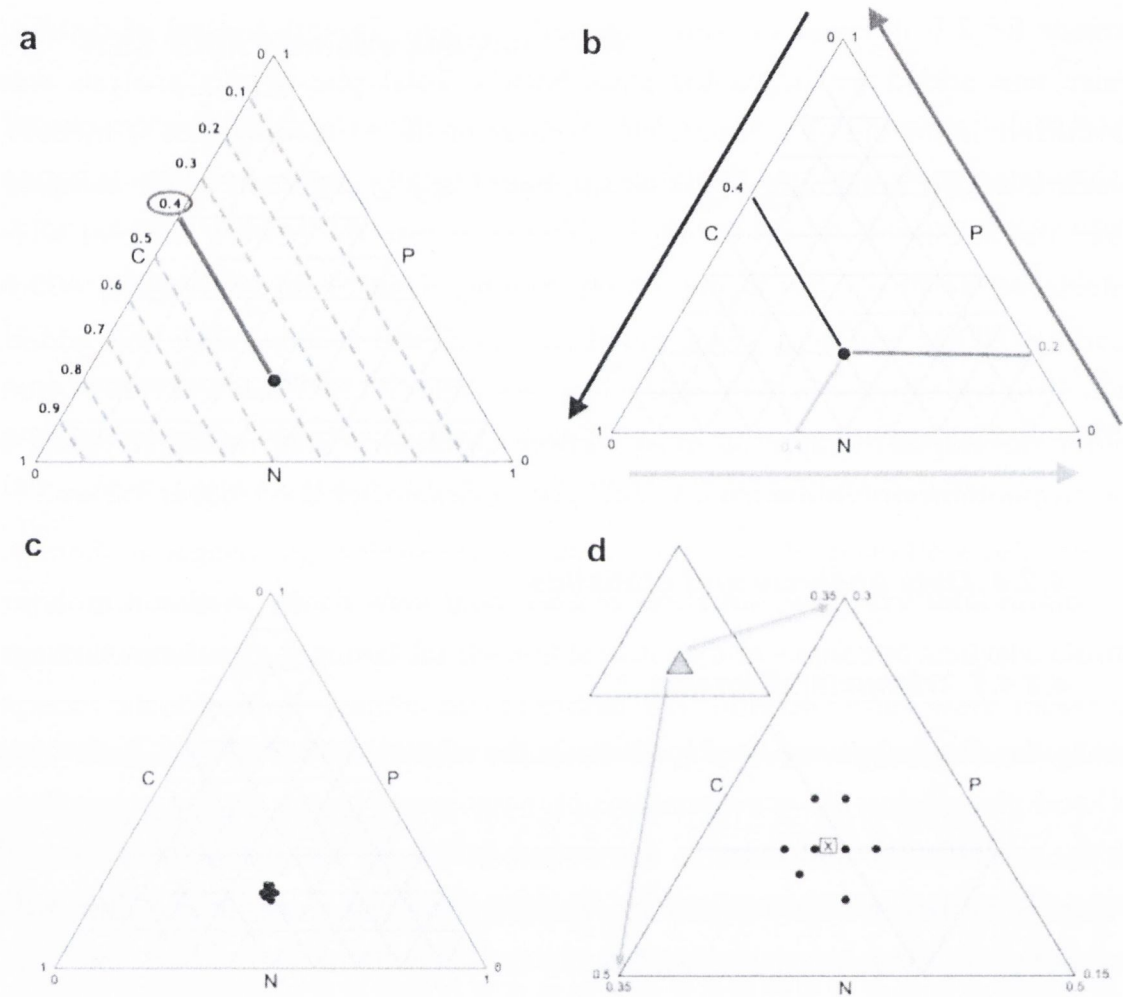


Figure 4.1 Example triangular (ternary) diagrams. **a)** Estimating the relative C (Carbon) concentration of any data in the triangle can be done with the help of the dashed gridlines. The thick line from the given data point corresponds to 0.4 on the C scale. **b)** Thick gridlines in different shades of grey indicate the relative compositions of C, N and P of the given data point. Arrows show the direction of the increasing values for each scale. **c)** Example of a group of data clustered together in a complete triangle (0-1 scale). **d)** Magnified view of the same data cluster (in 1c) in an enlarged triangle, which corresponds to the shaded area within the smaller triangle on the top left. Square box (X) indicates the centre of gravity of the data points belonging to the same group.

In this study, the stoichiometry data clustered together with little variation among data points, as shown in Figure 4.1c. In order to overcome this, a higher resolution of data points are displayed in enlarged equilateral sub-triangles (as in Figure 4.1d) throughout this chapter. A diagram displaying the relative sizes of the sub-triangles compared with the complete triangles are also included next to the sub-triangle (Figure 4.1d). In this example, centres of gravity, which represent the central point of the group data values, are indicated in boxes with the initials of the group names.

All the triangular diagrams were plotted using the “ade4” package (Dray and Dufour 2007) in R software version 2.14.1 (R development core team, 2012).

#### **4.2.4.2 Macroinvertebrate C:N:P stoichiometry**

Differences of the elemental ratios (C:N, C:P and N:P) of individual macroinvertebrate species across lakes were tested using One-way Multivariate Analysis of Variances (MANOVA), while the effects of shoreline habitat types and species on the elemental ratios of macroinvertebrates in individual lakes were tested using two-way MANOVAs. The maximal two-way MANOVA models included the shoreline habitat type and species as the main explanatory variables, and their interaction terms. Model simplification was based on the principle of parsimony and consisted of the removal of the non-significant interaction term in order to achieve the minimal adequate models (Crawley 2007). MANOVA models were chosen because of the interdependent nature of the elemental ratios, so that the C:N, C:P and N:P values were treated as one multivariate response (Liess and Hillebrand 2005). Pillai's trace ( $\nu$ ) statistic was chosen as the multivariate test statistics, since it is considered to be the most robust to violations of MANOVA assumptions (*e.g.* data normality and homogeneity) in cases of reduced sample sizes and unbalanced designs (Johnson and Field 1993). When a significant difference was detected for a MANOVA, contribution of the individual elemental ratios to the significant multivariate effects was tested separately with an analysis of variance (ANOVA). Following that, Tukey's honestly significant difference (HSD) test was used to examine the significant differences between different lakes, shoreline habitat types and species.



## 4.3 Results

### 4.3.1 Spatial variation of macroinvertebrate C:N:P across lakes

Because *G. pulex* and *G. duebeni* did not occur in the same lakes, gammarid elemental stoichiometry data from all three lakes were grouped together as *Gammarus spp.* for among lake comparisons. Based on the triangular diagrams displaying the distribution of the relative molar concentrations of carbon, nitrogen and phosphorus of individual macroinvertebrate species according to lakes, no distinct clustering between lakes could be observed for *C. pseudogracilis* (Figure 4.2). This was statistically supported as the C:N:P stoichiometry of *C. pseudogracilis* showed no significant differences ( $p > 0.05$ ) between Brackley and Muckno (one-way MANOVA test, Table 4.1). The centres of gravity of the three study loughs with *A. aquaticus* were also separable on the triangular diagrams (Figure 4.2); however, these differences lacked statistical significance ( $p > 0.05$ ) (one-way MANOVA test, Table 4.1). On the other hand, elemental ratios of the *Gammarus spp.* were significantly different across the lakes (MANOVA,  $\nu = 0.676$ ,  $F_{6,32} = 2.7$ ,  $p = 0.03$ , Table 4.1).

In the triangular diagrams for *Gammarus spp.*, the centres of gravity of Loughs Fern and Muckno were overlapping, suggesting similar elemental compositions. However the separate position of Brackley Lough to both Fern and Muckno suggested differences of elemental compositions of *Gammarus spp.* in Brackley and the latter two lakes, particularly owing to distinct nitrogen concentrations (Figure 4.2). When individual elemental ratios were compared separately, the C:P ratio of *G. pulex* in Lough Muckno was found significantly higher than *G. duebeni* in Brackley Lough (One-way ANOVA, Tukey's HSD,  $p < 0.05$ ), even though it was not significantly different than *G. duebeni* in Lough Fern (Tukey's HSD,  $p > 0.05$ ) (Table 4.1). The N:P ratios of *Gammarus spp.* in Loughs Fern and Muckno were both significantly higher than in Brackley (Tukey's HSD,  $p < 0.01$ ). The C:N ratio of *Gammarus spp.* among lakes was not significantly different (ANOVA,  $p = 0.13$ ). Overall, no significant differences were observed for any of the molar elemental ratios in Loughs Muckno and Fern (Tukey's HSD,  $p = 0.05$ ) (Table 4.1).



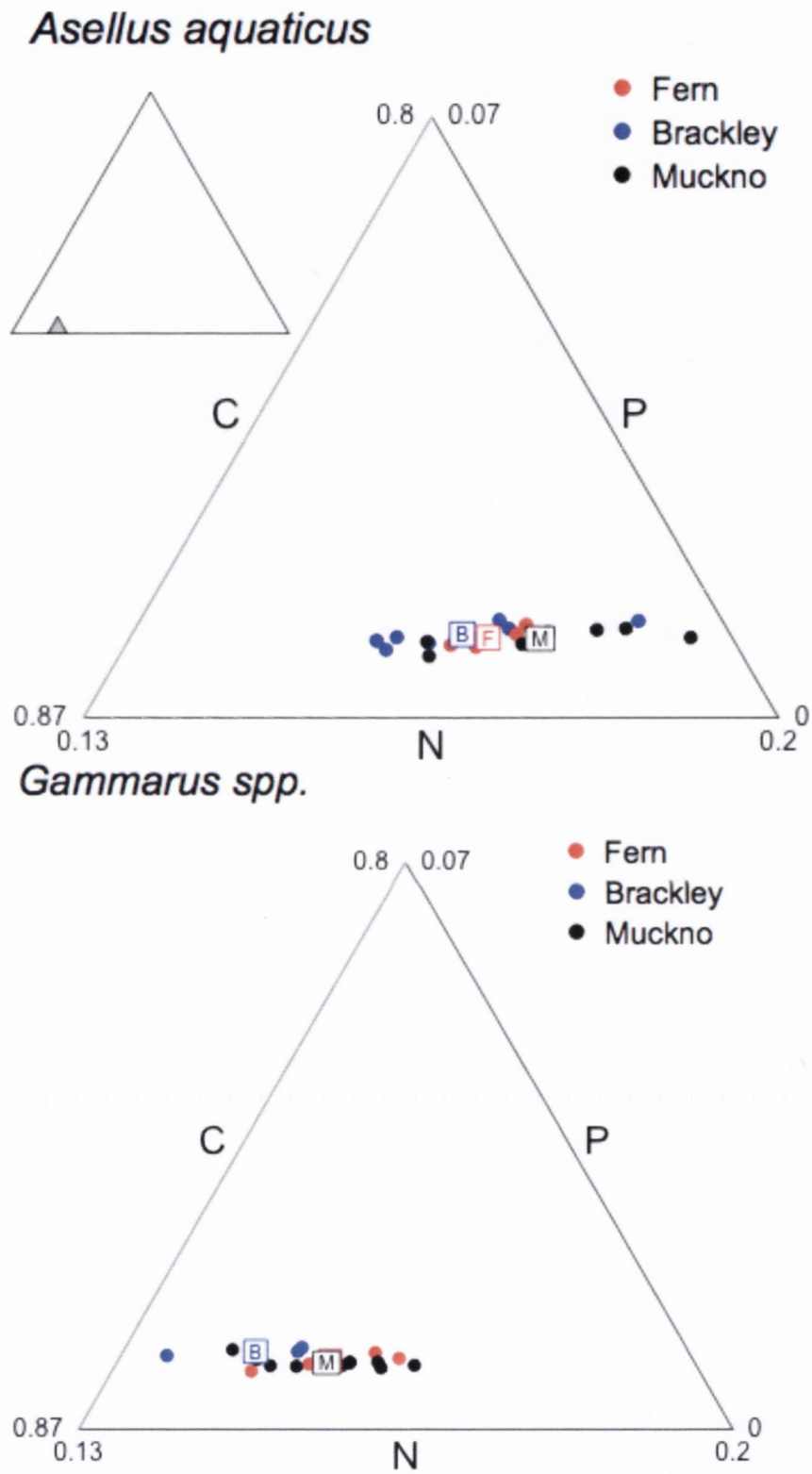


Figure 4.2 Triangular plots displaying the lake effect on the elemental composition of *A. aquaticus*, *Gammarus spp.* and *C. pseudogracilis*. Each point represents a pooled sample of individuals from single a littoral habitat. The centre of gravity of each lake group is indicated in boxes with its initials (see Figure 4.1 for a guideline to read the triangular diagrams).

*Crangonyx pseudogracilis*

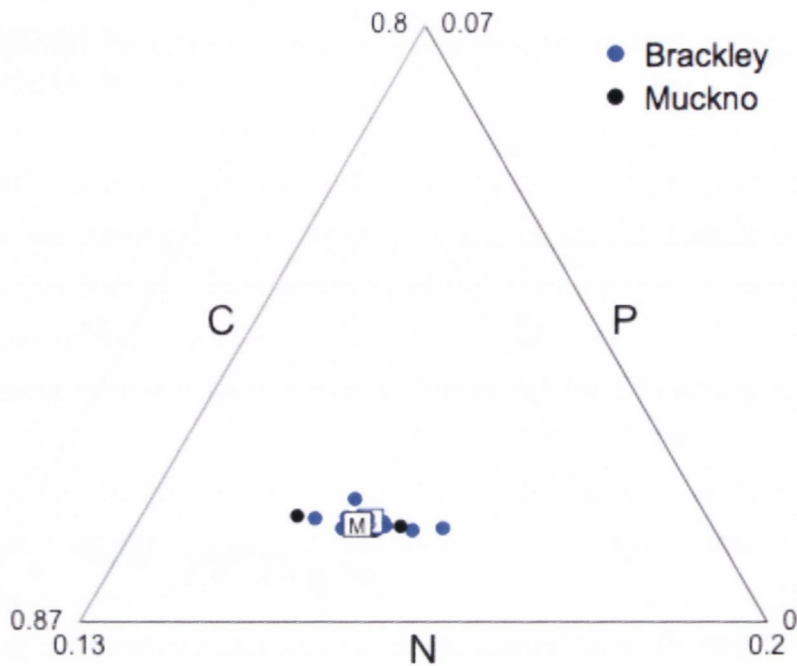


Figure 4.2 (continued) Triangular plots displaying the lake effect on the elemental composition of *A. aquaticus*, *Gammarus spp.* and *C. pseudogracilis*. Each point represents a pooled sample of individuals from single a littoral habitat. The centre of gravity of each lake group is indicated in boxes with its initials (see Figure 4.1 for a guideline to read the triangular diagrams).

Table 4.1 One-way Multivariate Analysis of Variance (MANOVA) results (Pillai’s trace ( $\nu$ )) for the effect of lakes on C:N:P molar ratios of the macroinvertebrate taxa. Lakes are ordered from highest to lowest mean ratios.  $\geq$  = no significant difference;  $>$  = significant difference between adjacent lakes. Significant  $p$  value and the lakes with significant differences are indicated in bold font (B = Brackley; F = Fern and M = Muckno).

| Taxa                     | Pillai’s trace ( $\nu$ ) | F   | Df    | p             | Comparison among loughs |                                              |                     |
|--------------------------|--------------------------|-----|-------|---------------|-------------------------|----------------------------------------------|---------------------|
|                          |                          |     |       |               | C:N                     | C:P                                          | N:P                 |
| <i>A. aquaticus</i>      | 0.433                    | 1   | 6, 32 | 0.218         | B $\geq$ F $\geq$ M     | M $\geq$ F $\geq$ B                          | M $\geq$ F $\geq$ B |
| <i>Gammarus spp.</i>     | 0.676                    | 2.7 | 6, 32 | <b>0.030*</b> | B $\geq$ M $\geq$ F     | <b>M<math>\geq</math>F<math>\geq</math>B</b> | M $\geq$ F $>$ B    |
| <i>C. pseudogracilis</i> | 0.163                    | 1   | 3, 8  | 0.680         | M $\geq$ B              | M $\geq$ B                                   | B $\geq$ M          |

\* $p < 0.05$ .

4.3.2 Taxonomic variation of macroinvertebrate C:N:P across lakes

The C:N ratios compared with the C:P ratios of the overall macroinvertebrate samples across the three lakes showed differences of species stoichiometry (Figure 4.3). *Crangonyx pseudogracilis* displayed the narrowest range in stoichiometry, while *G. duebeni* showed a broader range compared with *G. pulex*, *C. pseudogracilis* and *A. aquaticus* (Figure 4.3).

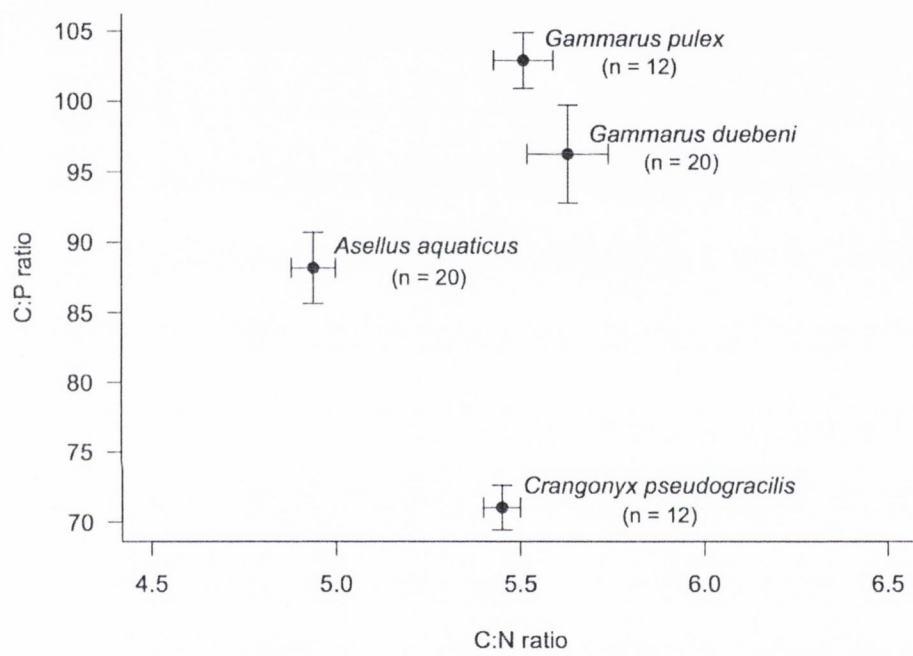


Figure 4.3 Molar C:N vs. C:P ratios of macroinvertebrate species across Loughs Fern, Brackley and Muckno. Data points represent means over all samplings  $\pm$  SE; where n = number of samples representing pooled individuals collected from different littoral habitats.

Comparison of the molar elemental ratios of macroinvertebrate species averaged across the three study lakes showed significant differences (MANOVA,  $\nu = 1.18$ ,  $F_{9, 144} = 10$ ,  $p < 0.001$ ) (Table 4.2). Individual examination of the elemental ratios showed significant results, *A. aquaticus* had the lowest mean ( $\pm$ SD) molar C:N ratios ( $4.9 \pm 0.3$ ,  $n=20$ ), and *Gammarus duebeni* the highest ( $5.7 \pm 0.3$ ,  $n=20$ ). Post hoc tests showed that only *A. aquaticus* had significantly lower C:N ratios compared with the other species (Tukey’s HSD,  $p < 0.01$ ). No significant differences were observed among the C:N ratios of *C. pseudogracilis* and the two gammarid species (Tukey’s HSD,  $p > 0.05$ ) (Figure 4.3, Table 4.2).



Table 4.2 Comparison of descriptive statistics (mean $\pm$ SD) for molar C:N, C:P and N:P ratios of the macroinvertebrates sampled across Loughs Brackley, Fern and Muckno; and results for One-way ANOVAs for individual elemental ratios; where n = number of samples representing pooled individuals collected from different littoral habitats. Multivariate Analysis of Variance (MANOVA) test results: Pillai's trace = 1.18,  $F_{9,144} = 10$ . Mean comparisons among species are based on TUKEY's HSD test ( $p < 0.05$ ) and presented in a decreasing order of mean elemental ratios.  $\geq$  = no significant difference;  $>$  = significant difference between adjacent species. Different symbols next to the species abbreviations indicate significant differences among species (**A** = *Asellus aquaticus*; **C** = *Crangonyx pseudogracilis*; **Gd** = *Gammarus duebeni*; **Gp** = *Gammarus pulex*).

| Elemental Ratios | Species Means ( $\pm$ SD)          |                                           |                                  |                                 | One – way ANOVA Results |           |            | Comparison among species (TUKEY's HSD Results)                                |
|------------------|------------------------------------|-------------------------------------------|----------------------------------|---------------------------------|-------------------------|-----------|------------|-------------------------------------------------------------------------------|
|                  | <i>Asellus aquaticus</i><br>(n=20) | <i>Crangonyx pseudogracilis</i><br>(n=12) | <i>Gammarus duebeni</i><br>(n=9) | <i>Gammarus pulex</i><br>(n=12) | <i>F</i>                | <i>Df</i> | <i>p</i>   |                                                                               |
| C:N              | 4.9 $\pm$ 0.3                      | 5.5 $\pm$ 0.2                             | 5.7 $\pm$ 0.3                    | 5.5 $\pm$ 0.3                   | 19.17                   | 3, 48     | <0.001 *** | Gd <sup>†</sup> $\geq$ Gp <sup>†</sup> $\geq$ C <sup>†</sup> > A <sup>*</sup> |
| C:P              | 88.2 $\pm$ 11.3                    | 71.0 $\pm$ 5.5                            | 96.3 $\pm$ 10.4                  | 102.9 $\pm$ 6.6                 | 25.57                   | 3, 48     | <0.001 *** | Gp <sup>†</sup> $\geq$ Gd $\geq$ A <sup>*</sup> > C <sup>0</sup>              |
| N:P              | 17.8 $\pm$ 1.8                     | 13.1 $\pm$ 1.3                            | 17.1 $\pm$ 1.9                   | 18.7 $\pm$ 1.8                  | 25.87                   | 3, 48     | <0.001 *** | C <sup>†</sup> > Gd <sup>*</sup> $\geq$ A <sup>*</sup> $\geq$ Gp <sup>*</sup> |

\*\*\*  $p < 0.001$ .

C:P and N:P ratios were also highly significantly different among species (ANOVA,  $F_{3, 48} = 25.57$ ,  $p < 0.001$  and  $F_{3, 48} = 25.87$ ,  $p < 0.001$ , respectively). *Crangonyx pseudogracilis* had the lowest mean molar C:P and N:P ratios ( $71.0 \pm 5.5$  and  $13.1 \pm 1.3$ , respectively), while they were highest for *G. pulex* ( $102.9 \pm 6.6$  and  $18.7 \pm 1.8$ , respectively). Post hoc tests revealed no significant differences for the C:P or N:P ratios between the two species of gammarids (Tukey's HSD,  $p > 0.05$ ). On the other hand, C:P and N:P ratios for *C. pseudogracilis* was significantly lower compared with the rest of the species. *Asellus aquaticus* had significantly lower C:P ratios than *G. pulex*, while the difference between the N:P ratios of *A. aquaticus* and either of the *Gammarus* spp. was not significant (Tukey's HSD,  $p < 0.05$ ) (Figure 4.3, Table 4.2).

#### 4.3.3 Within-lake variation of macroinvertebrate C:N:P

Distribution of the relative elemental concentrations of macroinvertebrates based on shoreline habitat types and species are shown in triangular diagrams for the three study lakes separately (Figure 4.4 - Figure 4.6) The effects of species and the shoreline habitat type (woodland and grassland) on the macroinvertebrate C:N, N:P and N:P ratios for each study lake were determined using two-way multivariate analysis of variance (MANOVA) tests. Summary data for the macroinvertebrate elemental ratios (mean  $\pm$  SE) based on shoreline habitat types are displayed in Table 4.3.

Based on the triangular diagrams, samples from the woodland and the grassland shorelines showed no obvious separation in Loughs Fern (Figure 4.4) or Brackley (Figure 4.5), suggested by the overlapping or very close centres of gravity, respectively. The most parsimonious MANOVA model also showed that shoreline habitat type main effect was not significant for any of the lakes ( $p > 0.05$ ) (Table 4.4). On the other hand, relative elemental compositions of different species separated clearly from each other in the triangular diagrams for all lakes, with a consistency for each species' relative trophic positions in each lake (Figure 4.4 - Figure 4.6). *Asellus aquaticus* samples were positioned on the right hand corner of the triangle compared within either of the *Gammarus* spp. in all lakes, indicating lower C:N ratios in *A. aquaticus*. *Crangonyx pseudogracilis* was positioned above other species in Brackley and Muckno, indicating relatively higher P contents, hence lower C:P and N:P ratios.

Table 4.3 Comparison of descriptive statistics (mean±SE) for molar C:N, C:P and N:P ratios of the macroinvertebrates sampled across the woodland (W) and grassland (G) shoreline habitats of Loughs Fern, Brackley and Muckno; where n = number of samples representing pooled individuals collected from different littoral habitats. \* *Gammarus spp.* = *G. duebeni* in Loughs Fern and Brackley, and *G. pulex* in Lough Muckno.

| Lough    | Elemental Ratio | Shoreline Habitat | n | <i>Asellus aquaticus</i> | n | <i>Gammarus spp.*</i> | n | <i>Crangonyx pseudogracilis</i> |
|----------|-----------------|-------------------|---|--------------------------|---|-----------------------|---|---------------------------------|
| Fern     | C:N             | W                 | 2 | 4.9 ± 0.1                | 3 | 5.4 ± 0.1             |   |                                 |
|          |                 | G                 | 3 | 5.0 ± 0.1                | 3 | 5.6 ± 0.2             |   |                                 |
|          | C:P             | W                 | 2 | 81.8 ± 6.5               | 3 | 99.2 ± 2.7            |   |                                 |
|          |                 | G                 | 3 | 93.5 ± 4.9               | 3 | 101.3 ± 8.9           |   |                                 |
|          | N:P             | W                 | 2 | 16.6 ± 1.0               | 3 | 18.3 ± 0.3            |   |                                 |
|          |                 | G                 | 3 | 18.7 ± 0.8               | 3 | 18.1 ± 1.1            |   |                                 |
| Brackley | C:N             | W                 | 5 | 5.0 ± 0.2                | 1 | 5.6                   | 5 | 5.5 ± 0.1                       |
|          |                 | G                 | 2 | 5.1 ± 0.2                | 2 | 6.0 ± 0.3             | 4 | 5.4 ± 0.1                       |
|          | C:P             | W                 | 5 | 85.4 ± 6.6               | 1 | 83.4                  | 5 | 70.1 ± 0.1                      |
|          |                 | G                 | 2 | 85.8 ± 6.9               | 2 | 90.8 ± 3.2            | 4 | 71.1 ± 4.6                      |
|          | N:P             | W                 | 5 | 17.0 ± 0.9               | 1 | 14.8                  | 5 | 12.8 ± 0.3                      |
|          |                 | G                 | 2 | 16.6 ± 0.5               | 2 | 15.2 ± 0.3            | 4 | 13.3 ± 1.0                      |
| Muckno   | C:N             | W                 | 6 | 4.8 ± 0.1                | 6 | 5.5 ± 0.1             | 2 | 5.3 ± 0.1                       |
|          |                 | G                 | 2 | 4.9 ± 0.3                | 5 | 5.6 ± 0.1             | 1 | 5.8                             |
|          | C:P             | W                 | 6 | 87.0 ± 2.8               | 6 | 103.5 ± 1.5           | 2 | 75.4 ± 1.8                      |
|          |                 | G                 | 2 | 97.1 ± 18.0              | 5 | 102.3 ± 4.2           | 1 | 67.1                            |
|          | N:P             | W                 | 6 | 18.3 ± 0.5               | 6 | 19.0 ± 0.5            | 2 | 14.1 ± 0.2                      |
|          |                 | G                 | 2 | 19.7 ± 2.6               | 5 | 18.5 ± 1.1            | 1 | 11.6                            |



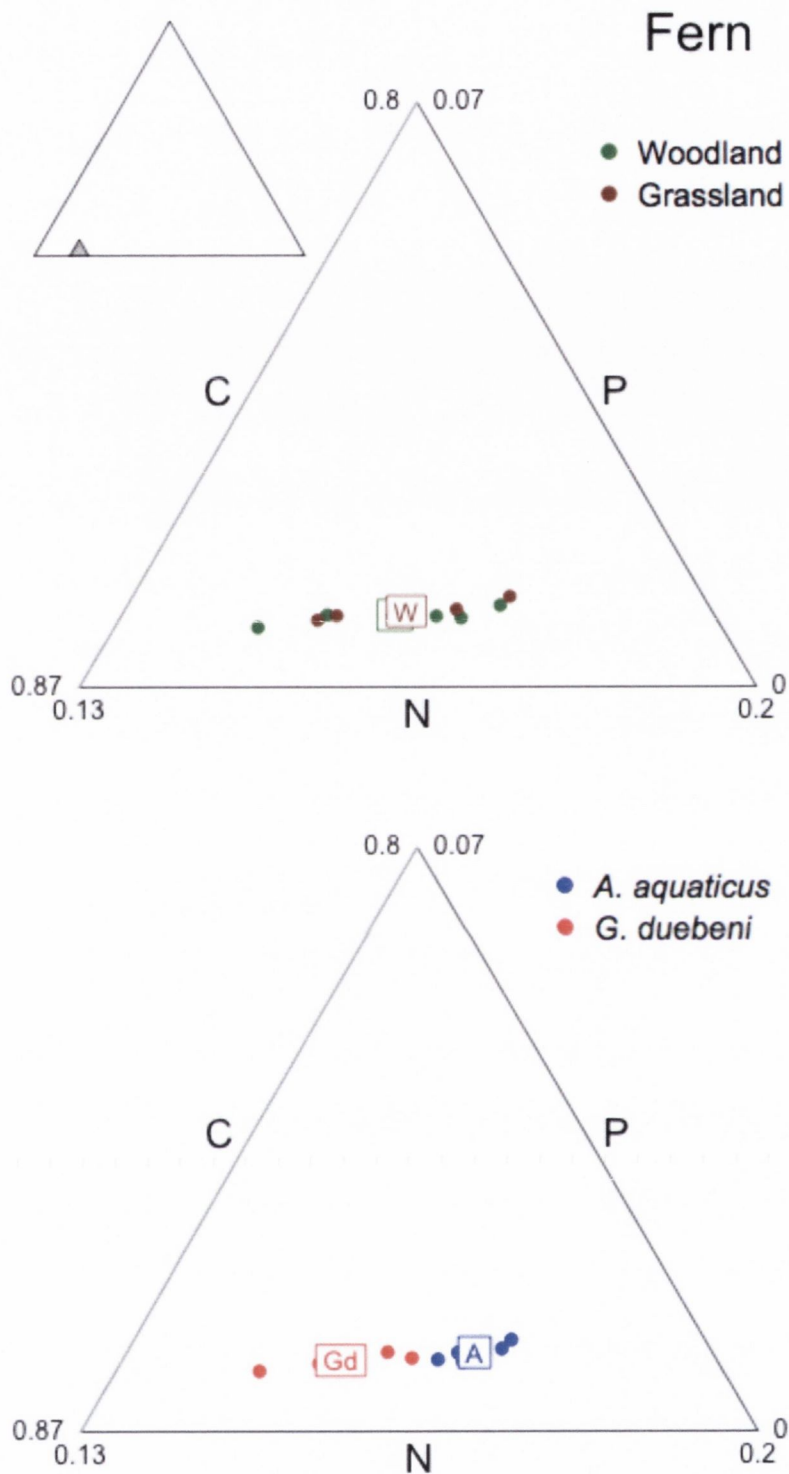


Figure 4.4 Triangular plots showing the relative molar concentrations of C, N and P of macroinvertebrates in Lough Fern, classified according to shoreline habitat types (top) and to species (bottom). Each point represents a pooled sample of individuals from a single littoral habitat. The centre of gravity of each group is indicated with its initials in boxes. See Figure 4.1 for how to read triangular diagrams.

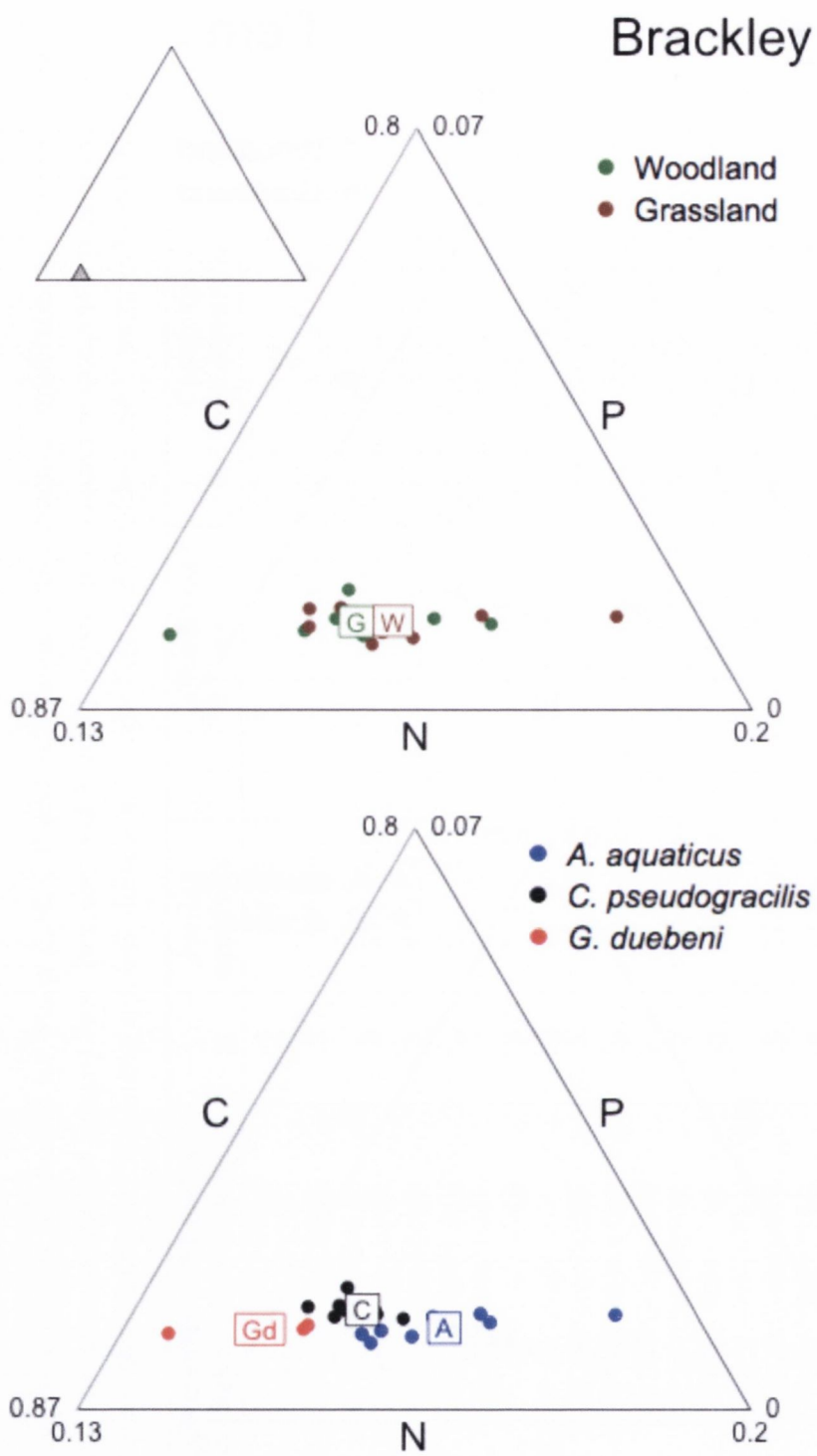


Figure 4.5 Triangular plots showing the relative molar concentrations of C, N and P of macroinvertebrates in Brackley Lough, classified according to shoreline habitat types (top) and to species (bottom). Each point represents a pooled sample of individuals from a single littoral habitat. The centre of gravity of each class is indicated with its initials in boxes. See Figure 4.1 for how to read triangular diagrams.

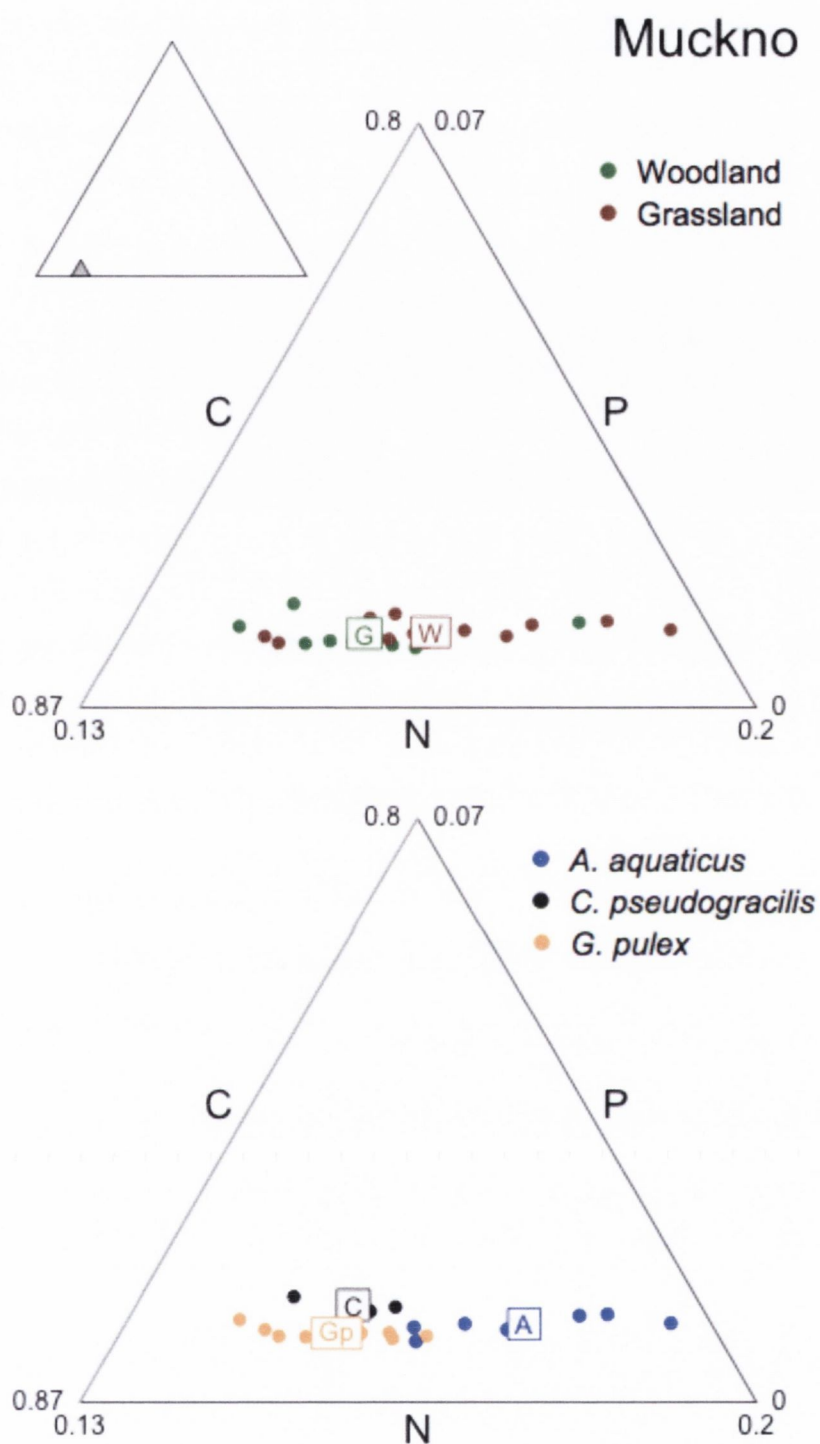


Figure 4.6 Triangular plots showing the relative molar concentrations of C, N and P of macroinvertebrates in Lough Muckno, classified according to shoreline habitat types (top) and to species (bottom). Each point represents a pooled sample of individuals from a single littoral habitat. The centre of gravity of each class is indicated with its initials in boxes. See Figure 4.1 for how to read triangular diagrams.



Results of the MANOVA tests also supported the separation of the elemental concentrations among invertebrate taxa observed in the triangular diagrams (Figure 4.4 - Figure 4.6, Table 4.4), while significant differences were found among the C:N:P molar ratio of the macroinvertebrate species for all of the three lakes: Fern ( $\nu = 0.84$ ,  $F_{3,6} = 10.0$ ,  $p < 0.01$ ), Brackley: ( $\nu = 1.12$ ,  $F_{6,28} = 6.0$ ,  $p < 0.001$ ), Muckno: ( $\nu = 1.21$ ,  $F_{6,34} = 8.7$ ,  $p < 0.001$ ) (Table 4.4).

When each elemental ratio was analysed individually, using univariate analyses of variance (ANOVA) for each lake as follow up tests to MANOVA, all of the dependent variables (C:N, C:P and N:P) were significant ( $p < 0.01$ ), except the C:P and N:P molar ratios in Lough Fern (Table 4.4). Post hoc analysis for the C:N molar ratios showed that the rank of the species remained the same for all of the three lakes, as was suggested by the triangular diagrams; while the significance varied among species in different lakes (Table 4.4, Figure 4.7). *Gammarus spp.* had the highest C:N molar ratios among all species with significant differences to *A. aquaticus* in Lough Fern ( $F_{1,8} = 22.21$ ,  $p < 0.01$ ) and to *C. pseudogracilis* and *A. aquaticus* in Brackley Lough ( $F_{2,15} = 8.98$ ,  $p < 0.01$ ). On the other hand, *A. aquaticus* had the lowest C:N molar ratios among all taxa, with significant differences to *C. pseudogracilis* and *G. pulex* in Lough Muckno ( $F_{2,18} = 16.29$ ,  $p < 0.001$ ).

The molar C:P ratio of *C. pseudogracilis* was significantly lower than the other two species both in Brackley ( $F_{2,15} = 7.45$ ,  $p < 0.01$ ) and in Muckno ( $F_{2,18} = 14.4$ )  $p < 0.001$ ). Similarly, the molar N:P ratio of *C. pseudogracilis* was significantly lower than *A. aquaticus* in Brackley ( $F_{2,15} = 14.2$ ,  $p < 0.001$ ) and it was significantly lower than both of the other two species in Muckno ( $F_{2,18} = 11.43$ ,  $p < 0.001$ ) (Table 4.4), Figure 4.7).

Table 4.4 Multivariate Analysis of Variance (MANOVA) and follow up one-way Analysis of Variance (ANOVA) results for the species main effect on the C:N:P stoichiometry of macroinvertebrate in the three study lakes.  $\geq$  = no significant differences among species,  $>$  = significant differences among species. Significant  $p$  values were indicated in bold font. **A** = *Asellus aquaticus*, **C** = *Crangonyx pseudogracilis*, **Gd** = *Gammarus duebeni*, **Gp** = *Gammarus pulex*.

|                 |       |                          | Fern            | Brackley                                      | Muckno                           |
|-----------------|-------|--------------------------|-----------------|-----------------------------------------------|----------------------------------|
| MANOVA          |       | Pillai's trace ( $\nu$ ) | 0.84            | 1.12                                          | 1.21                             |
| Test Results    | C:N:P | $F$                      | 10.0            | 6.0                                           | 8.7                              |
|                 |       | Df                       | 3, 6            | 6, 28                                         | 6, 34                            |
|                 |       | $p$                      | <b>0.008 **</b> | <b>0.004 **</b>                               | <b>&lt;0.001 ***</b>             |
|                 |       |                          |                 |                                               |                                  |
| Follow-up ANOVA | C:N   | Df                       | 1, 8            | 2, 15                                         | 2, 18                            |
|                 |       | $F$                      | 22.21           | 8.98                                          | 16.29                            |
|                 |       | $p$                      | <b>0.002 **</b> | <b>0.003**</b>                                | <b>&lt;0.001 ***</b>             |
|                 |       | Rank                     | <b>Gd&gt;A</b>  | <b>Gd&gt;C<math>\geq</math>A</b>              | <b>Gp<math>\geq</math>C&gt;A</b> |
|                 | C:P   | Df                       | 1, 8            | 2, 15                                         | 2, 18                            |
|                 |       | $F$                      | 3.51            | 7.45                                          | 14.40                            |
|                 |       | $p$                      | 0.098           | <b>0.006 **</b>                               | <b>&lt;0.001 ***</b>             |
|                 |       | Rank                     | Gd $\geq$ A     | Gd $\geq$ A>C                                 | Gp $\geq$ A>C                    |
|                 | N:P   | Df                       | 1, 8            | 2, 15                                         | 2, 18                            |
|                 |       | $F$                      | 0.15            | 14.20                                         | 11.43                            |
|                 |       | $p$                      | 0.710           | <b>&lt;0.001 ***</b>                          | <b>&lt;0.001 ***</b>             |
|                 |       | Rank                     | Gd $\geq$ A     | <b>A<math>\geq</math>Gd<math>\geq</math>C</b> | <b>Gp<math>\geq</math>A&gt;C</b> |

\*\*  $p < 0.01$ . \*\*\*  $p < 0.001$ .

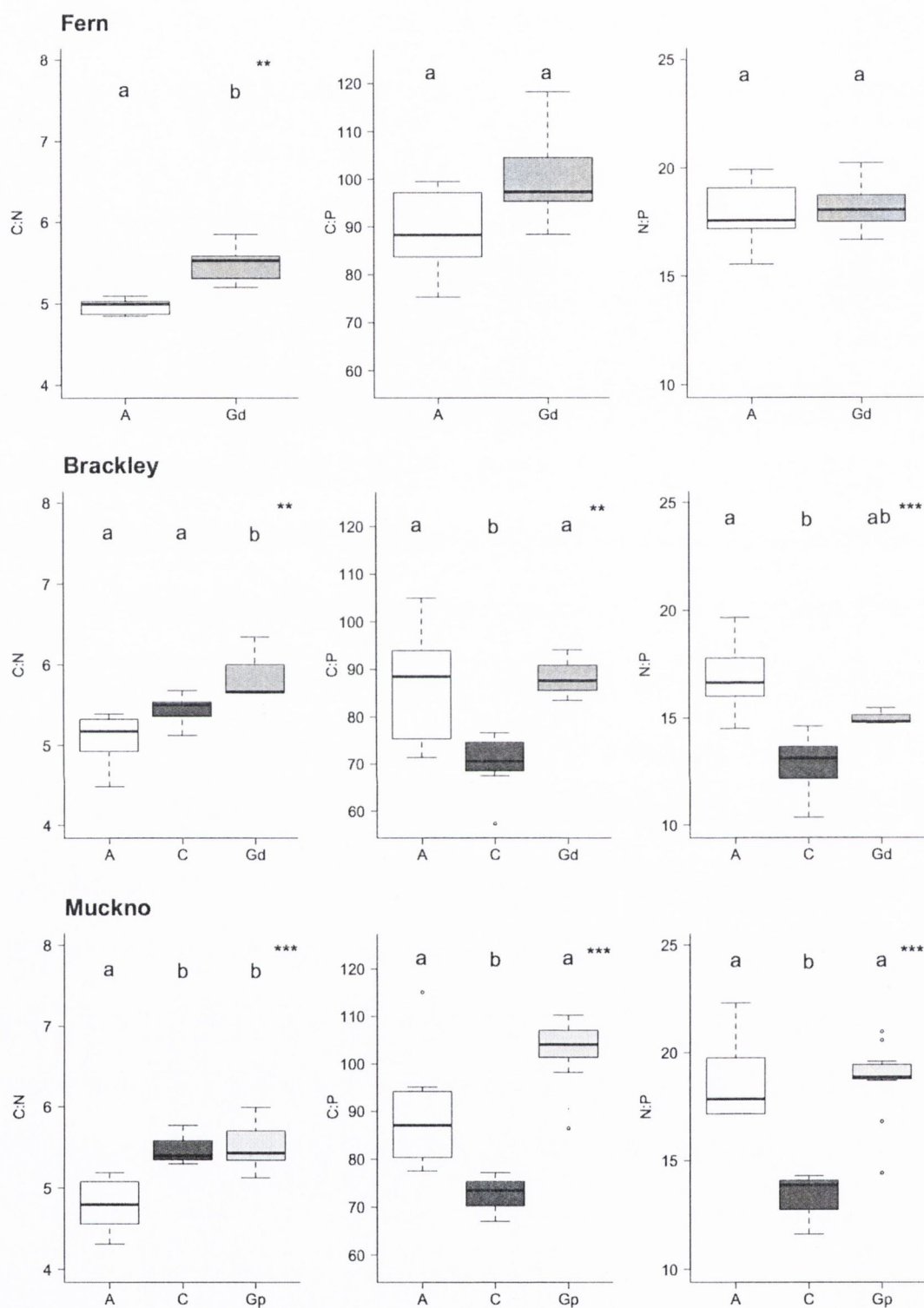


Figure 4.7 Variation of macroinvertebrate molar elemental ratios in study lakes. Different letters above the boxes indicate significant differences among species. Mean comparisons are based on TUKEY's HSD. The line in the middle of the box represents the median and the lower and upper ends of the box represent the 25% and 75% quartiles respectively. A = *Asellus aquaticus*; C = *Crangonyx pseudogracilis*; Gd = *Gammarus duebeni*, Gp = *Gammarus pulex*. Significance levels are shown on the top right corners: \*\*  $p < 0.01$ . \*\*\*  $p < 0.001$ .



## 4.4 Discussion

### 4.4.1 Spatial variation of macroinvertebrate stoichiometry

#### 4.4.1.1 Among-lake variation

Effect of lake on the macroinvertebrate stoichiometry was species dependent. Comparison of elemental ratios of individual invertebrate species across the three study lakes revealed no significant differences for *A. aquaticus* or *C. pseudogracilis*, while significant differences were observed for *Gammarus* spp. Therefore, the first hypothesis of this chapter, which predicted significant spatial differences among macroinvertebrate elemental stoichiometry across lakes, could be accepted only for *Gammarus* spp.

Relatively consistent elemental ratios of *A. aquaticus* and *C. pseudogracilis* across lakes suggest relatively stable elemental ratios (strong homeostasis) for these species. Frost *et al.* (2003) also found only few differences among within-lake averages of C, N and P contents of all the invertebrates collected across eight Canadian lakes along a large trophic gradient from which the authors concluded that the average elemental requirements for growth and reproduction are relatively similar among invertebrates (Frost *et al.*, 2003). According to Evans-White *et al.* (2005), comparison of nutrient ratios within macroinvertebrate taxa collected from 35 streams in two different regions (Indiana-Michigan and Wisconsin in USA) revealed no significant differences. However, the comparison of the elemental stoichiometry of macroinvertebrates in these earlier studies involved higher taxonomic levels and without reports of among-lake stoichiometric variations of individual macroinvertebrate species.

Significant differences in the elemental ratios of *Gammarus* spp. across lakes, owing to the variation in their C:P and N:P ratios, can be attributed to relatively higher P concentrations of gammarids in Brackley Lough (see Table 4.2). It is unlikely that these differences are attributable to different species of gammarids found in different lakes, as the C:P and N:P ratios were not significantly different between the *G. pulex* in Muckno and *G. duebeni* in Fern while the N:P ratios between *G. duebeni* in Loughs Brackley and Fern were different (see Table 4.1). In a study of two detritus based headwater streams with distinct trophic states (in North Carolina, USA), Cross *et al.* (2003) also found significantly higher %P and lower

C:P and N:P ratios in the nutrient-enriched than in the reference stream. There were up to four fold differences among C:P and C:N ratios for similar sized insect taxa (mainly Trichoptera), based on comparisons at the genus level. This indicated deviations from strict homeostasis for certain taxa (Cross *et al.* 2003). Increased P storage as a prevention mechanism against short-term decreases in P availability (Woods *et al.* 2002) or increased rRNA linked to the higher growth rates in the nutrient-enriched conditions (Frost and Elser 2002) were the two explanations suggested by authors (Cross *et al.* 2003). The increased N:P ratios in *G. duebeni* in Brackley Lough than in Lough Fern could be associated with relatively higher nutrient levels in the former lake (see Table 2.1), but, these results have to be interpreted cautiously both because of restricted number of lakes being compared, and as *G. duebeni* was found only in 3 out of 13 littoral habitat samples, and was the uncommon amphipod species after *C. pseudogracilis* in Lough Brackley, while it was the only gammarid species widely distributed (found in 6 out of 7 samples) in Lough Fern (Table A 8, Appendix III). Also, it should be noted that, despite higher P concentrations in Lough Muckno than Brackley, N:P ratios of *G. pulex* was not significantly different compared with *G. duebeni*.

#### **4.4.1.2 Within-lake variation**

In this study, no evidence could be found for within-lake variations of the littoral consumer elemental ratios in relation to shoreline habitats based on the ANOVAs. Therefore, the second hypothesis, which predicted significant spatial differences within lakes reflecting differences in shoreline habitats, could not be supported by the findings of the current study for any of the species. To date, no studies have reported the within-lake variation of littoral macroinvertebrate elemental ratios in relation to lake shoreline habitats. Results based on a few previous studies, which compared the elemental stoichiometry of *A. aquaticus* and *Gammarus* species across different sites of a lake (or a stream), suggest homeostasis for these species (Liess and Hillebrand 2005, Fink *et al.* 2006, Gücker *et al.* 2011). Fink *et al.* (2006) observed significant differences neither for *A. aquaticus* L. (Isopoda, Asellidae) collected from two different sampling sites, nor for *G. roeseli* Gervais (Amphipoda, Gammaridae) in Lake Constance (Germany), despite significant differences between the stoichiometry of periphyton from these two sites (Fink *et al.*, 2006). According to Liess & Hillebrand (2005), elemental ratios of the only isopod species *A. aquaticus* were not significantly different across five sampling sites in Lake



Erken (Sweden), while they were significant for some species belonging to Bivalvia, Gastropoda, Trichoptera and Diptera families (Liess and Hillebrand 2005). Comparison of the nutrient stoichiometry of invertebrate consumers, including isopod *A. aquaticus* and gammarids *G. pulex* and *G. roeseli* revealed no significant distinction between agricultural and urban sites in a German stream (Gücker *et al.* 2011).

It should also be noted that high water levels were observed during the sampling period, owing to extremely high levels of rainfall in the summer of 2009 (MET Annual Report 2009). As a result of this, some samples from sites particularly adjacent to grassland habitats were collected from inundated vegetation, especially in Loughs Brackley and Muckno (Table A 8, Appendix III). Even though the inundated vegetation habitats may have reduced the distinctiveness of the elemental signals, wooded sites still reflect habitats with more allochthonous matter, such as leaf litter.

#### **4.4.2 Taxonomic variation**

Results based in this chapter suggest that mainly taxonomy, rather than spatial differences, govern the stoichiometric variation among invertebrates both within and across lakes, supporting my third hypothesis. Significant taxonomic variation of C:N:P elemental stoichiometry has been reported among various littoral macroinvertebrate groups before (Frost *et al.* 2003, Liess and Hillebrand 2005, Evans-White *et al.* 2005, Fink *et al.* 2006, Donohue 2008). However, this is the first study to demonstrate significant stoichiometric differences among freshwater amphipod species that are common to Irish lakes (see Appendix IV for a compilation of elemental stoichiometry of amphipod and isopod crustacean species based on this and other studies). The relative spatial distributions of species on the triangular diagrams based on their molar C, N and P compositions were consistent for individual lakes (see Figure 4.7), revealing general patterns about species food partitioning and trophic positions despite spatial distinctions.

The range of the nutrient elemental ratios can also be used as an indicator to predict the relative nutrient requirements of species and potential nutrient limitations they may encounter. According to homeostasis theory, consumers have specific nutrient needs and can regulate their nutrient concentrations despite the high variability in the elemental composition of their food sources as a result of



changing environmental conditions. In this study, *C. pseudogracilis* demonstrated a narrow range of body elemental ratio, which suggests that it is a more homeostatic species compared with *A. aquaticus* or either of the *Gammarus* spp. (Figure 4.3). On the other hand, *Gammarus duebeni*, native to Ireland, shows a broader range in its elemental ratios than the rest of the species (Figure 4.3), which may indicate higher tolerances to fluctuations in the nutrient contents of its food sources (highest plasticity).

Comparisons of individual elemental ratios among species demonstrated that *Gammarus* spp. had the highest mean C:N ratios among all the species in all of the lakes; whereas they were significantly lower for *A. aquaticus* among other crustacean species. These results support the findings of Donohue (2008), who showed significant differences between the mean C:N and C:P ratios of *G. duebeni* and *A. aquaticus* across nine Irish lakes. Liess & Hillbrand (2005) also reported relatively low elemental ratios for *A. aquaticus* in comparison with other benthic invertebrates in a Swedish lake. On the other hand, no significant differences were found between the elemental composition of isopod *A. aquaticus* (n=14) and another amphipod species *G. roeseli* (n=44) in Lake Constance (Germany) despite significant variations among some other invertebrate taxonomic groups (Fink *et al.* 2006). Earlier studies found no evidence for competition for space (Graca *et al.* 1994b) or food (Graca *et al.* 1994a) between *G. pulex* and *A. aquaticus*, which suggested that differences in physical and chemical tolerances rather than exploitive competition can explain the distribution patterns of these two species (Graca *et al.* 1994a). The relatively higher C:N ratios in amphipods compared with *A. aquaticus*, could be linked to the relatively bigger size of gammarids, which have relatively higher proportions of external skeleton with high C and low N and P contents (Liess and Hillebrand 2005). Chitin, which is an important structural polysaccharide that makes up the external skeleton of insects and the carapaces of crustaceans, have high C, low N and no P content (Liess & Hillebrand, 2005; Elser *et al.*, 1996). Liess & Hillebrand (2005) also explained the variations among the elemental ratios of classes and orders of invertebrates by differences in the structural biomolecules between the soft-bodied invertebrates and the ones with heavy exoskeletons.

In my study, *Crangonyx* showed the lowest C:P and N:P ratios among all species, with significant differences in Loughs Brackley and Muckno, which were the only

lakes with *C. pseudogracilis*. This further supports the findings of Chapter 3 based on stable isotope signatures suggesting distinction of the trophic niches between *C. pseudogracilis* and other *Gammarus* spp. Previous studies showed that these two species generally occupy discrete patches even though they were found to co-occur in certain habitats (Macneil *et al.* 1997). In their laboratory experiment, MacNeil, Elwood & Dick (1999) observed *Gammarus* staying more on the bottom substratum, with *C. pseudogracilis* higher in the water column. In the current study, based on species presences across sampling sites, *C. pseudogracilis* co-occurred with *G. pulex* in Lough Muckno more often, in comparison with *G. duebeni* in Lough Brackley (Table A 8, Appendix III), suggesting more overlapping niches between *G. pulex* and *C. pseudogracilis*.

Being similar in body size, consuming similar food sources and mostly inhabiting separate freshwater systems, *G. duebeni* and *G. pulex* have been considered as potential competitors and ecological equivalents (Hynes 1954, Dick *et al.* 1993). Similar C:N:P ratios between *G. duebeni* and *G. pulex* found in this study indicate similar nutritional needs and support potentially overlapping trophic niches. While the competitive exclusion principle predicts the coexistence of two competitors consuming the same resource as impossible (Hardin 1960), it is possible according to the stoichiometrically explicit niche model developed by Hall (2009). Hall's model predicts that variation in the food quality can promote species co-existence by shifting species interactions from competition (food is good quality but low in abundance) to facilitation (food is abundant but of low quality) (Hall 2009). This was also supported by Urabe (2002), who showed that low food quality enabled coexistence of two *Daphnia* species. Therefore, whether similar body stoichiometry among the two *Gammarus* spp. would be an indication of competitive exclusion of either species depends on the abundance and nutritional quality of their food sources (Moe *et al.* 2005). Both *G. duebeni* and *G. pulex* showed identical micro-distribution patterns based on a mesocosm study, such that they both occurred in 'confined' habitats (e.g. on sand or among pondweed), unlike *Crangonyx* which preferred open water columns (MacNeil, Elwood & Dick 1999). Gammarids are potentially omnivorous macroinvertebrates, showing a variety of feeding modes (Macneil *et al.* 1997). From a stoichiometric perspective, omnivory occurs more frequently among coexisting consumer species when they are nutrient limited (Diehl 2003), because nutrient limitation may induce a switch from herbivory to predation on another consumer which would



have higher nutritional quality than plant or detritus based food sources (Moe *et al.* 2005). Predation and cannibalism between these *Gammarus* species has also been documented in laboratory studies (Dick *et al.* 1993, Macneil *et al.* 1997) which found that *G. pulex* was more successful in avoiding predation by *G. duebeni* and also to engage in predation on *G. duebeni*. According to a more recent review by Dick (2008), behavioural mechanisms mediated by biotic and abiotic factors were suggested as possible explanations for the success of the non-native *G. pulex* over native *G. duebeni*.

## 4.5 Conclusions

The results of this chapter can lead to the following overall conclusions:

- Taxonomy, rather than the spatial differences is more likely to be the primary explanation of among lake variation of macroinvertebrate elemental stoichiometry. Among the four crustacean taxa, *C. pseudogracilis* was the most homeostatic species, with the smallest range of body stoichiometry, while *G. duebeni* was the most plastic. No significant differences between the C:N:P ratios of *G. pulex* and *G. duebeni* supported earlier findings proposing that the two gammarids are ecological equivalents. *Asellus aquaticus* and *C. pseudogracilis* had significantly lower C:N and C:P ratios than other species across lakes, respectively.
- Within-lake differences in the elemental stoichiometry of macroinvertebrates were also a result of taxonomy. Based on the comparisons from sites adjacent to woodland and grassland shoreline habitats, no relation between the shoreline habitat type and elemental stoichiometry of macroinvertebrates could be found.



## **CHAPTER 5**

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### **5. GENERAL DISCUSSION AND CONCLUSIONS**



## 5.1 Comparison of functional indicators

Anthropogenic activities have adverse effects on lakes as a result of *inter alia* eutrophication, alteration of hydrological regimes and structural degradation of riparian habitats. Much effort has been spent investigating the mechanisms of eutrophication for many decades (Beklioglu *et al.* 2011). Recently, modification of lake riparian and shoreline structures is increasingly recognised as a serious threat for lake ecosystems worldwide (Rosenberger *et al.* 2008, Brauns *et al.* 2011, Jurca 2012). Lake littoral zones comprise diverse habitat patches with strong lateral and vertical gradients of environmental conditions, influencing the structure and function of macroinvertebrate communities (Brauns 2008). They also act as important regulators of nutrient dynamics in lake ecosystems through coupling pelagic (*e.g.* horizontal movement of fish or zooplankton (Vadeboncoeur *et al.* 2002)) and terrestrial habitats (Brauns *et al.* 2011). Therefore, changes in the habitat and trophic structure of the lake littoral do not only have consequences for the littoral food webs but for the whole lake trophic structure.

In Ireland, a common type of lake shoreline habitat modification has been the change from structurally complex woodland habitats to much simpler grassland habitats that are mainly grazed by livestock. Multiple pressures are associated with modified grassland shorelines, including degradation of physical habitats, disruption of the energy coupling between aquatic-terrestrial linkages and being sources of non-point loadings contributing to nutrient enrichment in lakes (Brauns 2008). Measuring effects of these changes on littoral communities is complex. According to Jurca (2012), univariate diversity measures, such as taxon richness and Margalef diversity, of littoral macroinvertebrate assemblages were affected by shoreline habitat modifications, but overall macroinvertebrate community composition was not. Macroinvertebrate taxa indicative of morphological alterations were limited to a few habitat specialists in lakes without high nutrient enrichment (Jurca 2012). While the taxa composition of macroinvertebrates provides an emergent property of the littoral zones of lake, it may not reveal much about functional responses to habitat, nutrients and food webs. In order to provide greater understanding of these aspects within littoral zones, this study investigated the use of elemental signatures, provided by stable isotopes and C:N:P stoichiometry as functional indicators of the response of common and



abundant model species to physical and chemical properties of littoral zones across a series of lakes. The thesis represents a novel approach to investigating habitat effects on lake communities and attempts to draw out salient features related to ecosystem function rather than the more commonly applied measures and metrics of the structure of communities.

### **5.1.1 Linear mixed effects models for isotopic data analysis**

The thesis has investigated three approaches to better understand to functional relationships within the littoral communities and their response to riparian habitat differences. In Chapter 2, application of linear mixed effects-models provided a useful tool for habitat assessment across lakes with different nutrient status. Adopting lake as a random effect allowed generalisation of findings from all study lakes, thus the conclusions were not only limited to a single study lake. Moreover, using linear models with Type III residuals in the mixed-effects models allows the method to cope with the unbalanced data commonly encountered in many field-based observations in ecology (Crawley 2007). All models of carbon and nitrogen stable isotope signatures of macroinvertebrate species, except for the  $\delta^{13}\text{C}$  model for *C. pseudogracilis*, indicated differences between the woodland and grassland shoreline habitat types, suggesting their potential as functional indicators of habitat modifications in aquatic systems. Also, using lake random effect on  $\delta^{13}\text{C}$  models of *A. aquaticus* and *C. pseudogracilis* was ineffective, as a higher level of variation was attributed to residuals.

Previous studies that examined the impacts of increased riparian agricultural land use on littoral or stream invertebrates using linear (Bergfur *et al.* 2009) or stepwise multiple (Vander Zanden *et al.* 2005) regressions for the stable isotope data have suggested elevated  $\delta^{15}\text{N}$  signatures in response nutrient enrichment. In contrast, the results of the current study showed no consistent pattern of isotopic responses among species to changes in land use. One reason for distinct findings could be related to the use of pooled invertebrate data across different taxa (*e.g.* Bergfur *et al.*, 2009; Vander Zanden *et al.* 2005) rather than individual species used in this study. The species-specific response of invertebrates to shoreline modifications can be explained by their specific habitat use and dietary needs. Given the significant effect of shoreline habitat structure on the isotopic signatures of the

study species, future work could usefully test specific hypothesis related to shoreline modifications on selected taxa.

The significant effect of PC2 axis, primarily representing the colour gradient, and/or its significant interaction with shoreline habitat type effect in all models, indicate the importance of water colour as an key factor for the isotopic signatures of macroinvertebrates in the lake littoral zones. Even though elevated levels of dissolved organic carbon and water colour, associated with respired CO<sub>2</sub> through heterotrophic pathways, has been shown to reduce the  $\delta^{13}\text{C}$  values in primary producers, including periphyton (France and Cattaneo 1998) and phytoplankton (Grey *et al.* 2000), more work is required to understand its impacts on macroinvertebrate elemental signatures. Besides, it would be important to differentiate sources of high water colour in lakes (*e.g.* eutrophication vs. high dissolved organic carbon). High colour observed in some of the study lakes with low nutrient concentrations (*e.g.* Fern and Akibbon, Table 2.1) most likely relates to the high percentage of peat in their catchments.

### 5.1.2 Isotopic niche metrics

The second approach involved utilising macroinvertebrate stable isotope data to compute isotopic niche metrics as presented in Chapter 3. The crustacean species used in this study have been demonstrated to engage in predatory interactions and the mechanisms for their coexistence is still debated (MacNeil *et al.* 2013). One of the main advantages of the isotopic niche approach is its potential for the assessment of species' competitive interactions across different habitat settings and environmental conditions through comparing their isotopic niche widths, niche overlaps, and relative isotopic positions that can suggest their relative trophic roles as well as differences in utilisation of carbon sources. By comparing lakes with different combinations of the native and invasive crustacean species with different abundances (Table A 8, Appendix III), it was possible to suggest further insights about their trophic interactions and function. For example, *C. pseudogracilis* demonstrated the narrowest isotopic niche width and dispersions across species, suggesting more specific feeding behaviour compared with *Gammarus spp.* or *A. aquaticus*; therefore, its categorisation as a generalist feeding amphipod (Leslie *et al.* 1997) similar to *Gammarus spp.* may not be appropriate. Moreover, the lack of isotopic niche overlap between *C. pseudogracilis* and *A.*



*aquaticus* both at the lake and local habitat level (Figure 3.7 and, Figure A 9, Appendix III), suggested a very likely separation between ecological niches of these species. Even though overlapping isotopic niches may not reflect ecological similarities, it is more likely that non-similar isotopic niches between two individuals represent non-overlapping ecological niches (Layman *et al.* 2012). Further comparison of isotopic niche overlaps among amphipod species indicated a higher overlap between *C. pseudogracilis* and *G. pulex* in Muckno, compared with *C. pseudogracilis* and *G. duebeni* in Lough Brackley, which is supportive of MacNeil *et al.* (2013) who proposed a greater negative field association of *C. pseudogracilis* with the previous invader *G. pulex* compared to the native *G. duebeni*.

Another advantage of the isotopic niche metrics can be related to its practicality as its application does not rely on the knowledge of species' consumed food sources, hence it can be more cost-efficient compared with, for example, using mixing models that require identification of species' potential food sources in order to construct food webs (Brauns *et al.* 2011). This can be especially relevant because differentiation between allochthonous and autochthonous food sources is not always possible owing to overlapping signatures in many cases (Finlay and Kendall 2007, Bergfur *et al.* 2009). Therefore, the use of the isotopic niche metrics represents a novel and pioneering approach to the better understanding of species interactions in natural communities. Naturally, the research also raised some limitations and questions to be addressed in future developments of niche-width metrics.

Even though they were not used to illustrate the effects of shoreline habitat modifications, application of isotopic niche metrics in this study exemplifies a novel approach for testing changes in species trophic interactions in spatially distinct habitats. It is valuable especially when investigating niche partitioning among potentially interacting species, such as the native and invasive crustacean species commonly occurring in Irish freshwaters. In this study, the smaller sample sizes of the less common amphipod species in certain lakes (*G. duebeni* in Brackley and *C. pseudogracilis* in Muckno), limits the comparison of isotopic niche metrics between the two shoreline habitat types which, despite being less sensitive to sample sizes in comparison with Layman metrics, nevertheless risks underestimating the population standard ellipse areas (SEA) (Jackson *et al.* 2011). For example, simulation of isotope data suggested that SEA asymptotes to a



constant value at around  $n=30$  and a comparison between SEA of two populations under  $n=6$  can lead to significant error (Jackson *et al.* 2011).

### 5.1.3 C:N:P elemental stoichiometry

The third functional signature included in this study was the elemental stoichiometry of macroinvertebrates, expressed as C:N:P ratios, that were used to test the influence of shoreline habitats in three study lakes (Chapter 4). The concept of ecological stoichiometry is based on the fact that each species has specific requirements of nitrogen and phosphorus that are key for their growth and variations in the availability of nutrients in their environment can result in nutrient limitations (Sterner and Elser 2002). Even though most consumer species are considered to be homeostatic compared with their food sources, there can be variations in their homeostasis, such that generalist species are hypothesised to have higher tolerances to elemental deficiencies (Anderson *et al.* 2005). Therefore, one advantage of using a stoichiometric approach is the comparison of elemental homeostasis among species. The results in Chapter 4 suggested that *G. duebeni* had the widest range of C:N:P ratios, while, *C. pseudogracilis* had the narrowest. This suggested more conservative elemental stoichiometry and stronger homeostasis for *A. aquaticus* and *C. pseudogracilis* in comparison with *G. duebeni*. These results are parallel with the findings of Chapter 3 on species' isotopic dispersion and niche widths, which also indicated that *C. pseudogracilis* was the species with the narrowest isotopic range, suggesting relatively low generalist feeding tendencies. However, in contrast to isotopic signatures, species' elemental ratios generally showed significant spatial variation within or among lakes. An exception was *G. duebeni*, which showed distinct N:P and C:P ratios between Loughs Fern and Brackley.

Significant taxonomic differences between the elemental ratios of *C. pseudogracilis* and *Gammarus spp.* suggested distinct elemental requirements and/or physiological adaptations (Figure 4.7). Such functional differences between the two species can have implications on the long-term conservation and management strategies. Moreover, findings of this study suggested similarities in the C:N:P ratios and isotopic niche widths between *G. duebeni* and *G. pulex*, collected from two different lakes, indicating similar nutritional needs and potentially overlapping trophic niches. These findings support the highly competitive

relationship between the two gammarid species suggested by the earlier studies (Dick *et al.* 1993). Whereas, differences observed in their isotopic dispersions, niche overlaps with other study species and the horizontal isotopic positions relative to *A. aquaticus* (Table 3.6) indicate potential variation in the trophic relationships of the two *Gammarus spp.* with other invertebrate species (*e.g.* *A. aquaticus*).

Finally, future studies should consider looking at the elemental imbalances between the C:N:P stoichiometry of these crustacean species and their potential food sources, such as detritus and benthic algae in different habitat settings, to better understand their potential functional differences and competitive success with regard to their respective environments.

## 5.2 Relative distribution of species across lakes

Chapter 2 was an attempt to disentangle the multiple factors influencing the within- and among-lake variation of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of three common littoral crustacean species across eight Irish lakes, using linear mixed-effects models. Native *A. aquaticus*, present in all eight-study lakes, with the exception of Lough Killooris, was the most widely distributed of the crustacean species included in this study (Table A 7, Appendix III). On the other hand, the other native species, *G. duebeni*, was found in six lakes, including Loughs Killooris, Akibbon, Fern, Brackley and Scur (Table A 7, Appendix III). The more widespread-distribution of *A. aquaticus* in comparison with *G. duebeni* in this study can be explained by higher tolerances of the former species to physico-chemical variations among lakes compared with the latter (MacNeil *et al.* 2002). On the other hand, the non-native amphipod *C. pseudogracilis* was restricted to Loughs Brackley, Muckno, Sillan and Scur, which were mainly characterised with medium to high nutrient concentrations (TP: 59-329  $\mu\text{g l}^{-1}$ , Figure 2.1), while not occurring in lakes with relatively low nutrient concentrations (TP: 23-35  $\mu\text{g l}^{-1}$ ), such as Loughs Carrigeencor, Killooris, Akibbon and Fern (Table A 7, Appendix III). In contrast, a recent study on another set of nine Irish lakes along a nutrient gradient reported the distribution of *C. pseudogracilis* in all study lakes, which also included Loughs Brackley, Muckno and Scur (Jurca 2012). *Crangonyx pseudogracilis* have been associated with aquatic vegetation (Costello 1993, Gledhill *et al.* 1993). Therefore the absence of *C. pseudogracilis*, particularly in Loughs Fern and



Akibbon, with mostly stony substrates were observed, can be a result of a lack of obvious macrophyte habitats.

Relative distribution patterns of *C. pseudogracilis* and *Gammarus* spp. in this study of eight lakes were mostly consistent with early studies suggesting strong negative field associations of *Gammarus* spp. with *C. pseudogracilis* (MacNeil *et al.* 2013). The co-existence of *C. pseudogracilis* with *Gammarus* spp. was limited to 3 out of 8 of my study lakes (Loughs Brackley, Muckno and Scur) (Table A 8, Appendix III). Differences in their availability across lakes could be partially related to higher tolerances of *C. pseudogracilis* to lower water quality and higher dispersal ability compared with *Gammarus* spp. (Holland 1976). However, more recently, the ability of *C. pseudogracilis* to tolerate physicochemical conditions in *Gammarus* dominated areas was demonstrated by Macneil, Dick, & Elwood (2000). Therefore, an alternative explanation for the different distribution patterns of *C. pseudogracilis* with *G. duebeni* is required. For example, high predation rates of *Gammarus* spp. on *C. pseudogracilis* has been reported based on laboratory experiments (Dick 1996, MacNeil *et al.* 1999). Contrasting with many earlier studies based on laboratory mesocosm experiments, the study on these species' competitive isotopic niches based on field samples (Chapter 3) showed a significantly higher trophic level occupied by *C. pseudogracilis* compared with *G. duebeni* or *G. pulex* in Loughs Brackley and Muckno, respectively. This suggests that an alternative trophic pathway for *C. pseudogracilis* compared with *G. pulex*. While *G. pulex* could be a predator on *C. pseudogracilis*, the latter could have a higher trophic signal from feeding on, for example, carrion or other invertebrates.

Species distribution and abundances showed that the co-existence of *C. pseudogracilis* with *G. duebeni* at the meso-habitat scale was more constrained in Lough Brackley, while it was more common with *G. pulex* in Muckno (Table A 8, Appendix III). Standard ellipses based on samples from these habitats suggested separation of these species' isotopic niches in the local habitat scale (Figure A 9, Appendix III). Another mesocosm experiment supported differences of micro-distribution between *C. pseudogracilis* and *Gammarus* spp. (MacNeil *et al.* 1999). In mesocosm set ups of 5 l tanks, open water spaces and tank walls seemed to be particularly critical for *C. pseudogracilis*, which appeared to move faster (at least over small distances) than *Gammarus* spp. and could avoid its attacks by performing a rapid jumping movement (MacNeil *et al.* 1999). Also, given the



distinct movements of the two species in water, *Crangonyx* moving upright as opposed to *Gammarus* that is always on its side (Dick *et al.* 1999), both their physiological and behavioural traits should be considered in different habitat settings in order to better understand their relative distributions and predatory interactions.

### 5.3 Study limitations and suggestions for future work

#### *Seasonal variation*

The design of the study was to compare species in habitats across lakes, at a represented time of the year and, hence, based on a single sampling occasion. Seasonal variations of the abundance and isotopic signatures of aquatic crustacean macroinvertebrates can occur owing to changes in food and habitat availability, or direct seasonal effects on physiology (Hellmann *et al.* 2013). Although the domain of this study was comparisons across lakes in relation to riparian habitats, it is recognised that isotopic and stoichiometric signatures might change over seasonal time scales that extend beyond durations of physiological changes mediated by shifts in diet. Considering that the main leaf-fall of many deciduous trees occurs in September-October, presence of conditioned leaf litter from the surrounding trees were observed in the wooded sites (Table A. 8, Appendix III).

#### *Shoreline habitat features*

Studying the impacts of shoreline habitats on lakes poses limitations, including finding comparable sites as replicates within and among lakes. In particular, locating three different sites with wide stretches of natural woodland shorelines can be challenging for lakes in agricultural settings. Rarely, it can be the opposite situation where woodlands would be abundant and access to modified shorelines is difficult. It is especially important to recognise possible variations within the broad categorisations of shoreline habitats associated with differences in vegetation and intensity of land use. Although not taken into consideration in this study, it is likely that grassland management features, including the number and density of livestock or applications of fertiliser have varied across sites in grassland shorelines. From a management point of view, it would be interesting to evaluate the influence of riparian buffer zones at lakeshores on the functioning of littoral communities in agricultural settings, following this study.

*Wave exposure*

The relative exposure of the sampling sites to wave action was not taken into account during the analysis of elemental signatures in the current study. Even though water movement has been linked to the  $\delta^{13}\text{C}$  signatures of primary producers in previous experimental and field studies (Trudeau and Rasmussen 2003, Plafkin and Keller 2007, Finlay *et al.* 2010), no study could be found on the direct effects of water movement to macroinvertebrate stable isotope or stoichiometric signatures. In studies presented in Chapters 3 and 4, selection of six sites (3 of each type of shoreline habitats) provided a range of exposure to wave action in Loughs Fern, Brackley and Muckno. However, there were differences of wave exposure between sites adjacent to woodland and grassland sites in lakes, where only two samples were collected. In Ireland, northeast shorelines of lakes are usually exposed to wind-induced waves owing to dominant south-easterly winds, which can be related to total lake and fetch area, and water depth. In Loughs Carrigeencor and Akibbon, both woodland and grassland shoreline sites had the same wind aspect, in contrasting directions between lakes (Figure 2.2). On the other hand, the wind aspects of the two sites in Loughs Kiltorris, Scur and Sillan differed. Neither of the sampling sites in Lough Kiltorris was facing south-easterly winds (Figure 2.2). However, the wooded sites in Loughs Scur and Sillan were situated in the more wave-exposed aspects of the lakes and grassland sites were more secluded from prevalent winds (Figure 2.4).

In the long term, wind exposure can determine littoral habitat characteristics, such that accumulation of sediment is often prevented by wind-induced waves and stony substrates without macrophyte beds can be a characteristic of wind-exposed shores (Harrison and Hildrew 1998, McGoff and Irvine 2009). In the short term, extreme wind exposure can lead to reduction of macroinvertebrate total abundance and species richness (Barton and Carter 1982). According to an experimental study by Gabel *et al.* (2008), different lake littoral habitats varied in the extent in providing refuges against wave disturbance for littoral invertebrates. Complex three dimensional habitats, including tree roots and to a lesser degree reed beds, provided the more efficient protection for invertebrates, including gammarid *G. roeseli*, against wave-induced disturbance, when compared with structurally less complex littoral habitats such as sand, coarse woody debris and stones (Gabel *et al.* 2008). In my study, more heterogeneous and complex habitats



(e.g. tree roots or intact reed beds) were associated with more natural woodland shorelines, particularly in Loughs Brackley and Muckno, while more homogenous and structurally less complex littoral habitats (stony and inundated vegetation (see below)) were observed in grassland shorelines (Table A 7, Appendix III).

#### *Littoral habitat characteristics*

Because of a lack of replication within lakes, littoral habitat characteristics (Table 7 A, Appendix III) were not included in the mixed effects models. Strong lateral and vertical gradients of environmental conditions in lake littoral zones comprise diverse habitat patches, influencing both the structure (Jurca 2012) and the functioning (Brauns *et al.* 2011) of macroinvertebrate communities. For example, according to Jurca (2012), macrophyte and substrate related diversity features were most important habitat features for structuring the macroinvertebrate assemblages across nine Irish lakes. Also, in a multi-lake study of Irish lakes, White & Irvine (2003) reported major differences between macroinvertebrate assemblages from predominantly cobble/pebble and macrophyte communities. My study suggested indication of variation of species' isotopic signatures even at the meso-habitat scale, which may indicate changes of species functional roles among different habitat patches or the degree (or lack of) mobility (Figure A 10, Appendix III). However, this requires further investigation. Particularly, the use of a species' isotopic niche metrics to make comparisons across different littoral habitats can contribute to the understanding of the spatial isotopic resolution in benthic consumers and their rate of mobility and use of different habitats.

Owing to high rainfall during the summer of 2009 (MET Annual Report 2009), high water levels were observed in some of the study lakes during the sampling period, particularly in Loughs Brackley and Muckno. This resulted in the sampling of the littoral zone of some grassland sites, which consisted of inundated vegetation (Table A 8, Appendix III). Therefore the interpretation of the data requires the consideration of that. However, the study nevertheless compared the effect of different substrata on the respective functional signals, although cannot say anything specifically about influences of more typical in-aquatic habitats. Furthermore, recent studies by Evtimova and Donohue (2014) have demonstrated large seasonal water level fluctuation even in unregulated lakes. The field conditions during the study field period, which were constrained in their timing by project logistics, can be considered to represent one end of a gradient of lake



water levels. The common dynamic nature of all Irish lakes implies a heretofore-unacknowledged awareness of species adaptability to water level fluctuations, including the colonisation of bank side habitat at times of high water level. While this has some impact on the present study, of greater global significance is the implications for wider routine monitoring as occurs under the European Water Framework Directive. For the current study, the limitations of the comparison of study sites with different littoral habitat characteristics and wind exposure are recognised, but the stoichiometric and isotopic signals of the model organisms used in this study nevertheless represent valid comparisons of functional metrics, and does not detract from the overall thrust of the thesis and the validity of its main findings.

#### *Species presence and sample size*

The among-lake isotopic variation of *G. pulex* could not be assessed in this study as the species occurred only in one of the study lakes (Lough Muckno). Furthermore, studies on species' isotopic niche metrics (Chapter 2) and C:N:P stoichiometry (Chapter 4) were conducted across three lakes and the impacts of different environmental variables were not addressed effectively mainly because of small sample sizes. However, results suggested differences between species' C:N:P stoichiometry (for *G. duebeni*) and isotopic niche metrics (*e.g.* niche widths and isotopic dispersion) particularly between Lough Fern, with relatively low nutrient status, and Loughs Brackley and Muckno. Given that, further studies exploring the potential of isotopic niche metrics and stoichiometry as indicators of lake nutrient status would provide improved understanding of what species do in habitats, and how nutrient enrichment, a pervasive pressure on Irish lakes, affects community dynamics of the littoral zone, and wider influences through littoral-pelagic coupling. Nevertheless, this study has also demonstrated the importance of using multiple lakes to provide a more comprehensive response that would have been obtained from use of a single lake, with its particular idiosyncrasies.

Finally, the important information that this study has provided on the composition of the littoral communities at meso scales and across lakes is a useful starting point to guide further experimental approaches using mesocosms and model habitats, as well as taxa.

## 5.4 Conclusions

The results of this study lead to the following key conclusions:

- Shoreline habitat type and its interaction with PC2 axis had significant effects on the  $\delta^{15}\text{N}$  signatures of all species, whereas the response of *G. duebeni*  $\delta^{13}\text{C}$  to PC gradients varied significantly between the woodland and grassland shorelines. No impact of shoreline habitat on the  $\delta^{15}\text{N}$  signatures of *C. pseudogracilis* could be detected. Also, the response of isotopic signatures to the shoreline habitat types varied among species.
- Nutrient enrichment had a significantly negative effect on the  $\delta^{13}\text{C}$  signatures of *A. aquaticus* and *C. pseudogracilis*, irrespective of the shoreline habitat types and an overall positive effect on *G. duebeni*  $\delta^{13}\text{C}$  signatures. Also, there was a negative influence of the nutrient gradient on the  $\delta^{15}\text{N}$  signatures of *A. aquaticus*, but not *G. duebeni* or *C. pseudogracilis*, suggesting limited ability of littoral macroinvertebrate isotope signatures to act as indicators of nutrient enrichment in the studied lakes.
- Water colour was a major component of the water chemistry acting on species along the secondary axis of the PCA. Based on all the linear mixed effects models with species isotopic signatures, PC2 axis and/or its interaction with shoreline habitat type had highly significant effects. While the  $\delta^{13}\text{C}$  signatures generally demonstrated a negative response along the colour gradient, there was a species-specific response of the PC2 axis on species'  $\delta^{15}\text{N}$  signatures. Future research is required to elucidate direct and indirect influences of water colour on macroinvertebrate, and their associated elemental signatures, in freshwater lakes.
- Body length was significantly important in explaining the isotopic variation of amphipods *G. duebeni* and *C. pseudogracilis*, with species-specific responses. *C. pseudogracilis*, which are typically smaller than females, were significantly  $\delta^{15}\text{N}$ -depleted compared with female individuals, suggesting a likely influence of size in the trophic position of *C. pseudogracilis*.
- *Gammarus duebeni* and *A. aquaticus* demonstrated narrower niche widths and isotopic dispersions in Lough Fern than in Brackley and Muckno. This is likely related to basal food sources with less diversity and/or within limited



carbon and nitrogen ranges associated with lower nutrient status. Brackley provided food sources with highest diversity and/or widest range of  $\delta^{13}\text{C}$ , which likely supported increased generalist-feeding behaviour for all studied species across lakes. However,  $\delta^{15}\text{N}$  signatures of both *A. aquaticus* and *G. duebeni* were significantly depleted in Lough Brackley compared with Fern, also coinciding with lower N:P and C:P elemental ratios in Brackley in comparison with Fern.

- Both isotope and stoichiometry results suggested that *C. pseudogracilis* had the least generalist feeding tendencies with the narrowest range of elemental ratios and  $\delta^{13}\text{C}$  dispersions, and a smaller isotopic niche than other species. There was no overlap between the isotopic niches of *C. pseudogracilis* and *A. aquaticus*, indicating distinct trophic positions occupied by the two species. Also, there was a higher isotopic niche overlap between *C. pseudogracilis* and *G. pulex* in Muckno, compared with *C. pseudogracilis* and *G. duebeni* in Brackley. *Crangonyx pseudogracilis* was associated with highest  $\delta^{15}\text{N}$  signatures, reflecting higher trophic positions among other species and some form of predatory diet in four of the study lakes that it was present. The isotope study revealed limited evidence for the predation of *Gammarus* spp. on *C. pseudogracilis*, contradicting earlier laboratory experiments.
- Despite non-overlapping distributions between *G. duebeni* and *G. pulex* across lakes, computing isotopic niche metrics in reference to *A. aquaticus* enabled a comparison of their functions isotopic dispersion and niche widths, as well as niche overlaps with other species. Results suggest a broader diversity of carbon sources utilized by *G. duebeni* in comparison with a broader range of trophic position and greater omnivory by *G. pulex*. *Gammarus pulex* was more  $\delta^{13}\text{C}$ -enriched and showed a higher trophic overlap with *A. aquaticus* compared with *G. duebeni*. Similar C:N:P elemental ratios between *G. duebeni* and *G. pulex* across three different lakes suggested shared nutritional needs by the two *Gammarus* species.
- Taxonomy, rather than the spatial differences is more likely to be the primary driver of among and within lake variation of C:N:P stoichiometry among study species. Significant stoichiometric differences among *A. aquaticus*, *Gammarus* spp. and *C. pseudogracilis* were demonstrated. The elemental



stoichiometry of *C. pseudogracilis* and significant stoichiometric differences among amphipod species was reported for the first time.

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APPENDIX I

Table A 1 Model simplification steps for the  $\delta^{13}\text{C}$  data with *A. aquaticus*. Model 2 represents the minimal adequate model. (SHab=Shoreline habitat type; BL=Body Length; K=Number of parameters; AICc=corrected Akaike’s information criterion; ‘x’ denotes interaction between terms; ‘+’ denotes where a variable was remained; ‘-’ denotes where a variable was eliminated).

| Model ID | PC1 | PC2 | SHab | BL | PC1 x SHab | PC2 x SHab | K | AICc  |
|----------|-----|-----|------|----|------------|------------|---|-------|
| 0        | +   | +   | +    | +  | +          | +          | 6 | 676.5 |
| 1        | +   | +   | +    | +  | -          | +          | 5 | 675.1 |
| 2        | +   | +   | +    | -  | -          | +          | 4 | 673.0 |

Table A 2 Model simplification steps for the  $\delta^{15}\text{N}$  data with *A. aquaticus*. Model 1 represents the minimal adequate model. (SHab=Shoreline habitat type; BL=Body Length; K=Number of parameters; AICc=corrected Akaike’s information criterion; ‘x’ denotes interaction between terms; ‘+’ denotes where a variable was remained; ‘-’ denotes where a variable was eliminated).

| Model ID | PC1 | PC2 | SHab | BL | PC1 x SHab | PC2 x SHab | K | AICc  |
|----------|-----|-----|------|----|------------|------------|---|-------|
| 0        | +   | +   | +    | +  | +          | +          | 6 | 423.7 |
| 1        | +   | +   | +    | -  | +          | +          | 5 | 421.7 |

Table A 3 Model simplification steps for the  $\delta^{13}\text{C}$  data with *G. duebeni*. The maximum model (Model 0) also represents the minimal adequate model. (SHab=Shoreline habitat type; BL=Body Length; K=Number of parameters; AICc=corrected Akaike’s information criterion; ‘x’ denotes interaction between terms; ‘+’ denotes where a variable was remained).

| Model ID | PC1 | PC2 | SHab | BL | PC1 x SHab | PC2 x SHab | K | AICc  |
|----------|-----|-----|------|----|------------|------------|---|-------|
| 0        | +   | +   | +    | +  | +          | +          | 6 | 239.4 |



Table A 4 Model simplification steps for the  $\delta^{15}\text{N}$  data with *G. duebeni*. Model 2 represents the minimal adequate model. (SHab=Shoreline habitat type; BL=Body Length; K=Number of parameters; AICc=corrected Akaike’s information criterion; ‘x’ denotes interaction between terms; ‘+’ denotes where a variable was remained; ‘-’ denotes where a variable was eliminated).

| Model ID | PC1 | PC2 | SHab | BL | PC1 x SHab | PC2 x SHab | K | AICc  |
|----------|-----|-----|------|----|------------|------------|---|-------|
| 0        | +   | +   | +    | +  | +          | +          | 6 | 168.3 |
| 1        | +   | +   | +    | +  | -          | +          | 5 | 167.7 |
| 2        | -   | +   | +    | +  | -          | +          | 4 | 165.7 |

Table A 5 Model simplification steps for the  $\delta^{15}\text{N}$  data with *C. pseudogracilis*. Model 3 represents the minimal adequate model. (SHab=Shoreline habitat type; BL=Body Length; K=Number of parameters; AICc=corrected Akaike’s information criterion; ‘x’ denotes interaction between terms; ‘+’ denotes where a variable was remained; ‘-’ denotes where a variable was eliminated).

| Model ID | PC1 | PC2 | SHab | BL | PC1 x SHab | PC2 x SHab | K | AICc  |
|----------|-----|-----|------|----|------------|------------|---|-------|
| 0        | +   | +   | +    | +  | +          | +          | 6 | 422.0 |
| 1        | +   | +   | +    | +  | +          | -          | 5 | 419.7 |
| 2        | +   | +   | +    | +  | -          | -          | 4 | 419.4 |
| 3        | +   | +   | -    | +  | -          | -          | 3 | 417.1 |

Table A 6 Model simplification steps for the  $\delta^{15}\text{N}$  data with *C. pseudogracilis*. Model 2 represents the minimal adequate model. (SHab=Shoreline habitat type; BL=Body Length; K=Number of parameters; AICc=corrected Akaike’s information criterion; ‘x’ denotes interaction between terms; ‘+’ denotes where a variable was remained; ‘-’ denotes where a variable was eliminated).

| Model ID | PC1 | PC2 | SHab | BL | PC1 x SHab | PC2 x SHab | K | AICc  |
|----------|-----|-----|------|----|------------|------------|---|-------|
| 0        | +   | +   | +    | +  | +          | +          | 6 | 376.3 |
| 1        | +   | +   | +    | +  | -          | +          | 5 | 374.4 |
| 2        | -   | +   | +    | +  | -          | +          | 4 | 374.1 |

APPENDIX II

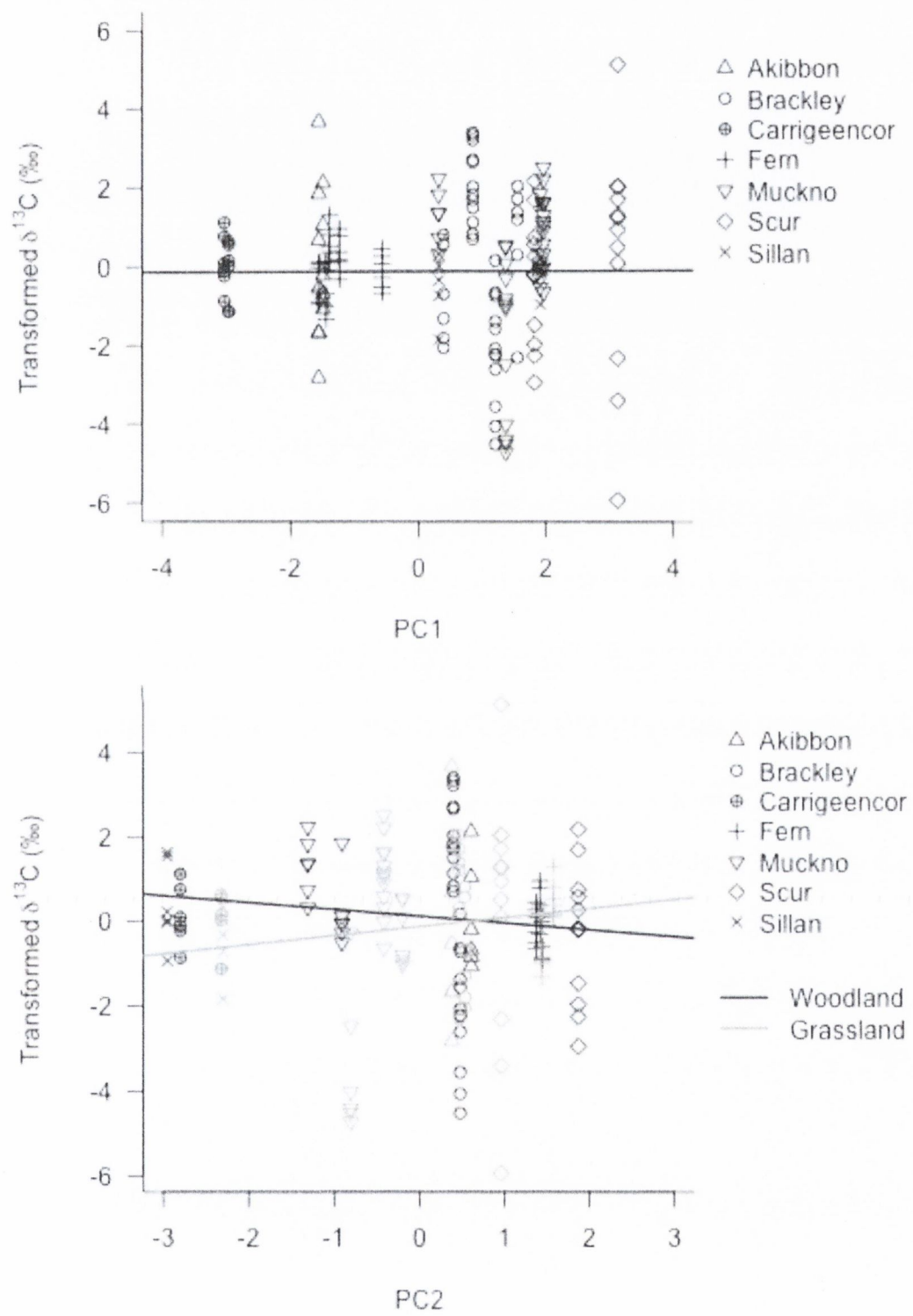


Figure A 1 The lake-corrected version of Figure 2.9 displaying the distribution of transformed  $\delta^{13}\text{C}$  signatures of *A. aquaticus* along the PC1 (above) and PC2 (below) axes. (See Section 2.2.5.2 for detailed information on the transformation method).

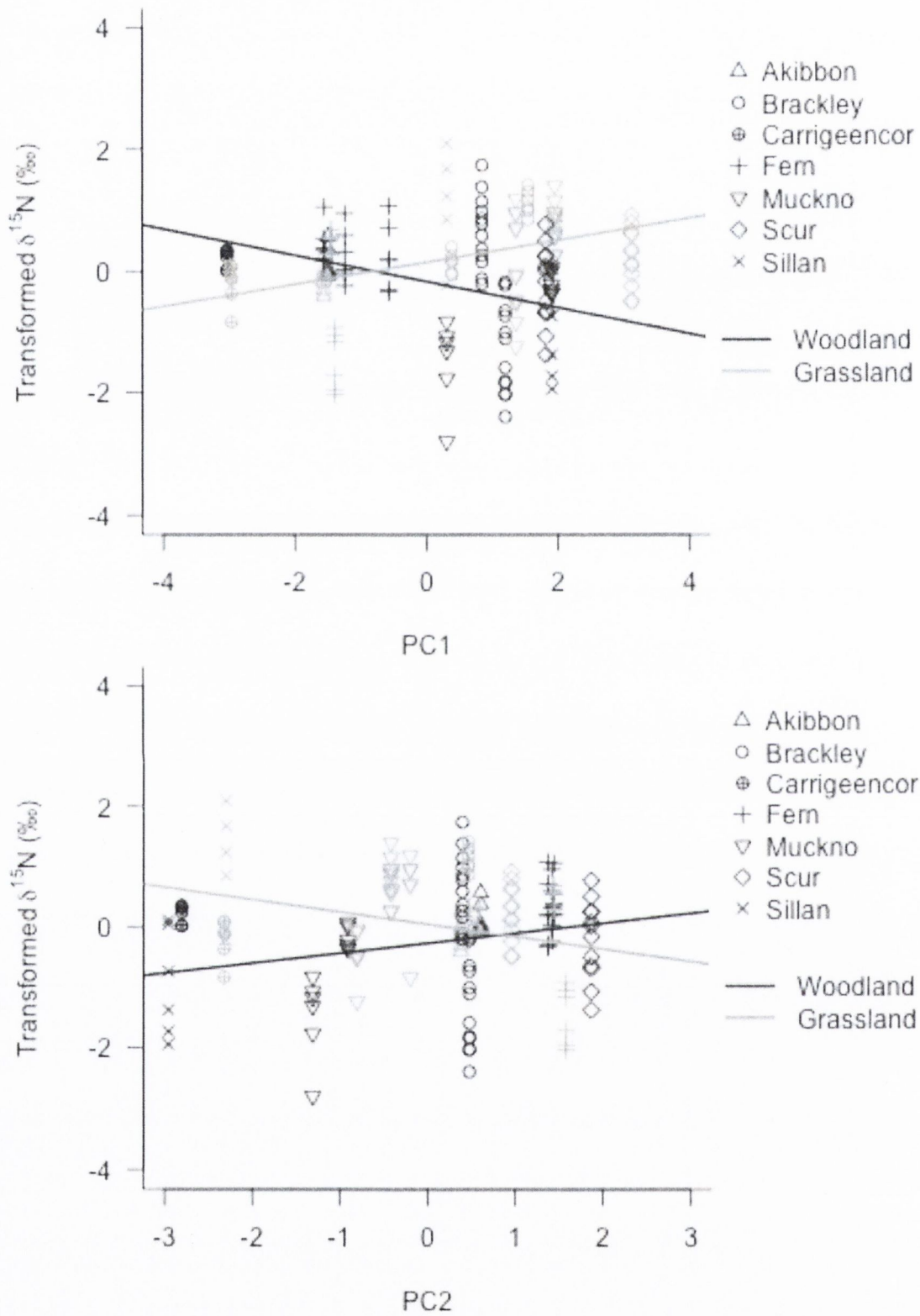


Figure A 2 The lake-corrected version of Figure 2.10 displaying the distribution of transformed  $\delta^{15}\text{N}$  signatures of *A. aquaticus* along the PC1 (above) and PC2 (below) axes. (See Section 2.2.5.2 for detailed information on the transformation method).



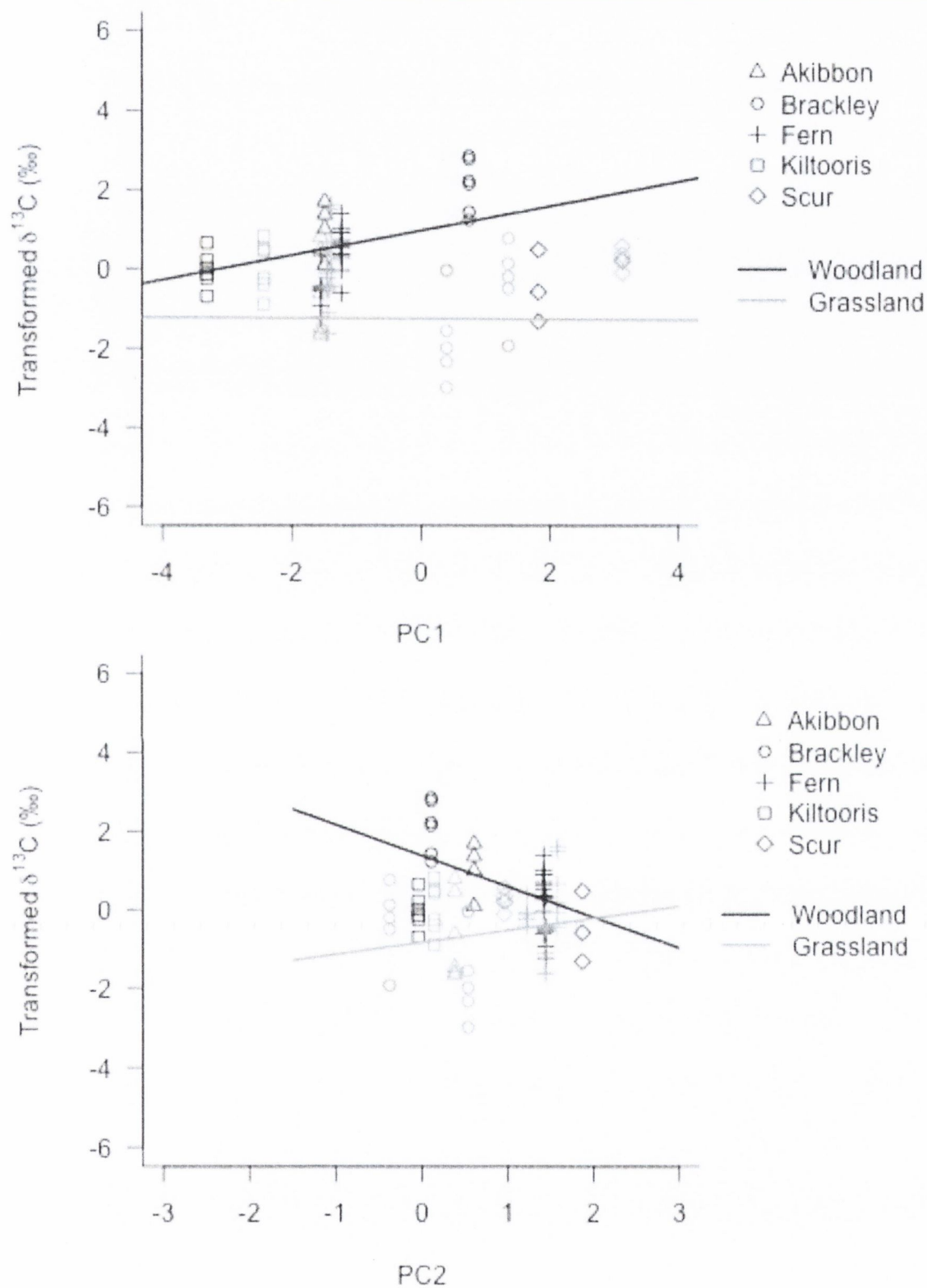


Figure A 3 The lake-corrected version of Figure 2.11 displaying the distribution of transformed  $\delta^{13}\text{C}$  signatures of *G. duebeni* along the PC1 (above) and PC2 (below) axes. (See Section 2.2.5.2 for detailed information on the transformation method).

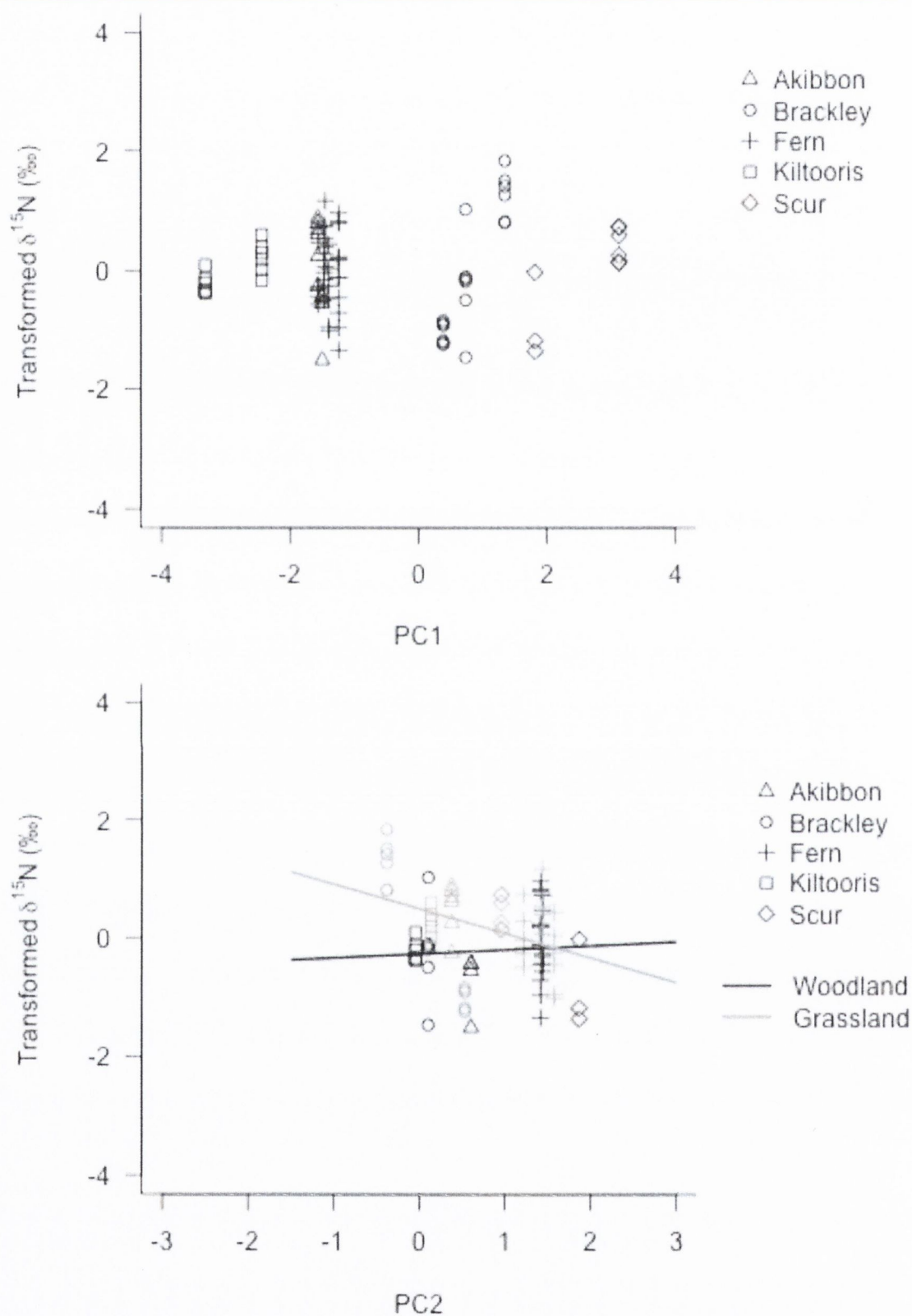


Figure A 4 The lake-corrected version of Figure 2.13 displaying the distribution of transformed  $\delta^{15}\text{N}$  signatures of *G. duebeni* along the PC1 (above) and PC2 (below) axes. (See Section 2.2.5.2 for detailed information on the transformation method).

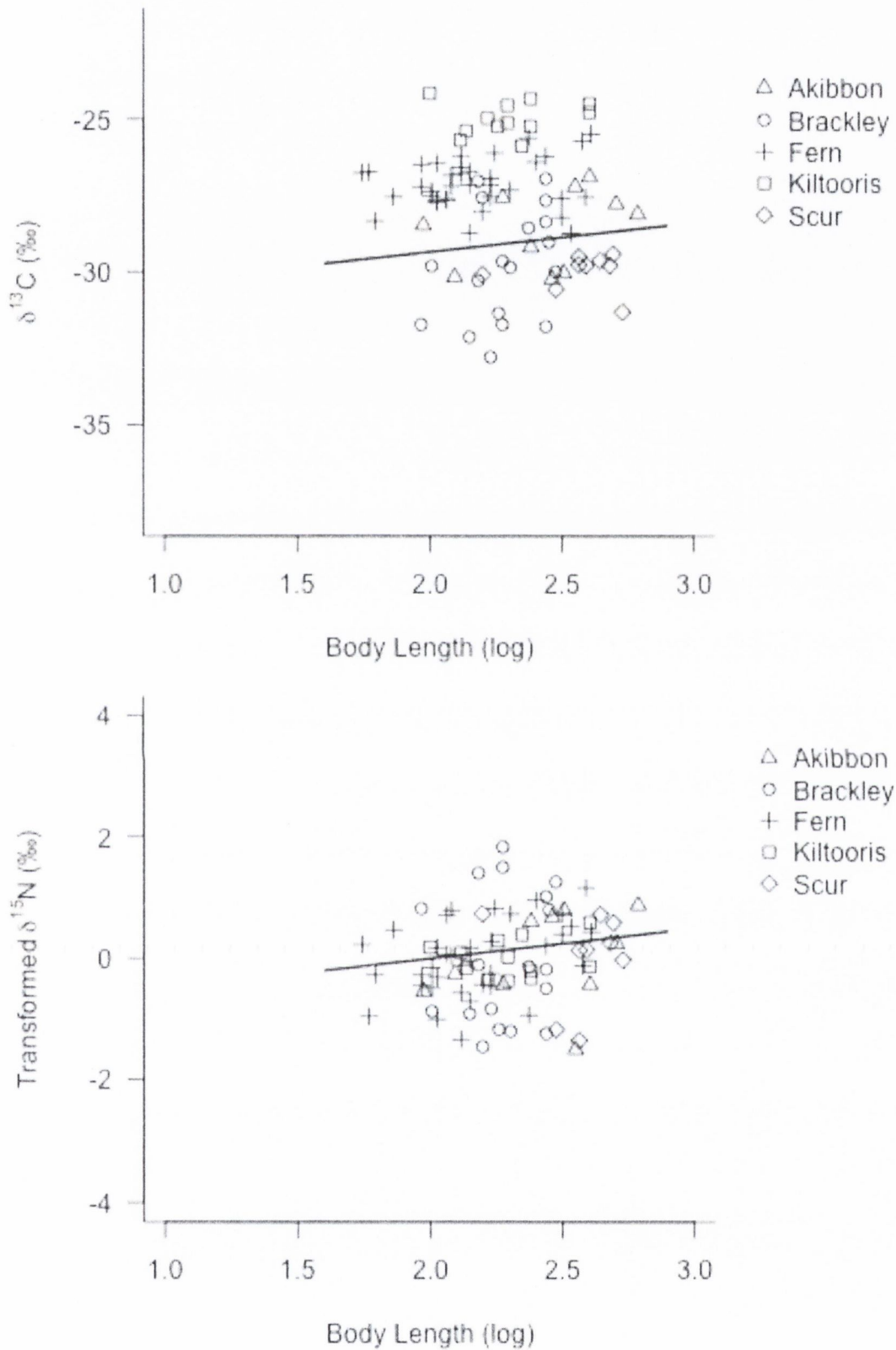


Figure A 5 The lake-corrected version of Figure 2.12 and Figure 2.14 displaying the distribution of transformed  $\delta^{13}\text{C}$  (above) and  $\delta^{15}\text{N}$  (below) signatures of *G. duebeni* along the body size (log) gradient, respectively. (See Section 2.2.5.2 for detailed information on the transformation method).



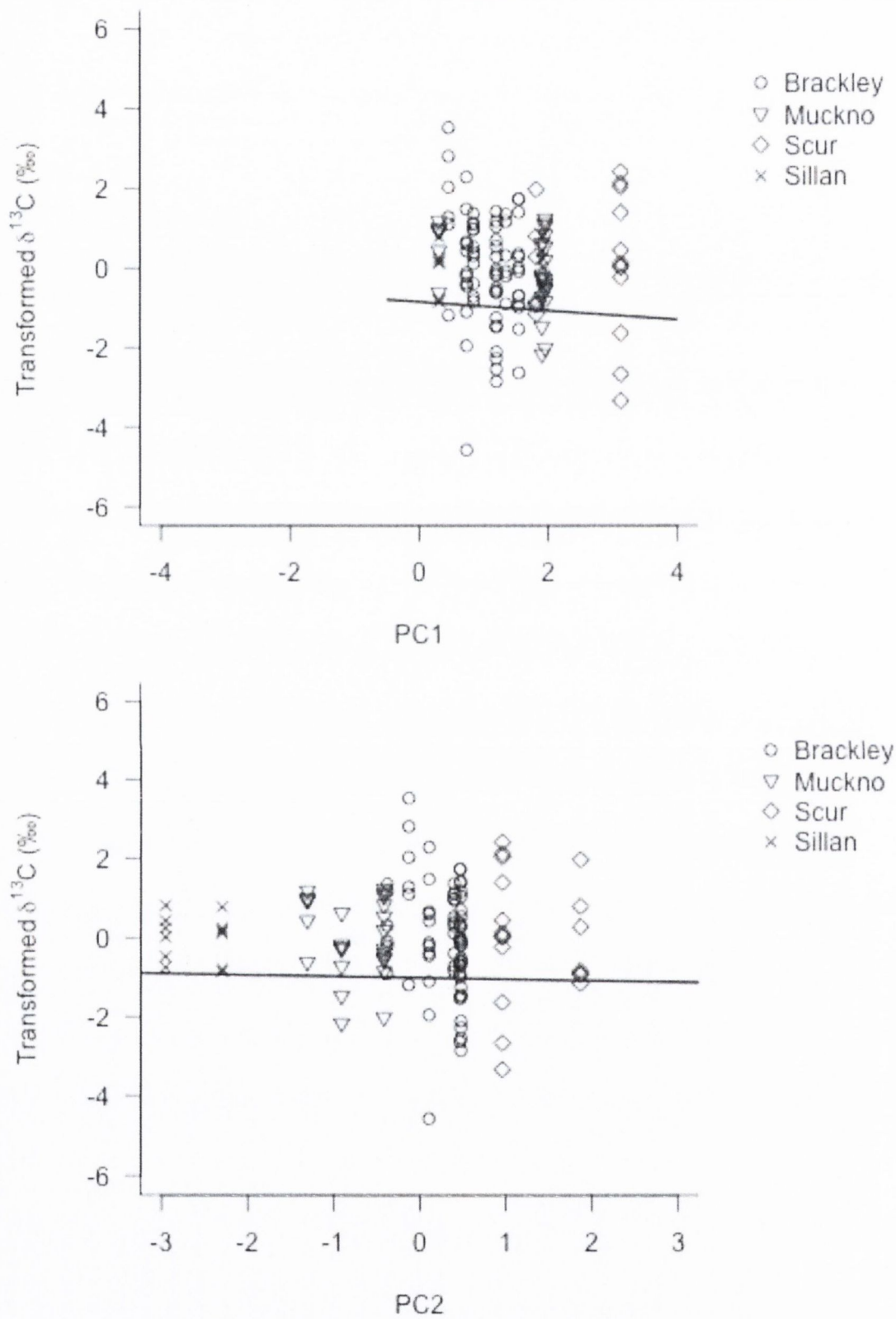


Figure A 6 The lake-corrected version of Figure 2.15 displaying the distribution of transformed  $\delta^{13}\text{C}$  signatures of *C. pseudogracilis* along the PC1 (above) and PC2 (below) axes. (See Section 2.2.5.2 for detailed information on the transformation method).

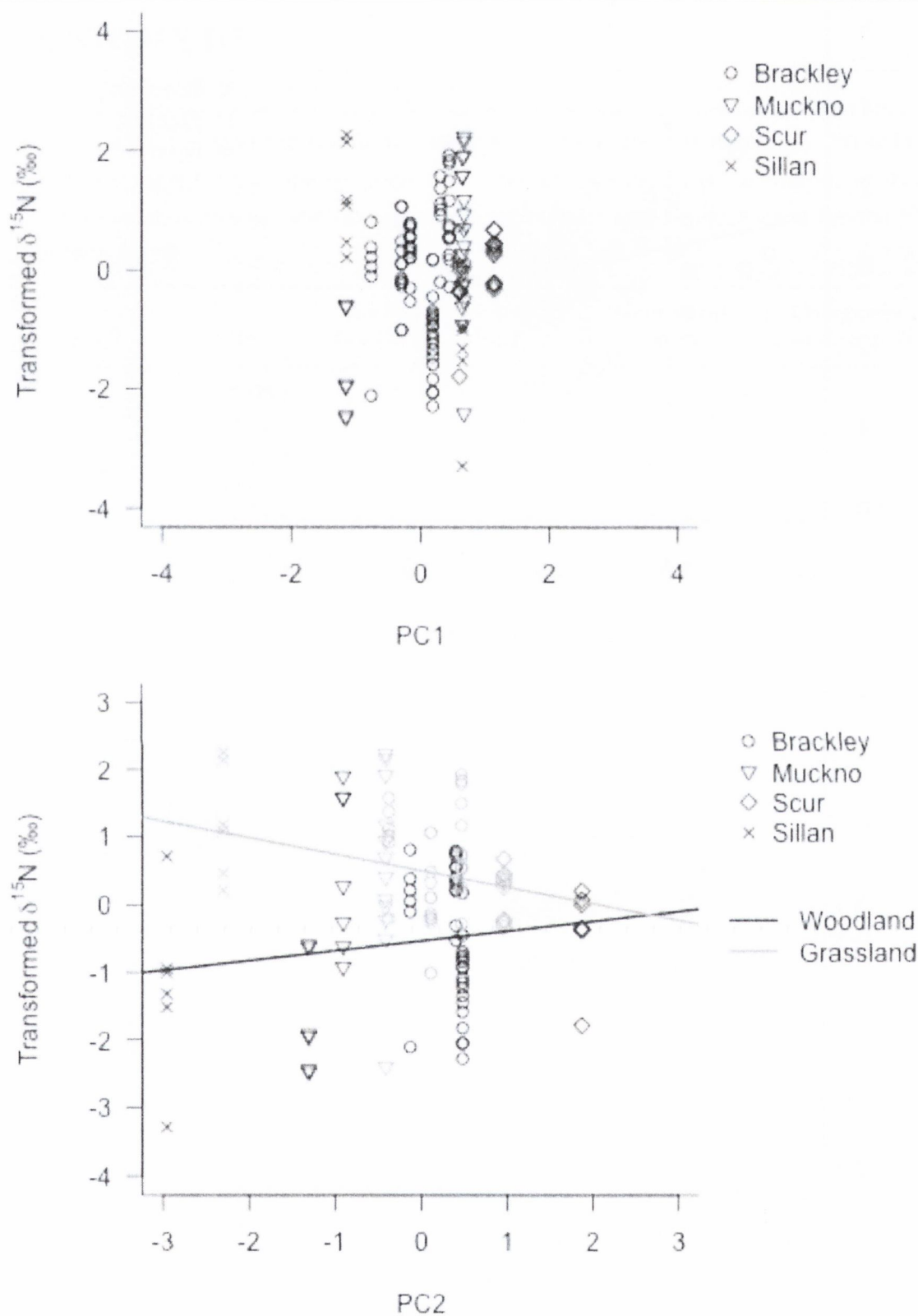


Figure A 7 The lake-corrected version of Figure 2.17 displaying the distribution of transformed  $\delta^{15}\text{N}$  signatures of *C. pseudogracilis* along the PC1 (above) and PC2 (below) axes. (See Section 2.2.5.2 for detailed information on the transformation method).

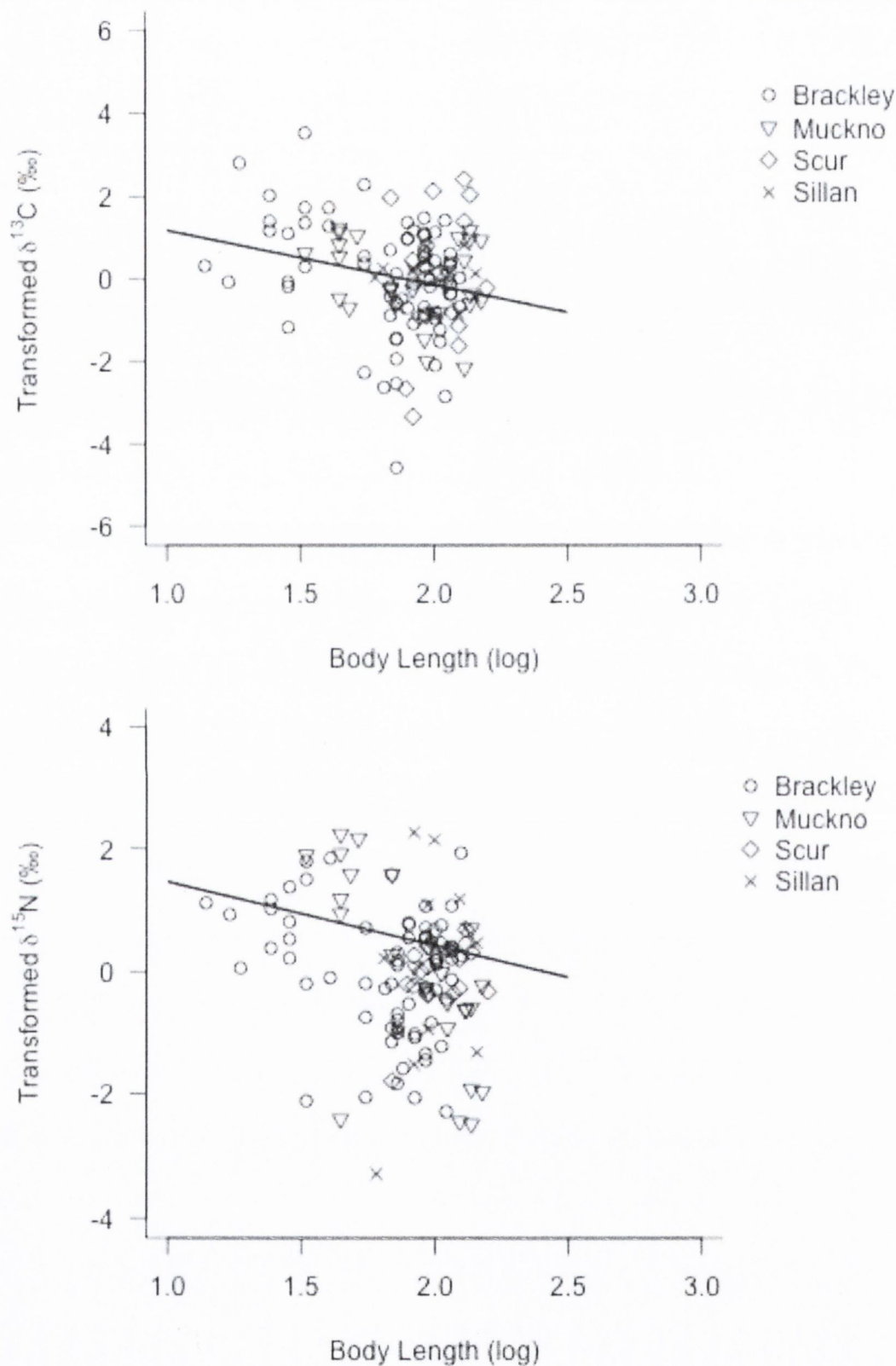


Figure A 8 The lake-corrected version of Figure 2.16 and Figure 2.18 displaying the distribution of transformed  $\delta^{13}\text{C}$  (above) and  $\delta^{15}\text{N}$  (below) signatures of *C. pseudogracilis* along the body size (log) gradient, respectively. (See Section 2.2.5.2 for detailed information on the transformation method).



APPENDIX III

Table A 7 Presences of macroinvertebrate species across sampling sites of the eight study lakes. Shoreline habitats adjacent to sampling sites are indicated after site names; W=Woodland and G=Grassland. '✓' indicates species present at a site but not used for the analysis in this study owing to low abundances; '✓✓' indicates species present at a site and used for the SIA and elemental analysis.

| Lough        | Site    | <i>Asellus aquaticus</i> | <i>Gammarus duebeni</i> | <i>Gammarus pulex</i> | <i>Crangonyx pseudogracilis</i> |
|--------------|---------|--------------------------|-------------------------|-----------------------|---------------------------------|
| Carrigeencor | C1 (W)  | ✓✓                       |                         |                       |                                 |
|              | C2 (G)  | ✓✓                       |                         |                       |                                 |
| Akibbon      | A1 (W)  | ✓✓                       | ✓✓                      |                       |                                 |
|              | A2 (G)  | ✓✓                       | ✓✓                      |                       |                                 |
| Kiltooris    | K1 (W)  |                          | ✓✓                      |                       |                                 |
|              | K2 (G)  |                          | ✓✓                      |                       |                                 |
| Fern         | F1 (W)  | ✓✓                       |                         |                       |                                 |
|              | F2 (W)  | ✓✓                       | ✓✓                      |                       |                                 |
|              | F3 (W)  | ✓✓                       | ✓✓                      |                       |                                 |
|              | F4 (G)  | ✓✓                       | ✓✓                      |                       |                                 |
|              | F5 (G)  | ✓                        | ✓✓                      |                       |                                 |
|              | F6 (G)  | ✓✓                       | ✓✓                      |                       |                                 |
| Brackley     | B1 (W)  | ✓✓                       |                         |                       | ✓✓                              |
|              | B2 (W)  | ✓✓                       |                         |                       | ✓✓                              |
|              | B3 (W)  |                          | ✓✓                      |                       | ✓✓                              |
|              | B4 (G)  | ✓✓                       | ✓✓                      |                       | ✓✓                              |
|              | B5 (G)  | ✓                        | ✓✓                      |                       | ✓✓                              |
|              | B6 (G)  | ✓✓                       |                         |                       | ✓✓                              |
| Muckno       | M1 (W)  | ✓✓                       |                         | ✓✓                    | ✓✓                              |
|              | M2 (W)  | ✓✓                       |                         | ✓✓                    | ✓✓                              |
|              | M3 (W)  | ✓                        |                         | ✓✓                    | ✓                               |
|              | M4 (G)  | ✓✓                       |                         | ✓✓                    | ✓                               |
|              | M5 (G)  | ✓✓                       |                         | ✓✓                    | ✓                               |
|              | M6 (G)  | ✓✓                       |                         | ✓✓                    | ✓✓                              |
| Sillan       | Si1 (W) | ✓✓                       |                         |                       | ✓✓                              |
|              | Si2 (G) | ✓✓                       |                         |                       | ✓✓                              |
| Scur         | Sl1 (W) | ✓✓                       | ✓✓                      |                       | ✓✓                              |
|              | Sl2 (G) | ✓✓                       | ✓✓                      |                       | ✓✓                              |

Table A 8 Abundances of macroinvertebrate species at the meso-habitat scale in Loughs Fern, Brackley and Muckno. Individuals of species from any given littoral sample that were used for the SIA are indicated in bold font by their respective abundances, which are comparable among species within samples but not across samples as sampling regime was not standardised. Littoral habitat characteristic indicates a broad category of substrate and macrophyte presence at each littoral habitat.

| Lough    | Shoreline<br>Habitat Type | Site | Littoral<br>Sample | <i>Asellus<br/>aquaticus</i> | <i>Gammarus<br/>duebeni</i> | <i>Gammarus<br/>pulex</i> | <i>Crangonyx<br/>pseudogracilis</i> | Littoral Habitat<br>Characteristic              | Common Trees<br>at Woodland<br>Shores                                                                         |
|----------|---------------------------|------|--------------------|------------------------------|-----------------------------|---------------------------|-------------------------------------|-------------------------------------------------|---------------------------------------------------------------------------------------------------------------|
| Fern     | Woodland                  | F1   | F1.1               | 29                           | 0                           | -                         | -                                   | stony/emergent<br>macrophytes                   | <i>Salix spp.</i> (Willow)                                                                                    |
|          |                           |      | F1.2               | <b>12</b>                    | 0                           | -                         | -                                   | stony                                           |                                                                                                               |
|          |                           | F2   | F2.1               | <b>60</b>                    | <b>18</b>                   | -                         | -                                   | stony/submerged<br>macrophytes                  | <i>Salix spp.</i> (Willow)                                                                                    |
|          |                           |      | F2.2               | 0                            | <b>50</b>                   | -                         | -                                   | muddy/emergent<br>macrophytes                   |                                                                                                               |
|          | Grass-<br>land            | F3   | F3.1               | <b>19</b>                    | <b>30</b>                   | -                         | -                                   | tree roots                                      | <i>Salix spp.</i> (Willow)                                                                                    |
|          |                           | F4   | F4.1               | <b>99</b>                    | <b>12</b>                   | -                         | -                                   | stony                                           | -                                                                                                             |
|          |                           | F5   | F5.1               | 20                           | <b>21</b>                   | -                         | -                                   | inundated vegetation                            | -                                                                                                             |
|          |                           | F6   | F6.1               | <b>15</b>                    | <b>21</b>                   | -                         | -                                   | stony/emergent<br>macrophytes                   | -                                                                                                             |
| Brackley | Woodland                  | B1   | B1.1               | <b>6</b>                     | 0                           | -                         | <b>16</b>                           | stony/emergent<br>macrophytes                   | <i>Betula pendula</i><br>(Birch); <i>Alnus<br/>glutinosa</i> (Alder);<br><i>Fraxinus exelsior</i><br>(Ash)    |
|          |                           |      | B1.2               | <b>25</b>                    | 0                           | -                         | <b>32</b>                           | stony                                           |                                                                                                               |
|          |                           |      | B1.3               | 4                            | 0                           | -                         | 25                                  | tree roots                                      |                                                                                                               |
|          |                           | B2   | B2.1               | <b>20</b>                    | 0                           | -                         | <b>114</b>                          | submerged<br>macrophytes                        | <i>Alnus glutinosa</i><br>(Alder);<br><i>Salix spp.</i> (Willow)                                              |
|          |                           |      | B2.2               | <b>15</b>                    | 0                           | -                         | <b>70</b>                           | muddy/emergent<br>macrophytes                   |                                                                                                               |
|          |                           |      | B2.3               | 6                            | 0                           | -                         | <b>87</b>                           | tree roots/inundated<br>grass                   |                                                                                                               |
|          | Grassland                 | B3   | B3.1               | 0                            | <b>26</b>                   | -                         | <b>19</b>                           | stony, emergent<br>macrophytes                  | <i>Corylus avellana</i><br>(Hazel); <i>Salix spp.</i><br>(Willow);<br><i>Crataegus monogyta</i><br>(Hawthorn) |
|          |                           | B4   | B4.1               | <b>25</b>                    | <b>6</b>                    | -                         | <b>114</b>                          | inundated vegetation                            | -                                                                                                             |
|          |                           |      | B4.2               | 0                            | 0                           | -                         | <b>62</b>                           | stony/emergent<br>macrophytes                   |                                                                                                               |
|          |                           | B5   | B5.1               | 13                           | <b>39</b>                   | -                         | 9                                   | inundated<br>vegetation/emergent<br>macrophytes | -                                                                                                             |
|          |                           |      | B5.2               | 14                           | 0                           | -                         | 8                                   | emergent<br>macrophytes                         |                                                                                                               |
|          |                           | B6   | B6.1               | <b>18</b>                    | 0                           | -                         | <b>107</b>                          | stony/inundated<br>vegetation                   | -                                                                                                             |
|          |                           |      | B6.2               | 4                            | 0                           | -                         | <b>15</b>                           | emergent<br>macrophytes                         |                                                                                                               |

Table A 8 (continued) Abundances of macroinvertebrate species at the meso-habitat scale in Loughs Fern, Brackley and Muckno. Individuals of species from any given littoral sample that were used for the SIA are indicated in bold font by their respective abundances, which are comparable among species within samples but not across samples as sampling regime was not standardised. Littoral habitat characteristic indicates a broad category of substrate and macrophyte presence at each littoral habitat.

| Lough  | Shoreline<br>Habitat Type | Site | Littoral<br>Sample | <i>Asellus<br/>aquaticus</i> | <i>Gammarus<br/>duebeni</i> | <i>Gammarus<br/>pulex</i> | <i>Crangonyx<br/>pseudogracilis</i> | Littoral Habitat<br>Characteristic            | Common Trees<br>at Woodland<br>Shores     |
|--------|---------------------------|------|--------------------|------------------------------|-----------------------------|---------------------------|-------------------------------------|-----------------------------------------------|-------------------------------------------|
| Muckno | Woodland                  | M1   | M1.1               | 0                            | -                           | 6                         | 6                                   | stony                                         | <i>Acer pseudoplatanus</i><br>(Sycamore); |
|        |                           |      | M1.2               | 5                            | -                           | 41                        | 10                                  | stony/submerged<br>macrophytes                | <i>Salix</i> spp. (Willow);               |
|        |                           |      | M1.3               | 6                            | -                           | 24                        | 3                                   | tree roots                                    | <i>Fraxinus excelsior</i><br>(Ash)        |
|        |                           | M2   | M2.1               | 12                           | -                           | 0                         | 12                                  | stony/emergent<br>macrophytes                 | <i>Alnus glutinosa</i><br>(Alder);        |
|        |                           |      | M2.2               | 8                            | -                           | 24                        | 24                                  | emergent macrophytes                          | <i>Fraxinus excelsior</i><br>(Ash)        |
|        |                           |      | M2.3               | 0                            | -                           | 7                         | 2                                   | tree roots                                    |                                           |
|        |                           | M3   | M3.1               | 52                           | -                           | 19                        | 1                                   | stony                                         | <i>Alnus glutinosa</i><br>(Alder);        |
|        |                           |      | M3.2               | 2                            | -                           | 60                        | 0                                   | emergent macrophytes                          | <i>Crataegus monogyna</i><br>(Hawthorn);  |
|        |                           |      | M3.3               | 3                            | -                           | 44                        | 3                                   | tree roots                                    | <i>Hedera helix</i> (Ivy)                 |
|        | Grassland                 | M4   | M4.1               | 9                            | -                           | 30                        | 9                                   | stony                                         | -                                         |
|        |                           |      | M4.2               | 1                            | -                           | 7                         | 0                                   | emergent macrophytes                          |                                           |
|        |                           | M5   | M5.1               | 21                           | -                           | 12                        | 13                                  | inundated vegetation/<br>emergent macrophytes | -                                         |
|        |                           |      | M5.2               | 1                            | -                           | 0                         | 3                                   | muddy/floating<br>macrophytes                 |                                           |
|        |                           | M6   | M6.1               | 6                            | -                           | 3                         | 97                                  | muddy/submerged<br>macrophytes                | -                                         |
|        |                           |      | M6.2               | 7                            | -                           | 36                        | 7                                   | stony/emergent<br>macrophytes                 |                                           |



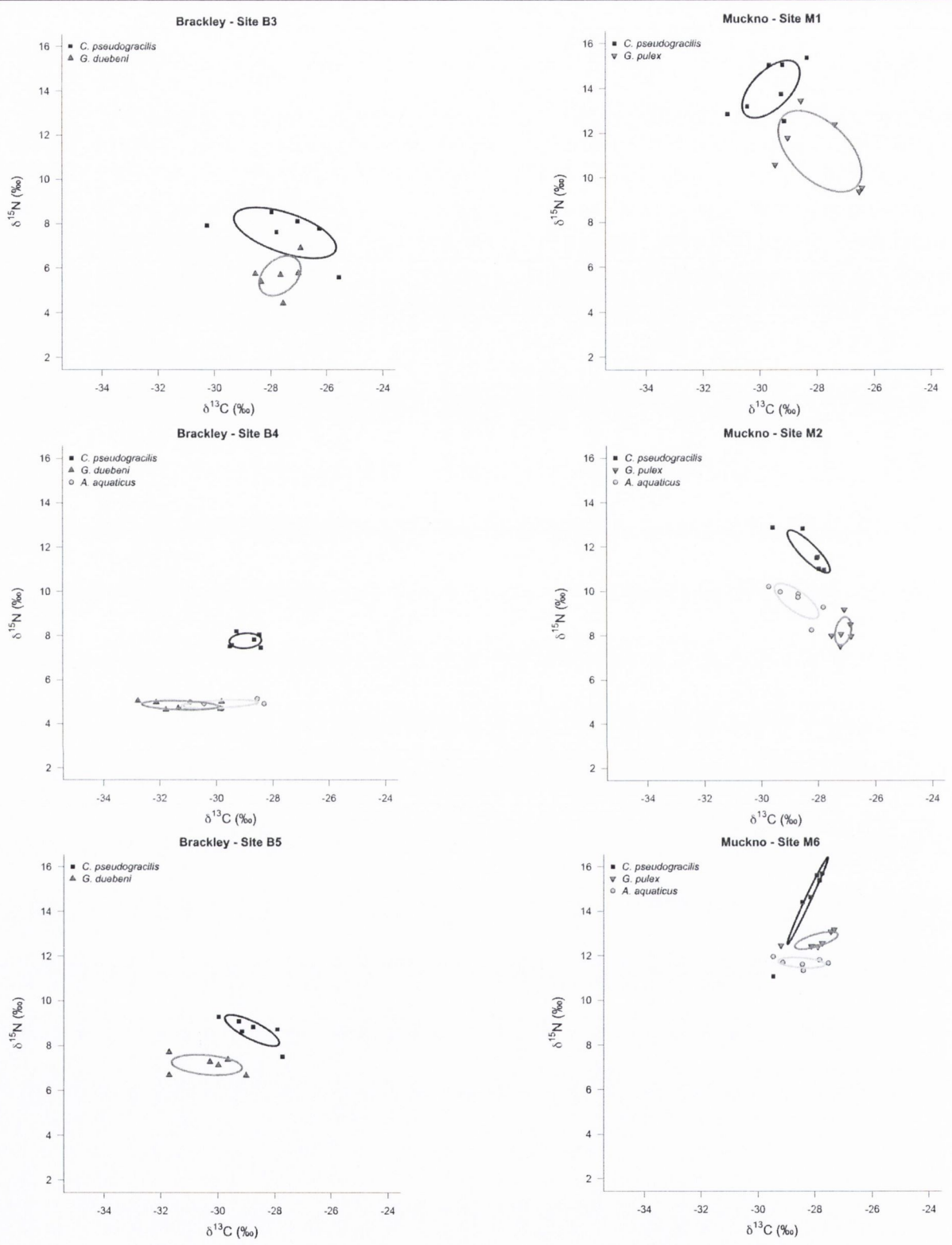


Figure A 9 Examples of standard ellipses (SEA<sub>B</sub>) estimated for macroinvertebrate species in different sites of Loughs Brackley and Muckno. Each data point represents an individual macroinvertebrate sample in the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  bi-plot and each SEA<sub>B</sub> is generated using a min. of 6 samples of a species from the same littoral habitat (B3.1, B4.1, M1.2, M2.1 and M6.2) except for B5 (see Section 3.2.4 for further explanation and for a list of sample details).

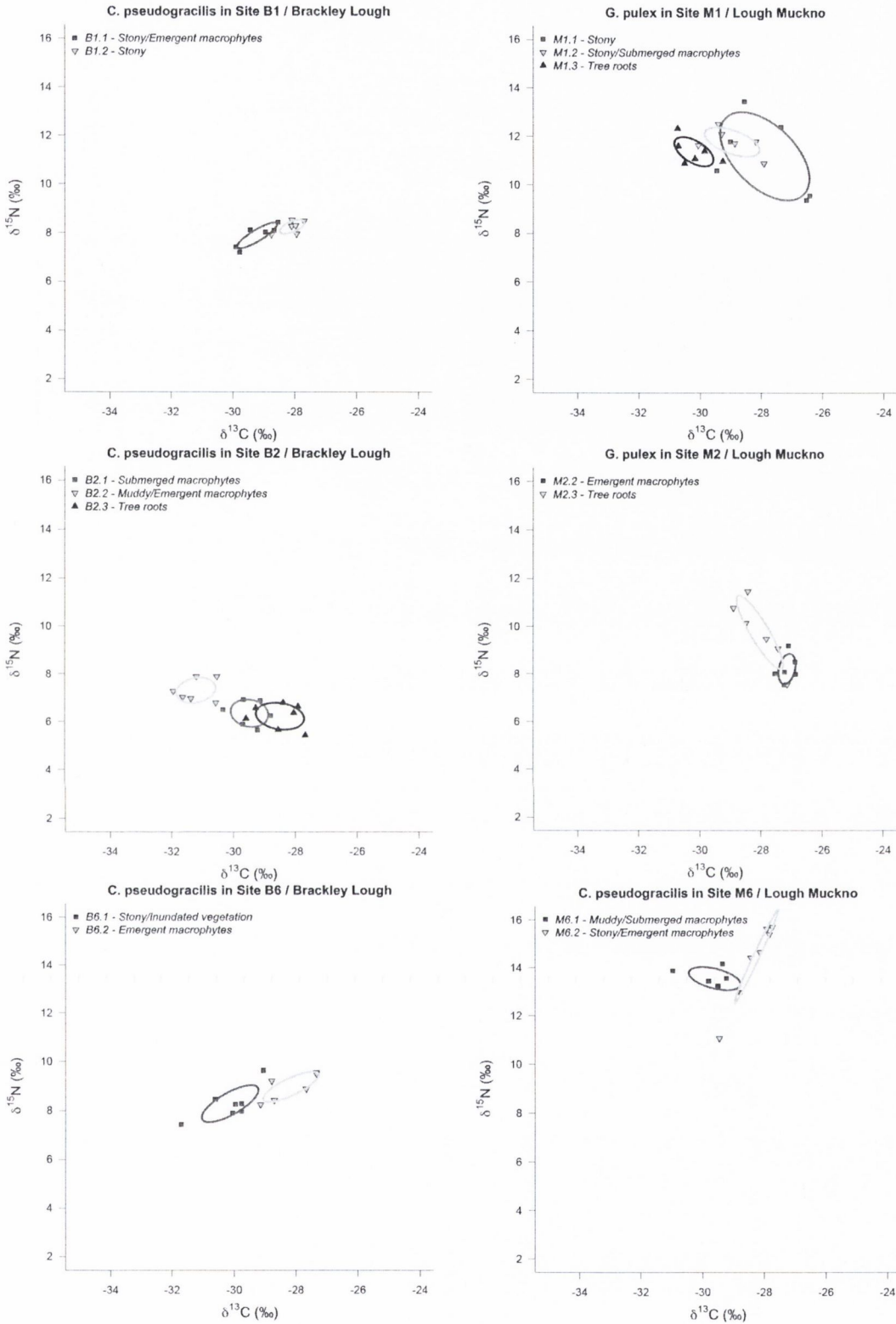


Figure A 10 Examples of standard ellipses (SEA<sub>B</sub>) estimated for a species in two different littoral habitat types of the same sites of Loughs Brackley and Muckno. Each data point represents an individual *C. pseudogracilis* or *G. pulex* in the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  bi-plot and each SEA<sub>B</sub> is generated using a min. of 6 samples of a species from the same littoral habitat (see Section 3.2.4 for further explanation and for a list of sample details).



## APPENDIX IV

Table A 9 Comparison of the molar C:N, C:P and N:P elemental ratios (mean±SE) of freshwater macroinvertebrate species of Amphipoda and Isopoda families between the current and previous studies. n=number of replication. Species and elemental ratios based on this study are shown in bold font.

| Family    | Species                                         | Study Type (Lakes/Streams)   | Country         | C:N            | C:P              | N:P             | n  | References                      |
|-----------|-------------------------------------------------|------------------------------|-----------------|----------------|------------------|-----------------|----|---------------------------------|
| Amphipoda | <i>C. pseudogracilis</i>                        | Field study (Lakes)          | Ireland         | <b>5.5±0.2</b> | <b>71.0±5.5</b>  | <b>13.1±1.3</b> | 12 | Present study                   |
|           | <i>G. pulex</i>                                 | Field study (Lakes)          | Ireland         | <b>5.5±0.3</b> | <b>102.9±6.6</b> | <b>18.7±1.8</b> | 12 | Present study                   |
|           |                                                 | Laboratory experiment        | The Netherlands | 5.4±0.1        | 78.3±1.0         | 14.4±0.2        | 25 | (Dorenbosch and Bakker 2011)    |
|           | <i>G. duebeni</i>                               | Field study (Lakes)          | Ireland         | <b>5.7±0.3</b> | <b>96.3±10.4</b> | <b>17.1±1.9</b> | 9  | Present study                   |
|           |                                                 | Field study (Lakes)          | Ireland         | 5.2±0.2        | 112.9±4          | 23.3±1.8        | 27 | (Donohue 2008)                  |
|           |                                                 | Mesocosm experiment (Stream) | Ireland         | 7.1            | 56.7             | 8.6             | 6  | (Hladyz <i>et al.</i> 2009)     |
|           | <i>G. roeseli</i>                               | Field study (Lake)           | Germany         | 4.6±0.1        | 47.3±1.2         | 10.2±0.3        | 44 | (Fink <i>et al.</i> 2006)       |
|           | <i>Hyalella azteca</i> /<br><i>G. lacustris</i> | Field study (Lakes)          | Canada          | 5.8±0.6        | 93.8±12.3        | 16.2±2.5        | 8  | (Frost <i>et al.</i> 2003)      |
|           | <i>G. pseudolimneus</i>                         | Field study (Streams)        | USA             | 4.7±0.3        | 39.4±5.9         | 8.4±1.3         | 19 | (Evans-White and Lamberti 2005) |
| Isopoda   | <i>A. aquaticus</i>                             | Field study (Lakes)          | Ireland         | <b>4.9±0.3</b> | <b>88.2±11.3</b> | <b>17.8±1.8</b> | 20 | Present study                   |
|           |                                                 | Field study (Lakes)          | Ireland         | 4.0±0.2        | 104±3.6          | 28.5±2.3        | 27 | (Donohue 2008)                  |
|           |                                                 | Field study (Lake)           | Germany         | 4.3±0.1        | 44.6±1.7         | 10.5±0.4        | 14 | (Fink <i>et al.</i> 2006)       |
|           |                                                 | Field study (Lake)           | Sweden          | 4.5±0.7        | 47.9±4.9         | 10.5±1.1        | 35 | (Liess and Hillebrand 2005)     |
|           |                                                 | Field study (Lakes)          | Germany         | 5              | 64               | 14              | 2  | (Brauns 2008)                   |
|           | <i>A. militaris</i>                             | Field study (Streams)        | USA             | 4.6±0.4        | 35.3±3.0         | 7.7±0.5         | 2  | (Evans-White and Lamberti 2005) |