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“Aquatic plant diversity in hardwater streams across global and local scales”

Julissa Tapia Grimaldo

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University of Glasgow in collaboration with the Centre for Ecology and Hydrology
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And God said, "Behold, I have given you every plant yielding seed which is upon the face of all the earth, and every tree with seed in its fruit; you shall have them for food. And to every beast of the earth, and to every bird of the air, and to everything that creeps on the earth, everything that has the breath of life, I have given every green plant for food." And it was so. (Gen 1:29-30).

ABSTRACT

“Aquatic plant diversity in hardwater streams across global and local scales”

The variety of life forms within a given species, ecosystem, biome or planet is known as biodiversity. Biodiversity can also be referred as species diversity and species richness. Understanding the drivers of biodiversity requires an understanding of intertwined biotic and abiotic factors, including climate patterns over the earth, primary productivity processes, e.g. photosynthetic pathways which change with climate and latitude; latitude, geology, soil science, ecology and behavioural science.

Diversity of living organisms is not evenly distributed; instead it differs significantly across the globe as well as within regions. The aim of my study is to try to understand the diversity patterns of aquatic plants, using both information derived from previous studies and by collecting new data across the globe, allowing me to examine the underlying mechanisms driving biodiversity at regional and local scales. Both geographical location and local environmental factors were found to contribute to variation in macrophyte assemblage and alpha diversity (i.e. number of species in a locality), with important roles being played by local biotic interactions and abiotic environmental factors.

Overall aquatic plants, or macrophytes, play a significant role in the ecology of large numbers of freshwater ecosystems worldwide. For the purpose of my study only calcareous steams, located in both temperate and tropical/subtropical regions were included. Such streams are common in catchments throughout the world because approximately one fifth of the earth’s surface is underlain by carbonate-containing rock.

Overall my findings in **Chapter 3** provide evidence that there is a high variation in macrophyte assemblages of calcareous rivers across the different countries included in my study, broadly agreeing with information from the literature. I found two large groups based on species assemblages across the different countries included, i.e. a subtropical/tropical and a temperate group. As demonstrated in different parts of **Chapter 4**, it is possible to identify different

diversity responses of macrophyte functional groups to environmental conditions, at local scale, in hardwater rivers. Width and flow were found to be significantly affecting the distribution patterns of diversity of free-floating and floating-leaved rooted species, whereas diversity of marginal species was significantly related to alkalinity and width, and floating-leaved rooted diversity was significantly related to alkalinity. Last but not least submerged species were related to shading. **Chapter 5** shows that variation in richness and community structure for hardwater river macrophytes can be partly explained by environmental variation relative to spatial processes in the British Isles (temperate scenario) and in Zambia (tropical scenario). Among the environmental variables, climatic ones explained a great part of species richness and composition distribution for the British Isles. Conversely in Zambia spatial processes made the greatest contribution to variation in hardwater river macrophyte species richness and community structure. Moreover **Chapter 6** illustrates how macrophyte species richness, measured as alpha-diversity in calcareous rivers, was at best only very weakly attributed to latitudinal gradient. This is most likely due to the effect of other physical, chemical and biotic variables overriding broader-scale influences on species richness, at more local scales.

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Totus Tuus

Author’s declaration

I confirm that the work presented in this thesis is my own work with the following exceptions.

Some part of the data been used in my study has been kindly given access by different people.

Chapter 4 includes STAR and MTR datasets that were provided by Matthew O’Hare.

Chapter 5 includes MTR dataset provided by Matthew O’Hare and some of the data included on the analysis for Zambia includes some sites from SAFRASS project, provided by Mike Kennedy and Steve Lowe.

Chapter 6 includes MTR dataset provided by Matthew O’Hare.

Chapter 1. The role of aquatic plants in their environment.

This introduction outlines the current understanding of plants living in calcareous streams (Figure 1-1) across the globe. Details of the chosen study will be given as well as setting out the main questions that will be addressed in my thesis.

1.1 THE IMPORTANCE OF AQUATIC PLANTS IN ECOSYSTEMS

The presence of plants diversifies the habitat within an ecosystem, by providing habitat and food resources to other organisms (Person and Crowder, 1998 Baattrup-Pedersen, 2006, Bouchard, 2007; Petr, 2000). Plants act as architects of their own habitat. The architecture or spatial complexity provided by plant species may incorporate a variety of microhabitats and as a consequence support a more diverse community (Petr, 2000).

Figure 1-1. Knockan Burn, in the Durness Limestone region of north-west Scotland: an example of a small calcareous stream.

Overall aquatic plants, or macrophytes, play a significant role in the ecology of very large numbers of freshwater ecosystems worldwide. Thus they are one of the groups of aquatic organisms used to establish ecological quality of freshwater systems in bioassessment programmes required by legislation in numerous countries of the world (e.g. within the European Union the EC Water Framework Directive (WFD) specifies that macrophytes, benthic algae, and benthic invertebrates are to be used as indicators of river and lake biointegrity: Baattrup-Pedersen et al., 2006). Macrophytes are referred to as one of the major components of freshwater environments because they help to maintain both biodiversity (Theel et al., 2008) and ecosystem functions (Bouchard et al., 2007). In freshwater stream ecology plants provide refuge from predators and adverse environmental conditions, e.g. flow and temperature (Moss et al. 1998; Allouche, 2002; Lambert and Sommer, 1998). Previous studies found marophyte cover to be positively related to fish abundance (e.g. Esox lucius) (Caffrey, 1993; Casselman, 1978). Another example involving fish showed cichlids habitat selection to be related to substrate type and submersed vegetation (Gamboa-Perez and Schmitter-Soto, 1999). In addition, aquatic plants were found to provide fish with spawning habitat (Allouche, 2002).

Despite their ecological importance relatively few studies have been undertaken to determine what environmental and anthropogenic influences act as drivers of the diversity of macrophyte communities.

1.2 MACROPHYTE DISTRIBUTION

Geographic patterns of species distribution are central to ecology (Currie, et al., 2004). In terms of global-scale latitudinal patterns, long-term studies of the terrestrial floras of tropical countries such as Panama, Costa Rica, Ecuador, have shown that biodiversity in tropical zones greatly exceeds that known from temperate regions (Crow, 1993). However, very little work has been done to examine such patterns in the context of aquatic vegetation. For instance Crow

(1993) investigated freshwater macrophytes in Costa Rica and showed that diversity in the tropical aquatic environments there was far lower than anticipated, at a level of freshwater plant species diversity equal to that found in temperate aquatic systems. More recently a study on the numbers and global distribution of vascular macrophytes (Chambers et al., 2008) has shown that though many species have broad ranges, macrophyte species diversity is highest in the Neotropics, intermediate in the Oriental, Nearctic and Afrotropics, lower in the Palearctic and Australasia, lower again in the Pacific Oceanic Islands, and lowest in the Antarctic region. Some 39% of the c. 412 genera containing aquatic vascular macrophytes were found by this study to be endemic to a single biogeographic region, with 61-64% of all aquatic vascular macrophytes found in the Afrotropics and Neotropics being endemic to those regions (Chambers et al. 2008). Apart from the studies by Chambers et al. (2008), Crow (1993), Baattrup-Pedersen et al. (2006); Rorslett (1991), and a new study, as yet unpublished which I know about, from Belgium (the latter four studies all being limited in geographical coverage) there has been nothing previously published on drivers of freshwater macrophyte diversity at a worldwide scale. My work hence makes a start to the task of establishing the importance of global versus local scale environmental drivers of macrophyte diversity, taking hardwater rivers as the target habitat type on a worldwide basis.

1.3 BIODIVERSITY OF AQUATIC MACROPHYTES ON A TAXONOMIC BASIS

One approach that has been used to evaluate diversity in relation to latitude is to compare regional aquatic-wetland floras on a taxonomic basis. Of interest is whether variations in aquatic plant assemblages reflect real latitudinal geographic patterns or whether regional distributions are just consequences of taxonomic variation. The only study that has attempted to investigate this in detail macrophytes is that of Crow (1993). As expected there are several groups of macrophytes that are better adapted to, or are characteristic of either tropical or temperate conditions. For instance aquatic plant families such as Podostemaceae, Nymphaeaceae, Limnocharitaceae, Hydrocharitaceae, Xyridaceae, Mayacaceae, Eriocaulaceae, Pontederiaceae, and the Old World Aponogetonaceae reveal the highest diversity in the tropics. In contrast the families of Potamogetonaceae, Hippuridaceae, Sparganiaceae, Juncaginaceae, Callitrichaceae, Elatinaceae, Haloragaceae and *Ranunculus* subgenus *Batrachium* of the Ranunculaceae show higher diversity in temperate regions (Tables 1 and 2 in Crow, 1993). Crow's (1993) findings concerning geographical patterns of aquatic plants based on taxonomic groups suggested an increase of macrophyte biodiversity in temperate regions, in other words a higher diversity of macrophytes at higher latitudes.

On the other hand the findings of Chambers et al. (2008) depict macrophyte species richness to be broadly inversely correlated with latitude. Their results showed a tendency to find higher diversity of macrophyte species in tropical areas than in temperate latitudes. The contradictory nature of previous studies may partly be based on the different approaches used, but this emphasises the need to look in more detail into the relative difference of spatial and local factors that may be driving macrophyte species diversity on a global scale.

1.4 BIODIVERSITY OF AQUATIC MACROPHYTES ON A HABITAT BASIS

My study examined the biodiversity question on a latitudinal basis using habitat comparisons and field studies to see if the macrophyte diversity of hardwater river habitats in the tropics parallels the richness of plant diversity observed in many tropical terrestrial habitats. For instance habitat area, water quality, altitude and trophic state have been found to be good quality predictors of macrophyte species richness in north European softwater lakes (Murphy, 2002). Rørslett, (1991) studied the determinants of macrophyte richness in Northern European lakes and found that both latitude and altitude were strong predictors of species richness, probably as a function of influencing the length of the growing season. Macrophyte growth rate decreased at higher altitudes (Rørslett and Hvoslef, 1986; Rørslett, 1989, 1991). Lake pH has also been found to be a principal determinant of macrophyte richness (Iversen, 1929; Rørslett, 1991).

The pH of natural waters generally correlates with a number of other factors such as conductivity, dissolved inorganic carbon, and macro-nutrients (Rørslett 1991). The effects of pH on macrophyte richness occur on a large regional scale, thus many sites are needed before this pattern can be observed from the background noise. This can explain some contrasting conclusions on pH species richness relationships obtained from more restricted surveys (e.g. Grahn, 1977; Roberts et al., 1985; Yan et al., 1985). A relationship between lake pH and fish species richness was found in Ontario lakes (Matuszek and Beggs 1988), and Rørslett (1991) obtained similar pH relationships between lake pH and macrophyte species richness, perhaps suggesting a more general importance of pH in influencing lacustrine species assemblage diversity. Where the observed species richness was closely related to the trophic state of the lakes, i.e. meso-eutrophic and eutrophic lakes supported significantly more species than did dystrophic or oligotrophic waters (Huston, 1979; Rorslett, 1991). Last but not least the variation with stream order (moving from small-sized streams to medium-sized streams in the mountains, or to lowland streams) is to be expected as an influence on species diversity, richness and community structure (Baattrup-Pedersen et al., 2006).

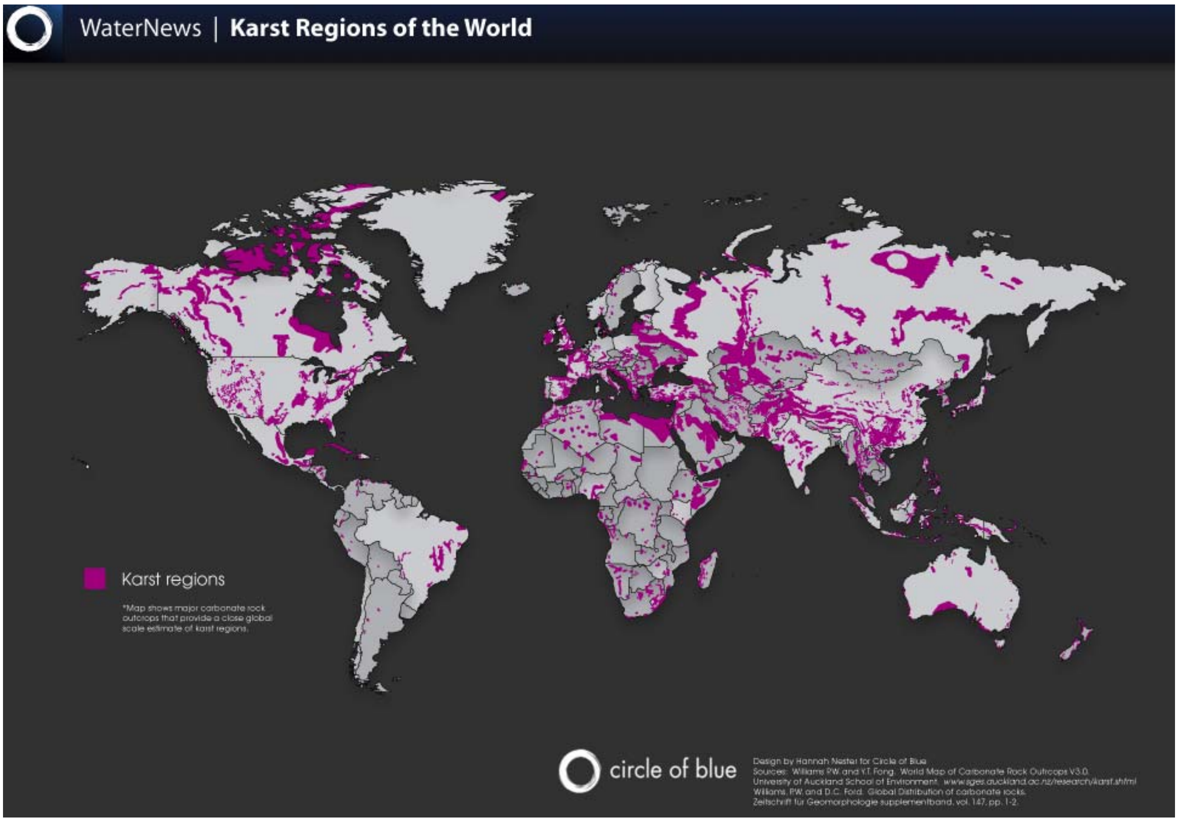
Based on factors known from previous studies to affect organisms living in freshwater ecosystem my study looks at a specific habitat type i.e. hardwater rivers and streams (and closely-associated riverine water bodies, including floodplain lagoons, oxbows, and other waterbodies which show close connectivity to the river system). For a study focusing on river vascular macrophytes (bryophyte and macroalgal diversity was not included here) such systems are ideal because they are well known to support macrophyte growth (e.g Haslam 1978).

Approximately one fifth of the earth's surface is underlain by carbonate rocks, which produced a diverse topographic feature by weathering under varied climate conditions (Lamoreaux, 1991) (Figure 1-1). Some karst terrains are covered by fertile soils, in others soils are missing. Carbonate rocks are a source of abundant minerals, water supplies and gas and oil. Rapid dynamic ecological changes within the karst are usual as a function of the synergistic relation between the solution of the rock and the circulation of water. The greater the solubility of the rock the faster the rates in changes in or progressive lowering of base levels, water tables, progressive cave enlargement and changes in karst topography may occur very soon (Lamoreaux, 1991). The most important property of an aquifer of karst system is its porosity and permeability within its three components: the matrix of permeability of the bedrock itself, the permeability due to conduits and the permeability produced by fractures. Limestone and dolomites are brittle rocks and affected by fracturing by tectonic forces and the stress relief caused by either glacier unloading or erosion (Lamoreaux, 1991). Moreover the hydrology of each karst drainage basin varies and is controlled mainly by the underlying stratigraphy and structure (e.g. the thickness of karstic rock units, detailed lithology (shaley limestone, crystalline limestone), bulk lithology (limestone, dolomite or gypsum) or other smaller fractures or large scale faults or folds (White, 2007).

I. Site selection

Sampling sites of (each approximately a 100m length) were selected from calcareous streams across the world from 3 different types of riverine floodplain water bodies:

- flowing channels (main river, distributary channels and tributaries);
- static to slow-flowing water channels;
- permanent lagoons, cenotes etc.: lentic but reasonably closely connected to the river channel (relatively few sites were used from this type of system).


WaterNews | Karst Regions of the World

*Map shows major carbonate rock outcrops that provide a close global scale estimate of karst regions.

Design by Hannah Nester for Circle of Blue
Sources: Williams P.W. and Y.T. Fong. World Map of Carbonate Rock Outcrops V3.0.
University of Auckland School of Environment. www.sges.auckland.ac.nz/research/karst.shtml
Williams, P.W. and D.C. Ford. Global Distribution of carbonate rocks.
Zeitschrift für Geomorphologie supplementband, vol. 147, pp. 1-2.

Figure 1-2. Karst regions across the world. (http://www.circleofblue.org/waternews/wp-content/uploads/2010/01/world-karst-map-web-1.12.jpg).

"Hardwater" is defined here as streams and rivers with a moderate to high concentration of dissolved calcium carbonate ($CaCO_3$). Calcium carbonate, a widespread constituent of many rock types, is almost insoluble in water, but it dissolves easily, as bicarbonate HCO_3^-, in carbonic acid, and it neutralizes the soil water where it occurs (Hynes, 1970). Spring water in limestone regions is often very rich in calcium bicarbonate where it emerges to the surface. As it flows downstream carbon dioxide (CO_2) will be lost through photosynthesis processes and to the atmosphere, therefore causing a loss in the equilibrium of CO_2 causing the deposition of calcium carbonate, which is a common feature of streams in limestone areas (Hynes, 1970).

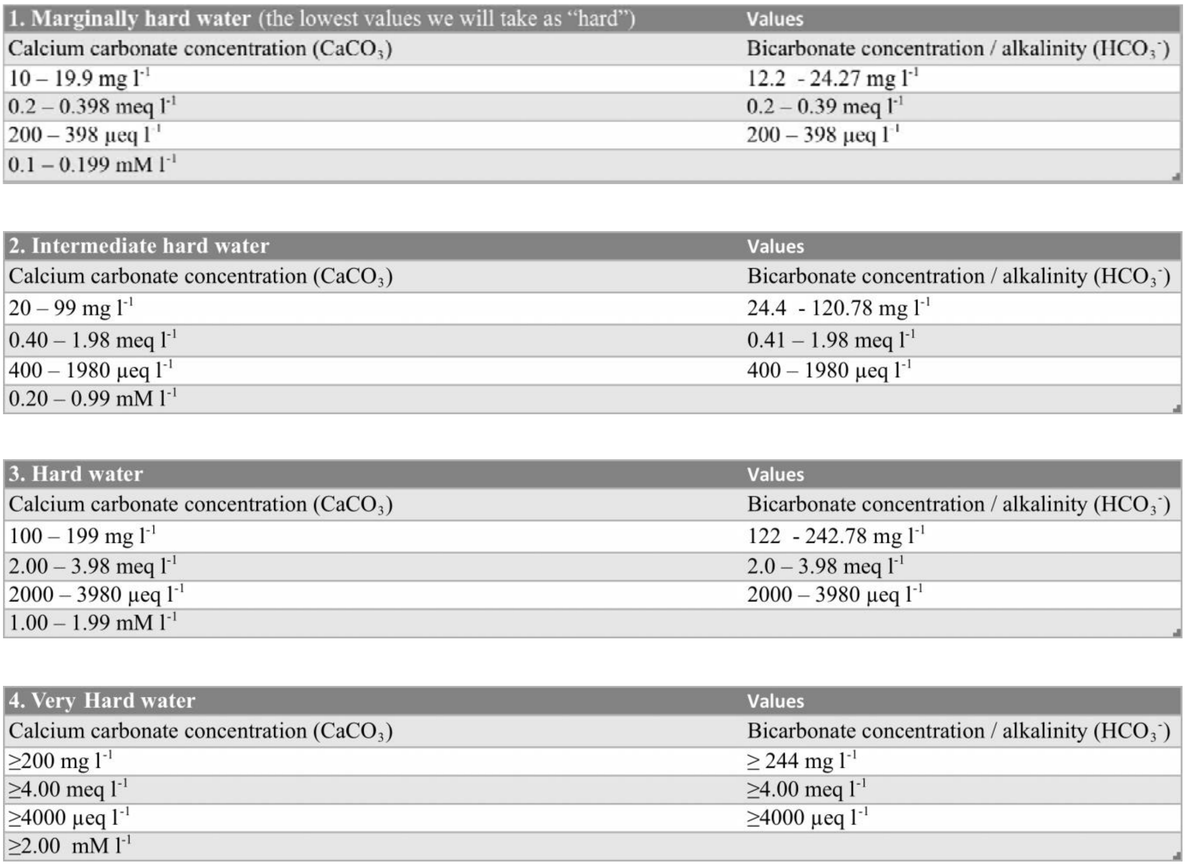
$$Ca\,CO_3 + H_2CO_3 \longrightarrow Ca(HCO_3)_2 \rightleftarrows Ca^{++} + 2HCO_3^-$$

In hard waters, especially those that are fed by limestone springs, deposits of calcium carbonate are often laid down. These can form large solid structures, which block up the stream, producing waterfalls or even raise the streambed above the level of the surrounding land (Haslam and Wolseley, 1981).

The alkalinity of the water (Neal, 2001) or some associated parameter such as pH or hardness, has often been considered to apply a considerable control on algal and macrophyte production (Hynes, 1970). This also has some implications on the performance of different species assemblages as there are some aquatic plants that are more suitable than those that are carbon-limited (i.e. cannot tolerate high concentrations of calcium and have life-strategies to uptake carbon from other sources like converting CO_2 from the atmosphere). Species distribution is related to their ability to use bicarbonate and extract inorganic carbon, however there is also an influence of phenotypic plasticity and local environmental heterogeneity in influencing this (Vestergaard and Sand-Jensen, 2000).

Butcher (1933) was the first to describe macrophyte assemblages typical of different hardness-status rivers in the UK (i.e. very slightly calcareous but alkaline rivers, through moderately calcareous, to highly calcareous rivers). Based on this and Ratcliffe (1977) I subdivided hardwater rivers into 4 categories of hardness (Table 1-1). Softwater rivers were not included in my study.

Table 1-1. Criteria for classifying sites into four categories of water hardness (based on Butcher, 1993 and Ratcliffe, 1977 classification of river hardness).



1. Marginally hard water (the lowest values we will take as "hard")	Values
Calcium carbonate concentration ($CaCO_3$)	Bicarbonate concentration / alkalinity (HCO_3^-)
10 – 19.9 mg l^{-1}	12.2 - 24.27 mg l^{-1}
0.2 – 0.398 meq l^{-1}	0.2 – 0.39 meq l^{-1}
200 – 398 μeq l^{-1}	200 – 398 μeq l^{-1}
0.1 – 0.199 mM l^{-1}	

2. Intermediate hard water	Values
Calcium carbonate concentration ($CaCO_3$)	Bicarbonate concentration / alkalinity (HCO_3^-)
20 – 99 mg l^{-1}	24.4 - 120.78 mg l^{-1}
0.40 – 1.98 meq l^{-1}	0.41 – 1.98 meq l^{-1}
400 – 1980 μeq l^{-1}	400 – 1980 μeq l^{-1}
0.20 – 0.99 mM l^{-1}	

3. Hard water	Values
Calcium carbonate concentration ($CaCO_3$)	Bicarbonate concentration / alkalinity (HCO_3^-)
100 – 199 mg l^{-1}	122 - 242.78 mg l^{-1}
2.00 – 3.98 meq l^{-1}	2.0 – 3.98 meq l^{-1}
2000 – 3980 μeq l^{-1}	2000 – 3980 μeq l^{-1}
1.00 – 1.99 mM l^{-1}	

4. Very Hard water	Values
Calcium carbonate concentration ($CaCO_3$)	Bicarbonate concentration / alkalinity (HCO_3^-)
≥200 mg l^{-1}	≥ 244 mg l^{-1}
≥4.00 meq l^{-1}	≥4.00 meq l^{-1}
≥4000 μeq l^{-1}	≥4000 μeq l^{-1}
≥2.00 mM l^{-1}	

1.5 PLANT ECOLOGY OF HARDWATER RIVERS

The drivers of variation in macrophyte species richness within the envelope of environmental conditions typical of hardwater streams and rivers (typified by high concentration of calcium, high alkalinity, and high water clarity; and supporting species-rich plant communities, which in turn play important ecosystem-support roles in such rivers) are poorly understood at local scale, let alone on a global basis. Latitudinal diversity gradients certainly exist in aquatic plant communities (e.g. Crow 1993), but their precise nature, and importance in relation to local-scale factors (including anthropogenic impacts such as eutrophication) remain inadequately known for this group of plants.

Major threats to the survival of hardwater stream vegetation include eutrophication (e.g. O'Hare et al., 2009; Lachavanne, 1985), acidification, and increased use of rivers for recreational purposes, or change of water flow for hydro-electric schemes. Additionally the possible impacts of global CO_2 increase might change the distribution of macrophyte assemblages causing loss of species sensitive to change in temperature, hydrology or dissolved inorganic carbon status soft water systems all likely to result from predicted climate change scenarios.

A major aim of my study was to build on existing knowledge, usually of geographically-limited extent, such as that summarised above, to determine how much variation in macrophyte richness and community composition can be explained by local environmental factors such as water conductivity, pH, water hardness, flow, shading and how much variation is determined by spatial factors associated with underlying latitudinal gradients. The work undertaken helps form a baseline of knowledge about the current worldwide status of hardwater river macrophyte diversity, its likely response to climate change, and the potential needs for future work in this area.

1.6 OVERALL AIMS

The overall aim of my project was to investigate the relative importance of global-scale (latitudinal) drivers, versus the impact of more local-scale environmental and anthropogenic drivers of freshwater vascular macrophyte diversity, specifically addressing one type of freshwater habitat, hardwater (calcareous) rivers, which are to be found in many different parts of the world, both tropical and temperate.

The specific objectives of the study were:

(1) To establish the geographical patterns of species and genus diversity in aquatic macrophyte taxa, emphasizing latitudinal relationships;

(2) To establish, and describe macrophyte assemblages which occur in different types of calcareous streams across the world, and to assess their variability in terms of a range of structural and ecological metrics within these types;

(3) to test hypotheses about the relative importance of latitude (as a global scale factor) and more local factors (such as altitude and water physico-chemistry variables) as predictors of hardwater river macrophyte diversity and assemblage.

CHAPTER 2. Methods

2.1 INTRODUCTION

The analyses presented in the results chapters of this thesis are based on field survey data collected using standard methods, which are presented here.

A combination of personally collected new survey data plus data from appropriate existing databases was used for this study. For example standard macrophyte surveys and supporting environmental data were available from the EC STAR project for Italy, Greece, Germany, UK, France, Latvia, Czech Republic, and Portugal.

The pre-existing data were supplemented by field work conducted during the three years of my PhD, at selected locations with calcareous rivers in the UK, northern Scotland; Yorkshire Dales (northern England) and abroad (including Zambia; Bonito, Upper Paraná, Pantanal and Chapadas regions of Brazil; northern and eastern Argentina; northern upland streams of Trinidad; northern Florida; western Ireland; Yucatan region of Mexico; and South Africa) which were surveyed in order to fill perceived gaps in the available data.

Owing to the relative lack of pre-existing data from field studies in calcareous rivers in tropical and sub-tropical areas, the data for such regions necessarily drew quite heavily on my own aquatic field work in such areas: e.g. Zambia, Mexico, Trinidad and Brazil.

This methods chapter covers site selection, sampling methods, and data processing and analysis techniques. Brief background data are provided for the regions sampled by myself and information is provided on the sources of pre-existing data.

2.2 SITE SELECTION

Sampling sites were selected from calcareous streams across the world, from three different types of riverine floodplain water bodies (Table 2-1):

- flowing channels (main river, distributary channels and tributaries);
- static to slow-flowing water channels associated with rivers (e.g. backwaters);
- permanent lagoons, oxbows, cenotes (sinkholes, produced from the collapse of limestone bedrock filled with groundwater derived from underground rivers) etc.: lentic but reasonably closely connected to the river channel (relatively few sites were used from this type of system).

The following criteria were used for site selection within these habitats:

- Degree to which sites filled known gaps in the pre-existing data;
- Presence of calcareous rock or soil types; (e.g. limestone, chalk, marine shell soil “machair” habitats, calcareous alluvial soils), within the catchment of the sites sampled;
- Accessibility and safety: ease of access and risks of dangerous wildlife (especially at African sites);
- All sites were located within 2-3 hours travel by car or boat, as appropriate, from base sites for individual survey areas, sampled within the different regions studied across the world.

Figure 2.1 illustrates the locations of data collected across the planet’s latitudinal gradient.

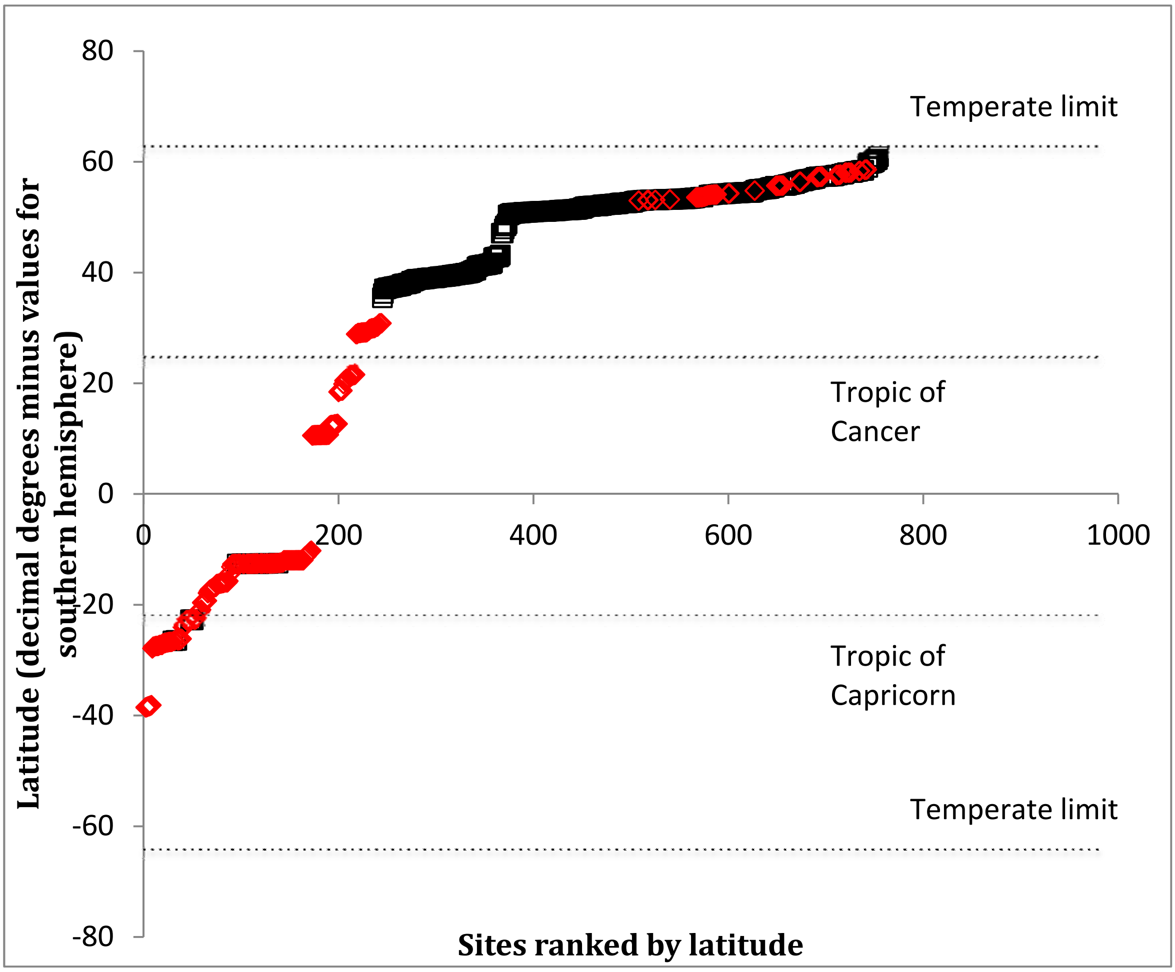


Figure 2-1. Sites ranked by latitude versus latitude. The circles in red represent personally-sampled sites, partly chosen to fill the gaps in pre-existing data.

Table 2-1. Number of river types based on their water flow and width values across all countries sampled.

	River flow (number of sites per country)			Width (m) (number of sites per country)			
Country	Still/slow	Moderate	Fast	<1	<10	<100	>100
Scotland	15	10	2	8	19		
England	3	2	5	1	6	3	
Ireland	4	10	3		9	8	
USA	16	9	2		3	23	1
México	18	1			3	1	14
Trinidad	2	7	9		18		
Zambia	47	38	18	13	40	39	11
Brazil	16	6	2	1	10	6	7
South Africa	7	6	4	10		3	1
Argentina	12	3	3		6	8	4
Total	140	92	48	33	114	91	38

The initial intention was to produce a complete dataset, which stretched between the two temperate latitudinal limits. Within the limits of the project however this was not entirely possible and gaps occurred in the northern tropics and the southern end of the temperate zone. The northern tropics, where calcareous rivers occur in both Africa and Central America are politically turbulent and difficult to sample. I did attempt to get both data and samples from Australia (in New South Wales and Tasmania) to cover the southern temperate zone but visa restrictions and time limitations made the trip impossible.

Below basic summary information on the different countries sampled is given.

Scotland

In Scotland I sampled rivers in two distinct types of calcareous areas: karstic limestone and machair (marine shell derived soils, part influenced by upland peat, occurring in coastal areas in north west Scotland) in this temperate region of the globe.

The karstic geology of Scotland is formed of limestone and to a lesser extent dolomite (magnesium-rich limestone). It is a small component of Scotland's landscape, found mainly in Durness, near Knockan and the Achmore plateau. The Durness dolomite covers from north to south of Assynt from Smoo Cave on the north coast, to Loch Slapin on Skye, and at Glen Creran. Disappearing underground flowing streams are a feature of parts of the Durness area, and one stream sampled (Knockan Burn) was of this type. Limestone also occurs elsewhere in Scotland, for example in Caithness, around Oban on the west coast of Argyll, and parts of the upper Clyde catchment, though usually in combination with other rock types.

Another unique and distinctive type of landscape in Scotland is machair, a low-lying fertile plain (soils derived from seashells, but also influenced by peaty upper catchment conditions, providing an unusual combination of soil and sediment conditions) with long ranges of sandy plains along the Atlantic coast of the Outer Hebrides allowing the formation of foredune, machair plain and transitions to saltmarsh and saline lagoons, calcareous lochs, acidic grasslands, and heath. This type of ecosystem is found only in the northwest and west coast of Ireland, and in the Outer Hebrides of Scotland, mainly on Barra, Uist and Tiree and provides a habitat with many small calcareous streams suitable for aquatic plants.

At the sites sampled in north-west Scotland (Table 2-2) land uses included small scale sheep farming and some housing around the area, mostly crofts, with streams mainly used for recreational purposes such as fishing (Figure 2-2). All these rivers were characterized mainly by slow-moderate flow, limestone rocks, or shell-sand substrates, with overall clear waters, allowing aquatic macrophytes to inhabit these waters. The sites in the upper Clyde catchment (Mouse, South Medwin) were in stream catchments draining sheep grazed farmland, or moorland. The Lonan sites had cattle grazing and coniferous forestry in the river catchment. The altitude range for the Scottish sites collected in this area was 6-300 m a.s.l., and pH range: 6.78 - 8.45. Width varied from <1m to <10m, mostly with no or little shade cover. Alkalinity was intermediate hard - hard water.

Table 2-2 Rivers surveyed in Scotland

River and site number	Latitude (decimal degrees North)	Longitude (decimal degrees West)	Altitude (m)
Mouse Water 1	55.7285	3.6944	300
Mouse Water 2	55.6777	3.6963	300
Mouse Water 3	55.7215	3.6788	300
1 South Medwin	55.7048	3.6788	264
2 South Medwin: Newholm Bridge	55.7147	3.4696	272
3 South Medwin	55.6828	3.5573	261
4 South Medwin: furthest d/s	55.6794	3.6222	242
1 Knockan Burn	58.0435	5.0145	226
4 KnocKan Burn	58.0516	5.0338	190
1 Croispol Burn u/s of loch c. 400m	58.5656	4.7676	65
2 Croispol Burn d/s	58.5753	4.7682	6
Siabost stream: Isle of Lewis	58.3316	6.6822	9
Morven stream, Isle of Lewis	58.372	6.5221	32
Berneray: Borgh stream	57.7146	7.191	6
North Uist: Loch Grogary stream outflow	57.6153	7.5122	8
North Uist: Leathbhal stream	57.6557	7.3437	3
North Uist: Machair Robach stream	57.66	7.2501	6
South Uist: Stilligarry stream	57.3229	7.3802	6
South Uist: Lòn Mòr stream	57.3275	7.3877	3
South Uist: Loch Olaidh Meadhanach outflow stream	57.2655	7.4012	4
South Uist: Loch Druidibeg outflow stream	57.3167	7.3183	9
South Uist: Bornish stream	57.2418	7.419	3
Oban: River Lonan	56.3993	5.3433	90
Oban River Lonan u/s	56.3994	5.3433	100
Urigill River: Na Luirgean	58.06093	4.99537	183
2 Knockan Burn	58.04670	5.01870	206
3 Knockan Burn	58.04720	5.02050	200

A) B)

Figure 2-2. Scottish sites: A) Knockan Burn on Durness limestone. B) South Uist: Bornish stream, on machair shell-sand.

England

The calcareous geology of England consists of southeast, west and central relatively low-lying upland limestone or chalk regions, together with some higher mountains which include limestone geology. In the southeast and southwest the hills are low and characterized by limestone or chalk river valleys. My sites were located in the Yorkshire Dales, which is a collection of river valleys draining east to the Vale of York, or westwards from the mountains of the main Pennine watershed in northern England. Other types of rocks present in this area are shale, sandstone and millstone grit. At the sites sampled (Table 2-3) land uses included small scale farming of sheep and cattle, plus some housing (villages and a small town). The water bodies were used for recreational fishing (Figure 2-3). All these rivers were characterized mainly by fast-moderate flow, limestone rocks, and overall clear waters, allowing aquatic macrophytes to inhabit these waters. Disappearing, underground-flowing, and re-appearing streams are common in the area and four of the sites were located on such streams. The altitude range for these sites collected in this area was 158 - 431 m a.s.l., with a pH range 7.39 - 8.32, conductivity 100 - 239 µS cm^{-1} and the width usually varied from <1m to <10m (one site was larger, at <100 m), mostly with no shade and with intermediate hard - hard water conditions.

Table 2-3 Rivers surveyed in England.

River and site number	Latitude (decimal degrees North)	Longitude (decimal degrees West)	Altitude (m)
Tongue Gill (tributary of River Ribble)	54.11343	2.250001	352
Inflow (minor stream) to Malham Tarn	54.09842	2.18448	431
Outflow stream from Malham Tarn	54.08811	2.16455	426
Gordale Beck: Malham	54.06897	2.13239	283
River Aire, Calton	54.02799	2.14763	211
River Aire, upstream of Gargrave	53.98044	2.12146	166
Kilnsey stream (Wharfe tributary)	54.103	2.03757	230
Bainbridge stream (near Hawes)	54.30008	2.18439	318
River Bain: Raydale	54.28502	2.1222	299
River Ure at Wensley	54.80109	1.84586	158

A)

B)

Figure 2-3. Yorkshire Dales sites (England): A) Tongue Gill. B) Inflow stream to Malham Tarn. Both on limestone.

Ireland

The geology of Ireland consists of a central lowland area, with extensive limestone, which is ringed by mountains of varied geology. In the south and west the mountains are characterized by limestone river valleys. My sites (Table 2-4) were located in the west of the country, near Galway and included the karstic limestone outcrop area of the Burren, internationally regarded as a botanical hotspot in the temperate region. In general the climate of Ireland is temperate, wet and oceanic providing mild growing conditions for a range of vegetation including aquatic macrophytes.

At the sites sampled land uses included small scale farming of sheep, a few households in the surrounding areas, ecotourism in some of the areas, and in terms of water usage some recreational fishing occurs (Figure 2-4). All these rivers were characterized mainly by fast - moderate flow, limestone rocks, overall clear waters, allowing aquatic macrophytes to inhabit these waters. The altitude range for these sites collected in this area was 71 -172 m a.s.l., with pH range 7.2 - 8.35, conductivity 73 - 481 µS cm^{-1} and the width varied from <10m to 100m, mostly with no to moderate shade and with hard - very hard water. As in Yorkshire, some sites were located on rivers which flow underground for part of their length.

Table 2-4 Rivers surveyed in Ireland

River and site number	Latitude (decimal degrees North)	Longitude (decimal degrees West)	Altitude (m)
Kilcolgun River tributary	53.21318	8.81671	79
Caher River 1	53.12434	9.26468	135
Caher River 2	53.10533	9.23553	172
Clare River at Kilcreevanty Br.	53.57503	8.91501	95
Tonmoyle Br. Clare tributary 1	53.58184	8.38962	100
Clare tributary 2	53.60476	8.84307	105
Sinking River: Cloonagh Br.	53.61861	8.84235	114
River Suck	53.77138	8.62331	132
Figh Br.: Lung River 1	53.85365	8.61069	130
Lung River 2	53.88516	8.56804	126
Ballychalan River	53.09902	8.75259	96
Beagh River: outflow from Lough Cotra	53.05666	8.78565	93
Castlelodge River	52.99545	8.89977	71
Marnagh River	53.05862	8.8905	77
Blach River	53.47966	9.46832	166
Robe River	53.66217	9.416047	89
Lough Mask inflow(N)	53.69494	9.31117	69

A) B)

Figure 2-4. Irish sites: A) River Suck. B) Blach River. Both on limestone.

Florida

The Florida Peninsula is a low-lying limestone peninsula, laid down many millions of years ago, when this part of the world was warm shallow sea, with deposits of small sea creatures building up to make a thick layer of limestone (as well as other calcareous deposits, such as gypsum - calcium sulphate dihydrate), which is up to several thousand meters thick. Geologically speaking Florida limestone is only 50-60 millions years old, to compare to other calcareous rocks in the USA, e.g. in Kentucky which has limestone about 430 million years old. At the sites sampled (Table 2.5) disturbance in the streams included recreational fishing and use of powerboats in the watercourses (Figure 2-5). The altitude range for the sites sampled in this area was 3 - 10 m a.s.l., with pH range 7.28 - 8.3, conductivity 127 - 3012 µS cm^{-1} (some sites had quite marked marine or brackish spring-fed saline influences) and the width varied from <10m to >100m, usually with no – moderate shade, and mostly water with intermediate hard conditions.

Table 2-5 Rivers sampled in United States (Florida)

River and site number	Latitude (decimal degrees North)	Longitude (decimal degrees West)	Altitude (m)
Rainbow Springs	29.4018	82.43753	3
KP Hole: Rainbow spring run	29.08718	82.4287	3
Three Sisters: Crystal River 1	28.88799	82.58962	3
Blue Springs	29.82975	82.68296	10
Santa Fe River	29.93292	82.80858	10
Manatee Springs	29.48917	82.97811	10
Silver River 2	29.20486	82.00375	10
Juniper Creek	29.18429	81.712	10
Fern Hammock	29.18438	81.70308	10
De Leon Springs	29.1349	81.86351	10
Alexander Springs 1	29.08177	81.57702	10
Alexander Springs 2	29.08102	81.566	10
Juniper Springs 2	29.8131	81.65468	10
Silver Glen	29.24532	81.643	10
Wacissa 1	30.83998	83.99145	10
Wacissa 2	30.82406	83.9872	10
Wacissa 3	30.22442	83.96933	10
Three Sisters: Crystal River 2	28.88215	82.59441	3
Ichetucknee 1	29.98399	82.76189	10
Ichetucknee 2	29.98061	82.75852	10
Silver River 1	29.20671	82.03058	10
Silver River 3	29.21114	81.99021	10
Wacissa 3	30.82797	83.9849	10
Wakulla Springs 1	30.23528	84.80138	10
Wakulla Springs 2	30.47597	84.24369	10
St Marks River	30.19932	84.17765	10
Ichetucknee 3	29.93257	82.80021	10

A) B)

Figure 2-5 Florida sites: A) St Marks. B) Juniper Springs. Both on limestone

Mexico

The cenotes of Yucatan are sinkholes forming the surface access to underground rivers (an extreme example of the karstic disappearing streams also encountered in Scotland, Yorkshire and Ireland), which are a prominent feature of the low-lying limestone geology of this tropical area, varying in size from small, to quite large systems. Though widely distributed across the landscape, many cenotes are particularly concentrated in an arc, to the south of the city of Mérida, which forms the landward perimeter of the giant Chicxulub impact feature produced by the Cretaceous “dinosaur-killer” meteor. Heavy showers, especially during the winter season, supply water which sinks through the permeable limestone to supply the underground rivers and their cenotes, which in turn provide a static to slow-flowing habitat (together with springs, riverine lagoons and a few surface rivers in the south of the region), and a relatively stable ecosystem for macrophytes. At the sites sampled (Table 2-6) disturbance caused in the waterbodies included recreational swimming with a few being heavily used for ecotourism and fishing purposes (Figure 2-6). In terms of land uses, there were small settlements and farms around the sites surveyed, with some sites being closer (within 100 km) to the ecotourism compounds, such as resorts and archaeological sites frequently visited by tourists. The altitude range for the sites sampled in this area was 1 – 27 m a.s.l., pH range 6.83 - 9.00, conductivity 415 - 4000 µS cm^{-1} (as in Florida some sites had pronounced coastal marine influence) and the width varied from <10m to >100m, usually with no – moderate shade, static to slow flowing, and mostly water with intermediate hard conditions.

Table 2-6 Sites sampled in Mexico, Yucatan

River/ cenote/ laguna name and site number	Latitude (decimal degrees North)	Longitude (decimal degrees West)	Altitude_m
Laguna de Coba	20.49414	87.73379	27
Laguna Macanxoc at Coba: near archaeological sites	20.48945	87.72769	26
Laguna Azul at Coba: near a cenote	20.6473	87.63448	23
Laguna Bacalar 1	18.68125	88.88406	3
Laguna Bacalar 2	18.67861	88.88725	3
Cenote Azul	18.64674	88.41324	3
Laguna Bacalar 3	18.6848	88.88526	3
El Palmar	18.44031	88.5273	3
Laguna Azul	19.87578	88.07871	3
El Zapotal: La Caña	21.86008	87.605	10
El Zapotal: Cenote	21.36939	87.60943	10
Laguna Tortugas	21.35332	87.6183	10
Agua da Abeja	21.34707	87.60628	10
San Felipe 1	21.56547	88.4793	1
San Felipe 2	21.48425	88.43049	2
San Felipe 3	21.47371	88.43102	3
Laguna Yalahau	20.65819	89.21879	19
Laguna Mosquito	20.853	90.26035	12

A) **B)**

Figure 2-6. Mexican sites: A) Laguna de Coba. B) Laguna Bacalar. Both on limestone

Trinidad

Located in the northern tropical region of Trinidad is a range of limestone hills running across the island, called the Northern Range. The range runs east - west at an average elevation of about 460m rising to 940m at Mount Aripo (El Cerro, del Aripo). The north range supports a large number of mountain streams while on the southern side of the range rivers run through foothills approximately 150m high, then descend to the low-lying Northern Plain.

The geological formations within this part of Trinidad consist mainly of sedimentary rocks such as schists and limestones while the eastern end of the range is of volcanic origin. At the sites sampled (Table 2-7) land uses surrounding the sampling streams included intensive and small crop farming (e.g. paddy rice fields, bananas, and some vegetables unique to the area) and forestry. Uses of streams include recreational fishing and swimming (Figure 2-7). The altitude range for these sites collected in this area was 4 – 180 m a.s.l., with pH range 6.49 - 8.28, conductivity 111 - 398 µS cm^{-1} and the width was mostly <10m. Most were fast flowing streams with moderate shade and mostly water with intermediate hard conditions.

Table 2-7 Rivers sampled in Trinidad

River name and site number	Latitude (decimal degrees North)	Longitude (decimal degrees West)	Altitude (m)
Aripo River	10.68576	61.22477	140
Arouca River tributary 1	10.63671	61.31666	10
Arouca River tributary 2	10.66148	61.33086	142
Arouca River 1	10.68862	61.825	73
Arouca River 2	10.710503	61	140
Arouca River 3	10.71473	61.82137	140
Arouca River 4	10.62412	61.83982	140
Arima River 1	10.68936	61.29093	40
Arima River 2	10.70013	61.28969	177
Plain stream	10.6386	61.81736	4
Quara River	10.61241	61.48948	8
Valencia River	10.65978	61.43157	180
River (unnamed): back water	10.66672	61.07206	42
River (unnamed)	10.66527	61.07256	8
Aripo Tributary 3	10.67991	61.22854	115
Aripo River 5	10.68891	61.22614	140
Cumaca River	10.65468	61.18079	104
Arima River 3	10.5767	61.82552	10

A) B)

Figure 2-7. Trinidad sites: A) Cumaca River.B) Arima River.

Zambia

Zambia is a tropical country mostly comprising relatively high altitude flat to gently rolling plateau, but with lower relief in the main river valleys of the Zambezi and its tributaries, in the more southerly parts of the country. There are extensive areas of riverine wetland, and a few relatively small lakes. Geologically the oldest rocks in Zambia are volcanic and granites with the inclusion of some sedimentary rocks such as calcite and carboniferous limestone. The Bangweulu Basin, which lies in the Congo catchment, in northern Zambia, with a general elevation of 158 m at the core, comprises many swamps, lakes, floodplains and flats, with 17 principal rivers flowing into the basin but only drained by the Luapula River (Symoens and Burgis, 1987). Climatic conditions in this part of the world vary in terms of rainfall, temperature and sunshine patterns as well as the quality of the soils. Sites within the southern valleys in and around the Zambezi River are characterized by a tropical climate, whereas the plateau in the north has more subtropical climate (because of its altitude), despite its proximity to the Equator. Sampling occurred during the period between 2008 – 2010 (some samples included were collected by me before the start of my PhD). Two sampling campaigns were carried out during the dry season (2008 and 2009), and one during the wet season (2010).

At the sites sampled (Table 2-8) land uses included small scale agriculture or unaltered bush. There is miombo forest burning (during the dry season) to control and avoid large fires during the summer. Watercourse usage was recreational, swimming, drinking water source and washing clothes, plus food preparation e.g. soaking cassava shoots. These rivers (plus some associated sinkhole and lake sites) were characterized mainly by slow - moderate flow, over calcareous rocks, which usually occurred in mixed geology catchments. The altitude range for these sites collected in this area was 1161 -1475 m a.s.l., with pH range 6.00 - 8.72, conductivity 12 - 507 µS cm^{-1} and the width varied from <10m to <100m, usually with no shade, and with intermediate hard - hard water conditions (Figure 2-8)..

Table 2-8. Rivers surveyed in Zambia

River and site number	Latitude (decimal degrees South)	Longitude (decimal degrees East)	Altitude (m)
Mulamba	17.88717	25.85909	901
Ngweze	17.55486	25.21376	951
Loanji (Tributary)	17.28507	24.66540	947
Machili	16.84221	25.11493	1102
Kalomo (Tributary)	16.98880	26.46459	1229
Bwenga (Tributary) 1	16.80875	26.96037	1305
Maamba River	17.23506	27.38632	520
Zinaza	17.05980	27.33320	890
Bwenga (Tributary) 2	16.78116	26.99362	1272
Munyeke River 1	16.26283	26.89906	1132
Munyeke River 2	16.08904	26.99233	1007
Kafue	15.94414	28.87635	373
Zambezi 1	15.93849	28.93860	360
Zambezi 2	15.94244	29.00674	370
Zambezi 3	15.94563	28.93142	372
Lusito 1	16.17856	28.75328	410
Lusito 2	16.23789	28.54224	464
Chongwe	15.70147	29.33167	358
Lusito 3	16.17850	28.83681	370
Zambezi 4	16.18979	28.83709	386
Zambezi 5	16.11072	28.85866	378
Makunka	16.05151	28.49882	675
Mulungushi	14.36360	28.63385	1105
Mkushi	14.37393	29.37051	1114
Kaombe 1	13.15423	30.70296	1524
Mulembo 1	12.53800	30.36639	1214
Mulaushi 1	12.55644	30.37620	1175
Luwombwa 1	12.50262	30.13149	1177
Kasanka 1	12.54075	30.21297	1175
Musola 10	12.59170	30.25194	1183
Mansa	11.19734	28.87369	1181
Mansa near school	11.20146	28.93551	1189
Luongo	10.70964	28.85094	1158
Luapula	10.57099	28.67515	953
Lupososhi	10.35560	29.48071	1243
Luososhi	10.21921	30.19875	1274
Chambeshi	10.92611	31.07715	1195
Kanchibia	11.49557	31.27983	1302
Musamfushi	12.45088	31.29500	1404
Mulaushi 2	12.59171	30.25195	1174

Musola 1	12.4754	30.14855	1166
Musola 2	12.502783	30.131583	1166
Musola 3	12.444	30.13158	1168
Mulembo 2	12.47845	30.1492	1166
Musola 4	12.669133	30.38271	1240
Luwombwa 2	12.6207	30.39395	1220
Luwombwa 3	12.5985166	30.39326	1212
Luwombwa 4	12.357	30.2312	1161
Lusenga	11.9512	30.2394	1160
Chitikilo	11.950183	30.2394	1160
Njelele	11.94905	30.2394	1160
Kasanka 2	11.946383	30.23746	1160
Kasanka 3	11.94556	30.23695	1160
Lukulu 1	11.941616	30.23348	1160
Lukulu 2	11.93905	30.2313333	1160
Lukulu 3	11.93985	30.229683	1160
Lukulu 4	11.953316	30.2465	1160
Lukulu 5	11.954516	30.248	1160
Lukulu 6	11.9545166	30.2499	1195
Lukulu 7	11.95486	30.25436	1184
Lukulu 8	11.954116	30.2468	1170
Lukulu 9	11.954116	30.24398	1105
Lukulu 10	11.94485	30.233866	1167
Lukulu 11	11.95995	30.25598	1183
Lukulu 12	11.95976	30.2557	1166
Lukulu 13	11.96005	30.2551	1167
Lukulu 14	11.958533	30.25413	1167
Lukulu 15	11.95766666	30.2542	1167
Lukulu 16	11.95545	30.253983	1161
Lukulu 17	12.363983	30.60518	1162
Lukulu 18	12.516216	30.60303	1160
Lukulu 19	12.6	30.248433	1160
Lukulu 20	12.587116	30.2391	1170
Lukulu 21	12.587116	30.24815	1175
Lulimala	12.5999666	30.37846	1209
Lake Wakawaka	12.50946667	30.2879	1204
Chilengwa na Lese sinkhole	12.5473	30.37838	1190
Musola 5	12.54773	30.37691	1190
Kapabi	12.54853333	30.37715	1175
Mulaushi 3	12.549116	30.37668	1166
Mulembo	12.57261	30.2312	1176
Mulembo	12.57238	30.233483	1164
Lulimala	12.574	30.2129	1196
Kaombe 2	12.53936	30.216	1158
Mufubushi	12.60653	30.2197	1221

Lukulu	12.64865	30.183816	1192
Mulembo 5	12.6207	30.39395	1475
Mulaushi 4	12.598516	30.39326	1420
Mulembo 6	12.55636	30.376083	1234
Musola 6	12.633666	30.27768	1231
Musola 7	12.59128	30.256866	1158
Musola 8	12.59168	30.25193	1160
Mulaushi 5	12.66193	30.244583	1160
Musola 9	12.47976	30.1904	1160
Kasanka 4	12.47855	30.18995	1160
Kasanka 5	12.54283	30.39091	1160
Kasanka 6	12.3556	30.605516	1160
Lusenga	12.6691	30.3827	1160
Luwombwa 5	12.4754	30.1485	1160
Luwombwa 6	12.5027	30.1315	1160

A) B)

Figure 2-8. Zambian sites: A) Musola River. B) Kaombe River: upstream of Kundalila Falls). Both on limestone.

Brazil

Brazil has one of the world's most extensive river systems with eight major drainage basins, all of which drain into the Atlantic Ocean. The basement of the South American Platform is formed out of metamorphic rocks, schist facies, sedimentary rocks and volcanic coverings. I collected samples from four calcareous areas. Chapada Diamantina (State of Bahia in northern Brazil), is mainly occupied by thick horizontal beds of clays and clayey sandstones, with soft deposits where the streams cut down through them to the harder and older limestone and other rocks below, making deep narrow valleys or cañons. Bonito (State of Mato Grosso do Sul in Central West of Brazil) is characterized by its extensive quantity of limestone where some of the rivers have the clearest and most transparent freshwaters (of blue - turquoise colour) to be found anywhere in the world. I also collected samples from rivers flowing through the nearby southern Pantanal wetland region. The Paraná River is formed by the union of the Grande and Paraníba rivers. It is the tenth longest river in the world (4,695 km) with a habitat type characterized by large river headwaters flowing through calcareous soft deposits, clay and sandstones. The Upper Paraná (States of Mato Grosso do Sul and State of Paraná in the Central South of Brazil) covers the first third of the Paraná River Basin where spectacular waterfalls and outstanding levels of freshwater biodiversity are found. The climate in this region is tropical/subtropical with annual average temperature of 15ºC.

At the sites sampled (Table 2-9) land uses included small-and often large scale farming of food crops (e.g. large fields of sugar cane), cattle, and horse grazing. Large ecotourism developments, where watercourses are mainly used for recreational purposes such as fishing, boating and swimming characterize Bonito. All four areas sustain a high biodiversity of birds, and aquatic mammals such as giant otters and capybaras. In the larger river systems (e.g. Paraná River) powerboats, large commercial shipping and fishing boats are to be found (Figure 2-9). The altitude range for these sites collected in this area was 78 - 400 m a.s.l., with pH range 7 - 7.95, conductivity 24 - 591 µS cm^{-1}, and the width varied from <10 to >100m, usually with no – moderate shade, and mostly water with intermediate hard to very hard water conditions.

Table 2-9 Rivers sampled in Brazil

River name and site number	Latitude (decimal degrees South)	Longitude (decimal degrees West)	Altitude (m)
Paraná River (main channel) Guaira	23.85909	54.03023	224
Lagoa Xambre (Guaira)	23.88412	54.00481	222
Pao Velho backwater (Porto Rico)	22.74915	53.25976	225
Lagoa São João (Guaira)	23.81251	53.99789	219
Ressaco Leopoldo (Porto Rico)	22.5559	53.26803	232
Baía River downstream	22.69225	53.22733	229
Baía River upstream	22.6636	53.20582	226
Santa Rosa (Porto Rico)	22.77262	53.30443	220
Ressaco do Manezinho (Porto Rico)	22.7795	53.34982	226
Ressaco do Valdo (Porto Rico)	22.76359	53.29441	225
Rio Formoso 2: Balnearias Municipal (Bonito)	21.17123	56.44658	277
Rio Formoso 1: Cabanas (Bonito)	21.17486	56.44861	279
Rio Bonito (Bonito)	20.89606	56.52877	400
Rio Sucuri (Bonito)	21.26635	56.55954	292
Rio da Plata (Bonito)	21.4384	56.44521	229
Rio Miranda: lagoon (Pantanal)	19.61025	56.98637	78
Rio Miranda: main channel (Pantanal)	19.58388	56.99014	86
Corixao: distributary of R. Miranda (Pantanal)	19.53697	57.05233	80
Rio Vermelho: vazante (secondary channel) of Vermelho (tributary of R. Miranda) (Pantanal)	19.62321	56.96017	80
Rio Negro: main channel, Bridge 61, km57.480 (Pantanal)	19.26061	57.18233	91
Santo Antonio: main channel (tributary of Paraguacu): Chapada Diamantina	12.4	41.2	224
Santo Antonio: secondary channel (tributary of Paraguacu): Chapada Diamantina	12.4	41.2	224
Lagoa Saraiva (Guaira)	24.0016	54.10866	217
Rio Paraguay: main channel Porto da Manga (Pantanal)	19.25982	57.23302	90

A) B)

Figure 2-9. Brazilian rivers: A) Bonito on limestone B) Pantanal, with capybaras on calcareous soft deposits.

South Africa

The northern part of South Africa, which I visited to obtain samples is subtropical and forms a quite high altitude plateau (the High Veld) rising to the south to the Drakensberg (Afrikaans: “Dragon Mountains”), which is the highest mountain range in South Africa rising to 3,482 m. The underlying geology is covered with sedimentary rock formations with layers of solid basalt. The Lower Vaal area is underlain by the Transvaal super group consisting of dolomite and subordinate limestone (DWAF, 2004). The high rainfall in this area generates many mountain streams and rivers, including the sources of the Orange River, southern Africa’s longest and the Tugela River.

At the sites sampled (Table 2-10) land uses included semi-intensive food crops and mining activities (Figure 2-10). The altitude range was 1343 – 1483 m a.s.l., with pH range 7.24 - 8.61, conductivity 239 - 1932 µS cm^{-1}, and the width varied from <10m to >100m, usually with no - moderate shade, and mostly water with intermediate hard conditions.

Table 2-10 Rivers sampled in South Africa

River name	Latitude (decimal degrees South)	Longitude (decimal degrees East)	Altitude_m
Goedspruit 1	26.70745	27.07775	1377
Goedspruit downstream 2	26.701	27.10293	1360
Mooi river 1	26.68469	27.10027	1362
Mooi downstream 2	26.75798	27.09828	1343
Mooi Source 3	26.14215	27.15136	1505
Wonderfontein	26.25285	27.1597	1479
Mooi dam 4	26.51449	27.12451	1427
Mooi river 5	26.97082	27.20976	1345
Mooi river 6	26.82919	27.17207	1388
Vaal River: Schoenansdrift 1	26.39547	26.9153	1459
Vaal River: Parys 2	26.41394	26.7924	1432
Rooihaaskraal River	26.67941	26.58326	1359
Rietspruit River	26.94003	25.92336	1483
Rietspruit River site 2	26.36711	27.27076	1482
Schoenspruit River	26.4451	27.11831	1434
R507	26.905255	27.4433	1397
Bamboesspruit River	26.79784	26.3748	1466
Mooi 7	26.4447	27.1197	1422

A) B)

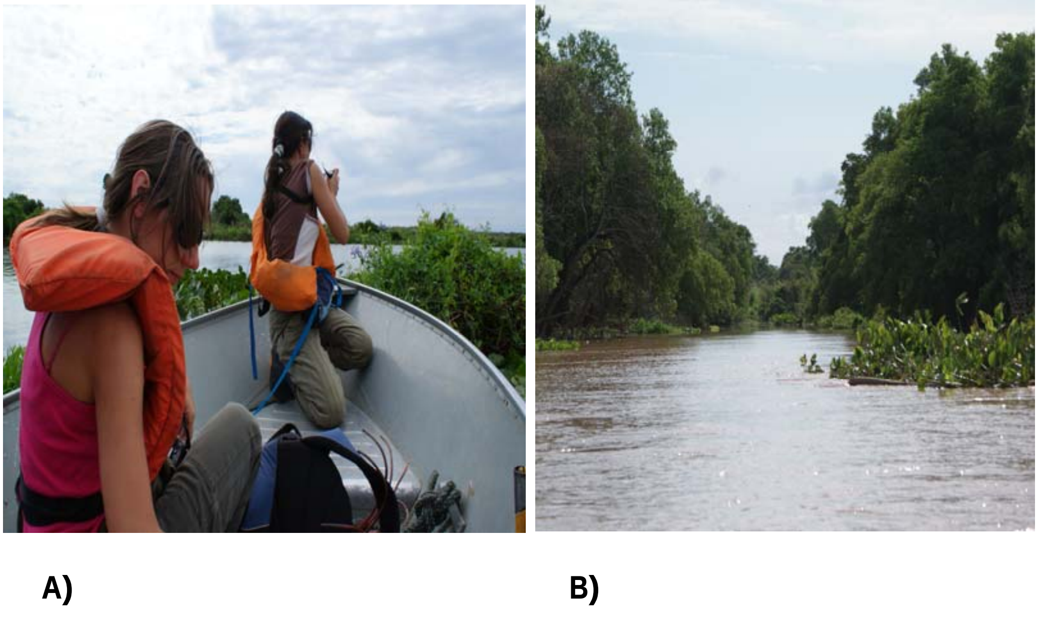
Figure 2-10. South African site and landscape:A) Vaal River Schoenansdrift. B) Limestone bedrock outcrop. Dry season.

Argentina

The basement of the South American Platform is formed out of metamorphic rocks, schist facies, sedimentary rocks and volcanic coverings. I surveyed rivers in two regions, both on calcareous alluvial soils. The first was within the Río de la Plata system, the largest river basin in northern Argentina, draining the whole of Paraguay, eastern Bolivia, most of Uruguay and a large part of Brazil. The second was a small river catchment draining the low hills in the southern part of the pampas region (Province of Buenos Aires), and flowing direct to the Atlantic. At the sites sampled (Table 2-11) land uses included intensive food crop farming (e.g. maize, cane sugar), and cattle rearing. In terms of usage of water streams, recreational fishing and usage of power boats were the main ones in the northern streams, with no apparent recreational use in the pampas stream system (Figure 2-11). The altitude range was 61 - 265 m a.s.l., with pH range 6.66 - 8.15, conductivity 56 - 928 μScm^{-1}, and the width varied from <10m to >100m, usually with no shade, slow-moderate flow, and mostly water with intermediate hard conditions.

Table 2-11 Rivers sampled in Argentina

River name and site number	Latitude (decimal degrees South)	Longitude (decimal degrees West)	Altitude (m)
Rio Negro 1	27.45996	58.91046	61
Rio Paraguay 1	27.2449	58.5811	66
El Divisorio	38.33787	61.60524	227
R. Sauce Grande	38.48615	61.7853	130
Cementeria: R. Sauce Grande	38.20108	61.75836	228
R. Negro affluent to the R. Sauce Grande	38.12795	61.7634	265
R. Zorro affluent to the R. Sauce Gde	38.28501	61.67835	222
El Divisorio downstream	38.40074	61.65678	180
Naposta Chica	38.53806	61.87571	149
Riachuelo	27.55318	58.75100	73
Riachuelo	27.55447	58.75034	73
Empedrado	27.86686	58.76300	66
Tragadero, Chaco	27.42809	58.87043	62
Rio Negro 2	27.42030	59.00601	76
Rio Negro 3	27.43691	58.98000	66
Rio Paraguay 2	27.23940	58.58123	66
Rio Paraguay 3	27.23610	58.58439	66
Rio Paraguay 4	27.28572	58.60564	66



A) B)

Figure 2-11. Argentine sites: A) and B) Rio Paraguay backwaters.

2.3 VEGETATION

2.3.1 SAMPLING METHOD

A standard 100 m length of river was used, sometimes with >1 sampling stretch per river, to provide a standardized quantitative dataset to determine inter-river variation in macrophyte diversity in response to both local and larger-scale drivers. Macrophytes were surveyed at my personally-sampled sites using an adapted version of the Mean Trophic Rank (MTR) field protocol developed in the United Kingdom (Holmes et al., 1999). The European Water Framework Directive (WFD) (Furse, 2006) included macrophytes as one of the major groups of organisms upon which an assessment should be made for the protection of surface waters. It was therefore important to know the reliability of the metrics and indices they provided. Staniszewski et al. (2006) tested the efficiency and precision of the MTR sampling method. They found MTR to be useful for estimating the ecological status of compiled rivers by the WFD.

The MTR survey procedure is based on the presence and abundance of species of aquatic macrophyte. The abundance of species is usually measured on a 5 point scale at each sampling point. MTR uses a 5 point and 9 point scale, based on a 100m sample reach subdivided into 5 equal subsections (O'Hare pers comm). As a variation on this, I recorded presence and absence of species at 5 random sampling points within the survey site, and then used the resulting score ("hits out of 5") to calculate a percentage frequency (%F) value for each species present at each site. At sites where it was safe to do so, the full survey length and channel width was surveyed by wading. At those sites that were not safe, where it was too deep to wade, or in the case of African sites, dangerous wildlife were present, then macrophyte records were made of those species that could be seen clearly walking along the bank and using a grapnel to access submerged and floating species, as necessary. Both techniques are allowable under the MTR methodology. On larger rivers (Paraguay, Paraná, Zambezi, Pantanal streams, and some Florida rivers) sampling was from powerboats, and in the Lukulu delta system in Zambia, and Chapada Diamantina in Brazil from hand-propelled boats).

Streams that were assessed by wading were done in a zigzag manner across the channel to try to incorporate all habitat types present as shown in Figure 2-12.

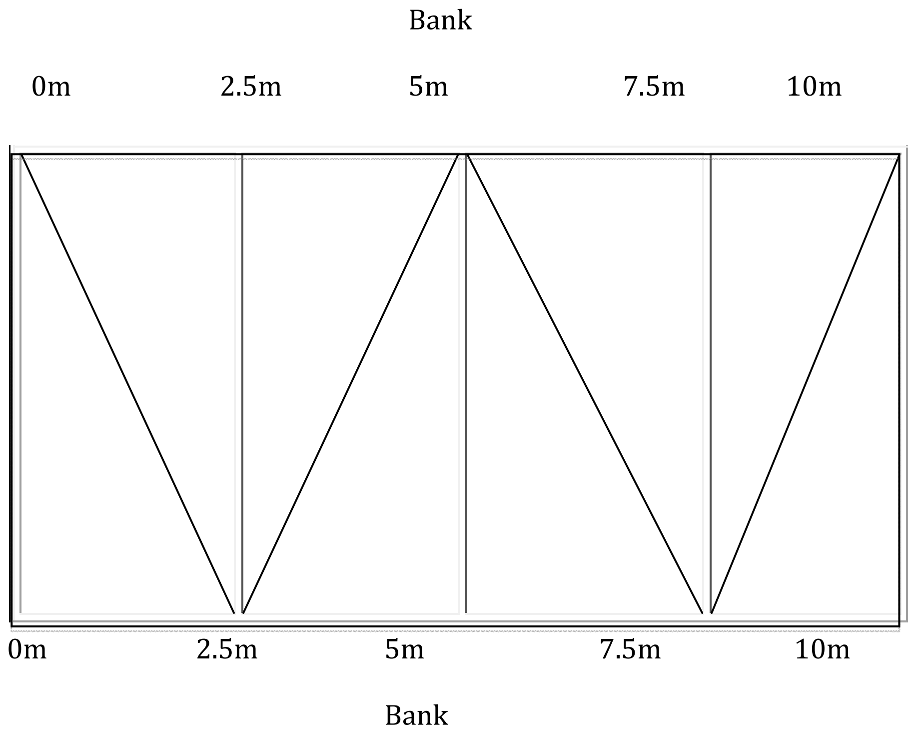


Figure 2-12. Diagrammatic representation of survey method (after Holmes, 1999).

2.3.2 TAXONOMY

The definition of a freshwater macrophyte is a plant that has its functional photosynthetic structures below or on the surface of a freshwater body (i.e. submerged and floating), or above the water surface (i.e. emergent) for at least 50% of the year (Chambers et al. 2008). Vascular aquatic plants present at each site and meeting the above definition were listed. Identification guides were used where appropriate (e.g. Cook, 2004; Haslam etal.1982; Spencer-Jones and Wade, 1986; Biggs 1996; Pott and Pott, 2000).

Identification was an issue in some regions (notably Mexico and Trinidad), where appropriate ID resources are very limited for macrophytes. The allocation of a name to some species was given at the family level when known, and by adding the name of the site it was collected at, for future reference. If there was no clear identification it was recorded as an unknown species and a code was created with a ? mark followed by its physical description and/ or by the code of the site where it was found. Table 2-11 shows the level of taxonomic resolution for each country sampled.

Table 2-12. Taxonomic resolution of species identification per country sampled.

Country	Number of species identified	Number of genera identified	Number of "unidentified species" codes
Scotland	63 (100%)	41 (100%)	0
England	22 (100%)	18 (100%)	0
Ireland	63 (100%)	44 (100%)	0
Florida	76 (92%)	54 (90%)	6
Yucatan	74 (47%)	27 (37%)	37
Trinidad	44 (40%)	21 (52%)	20
Zambia	80 (80%)	70 (95%)	4
Brazil	53 (96%)	36 (98%)	1
South Africa	60 (83%)	34 (96%)	2
Argentina	50 (96%)	33 (98%)	5

2.4 WATER PHYSICO-CHEMISTRY

All on-site measurements were taken during morning to early afternoon. At each site measurement was made of water pH and conductivity (μS cm^{-1}) (using a Schott Handylab pH 11/12 meter. Conductivity, which estimates the amount of total dissolved ions in the water, in streams and rivers is affected mainly by the geology of the region through which the water flows under natural conditions. Rain and rocks give most of the inorganic substances that reach fresh water (Gibbs, 1970). Waters flowing through igneous rocks (e.g. granite) tend to have lower conductivity due to the presence of inert minerals, in the order Na>Mg>Ca>K when cations present in the rain are included, which do not dissolve into ionic components when washed into the water. Conversely streams running through sedimentary rocks (e.g. limestone) are often porous, with larger surface for water to permeate and have binding materials that are usually soluble and easily weathered e.g. sulphate, carbonate and phosphate and high concentration of calcium carbonate (i.e. from shells of marine organisms) especially in the limestone and chalks. Moreover calcium and bicarbonate ions are released from this type of rocks by the acids in the rain, so that the flowing waters are neutral or alkaline (Moss 1998). The link between the ions available in the waters and soil, of a particular catchment and the organisms living in it, determines to an extent the productivity in the system. For instance phosphates (PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$), which are only soluble in neutral pH waters, are key nutrients in addition to nitrates, bicarbonate, and, in much smaller quantities, the minor nutrients, such as molybdenum, for many organisms including plants (Moss, 1998). pH of water is a measure of the concentration of hydrogen ions, that determines the solubility and biological availability of nutrients (e.g. phosphorus) and heavy metals, and also strongly influences the dissolved carbon equilibrium, influencing forms of C available for submerged photosynthesis.

Latitude and longitude positions, and altitude, were obtained with a Garmin GPS. Underwater light was measured with a single sensor SKYE SKP210 PAR system recording photosynthetically active radiation (PAR) (μE m^{-2} s^{-1}) just below the water line (0 m) and at a recorded depth (usually 20cm) sub-surface, and the values used to calculate underwater light attenuation coefficient (*k* m^{-1}) as an indicator of water clarity. All measurements were made without disturbing sediment.

Additionally water flow was assessed visually at each site in 3 categories 1 = slow (0 to circa 0.2 m s^{-1}), 2 = moderate (0.21 to circa 0.4 m s^{-1}), 3 (> 0.4 m s^{-1})= fast flow. River width was assessed on a scale of < 1m, >10 m (in some locations), <100 m, and > 100m.

One water sample was collected at each site (in an undisturbed sediment area) and taken back to the laboratory to measure alkalinity, using the Gran alkalinity titration method with the use of Alcagran software (Neal, 2001).

2.5 PRE-EXISTING DATA

The EU funded research project STAR (Standardisation of River Classifications) calibrated different biological survey outputs versus ecological quality classification for a number of EU countries (Furse et al., 2006). Macrophytes were surveyed for this study using a slightly adapted version of the MTR, carrying out most of the surveys between mid-June and mid-September after several days of low flow or low-normal flow.

For rivers considered in the STAR project, the WFD defined typology on the basis of ecoregions, the catchment area, catchment geology and altitude. Within a specific typology, it assumes that the biological communities, such as macrophytes, diatoms, fish and macroinvertebrates at almost zero disturbances would create a type-specific biological target and a measure of spatial variability in stream and river monitoring. For the STAR project a total of 233 sites were fully sampled. The dataset covers 13 countries and includes 22 stream types reflecting the three types of landscapes in Europe: Mountains, Lowlands and Mediterranean (Furse et al., 2006).

2.6 SAMPLING EFFORT

Awareness should be given to the limitations faced when doing a study of this magnitude in terms of sampling effort across different studies.

- There is not enough data for all the countries;
- Standardization of approach in sampling effort for other studies is outwith my control;
- Calcareous streams although widespread in some regions are less common in others, so inevitably there is a difference in availability of potential sites for sampling.

Table 2-13. List of the different countries included for Chapter 3. To compare different geographical locations versus presence and absence of species.

Chapter 2Country	Chapter 3Number of sites per country	Chapter 4Source of data
Chapter 5Scotland	Chapter 627	Chapter 7personally sampled
Chapter 8Ireland	Chapter 917	Chapter 10personally sampled
Chapter 11South Africa	Chapter 1217	Chapter 13personally sampled
Chapter 14Zambia	Chapter 1568	Chapter 16personally sampled
Chapter 17Trinidad	Chapter 1818	Chapter 19personally sampled
Chapter 20Florida	Chapter 2127	Chapter 22personally sampled
Chapter 23Mexico	Chapter 2418	Chapter 25personally sampled
Chapter 26Brazil	Chapter 2713	Chapter 28personally sampled
Chapter 29Argentina	Chapter 3013	Chapter 31personally sampled
Chapter 32UK	Chapter 3311	Chapter 34personally sampled
Chapter 35Portugal	Chapter 36100	Chapter 37Teresa Ferreira
Chapter 38UK	Chapter 3921	Chapter 40Matthew O'Hare, STAR
Chapter 41Germany	Chapter 425	Chapter 43Matthew O'Hare, STAR
Chapter 44Latvia	Chapter 4519	Chapter 46Matthew O'Hare, STAR
Chapter 47Sweden	Chapter 4812	Chapter 49Matthew O'Hare, STAR
Chapter 50Italy	Chapter 518	Chapter 52Matthew O'Hare, STAR
Chapter 53Denmark	Chapter 5412	Chapter 55Matthew O'Hare, STAR
Chapter 56France	Chapter 576	Chapter 58Matthew O'Hare, STAR
Chapter 59Greece	Chapter 6014	Chapter 61Matthew O'Hare, STAR
Chapter 62British Isles	Chapter 63213	Chapter 64Matthew O'Hare, MTR
Chapter 65England	Chapter 6642	Chapter 67Andrew Spink
Chapter 68Ireland	Chapter 6950	Chapter 70Joe Caffrey
Chapter 71Zambia	Chapter 7211	Chapter 73Sean Morrison
Chapter 74Brazil	Chapter 754	Chapter 76Roger Mormul
Chapter 77France	Chapter 781	Chapter 79Carbiener

Table 2-14. List of countries included for data analysis for Chapter 4. Selection of sites based on sites containing width category, water flow, shade and alkalinity data with presence and absence of species.

Chapter 80Country	Chapter 81Number of sites per country	Chapter 82Source of data
Chapter 83Scotland	Chapter 8426	Chapter 85personally sampled
Chapter 86Argentina	Chapter 8718	Chapter 88personally sampled
Chapter 89Brazil	Chapter 9024	Chapter 91personally sampled
Chapter 92England	Chapter 9310	Chapter 94personally sampled
Chapter 95Ireland	Chapter 9614	Chapter 97personally sampled
Chapter 98Mexico	Chapter 9918	Chapter 100personally sampled
Chapter 101South Africa	Chapter 10217	Chapter 103personally sampled
Chapter 104Trinidad	Chapter 10517	Chapter 106personally sampled
Chapter 107USA	Chapter 10828	Chapter 109personally sampled
Chapter 110Zambia	Chapter 111102	Chapter 112personally sampled and Michael Kennedy

Table 2-15. List of countries included for data analysis for Chapter 5. Selection based on large dataset availability.

Chapter 113Country	Chapter 114Number of sites per country	Chapter 115Source of data
Chapter 116British Isles	Chapter 1171025	Chapter 118Matthew O'Hare
Chapter 119Zambia	Chapter 120167	Chapter 121Personally sampled and Michael Kennedy

Table 2-16. List of countries included for data analysis for Chapter 6. Selection of sites caracterized by width category <10m, slow to moderate flow conditions with no shading at different latitudes with presence and absence of species.

Chapter 122Country	Chapter 123Number of sites per country	Chapter 124Source of data
Chapter 125Argentina	Chapter 1266	Chapter 127personally sampled
Chapter 128Brazil	Chapter 12910	Chapter 130personally sampled
Chapter 131Denmark	Chapter 13212	Chapter 133Matthew O'Hare, STAR
Chapter 134Greece	Chapter 13513	Chapter 136Matthew O'Hare, STAR
Chapter 137Ireland	Chapter 13810	Chapter 139personally sampled
Chapter 140Italy	Chapter 1418	Chapter 142Matthew O'Hare, STAR
Chapter 143Latvia	Chapter 14419	Chapter 145Matthew O'Hare, STAR
Chapter 146Mexico	Chapter 1473	Chapter 148personally sampled
Chapter 149Portugal	Chapter 15037	Chapter 151Teresa Ferreira
Chapter 152Scotland	Chapter 15310	Chapter 154personally sampled
Chapter 155South Africa	Chapter 1566	Chapter 157personally sampled
Chapter 158Trinidad	Chapter 15916	Chapter 160personally sampled
Chapter 161United Kingdom	Chapter 16260	Chapter 163Mattie O'Hare, MTR
Chapter 164USA	Chapter 1653	Chapter 166personally sampled
Chapter 167Zambia	Chapter 16831	Chapter 169personally sampled and Michael Kennedy

Table 2-17. List of countries included for the second large data analysis for Chapter 6. Selection of sites based on sites containing width (<10m, >10m, >100m), k, flow, and alkalinity data at different latitudes with presence and absence of species.

Chapter 170Country	Chapter 171Number of sites per country	Chapter 172Source of data
Chapter 173Argentina	Chapter 1743	Chapter 175personally sampled
Chapter 176Brazil	Chapter 1776	Chapter 178personally sampled
Chapter 179Denmark	Chapter 18012	Chapter 181Matthew O'Hare, STAR
Chapter 182Greece	Chapter 18313	Chapter 184Matthew O'Hare, STAR
Chapter 185Italy	Chapter 1868	Chapter 187Matthew O'Hare, STAR
Chapter 188Latvia	Chapter 18919	Chapter 190Matthew O'Hare, STAR
Chapter 191Mexico	Chapter 1923	Chapter 193personally sampled
Chapter 194Portugal	Chapter 19537	Chapter 196Teresa Ferreira
Chapter 197Scotland	Chapter 1989	Chapter 199personally sampled
Chapter 200South Africa	Chapter 2014	Chapter 202personally sampled
Chapter 203Trinidad	Chapter 2046	Chapter 205personally sampled
Chapter 206United Kingdom	Chapter 20764	Chapter 208Mattie O'Hare, MTR
Chapter 209USA	Chapter 2101	Chapter 211personally sampled
Chapter 212Zambia	Chapter 21318	Chapter 214personally sampled and Michael Kennedy

As shown above in Tables (2.13 to 2.17) there is hence inevitably variation in the sampling effort between datasets for different countries/ regions, which will equally inevitably contribute to the noise associated with analyzing these datasets.

Chapter 3 – the main objective was to address the difference in species assemblages at different latitudes with presence and absence species data. Some regions such as the British Isles have many more sampling sites compared to other areas, increasing the likelihood that reasonably accurate findings emerge from such datasets. In comparison, for other countries where there are few available data, such as Germany and Italy, it is quite probable that only a partial picture of calcareous river macrophyte diversity has emerged from the analysis. This should be taken into consideration when considering the results provided here, but nevertheless I consider that even a partial picture is better than no picture at all. Further research in the future may well, of course, if more data becomes available, alter the findings presented here for such countries.

Chapter 4 – in order to look at the environmental factors affecting the different functional groups of macrophytes, a more balanced dataset in terms of number of sites per country was included. Sampling effort in this case was the same as all data was collected personally, and the variation in number of sampling sites between countries/ regions was much less extreme than when anlysing the datasets examined in Chapter 3. Nevertheless, country was used as a random effect to account for the potential variation that may occur in the response variable between countries, due to unequal number of sites sampled within each country. This approach permitted me to know the variance of the response due to country, i.e. how the spread of the response variable of each country compare to each other.

Chapter 5 – For the purpose of these analyses large datasets were required and so only the data from the British Isles and Zambia were feasible cases for this study. Although the same analysis approach was used for both countries, each was dealt with separately, thus avoiding any problems relating to sampling effort. In addition, sampling procedures for both datasets followed similar MTR-based protocols (see Chapter 2 for methodology).

Chapter 6 – the main objective was to look at the effect of latitude on specific genera. To standardise for sampling effort, sites only with similar stream width (i.e. <10m) were included. A second analysis was carried out which included streams of different width categories and with k, flow and alkalinity data, excluding sites with high shade and no alkalinity or k values.

2.7 DATA PROCESSING AND ANALYSIS

The analyses carried out for each chapter are mentioned further below.

In the results (Chapter 3) I examine the assemblage structure of the macrophyte communities. The primary analytical approach utilized multivariate classification procedures (TWINSPAN) to establish groups of sites exhibiting similar assemblages of macrophyte species.

Once groupings of rivers or stretches were established by this approach the aim was to assess the significance of any variation in diversity between groupings in terms of S (species richness).

Detrended correspondence analysis (DCA) was undertaken, using Canoco (ter Braak and Smilauer, 1998), for unconstrained ordination of the vegetation data, with rare species downweighted.

In Chapter 4 I examine macrophytes' abiotic tolerance factors. I have included the data collected by myself, at selected calcareous rivers locations with in UK, Scotland; Yorkshire Dales (northern England) and abroad (including Zambia; Bonito, Pantanal, Upper Paraná and Chapadas regions of Brazil; northern and eastern Argentina; northern upland streams of Trinidad; northern Florida; western Ireland; Yucatan region of Mexico; and South Africa). Data were analyzed using a General Linear Model by the Laplace approximation using R software.

In Chapter 5 I examine the environmental and spatial drivers of species richness and community structure. To evaluate the spatial patterns species richness, eigenvectors-based spatial filters were created using PCNM (principal coordinates of neighbour matrices) eigenfunctions (Griffith and Peres Neto, 2006; Astorga et al. 2011). Spatial analyses were carried out with the geographical coordinates (longitude and latitude) from each stream river site in the British Isles and Zambia that were obtained using a Garmin GPS in the field. With the use of R Studio-software the coordinates for each 100m stream, river, sites sampled in British Isles (1151 sites) and Zambia (201 sites: personally sampled plus SAFRASS project data sites: Kennedy et al. 2012 in press); were used to create a matrix of Euclidean distances among the sites for each case study respectively, which makes it easier to look at the spatial patterns by commencing from the fine-scale relationships instead of the broad-scale trends (Borcard and Legendre, 2002).

PCNMs depict a spectral decomposition of spatial relationships among sampling sites, that can be seen for the data set of interest followed up by the set up of scales to which the data set responds to (Borcard et al., 2004; Dray et al., 2006; Blanchet et al., 2008).

To evaluate the climatic variables, mean values from 1950-2000 (www.worldclim.com, 2012) were obtained for the British Isles and Zambia. Climatic variables included in the analysis were 1) actual evapotranspiration, mean annual temperature, tempearature seasonal, maximum temperature of warmest month, minimum temperature of coldest month, mean temperature of wettest quarter, mean temperature of driest quarter, annual precipitation,precipitation seasonality (coefficient of variation) (Precipseason), precipitation of wettest quarter, precipitation of warmest quarter, precipitation of coldest quarter.

In results Chapter 6 I examine species richness on the basis of latitude. In order to do so I included species richness based on a qualitative species presence dataset utilizing data from MTR (UK), STAR (Italy, Latvia, Denmark and Greece), Portugal, Zambia, USA, Trinidad, South Africa, and Brazil. A simple regression analysis was carried out on this dataset.

Chapter 3. Aquatic macrophyte assemblages of hardwater rivers at global and national scales

3.1 INTRODUCTION

The purpose of this chapter is to examine the distribution of macrophytes in relation to each region sampled, to fill the gaps with new field data where published data were not available, and thus expand our understanding of the distribution of aquatic plants in different parts of the world. I first outline the species composition, the functional groups, and the number of species found in calcareous rivers around the world, forming this dataset.

Aquatic macrophytes play a significant role in the ecology of very large numbers of freshwater ecosystems worldwide and are one of the groups of aquatic organisms used to establish ecological quality of freshwater systems in bioassessment programmes required by legislation in numerous countries of the world (e.g. Baattrup-Pedersen et al., 2006). Macrophytes are one of the major components of freshwater environments as they help to maintain both biodiversity (Theel et al., 2008) and ecosystem functions (Bouchard et al., 2007). Despite their ecological importance relatively few studies have been undertaken to determine what environmental and anthropogenic influences act as drivers of the diversity of macrophyte communities.

When looking at gradients of biodiversity, based on the distribution of species over the land surface of the earth, we find that they are not evenly distributed. Biodiversity can be measured at different levels, from biome down to genome (Hawksworth, 1995; Roy and Foote, 1997). Usually the tropics contain many more species that a similar size area of the higher latitudes. This pattern is seen across a wide range different animal and plant groups. For example, Cox and Moore (1993) showed that this pattern is observed in birds, mammals and trees. In terms of vascular macrophytes, which have been found to have broad ranges, a higher species diversity has also been recorded in the Neotropics, intermediate in the Oriental, Nearctic and Afrotropics, lower in the Palearctic and Australasia, lower again in the Pacific Oceanic Islands, and lowest in the

Antarctic region (Chambers et al., 2008). Some 39% of the c. 412 genera containing aquatic vascular macrophytes were found to be endemic to a single biogeographic region, with 61-64% of all aquatic vascular macrophytes found in the Afrotropics and Neotropics being endemic to those regions (Chambers et al., 2008). Understanding the causes of geographic patterns of species presence or absence at a particular site is central to ecology (Schall and Pianka 1978; Rohde 1992; Rhode et al. 1993). The three main determining factors are: tolerance of abiotic environment, interactions with other biota, and dispersal (Petts and Calow, 1996). Apart from the studies by Chambers et al., (2008) and Crow (1993), there has been nothing previously published on freshwater macrophyte diversity at a worldwide scale although there are some national scale studies: Baattrup-Pedersen et al. (2006); Rorslett (1991). However there are no studies which focus on macrophyte assemblage structure in hardwater rivers at national or international scales. There is therefore a clear gap in the literature in this context. Specifically, previous studies have had to group data collected by a variety of different means into large spatial units.

In my study I used a uniform sampling methodology across a range of countries and latitude. In this chapter I focus on characterising large-scale patterns in assemblage structure at the international and national scale to inform the analyses of later chapters. Later chapters focus on the site level scale.

As the studies already cited suggest tropical, through subtropical to temperate changes in assemblage I tested for gradients in assemblage between countries. Overlap between assemblage-structure between countries could be attributable to species, which are naturally cosmopolitan or invasive. Invasive species often characterise freshwater systems and some of the most aggressive weeds are aquatic and many of them are capable of living in hardwater systems (Pieterse and Murphy 1990). Finally it is important to understand the structure of species assemblages at the country scale. As previously noted large spatial units equivalent in size to individual countries have been used as single reporting units. However other studies have described significant variation within countries attributable to environmental conditions. Before proceeding further with analyses it is important to understand therefore the degree of turnover in assemblage structure within individual countries. Unconstrained ordination analyses can quantify assemblage turnover. Based on these observations the following hypotheses have been formulated:

1) Are there distinct floras observable in calcareous rivers between temperate, subtropical and tropical regions?
2) Where the macrophyte communities of countries overlap in assemblage structure, can the overlap be attributed to either cosmopolitan species and/or widespread invasive species.
3) Can distinct species assemblages be observed within individual countries?

In addition the general character of sites within the individual countries sampled by myself is described here.

3.2 METHODS

Collection methods for data examined here are described in the previous chapter. A combination of personally-collected new survey data, plus data from appropriate existing databases was used for this study (Table 3-1, Appendix 2). e.g. MTR in UK, EC STAR project data (a database set for river condition assessment, from which samples useful for this study have been pulled out and analysed: including samples from Italy, Greece, Germany, UK, France, Latvia, Czech Republic, Portugal). The pre-existing data were supplemented by field work conducted across the three years of my PhD, at selected locations with calcareous rivers in the UK, northern and western Scotland; Yorkshire Dales (northern England); and abroad (including northern Zambia; Bonito, Upper Paraná, Pantanal and Chapadas regions of Brazil; northern and eastern Argentina; northern upland streams of Trinidad; northern Florida; western Ireland; Yucatan region of Mexico; and South Africa), these areas being surveyed in order to fill perceived gaps in the available data (see below). Owing to the relative lack of field studies in aquatic habitats in tropical and sub-tropical areas, the work drew quite heavily on my own aquatic field work in such areas: e.g. Florida, Zambia, Mexico, Trinidad and Brazil. Macrophytes were surveyed using the Mean Trophic Rank (MTR) field protocol developed in the United Kingdom (Holmes et al., 1999). The MTR survey procedure is based on the presence and abundance of species of aquatic macrophytes.

3.2.1 ANALYSIS PROCEDURES

Ordination analysis was carried out, which requires adjustments to the input data as inclusion or exclusion of species or samples affect the final result. Firstly a TWINSPAN analysis was conducted on 740 samples, which included 521 species to test for differences between tropical, sub-tropical and temperate regions. Detrended Correspondance Analysis (DCA) was then used to look at variation in assemblage structure within each country included in the dataset. As TWINSPAN and DCA use similar algorithms to order a species by site matrix this procedure is reasonable. DCA diagrams usually illustrate sites or species ordinated in a two-dimensional space according to their scores on a two-ordination axis (Figure 3.1). The relative positions of the points in the DCA are indicators of the likeness between them, hence the sites closer to each other are likely to have similar species assemblages and species that are close to each other are likely to co-occur. Sites by species abundance matrices were constructed from the data sources and personally-surveyed samples. The length of the gradient on DCA axis one indicates the amount of turnover in assemblage structure with 2.5 units of change along an axis (standard deviations of species turnover), indicating a near complete change in species composition (ter Braak and Smilauer, 1998).

Following initial analysis of all sites, some outlying sites were made supplementary (i.e. excluded from the analysis) allowing me to depict more easily the relationships between the remaining, less extreme sites. Outliers were sites GE655 (Germany), IT837 (Italy), 21541215 (Portugal) and A6, A46, A45, A4 and UK681 (British Isles). In regards to the grouping of species, species that occur in samples within each country less than ten times were removed for the purpose of this analysis. In addition the divisions made for the groupings were ten as minimum, and twenty as the maximum division level and with only three indicator species allowed per cut-level. Sample sites from Argentina and Brazil were amalgamated due to the proximity of river systems, which had the effect of increasing the number of sites in the analysis.

Table 3-1 Sampling sites (Personally sampled; other data: sources).

Source of Data	*Country*
Personally sampled	British Isles, South Africa, Zambia, Trinidad, Florida, Mexico, Brazil, Argentina
Teresa Ferreira (DEFISA)	Portugal
Matthew O'Hare (CEH)	British Isles, used for STAR project
Matthew O'Hare (CEH)	British Isles, used for MTR project
Andrew Spink (Glasgow University)	England (SK code)
Joe Caffrey (Fisheries Ireland)	Ireland
Sean Morrison (SEPA)	Zambia
Roger Mormul (UEM)	Brazil
Carbiener et al. 1990	France

3.3 RESULTS

Are there distinct calcareous river floras in temperate and tropical regions?

The TWINSPAN analysis depicts the separation of the entire dataset into two main distinct groups. At the first division of the ordination there is a division between samples from the tropics and those from Europe. The British Isles samples keep splitting progressively from the rest of Europe, which may be a reflection of the significantly greater sampling effort leading to discrete groups being identified. Samples from the tropics and sub-tropics have some overlapping species in common (Figure 3-1).

Moreover samples in Portugal were also discretely separated from the rest of the European sites and some Portuguese sites were grouped within the tropics groups, thus showing some species in common, shared between Portugal and the samples from tropical countries. Portuguese sites were singled out after several divisions, of which two species were identified as indicator species, namely *Nasturtium officinale* and *Rananculus peltatus.*

Samples representing mostly the tropical regions had fourteen species identified as indicator species, namely *Commelina* cf. *erecta, Colocasia esculenta, Cladium jamaicense, Vallisneria americana, Panicum repens, Lemna minor, Cyperus alopecuroides, Ottelia exserta, Persicaria senegalensis, Nymphaea nouchali* var. *caerulea, Ludwigia adscendens, Persicaria amphibia, Stuckenia pectinata*, and the unidentified grass Poa9T2.

Where the macrophyte communities of countries overlap in assemblage structure, can the overlap be attributed to either cosmopolitan species and/or widespread invasive species?

The distinct floras of temperate and sub tropical/ tropical regions

Two distinct groups of countries were identified; one mostly comprising samples from the temperate regions plus some neotropical samples, and the other group with mainly samples within the tropical region and associated subtropics.

Species found commonly across samples within the temperate regions were: *Alisma plantago-aquatica, Apium nodiflorum, Azolla filiculoides, Berula erecta, Butomus umbellata, Callitriche hamulata, Callitriche obtusangula, Callitriche stagnalis, Carex rostrata, Catabrosa aquatica, Ceratophyllum demersum, Eleogiton fluitans, Elodea canadensis, Equisetum fluviatile, Glyceria maxima, Hippurus vulgaris, Iris pseudacorus, Lemna minor, Myriophyllum alterniflorum, Myriophyllum spicatum, Myriophyllum verticillatum, Nasturtium officinale, Nuphar lutea, Persicaria amphibia, Phragmites australis, Potamogeton alpinus, Potamogeton berchtoldii, Potamogeton crispus, Potamogeton gramineus, Potamogeton lucens, Potamogeton natans, Potamogeton nodosus, Potamogeton perfoliatus, Potamogeton polygonifolius, Potamogeton praelongus, Potamogeton pusillus, Ranunculus peltatus, Ranunculus aquatilis, Ranunculus circinatus, Ranunculus flammula, Ranununculus fluitans, Ranunculus penicillatus, Rorripa amphibia, Rumex hydrolapathum, Sagitaria sagittifolia, Schoenoplectus lacustris, Sparganium emersum, Sparganium erectum, Spirodela polyrrhiza, Stuckenia pectinata, Typha latifolia , Veronica anagallis-aquatica, Veronica beccabunga,* and *Zanichellia palustris (*Appendix 4).

In contrast species found commonly across samples within the sub-tropical and tropical regions were: *Alternanthera philoxeroides, Azolla filiculoides, Bacopa monera, Brachiaria subquadripara, Ceratophyllum demersum, Colocasia esculenta, Commelina schomburgkiana, Cyperus alopecuroides, Cyperus difformis, Cyperus digitatus, Cyperus erythrorhizos, Echinochloa crusgalli, Echinodorus grandiflorus, Eichhornia azurea, Eichhornia crassipes, Eleocharis atropurpurea, Hydrocotyle bonariensis, Hydrocotyle ranunculoides, Hydrocotyle umbellata, Leersia hexandra, Lemna minor, Limnobium laevigatum, Ludwigia leptocarpa, Ludwigia palustris, Ludwigia peploides, Myriophyllum aquaticum, Myriophyllum spicatum, Nasturtium officinale, Nuphar luteum, Nymphaea amazonum, Panicum repens, Paspalum repens, Persicaria attenuata, Persicaria hydropiper , Persicaria lapathifolia, Persicaria senegalensis, Phragmites australis, Pistia stratiotes, Polygonum punctatum, Pontederia cordata, Potamogeton nodusus, Potamogeton pusillus, Potamogeton schweinfurthii, Salvinia minima, Stuckenia pectinata, Typha domingensis, Typha latifolia, Veronica anagallis-aquatica,* and *Zannichellia palustris* (Appendix 3).

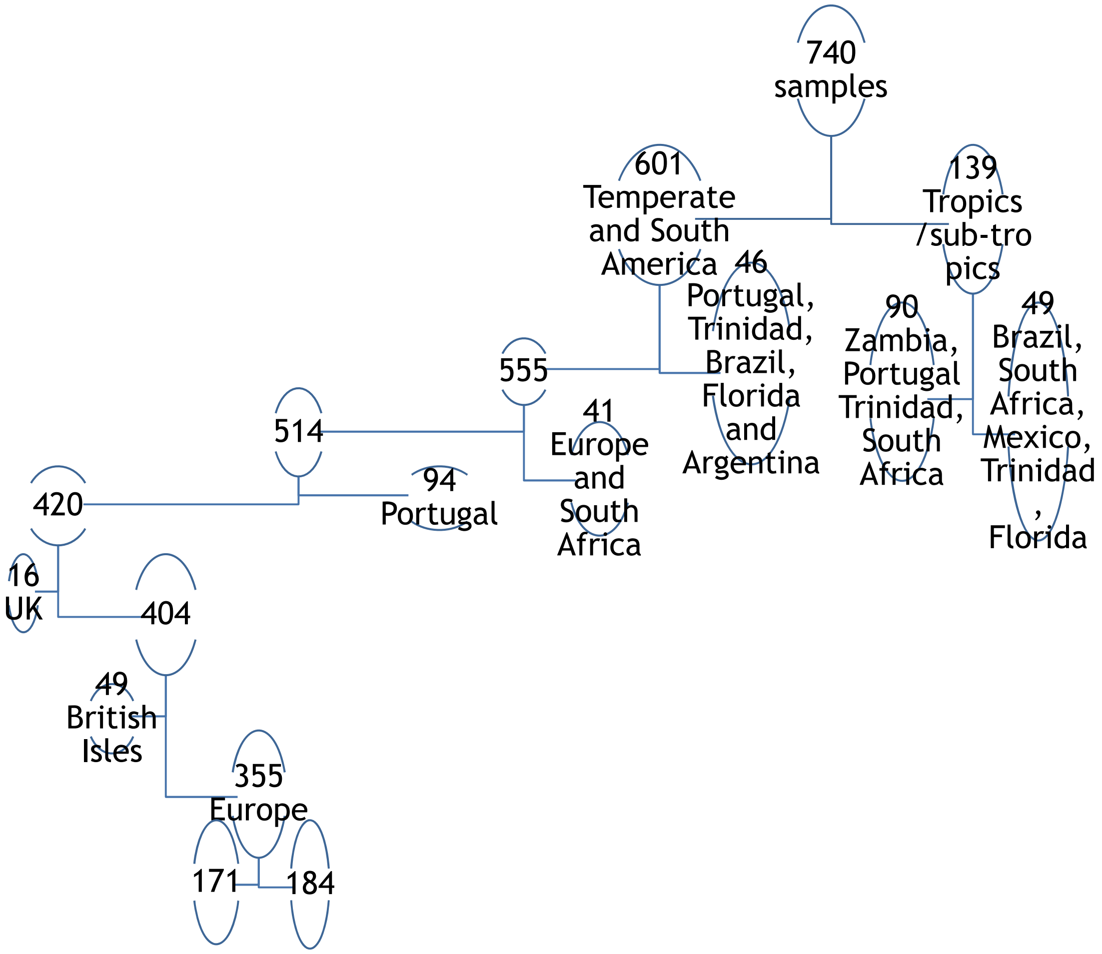


Figure 3-1 TWINSPAN tree with 8 end clusters. The number of samples is shown inside each circle. End clusters are named with the countries contained within the samples.

Can distinct species assemblages be observed within individual countries?

The DCA scatter plots (Figure 3.5 - 3.14) illustrate separately subsets of the data (i.e. species and samples) from 16 separate geographical regions (mostly individual countries). Some individual countries supported more than one species assemblage, (Table 3-3): with the criterion being a gradient length greater than 2.5 standard deviations of species turnover (SD) along axis 1, which depicts a complete turnover of species assemblages. This was the case in regions such as: Argentina and Brazil, France, Germany, Greece, Ireland, Latvia, Mexico, Portugal, South Africa, Sweden, Trinidad, British Isles, USA and Zambia. Calcareous streams sampled in countries such as Denmark and Italy, with values less than 2.5 SD on axis 1, effectively support only a single hardwater stream species assemblage, from these results, which probably at least in part reflects the limited data source available for these countries (i.e. STAR project).

Table 3-3. Axis length (SD of species turnover) and eigenvalues for the individual country ordination plots (Figs. 3.5 - 3.14).

Country	Metrics	Axis 1	Axis 2	Axis 3	Axis 4	Number of sites	Number of species	Total inertia
Argentina and Brazil	Eigenvalues	0.81	0.55	0.36	0.28	37	50	6.75
	Lengths of gradient	5.29	6.68	3.15	4.37			
Denmark	Eigenvalues	0.37	0.25	0.09	0.03	12	21	1.64
	Lengths of gradient	2.27	1.65	1.43	1.44			
France	Eigenvalues	0.59	0.28	0	0	7	44	1.95
	Lengths of gradient	2.76	2.42	1.82	1.65			
Germany	Eigenvalues	0.635	0.06	0	0	4	12	1.18
(removed sample 655) _	Lengths of gradient	2.81	1.70		0			
Greece	Eigenvalues	0.41	0.07	0	0	14	4	0.79
	Lengths of gradient	2.82	2.08	2.06	0			

Country	Metrics	Axis 1	Axis 2	Axis 3	Axis 4	Number of sites	Number of species	Total inertia
Ireland	Eigenvalues	0.51	0.34	0.25	0.17	67	82	5.60
	Lengths of gradient	3.83	3.95	3.58	2.63			
Italy	Eigenvalues	0.22	0.13	0	0	7	3	0.40
Removed sample IT837	Lengths of gradient	1.01	1.07	0	0			
Latvia	Eigenvalues	0.59	0.42	0.26	0.16	19	35	3.89
	Lengths of gradient	4.09	3.86	2.13	2.43			
Mexico	Eigenvalues	0.61	0.43	0.3	0.18	18	72	5.38
	Lengths of gradient	4.15	3.64	2.94	3.04			
Portugal	Eigenvalues	0.67	0.50	0.41	0.32	99	28	7.92
(removed sample 21541215)	Lengths of gradient	6.15	4.76	4.28	3.85			
South Africa	Eigenvalues	0.74	0.46	0.39	0.12	17	56	5.18
	Lengths of gradient	5.0	3.29	3.35	2.70			

Country	**Metrics**	**Axis 1**	**Axis 2**	**Axis 3**	**Axis 4**	**Number of sites**	**Number of species**	**Total inertia**
Sweden	Eigenvalues	0.53	0.38	0.16	0.04	12	27	3.18
	Lengths of gradient	4.93	2.99	2.68	3.25			
Trinidad	Eigenvalues	0.86	0.52	0.24	0.2	18	44	5.53
	Lengths of gradient	6.46	3.76	2.87	1.36			
British Isles	Eigenvalues	0.65	0.57	0.49	0.39	308	135	20.09
(removed samples UK681,A6, A46, A4, A45)	Lengths of gradient	6.52	6.48	6.71	5.73			
USA	Eigenvalues	0.52	0.36	0.23	0.19	27	77	5.82
	Lengths of gradient	4.61	3.36	2.46	2.24			
Zambia	Eigenvalues	0.79	0.51	0.45	0.36	79	80	11.90
	Lengths of gradient	7.44	6.188	4.216	3.826			

In the country by country DCA results given below, where samples were collected personally then site observations on macrophyte assemblage structure are also noted.

British Isles

The DCA ordination of the data collected for the British Isles is shown in Figure 3-5. Small to medium-sized lowland calcareous streams in the British Isles support a range of different macrophyte species assemblages. There was a high degree of macrophyte species turnover across axis 1 (Table 3-3), with a mixture of species representing all five functional groups shown in the diagram (FGs: simply defined here as submerged, free-floating, floating-leaved rooted, emergent, and marginal species: see Chapter 4, Section 4.1 Introduction for a fuller description)). The eigenvalues showed that the ordination diagram was explaining the variation for all British Isles samples in the species data well (though only moderately-well for Irish sites alone). Axis one for the British Isles all—samples analysis had an eigenvalue of 0.6 and explained 7 % of the total variation explained by the ordination. Axis 2 had an eigenvalue of 0.5 and explained 6% of the total variation explained by the ordination (Table 3-3). In addition a DCA ordination only using the data collected for Ireland (Figure 3-6) showed there was a complete macrophyte species turnover across the diagram, again with a mixture of functional groups present. Samples from the central part of Ireland are at the centre of the diagram whereas those from the west coast of Ireland are located more at the right side of the diagram, with one outlier at the bottom of the right corner. The outlier was similar to other sites in most of the physical parameters measured but this site was characterized by having a gravel and sand cobble substrate and a red tint to water, probably from peat within its catchment. The eigenvalues showed that the ordination diagram was explaining the variation in the species data well. Axis one had an eigenvalue of 0.5 and explained 4% of the total variation. Axis 2 had an eigenvalue of 0.3 and explained 4% of the total variation explained by the ordination (Table 3-3).

Additional notes are given below for the subsets of British Isles samples personally collected in Scotland, Yorkshire and western Ireland.

Species variation of macrophytes in calcareous streams in the north- west coast of Scotland, and in the Outer Hebrides, were characterized by having a moderate abundance of macrophytes across all the sites sampled. Across all sites sixty-three different species were recorded, with a mixture of species representing all functional groups. Invasive species found in Scotland were *Elodea canadensis and Elodea nuttallii.* Two small streams in the Island of South Uist (Lòn Mòr and Bornish streams), followed by Moven stream in the Island of Lewis had the highest diversity of macrophyte species. Lòn Mòr was characterized by a shell-sand substrate (typical of machair soils) with clear water, whereas the Bornish stream had also very clear water and green algae present, which is indicative of eutrophication. Moven stream had a peaty substrate, and clear water with some green algae. Part of this enrichment was probably as a result of the presence of cattle in the surrounding areas, which in turned may have enhanced macrophyte diversity. Examples of streams sampled in the Outer Hebrides are illustrated on Figure 3-4. Species that were common in Scottish streams were *Agrostis stolonifera, Equisetum fluviatile, Caltha palustris, Rorippa nasturtium-aquaticum,* and *Iris pseudacorus.*

Species variation of macrophytes in karstic streams in the Yorkshire Dales was characterized by having a relatively high abundance of macrophytes across all the sites sampled in this region of England. Across all sites twenty-two different species were recorded, with a mixture of species representing all functional groups. One invasive species was recorded in the Yorkshire Dales streams: *Impatiens glandulifera.* The outflow stream of Malham Tarn and Bain River near Hawes had the highest diversity of macrophyte species. The first site is a small stream with low flow, the second had very clear water with gravel substrate and with some runoff input due to the grazing pressure of sheep in this area. The enrichment of nutrients may have enhanced macrophyte diversity. Examples of streams sampled in the Yorkshire Dales are illustrated on Figure 3-2. Species common in Yorkshire Dales were *Agrostis stolonifera, Caltha palustris* and *Juncus effusus.*

Species variation of macrophytes in calcareous streams in the west coast of Ireland were characterized by having a high abundance of macrophytes across all the sites sampled in within the east coast of Ireland. Across all sites sixty-three different species were recorded, with a mixture of species representing all three functional groups. No invasive species were recorded for Ireland. The Lough Mask inflow stream, followed by Castlelodge River and Marnagh River had the highest diversity of macrophyte species. Both streams had green algae, which is indicative of eutrophication. flowing over stony and silt substrate. The enrichment of nutrients may have enhanced macrophyte diversity. Examples of stream sites sampled in the west coast region of Ireland are illustrated in Figure 3-3. Species common in Irish streams were: *Phalaris arundinacea, Sparganium erectum* and *Schoenoplectus lacustris.*

A) **B)**

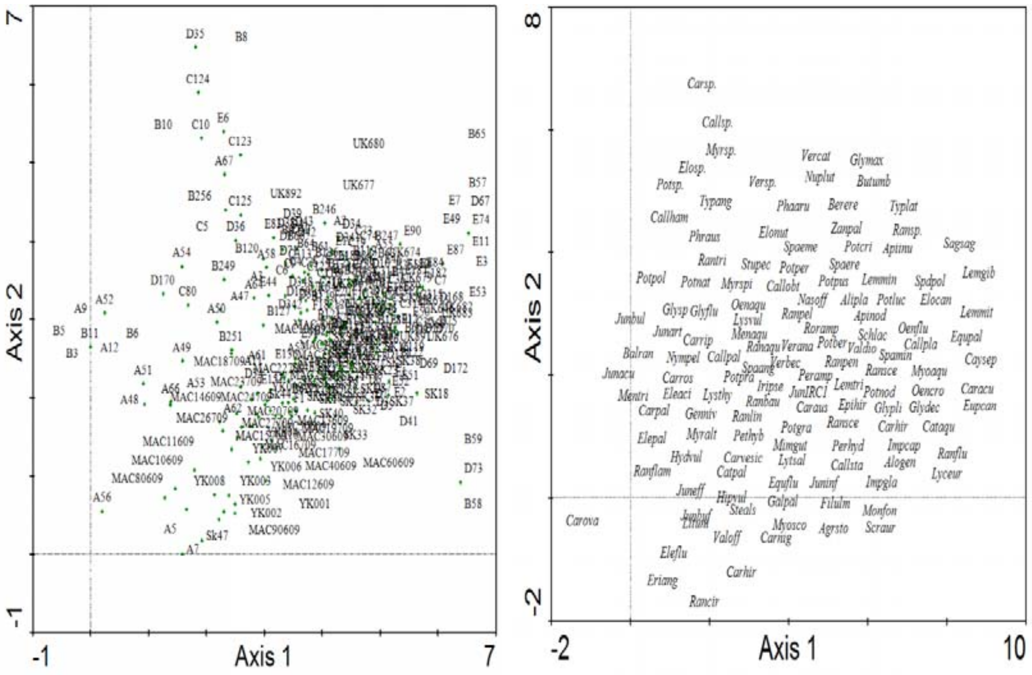
Figure 3-2. Yorkshire Dales streams: A) Gordale Beck, Malham;. B) Tongue Gill, near Stainforth

A) B)

Figure 3-3. Irish stream and limestone landscape:A) Beagh River outflow from Lough Cotra; B) Limestone bedrock in the Burren, west coast region of Ireland.

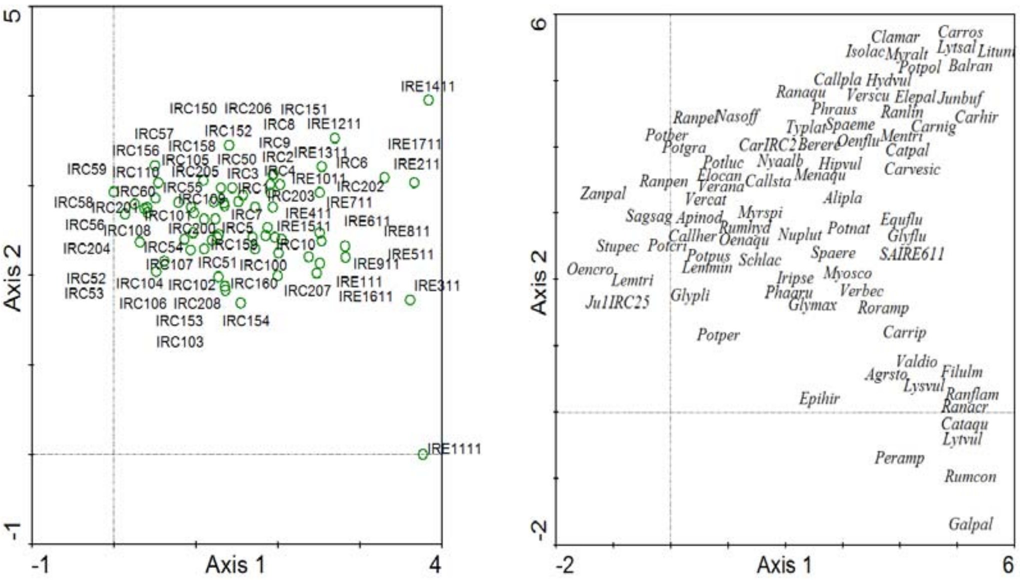
A) B)

Figure 3-4. Examples of Scottish machair streams: A) Lòn Mòr stream, island of South Uist; B) Leathbhal stream, island of North Uist



A) B)

Figure 3-5 DCA ordination diagrams for British Isles: A) samples, B) species.



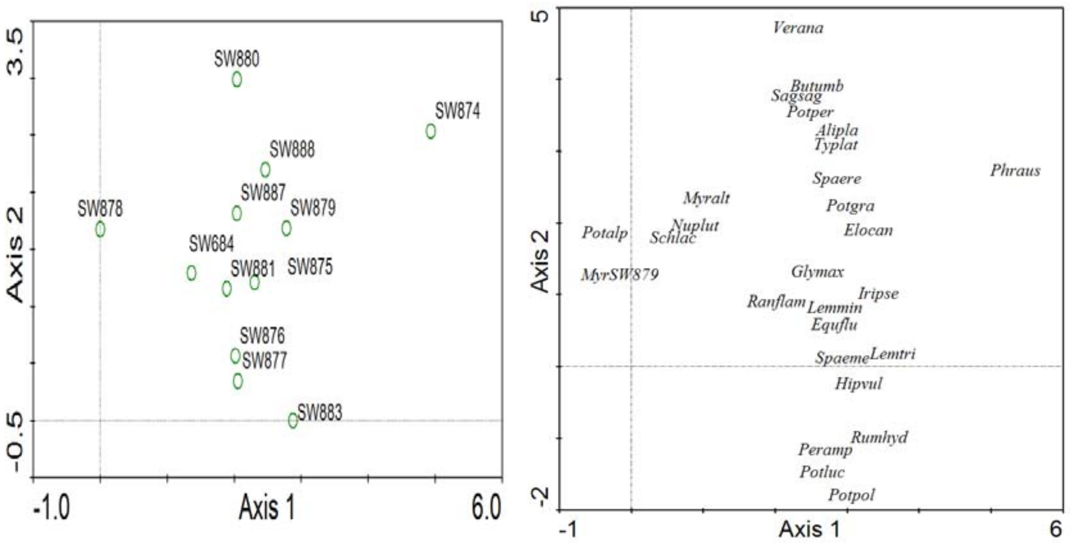
A)

B)

Figure 3-6 DCA ordination diagrams for Ireland: A) samples, B) species

Sweden

The outcomes of DCA ordination of the data collected for Sweden are shown in Figure 3-7. These were mostly medium-sized streams on calcareous soils, supporting a fairly wide range of macrophyte species assemblages. There was a moderately high degree of macrophyte species turnover across axis 1 (Table 3-3), with a mixture of species representing all functional groups showing in the diagram. Potamogetonaceae and Haloragaceae were well represented. The eigenvalues showed that the ordination diagram was explaining the variation in the species data moderately well. Axis one had an eigenvalue of 0.5 and explained 5 % of the total variation explained by the ordination. Axis 2 had an eigenvalue of 0.3 and explained 3% of the total variation explained by the ordination (Table 3-3). The only invasive recorded was *Elodea canadensis*.

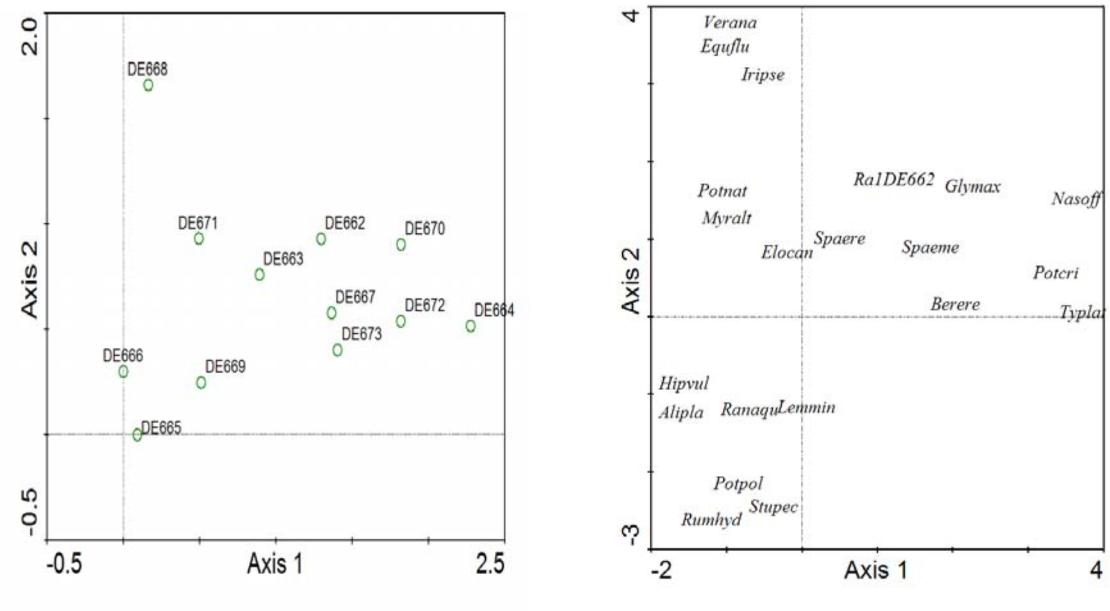


A) B)

Figure 3-7 DCA ordination diagrams for Sweden: A) samples, B) species.

Denmark

The outcomes of DCA ordination analysis of the data collected for Denmark are shown in Figure 3-8. Sites were all on medium sized lowland calcareous streams across Denmark, and effectively are represented by only one species assemblage, as shown in the diagram, with only a low degree of macrophyte species turnover across axis 1 (Table 3-3), but with a mixture of species representing all functional groups present. The eigenvalues showed that the ordination diagram was explaining the variation in the species data only poorly. Axis one had an eigenvalue of 0.3 and explained 2 % of the total variation explained by the ordination. Axis 2 had an eigenvalue of 0.2 and explained 2% of the total variation explained by the ordination (Table 3-3).

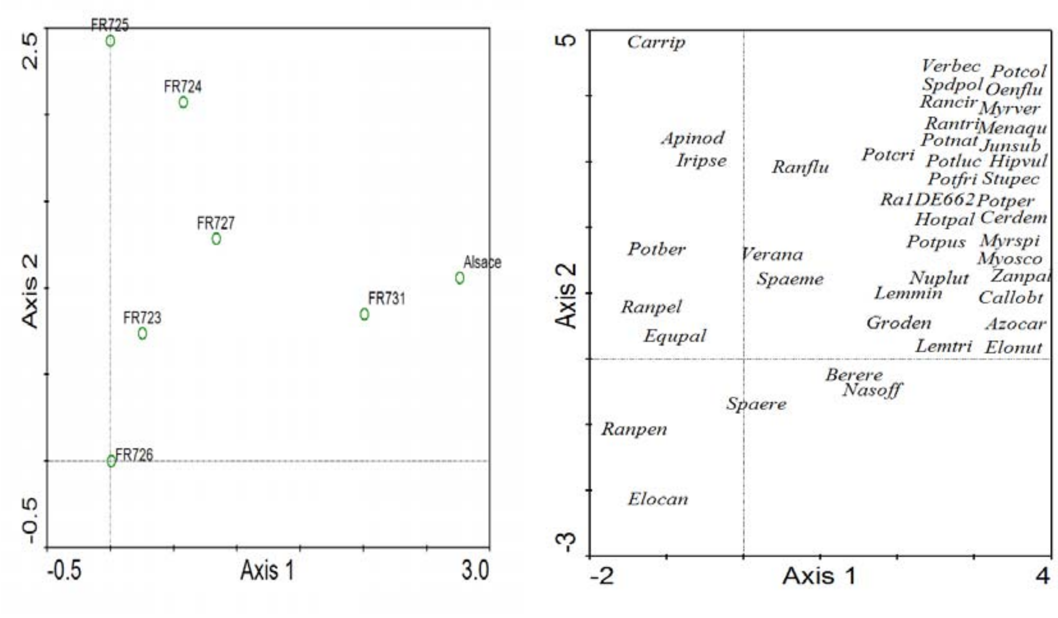


A) **B)**

Figure 3-8 DCA ordination diagrams for Denmark: A) samples, B) species.

France

The outcomes of DCA ordination analysis of the data collected for France are shown in Figure 3-9. The sites were mainly from small-sized shallow headwater streams in eastern France, supporting a low number of different species assemblages, but with a mixture of species representing all functional groups (present. There was a single complete macrophyte species assemblage turnover across axis 1 (Table 3-3). To the left of the diagram there are predominately floating species and a few emergent, while moving towards the right on the diagram many submerged and floating species occurred. The eigenvalues however suggested that the ordination diagram was explaining the limited variation in the species data quite well. Axis one had an eigenvalue of 0.5 and explained 3 % of the total variation explained by the ordination. Axis 2 had an eigenvalue of 0.2 and explained 2% of the total variation explained by the ordination (Table 3-3). Invasives are *Elodea canadensis* and *E. nuttallii*.

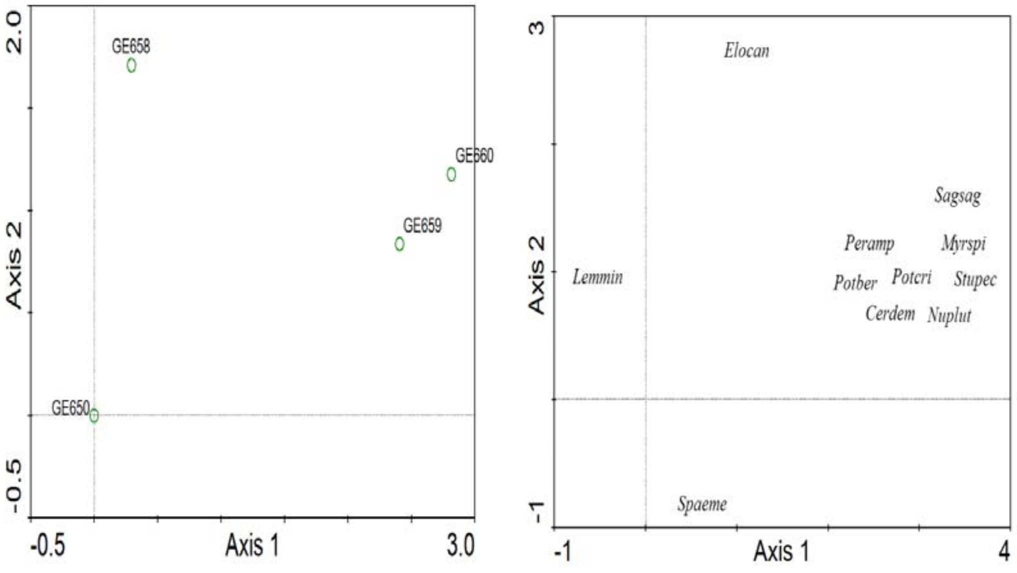


A) B)

Figure 3-9 DCA ordination diagrams for France: A) samples, B) species

Germany

The outcomes of DCA ordination analysis of the data collected for Germany are shown in Figure 3-10. Sites were mainly from small-sized Buntsandstein streams, supporting a moderate variation in species assemblage (though with a small total number of species present). There was a complete macrophyte species turnover (with a value similar to that seen for French streams), with a mixture of functional groups (marginal species are not present) represented in the diagram. To the left of the diagram one free-floating species occurs, towards the right along axis 1 there was a limited number of species representing all four of the FGs found in these streams. The eigenvalues showed that the ordination diagram was explaining the variation in the species data well. Axis 1 had an eigenvalue of 0.6 and explained 3% of the total variation explained by the ordination (Table 3-3). The only invasive is *E. canadensis.*

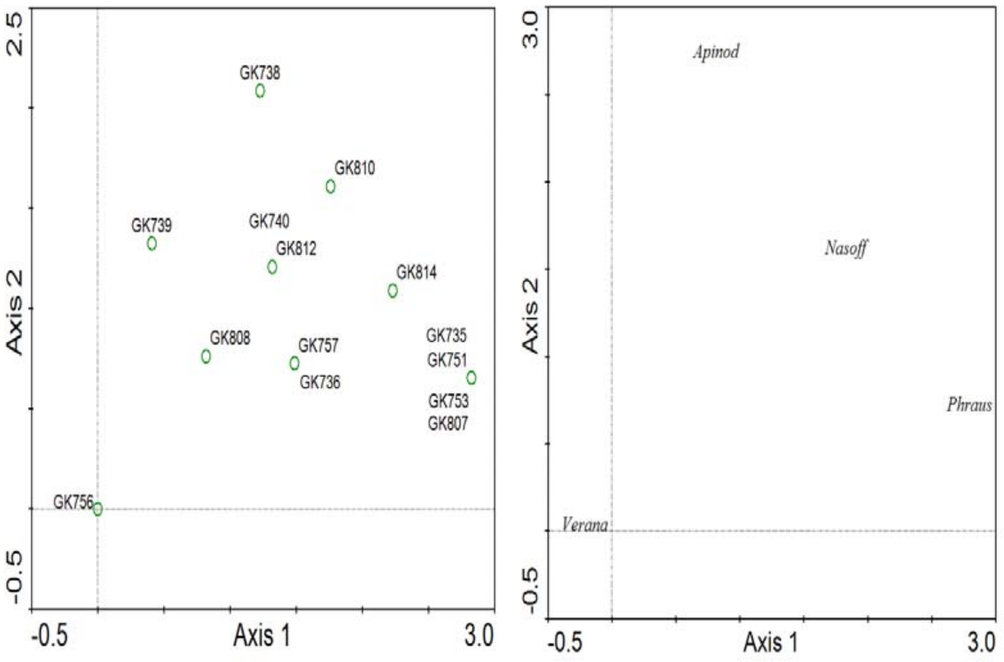


A) **B)**

Figure 3-10 DCA diagram a) samples, b) species for Germany.

Greece

The outcomes of DCA ordination analysis of the data collected for Greece are shown in Figure 3-11. Samples were from small calcareous mountain streams in western central and southern Greece, supporting only four species (all emergent) but still producing a complete macrophyte species turnover across axis 1, with a moderate eigenvalue of 0.4, explaining 3 % of the total variation (Table 3-3). No invasives were recorded.



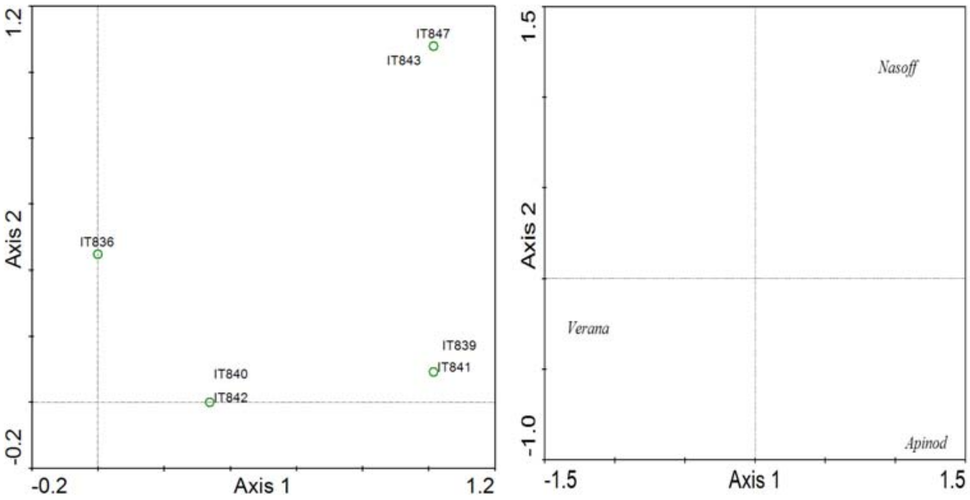
A)

B)

Figure 3-11 DCA ordination diagrams for Greece: A) samples, B) species.

Italy

The outcomes of DCA ordination analysis of the data collected for Italy are shown in Figure 3-12. Sites were located in small calcareous streams in the Central Apennines. The ordination results strongly resemble those seen for Greece, but are even more species-poor, and again entirely represented by emergents. Gradient length was very short, and the eigenvalue for axis 1 is very low (at 0.2, explaining only 1 % of the total variation): effectively there was only one assemblage present. No invasives were recorded.



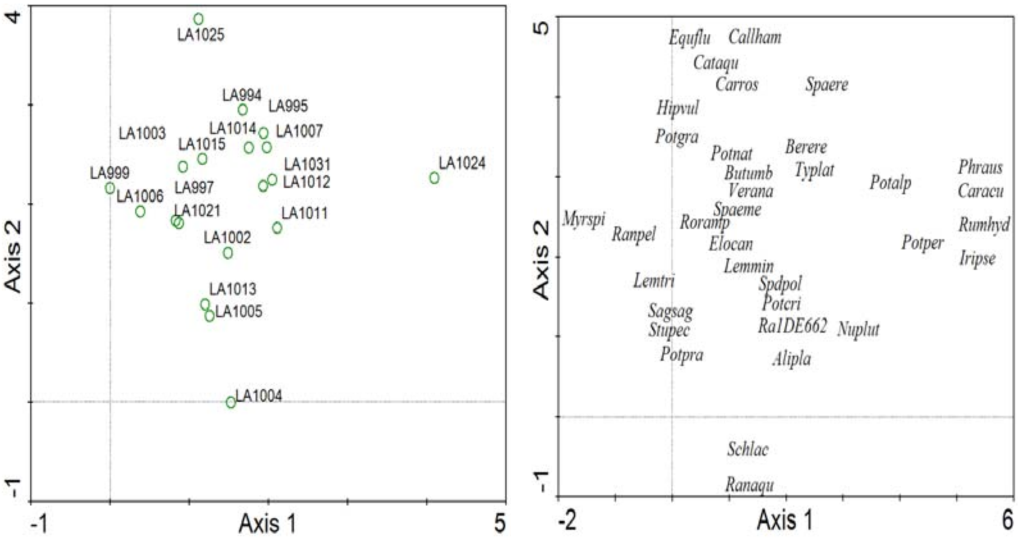
A)

B)

Figure 3-12 DCA ordination diagrams for Italy: A) samples, B) species.

Latvia

The outcomes of DCA ordination analysis of the data collected for Latvia are shown in Figure 3-13. Samples were from medium-sized lowland streams, and supported a range of species assemblages. There was a complete macrophyte species turnover across axis 1 with a mixture of species from all FGs shown in the diagram. The eigenvalues showed that the ordination diagram was explaining the variation in the species data well. Axis one had an eigenvalue of 0.5 and explained 4 % of the total variation. Axis 2 had an eigenvalue of 0.4 and explained 3.8 % of the total variation explained (Table 3-3). *E. canadensis* was the only invasive recorded.

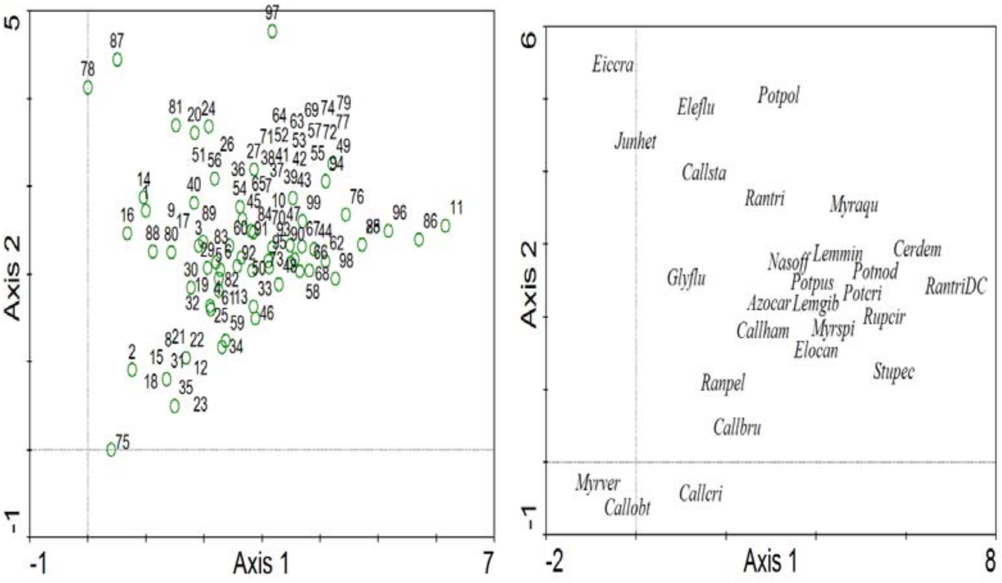


A) B)

Figure 3-13 **DCA ordination diagrams for Latvia: A) samples, B) species.**

Portugal

The outcomes of DCA ordination analysis of the data collected for Portugal are shown in Figure 3-14. Sites were from a mix of small to medium-sized streams. There was a lengthy gradient of macrophyte species turnover across axis 1 suggesting the presence of several assemblages, with four FGs represented (marginal were not present in the dataset), albeit with only a moderate total number of species present, and eigenvalues were high. Axis 1 had an eigenvalue of 0.6 and explained 6 % of the total variation. Axis 2 had an eigenvalue of 0.5 and explained 5 % of the total variation (Table 3-3). Notably well represented were Potamogetonaceae and Haloragaceae. Invasives present are *Elodea canadensis*, *Eichhornia crassipes* and *Myriophyllum aquaticum*



A) B)

Figure 3-14 DCA ordination diagrams for Portugal: A) samples, B) species.

USA

The outcomes of DCA ordination analysis of the data collected for the USA are shown in Figure 3-16. All data were personally collected, from a single state: Florida. Samples were from streams, medium-sized rivers, and spring runs, all on limestone. Axis 1 gradient length was high, suggesting substantial macrophyte species turnover across this axis, and with all FGs represented within several assemblages. Environmental variation was quite large in the Florida streams. For instance the sample furthest to the left in Figure 3-16A had a low conductivity, was highly shaded, with a moderate flow, and a width <10m. In contrast the sample located furthest right on axis 1 was from a much bigger river system, >100m wide, with slow flow and with a much higher conductivity. These environmental differences are reflected in the very different assemblages of species found in Florida. The eigenvalues showed that the ordination diagram was explaining the variation in the species data well. Axis one had an eigenvalue of 0.5 and explained 5 % of the total variation. Axis 2 had an eigenvalue of 0.3 and explained 3% of the total variation (Table 3-3).

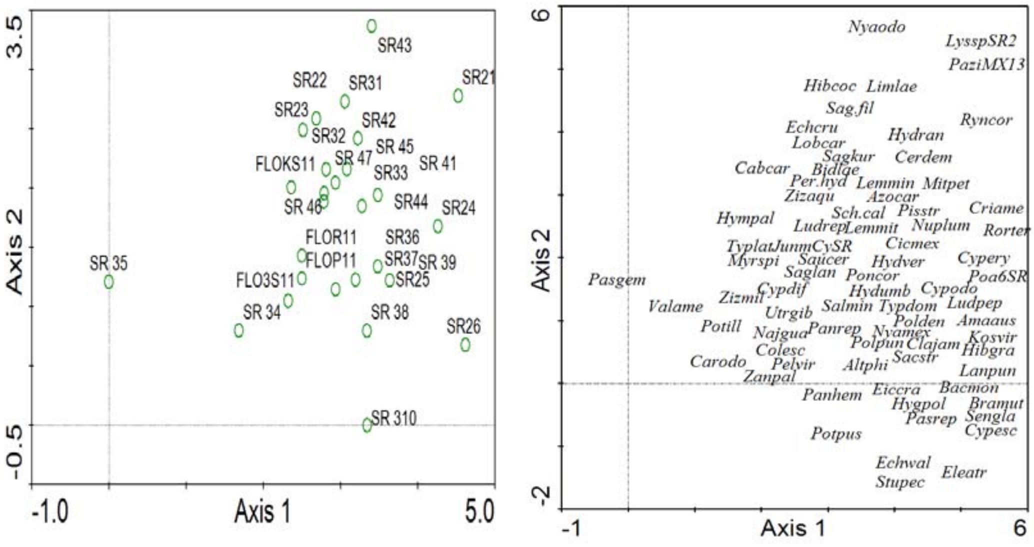
Species variation of macrophytes in karstic streams in Florida were characterized by having a moderate abundance of macrophytes. Across all sites seventy-six different species were recorded, with a mixture of species representing all functional groups present. Invasive species recorded in Florida were *Colocasia esculenta, Hydrilla verticillata Echinochloa crus-galli, Eichhornia crassipes, Hygrophila polysperma, Urochloa mutica, Senecio glabellus, Alternanthera philoxeroides, Myriophyllum spicatum, Pistia stratiotes, Salvinia minima* and *Landoltia punctata.*

Two spring runs, Fern Hammock and Rainbow Springs had the highest diversity of macrophyte species. Both streams had green algae, which is indicative of eutrophication. The enrichment of nutrients may have enhanced macrophyte diversity. The first site was experiencing recreational pressure, whereas the second site had been treated with herbicide for aquatic weed control (maintenance control of water hyacinth, most likely using 2,4-D).

Species that dominated (i.e. those species with a mean of 20 - 45 % in Florida were, *Vallisneria americana* (a species with a restricted world distribution: essentially limited to the Caribbean periphery, but locally abundant in calcareous streams), *Hydrilla verticillata* and *Hydrocotyle umbellata.*

A) B)

Figure 3-15. Examples of calcareous streams in northern Florida: A) Silver River; B) Rainbow Springs



A) B)

Figure 3-16 DCA ordination diagrams for Florida: A) samples, B) species.

Mexico

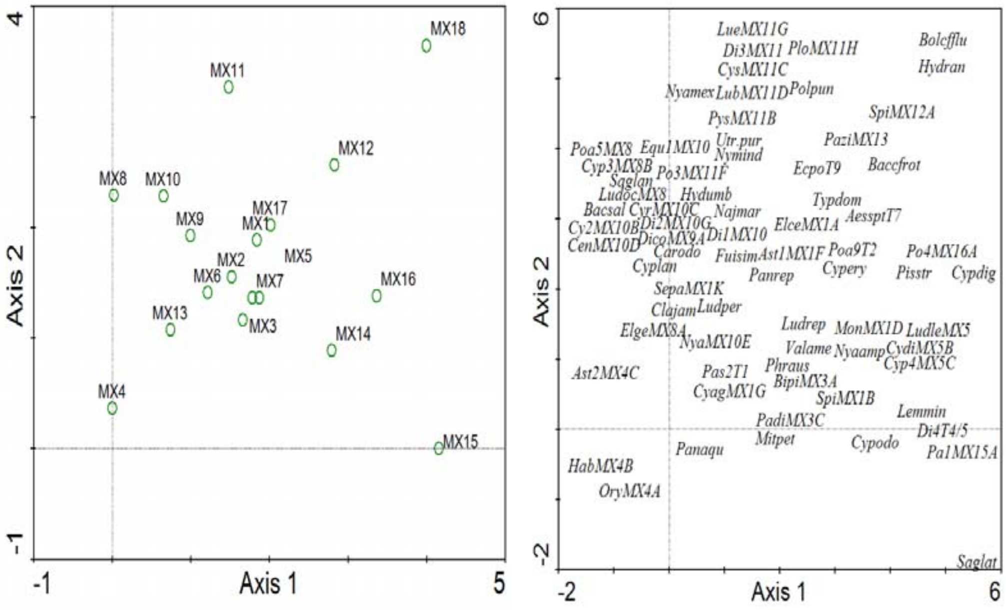
The outcomes of DCA ordination analysis of the data collected for Mexico are shown in Figure 3-18. All data were personally collected, from cenotes and small spring runs on limestone in the Yucatan peninsula. Axis 1 gradient length was high, suggesting substantial macrophyte species turnover across this axis, and with all FGs represented within several assemblages. Cyperaceae and Poaceae were well represented, and the vegetation is dominated mainly by emergent species, with a few floating plants, and few submerged species. Owing to the lack of identification resources available for Mexican macrophytes there are numerous "species" identified to only higher taxonomic levels for Mexico, though I am confident that such "species" are indeed taxonomically different from each other, and from those fully identified. The eigenvalues showed that the ordination diagram was explaining the variation in the species data well. Axis 1 had an eigenvalue of 0.6 and explained 4 % of the total variation. Axis 2 had an eigenvalue of 0.4 and also explained 4% of the total variation (Table 3-3). Species variation of macrophytes in the calcareous waters in the peninsula of Yucatan (areas within the perimeter of Mérida and Quintana Roo states) were characterized by a mixture of species representing all functional groups with a total of seventy-four different species recorded (but see note on identification problems, above). The presence of one invasive species; *Pistia stratiotes* was recorded for Mexico.

The sites called Laguna, Laguna de Coba and el Palmar had the highest diversity of macrophyte species. All sites are characterized by (usually very clear) water flowing on marl and over (or often under) solid lime-rich rock, and frequently appearing to have near-pristine condition, which may have enhanced macrophyte diversity. Examples of sites sampled in the Peninsula of Yucatan are illustrated in Figure 3-17.

Species common in Mexican samples were *Eleocharis* cf. *cellulosa, Cladium jamaicense, Typha domingensis* and *Spilanthes urens.*

A) B)

Figure 3-17. Examples of sites sampled in Yucatan: A) Unnamed laguna near San Felipe, north coast of Yucatan B) Laguna Tortugas



A) B)

Figure 3-18 DCA ordination diagrams for Yucatan in Mexico: A) samples, B) species.

Trinidad

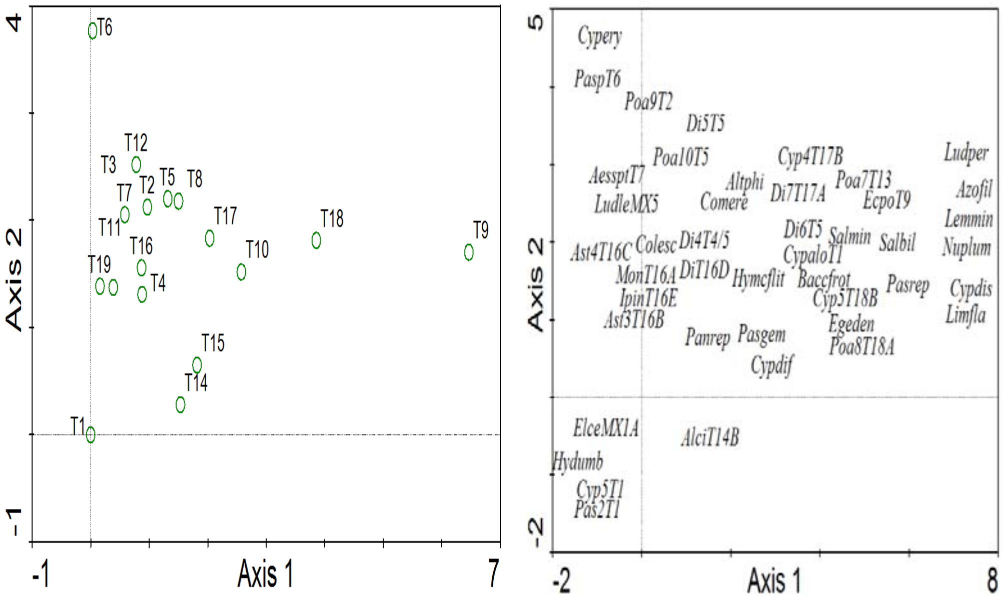
The outcomes of DCA ordination analysis of the data collected for Trinidad are shown in Figure 3-20. All data were personally collected, from 18 sites on rivers and small streams in the Northern Range limestone mountains of the island, and the adjoining low-lying plain through which these streams run to the sea. There was a complete macrophyte species turnover across axis 1, which showed a long gradient, with a mixture of functional groups shown in the diagram. As in Mexico, Cyperaceae and Poaceae were well represented, though the same note of taxonomic caution as raised for the Yucatan samples also applies to the Trinidad dataset. Samples occurring toward to the right have a relatively low pH and appeared to be mainly composed of floating species in comparison to a higher predominance of emergent species on the left side of the diagram. The eigenvalues showed that the ordination diagram was explaining the variation in the species data well. Axis one had a very high eigenvalue of 0.8 and explained 6 % of the total variation. Axis 2 had an eigenvalue of 0.5 and explained 4% of the total variation (Table 3-3).

Across all sites forty-four different species were recorded: with a mixture of species representing all functional groups present. Four invasive or introduced status species were found: *Panicum repens; Colocasia esculenta, Alternanthera philoxeroides* and *Limnocharis flava*.

The Arima River and tributary streams of the Aripo River, both drain South from the Northern Range in Trinidad, had the highest diversity of macrophyte species. The Arima River is a small lowland stream with soft sediment, and the Aripo tributary has sandy gravel substrate. Examples of sites sampled in the Northern Range of Trinidad are illustrated in Figure 3-19. Species common in the Trinidad streams were *Panicum repens, Commelina* cf. *erecta,* and an unidentified grass species coded as Poa9T2.

A) B)

Figure 3-19. Examples of sites in Trinidad: A) Arouca River. B) Aripo River



A) B)

Figure 3-20 DCA ordination diagrams for Trinidad: A) samples, B) species.

Argentina and Brazil

The outcomes of DCA ordination analysis of the data collected for Argentina and Brazil are shown in Figure 3-23. All data were personally collected, from both countries, and the two sets of data are combined for analysis (despite the large geographical extent of sample locations which results) because only a few samples were available from each country. Samples were taken from four widely separated river systems in Brazil, and from two systems, also far apart, in Argentina, all on limestone or calcium-rich alluvium. The eigenvalues are very high, and showed that the ordination diagram was explaining the variation in the species data well. Axis one had an eigenvalue of 0.8 and explained 5.2% of the total variation explained. Axis 2 had an eigenvalue of 0.6 and explained 6.6% of the total variation. Gradient length along axis 1 is also high, suggesting strong species turnover and multiple assemblages present (Table 3-3). There was some evidence for geographical separation being a strong influence on assemblage: for example the sites from Buenos Aires Province, all on Pampas calcareous alluvium cluster together closely at the left side of the sample ordination (Fig 3-22A). Sites from limestone spring-fed streams in the Bonito region of Brazil (very similar in appearance to Florida spring runs) also tend to cluster together at the right-hand end of Axis 1, and separated from the pampas streams by at least 5 SD of species turnover. The floating species tend to lie the centre of the diagram surrounded by different emergent species, but submerged plants are also well represented (good availability of identification resources for macrophytes in Brazil and Argentina, plus the availability of local expertise to assist ID of specimens meant that the ID problems encountered in Mexico and Trinidad were much less of an issue here). Overall total mean abundances of 0.75 -10.56 % occurred within each of the twenty-four sampled sites for Brazil (located in Chapada Diamantina National Park, State of Bahia, in north-eastern Brazil; and two separate locations, Bonito/ southern Pantanal area and the Upper Paraná floodplain system, both in the State of Mato Grosso do Sul, in southern Brazil). Across all sites fifty-three different species were recorded: with a mixture of species representing all three functional groups (submerged, floating and emergent) present. Introduced species in Brazil from this list are thought to include; *Lemna minor, Hydrilla verticillata, Cyperus* cf. *esculentus,* and *Nymphea lotus.* Of these only *Hydrilla* is truly invasive.

The Corixao River, a tributary of the River Miranda (Bonito), plus two sites from the Paraná floodplain, an upstream site in the Baía River (a distributary of the Paraná) and Ressaco do Valdo (a backwater of the main Paraná river channel) had the highest diversity of macrophyte species for Brazil. The last two rivers were found to flow through organic sediments. Examples of sites sampled in Brazil are illustrated n Figure 3-22. Species common in Brazil were: *Eichhornia azurea, Eichhornia crassipes* (native to Brazil, and only problematic there in habitats such as artificial impoundments), *Salvinia auriculata* and *Paspalum repens.*

Species abundance data were not collected at the Argentine streams but richness varied in the range 3 – 12 species per site for the 18 sites sample in rivers, both in the Paraguay system near the city of Corrientes in the north, and in the small pampas streams sampled near the city of Bahía Blanca, in eastern Argentina. Across all sites fifty different species were recorded from all FGs. Introduced species were *Lemna minor* and *Eichhornia crus-galli*, neither being considered particularly problematic in Argentina. Examples of sites sampled in Argentina are illustrated on Figure 3-10.

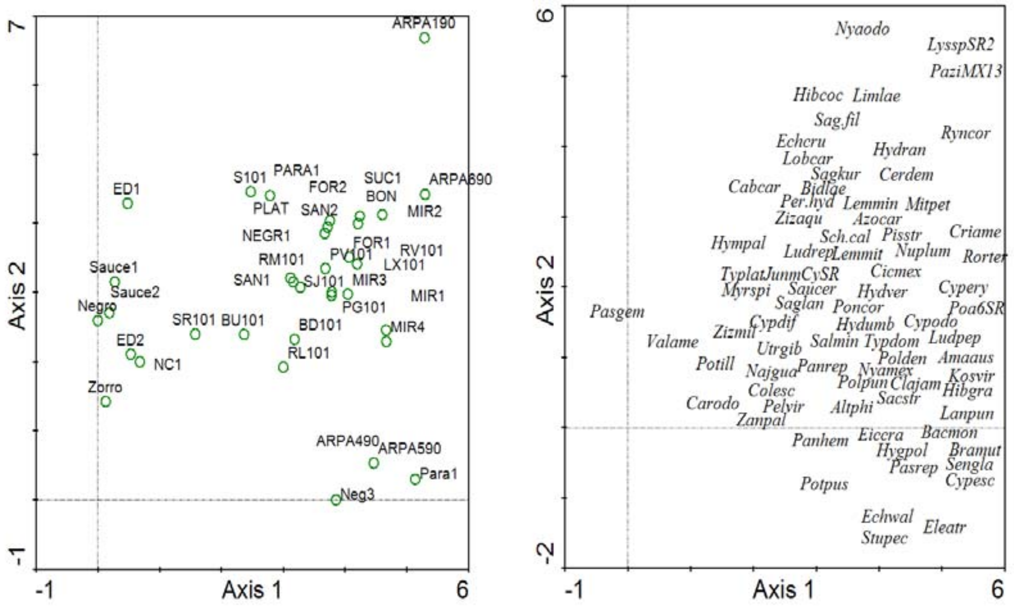
Common species in Argentina streams were *Ludwigia peploides, Polygonum acuminatum, Paspalum repens, Eichhornia azurea and Paspalidium geminatum.*

A) B)

Figure 3-21. Examples of sites sampled in Argentina A) Paraguay River main channel (at confluence with the Paraná River: note the change in water colour where the two streams meet and flow side by side for several kilometres downstream). B) Paraguay River backwater.

A) B)

Figure 3-22. Examples of sites sampled in Brazil: A) Rio Sucurri in Bonito, B) Rio Miranda (Pantanal)



A) **B)**

Figure 3-23. DCA ordination diagrams for Argentina and Brazil: A) samples, B) species.

Zambia

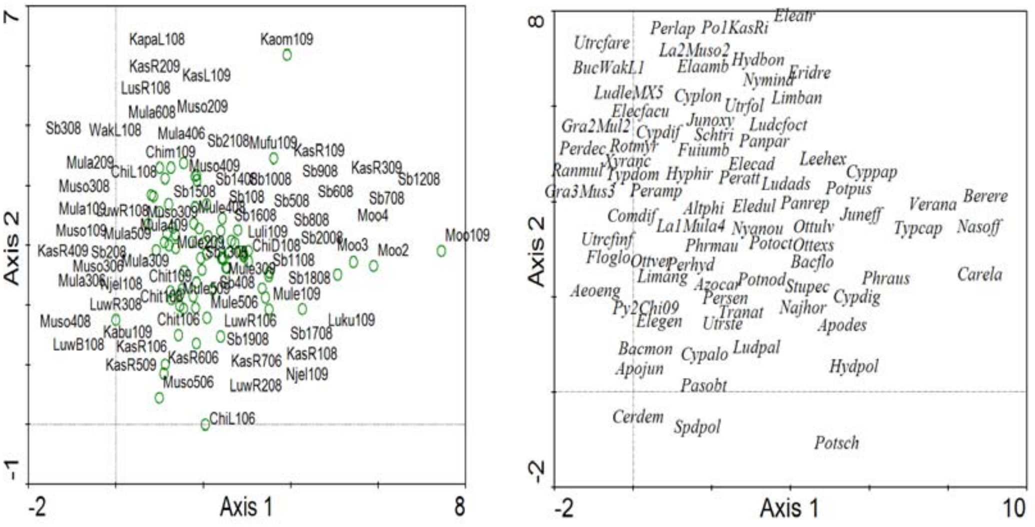
The outcomes of DCA ordination analysis of the data collected for Zambia are shown in Figure 3-25. Some of the dataset was personally collected. There was a complete macrophyte species turnover across with a mixture of functional groups shown in the diagram. The large eigenvalues (0.7 for axis one and 0.5 for axis two; respectively explaining 7% and 6% of total variation: (Table 3-3) and long gradient on axis 1 indicate the wide species variation of macrophytes in the 80 sites sampled in hardwater streams in Zambia. Across all sites eighty different species were recorded: with a mixture of species representing all functional groups present. There were no invasive species present in the sites sampled.

Four river sites, namely the Chitikilo, Mulembo, Lukulu (upstream in Lavushi Manda) and Lukulu (downstream, in the Bangweulu Swamp delta near Shoebill Camp) showed the highest diversity of macrophyte species. Examples of sites sampled in Zambia are illustrated in Figure 3-24.

Species common in Zambia were *Phragmites mauritianus, Nymphaea nouchali* var. *caerulea* and *Panicum repens.*

A) B)

Figure 3-24. Examples of sites sampled in Zambia: A) Zambezi River. B) Mulembo River



A) **B)**

Figure 3-25 DCA ordination diagrams for Zambia: A) samples, B) species .

South Africa

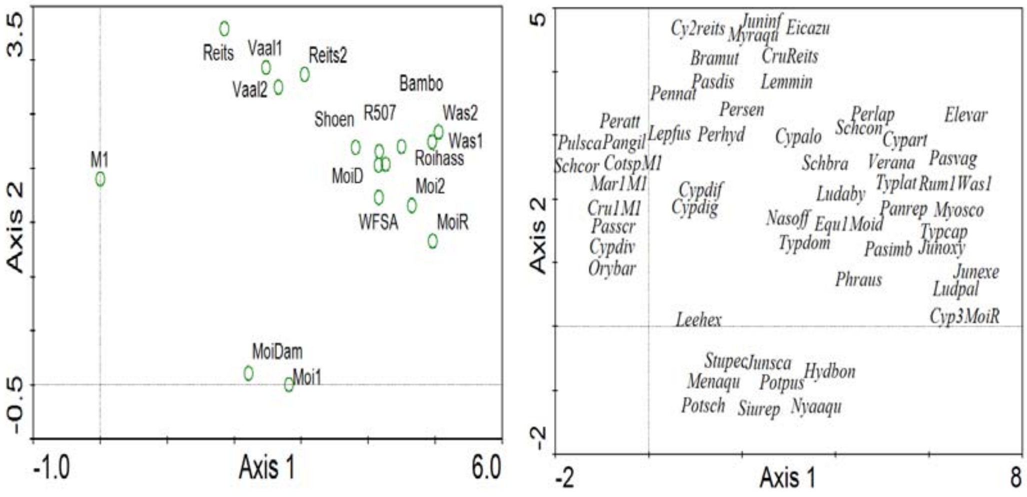
The outcomes of DCA ordination analysis of the data collected for South Africa are shown in Figure 3-27. All samples were personally collected. There was a complete macrophyte species turnover along axis 1 with a mixture of functional groups represented. The large eigenvalues (0.7 for axis one and 0.5 for axis two; respectively explaining 5% and 3% of total variation: Table 3-3) and long gradients indicate the wide species variation of macrophytes in hardwater South African rivers. To the left of the diagram there are predominately emergent species and a few submerged species at the bottom of the diagram; moving towards the right there are both emergent and floating species. Contrasting the sites at both extremes of the axis 1: the site on the left along with the two ones on the bottom are characterized by low conductivity and clear water compared to the one in the furthest right, which had higher conductivity and also polluted water with algae present. Sites were located in small to fairly large calcareous rivers within the vicinity of Potchefstroom, Vredefort, and Parys in the North-West and Free States, of South Africa. In total sixty different species were recorded: with a mixture of species representing all functional groups present. Three invasive species were recorded; *Paspalum vaginatum, Eichhornia crassipes,* and *Myriophyllum aquaticum*.

Two sites on the Mooi River, plus the Goedspruit stream had the highest diversity of macrophyte species. The Mooi downstream site was very close to a waste treatment outflow, and effluents from an abattoir polluted the Goedspruit stream. Streams in the target region of South Africa in general were likely to be under pollution stress, especially from heavy metals derived from mining. Examples of sites sampled in South Africa are illustrated in Figure 3-26.

Species common in South Africa were *Persicaria lapathifolia, Paspalum vaginatum, Cyperus alopecuroides* and *Phragmites australis.*

A) **B)**

Figure 3-26. Examples of South African sites: A) Mooi River. B) Wonder Fontein



A) B)

Figure 3-27 DCA ordination diagrams for South Africa: A) samples, B) species.

3.4 DISCUSSION

The results show a high degree of variability in community structure within calcareous streams at the international and national level.

Are there distinct floras between temperate and subtropical/ tropical regions?

I found that macrophytes were present in almost all the sampled stream and river types but also that there was a high degree of variability in community structure among the stream types investigated, with the exception of some cases such as streams in Germany and Italy (with very small lengths of gradient along axis 1 in the DCA). Small sample sizes may have influenced these results, or it could be that the typology used in my study is inappropriate in these countries to adequately describe their macrophyte assemblages (Baattrup-Pederson, et al., 2006). TWINSPAN classification carried out for my study identified two distinct groups of assemblages, defining the temperate regions and South America, and other groups with mainly samples within the subtropics, tropics and Portugal, with greater diversity found in the latter one. The previous large-scale study of freshwater macrophyte diversity by Crow (1993) also found distinctive geographical variation in taxonomic assemblages. Crow's study grouped vascular plant families into 3 main groups based on their predominant families: 1) cosmopolitan *Cyperaceae, Juncaceae, Poaceae* 2) pan tropical e.g. *Limnocharitaceae, Mayacaceae, Pontederiaceae, Aponogetonaceae, Podostemaceae, Hydrocharitaceae*; and 3) north temperate *Potamogetonaceae, Sparganiaceae, Haloragaceae,* which showed some similarity with my findings.

Bio-geographical distribution patterns are well known to occur across different taxa e.g. terrestrial plants, mammals, and birds (Cox and Moore, 1993). Distribution patterns of angiosperms, which tend to centre on the tropics, are made up of roughly an estimated 30 per cent of flowering plant families that are widespread in distribution, about 20 per cent mainly temperate and about 50 per cent mainly tropical, thus depicting a distinctive distributional pattern of family groups within bioregions with a greater diversity in the tropics (Cox and Moore, 1993).

Where the macrophyte communities of countries overlap in assemblage structure, can the overlap be attributed to either cosmopolitan species and/or widespread invasive species.

As shown above the two distinctive groups of assemblages (i.e. subtropical/ tropical v. temperate) had an overlap, with countries being represented in groups with other countries from outwith their bioregion e.g. Portugal and South America. My findings suggest that this could be certainly attributed to the presence of cosmopolitan or invasive species in these countries (i.e. those with percentage abundance >50% in at least 4 sites according to Bernez et al., 2006).

For instance Portugal apart for having some cosmopolitan species (Dodkins, 2012) is well known for having a high number of invasive species in its rivers (Aguiar, Moreira and Ferreira, 1996; Ferrerira et al., 1998; Ferrerira and Moreira, 2000; Aguiar et al., 2001). Invasive species reported in Portugal are: *Paspalum paspaloides, Azolla filiculoides, Apium nodiflorum, Panicum repens, Phragmites australis, Myriophyllum aquaticum, Eichhornia crassipes* and *Cyperus eragrostis* (Bernez et al., 2006; Moreira et al., 2002). However though possible nuisance weeds *Phragmites australis* and *Apium nodiflorum* are almost certainly native to Portugal, the former being a temperate cosmopolitan species, and the latter a west European endemic (see GBIF database: http://data.gbif.org/species/browse/taxon/5290149?qs=Phragmites australis; http://data.gbif.org/search/apium%20nodiflorum). Apart from *Paspalum paspaloides* these species were present in my Portuguese calcareous river study sites. Most of the invasive species present in Portugal are native to the sub-tropics and tropics region, thus explaining some of the overlap in macrophyte assemblage structure in these areas.

In South America, the presence of cosmopolitan species in the genera *Eleocharis, Cyperus, Oxycarum, Schoenoplectus,* and *Typha,* among other species which are widely distributed in warm-temperate to tropical areas. In addition the presence of *Potamogeton* species in South America, which are recorded from both warmer and cooler areas of the planet, may help account for the overlap in macrophyte assemblage structure seen in these areas. Moreover invasive species may also play a part this overlap. Many of the common invasives found in Old World countries, for example free-floating species like *Eichhornia* *crassipes*, are native to the Neotropics (e.g. Barret and Forno 1982) but now cause increasing problems in parts of Europe including southern Portugal (Ferreira and Moreira, 1998) and Spain (Trinidad et al., 2008). In addition there are records since the 1980s of species such as *Myriophyllum aquaticum, Ludwigia grandiflora,* and *Hydrocotyle ranunculoides* that are native to South America and invasive to Europe (Nehring and Kolthoff, 2011; Hussner, 2009; Dandelot et al., 2005; Gignon and Weber, 2005). All such cases are likely to increase the probability of assemblage overlap between macrophyte communities from temperate and South American countries.

Can distinct species assemblages be observed within individual countries?

There is evidence that species assemblages did vary within the countries typified by DCA plots with long axis 1 gradient values, though there was undoubtedly an effect of sampling effort. This was mainly a reflection of the small number of calcareous sites available for some countries sampled as part of the STAR project (Lorenz et al. 2012; Birk, Van Kouwen, and Willby, 2012) and Italy (Ceschin, Zuccarello, and Caneva, 2010).

Nonetheless my results on the distinct species assemblage observed within individual countries were supported by the literature for other countries and are likely to give a reasonable representation of the diversity in assemblage types. For instance a study which examined the macrophyte community present at 44 sites on the River Welland in Leicestershire, England, using the Mean Trophic Rank system, recorded some of the species found in my UK sites: e.g. *Apium nodiflorum, Callitriche stagnalis, Sparganium erectum, Potamogeton crispus, Lemna minor, Potamogeton perfoliatus, Glyceria maxima, Ranunculus penicillatus,* among others present within the catchment (Demars and Harper, 1998). In another study macrophytes were monitored in 79 small Danish lowland streams, in total 131 species were found of which 65 were found both in the stream and on the stream banks. Species that dominated the macrophyte communities in the streams were *Berula erecta*, species within the genus *Sparganium, Glyceria fluitans* and *Callitriche* (Baattrup-Pedersen, Larsen and Riis, 2003) representing taxa that I also found within Danish calcareous streams.

Lorenz et al. (2012) investigated the macrophyte community of 40 restored river reaches in the lowland and lower mountain areas of Germany and recorded *Veronica beccabunga, Alisma plantago-aquatica* together at the restored reaches. In the backwater areas created by the restoration they found *Spirodela polyrhiza, Lemna* sp., *Potamogeton berchtoldii,* and *Juncus* spp.; two of these also being found in my limited dataset for Germany

Ceschin, Zuccarello, and Caneva, (2010) surveyed the aquatic plant communities of the Tiber River basin, Italy and found an *Elodeo-Potametum crispi* association, plus *Nasturtium officinale* present in meso-eutrophic clean waters, a *Potamogeton nodosus* community plus *Ceratophyllum demersum* in eutrophic water of medium quality, and *Myriophyllum spicatum,* and a *Potametum pectinati* association, in hypertrorphic and poor water quality, just to illustrate some of the species present in this region. *Nasturtium officinale* was also present in my limited Italian dataset, in more upland streams.

Moreover the species assemblages recorded in my study for Brazil, were also found to be consistent with previous studies. Martins et al. (2008) recorded a total of 153 species of macrophytes in the Upper Paraná River floodplain, from which the predominant species were the free-floating *Eichhornia crassipes* and *Pistia stratiotes,* and floating-leaved rooted *Eichhornia azurea,* among others. Likewise in lagoons associated with the Paraná River species found to be dominant were *Eichornia azurea, Nymphaea amazonum, Paspalum repens, Hydrocotyle ranunculoides,* and *Eichhornia crassipes*. All of these were present in my dataset from Brazil. Other studies looking at waterbodies connected with the Paraná River system have also shown similar species to be dominant, among others, and showing substantial agreement with the Brazilian species included in my dataset (e.g. Milne, 2006; Murphy et al. 2003; de Souza et al. 2011; Varandas Martins et al. 2013 in press).

Overall my findings in **Chapter 3** provide evidence that there is a high variation in macrophyte assemblages of calcareous rivers across the different countries included in my study, broadly agreeing with information from the literature. Outlining the presence, absence or predominance of certain type of macrophytes across the different counties, and stressing the existence of species distribution ranges. I found two large groups based on species assemblages across the different countries included, i.e. a subtropical/tropical and a temperate group. In addition these two groups were found to overlap in macrophyte assemblages within some countries, which could at least in part be attributed to the presence of invasive and cosmopolitan species. Spreading of aquatic plants across countries is known (Hussner, 2009) and is a well-documented aspect of global change (Chapin et al., 2000). Kercher and Zedler (2004) suggest that 24 per cent of the world's most invasive plants are wetland species, despite the fact that they only represent 6% of earth's land mass, thus showing the potential of at least some macrophyte species to spread over large areas of the planet.

Further examination of macrophyte diversity global patterns is undertaken in the next chapters, taking into consideration local scale factors (i.e. physic-chemical factors **Chapter 4)** and spatial scale factors, (i.e. latitudinal gradient, climatic variables: **Chapter 5)**, and their effects on macrophyte diversity distribution patterns.

Chapter 4. The influence of local environmental variables on hardwater river macrophyte functional groups

4.1 INTRODUCTION

Aquatic vascular plants comprise a diverse assemblage of species that have adapted from terrestrial origins to aquatic systems. At least 2600 aquatic macrophyte species are recognised (as a very conservative estimate: Chambers et al. 2008), though not all of these occur in rivers, and the species subset which is found in hardwater rivers will be smaller again. This still leaves a large number of individual species, each with its own ecological preferences, to deal with in the context of my study. However, as an alternative to taxonomic classification, all aquatic macrophytes can be conveniently classified by their "life form" (Sculthorpe 1967), into a small set of functional groups (FGs). A simple five-group system, defined by position of roots and photosynthetic structures relative to their water surface, is widely accepted (Sculthorpe, 1967; Cronk and Fennesy 2001); *Marginal macrophytes* are plants that live in habitats only occasionally inundated by water, though usually with their roots in very wet conditions (e.g. *Juncus effusus*). *Emergent macrophytes* are rooted plants with most of their stem and leaves above the water surface (e.g. *Phragmites australis*.). *Floating-leaved rooted macrophytes* comprise those species rooted in (or, rarely, attached to) the substratum, with photosynthetic structures lying on the water surface (e.g. *Nymphaea amazonum*). *Free-floating macrophytes* may or may not have roots, which dangle free in the water, but live unattached to the substrate (though they may be closely associated with it in some cases) with their photosynthetic structures below or upon the water surface (e.g. *Utricularia* spp., *Ceratophyllum demersum, Eichhornia crassipes, Lemna* spp.). *Submerged macrophytes* are rooted plants (or sometimes attaching to solid surfaces, as in the case of many species of Podostemaceae) with all or most of their non-root tissue beneath the water surface (e.g. *Hydrilla verticillata*) (Fox, 1992). Some species have morphological features that could place them in more than one single FG, e.g. submerged and emergent-leaved at maturity in different proportions, often according to changing water depth (Spence et al., 1987). The diversity of traits, in each FG, represents the species' niches or functions that characterise the group as a whole (Petchey, Hector and Gaston, 2004; McGill et al., 2006). Species FGs have been previously used as the basis for understanding how species richness or diversity relates to ecosystem function (Petchey, Hector and Gaston, 2004; Flynn et al., 2011) and to find out how diversity responds to environmental disturbance or stress (Suding et al., 2008; Cadotte, Carscadden and Mirotchnick, 2011). Thus in order to understand plant assemblages and their adaptations in this context for river plants, which face a unique set of physico-chemical pressures on survival, not least as a result of water movement within their habitat, it is necessary to have an understanding of the effects of different physical and associated chemical features of the river environment on the ecology of plant FGs growing in calcareous river habitats. This chapter aims to look at the local environmental factors affecting calcareous river macrophyte FGs, regardless of their location in the world (impacts of large-scale spatial factors are considered in later chapters).

The most important physical variables found to affect river macrophyte assemblages are: 1) slope, which is a surrogate for water velocity, and 2) substratum, light regime, temperature, water chemistry, and water level fluctuations (Fox, 1992; Sandjensen, 1989; Lacoul and Freedman, 2006b).

Fox (1992) describes a hierarchy of three factors, related to the individual traits of the plants, which further control the presence, or absence of aquatic vegetation at a site as:

1) Dispersal factor: has the species reached the site yet? If yes then it is present.
2) Abiotic tolerance factor: can it tolerate the physical environment? If yes then it is present.
3) Biotic interactions factor: is it competitively excluded by other macrophytes or eliminated by herbivores, pathogens or selective human management? If no then it is present.

In this section I look in more detail at the abiotic tolerance factors of hardwater river macrophytes. Firstly water movement will influence the establishment of macrophytes, in terms of their physical adaptations to water turbulence and other disturbance pressures associated with moving water (e.g. scouring of substrate during high flow events). The association of particular macrophyte species with certain ranges of water velocity has frequently been based on qualitative observations (e.g. Holmes, 1983; Sand-Jensen, 1989; Baattrup-Pedersen et al., 2005; Baattrup-Pedersen, et al., 2006). Free floating macrophytes will be usually limited to areas or periods of slow flow (except where they can find refuges from high flow in faster-flowing rivers, for example amongst marginal beds of emergent vegetation), whereas rooted river plants have better inherent resistance to various types of mechanical damage imposed by the water current. The hydraulic resistance of individual plants depends on each species dimensions in relation to the flow direction, and to morphological factors such as their leaf size and shape, branching, shapes and stem strength and flexibility. For instance submerged species with bushy or broad leaves (e.g. *Myriophyllum spicatum, Potamogeton lucens)* will create some resistance to flow, and are likely to be more susceptible to uprooting and battering than submerged plants with streamlined leaf morphology (e.g. *Vallisneria americana*), or plants with strong, well-developed root and rhizome systems to resist flow disturbance (e.g. *Sparganium erectum*) (Fox, 1992; Sabbatini and Murphy, 1996).

In addition to physical impacts of water movement, submerged and free – floating macrophytes (but to a much lesser extent floating-leaved rooted and emergent species) are also influenced by the fact that moving water around their tissues constantly replenishes dissolved materials, enhancing the supply of nutrients and dissolved carbon dioxide (and bicarbonate, for those species able to utilise the latter). Because the rate of CO_2 diffusion through water is 10,000 times slower than in air, water flow can be a very important factor affecting directly the gas exchange needed for the photosynthetic processes in plants with little or no direct access to the air for their leaves (Fox, 1992).

Secondly, for the plants' photosynthetic process in all macrophytes (there are no aquatic equivalents of the parasitic plants occurring in some terrestrial habitats) the availability of light is crucial for their survival. Emergent species and plants with surface-floating leaves are not affected by underwater light regime (except during stages of their life cycle when their leaves may be underwater, such as during seedling or young plant growth, or during flood events when mature leaves may become submerged). The rest of the time light regime influences on plants within these FGs are akin to those faced by terrestrial species (e.g. effects of shade by taller growing species on shorter ones). However the situation is very different for plants of the submerged FG (as well as those free-floating species which live below the water surface). Not only do they experience potential losses of incoming light energy reaching the surface of the water (for example due to shade by floating leaves or tall emergents, or bankside vegetation, as well as surface reflection), but also within the water column light is attenuated logarithmically with depth, due to absorption of light by water molecules, dissolved coloured compounds, suspended solids, and biological particles such as phytoplankton cells (Jerlov, 1976). In addition to this there is frequently competition for light between taller- and shorter-growing submerged species, beneath the water surface. In fact light is a key factor that sets the depth limit of plant distribution in water and applies a major control on macrophyte photosynthesis (Sand-Jensen, 1989; Skubinna et al., 1995; Vestergaard and Sand-Jensen, 2000). A previous study showed from a survey of macrophytes, (principally from temperate lakes), that the mean percentage of photosynthetically-active surface light energy present at the maximum depth of submerged macrophyte colonization was 21.4 ± 2.4% (SE) for submerged rooted plants, and 10.5 ± 1.6% for charophytes, which have a lower proportion of non-photosynthetic tissue within their structure and are hence inherently more shade-tolerant than vascular plants (Chambers and Kalff, 1985).

This chapter looks at local scale site variables in isolation, as predictors of macrophyte community structure. The project was limited to calcareous rivers, but within that habitat type I collected data from a geographically extensive set of sites, which consequently covered a wide range of physical and chemical habitat conditions. These local scale factors could potentially explain a significant amount of variation in the distribution and diversity of macrophyte vegetation in hardwater rivers. Therefore this variation needs to be examined and understood before proceeding to address the influence of larger spatial scale latitudinal gradients, and the analysis of those large scale factors gradients could be confined to sites comparable in terms of local conditions. This topic has of course been studied previously for river macrophytes, but usually only at most at a regional or national scale, and most preceding cognate studies are at smaller spatial scales than that (e.g. Baattrup-Pedersen et al., 2005; Baattrup-Pedersen et al., 2011; Baattrup-Pedersen et al., 2003; Baattrup-Pedersen and Riis 1999; Baattrup-Pedersen et al., 2006; Murphy 2002; Murphy et al., 2003; Sand-Jensen 1989). To the best of my knowledge there has never been a previous comparison of local scale physico-chemical drivers of river macrophyte ecology, at the geographic extent covered by my study, so analysis of the data collected here presents a novel opportunity to examine local scale plant-environment interactions in hardwater rivers across a gradient of temperate, sub-tropical and tropical conditions.

A practical reason that has prevented expanding analyses further is that only a few river macrophyte species show very widespread dispersal (*Ceratophyllum demersum* is arguably one of the very few such aquatic plants for which a case for near-worldwide distribution can be made: see Figure 4-1), making species level analyses difficult or even impractical.

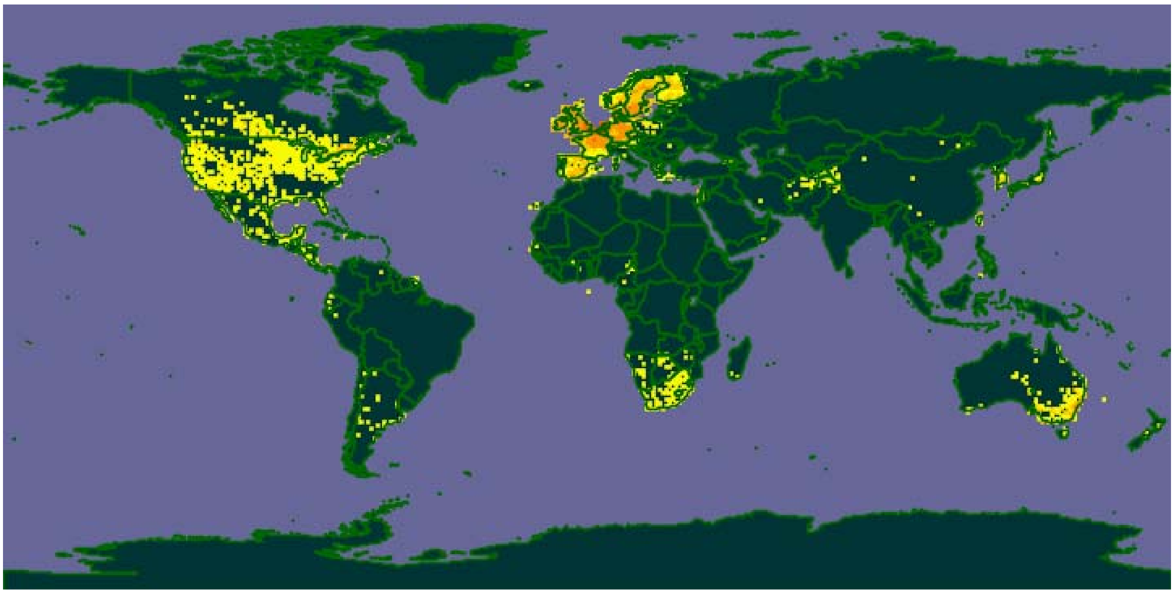


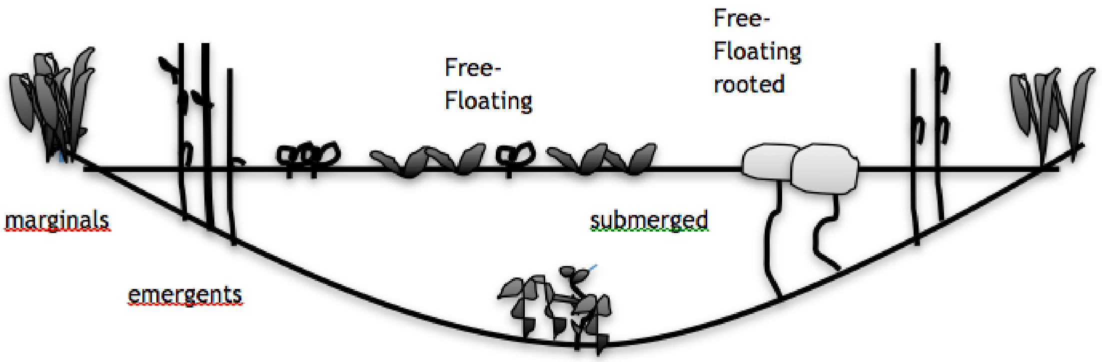
Figure 4-1. Worldwide distribution (tropical, subtropical and temperate) of *Ceratophyllum demersum*.

Origin of map: data.gbif.org/search/ceratophyllum%20demersum

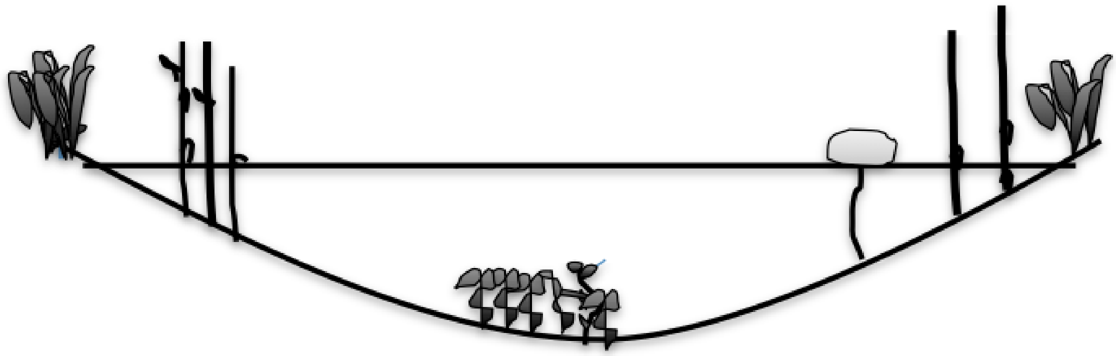
For aquatic vegetation however the occurrence of species within a small set of well-recognised FGs, produced by parallel evolution of river plant species in response to the sets of conditions common to river environments, in all river systems supporting macrophyte vegetation (though of course not all FGs may be represented at a given site) provides an alternative means of comparing the vegetation of river sites. The set of species making up individual FGs may differ between different rivers depending on local conditions (e.g. temperate v. tropical rivers) but it is now clearly established (from a large body of evidence, which originated as long ago as the work of Butcher in the 1930s and which is summarised in detail both by Sculthorpe (1967) and Hutchinson (1975) that each of the five macrophyte FGs, commonly described and defined by their recognizably different “life forms” has specific habitat associations which differ little between rivers, regardless of their geographical location.

Based on the literature cited above on the habitat preferences of the five macrophyte functional groups (Table 4.1; Appendix 1), and my own field observations I developed a series of hypotheses about likely FG occurrence, and species diversity in hardwater rivers, primarily related to flow regime, illustrated in Figure 4.2. At slow flowing sites I would expect the river to have a greater diversity compared to sites with faster flows, and with the presence of all five FGs. At sites with moderate flow, I would expect free-floating species to be absent, and the floating-leaved rooted FG to be less well represented, with more submerged species and with marginal and emergent species dominating the macrophyte community present. At fast flowing sites, I would expect to encounter marginal and emergent species mainly, together with a few specialist fast-flow adapted submerged species (e.g. Batrachian *Ranunculus* species in fast-flowing temperate rivers (up to a certain velocity limit); or species of Podostemaceae in fast-flowing tropical rivers). Because my study was limited to vascular macrophyte species, fast-flowing river habitats typical of high-altitude and/or high-latitude streams were largely excluded from the study (with a few exceptions such as the Greek, and Italian Apennine hill rivers, which do support vascular macrophytes: see previous Chapter) because in such rivers vascular macrophytes are largely absent, with their place instead being occupied by cryptophyte non-vascular species: mainly mosses and liverworts, plus algal periphyton (e.g. Lang and Murphy 2011).

a) Slow flowing



b) Medium flowing



c) Fast flowing

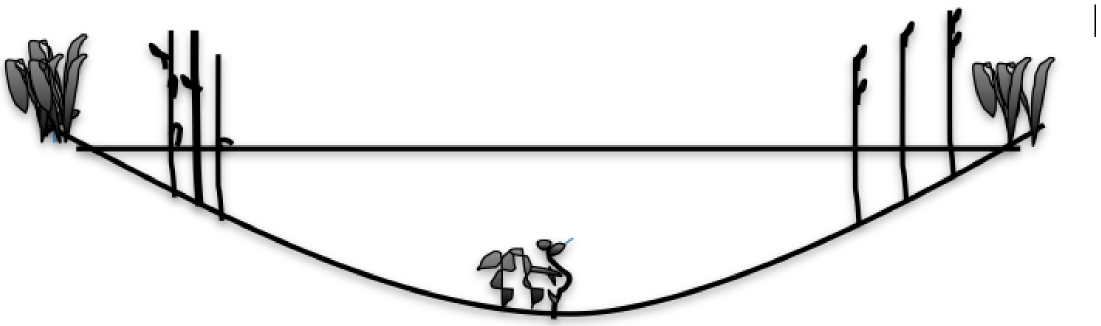


Figure 4-2. Diagrams depicting likelihood of occurrence of river macrophyte FG according to water velocity of the stream: a) slow flowing streams have a potential for large biomass and cover of all groups; b) medium flowing streams do not support free floating species, and emergent and submerged species are dominant, submerged species may be present as a reflection of habitat complexity, i.e. local scale variation with slow areas present in the river system; c) fast flowing waters have fewer FGs present, mainly marginal and emergent present and a few specialist submerged species, again if factors such as presence of physical features such as boulders provided sheltered habitat for them to colonise, or direct habitat for attachment (in the case of tropical Podostemaceae).

Table 4-1. Macrophyte FGs with their physical habitat preferences.

Group	Substrate	Flow and width	Depth	Light availability	Example species (and family)
Marginal	Thick layers of fine sediments, and coarser particles	Moderate	Shallow	High	*Phragmites mauritianus, Vossia cuspidata* (Poaceae)
Emergent	Thick layers of fine sediments, and coarser particles	Moderate	Shallow	High	*Cyperus difformis* , *Pycreus unioloides*(Cyperaceae), *Juncus effusus* (Juncaceae), *Phalaris arundinacea* (Poaceae)
Submerged	Thick layers of fine sediments, and coarser particles	Slow flowing water, deep water and wide channel	Shallow to deep	Low	*Stuckenia pectinata* (Potamogetonaceae), *Callitriche stagnalis* (Callitrichaceae)
Free Floating	Any	Reduced flow	Potentially any, but usually shallow to moderate	Moderate to high	*Lemna gibba* (Lemnaceae)
Floating Leaved Rooted.	Usually as for emergent	Reduced flow	Usually shallow	High	*Nuphar lutea* (Nymphaeaceae)

Hypotheses

1) If light conditions are good slow waters support greater number of species of all FGs than medium or fast sites.
2) Free floating and floating rooted species are absent from medium and fast sites unless suitable sheltered microhabitat is available
3) Successful FGs are not rooted and rooted floating or submerged in slow systems, submerged and emergent in medium and emergent and marginal in fast systems.

4.2 METHODS

Collection methods for data examined here are described in the Methods chapter. To ensure that values of alpha-diversity for each site were directly comparable, i.e. calculated for similar lengths of stream in all cases, in this section I have only included the data personally collected, at selected locations on calcareous rivers in the UK northern and central Scotland; Yorkshire Dales (northern England);, and elsewhere in the world (including Zambia; Bonito, Upper Paraná, Pantanal and Chapadas regions of Brazil; northern and eastern Argentina; Trinidad; northern Florida; western Ireland; Yucatan region of Mexico; and South Africa.

4.3 ANALYSIS PROCEDURES

The number of species per site, within each FG present, were counted, and box plots were created out of the 273 samples across different sites each with measures on width (narrow usually <10m; medium <50m; broad a mean of ≥ 100m), water velocity (slow, moderate, fast), shade (no cover, moderate cover). High shade cover sites were excluded due to the lack of sufficient samples in this category. Firstly sites were grouped on the basis of their width category to make comparisons of which FGs are favoured under certain shade and flow categories. The 480 species were split according to their functional group (Appendix 1).

MIXED EFFECTS MODELS: FUNCTIONAL GROUPS V. ENVIRONMENTAL SITE FACTORS

A linear mixed effects model for each FG was used with number of species as response variable. The fixed effects tested were all ordinal variables and included width, velocity, shading and alkalinity. Model assumptions were met in all analyses.

Country was used as a random effect to account for the potential variation which may occur in the response variable between countries, due to unequal number of sites sampled within each country. This approach permitted me to know the variance of the response due to country, i.e. how the spread of the response variable of each country compare to each other.

For all FGs, the models with the number of species as a response variable were fitted with generalised linear mixed-effects models with a Poisson error structure and a log link function. Tests for over-dispersion were carried out and accounted for within the model structure where appropriate

A backwards model selection procedure was performed for all models using deviance and AIC criteria for examining the significance of the fixed effects. The final models presented include only significant variables. A model fit such as AIC values or deviance value, compares models that are nested, i.e. uses the same dataset and model structures, but the variables included in the model will differ. The best model is the one with the lowest AIC value. The percentage variance explained by random effect is added to the residual value and working out the percentage that the country random effect can explain. In all cases the percentage of variance explained by the random effect was minimal compared to the residual variance (Appendix 4).

When no variables were significant a null model, with no fixed effects and only the random effect, is given in the chapter appendix (5).

Missing rows were removed prior to the analysis to carry out model selection procedures – this reduced the dataset to 234 observations. All analyses were carried out in R.

Boxplots for all FGs against the main environmental variables are provided in Appendix (6-8). Only significant relationships are illustrated.

4.4 RESULTS

My findings showed that in most cases all macrophyte FGs were present in rivers sampled across each of the 10 countries sampled (refer to **Chapter 3** for details of macrophyte assemblages and FGs present in each country). For an example of the different species forming FGs within different countries, in Florida *Althernanthera philoxeroides* (emergent), *Bacopa monnieri* (emergent), and *Eichhornia crassipes* (free-floating) were all species present in these FGS, in Florida but not the British Isles, while *Myriophyllum spicatum* (submerged) was recorded in this FG in both Florida and the British Isles. On the other hand in the British Isles, *Potamogeton natans* (floating-leaved rooted), *Ranunculus penicillatus* (submerged), and *Rumex hydrolapathum* (emergent) were all recorded in these FGs here, but not in Florida (Appendix 3).

Macrophyte number (S: alpha-diversity) was found to be different across the 10 countries and to be significantly related to some environmental variables; such as water velocity, alkalinity and width. Below is a more detailed description of the relationships and effects of environmental variables on macropyte diversity. The physico-chemical parameters that I measured at each site did manage to explain part of the variation in macrophyte diversity.

Overall results from the analyses indicate that the diversity of certain FGs may indicate the environmental conditions at a site. For instance more marginal species were found at sites with low alkalinity and width (narrow) categories than those sites with high alkalinity and width (medium and broad). Also velocity was proved in my study to be important environmental variable for free floating and floating rooted FGs. Last but not least, shading was found to be an important environmental variable for submerged species only.

If light conditions are good slow waters support higher cover of all groups than medium or fast sites.

Shading was only found to be a significant variable for submerged species. A linear mixed effects model (GLM fitted by Laplace) demonstrated that there were significant differences between velocity categories for number of free-floating species (Table 4.2) and floating rooted (Table 4.3) species within the 10 countries sampled.

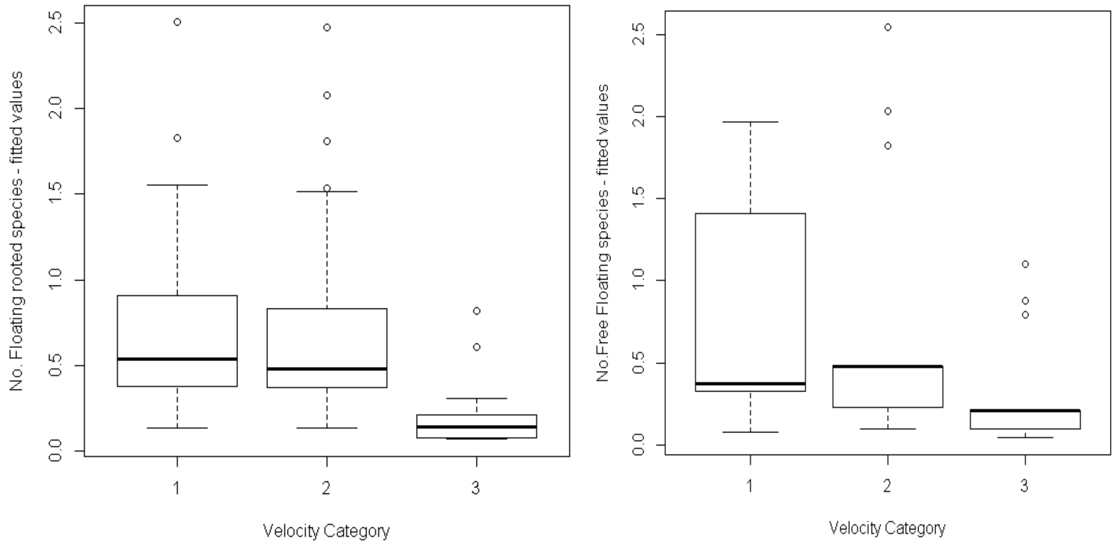
For instance slow water velocity was significantly related to the higher number of free floating and floating-leaved rooted species in streams with relatively low shading (Figure 4-3). However this was not always the case, from my fieldwork observations, I know that some sites, e.g. in South Africa, with slow flow conditions (e.g. Goedspruit, Roihass (Mooi river) did not support any free-floating species. In such cases other environmental factors, e.g. heavy metal water pollution, may have influenced the species assemblages.

Table 4-2. **Statistical results for the final model relating number of free-floating species to environmental variables (General Linear Mixed Model fitted by the Laplace approximation). Significance is coded as follows: P < 0.001***', P < 0.01'**', P <0.05 '*'. The variance explained by country was X^2= 1.8716 ± SD 1.36,based on number of observations = 234, in 10 countries.**

	Estimate	Std. Error	z value	Pr(>|z|)	Significance
(Intercept)	-1.2628	0.4783	-2.640	0.00828	**
Velocity.category2	0.2567	0.1738	1.477	0.13969	
Velocity.category3	-0.5801	0.3286	-1.766	0.07748	

Table 4-3. **Statistical results for the final model relating number of floating rooted species to environmental variables (General Linear Mixed Model fitted by the Laplace approximation). Significance is coded as follows: P< 0.001***', P < 0.01'**', P <0.05 '*'. The variance explained by country was X^2= 1.0195 ± SD 1.0097,based on number of observations = 234, in 10 countries.**

	Estimate	Std. Error	z value	Pr(>|z|)	Significance
(Intercept)	-0.37944	0.40401	-0.939	0.34764	
Velocity.category2	-0.01292	0.19567	-0.066	0.94736	
Velocity.category3	-0.94424	0.40966	-2.305	0.02117	*



a) b)

Figure 4-3. Boxplots of fitted data for a) number of floating rooted species and b) free floating across three velocity categories. 1) slow, 2) moderate, 3) fast.

Successful groups are floating or submerged in slow systems, submerged and emergent in medium and emergent and marginal in fast systems.

Using statistical tools, the numbers of free-floating and floating rooted species were significantly related to the water velocity, and were favoured by slow water flow (Figure 4-3). However velocity was not a significant variable influencing diversity of any other FGs (Appendix 5). For instance the mean number of submerged species did not significantly change with flow, nonetheless submerged species number decreased significantly at moderate shading (Table 4-4, Figure 4-4).

Table 4-4. Statistical results for the final model relating number of submerged species to environmental variables (General Linear Mixed Model fitted by the Laplace approximation). Significance is coded as follows: P< 0.001*', P < 0.01'**', P < 0.05 '*'. The variance explained by country was X^2= 1.3228 ± SD 1.1501 based on number of observations = 234, in 10 countries.**

	Estimate	Std. Error	z value	Pr(>|z|)	Significance
(Intercept)	-0.5008	0.3917	-1.278	0.20108	
Shading.category 2	-0.1586	0.1428	-1.111	0.26659	
Shading.category 3	--0.9548	0.3202	-2.982	0.00286	**

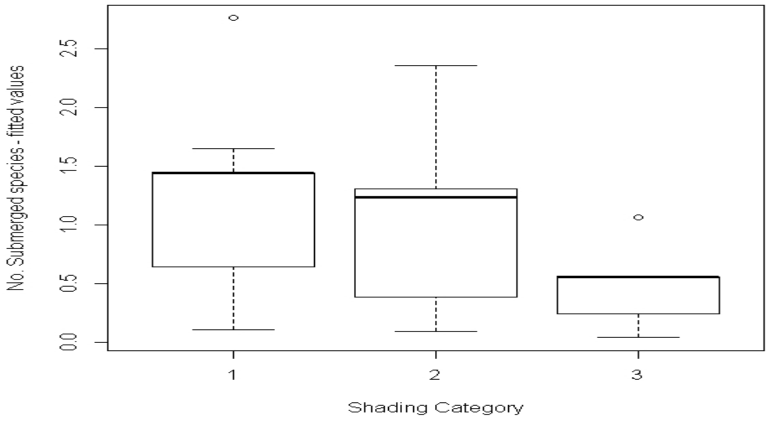


Figure 4-4. Boxplots of fitted data for number of submerged species across three shading categories. 1) none 2) moderate 3) high.

In addition my analysis of the raw data showed marginal and emergent species to be the most successful FGs across all width and flow categories (Appendix 5-8). Similarly free floating FGs were found to be most successful in slow flowing narrow streams, <10m, and floating-leaved rooted species were most successful in slow flowing wider streams.

That velocity was not a significant variable for any other FGs, may have been influenced by my sampling methodology and effort (Figure 4-5 and Figure 4-6: it is clear that the species-effort relationship shows little sign of asymptoting, suggesting that more species would have been found had more sites been sampled) and the influence of other environmental parameters not measured here. For example, from fieldwork observations on sites that were in intensive-agricultural catchments, input of nutrients from runoff and drainage into the target steams is likely to have been an important local driver of diversity, possibly overriding other environmental factors, e.g. water velocity, at these sites.

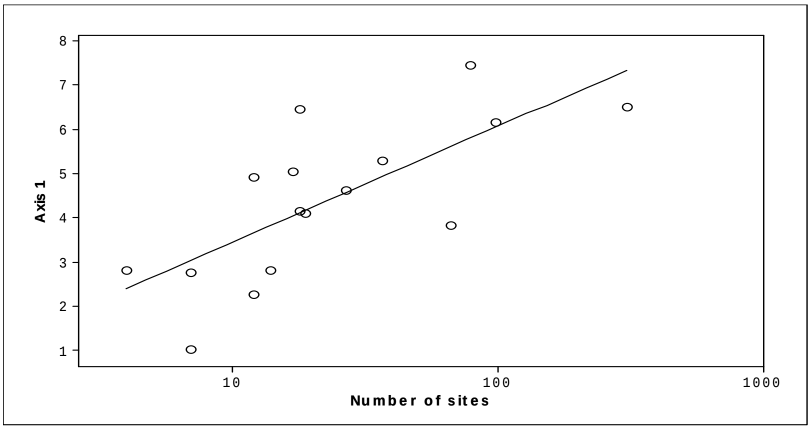


Figure 4-5 Regression analysis **relating Axis 1 to number of sites. Adj R^2 value = 52.8%; $P < 0.001$.**

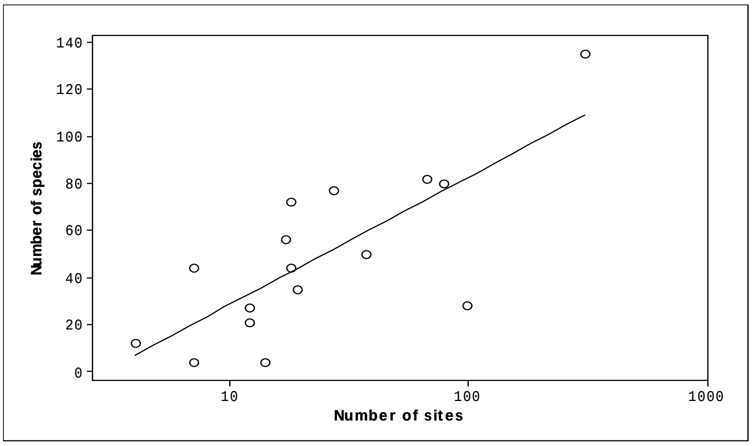


Figure 4-6 Regression analysis relating cumulative number of species to cumulative number of sites sampled. Adj R^2 value = 54.6%; $P < 0.001$.

Free-floating or floating rooted are absent from medium and fast sites unless suitable microhabitat available.

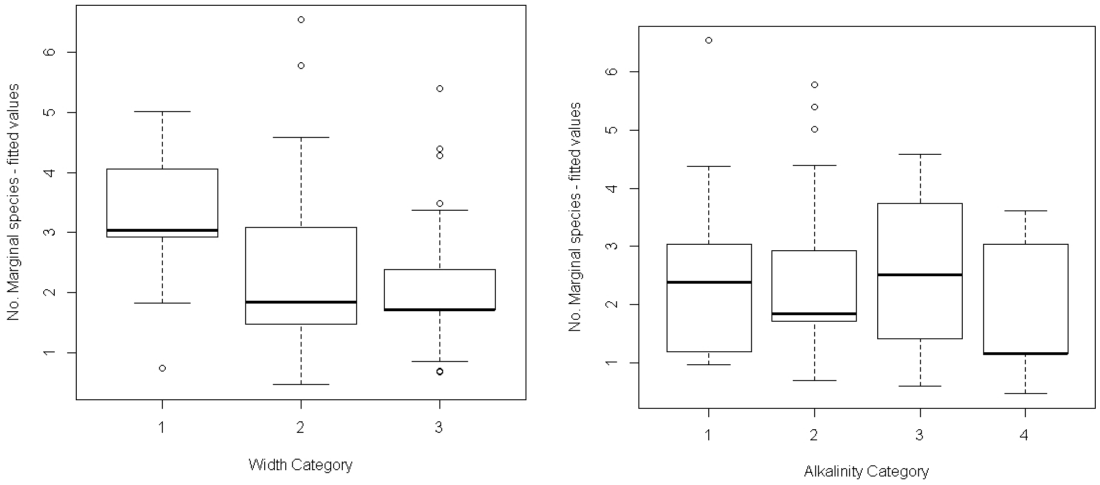
The statistical analysis showed that the number of free floating and floating-leaved rooted species did significantly change with the environmental variables tested (Appendix 5). In addition the boxplots for the raw data showed that free-floating and floating-leaved rooted FGs were present at medium and fast sites (Figure 4-3). This suggests that the presence of a microhabitat for them to utilise may have influenced their presence.

Alkalinity and width

The number of marginal species was significantly negatively related to both alkalinity and width (Figure 4-7). My findings suggest that at higher alkalinities macrophytes have a greater variance and mean. Marginal species were significantly less in number at sites with high alkalinity. Categories 2 and 3 had lower diversity than sites with low alkalinity (category 1); and there were also significantly fewer species in sites with high alkalinity (category 3 and 4) compared to low alkalinity sites (category 1) (Table 4-5, Figure 4-7). Floating rooted species also significantly decrease between alkalinity category 1 and 3 (Table 4-8, Figure 4-9). Emergent FG species similarly decreased in number with increase of width (from category 1 and 3) (Table 4-6, Figure 4-8).

Table 4-5. Statistical results for the final model relating number of marginal species to environmental variables. It is a General Linear Mixed Model fitted by the Laplace approximation. Significance is coded as follows: P< 0.001*', P < 0.01'**', P < 0.05 '*'. The variance explained by country was X^2= 0.43026 ± SD 0.65595 based on number of observations = 234, in 10 countries.**

	Estimate	Std. Error	z value	Pr(>|z|)	Significance
(Intercept)	1.5707	0.2715	5.784	7.28e-09	***
Width.category 2	-0.4626	0.1497	-3.090	0.002000	**
Width.category 3	-0.5326	0.1610	-3.308	0.000940	***
Alkalinity.category 2	-0.3275	0.1200	-2.728	0.006366	**
Alkalinity.category 3	-0.5587	0.1616	-3.458	0.000545	***
Alkalinity.category 4	-0.7968	0.1967	-4.051	5.09e-05	***



a) **b)**

Figure 4-7. Boxplots of fitted data for the a) number of marginal species across four alkalinity categories 1) Marginally hard water (12.2 - 24.27 mg l^{-1}) 2) Intermediate hard water (24.4 - 120.78 mg l^{-1}) 3) Hard water (122 - 242.78 mg l^{-1}) 4) Very hard water (>244 mg l^{-1}) HCO_3 and b) width category 1) narrow, 2) medium, 3) broad.

Table 4-6. Statistical results for the final model relating number of emergent species to environmental variables (General Linear Mixed Model fitted by the Laplace approximation). Significance is coded as follows: P< 0.001*', P < 0.01'**', P < 0.05 '*'. The variance explained by country was X^2= 0.45332 ± SD 0.21291 based on number of observations = 234, in 10 countries.**

	Estimate	Std. Error	z value	Pr(>|z|)	Significance
(Intercept)	1.6134	0.1383	11.662	< 2e-16	***
Width.category 2	-0.3813	0.1295	-2.945	0.00323	**
Width.category 3	-0.3535	0.1335	-2.648	0.00809	**

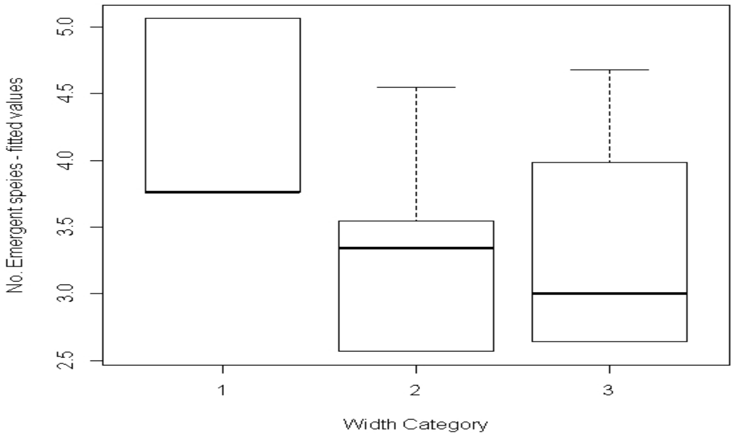


Figure 4-8. Boxplots of fitted data for a) number of emergent species across width category 1) narrow, 2) medium, 3) broad.

Table 4-7. **Statistical results for the final model relating number of floating rooted species to environmental variables (General Linear Mixed Model fitted by the Laplace approximation). Significance is coded as follows: P< 0.001***', P < 0.01'**', P < 0.05 '*'. The variance explained by country was X^2= 1.095 ± SD 1.0097 based on number of observations = 234, in 10 countries.**

	Estimate	Std. Error	z value	Pr(>|z|)	Significance
(Intercept)	-0.37944	0.40401	-0.939	0.34764	
Alkalinity.cat2	-0.36419	0.23392	-1.557	0.11950	
Alkalinity.cat3	-0.84292	0.32250	-2.614	0.00896	**
Alkalinity.cat4	-0.67789	0.35120	-1.930	0.05358	

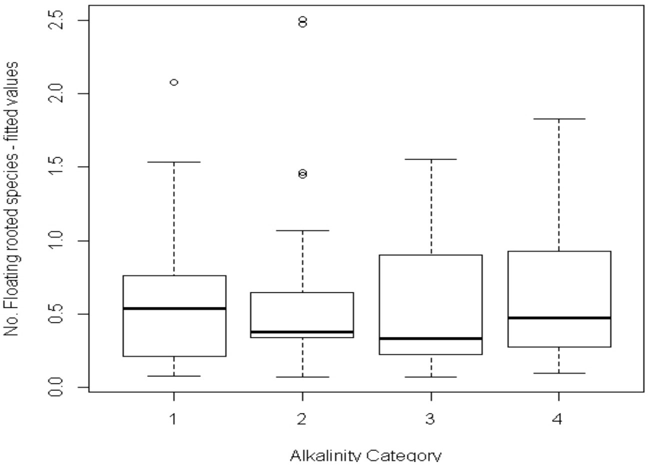


Figure 4-9 Boxplots of fitted data for the number of floating rooted species across four alkalinity categories 1) Marginally hard water (12.2 - 24.27 mg l^{-1}) 2) Intermediate hard water (24.4 - 120.78 mg l^{-1}) 3) Hard water (122 - 242.78 mg l^{-1}) 4) Very hard water (>244 mg l^{-1}) HCO_3.

4.5 DISCUSSION

My results demonstrate that the diversity of macrophyte functional group assemblages is influenced by local environmental factors. Physical factors shown to influence macrophyte assemblages significantly were: water velocity for free-floating and floating rooted species; width for marginal and emergent species and shade for submerged species.

As for chemical factors alkalinity was found to have a significant relationship with diversity of marginal and floating rooted species.

If light conditions are good slow waters will support greater number of all groups than medium or fast sites.

I was able to show how slow flow conditions enhanced the number of free-floating and floating rooted species. Free floating species such as *Eichhornia crassipes* are likely to be found in greater numbers in slow flow conditions as they do not posses any anchoring root-system that would allow them to withstand faster flows and as a result tend to be washed away. In riverine systems where water velocities can exceed 1 m s^{-1}, *Eichhornia crassipes* is expected to accumulate at a greater rate in hydrodynamically (i.e. as a function of water currents) less-active environments such as embayments or coves. At low water velocities wind can dominate transport given sufficient air velocity (Downing-Kinz and Stacey, 2011). Previous qualitative descriptions of *Eichhornia crassipes* transport in the environment state wind as the primary forcing mechanism (Penfound and Earle, 1948; Bock, 1969). Most of the sites sampled for my study have a low gradient (i.e. a more nearly level streambed, and sluggishly moving water, compared to a high gradient (i.e. a steep slope and rapid flow of water), which has more ability to erode than a low gradient streams.

The distribution of macrophytes is also related to their large-scale ability to disperse vegetative or sexual propagules as well as their ecological tolerance (Hutchinson, 1975). For example free-floating plants, e.g. *Eichhornia crassipes, Salvinia molesta, Pistia stratiotes,* can benefit from slow waters by allowing them to reproduce clonally, rapidly forming massive standing mats covering large areas of the water and increasing the drag force (Lacoul and Freedman, 2006b; Downing-Kinz and Stacey, 2011).

In terms of the other FGs, their diversity was not found to be significantly related to flow. Unlike free-floating species, submerged species do possess an anchoring root-system that enables them to live in areas with greater flow, allowing them to exploit other suitable habitats. Some submerged species are better adapted to withstand greater shear friction than others e.g. *Ranunculus* species are almost entirely submerged and can compress and bend to reduce drag force (O'Hare et al. 2012). Contrary to my findings, other studies do find submerged species to be favoured in faster flowing streams. This has to do with the lower underwater gas rate exchange and hence carbon uptake in slower flowing waters compared to faster flowing streams. Thus limiting photosynthesis processes can occur under slow flow conditions (Madsen and Sand-Jensen, 2006).

In terms of diversity of marginal and emergent FGs, I did not find a significant relationship with flow. Previous studies have found such species to be related with water depth (i.e. flooding duration), which in turn is also affected by water flow (O'Hare et al. 2011). Auble, Friedman and Scott (1994) also showed riparian vegetation to substantially change accordingly to the duration of the flow, which in turn is correlated with sediment deposition, erosion and shear stress to name a few relevant variables. Moreover marginal and emergent species have also been found to be very sensitive to changes in flow boundaries, e.g. at high inundation duration riparian vegetation is likely to have greater and more frequent shear stress than sites with low inundation duration (Hupp and Osterkamp, 1985; Auble, Friedman and Scott, 1994; Chapin, Beschta and Wen Shen, 2002).

Free-floating and floating-leaved rooted species are absent from medium and fast sites unless suitable microhabitat available.

Surprisingly floating-leaved rooted and free-floating species were not entirely absent from sites categorised as medium and fast flowing. Field observations indicated the presence of suitable microhabitats for this FG within some fast flowing river systems. Such is the case for a number of sites in Zambia (e.g. Zambezi, Kafue, Lupososhi rivers), Scotland (e.g. Siabost stream, Lewis; South Medwin river), Ireland (Beagh River) where slow flowing sections of the stream provided refugia, by decreasing the inflicted shear force, and as a result plants of this FG are not washed away. Lesser erosion and more stable conditions provided in these microhabitats will also provide more suitable substrate (e.g. a mixture of material, including rocks) for floating-leaved rooted species to root into at the edges of the riverbank.

One other explanation for this finding would be the role of connectivity between water bodies. Such is the case for the Zambezi, an extensive water body with microhabitats connected with the main channel, where reproductive dispersal and connectivity mechanisms, i.e. connection between sites, enhance FG distribution between microhabitats (e.g. slow flow waters). This could sustain macrophyte populations in otherwise unfavourable habitats; and may suggest a spatial component to the distribution of macrophytes (French and Chambers, 1996; Lacoul and Freedman, 2006a). Previous studies have found how the proximity of other waterbodies has an impact on the local species composition and richness of macrophyte communities (Van den Brink et al., 1991; Bornette et al. 1998); with an exception in floodplain lakes in the Netherlands (Van Geest et al. 2003). Similarly a study carried out in British ponds looking at macrophyte richness found a positive correlation between richness and neighbouring waterbodies (Linton and Goulder, 2000). Furthermore microhabitat heterogeneity is related to substrate quality, local anthropogenic influences and flow regime (itself related to topography) can also enhance macrophyte richness (Ormerod et al. 1994; Suren and Ormerod, 1998). Conversely facilitated dispersal by hydrologic connectivity can result in more homogenous species communities of aquatic plants in lotic habitats compared with lentic ones (Bornette et al. 1998; Williams et al. 2003).

Successful groups are floating or submerged in slow systems, submerged and emergent in medium, and emergent and marginal in fast systems.

My analysis of the raw data showed marginal and emergent species to be the most successful species across all flow categories (Appendix 5). Similarly free-floating and floating-leaved rooted species were found to be successful in slow flowing streams. Success of a specific FG can be explained in terms of mechanical stresses produced by water (tidal flows, current, wind) that can have a great impact on species distribution and community dynamics (Vogel, 1994; Denny, 1988). Puijalon et al. (2005) found that plants' phenotypic plasticity or local selection were a function of hydrodynamic dynamics (i.e. the capacity to minimize mechanical forces). For example alterations to the root system (e.g. increased root development) can increase plants' resistance to uprooting, e.g. *Ranunculus* spp. (Crook and Ennos, 1996; Niklas, 1996). My recordings of *Ranunculus* species in moderate and fast flowing waters corroborate this.

Overall large biomass and richness of macrophyte communities has been previously demonstrated to be linked with water velocities of 0.3 - 0.4 m s^{-1}, declining at water velocities of 0 6 m.s^{-1}, and at >1.0 m s^{-1} rivers are inhospitable habitat for most aquatic vascular plants (Chambers et al. 1991; Riis and Biggs, 2003). Others have found that macrophyte communities in running waters are best developed in moderate flow waters with tolerable physical stress and enhanced nutrient supply (Lacoul and Freedman, 2006b). Moreover species respond in different ways to high-flow conditions. Species recorded in water flows up to 0.4 m s^{-1}(sometimes even faster) include *Elodea canadensis, Potamogeton cheesemanii, Rorippa nasturtium-aquaticum* and *Ranunculus aquatilis* (French and Chambers, 1996; Riis and Biggs, 2003). Puijalon (2007), in a study focusing on four aquatic plant species (*Luronium natans, Mentha aquatica, Potamogeton coloratus, Sparganium emersum)* chosen for ability to colonize both running and standing waters, found plastic differences that enhanced their hydrodynamic performance in different ways under running water conditions.

Although my results did not find submerged species to be the most successful FGs at moderate flows, I did record them in some sites e.g. Scotland (Mouse

Water), Zambia (Kasanka River, Ngweze), USA (Silver River, Silver Glen, Santa Fe River) as the most dominant group under these conditions. Despite the fact that water flow is a key factor for macrophyte distribution, other factors can also influence their presence, accounting for part of my findings. For instance in large rivers gradients of turbidity have been shown to be important in predicting the distribution and abundance of aquatic plants (e.g. Murphy et al. 2003). Turbidity, shading of riparian vegetation, and water colour are factors that affect the depth of the euphotic zone (where sufficient light is available for photosynthesis to take place) limiting the presence of submerged species, some submerged species being more shade-tolerant than others (Murphy & Eaton, 1983; Sand-Jensen and Borum, 1991, Kalf, 2001).

Thus water depth can be used to a certain extent as a surrogate of light availability (affected by water turbidity), nonetheless light availability depends heavily on turbidity (Chambers and Kalff, 1985; Squires et al. 2002); and the exponential attenuation of irradiance with depth (Sand-Jensen and Borum, 1991). Macrophyte FG dominance is to some extent related to the light availability conditions. For instance in low-light conditions in shallow littoral zones, emergent species are the dominant group, while free-floating species dominate deeper waters (Bini et al., 1999; Vestergaard and Sand-Jensen, 2000; Squires et al. 2002). To determine light availability conditions at my sites I took into consideration riparian shading effect within all FGs, and underwater light water attenuation (k), i.e. clarity of water effect, only for submerged species. For my study submerged species were significantly related to shading. Previous studies have also found shading by riparian trees to reduce the abundance of all types of macrophytes in narrow river channels (Canfield and Hoyer, 1988). Moreover marginal and emergent species did not show a significant relationship with light (i.e. riparian shading). Previous studies have found such species to be related with water depth (i.e. flooding duration) (O'Hare et al. 2011). Usually emergent species and floating-leaved aquatic plants rarely grow in water deeper that 3 m (Canfield and Hoyer, 1992), with few exceptions e.g. *Trapa bispinosa* recorded to be rooted in hydrosoil as deep as 5m below the surface (Lacoul, 2004). This highlights the importance of measuring depth for future studies for a better picture of these FGs.

Last but not least, chemical factors can also have an impact on macrophyte survival. In my results I found marginal and floating rooted species to be related to differences in water alkalinity, despite the fact that this study deliberately concentrated only on hardwater systems. Higher number of marginal species was found at higher alkalinities. There is good evidence showing that some submerged species have an ability to use bicarbonate in photosynthesis (e.g. *Potamogeton sp.*) while others have a weaker, or no, ability to use this form of dissolved C e.g. *Myriophyllum alterniflorum* (Spence and Maberly, 1985; Madsen and Sand-Jensen, 1994; Riis, Sand-Jensen and Vestergaard, 2000). High concentrations of carbon dioxide are available in most streams, however, high concentration of bicarbonates in alkaline streams are used by species to keep high photosynthesis throughout the day, which can be extremely important for sites with dense macrophyte stands (Sand-Jensen and Frost-Christensen, 1999). Although field observations suggest the marginal vegetation of alkaline systems, such as chalk streams, can be particularly productive (O'Hare *pers comm.*) there is no direct evidence from the literature to suggest why this may be the case.

Conclusions

As demonstrated in different parts of this chapter, it is possible to identify different diversity responses of macrophyte FGs to environmental conditions, at a local scale, in hardwater rivers. Taking into consideration that each species will have specific response thresholds to different environmental factors, macrophytes have the potential to be used as an indicator of environmental changes within a study region. Knowledge of the environmental factors within a habitat, allowed me to show the effects they have on macrophyte diversity distribution. Width and flow were found to be significantly affecting the distribution patterns of diversity of free-floating and floating-leaved rooted species, whereas diversity of marginal species was significantly related to alkalinity and width, and floating-leaved rooted diversity was significantly related to alkalinity. Last but not least submerged species were related to shading.

For future studies, it is worth considering allocating some effort to the number of sites, e.g. based on their width, for a more balanced dataset. As the sites being sampled were being visited for the first time it was impossible to impose a carefully balanced design. In addition it would be beneficial to record environmental variables such as: flow, width, as continuous data rather than categorical data, giving more flexibility for statistical analysis. However this may not always be possible, due to technical problems. For example in rivers like the Paraná, in Brazil, this may not be feasible because of the size of the catchment. In other places like the Zambezi, in Zambia, due to the presence of wild life, e.g. crocodiles, hippopotamus, elephants, it may not be safe to do so.

It would also be cost-effective to try to run some nutrient (e.g. phosphorus, nitrogen) analysis on the water samples. Nutrients are often found to be successful indicators of aquatic plant community structure. In my study, carried out at remote locations without access to laboratory facilities, this was not feasible. Phosphorus in particular is labile and samples taken from hard water systems must be analysed soon after collection (Wetzel, 2001).

In addition inclusion of other variables like slope, substrate and depth (Sand-Jensen, 1989; Gordon, McMahon, and Finlayson, 1992; Auble, Friedman and Scott, 1994; Skubinna et al. 1995; Vestergaard and Sand-Jensen, 2000), can also improve our understanding of the factors influencing macrophyte distribution, as in previous studies.

So far I have looked at the effect of local environmental factors on macrophyte distribution, explaining some of the variation in the distribution of vegetation diversity. Knowledge about the possible impacts of local conditions enables me to address latitudinal gradient effects (regional factors), utilising sub-sets of sites with comparable local conditions. In **Chapter 5**, I aim to compare a wide range of habitats sampled using the same techniques across a wide geographic area to look at the effect of latitudinal gradients on macrophyte diversity distribution.

Chapter 5. Testing regional versus local factors as drivers of calcareous river diversity of macrophytes: case study of the British Isles and Zambia

5.1 INTRODUCTION

Geographic patterns of species distribution are central to ecology (Currie, et al. 2003). As illustrated in previous chapters aquatic plant distribution across different parts of the world varies considerably in species richness and assemblage patterns. Recently, considerable progress has been made toward documenting broad-scale patterns of plant richness (Mutke and Barthlott, 2005); Barthlott et al. 2005; Kreft and Jetz, 2006). Species richness, the most basic index of biodiversity, differs significantly over extensive spatial scales (Gaston, 1991; Francis and Currie, 2003). Many theories have been proposed to explain the observed geographical patterns of species richness. Even amongst closely-related aquatic plant species there may be wide variation in their extent of distribution. Some are widespread, occurring on more than one continent, in part due to their several dispersal mechanisms, with a good example being *Phragmites australis* (Figure 5.1). Others have very restricted distributions, an example being *Phragmites mauritianus*, the world distribution of which is limited to southern to central Africa (Figure 5.2).

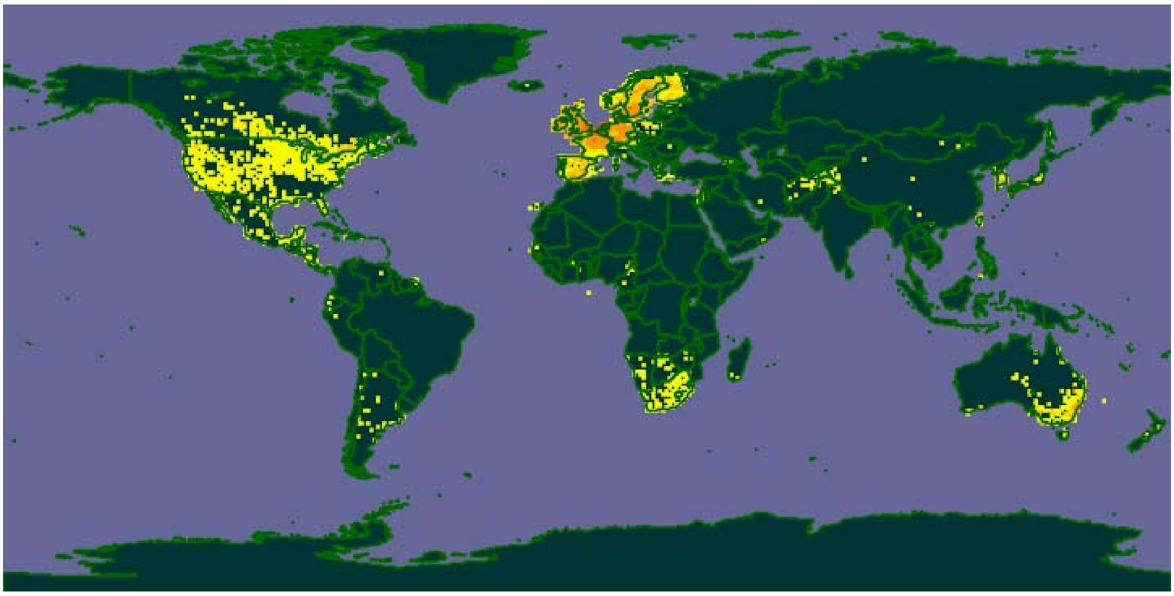


Figure 5-1.World distribution of *Phragmites australis*. **Origin of map: data.gbif.org/search/phragmites%20australis**

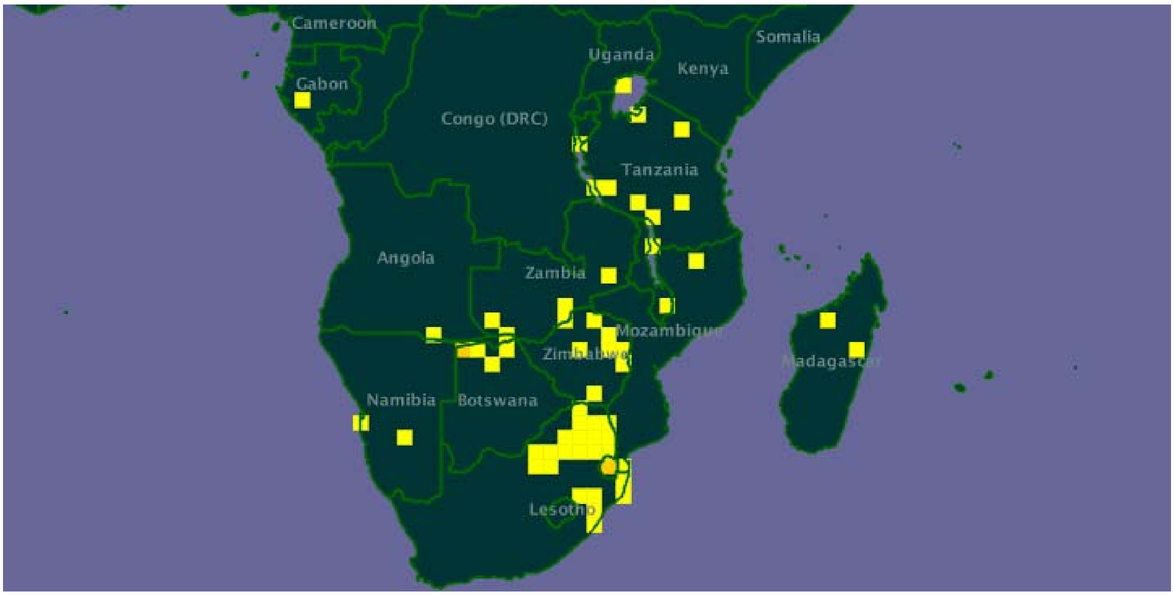


Figure 5-2. World distribution of *Phragmites mauritianus.* **Origin of map: data.gbif.org/search/phragmites%20mauritianus**

Factors interacting with macrophytes can be considered at various scales. One is the regional scale related to geography (e.g. temperate versus tropical) and environmental interactions (e.g. alkalinity). This is followed by catchment or medium scale, where hydrological ecosystems and the conditions of the system are considered. Lastly the local scale, related to specific habitats and communities, and the biological interactions which go on at this level, such as herbivory and competition (Lacoul and Freeman, 2006). Environmental factors affecting species' distribution and richness differ and interact (spatially and temporally) according to biogeography (e.g. latitude and altitude), climate (e.g. temperature) and geomorphology (e.g. basins attributes, topography). Biodiversity distributional patterns have been variously explained by hypotheses on niche space and interspecific interactions (Chase and Leibold, 2003); habitat heterogeneity and area (Prestons, 1962; Kerr and Packer, 1997); habitat stability (Mac Arthur, 1965), ecosystem function (Ehrlich and Ehrlich, 1981); species energy interaction (Allen et al. 2002); invasive species interactions (Elton, 1958); intermediate disturbance and dispersal potential (Grime, 1973); and landscape filter concept (Poff, 1997). I hereafter describe a few, for example the niche theory looks at each species' ecological preferences, i.e. the habitat that provides each species with their optimal living conditions and thus maximizing its survival (Hutchinson, 1975). The landscape filter concept emphasizes the structure of local river communities as a result of a set of environmental factors that shape certain biodiversity patterns (Poff, 1997). Species richness patterns explained on the basis of area suggest species richness to increase with large areas (Arrhenius, 1921; Preston 1962). In terms of species-energy interactions, previous studies have shown how variation in species richness can be explained in terms of temperature on species metabolism (Allen et al. 2002).

In addition, there has been a recent consensus that community structure is affected by the sum and interactions of several processes occurring at various spatial scales (Borcard et al., 2004). Spatial relationships, combining local processes and dispersal in shaping community structure have mainly given rise to metacommunity ecology (Hanksi and Gilpin, 1991; Holyoak et al., 2005, Leibold et al., 2004). Reports based on the spatial variation of organisms across different latitudes have increased substantially our understanding of the geographic distribution of species richness (Hillebrand, 2004). Hence modelling spatial patterns at multiple temporal and spatial scales can be an important approach to understand the functioning of ecological communities (Borcard et al., 2004).

At a small spatial scale, species richness is normally assessed using survey data, linked to local factors, such as environmental variables, interspecific interactions and habitat complexity. Whereas at a broad-scale, grid-based data are required in order to see the richness gradients and their interactions with climate (Hillenbrand, 2004). Modelling spatial patterns at multiple temporal and spatial scales has been carried out previously in stream research (Poff, 1997). However information of large-scale richness patterns in freshwater ecosystems is less well developed, with the exception of, perhaps, fish (Hof et al., 2008). Streams provide a challenge when studying species richness, as they are organized as natural spatio-temporal hierarchies, meaning that species richness is influenced by local in-stream variables, regional environmental factors, and catchment characteristics.

Two previous studies have assessed the relationships between environmental factors and assemblage of aquatic vascular plants on a global scale (Chambers et al., 2008; Crow, 1993). Other studies have shown a variation in species richness (as a measure of diversity) in freshwater vascular plants as a function of a limited latitudinal gradient in the northern hemisphere only (Baattrup-Pedersen et al., 2006; Rorslett, 1991).

My case study aims to address how local (e.g. pH, conductivity, shade cover, flow, alkalinity), regional (e.g. range in elevation, temperature and precipitation) and spatial factors may interact with each other and affect macrophyte species richness, contrasting a temperate (British Isles) versus a tropical (Zambia) case scenario. Despite the recent success in this field, combined analysis of spatial and environmental factors has never been applied to macrophyte communities of designated conservation value (Capers et al., 2010). I aim to illustrate the geographical interplay of different environmental and spatial factors as predictors of macrophyte species richness. The outcome is likely to prove useful for identifying richness patterns of aquatic plants that still escape our understanding. This type of analysis can then further be used to verify if the patterns detected in terrestrial systems are similar to those detected in aquatic systems.

Hypotheses:

1) Can variance in macrophyte distribution patterns be attributed to spatial and environmental factors in the British Isles and Zambia?
2) Are spatially structured environmental variables important?
3) Are there any differences in the influence of climatic factors between a temperate region such as the British Isles, and a tropical region, Zambia attributable to their climatic regions?

5.2 GENERAL METHODS

Large datasets were required for this section of my study. Thus my analysis was carried out for the British Isles with 1151 sites and 106 species and for Zambia with 203 sites and 260 species. Spatial variables were created using an eigenfunction spatial analysis procedure called principal coordinates of neighbour matrices (PCNMs) (Borcard and Legendre, 2002; Griffith and Peres Neto, 2006). For the environmental variables, local conditions (pH, alkalinity) and climatic factors (e.g. temperature seasonality, annual precipitation) were included (refer to methods section).

5.2.1 DATA ANALYSIS

Spatial variation of macrophyte species richness and community structure in hardwater streams in river basins of the British Isles were assessed at two spatial extents (i.e. national (Britain plus Ireland combined) and local: River Basin Units). Spatial variation of macrophyte species richness and community was assessed at a national level only in Zambia, due to the smaller dataset available for this case study. To evaluate the spatial patterns in species richness, eigenvector-based spatial filters were created using PCNM (principal coordinates of neighbour matrices) eigenfunctions (Griffith and Peres Neto, 2006; Astorga et al. 2011; O'Hare et al. 2012a). Spatial analyses were carried out with the geographical coordinates (longitude and latitude) from each stream and river site in the British Isles and Zambia, that were obtained using a Garmin GPS in the field. Each analysis aims to address how local, regional and spatial factors may interact with each other and affect macrophyte species richness, while contrasting a temperate (British Isles) versus a tropical (Zambia) case scenario. Partitioning of variance (i.e. pure environmental, pure spatial, environmental spatially structure) was carried out for each model as done in previous studies (Peres-Neto, et al., 2006).

5.3 RESULTS

My findings support the existence of spatial components attributed to the distribution of macrophytes in the British Isles and Zambia. PCNM analysis illustrated macrophyte species richness and community variation to be significantly related partially to pure environmental, pure spatial and environmental spatially structured factors (Table 5-1, 5-2). In the case of the British Isles, pure environmental factors and environmental spatially structured factors were found to explain some of the variation observed in species richness and community structure. In Zambia, species richness was explained only by pure spatial factors, whereas at the community level, space factors and some environmental factors explained some of the variation observed.

British Isles

National scale - species richness

The total species number for the British Isles was 106 species consisting of 58 emergent species, 14 floating species and 34 submerged species. Macrophyte species richness variance, across all the six recognised major River Basin Units in the British Isles (Table 5-1, Figure 5-3) was mainly explained by the spatially structured environmental component (11.4%). The pure environmental component (e.g. alkalinity, temperature seasonality) explained 2.1% of the variation and the pure spatial component explained 8.8% (PCNMs 4, 20, 100). Both fractions were statistically significant (Table 5-1, Figures 5.3-5.7).

National scale – community structure

Analysis at the community variation level in the British Isles was explained by the shared fraction of environmental and spatial factors (3.9%). Pure spatial factors (PCNMs 1, 4, 2) explained 5.4% of the variance. In contrast pure environmental factors (e.g. Annual precipitation, Min temperature of coldest month, precipitation of warmest quarter) taken into account only managed to explain 1% of the variance (Table 5-2 and Figures 5.4-5.7).

Regional (RBU) scale – species richness

Macrophyte richness variation within each of the six individual River Basin Units comprising the British Isles was explained by spatial factors across RBUs. For instance spatial factors explained some of the richness variation observed in N England (14%), SE England (5%), SW England and Wales (10%), and N Ireland (13%). In addition spatial richness for N England and SE England retained high spatial variables indicating patterns at broad scales; conversely SW England and Wales and N Ireland retained low PCNMs numbers indicating finer spatial patterns. Species variation in Scotland and S Ireland remained unexplained for my study (Table 5.1). In terms of environment "effect" this was only shown at broad-scales (Table 5.1).

Regional (RBU) scale – community structure

Macrophyte community variation within basins differed among regions. For instance in Scotland variance observed at a community level was explained by a shared fraction of environmental spatially structured factors (6.9%). The pure environmental component (e.g. alkalinity, temperature seasonality and min temperature of coldest month) was significant and explained 2.8% of the variation, spatial factors (e.g. PCNMs 3, 1, 4) contributed to 1.3% of the variation. N England river basin community variation was explained by pure environment factors (max temperature of warmest quarter) 2.5%, pure spatial factors (e.g. PCNMs 1, 6, 4) 3.6% and environmental spatially structured factors (e.g. max temperature of warmest quarter, altitude, min temperature of coldest quarter) (4.5%). Community structure for SE England was explained by pure environment (e.g. precipitation of coldest quarter, max temperature of warmest month, precipitation seasonality) (1%), pure space (e.g. PCNMs 8, 1, 21) (2.0%) and environmental spatially structure factors (7.0%). In the SW England and Wales RBU community variation was explained by different factors i.e. pure environmental (e.g. precipitation of coldest quarter, precipitation of warmest quarter, altitude) (1.5%), pure spatial (e.g. PCNMs 2, 1, 8) (4.2%), and environmental spatially structured contributed too (2.3%). Macrophyte community variation in the S Ireland RBU remained unexplained and N Ireland basin community variation was only explained by spatial variation (e.g. PCNMs 4, 1) (4.1%). Spatial community variance observed for SE England and SW England and Wales retained vectors high PCNMs numbers indicating patterns at broad scales in combination with some low numbers too; conversely river basins in Scotland, N England and N Ireland retained low PCNM's numbers indicating finer spatial patterns (Table 5.2 and Figures 5.4-5.7).

Table 5-1. Spatial and environmental models for macrophytes species richness in the British Isles as a whole and for each Regional Basin Unit (RBU). The order of the spatial and environmental models is given according to the level of importance.

Region	Environmental variables in final model	Spatial variables in final model (PCNM)	P Global Environment	P Global Spatial	P environment	P spatial	AdjR^2 shared	AdjR^2 environment	AdjR^2 spatial
British Isles	Alkalinity Temperature Seasonality, Max. Temperature of Warmest Month, Min Temperature of Coldest Month, Mean Temperature of Wettest Quarter	4, 20, 100, 6, 16, 21, 8, 525, 166, 99, 23, 383, 42, 39, 101, 438, 135, 102, 320	**0.0002**	**0.0002**	**0.0002**	**0.0002**	0.114	0.021	0.088
Scotland	None	none	0.9016	0.6472	-	-	-	-	-
N England	None	81, 7, 16, 19, 65, 61, 75	0.0810	**0.0344**	-	**0.0002**	-	-	0.144
SE England	None	106	0.3656	**0.0054**	-	**0.0002**	-	-	0.059
SW England and Wales	None	1	0.1078	**0.0002**	-	**0.0298**	-	-	0.109
N Ireland	None	4, 6	0.1888	**0.0004**	-	**0.001**	-	-	0.138
S Ireland	None	none	0.4012	0.5122	-	-	-	-	-

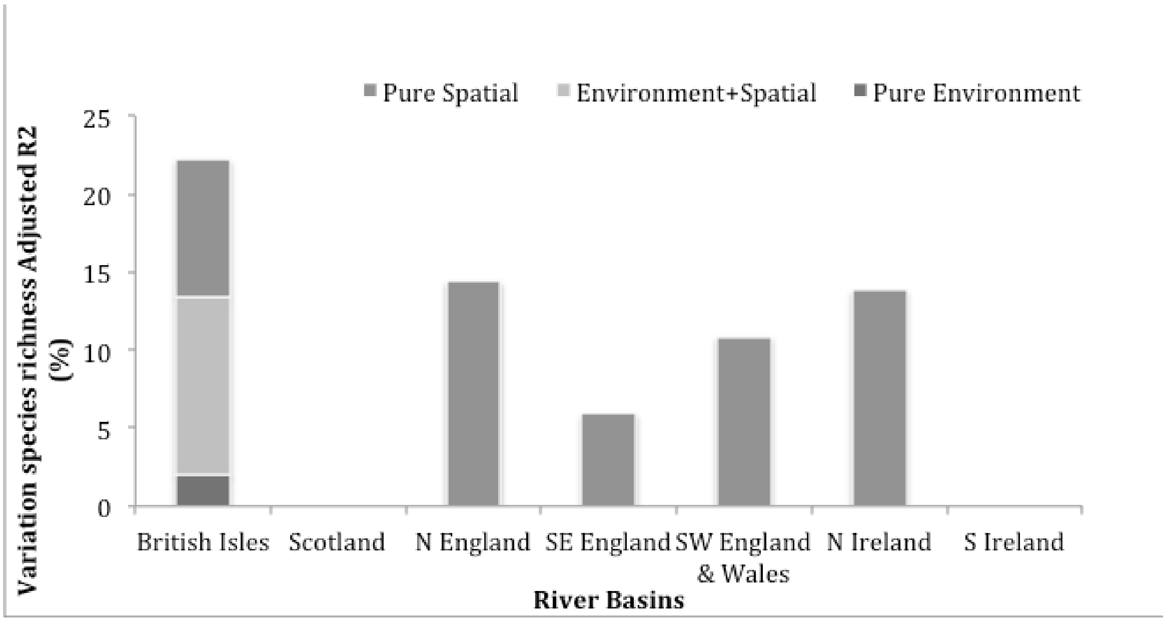


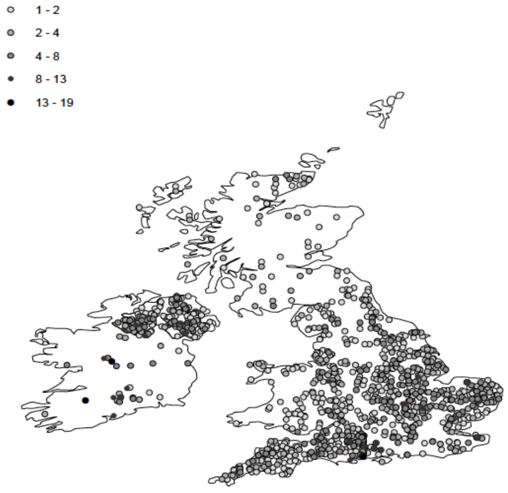
Figure 5-3. Partitioning of variation in macrophyte species richness for the British Isles and for each RBU.

Table 5-2. Spatial and environmental models for macrophytes species community at the British Isles as a whole and for each Regional Basin Unit (RBU).

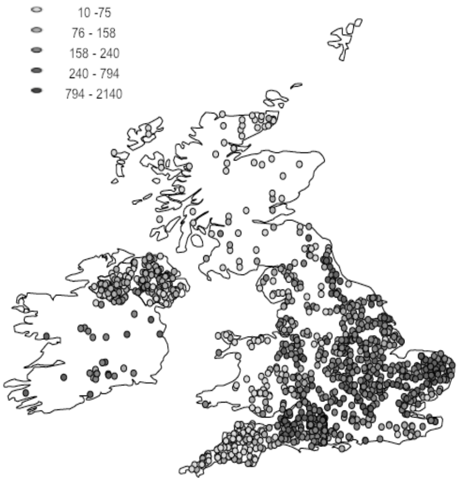
Region	Environmental variables in final model	Spatial variables in final model (PCNM)	P Global Environment	P Global Spatial	P environment	p spatial	Adj R2 environment	AdjR2 shared	Adj R2 spatial
British Isles	Annual Precipitation, Precipitation of Warmest Quarter, Min Temperature of Coldest Month, Temperature seasonality, Max Temperature of Warmest Month, Altitude, Alkalinity, Precipitation Seasonality, Mean Temperature of Wettest Quarter, Precipitation of Coldest Quarter, Mean temperature of Wettest Month, Precipitation of Coldest Quarter, Annual Mean Temperature, Actual evapotranspiration	1, 4, 2, 3, 5, 20, 10, 9, 6, 16, 7, 14, 12, 8, 11, 15, 19, 18, 24, 193, 21, 22, 17, 28, 53, 25, 54, 27, 47, 45, 23, 41, 338, 56, 65, 387, 26, 522, 51	**0.005**	**0.005**	**0.005**	**0.005**	**0.011**	**0.039**	0.054
Scotland	Alkalinity, Temperature Seasonality, Min temperature of Coldest Month, Mean Temperature of Wettest Quarter, Precipitation of Coldest Quarter	3, 1, 4, 28	**0.028**	**0.005**	**0.018**	0.103	**0.028**	0.069	**0.013**
N England	Max temperature of Warmest Quarter, Altitude, Min Temperature of Coldest Quarter, Temperature seasonality, Mean temperature of Wettest Quarter, Alkalinity, Precipitation Seasonality, Precipitation Seasonality, Precipitation of Coldest Quarter, Temperature Seasonality Mean temperature of Wettest Quarter, Alkalinity , Precipitation of Coldest Quarter, Precipitation of Warmest Quarter, Annual Precipitation	1, 6, 4, 11, 9, 14, 3, 13, 7, 2, 15, 52	**0.005**	**0.005**	**0.005**	**0.005**	**0.025**	**0.045**	**0.036**

Region	Environmental variables in final model	Spatial variables in final model (PCNM)	P Global Environ ment	P Global Spatial	P environ ment	p spatial	Adj R2 environment	AdjR2 shared	Adj R2 spatial
SE England	Precipitation of Coldest Quarter, Max Temperature of Warmest Month, Precipitation Seasonality, Max Temperature of Warmest Month , Precipitation Seasonality, Altitude, Alkalinity, Temperature Seasonality, Mean Temperature of driest Month, Annual Precipitation, Min Temperature of Coldest Month	8, 1, 21, 2, 7, 13, 18, 19, 30, 10, 3, 120, 6, 147, 108, 11, 24, 97, 23, 31, 9, 52, 25	**0.005**	**0.005**	**0.005**	**0.005**	**0.013**	**0.021**	0.071
SW England and Wales	Precipitation of Warmest Quarter, Precipitation of Coldest Quarter, Altitude, Max Temperature of Warmest Month, Annual Precipitation, Alkalinity	2, 1, 8, 6, 47, 4, 37, 89, 3, 5, 130, 7, 94, 67, 54	**0.005**	**0.005**	**0.005**	**0.005**	**0.015**	**0.023**	**0.042**
N Ireland	None	4, 1, 2	0.082	**0.005**	-	**0.005**	**0**	**0**	**0.041**
S Ireland	None	none	0.22	0.65	-	-	**0**	**0**	**0.031**

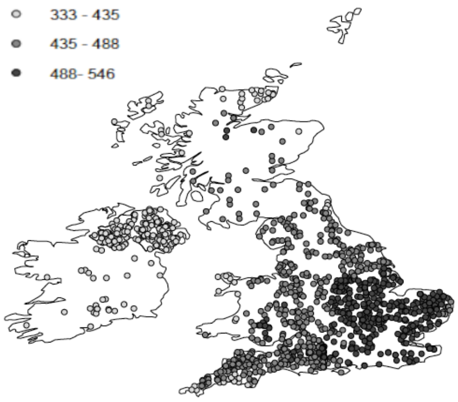
Richness per 100m (S)



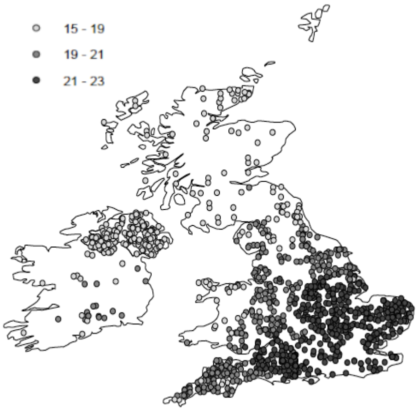
Alkalinity µg.L^{-1}



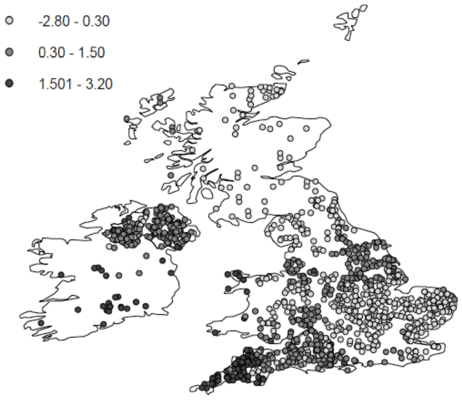
Temperature Seasonality (SD * 100) ° C



Max temperature warmest Quarter ° C



Min temperature coldest Quarter ° C



Annual precipitation (mm)

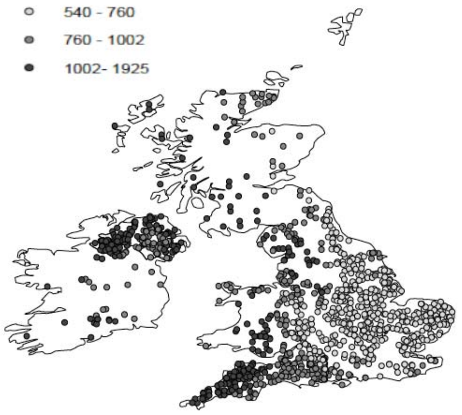
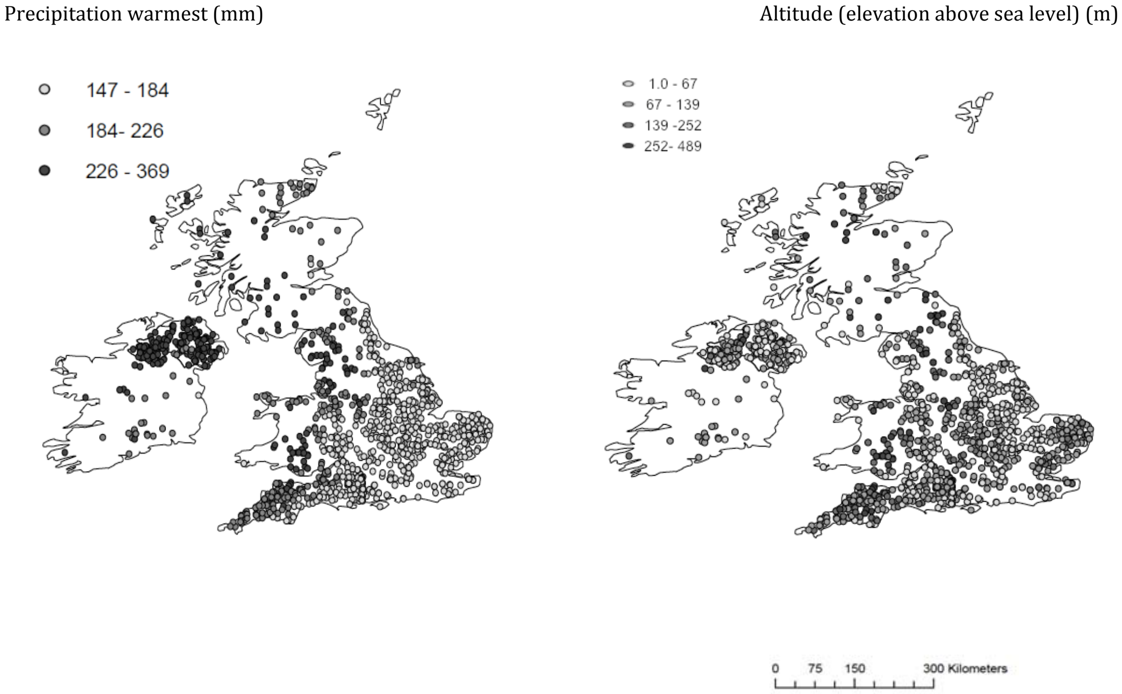


Figure 5-4. Environmental variables across the British Isles. Values starting above zero reflect the lowest records starting point. Scale bar in Figure 5.5



Precipitation warmest (mm)

Altitude (elevation above sea level) (m)

Figure 5-5. Environmental variables across the British Isles. Values starting above zero reflect the lowest records starting point.

Figure 5-6 Spatial variables across the British Isles. The value of the symbol is associated with eigenvector values from negative (bright) to strongly positive values (darker).

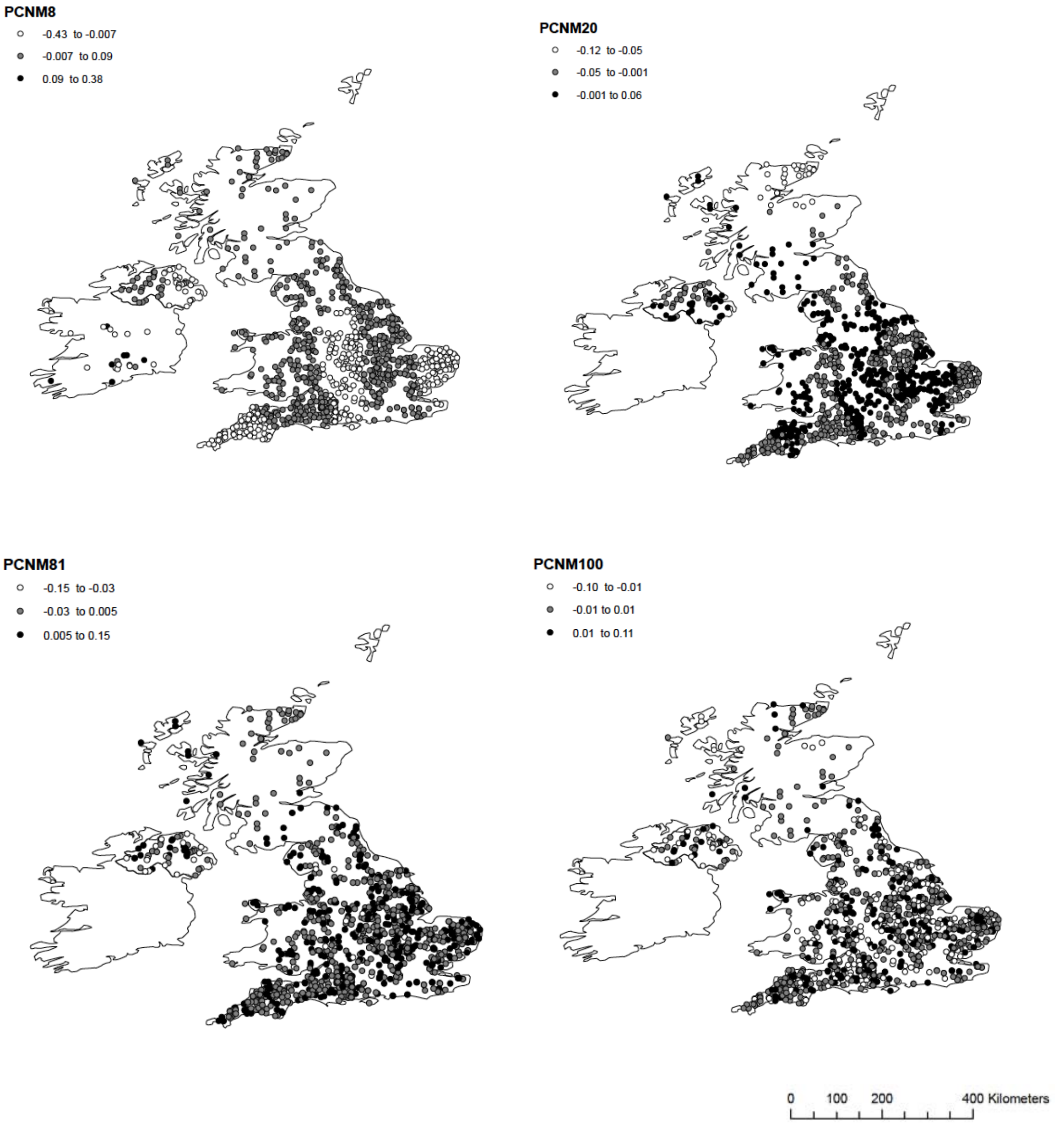


Figure 5-7. Spatial variables across the British Isles. The value of the symbol is associated with eigenvector values from negative (bright) to strongly positive values (darker).

Zambia

National scale - species richness

The total species number in Zambia was 260 species consisting of 186 emergent species, 18 floating species and 51 submerged species. Macrophyte species richness variation within Zambian streams was accounted for by the pure spatial component, which explained 26% and was statistically significant. Spatial richness retained low numbers for PCNMs indicating finer spatial patterns (Table 5.3). Species richness across different sites in Zambia ranged between 9 - 21 species per site.

Table 5-3. Spatial and environmental models for macrophytes species richness in Zambian freshwater bodies.

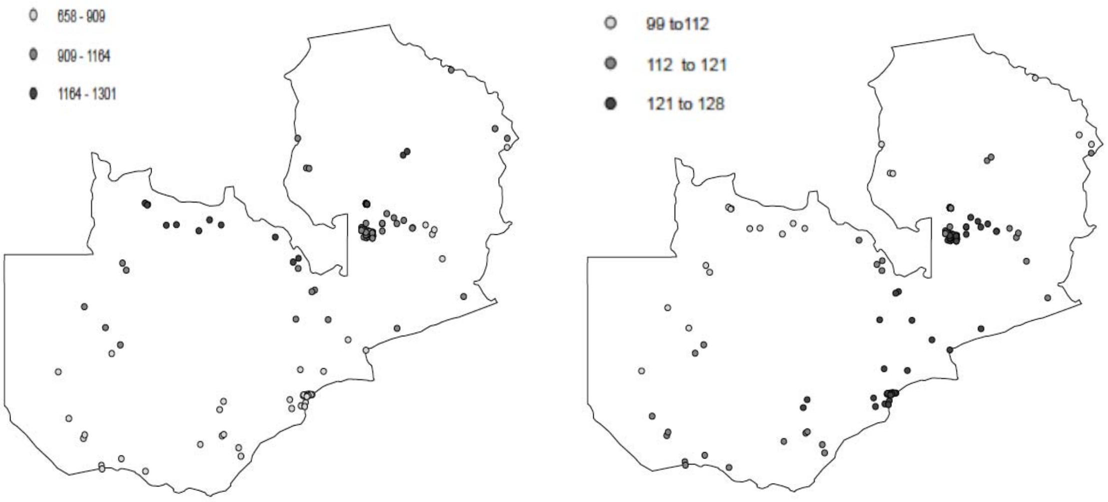
Region	Environmental variables in final model	Spatial variables in final model (PCNM)	P Global Environment	P Global Spatial	p environment	p spatial	Adj R2 environment	AdjR2 shared	AdjR2 spatial
Zambia	none	9, 7, 3, 8, 2, 1, 21	0.11	**0.01**	-	**0.005**	-	-	0.258

National scale – community structure

Macrophyte community variance, within freshwater bodies in Zambia, was partly explained by the spatially structured environmental fraction (4.6%). Additionally the pure environmental component explained 2.7% of the variation and the pure spatial component explained 3.8%. All three fractions were statistically significant (Table 5.4). Regional variables which explained the variation observed in macrophyte community (response matrix) were: Annual precipitation, Precipitation seasonality, Actual Evapotranspiration. Local variables retained in the final model explaining community structure in Zambia were altitude and alkalinity. The mean value for annual evapotranspiration in Zambia was 800.22 mm, while the mean value for altitude was 664 m a.s.l. Other environmental variables were also incorporated into the analysis but forward selection had not retained them. PCNMs retained under this model had low numbers, indicating finer spatial patterns (Table 5.4 and Figures 5.8-5.9).

Table 5-4. Spatial and environmental models for macrophytes species community in Zambian freshwater bodies.

Region	Environmental variables in final model	Spatial variables in final model (PCNM)	P Global Spatial	P Global Environment	p environment	p spatial	Adj R2 environment	Adj R2 shared	AdjR2 spatial
Zambia	Annual Precipitation, Precipitation Seasonality Actual Evapotranspiration, Altitude, Alkalinity.	1, 2, 7, 4, 34, 6, 41, 32, 24, 39	**0.005**	**0.005**	**0.005**	**0.005**	**0.027**	**0.046**	**0.038**

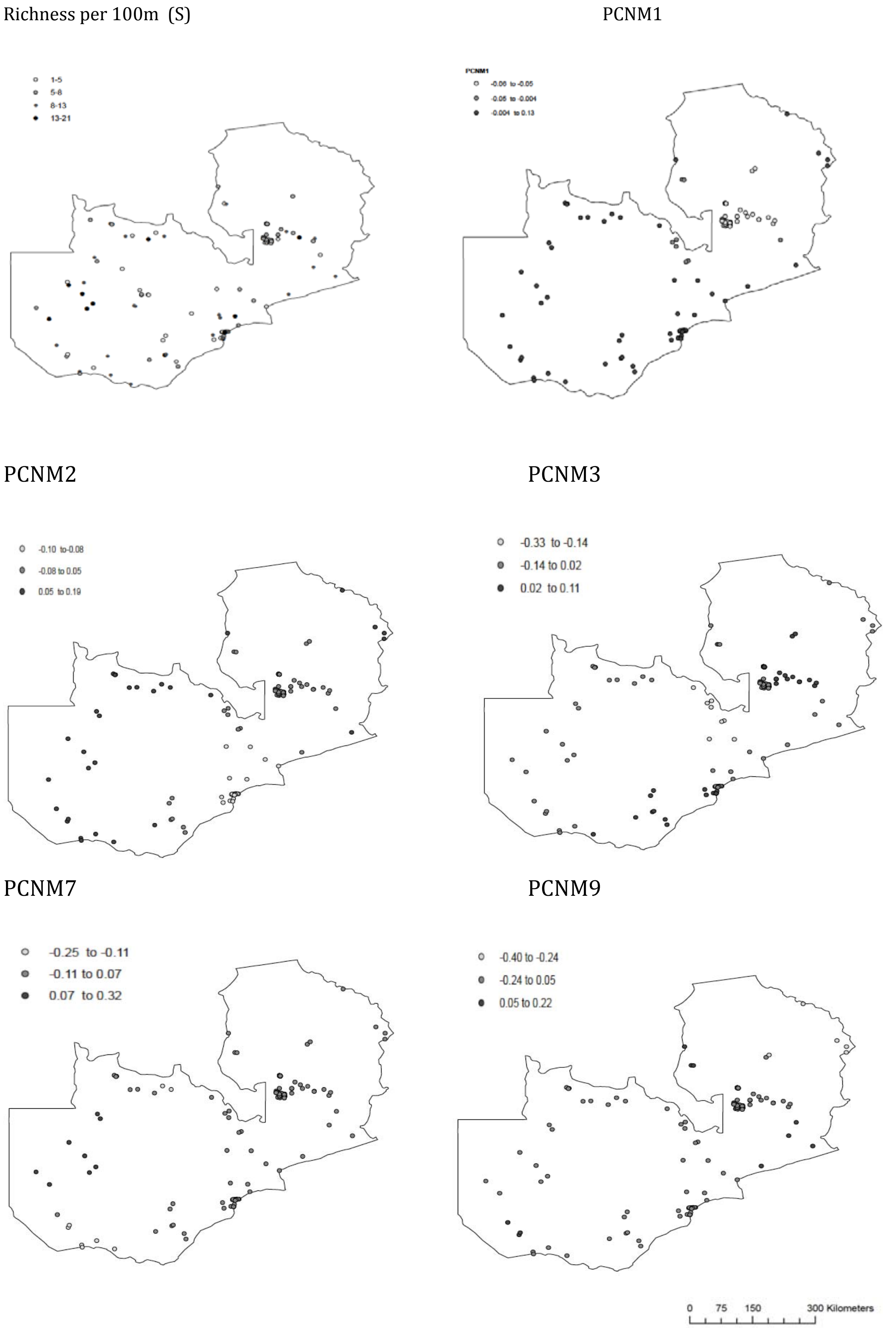


Annual precipitation (mm)

Precipitation seasonality (mm)

Annual evapotranspiration (mm)

Figure 5-8 Environmental variables across Zambia. Values starting above zero reflect the lowest records starting point.



Richness per 100m (S)

PCNM1

PCNM2

PCNM3

PCNM7

PCNM9

Figure 5-9. Spatial variables across Zambia. The value of the symbol is associated with eigenvector values from negative (bright) to strongly positive values (darker).

5.4 DISCUSSION

My findings illustrate some of the spatial and environmental factors that influenced species richness and community structure at a regional (river basins in the British Isles only) and national scale (both British Isles and Zambia). Inclusion of spatial factors in my analysis did explain the greater part of the variation observed in species richness and community structure in the British Isles and Zambia. This demonstrated the importance of including spatial variables when examining species distributional patterns.

The overall variance explained by my analysis on species richness may seem low at Adj R^2 22.3% for the British Isles and Adj R^2 25.8% for Zambia; and for community Adj R^2 10.4% for the British Isles and Adj R^2 11.1% for Zambia. However these results are of comparable magnitude to those recorded in similar studies elsewhere (Dray et al. 2006; O'Hare et al. 2012a). This recorded low explained variance reflects technical issues with the analyses which is best illustrated by highlighting that the variance explained essentially equates to a half to a third of that explained by an equivalent unconstrained ordination analysis.

Is the variation of macrophyte distribution patterns attributable to spatial and environmental factors in the British Isles and Zambia?

My results suggest that variation in macrophyte richness and community structure for hardwater rivers in the British Isles are related to 1) pure spatial, 2) pure environmental and 3) environmental spatially structured factors at a national scale. At a regional level (i.e. RBUs in N England, SE England, SW England and Wales, N Ireland, but not Scotland or Southern Ireland) species richness was explained only by spatial variables.

In the case of Zambia, at a national level, species richness was only attributed to spatial variables, but community structure was partially explained by the pure environmental variables taken into consideration for my study, in addition to the pure spatial and environmental spatially structured factors. I now discuss these patterns in detail addressing the importance of environmental variables first, then spatial variables and finally spatially structured environmental variables.

Relative importance of environmental variables

My findings confirmed the fact that large scale patterns are described mainly by climate (Hill, 1994; Capers et al. 2010; Sweetman et al. 2010; O'Hare, 2012a). With the exception of alkalinity and altitude which were found to contribute to part of the variation in species distribution, the rest were climatic variables. Climate variables are strongly correlated with one another, thus simplicity and selection of bioclimatic variables was done as suggested in previous studies (Prentice et al. 1992).

In the British Isles species richness was explained at a national level by alkalinity, temperature seasonality, max temperature warmest quarter, min temperature coldest quarter, and mean temperature wettest quarter. For community structure, similar variables were found to interact with species distribution with the addition of few more such as: annual precipitation, precipitation of warmest month, altitude, to mention a few at a national and regional level.

In Zambia environmental variables such as: annual precipitation, precipitation seasonality, annual evapotranspiration, altitude, alkalinity were found to influence community structure. The effect of precipitation on community structure has been previously recorded (O'Hare, 2012a). My results confirm past studies showing the importance of environmental factors i.e. altitude, climatic factors (e.g. temperature, precipitation), as key determinants of species richness (Hill, 1994; Jones et al., 2003; Brown et al. 2007; Hawkings 2007; Vestergaard and Sand-Jensen, 2000, Vinson and Hawkins 2003; Astorga et al. 2011).

Spatial variables

Spatial variables did explain variation not attributable to environment variables only. Due to spatial processes, such as dispersal, differential mortality, species interactions and organization, species tend to be spatially organized (Keitt et al. 2002; Cottenie, 2005). As illustrated in my PCNMs outputs, a non-random distribution of species richness was found across the British Isles and Zambia.

In the British Isles species richness (8.8%) and community structure (5.4%) were strongly related to pure spatial variation at a national level, displaying not only broad-scale variation (i.e. large PCNMs values), but also fine-scale spatial variation (i.e. low PCNMs values). PCNMs output (e.g. PCNMs 2, 3, 8) illustrated distinctive spatial patterns influencing the central part of England and setting it apart from the rest of the sites. In addition other PCNMs e.g. PCNM4 illustrate a north to south gradient pattern. Such spatial factors could also act as surrogates of unmeasured ecological drivers and could be taken into consideration for future analyses.

In Zambia species richness (25.8%) and community structure (10.4%) at a national level were also strongly related to pure spatial variation at fine-scale. Fine-scale patterns illustrate spatial autocorrelation created by dynamic processes controlling species richness (e.g. biotic interaction, dispersal), or unmeasured abiotic factors (e.g. land use) (Astorga et al. 2011). PCNMs outputs illustrate a fine-scale spatial component between the two major river basins which comprise Zambia (all rivers in Zambia flow either north and west to the Congo, or south and east to the Zambezi). Higher diversity was generally recorded for sites in the Congo River basin compared to the Zambezi River basin with only a few exceptions. A recent study illustrated that the spatial autocorrelation of species abundance is often due to dispersal constraints, competition, or aggregation on small to intermediate scales (Legendre, 1993), suggesting that the spatial distribution may also arise by neutral mechanisms (Hubbell, 2001; Yuan, Ma, Wang, 2012).

Are spatially structured environmental variables important?

Environmental factors responsible for species richness and community structure in the British Isles and Zambia were shown to be spatially organized; imposing a spatial structure, called *induced spatial dependence* (Peres-Neto and Legendre, 2010). That is non-random organization across space, in either species distribution or environmental processes, were observed for Zambia and the British Isles.

In the British Isles, species variation was attributed to spatially structured environmental variables (11.4%) at a national level, where spatial factors (PCNMs) depicted a large climate gradient across river samples in the British Isles. For instance hardwater river macrophyte species richness in the British Isles increased along a North-West to South-East gradient. Environmental parameters such as alkalinity, temperature seasonality, max temperature of warmest quarter also increased in value from north to south. On the other hand min temperature coldest quarter, annual precipitation, precipitation of warmest month, increased in values along an east to west gradient. In terms of community structure, variation in the British Isles was attributed to spatially structured environmental variables at a national (3.9%) and regional level where a large proportion of the variation was attributed to spatially structured environmental variables. Haslam (1978) in a qualitative analysis emphasised the importance of variation with geographical location, with both geology and topography acting as fundamental drivers. She found, for instance, that more southerly areas in Britain had lower water flow, yielding denser vegetation (e.g. *Ranunculus* spp.) in both upland and lowland stream types. Conversely many streams in north-west England, are mountainous and empty of macrophytes, while those in north-east England tend to have less water force and support macrophyte vegetation.

In the case of Zambia, species richness variation was explained only by spatial factors along the two river basins gradient (i.e. the Zambezi and the Congo River Basins), which can function as surrogates or proxies of environmental factors that were not taken into account in my study (e.g. dispersion).

In terms of community structure variation in Zambia, this was strongly related to climatic spatially structured environmental variables, e.g. annual precipitation, precipitation seasonality, annual evapotranspiration, along a south to north pattern of changing values.

Are there any differences in the influence of climatic factors between the two countries attributable to their climatic regions (temperate region, British Isles and a tropical region, Zambia)?

Differences in the influence of climatic factors between the British Isles and Zambia were seen. Broad-scale richness gradients and their relationship to climate were apparent for the British Isles; but this was not the case for Zambia. Although similar climatic variables were tested for both countries, the model did not retain the same climatic variables to explain species distribution patterns at each country. More stable climatic conditions and larger gradients across Zambia may have contributed to my results. Similarly previous studies looking at richness of angiosperms were found to co-vary with heat in cold areas but not strongly so in warm areas, suggesting that richness-climate relationships may differ significantly among geographic regions (Francis and Currie, 2003).

In addition the fact that fine-scale spatial patterns contributed to macrophyte species richness distribution in Zambian hardwater rivers may be indicative of more localized effects as important drivers, and should be considered further to gain a better understanding.

Conclusion

My findings show that variation in richness and community structure for hardwater river macrophytes can be partly explained by environmental variation relative to spatial processes in the British Isles (temperate scenario) and in Zambia (tropical scenario). Among the environmental variables, climatic ones explained a great part of species richness and composition distribution for the British Isles. Conversely in Zambia spatial processes made the greatest contribution to variation in hardwater river macrophyte species richness and community structure. These results increase our knowledge of the processes influencing calcareous river macrophyte ecology, butclearly it is important to consider as wide a range as possible of potential structuring influences on river communities, environment and space (O'Hare, et al. 2012a; Borcard and Legendre, 2002; Jombart, Dray and Dufour, 2009). Therefore illustrating a multivariate analysis that incorporates all associated predicting factors into a single analysis is of extreme importance. A key finding here was the difference in spatial structuring of environmental variables at different scales (both national and regional) of the British Isles and Zambia. The incorporation of connectivity analysis between sites in Zambia, and data records on local environmental variables, such as nutrients, biomes (e.g. Kennedy et al. 2012 in press) and anthropogenic impacts, might help explain in more detail the spatially structured environmental variables that were shown in my study to be determinants of macrophyte species richness patterns in hardwater rivers in the two areas compared.

Chapter 6. A macroecological approach to study aquatic plant distribution patterns in calcareous rivers: a latitudinal gradient analysis.

6.1 INTRODUCTION

As shown in previous chapters aquatic plant distribution patterns in calcareous rivers can be attributed to both spatial and environmental factors across local and global scales. At a global scale, latitudinal drivers have a potential to explain part of the variation shown in macrophyte species richness. The study of relationships between organisms and their environment at large temporal and/or spatial scales aiming to explain the patterns of abundance diversity and distribution is known as macroecology. Macroecology can be a useful tool to look at species distribution patterns, including topics like gradients in species richness, structure of geographical ranges and species-abundance distributions (Carvalho et al. 2009; Brown 1995). Macroecology studies date back to the late 1960s and early 1970s (MacArthur and Wilson, 1967; MacArthur, 1972) with a rapid expansion in this field in recent decades (Rosenzweig, 1995; Gaston and Blackburn, 2000). However greater attention has been paid to terrestrial vertebrates and higher plants compared to marine and freshwater systems which have been examined less commonly (Diniz-Filho, De Marco and Hawkins, 2010; Heino, 2009).

The analysis of latitudinal gradient effects on global patterns of species richness, has usually focused on specific taxonomic groups and their relationships between local abundance and regional distribution (Lawton 1993; Lawton et al, 1993), where the size of the habitat and the diversity of species are interrelated (Brown, 1984; Rosenzweig, 1995; Edwards et al. 1993; Hewitt et al. 2005). The usual hypothesis tested is that there is greater biogeographic heterogeneity in the tropics compared to the temperate zones, because the tropics provide more habitats and refuges, enhancing the occurrence of larger populations, higher speciation rate and lower extinction rates there (Terborgh, 1973; Rosenzweig, 1995; Guegan et al. 1998; Hewitt et al. 2005). In terms of riverine systems this could be linked to the higher rainfall and higher run-off condition in the tropics that may present a broader range of habitats from headwaters to river mouth than their higher-latitude equivalents (Hugueny et al. 2010). Thus habitat area for freshwater organisms living in rivers could be partly related to latitudinal gradient.

Other hypotheses such as the evolutionary hypothesis mechanistically link the rate and time available for speciation at different latitudes (Mittelbach et al. 2007). They suggest that more stable conditions observed in the tropics compared to the higher-latitude zones could facilitate speciation and thus lead to higher species richness. However speciation rate in the tropics can only be linked to latitude if the large-scale dispersal of species from the tropics to temperate regions is limited (Hillebrand, 2004).

The historical hypothesis links the glaciation periods to organisms present in temperate regions (Whittaker, 1977). This highlights the presence or absence of species in higher latitudes as a function of species re-colonization after the most recent glacial event. The hypothesis suggests that higher species richness will occur in the tropics, because they have experienced long periods of relatively stable conditions compared to the temperate zones, and were not glaciated during the last ice age (e.g. study of freshwater fish in North America: Griffiths, 2010). A previous study on macrophyte species and subspecies endemic to Europe and parts of North Africa bordering the Mediterranean proposed that c. 75% of 61 endemic taxa evolved after the ice age whereas only c. 25% were relicts left by extinction (Cook, 1983).

Species richness has also classically been explained in relation to a latitudinal gradient (Wallace, 1878). To define the occurrence of species is not that straight forward because some species will be distributed across different geographical isolated groups (i.e. in terms of scale). A simple way to interpret the geographical distribution of a species is to look at the resources that it is able to exploit (Brown, 1984; Edwards, et al. 1993). Large areas, invasion ability and high abundances are interlinked characteristics of species (Edwards et al. 1993). Demographic rates, birth, death, immigration and emigration, will also play a key role in the distribution of species population dynamics. In general, regions close to he equator are shown to have the highest productivity possibly as a consequence of the the prevailing climate which is hot, wet and relatively free from seasonal variaton (Wright, 1983; Currie, 1991; Cox and Moore 1993). In fact the world’s distribution of plant productivity has been shown as an estimate of over 800 g carbon m^{-2} per year of organic dry matter, that accumulates during a single growing season, in areas close to the Equator and within the tropics (Cox and Moore 1993) (see Figure 6-1).

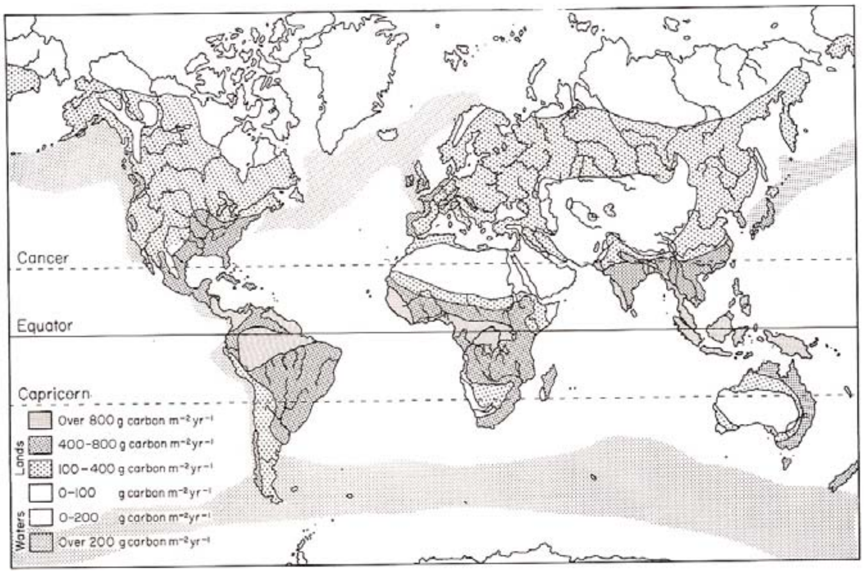


Figure 6-1. **World distribution of plant productivity. The data displayed here are simple estimates of the amount of organic dry matter that accumulates during a single growing season. Full adjustments for the losses due to animal consumption and the gains due to root production have not been made. Map compiled by H.Leith in Cox and Moore 1993).**

Higher terrestrial plant biomass in the tropics, could help to create a greater spatial complexity in the environment and in turn increase the potential for higher diversity in the living organisms that dwell in the region (Cox and Moore, 1993). However the amount of metabolic energy that an area can sustain is limited, thus limiting the total number of species that can coexist (Hutchinson, 1959). This hypothesis has been criticised because it only provides a link between higher energy and higher biomass but not a clear link between higher energy and higher species richness (Gaston and Blackburn, 2000).

In terms of global-scale latitudinal patterns, long-term studies of the terrestrial floras of tropical countries such as Panama, Costa Rica, Ecuador, have shown that biodiversity in tropical zones greatly exceeds that known from temperate regions (Crow, 1993). However, very little work has been done to examine such patterns in the context of aquatic vegetation. One approach that has been used to evaluate diversity in relation to latitude is to compare regional aquatic-wetland floras on a taxonomic basis.

An interesting question is whether variations in aquatic plant assemblages reflect real latitudinal geographic patterns or whether regional distributions are just consequences of taxonomic variation. A study comparing aquatic plant diversity of representative aquatic families on a latitudinal basis found a higher level of diversity at warmer temperate latitudes and a high, if not highest, level at cool temperate latitudes (Crow, 1993). Aquatic plants represent a small fraction of the total plant species on earth (<1% for true freshwater species, though considerably more if wetland species are included (Chambers et al., 2008). These plants must possess a specialist set of morphological and physiological features, to allow adaptation to water habitat conditions (Chambers et al. 2008). For instance macrophyte adaptations on seed buoyance and fragmentation of body parts; are essential mechanisms in species relying on water drift for dispersal (Bornette and Puijalon, 2009). This stresses the importance of species adaptations to live in water habitats, with some species favoured over others. Chappuis et al. (2012) found, for instance, a relative higher abundance of hydrophytes (i.e. floating-leaved rooted, submerged and free-floating species) compared to helophytes (i.e. emergent species) at higher latitudes as a function of increased water levels at northern latitudes contrasted by water scarcity at near-equator latitudes. This suggests a relationship between species morphological growth form and habitat availability in relation to latitude. Last but not least a previous study classifying different aquatic vascular plant families has classed them into three floristic groups on the basis of species richness: cosmopolitan (e.g. Cypereceae, Juncaceae, Poaceae), north-temperate (e.g. Potamogetonaceae, Sparganiaceae, Haloragaceae) or pan-tropical (e.g. Podostemaceae, Hydrocharitaceae, Limnocharitaceae, Aponogetonaceae) (Crow, 1993).

Hypotheses

1) Is the aquatic plant richness of calcareous rivers related to latitude?
2) Are some aquatic groups of macrophyte better adapted to, or characteristic of either tropical or temperate conditions?
3) Are some functional groups of macrophyte dominant in, or characteristic of either tropical or temperate conditions?

This chapter aims to address latitude as a predictor of macrophyte richness, and also examines functional group distribution across latitude, for calcareous rivers. The project collected data from sites which covered a wide range of physical and chemical habitat types, and could therefore potentially explain a significant amount of variation in the distribution of calcareous river vegetation. By taking into account this variation, and grouping sites with similar abiotic characteristics (as explained in Chapter 3 and 4), it is possible then to address the influence of latitudinal gradients, as the analysis of those gradients could be confined to sites, which were comparable in their local conditions. However I also examine the richness-latitude/environment relationship for a wider subset of my data. Based on the literature cited above, I aim to consider the interplay of previous hypotheses looking at species richness in relation to latitudinal gradients, and observe any similarities or discrepancies with other species richness patterns.

6.2 METHODS

Collection methods for data examined here are described in the methods chapter. In this section I have included the data collected by myself, at selected locations with calcareous rivers in the UK, Yorkshire Dales (northern England) and abroad (including northern Zambia; Bonito and Chapadas regions of southern Brazil; northern Argentina; northern upland streams of Trinidad; northern Florida; Yucatan region of Mexico; and South Africa). I have also included the data for calcareous rivers for the British Isles drawn from the MTR database; and similar data for Greece, Italy, Denmark, and Latvia based on the STAR dataset, plus data for Portugal based on an unpublished dataset (T. Ferreira pers comm).

6.3 ANALYSIS PROCEDURES

A total of 244 sites were included, for the first analysis, with criteria for inclusion on the basis of width category of <10m, with slow-moderate flow conditions, and with no shading. Species counts were split accordingly to their functional group and grouped at genus level. Genera illustrated below were selected on the basis of their higher occurrence across sites with the exception of *Eichhornia*. For the second analysis sites of greater width and fast flow, were also included to look at the relationship between number of macrophyte species per site (alpha-diversity) and latitude.

6.4 RESULTS

My findings showed that all macrophyte functional groups were present across the latitudinal gradient (Figures 6.2 - 6.9).

Is aquatic plant richness related to latitude?

Latitude was not significantly related to species richness in small calcareous, unshaded slow flowing streams using standard regression techniques (Table 6-1). That is species richness did not significantly vary from low to high latitudes (Figure 6-3).

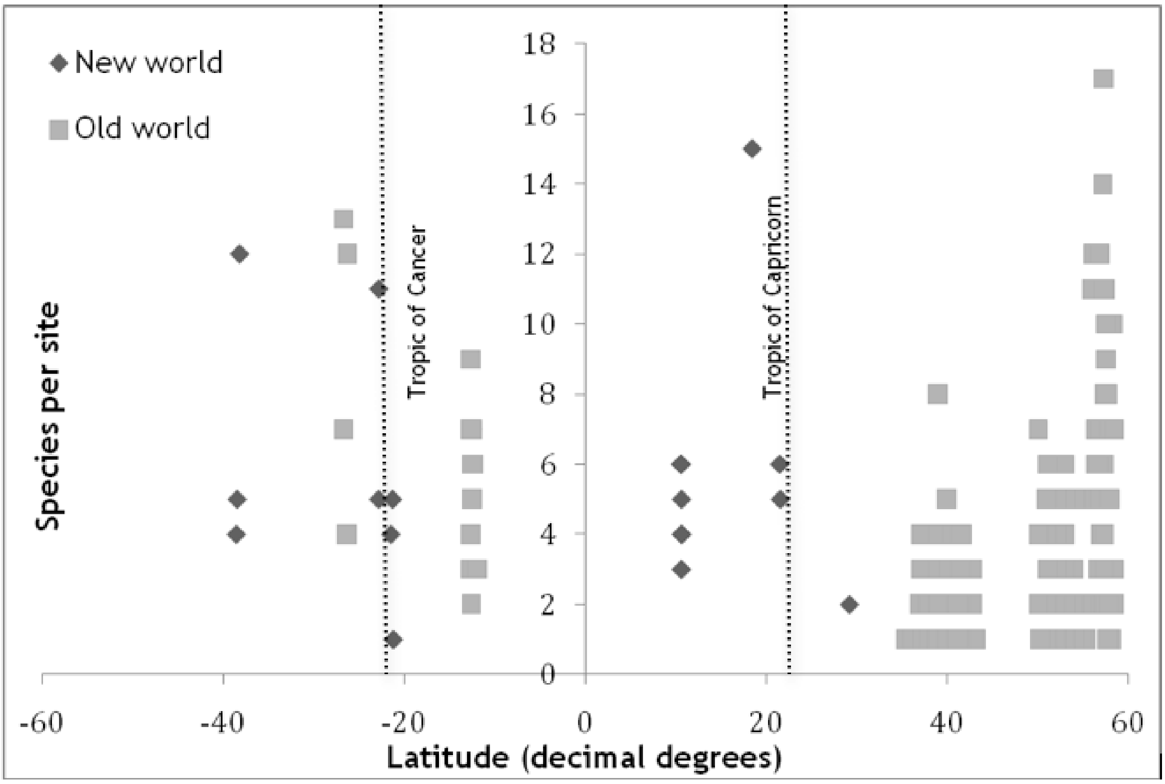


Figure 6-2. Macrophyte richness across latitudinal gradient in the New World and Old World.

A regression analysis for the first subset of the data (small calcareous, unshaded slow flowing streams) showed only a non-significant and very weak negative relationship between number of species per site and latitude (Table 6-1). A second regression analysis was carried out with the personally collected data, minus sites with no light availability (k) or no alkalinity 1) Marginally hard water (12.2 - 24.27 mg l^{-1}) 2) Intermediate hard water (24.4 - 120.78 mg l^{-1}) 3) Hard water (122 - 242.78 mg l^{-1}) and 4) Very hard water (>244 mg l^{-1}) HCO_3. Alkalinity and k both needed log10 normalisation. This showed a very weak but significant positive influence of latitude on diversity (Table 6-2).

Table 6-1. Multiple regression analysis of latitude versus number of species per site, for small calcareous, unshaded slow flowing streams

```
The regression equation model is:

Richness = 4.70 - 0.0137 absolute latitude value

Predictor        Coef  SE Coef      T      P
Constant       4.6978  0.7063   6.65  0.000
absolute lat  -0.01371  0.01552  -0.88  0.378
S = 3.30167   R-Sq = 0.4%   R-Sq(adj) = 0.0%

Analysis of Variance

Source          DF      SS     MS     F      P
Regression       1    8.51   8.51  0.78  0.378
Residual Error  201  2191.11  10.90
Total          202  2199.62
```

Table 6-2. Multiple regression analysis of number of species per site versus latitude, water velocity, log10 light availability (k), log10 alkalinity for all calcareous streams personally sampled minus sites with no light availability or no alkalinity 1) Marginally hard water (12.2 - 24.27 mg l^{-1}) 2) Intermediate hard water (24.4 - 120.78 mg l^{-1}) 3) Hard water (122 - 242.78 mg l^{-1}) and 4) Very hard water (>244 mg l^{-1}) HCO_3.

```
The regression equation is

Total S_1 = 8.27 + 0.0711 Abs lat_1 - 0.570 Velocity category_1 + 0.124 logtK
          -   0.390 logtalk

Predictor              Coef  SE Coef      T      P
Constant              8.269    1.938   4.27  0.000
Abs lat_1           0.07112  0.01701   4.18  0.000
Velocity category_1  -0.5705   0.3606  -1.58  0.115
logtK                0.1244   0.9345   0.13  0.894
logtalk             -0.3900   0.5839  -0.67  0.505
S = 3.92692   R-Sq = 8.9%   R-Sq(adj) = 7.2%

Analysis of Variance
Source          DF       SS     MS     F      P
Regression       4   316.88  79.22  5.14  0.001
Residual Error  210  3238.36  15.42
Total           214  3555.24
```

Are some aquatic groups of macrophyte better adapted or are characteristic of either tropical or temperate conditions?

Several groups of macrophytes were better adapted or were characteristic of either tropical or temperate conditions. For instance aquatic plant genera such as *Cyperus*, *Ludwigia* and *Panicum* were generally restricted to the tropics and subtropics (low latitudes). In contrast genera such as *Nasturtium*, *Berula* and C*allitriche* were mostly recorded in temperate regions (high latitudes) for my datasets (Figures 6-4, 6-9). Genera typical of different functional groups recorded across the latitudinal gradient are mentioned below: (though it should be noted that some genera contain species representative of >1 FG: e.g. *Sparganium*).

A) Marginal genera were mainly found at high latitudes (e.g. *Berula, Apium, Nasturtium*) with a few present at both high and low latitudes (e.g. *Persicaria, Juncus)*. *Ludwigia* and *Panicum* occurred only at low latitudes.

B) Emergent genera were mainly found at high latitudes (e.g. *Phalaris*, *Glyceria* and *Sparganium*) with a few present at both high and low latitudes (e.g. *Phragmites* and *Schoenoplectus*). *Cyperus* was only present at low latitudes.

C) Submerged genera were recorded mainly at high latitudes (e.g. *Callitriche*, *Elodea* and *Ranunculus*) whereas *Myriophyllum* and *Potamogeton* were present at both high and low latitudes.

D) Free-floating genera: *Eichhornia* was only present at low latitudes, whereas *Lemna* was found both at low and high latitudes.

E) Floating-leaved rooted genera such as *Nuphar* were present at both high and low latitudes.

Are some functional groups of macrophyte dominant or are characteristic of either tropical or temperate conditions?

In my findings few genera occurred in higher numbers at some regions. *Cyperus* was found with higher numbers in the tropics, whereas *Callitriche* and *Ranunculus* occurred in higher numbers in the temperate regions. Other genera such as *Potamogeton* and *Juncus* occurred in higher numbers at both high and low latitudes.

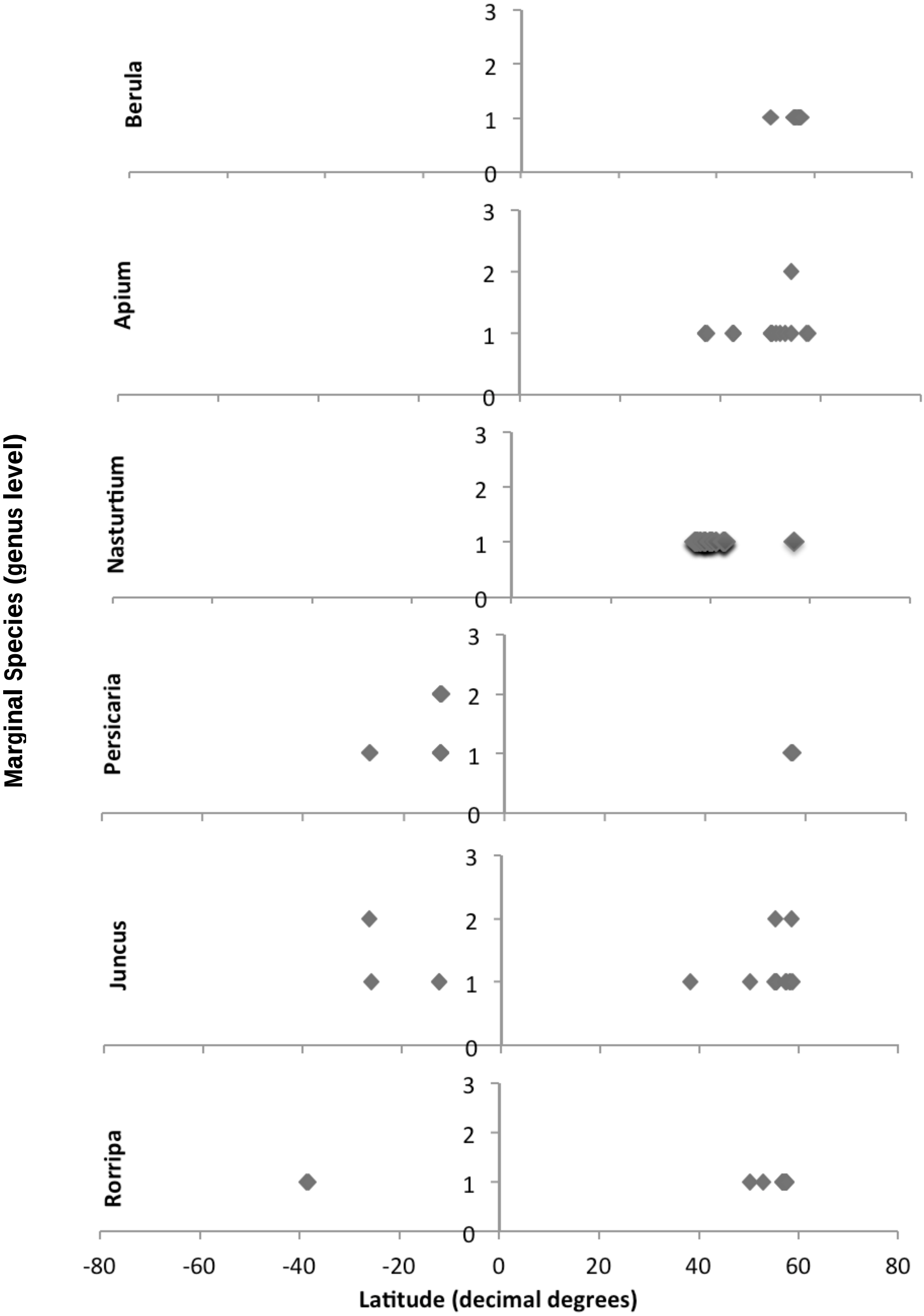


Figure 6-3 Marginal species distribution across latitude.

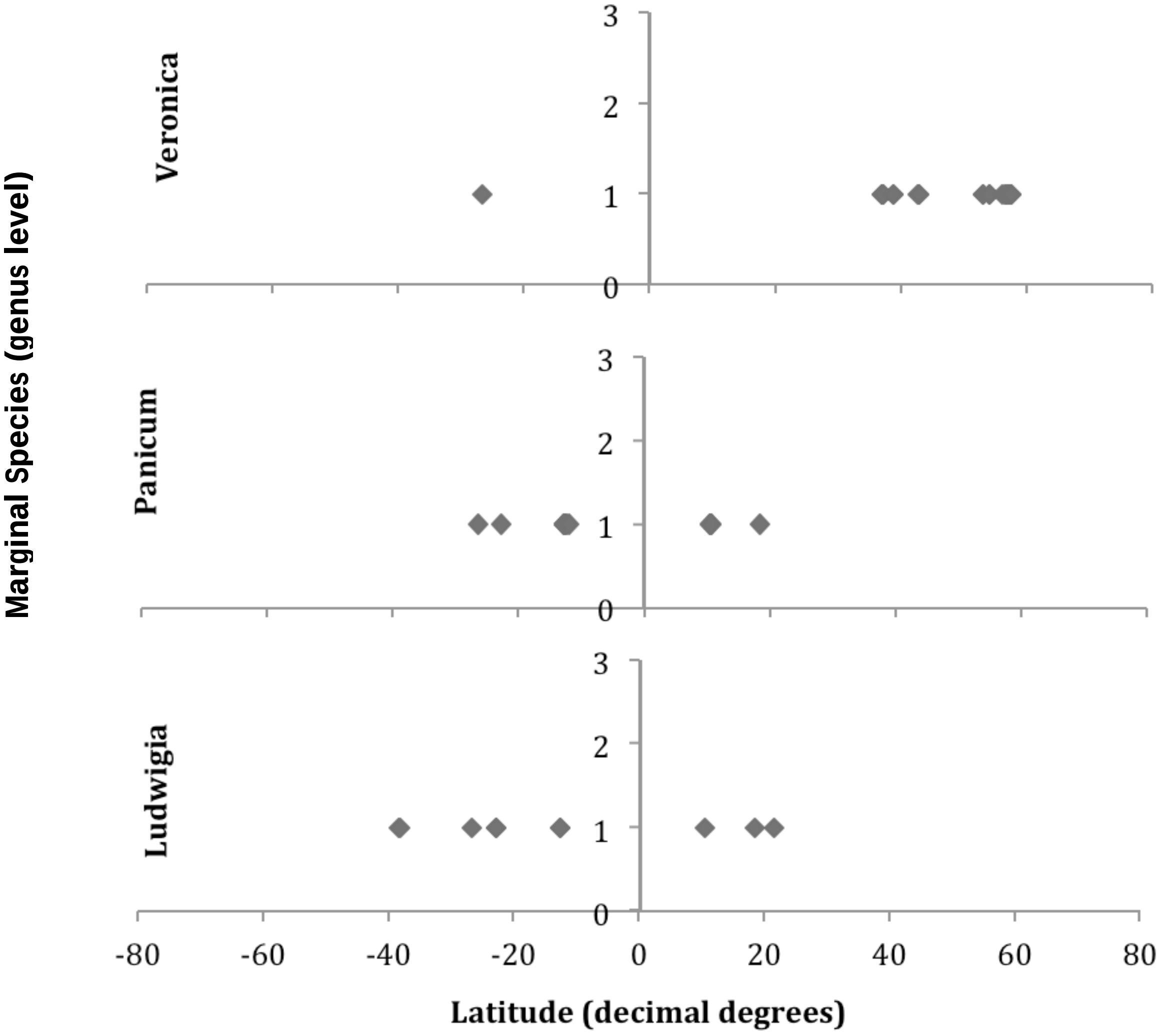


Figure 6-4 Marginal species distribution across latitude.

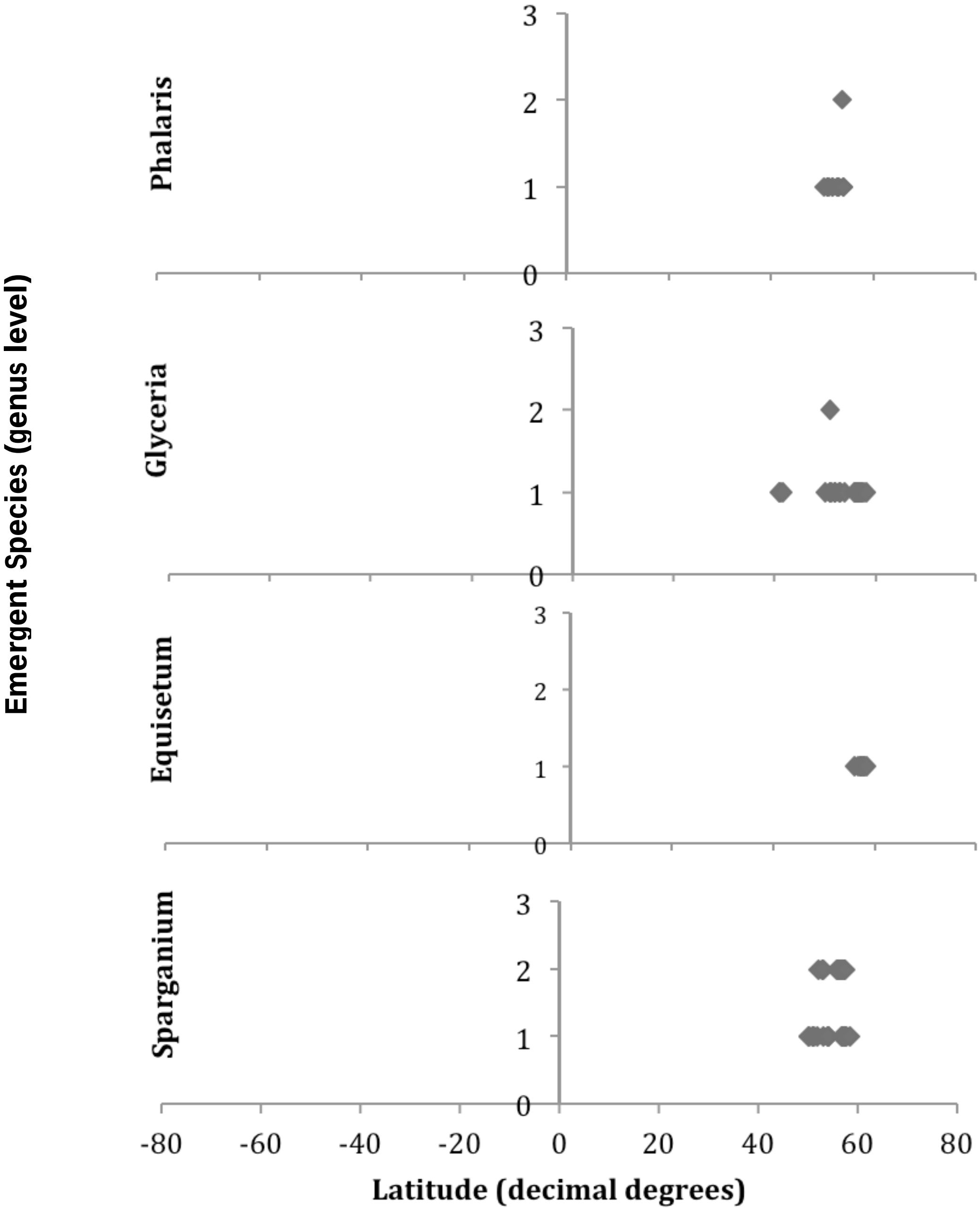


Figure 6-5 Emergent species distribution across latitude.

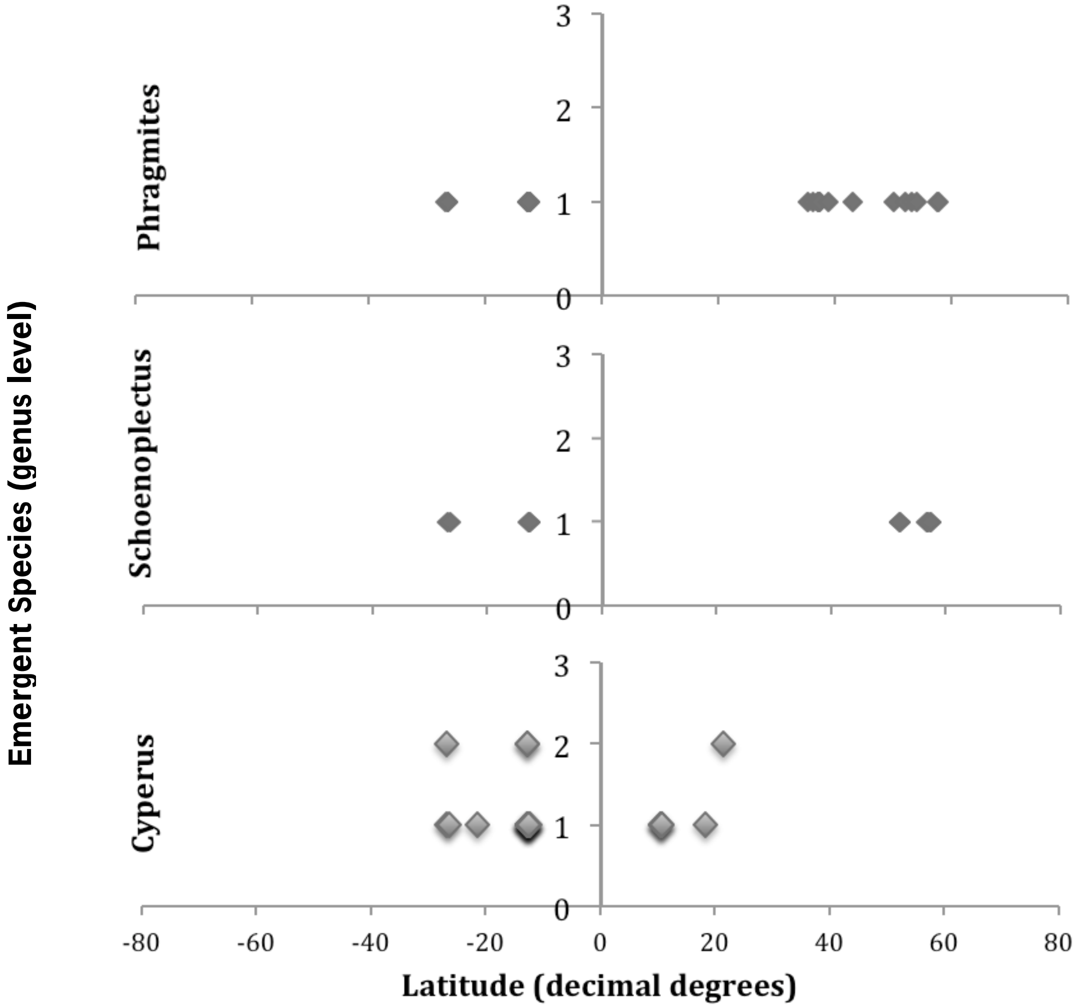


Figure 6-6 Emergent species distribution across latitude.

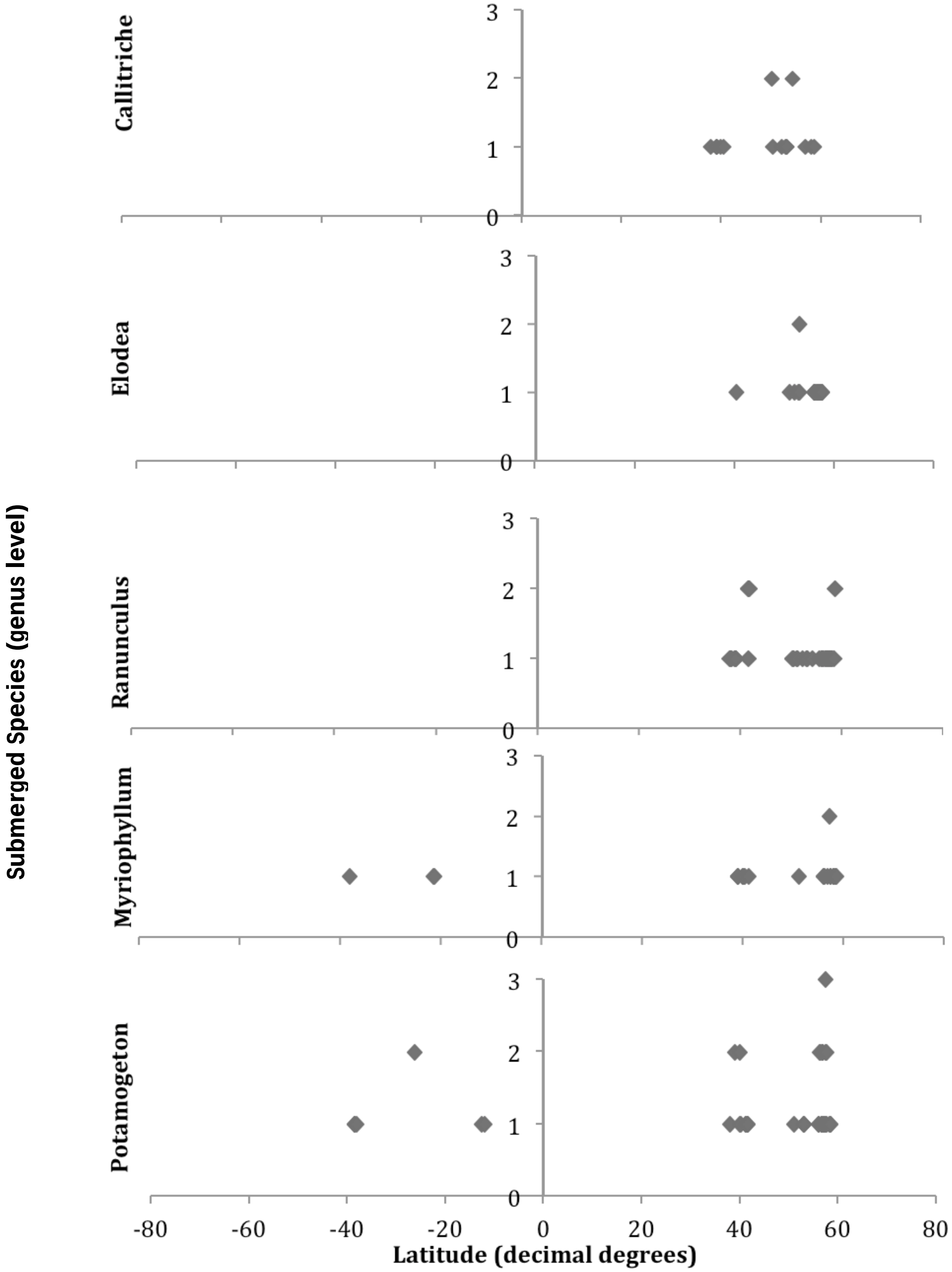


Figure 6-7. Submerged species distribution across latitude.

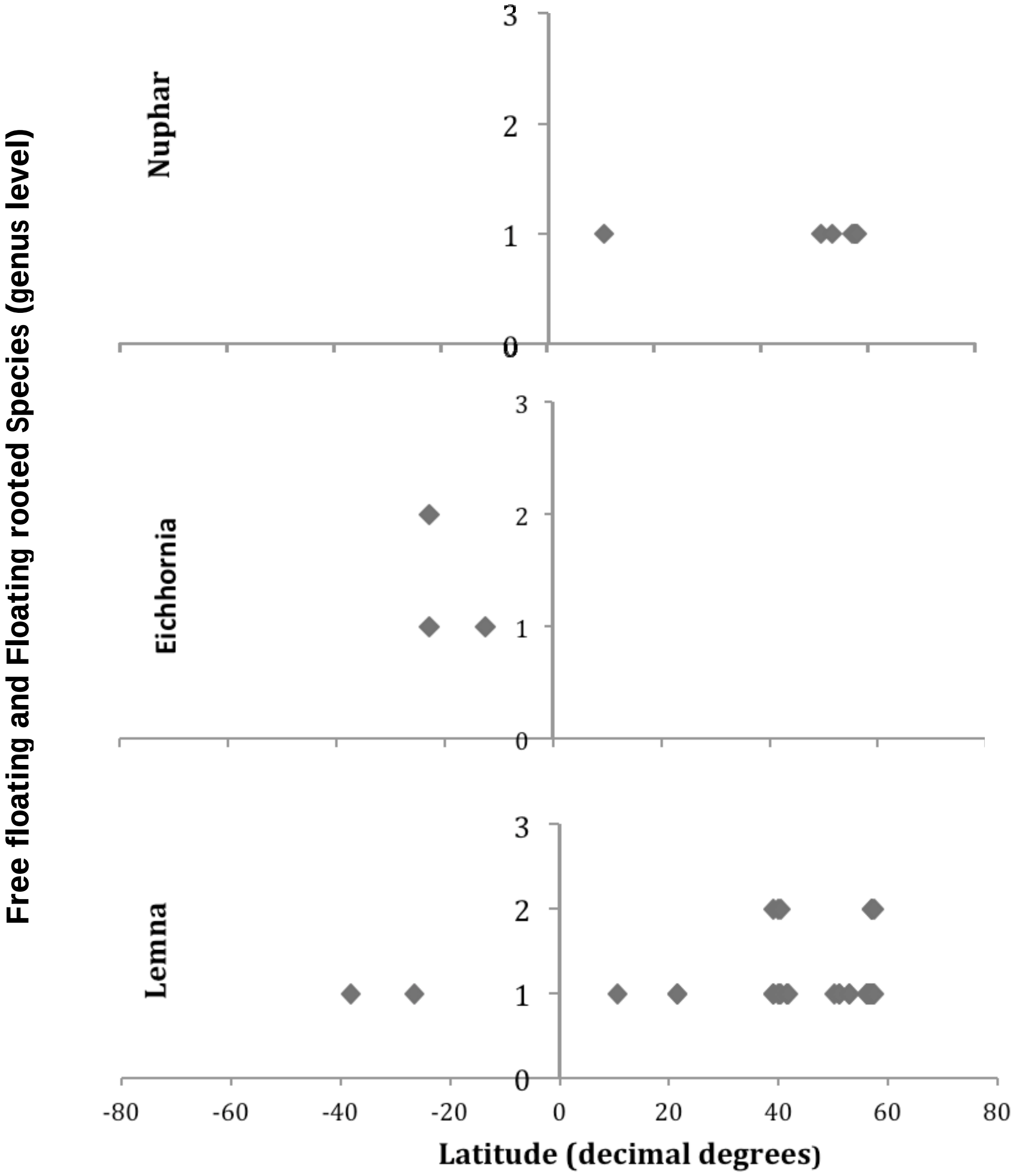


Figure 6-8. **Free-floating and floating rooted species distribution across latitude.**

6.5 DISCUSSION

Is aquatic plant richness related to latitude?

My findings show that latitude does not predict aquatic macrophyte diversity, for the regions included in my study. I was able to find only weak and very limited evidence for any influence of latitude as a factor influencing alpha-diversity of marophytes in calcareous rivers as a whole (though the weak trend observed was for increasing diversity at high latitudes, agreeing with the findings of Crow (1993), and none for a subset of the data comparing similar types of calcareous river (small, slow flowing, unshaded streams), across the world. In other words macrophyte species richness variation observed in calcareous rivers in both the tropics and temperate regions is probably more influenced by local conditions, than by spatial factors influenced by latitude, acting at a global scale. Similar findings for aquatic plants have been shown in previous studies (Crow, 1993; Covich, 2009; Chappius, 2012). Other biota such as freshwater birds (at a regional scale, Buckton and Ormerod, 2002) caddisflies, and salamanders (at a global scale, Pearson and Boyero, 2009) similarly show little or no evidence for a diversity response related to a latitudinal gradient. Conversely fish, and benthic macroinvertebrates do show the classical patterns of richness decrease at high latitudes (Oberdorff et al. 2001; Castella et al. 2001).

The absence of any strong latitudinal diversity gradient for macrophytes in calcareous rivers can be linked to Linnean and Wallacean shortfalls that are prevalent at low latitudes (Whittaker et al., 2005; Bini, 2006). The Linnean explanation refers to the fact that most species are not adequately described, and the Wallacean explanation refers to the fact that species distribution is inadequately known. As mentioned before in previous chapters, and in preliminary studies, the lack of taxonomic and floristic/faunistic knowledge in the tropics and elsewhere does in part contribute to the lack of understanding of latitudinal richness gradients in freshwater taxa (Bini, 2006). Unlike terrestrial plants the addition of records of aquatic species in the tropics may still not reflect a change in latitudinal gradient effect. Because of the conditions favouring greater richness in tropical regions may be counterbalanced by increased precipitation in tropical regions (i.e. more water fluctuation, less light availability); and greater inorganic carbon availability in temperate regions (Payne, 1986).

Are some aquatic groups of macrophyte better adapted to or characteristic of either tropical or temperate conditions?

My findings did show overall how some functional groups occurred in either tropical or temperate region or in both, and also found evidence that certain macrophyte genera are better represented at some latitudes than others, in calcareous rivers. The addition of more data in the tropics would give a better insight on aquatic plant species richness in hardwater streams. Previous studies, have found that some families are better represented at some particular latitudinal range. One of the few studies done on macrophyte species diversity has shown that families such as the Podostemaceae, Hydrocharitaceae, Limnocharitaceae, have strong affinities with the tropical latitudes, whereas groups such as Sparganiaceae and Haloragaceae usually have most of their component species distributed in the temperate regions (Crow, 1993). Working from such taxonomic generalisations has inherent dangers though: the common and highly invasive *Myriophyllum aquaticum* is a tropical member of the Haloragaceae, though it has penetrated as far north as the British Isles. *Pistia stratiotes*, a member of the Araceae (a family which is most diverse in the New World tropics, although also occurring in the Palaeotropics and north temperate regions) shows a similar invasive pattern away from its tropical origins into higher latitudes (for distribution of *Pistia stratiotes* see Figure 6-9).

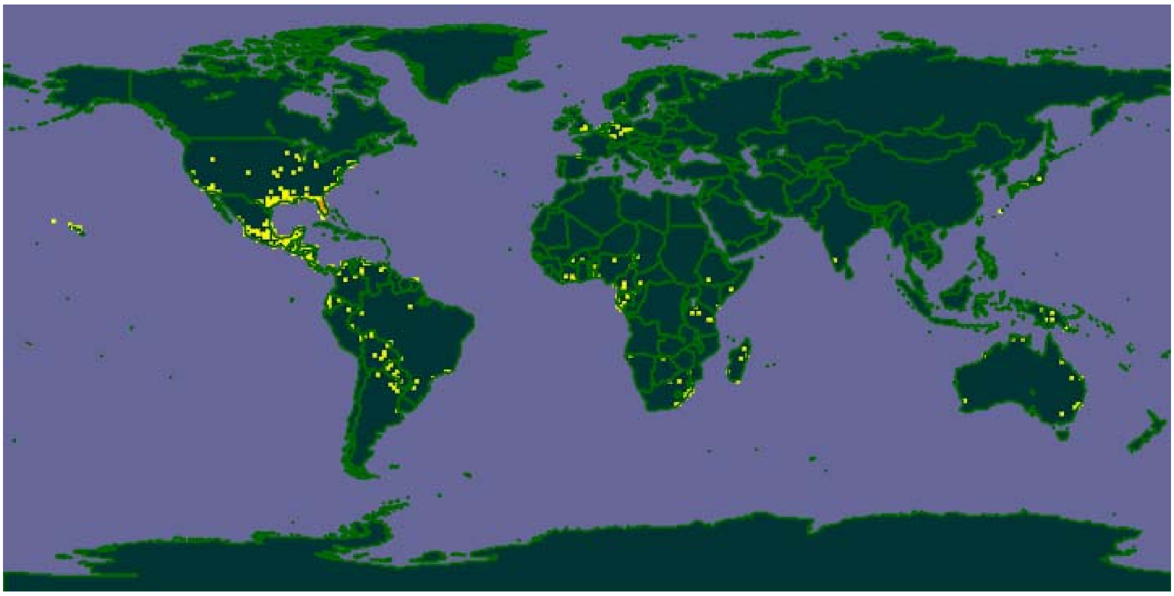


Figure 6-9.World distribution of *Pistia stratiotes*: centred in the Tropics, but invasive into higher latitudes in both northern and southern Hemispheres. Map Origin: http://data.gbif.org

It has been recognised that in terms of physical habitat preferences aquatic macrophyte species show strong parallel evolution, and species can hence be assigned to quite robustly-defined functional groups each of which has a specific habitat association. As well as the structurally-defined (zoned) functional groups utilised in my study, another well-known example (though not common in rivers) is the isoetids: a very clearly-distinct but taxonomically-varied functional group (members include a range of families from ferns, through Campanulaceae, to Plantaginaceae) mainly found in high latitude lakes, which is heavily adapted to low dissolved carbon, oligotrophic conditions (Rørslett 1991). It would be interesting to use macroecological methods to examine the relative impacts of large spatial v. local factors in influencing the distribution and diversity of such FGs (usually defined on combinations of morphological and/or physiological traits: e.g. Hills & Murphy, 1996) in rivers (and other freshwater systems).

Future macroecological studies in freshwater habitats may benefit from species-level information on well understood groups or use surrogates for species level patterns (e.g. families) (Heino, 2008).

Are some functional groups of macrophyte dominant in or characteristic of either tropical or temperate conditions?

My findings suggest that there is no specific functional group dominance across latitude. The reason behind this is probably largely to do with the universality of occurrence of the basic physical conditions defining the FGs used in my study. However the broad ecological tolerances and plastic responses of many aquatic plants, plus their clonal growth and abundance of easily dislodged propagules certainly facilitate their successful long distance dispersal as compared to other freshwater organisms, and hence contribute to the likelihood of their arrival in widely-geographically varied river habitats (Santamaria, 2002). A recent study has shown a relative higher abundance of hydrophytes (i.e. floating-leaved submerged and free-floating species) over helophytes (i.e. emergent species) at higher latitudes (Chappuis et al. 2012) suggesting a relationship between species morphological life form and habitat availability in relation to latitude. The scope of this study is more restricted in geographic range, than mine, which may have contributed to their findings. Since the scale of study does affect the relationship between latitude and species richness, clear latitudinal gradients present in regional studies may not be not present in global-scale studies. The predominant effect of large scale factors on local communities may overshadow latitudinal gradients (Heino, 2011).

My work is a focused study of freshwater macrophyte richness at a global scale, and it considered only one type of freshwater macrophyte-supporting habitat. It remains to be seen whether incorporation of a wider range of freshwater habitats would indicate any stronger latitudinal effects on macrophyte diversity than were detected for calcareous streams alone. Furthermore, future studies considering species distributional range in relation to latitude (Rapoport 1975) can also extend our understanding of how global spatial factors may affect freshwater macrophyte species richness.

Conclusions

Macrophyte species richness, measured as alpha-diversity in calcareous rivers, was at best only very weakly attributed to latitudinal gradient. This is most likely due to the effect of other physical, chemical and biotic variables overriding broader-scale influences on species richness, at more local scales. The expansion of knowledge of species richness and diversity in the tropics would also provide stronger evidence to support or reject my preliminary findings for macrophyte richness in hardwater stream systems.

Chapter 7. Diversity of macrophytes in calcareous streams across regional and local scales: discussion and conclusions.

7.1 INTRODUCTION

My study widens our current understanding of the diversity patterns observed in macrophyte ecology. This was made possible with the support of past studies and the supplement of additional surveys that I carried out during my study in areas where few or no previous records were available for hardwater river macrophytes.

Both global and local drivers were found to influence calcareous riverine macrophyte diversity across the world. My results show that geographical location is a good predictor of macrophyte diversity in the world, but the results showed thatlatitude *per se* showed only a weak, and somewhat contradictory association with species richness, despite the fact that geographical location was found to explain part (though only a small part) of the variation observed in macrophyte distribution. Furthermore spatial variables on their own plus spatially structured environmental variables were found to explain some part of the variation on macrophyte species richness and community structure, for the large datasets studied from a temperate and a tropical set of rivers. This is no surprise, as one would expect environmental factors to be correlated with geographic location. But one novelty in my results is that the model used to show these results may in future analyses allow us to partition the variation due to environment and spatial factors in much ghreater detail than was possible in my study (once suitable datasets become available: increasingly probable given, for example the increasing application of remote-sensing technology in freshwater ecology). Such an approach may prove to be a valuable tool to investigate and manage riverine species richness and community structure.

Last but not least local scale factors were found to be important in explaining hardwater river macrophyte species richness and community structure. In my study relatively few variables were included in the analysis but nevertheless water flow, pH, shade and alkalinity were shown to be co-related to species richness observed at a specific site. Moreover the distinct functional groups, into which macrophytes are usually split, as a function of their ecophysiology, did explain some of the expected variation observed at different sites.

7.2. MACROPHYTE DISTRIBUTION PATTERNS IN CALCAREOUS STREAMS

The basic unit to measure individual organisms of animals, plants or microbes is the species. Species are then classified into higher units, such as genera and families. Past studies on the geographical distribution of species show that there no two species have an exactly identical range range. Some species may be widespread within a given geographical area, and yet occupy different habitats and or microhabitat (Cox and Moore, 1993). Thus showing the complexity of defining a species distribution range and the importance of considering scale when studying distribution patterns. Angiosperms are first recorded in the Early Cretaceous, 120 million years ago. Many modern angiosperm families are known in the Northern Hemisphere, 95 million years ago, during the Early/Late Cretaceous boundary, depicting rapid speciation of flowering plants (Crane and Lidgard, 1989; Cox and Moore, 1993). The basic patterns of distribution of angiosperms have been explained by the Russian botanist Armen Takhtajan (1986), illustrated in the book edited by a British botanist, Vernon Heywood (1978) (Figure. 7-1). Biogeographical patterns of macrophyte distribution are not that straight forward however. Angiosperms are composed of 300 living families and 12 500 genera have been described compared to only 100 families and 1000 genera of living mammals. Greater diversity in plants may be due partly because flowering plants are much better at dispersal across ocean barriers compared to mammals, since dispersal may require as little as a single air-borne seed to colonise and successfully establish in a new place, instead of a breeding pair of mammals (or at least a single pregnant female: Cox and Moore, (1993). The aquatic macrophyte flora comprises a diverse assemblage of plants, which are adapted wholly or partially to life in fresh water. The majority are angiosperms (with very few or even no gymnosperms, depending on definition of freshwater habitat) as well as a few pteridophytes and a number of cryptogams.

Macrophytes have evolved physiological and morphological traits that allow them to live permanently, or at least for several months each year submerged in, floating on, or growing up out of fresh water habitats (Cook 1974). Few studies have looked in detail at the global distribution of macrophytes with the exception of Chambers et al. (2008), who found that though many species have broad ranges, macrophyte species diversity is highest in the Neotropics, intermediate in the Oriental, Nearctic and Afrotropics, lower in the Palearctic and Australasia, lower again in the Pacific Oceanic Islands, and lowest in the Antarctic region (note the differences in biogeographical regions used in this study, compared with Takhtajan's (1986) map). Some 39% of the c. 412 genera containing aquatic vascular macrophytes were found by this study to be endemic to a single biogeographic region, with 61 - 64% of all aquatic vascular macrophytes found in the Afrotropics and Neotropics being endemic to those regions (Chambers et al. 2008). Moreover Crow, (1993) shows global-scale latitudinal patterns on tropical floras.

Overall my findings in **Chapter 3** provide evidence that there is substantial variation in macrophyte assemblages present in calcareous rivers across the different countries included in my study, from temperate to tropical regions, broadly agreeing with information from the literature. Outlining the presence, absence or predominance of certain types of macrophytes across the different counties, and stressing the existence of species distribution ranges, I found two large groups based on species assemblages across the different countries included, i.e. a subtropical/tropical and a temperate group. In addition these two groups were found to overlap in macrophyte assemblages within some countries, which could at least in part be attributed to the presence of invasive and cosmopolitan species. Spreading of aquatic plants across countries is well documented (Hussner, 2009) and is a well-documented aspect of global change (Chapin et al., 2000).

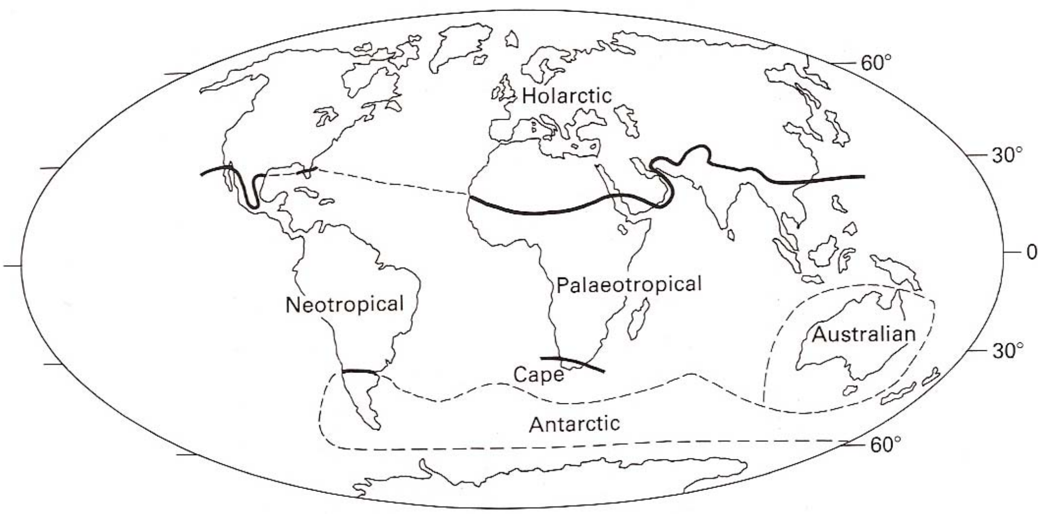


Figure 7-1. Floral regions of the world today. After Takhtajan (1986).

7.3 FACTORS INFLUENCING VEGETATION PATTERNS

Both geographical location and local environmental factors contribute to variation in alpha-diversity in the freshwater realm (Heino, 2011). Aquatic plants are sensitive to both longer and shorter-term changes in environmental factors and thus can be used as an indicator of temporal, spatial, chemical, physical and biological qualities of their ecosystem. The importance of a specific environmental factor depends on temporal and spatial scales (French and Chambers, 1996; Suren and Ormerod 1998). Aquatic plants may be grouped into five functional groups (marginal, emergent, free floating, floating-rooted and submerged species: Sculthorpe. 1967).

7.4 ENVIRONMENTAL FACTORS INFLUENCING SPECIES DISTRIBUTION

Multivariate analyses have been much used to assess the influence of physical-chemical, and other abiotic and biotic environmental factors potentially influencing macrophyte distribution, assemblage, and abundance in many types of freshwater habitat (e.g. Mackay et al. 2003; Murphy et al. 2003; Lacoul and Freedman, 2006b). Difference in environmental factors influences the distribution and abundance of aquatic plants, as is true of all organisms (Lacoul and Freedman, 2006b). Climatic factors of particular relevance to macrophytes includetemperature (Hutchinson, 1975; Spencer et al. 2000); wind (Andersson, 2001); precipitation (Matias and Irgang, 2006); climatic conditions associated with latitude (Chapin et al, 2002; Virola et al. 2001); altitude (Rorslett, 1991), hydrology associated with disturbance and drought (Mitsch and Gosselink, 2000; Anderssson, 2001); substrate (Ferreira, 1994); nutrients and trophic status (Chambers, 1987; Schneider and Melzer, 2003); pH and alkalinity (Murphy, 2002; Vestergaard and Sand-Jensen, 2000; Riis et al. 2000; Arts, 2002); and light availability linked directly to photosynthesis processes (Madsen and Maberly, 1991; Squires et al. 2002; Madsen and Sand-Jensen, 1994; Tavechio and Tomaz, 2003). The ability of aquatic plants to survive under various environmental conditions is partly related to their life form (isee functional group definition in **Chapter 2** and **Chapter 4**).

As demonstrated in different parts of **Chapter 4**, it is possible to identify different diversity responses of macrophyte FGs to environmental conditions, at local scale, in hardwater rivers. Taking into consideration that each species will have specific response thresholds to different environmental factors, macrophytes have the potential to be used as an indicator of environmental changes within a study region. Width and flow were found to be significantly affecting the distribution patterns of diversity of free-floating and floating-leaved rooted species, whereas diversity of marginal species was significantly related to alkalinity and width, and floating-leaved rooted diversity was significantly related to alkalinity. Last but not least submerged species were related to shading. Knowledge about the possible impacts of local conditions enables me to address latitudinal gradient effects (regional factors), utilising sub-sets of sites with comparable local conditions e.g. **Chapter 5**.

7.5 SPATIAL FACTORS INFLUENCING SPECIES DISTRIBUTION

Generally, the number of species present increases with the increase of habitat suitability (Arrhenius, 1921; Weiher and Boylen, 1994) and decreases with the isolation of habitat "islands" (Mac Arthur and Wilson, 1967). The Arrhenius equation basically looks at the relationship of species richness and habitat area.

[1] $S = cA^z$

where S is the number of species, c is a constant, A is habitat area, and z is the slope of a log/log relationship of S and A (Rosenzweig, 1995; Lacoul and Freedman, 2006b). Previous studies have shown how the surface area of a waterbody is related to the richness of aquatic plants present in terms of diversity, and area of habitat occupied by different species (Rørslett, 1991; Rosenzweig, 1995). Moreover species richness can also be affected by species limited dispersal at some spatial scales, becoming more important at larger scales (Hubbell, 2001).

A better understanding of the mechanisms of species diversity patterns may be gained based on the integration of large-scale macroecological and landscape-scale metacommunity research. Large-scale studies will illuminate patterns of species diversity across regional and local scales in the freshwater realm (Heino, 2011). In **Chapter 5** I illustrate the importance of including spatial factors as a way to describe some of the patterns observed in macrophytes across regional and local scales as found in previous studies (Heino, 2009; Heino, 2011; Bini, Thomaz and Souza, 2001; Kreft and Jetz, 2007; Carvalho, et al. 2009; Thomaz et al. 2009). My findings show that variation in richness and community structure for hardwater river macrophytes can be partly explained by environmental variation relative to spatial processes in the British Isles (temperate scenario) and in Zambia (tropical scenario). Among the environmental variables, climatic ones explained a great part of species richness and composition distribution for the British Isles. Conversely in Zambia spatial processes made the greatest contribution to variation in hardwater river macrophyte species richness and community structure.

It should be noted that my study made no attempt to identify what the actual factors were, acting at different spatial scales, in influencing these results, but simply showed that one or more such factors, associated with each relevant PCNM vector, differentially influenced macrophyte assemblages present in (for example) different parts of the British Isles. A considerable amount of further work is needed to tease out what exactly is responsible for these observed results, but it is highly likely to be due to spatial variation with latitude, longitude, both, or (most likely) a more complex combination of spatial factors. For example (refer to Figure 5-6), the small-scale spatial vector PCNM4 shows a strong north to south spatial trend in Britain, but less so in the island of Ireland. The intermediate spatial-scale vector PCNM8 shows a curious east to west bimodal pattern, with a hot spot for importance of this vector at sites in the south of Ireland. In contrast to these rather clear geographical patterns, both the largest-scale PCNM vectors (PCNM81 and PCNM100) showed a much more mixed distribution across the UK, and appear to be of no importance at all in the Republic of Ireland sites. Are these patterns really expressing differences in spatial drivers of calcareous river vegetation assemblage and diversity, and if so in what way? These are questions beyond the scope of my study to address, but at least my results indicate some possible directions for future work to address these issues, perhaps of particular relevance in the context of climate change and how it may affect river plants.

My results increase our knowledge of the processes influencing calcareous river macrophyte ecology, but clearly it is important to consider as wide a range as possible of potential structuring influences on river communities, environment and space (O'Hare, et al. 2012a; Borcard and Legendre, 2002; Jombart, Dray and Dufour, 2009). Therefore illustrating a multivariate analysis that incorporates all associated predicting factors into a single analysis is of extreme importance. The incorporation of connectivity analysis (e.g. in Astorga, 2011) for the British Isles explained in more detail the spatially structured environmental variables that were shown in my study to be determinants of macrophyte species richness patterns in hardwater rivers in the two areas compared, which could also be done in the future for Zambia or any other relevant regions for which sufficient distribution data exist.

The idea that latitudinal gradients defining regional species richness (RSR) patterns date back to the early 1800s and are considered to be the oldest recognised ecological pattern (Hawkings, 2007), with RSR normally decreasing with higher latitude. Such patterns have been shown constantly for different terrestrial taxa and marine taxa (Hillebrand, 2004a). There is more limited knowledge for freshwater taxa until recently (Balian et al. 2008). Nonetheless there is now some evidence, including my own results, to suggest that latitude gradients is not related to RSR for freshwater organisms at the global scale (Crow, 1993; Covinch, 2009). This is a topic clearly in need of further explanation, and a topic where further work is certainly required.

My own data, In **Chapter 6** illustrate that macrophyte species richness, measured as alpha-diversity, in calcareous rivers, could at best be only very weakly attributed to latitudinal gradient. This is most likely due to the effect of other physical, chemical and biotic variables overriding broader-scale influences on species richness, at more local scales. The expansion of knowledge of species richness and diversity in the tropics would also provide stronger evidence to support or reject my preliminary findings for drivers of macrophyte richness in hardwater stream systems.

7.6 CONCLUSIONS

The overall aim of my study was to widen current knowledge of the geographical patterns of species and family diversity in aquatic macrophyte taxa, targetting a defined type of freshwater system. This aim was achieved by gaining data to illustrate the different macrophyte assemblages found across different calcareous streams in temperate and tropical/subtropical regions. Macrophytes were found to be widespread in hardwater streams, across the world, though with different families prevailing in some parts of the globe. Due to the high level of polymorphism and phenotypic plasticity in their response to variation of environmental variables, many macrophytes can occur over a wide range of conditions. Moreover spatial factors were also shown to interact with species diversity and environmental factors in hardwater stream macrophyte communities, depicting the complex interactions determining species diversity and richness, which should be taken into further consideration for management of these aquatic ecosystems.

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APPENDICES

Appendix 1 Macrophyte species name, abbreviation and functional group allocation (E= emergent, m=marginal, FL= free-floating, FLR= floating-rooted, Sub= submerged.

Species name	Abbreviations	Function-al group
?Lysimachia sp. unknown emergent	LysspSR2	E
?Andropogon sp	AndspEMP	E
?Bidens cf. pilosa Yellow flower dicot hairy leaf MX3A	BipiMX3A	E
?Centella sp. MX10D small Rorippa like MX10D	CenMX10D	E
?Echinochloa polystachya smooth white stripe grass T9 T10 MX11F (mex specimen with flower)	EcpoT9	E
?Habenaria purple orchid MX4B ? Habenaria sp.	HabMX4B	E
?Ipomoea indica creeping trifoliate leaf T16E	IpinT16E	E
?Luziola bahiensis tall grass short leaf closed panicle MX11D	LubMX11D	m
?Panicum sp. T6 Small smooth ?Panicum	PaspT6	m
?Panicum zizanioides Grass with auricles MX13A	PaziMX13A	m
?Pycreus sp. sedge long peduncle flowers shorter stem MX11B in press (don't confuse with "Cyperus pale MX11B" these are different plants!)	PysMX11B	m
Acroceras macrum	Acrmac	m
Aeolanthus abyssinicus	Aeoaby	m
Aeollanthus engleri	Aeoeng	m

Aeschynomene fluitans	Aesflu	m
Aeschynomene sp.	AessptT7	m
Agrostis stolonifera	Agrsto	E
Alisma plantago-aquatica	Alipla	E
Alternanthera sessilis	Altses	E
Alternanthera philoxeroides	Altphi	E
Amaranthus australis	Amaaus	E
Ammannia senegalensis	Ammsen	E
Apium nodiflorum	Apinod	E
Aponogeton desertorum	Apodes	Sub
Aponogeton junceus	Apojun	Sub
Aponogeton rehmanii	Aporeh	Sub
Asteraceae	Ast	E
Asteraceae MX1F orange flower dicot MX1F	Ast1MX1F	E
Asteraceae MX4C yellow composite MX4C	Ast2MX4C	E
Asteraceae T16B (hairy composite purple flower T16B = Broad leaf dicot T11A)	Ast3T16B	E
Asteraceae T16C Yellow flower composite cerrated leaf T16 C	Ast4T16C	E
Axonopus compressus	Axocom	E
Azolla filiculoides	Azofil	Fl
Azolla caroliniana	Azocar	Fl

Azolla mexicana	Azomex	Fl
Bacopa floribunda	Bacflo	sub
Bacopa? salzmannii	Bacsal	sub
Bacopa australis	Bacaus	Sub
Bacopa cf.rotundifolia	Baccfrot	E
Bacopa monnieri	Bacmon	E
Baldellia ranunculoides	Balran	FLR
Berula erecta	Berere	E
Bidens laevis	Bidlae	E
Bolbitis heudelotii	Bolheu	Sub
Bolboschoenus cf. fluviatilis	Bolcfflu	E
Bracharia mutica	Bramut	E
Brachiaria subquadripara	Brasub	E
Buchnera sp.	BucWakL1	E
Cabomba caroliniana	Cabcar	Sub
Cabomba haynesii	Cabhay	Sub
Callitriche hamulata	Calham	sub
Callitriche platycarpa	Calpla	Sub
Callitriche stagnalis	Calsta	Sub
Caltha palustris	Calpal	m
Caperonia bahiaensis	Capbah	E
Caperonia castaneifolia	Capcas	E

Cardamine palustris	Carpal	m
Cardamine pratensis	Carpra	m
Carex nigra	Carnig	m
Carex riparia	Carrip	m
Carex rostrata	Carros	m
Carex spp 1	CarIRC2	m
Carex spp 2	Car2022	m
Carex vesicaria	Carves	m
Carphephorus odoratissimus	Carodo	E
Catabrosa aquatica	Cataqu	m
Cayaponia podanthe	Caypod	E
Ceratophyllum demersum	Cerdem	Fl
Ceratopteris thalictroides	Certha	E
cf. Cyperus alopecuroides open flower T10	CypaloT10	E
Cicuta mexicana	Cicmex	E
Cladium jamaicense	Clajam	E
Cladium mariscus	Clamar	E
Colocasia esculenta	Colesc	E
Commelina diffusa	Comdif	E
Commelina fluviatilis	Comflu	E
Comelina cf. erecta	Comere	E
Commelina schomburgkiana	Comsch	E
Cortaderia selloana	Corsel	E

Cotula sp	CotspM1	E
Crinum macowanii	Crimac	E
Crinum americanum	Criame	E
crucifer white flower	CruReits	E
Cyperaceae 1, chaco	Cyp17417	E
Cyperaceae MX10B yellow cyperus MX10B	Cy2MX10B	E
Cyperaceae MX8B small brown cyperus MX8B	Cyp3MX8B	E
Cyperaceae T17B small sedge T17B	Cyp4T17B	E
Cyperaceae T18B	Cyp5T18B	E
Cyperaceae with sword tooth edge	Cypspp1	E
Cyperus	Cypspp2	E
Cyperus alopecuroides	Cypalo	E
Cyperus articulatus	Cypart	E
Cyperus difformis	Cypdif	E
Cyperus digitatus	Cypdig	E
Cyperus involucratus	Cypinv	E
Cyperus longus	Cyplon	E
Cyperus papyrus	Cyppap	E
Cyperus procerus	Cyppro	E
Cyperus? reflexus MX10C	CyrMX10C	E
Cyperus? surinamensis sedge bright green v long bracts MX11C in press	CysMX11C	E
Cyperus aggregatus Cyperus long thin leaf brown flower MX1G	CyagMX1G	E

Cyperus aggregatus (= C. flavus)	Cypagg	E
Cyperus alopecuroides	Cypalo	E
Cyperus articulatus	Cypart	E
Cyperus B	Cy1para1	E
Cyperus b. Spec	Cy2reits	E
Cyperus cf. esculentus	Cypesc	E
Cyperus cf. gardneri	Cypcfgar	E
Cyperus difformis	Cypdif	E
Cyperus diggitatus	Cypdig	E
Cyperus distinctus compact flower MX5B (= C. distinctus = C. virens)	CydiMX5B	E
Cyperus dives	Cypdiv	E
Cyperus eragrostis	Cypera	E
Cyperus erythrorhizos [Sedge like cyperus? aleopecuroides MX1C (= T6 Cyperus? alopecuroides) = Cyperus erythrorhizos	Cypery	E
Cyperus esculentus	Cypesc	E
Cyperus giganteus	Cypgig	E
Cyperus lanceolatum pale small flower MX6A	Cyplan	E
Cyperus odoratus (= C. ferrugineus)	Cypodo	E
Cyperus sp.	Cyp3MoiR	E
Cyperus sp. MX11B pale	CypMX11B	E
Cyperus sp. MX5C fluffy inflorescenc brown stem MX5C	Cyp4MX5C	E
Cyperus sp. T1 in press T1	Cyp5T1	E

Cyperus? distichum	Cypdis	E
Dichromena colorata "star" cyperus white bract MX9A	DicoMX9A	E
Dicot creeper unknown T16D creaping broad leaf glabrous T16 D	DiT16D	m
Dicot unknown MX10 small pink flower	Di1MX10	m
Dicot unknown MX10G small shiny green leaf MX10G	Di2MX10G	m
Dicot unknown MX11 another Rorippa-like plant…	Di3MX11	m
Dicot unknown T4/T5 Rorippa like	Di4T4/5	m
Dicot unknown T5 big grass-like dicot	Di5T5	m
Dicot unknown T5 Smooth dicot small leaf	Di6T5	m
Dicot unkown T17A	Di7T17A	m
Echinochloa jubata	Echjub	E
Echinochloa ugandensis	Echuga	E
Echinochloa crus-galli	Echcru	E
Echinochloa walteri	Echwal	E
Echinodorus ashersonianus	Echash	Sub
Echinodorus bolivianus	Echbol	Sub
Echinodorus grandiflorus	Echgra	Sub
Egeria densa	Egeden	Sub
Eichhornia azurea	Eicazu	FLR
Eichhornia crassipes	Eiccra	Fl

Elatine ambigua	Elaamb	E
Eleocharis atropurpurea	Eleatr	E
Eleocharis caduca	Elecad	E
Eleocharis dulcis	Eledul	E
Eleocharis geniculata	Elegen	m
Eleocharis naumanniana	Elenau	E
Eleocharis? pachycarpa (needle like)	Elepac	E
Eleocharis? quinqueflorus (tubular)	Elequi	m
Eleocharis ?geniculata MX8A ?geniculata	ElgeMX8A	E
Eleocharis 1	Ele1	E
Eleocharis acicutans? = Eleocharis acicularis	Eleaci	Sub
Eleocharis atropurpurea	Eleatr	E
Eleocharis cf. acutangula	Elecfacu	E
Eleocharis cf. atropurpurea	Elecfatr	m
Eleocharis cf. cellulosa MX1A	ElceMX1A	m
Eleocharis cf. cellulosa tall, narrow flower NOT ?dulcis	Eledul	m
Eleocharis cf. nudipes	Elecfnud	m
Eleocharis palustris	Elepal	m
Eleocharis variegata	Elevar	m
Eleogiton fluitans	Eleflu	Sub
Elodea canadensis	Elocan	sub
Elodea nuttallii	Elonut	Sub
Enydra anagallis	Enyana	m
Enydra radicans	Enyrad	m

Epilobium hirsutum	Epihir	m
Epilobium sp Kund	Episp	m
Equisetum sp. MX10	Equ1MX10	m
Equisetum fluviatile	Equflu	m
Equisetum spp	Equ1Moid	m
Eriocaulon abyssinicum	Eriaby	Sub
Eriocaulon dregei	Eridre	Sub
Eriophorum angustifolium	Eriang	m
Filipendula ulmaria	Filulm	m
Floscopa glomerata	Floglo	m
Fuirena pubescens	Fuipub	m
Fuirena umbellata	Fuiumb	m
Fuirena simplex Green "grass/ cyperus"	Fuisim	m
Galium palustre	Galpal	m
Gentiana nivalis	Genniv	m
Glyceria declinata	Glydec	FLR
Glyceria fluitans	Glyflu	FLR
Grangea anthemoides	Graant	m
Grass 1 (indet)	Gra17077	m
grass Luap	GrassL	m
Grass white midrib	Gra2Mul2	m
Grass with hairy auricles	Grass3	m
Heteranthera zosteriformis	Hetzos	Sub
Hibiscus coccineus	Hibgra	m

Hibiscus grandiflorus	Hibgra	m
Hibiscus striatus	Hibstr	m
Hippurus vulgaris	Hipvul	E
Hydrilla verticillata	Hydver	Sub
Hydrocotyle sibthorpiodes	Hydsib	E
Hydrocotyle bonariensis	Hydbon	E
Hydrocotyle ranunculoides	Hydran	FLR
Hydrocotyle umbellata	Hydumb	E
Hydrocotyle vulgaris	Hydvul	E
Hydrostachys polymorpha	Hydpol	Sub
Hygrophila polysperma	Hygpol	Sub
Hymenachne amplexicaulis	Hymamp	m
Hymenocallis cf. littoralis	Hymcflit	m
Hymenocallis palmari	Hympal	m
Hyparrhenia hirta	Hyphir	m
Impatiens glandulifera	Impgla	m
Ipomea fistulosa	Ipofis	m
Ipomoea carnea	Ipocar	m
Iris pseudacorus	Iripse	E
Isoetes lacustris	Isolac	Sub
Isolepis prolifera? prolifera?	Isopro	E
Juncus 1	Ju1IRC25	m
Juncus articulatus	Junart	m
Juncus bufonius	Junbuf	m
Juncus bulbosus	Junbul	Sub

Juncus cf. oxycarpus	Juncfoxy	m
Juncus effusus	Juneff	m
Juncus exertus	Junexe	m
Juncus inflexus	Juninf	m
Juncus megacephalus "Cyperus D". SR4.6, SR4.7	JunmCySR	m
Juncus oxycarpus	Junoxy	m
Juncus scabriusculus	Junsca	m
Kosteletkzya virginica	Kosvir	m
Lagarosiphon ilicifolius	Lagili	Sub
Landoltia punctata (= Spirodela punctata)	Lanpun	Fl
Leersia hexandra	Leehex	m
Lemna gibba	Lemgib	Fl
Lemna minor	Lemmin	Fl
Lemna minuta	Lemmin	Fl
Lemna valdiviana	Lemval	Fl
Leptocloa fusca	Lepfus	E
Limnobium laevigatum (= L. spongia)	Limlae	Fl
Limnocharis flava	Limfla	m
Limnophila bangweolensis	Limban	m
Limnophila cf. indica	Limcfind	m
Limnophila indica	Limind	m
Limnophyton angolense	Limang	m
Limosella australis	Limaus	m
Littorella uniflora	Lituni	Sub

Lobelia cardinalis	Lobcar	E
Ludwigia ?erecta tall red stem MX11G	LueMX11G	E
Ludwigia ?octovalvis hairy stem MX8	LudocMX8	E
Ludwigia 2 chaco	Lud2Neg1	E
Ludwigia abysinica	Ludaby	E
Ludwigia adscendens	Ludads	E
Ludwigia cf. Octovalvis	Ludcfoct	E
Ludwigia erecta	Ludere	E
Ludwigia helminthorrhiza	Ludhel	E
Ludwigia leptocarpa	Ludlep	E
Ludwigia palustris	Ludpal	E
Ludwigia peploides	Ludpep	E
Ludwigia peruviana	Ludper	E
Ludwigia repens	Ludrep	E
Ludwigia senegalensis	Ludsen	E
Lysimachia thyrsiflora	Lysthy	m
Lysimachia vulgaris	Lysvul	m
Lythrum hyssopifolia	Lythys	m
Lythrum salicaria	Lytsal	m
Lythrum spp?	Lytspp	m
Lythrum vulgaris	Lytvul	m
Marsilea crotophora	Marcro	E
Marsilea spp.	Mar1M1	E
Mentha aquatica	Menaqu	E

Menyanthes trifoliata	Mentri	E
Mimulus gracilis?	Mimgra	m
Mimulus guttatus	Mimgut	m
Mitreola ?petiolata	Mitpet	m
Monocot unknown MX1D Big monocot like Acorus MX1D	MonMX1D	m
Monocot unknown T16A Broad leaf monocot T16 A	MonT16A	m
Myosotis scorpioides	Myosco	E
Myriophyllum ? elatinoides	Myrela	Sub
Myriophyllum alterniflorum	Myralt	Sub
Myriophyllum aquaticum	Myraqu	Sub
Myriophyllum spicatum	Myrspi	Sub
Najas guadalupensis	Najgua	Sub
Najas horrida	Najhor	Sub
Najas marina MX10A	NamaMX10	Sub
Najas microcarpa	Najmic	Sub
Narrow leaved grass spike inflorescence	Grass4	m
Nuphar lutea	Nuplut	FLR
Nuphar luteum	Nuplut	FLR
Nymphaea ?elegans MX10E ?elegans (blue/white flowers)	NyeMX10E	FLR
Nymphaea amazonum	Nymama	FLR
Nymphaea ampla white flower serrated leaf	Nymamp	FLR
Nymphaea gairdnerianum	Nymgai	FLR
Nymphaea lotus	Nymlot	FLR

Nymphaea nouchali var. caerulea	Nymnou	FLR
Nymphea aquatica	Nymaqu	FLR
Nymphea mexicana	Nymmex	FLR
Nymphea odorata	Nymodo	FLR
Nymphoides indica occidentalis	Nyminocc	FLR
Nymphoides indica white flower MX11A	NyiMX11A	FLR
Oenanthe aquatica	Oenaqu	Sub
Oenanthe fluviatilis	Oenflu	Sub
Oryza barthii	Orybar	E
Oryza? glumaepatula open grass smooth open panicle MX4A	OryMX4A	E
Osmunda regalis	Osmreg	E
Ottelia exserta	Ottexs	Sub
Ottelia ulvifolia	Ottulv	Sub
Ottelia sp	Ottspp	Sub
Ottelia verdickii	Ottver	Sub
Oxycaryum cubense	Oxycub	E
Panicum ?aquaticum	Panaqu	m
Panicum ?dichotomiflorum tall grass long leaf smooth big panicle MX3C	PadiMX3C	m
Panicum elephantipes	Panele	m
Panicum gilvum	Pangil	m
Panicum graminosum	Panigra	m
Panicum hemitomon	Panhem	m
Panicum parvifolium	Panpar	m
Panicum pernambucense	Panper	m

Panicum prionitis	Panpri	m
Panicum repens	Panrep	E
Panicum rivulare	Panriv	m
Panicum subalbidum	Pansub	E
Paspalidium geminatum	Pasgem	E
Paspalum distichum	Pasdis	E
Paspalum inbaliculatum	Pasinb	m
Paspalum repens	Pasrep	E
Paspalum scrobiculatum	Passcr	E
Paspalum sp. MX15A	Pa1MX15A	m
Paspalum sp. T1 In press T1 MX5 MX6	Pas2T1	m
Paspalum vaginatum	Pasvag	m
Peltandra virginica	Pelvir	m
Pennisetum natelense	Pennat	m
Persicaria amphibia	Peramp	FLR
Persicaria attenuata	Peratt	E
Persicaria attenuata ssp. africana	Peratt	E
Persicaria cf hydropiper	Percfhyd	E
Persicaria decipiens	Perdec	E
Persicaria hydropiper	Perhyd	E
Persicaria lapathifolia	Perlap	E
Persicaria limbata	Perlim	E
Persicaria meisneriana beyrichiana	Permebey	E
Persicaria senegalensis	Persen	E
Petasites hybridus	Pethyb	m

Phalaris arundinacea	Phaaru	m
Phragmites australis	Phraus	m
Phragmites mauritianus	Phrmau	m
Phyllanthus fluitans	Phyflu	Fl
Pistia stratiotes	Pisstr	Fl
Pluchea odorata Dicot white and pink flower MX11H	PloMX11H	m
Poaceae 1	Poa1Ria1	m
Poaceae 2 white mid-rib (kj photo)	Poa2Neg3	m
Poaceae unknown MX11F Small white stripe grass smooth with flower MX 11F	Po3MX11F	m
Poaceae unknown MX16A	Po4MX16A	m
Poaceae unknown MX8 grass thin pale green MX8C	Poa5MX8	m
Poaceae unknown SR2.4 Grass E open panicle SR2.4	Poa6SR	m
Poaceae unknown T13 Fine grass T13	Poa7T13	m
Poaceae unknown T18A red flower grass T18A	Poa8T18A	m
Poaceae unknown T2 White striped grass rough in press T2 T3 T5 T11	Poa9T2	m
Poaceae unknown T5 small white stripe grass	Poa10T5	m
Podostemaceae		Sub
Polygonum ?hydropiperoides	Polhyd	E
Polygonum acuminatum	Polacu	E
Polygonum densifolia (= Persicaria)	Polden	E

Polygonum ferrugineum	Polfer	E
Polygonum hydropiper	Polhyd	E
Polygonum lapathifolium	Pollap	E
Polygonum punctatum	Polpun	E
Polygonum senegalensis	Polsen	E
Polygonum stelligerum	Polste	E
Pontederia cf.rotundifolia	Poncfrot	m
Pontederia cordata	Poncor	m
Potamogeton nodosus	Potnod	FLR
Potamogeton berchtoldii	Potber	Sub
Potamogeton gramineus	Potgra	Sub
Potamogeton illinoensis	Potill	Sub
Potamogeton natans	Potnat	FLR
Potamogeton octandrus	Potoct	Sub
Potamogeton perfoliatus	Potper	Sub
Potamogeton polygonifolius	Potpol	FLR
Potamogeton pusillus	Potpus	Sub
Potamogeton richardii	Potric	Sub
Potamogeton schweinfurthii	Potsch	Sub
Potamogeton striatus	Potstr	Sub
Prionium	Pri1	m
Pulicaria scabra	Pulsca	m
Pycreus sp.	Py1	m
Pycreus unioloides	Py2Chi09	E
Rannunculus aquatilis	Ranaqu	Sub

Rannunculus flammula	Ranfla	E
Ranunculus acris	Ranacr	m
Ranunculus lingua	Ranlin	m
Ranunculus multifidus	Ranmul	m
Ranunculus penicillatus	Ranpen	Sub
Ranunculus pseudofluitans	Ranpse	Sub
Ranunculus trichophyllus	Rantri	Sub
Riparian, Purple flower	Ast3T16B	m
Rorippa ?teres	Rorter	m
Rorippa nasturtium-aquaticum	Rornaaqu	E
Rorripa amphibia	Roramp	E
Rotala myriophylloides	Rotmyr	Sub
Rumex conglomeratus	Rumcon	m
Rumex sp.	Rum1Was1	m
Rynchospora corniculata	Ryncor	m
Sacciolepis striata	Sacstr	m
Sagittaria ?filiformis	Sagfil	E
Sagittaria kurziana	Sagkur	Sub
Sagittaria lancifolia	Saglan	E
Sagittaria latifolia (broad leafs)	Saglat	E
Sagittaria montevidensis	Sagmon	E
Salvinia auriculata	Salaur	Fl
Salvinia biloba	Salbil	Fl
Salvinia herzogii	Salher	Fl
Salvinia minima	Salmin	Fl

Salvinia molesta	Salmol	Fl
Saururus cernuus	Saucer	m
Schoenoplectus	Schspp	m
Schoenoplectus triqueter	Schtri	m
Schoenoplectus 1	Sch1 6655	m
Schoenoplectus brachycerus	Schbra	m
Schoenoplectus confusus	Schcon	m
Schoenoplectus corymbosus	Schcor	m
Schoenoplectus decipiens	Schdec	m
Schoenoplectus? californicus	Schcal	m
Schoenoplectus lacustris	Schlac	m
Scirpus californicus	Scical	m
Scirpus confusus spec.	Scicon	m
Senecio bonariensis	Senbon	m
Senecio glabellus	Sengla	m
Setaria cf. parviflora Phleum like purple leaf grass MX1K	SepaMX1K	m
Sium repandum	Siurep	m
Solanum glaucophyllum	Solgla	m
Sparganium angustifolium	Spaang	FLR
Sparganium emersum	Spaeme	FLR
Sparganium erectum	Spaere	E
Sphaerothylax algiformis	Sphalg	Sub
Spilanthes cf. uliginosa long stem serrated leaf brown axillary flower on stalks MX12A ("sacaton")	SpuMX12A	m
Spilanthes urens Serrated edge leaf dicot blue-white flower MX1B	SpuMX1B	m

Spirodela polyrhiza	Spipol	Fl
Stellaria alsine	Steals	m
Stukenia pectinata	Stupec	Sub
Thalia geniculata	Thagen	m
Torenia thouarsii	Tortho	m
Trapa natans	Tranat	FLR
Tristicha trifaria	Tritri	Sub
Typha capensis	Typcap	E
Typha domingensis	Typdom	E
Typha latifolia	Typlat	E
Uknown Polygonaceae 1	Po1KasRi	E
unknown Lamiaceae 1	La1Mula4	m
Unknown Lamiaceae 2	La2Muso2	m
Urochloa cf. fasiculatus Grass T14B	UrfaT14B	m
Urochloa mutica	Uromut	m
Utricularia ?purpurea (NOT gibba)	Utrpur	Fl
Utricularia australis	Utraus	FL
Utricularia cf. arenaria	Utrcfare	FL
Utricularia cf. inflexa	Utrcfinf	FL
Utricularia foliosa	Utrfol	Fl
Utricularia gibba	Utrgib	Fl
Utricularia inflexa	Utrinf	FL
Utricularia sp. Ba	Utrspp1	Fl
Utricularia spp 1	Utr2Ria2	fl

Utricularia stellaris	Utrste	FL
Valeriana dioica	Valdio	m
Valeriana officinalis	Valoff	m
Vallisneria americana	Vallame	Sub
Vallisneria spiralis	Valspi	Sub
Veronia glabra	Vergla	m
Veronica anagallis-aquatica	Veranaqu	E
Veronica beccabunga	Verbec	E
Veronica scutellata	Verscu	m
Vossia cuspidata	Voscus	m
Websteria confervoides	Webcon	m
Wiesneria schweinfurthii	Wiesch	m
Wolffia brasiliensis	Wolbra	Fl
Xyris anceps	Xyranc	m
Yellow crucifer	Cru1M1	m
Zannichellia palustris	Zanpal	Sub
Zizania aquatica	Zizaqu	m
Zizaniopsis miliacea	Zizmil	m

Appendix 2. Sample sites code. Full-dataset used for DCA and twinspan analysis in Chapter 3 and a subset of the data was used for further analyses carried out in Chapter 4,5,6.

Country	Site code	Sample site
Argentina	PARA1	Rio Paraguay
Argentina	Sauce1	R. Sauce Grande
Argentina	Sauce2	Cementerio R. Sauce Grande
Argentina	ED1	El Divisorio
Argentina	Negro	R. Negro affluent to the Sauce Gde
Argentina	Zorro	R. Zorro affluent to the R. Sauce Gde
Argentina	ED2	El Divisorio downstream
Argentina	NC1	Naposta Chica
Argentina	Neg3	Rio Negro
Argentina	Para1	Rio Paraguay
Argentina	ARPA190	Garças Lake
Argentina	ARPA490	Patos Lake
Argentina	ARPA590	Ventura Lake
Argentina	ARPA690	Osmar Lake
Brazil	S101	Lagoa Saraiva (Guaira)
Brazil	PG101	Chapter 7Parana River (main channel) Guaira
Brazil	LX101	Lagoa Xambre (Guaira)
Brazil	PV101	Chapter 8Pao Velho backwater (Porto Rico)
Brazil	SJ101	Lagoa Sao Joao Guaira)
Brazil	RL101	Ressaco Leopoldo (Porto Rico)
Brazil	BD101	Baia River downstream
Brazil	BU101	Baia River upstream
Brazil	SR101	Santa Rosa (Porto Rico)
Brazil	RM101	Chapter 9Ressaco do Manezinho (Porto Rico)
Brazil	RV101	Chapter 10Ressaco do Valdo (Porto Rico)
Brazil	FOR1	Chapter 11Rio Formoso 2: Balnearias Municipal (Bonito)
Brazil	FOR2	Rio Formoso 1: Cabanas (Bonito)
Brazil	BON	Rio Bonito (Bonito)
Brazil	SUC1	Rio Sucuri (Bonito)
Brazil	PLAT	Rio da Plata (Bonito)
Brazil	MIR1	Rio Miranda: lagoon (Pantanal)
Brazil	MIR2	Chapter 12Rio Miranda: main channel (Pantanal)
Brazil	MIR3	Corixao: distributary of R. Miranda (Pantanal)
Brazil	MIR4	Chapter 13Rio Vermelho: vazante (secondary channel) of Vermelho (tributary of R. Miranda) (Pantanal)
Brazil	NEGR1	Rio Negro: main channel, Bridge 61, km57.480 (Pantanal)

Brazil	SAN1	Santo Antonio: main channel (trib of Paraguacu): Chapada Diamantina
Brazil	SAN2	Santo Antonio: secondary channel (trib of Paraguacu): Chapada Diamantina
Denmark	DE662	Karstoft Aa, Noerre Grene
Denmark	DE663	Mattrup Aa, Stids Moelle
Denmark	DE664	Lindenborg Aa, Roede Moelle
Denmark	DE665	Sunds Noerre Aa, Noerre Linaa, Noerre Linaa
Denmark	DE666	Rind Aa, Hoegild
Denmark	DE667	Kastbjerg Aa, Edderup
Denmark	DE668	Fjederholt Aa, Okkels
Denmark	DE669	Tange Aa, Lillemoelle
Denmark	DE670	Skibsted Aa, Skibstedbro
Denmark	DE671	Skals Aa, Faarup
Denmark	DE672	Ry Aa, Jerslev bro
Denmark	DE673	Ryom Aa, Koed
France	FR723	Ignon upstream Fresnois
France	FR724	Aube at Aubepierre-sur-Aube
France	FR725	Chapter 14 Seine at the 'Ermitage du Val de Seine'
France	FR726	Aujon upstream Giey-sur-Aujon
France	FR727	Rognon at Montot-sur-Rognon
France	FR731	Madon at HagÈcourt (pont bleu)
France	Alsace	Alsace
Germany	GE650	Eltingmuehlenbach near Greven (NRW)
Germany	GE655	Chapter 15 Berkel SE of Vreden (NRW)
Germany	GE658	Dinkel near Heek (NRW)
Germany	GE659	Issel N of Loikum (NRW)
Germany	GE660	Stever near Hullern (NRW)
Greece	GK735	Peristeria, Artiki
Greece	GK736	Peristeria, Kalo nero
Greece	GK738	Tsouraki, SL 98
Greece	GK739	Krathis, Tsivlos
Greece	GK740	Peiros
Greece	GK751	Koiliaris, Aptera
Greece	GK753	Gadouras, Gadouras
Greece	GK756	Gorgopotamos, Gorgopotamos Bridge
Greece	GK757	Gorgopotamos, Gorgopotamos Village
Greece	GK807	Pamissos
Greece	GK808	Pamissos, Vrahopanagitsa
Greece	GK810	Parap. Pamissou, Aghios Floros
Greece	GK812	Pamissos, Aris
Greece	GK814	Pamissos, Messini
Ireland	IRC59	Ireland
Ireland	IRC50	Ireland

Ireland	IRC206	Ireland
Ireland	IRC150	Ireland
Ireland	IRC151	Ireland
Ireland	IRC204	Ireland
Ireland	IRC160	Ireland
Ireland	IRC56	Ireland
Ireland	IRC55	Ireland
Ireland	IRC58	Ireland
Ireland	IRC57	Ireland
Ireland	IRC152	Ireland
Ireland	IRC158	Ireland
Ireland	IRC153	Ireland
Ireland	IRC109	Ireland
Ireland	IRC101	Ireland
Ireland	IRC110	Ireland
Ireland	IRC103	Ireland
Ireland	IRC54	Ireland
Ireland	IRC52	Ireland
Ireland	IRC156	Ireland
Ireland	IRC104	Ireland
Ireland	IRC60	Ireland
Ireland	IRC108	Ireland
Ireland	IRC105	Ireland
Ireland	IRC106	Ireland
Ireland	IRC53	Ireland
Ireland	IRC107	Ireland
Ireland	IRC208	Ireland
Ireland	IRC159	Ireland
Ireland	IRC51	Ireland
Ireland	IRC200	Ireland
Ireland	IRC154	Ireland
Ireland	IRC201	Ireland
Ireland	IRC202	Ireland
Ireland	IRC203	Ireland
Ireland	IRC205	Ireland
Ireland	IRC100	Ireland
Ireland	IRC6	Ireland
Ireland	IRC9	Ireland
Ireland	IRC2	Ireland
Ireland	IRC4	Ireland
Ireland	IRC8	Ireland
Ireland	IRC5	Ireland
Ireland	IRC1	Ireland
Ireland	IRC102	Ireland
Ireland	IRC3	Ireland

Ireland	IRC7	Ireland
Ireland	IRC10	Ireland
Ireland	IRC207	Ireland
Ireland	IRE111	Kilcolgun River tributary
Ireland	IRE211	Caher River
Ireland	IRE311	Caher River
Ireland	IRE411	Clare River at Kilcreevanty Br.
Ireland	IRE511	Tonmoyle Br. Clare tributary I
Ireland	IRE611	Clare tributary II
Ireland	IRE711	Sinking River Cloonagh Br.
Ireland	IRE811	River Suck
Ireland	IRE911	Figh Br. Lung River
Ireland	IRE1011	Lung River II
Ireland	IRE1111	Ballychalan River
Ireland	IRE1211	Beagh River outflow from Lough Cotra
Ireland	IRE1311	Castlelodge River
Ireland	IRE1411	Marnagh River
Ireland	IRE1511	Blach River
Ireland	IRE1611	Robe River
Ireland	IRE1711	Lough Mask inflow (N)
Italy	IT836	Albegna Roccalbegna (GR) reference
Italy	IT837	Merse Monticiano (SI)
Italy	IT839	Lente downstream Pitigliano (GR)
Italy	IT840	Senna Piancastagnano (SI) SS 2
Italy	IT841	Paglia Piancastagnano (SI) SS 2
Italy	IT842	Fiora downstream farm S. Fiora (GR)
Italy	IT843	Fiora Cellena (GR)
Italy	IT847	Chapter 16Ente downstream Podere dei Frati (GR)
Latvia	LA994	Arona 1, Upper part
Latvia	LA995	Arona 2, Middle part
Latvia	LA997	Kekava
Latvia	LA999	Licupe, near farmstead "UpesMarkuti"
Latvia	LA1002	Mergupe 3, Lower part
Latvia	LA1003	Pededze 1, Upper part
Latvia	LA1004	Pededze 2, Middle part
Latvia	LA1005	Pededze 3, Lower part
Latvia	LA1006	Tumsupe, Above Podkajas farmstead
Latvia	LA1007	Veseta, Near by Vietalva
Latvia	LA1011	Rauza 1, Upper part
Latvia	LA1012	Rauza 2, Middle part
Latvia	LA1013	Rauza 3, Lower part
Latvia	LA1014	Strikupe 1, Upper part
Latvia	LA1015	Strikupe 2, Middle part
Latvia	LA1021	Iecava

Latvia	LA1024	Korge, ~500 m from river mouth
Latvia	LA1025	Amula 1, Upper part
Latvia	LA1031	Letiza, Middle part
Mexico	MX1	Laguna de Coba
Mexico	MX2	Laguna Macanxoc at Coba near arquelogical sites
Mexico	MX3	Laguna Azul at Coba near a cenote
Mexico	MX4	Laguna Bacalera 1
Mexico	MX5	Laguna Bacalera 2
Mexico	MX6	Cenote Azul
Mexico	MX7	Laguna Bacalera 3
Mexico	MX8	El Palmar
Mexico	MX9	Laguna Azul
Mexico	MX10	El Zapotal La Cana
Mexico	MX11	Laguna
Mexico	MX12	Laguna Tortugas
Mexico	MX13	Agua da Abeja
Mexico	MX14	San Felipe 1
Mexico	MX15	San Felipe 2
Mexico	MX16	San Felipe 3
Mexico	MX17	Laguna Yalahau
Mexico	MX18	Mosquito
Portugal	1174614	Lentiscais
Portugal	1174914	Vale da Azinheira
Portugal	11741415	Porto_tejo
Portugal	11741715	Ponte_nova
Portugal	1174215	Monte_pedra
Portugal	11742215	Crato
Portugal	1272114	São Romão
Portugal	1272115	Monte dos Corvos
Portugal	1272314	Ficalho
Portugal	1272515	Safara
Portugal	12721114	Terges
Portugal	1273214	Abela Montante
Portugal	1273215	Valverde
Portugal	1273314	Abela Jusante
Portugal	1273315	Galo Jusante
Portugal	1273414	São Domingos Jusante
Portugal	1273514	São Cristovão Montante
Portugal	1273614	Grândola
Portugal	1273714	Ribeira de São Domingos
Portugal	1273814	Afluente do Torgal
Portugal	1273815	Gomes Aires ETAR
Portugal	1273914	Rio Torto
Portugal	1273915	Gomes Aires Montante

Portugal	1273114	Luzianes
Portugal	12731314	São Cristovão Jusante
Portugal	12731514	Mira-Cola
Portugal	12731614	Torgal Jusante
Portugal	12731714	Sado -Corona
Portugal	1274214	Monte dos Arneiros
Portugal	1274314	Monforte
Portugal	12741114	Pavia
Portugal	12741214	Malhada
Portugal	12741314	Fronteira
Portugal	12742815	Antas
Portugal	1274315	Monte_aguias
Portugal	12743615	Montemor
Portugal	1275114	Arquitecto
Portugal	13743115	Barro
Portugal	13743215	Belas
Portugal	13743315	Serra_silveira
Portugal	13743415	Cacem
Portugal	13743515	Cabra_figa
Portugal	17741714	Monte dos Irmãos
Portugal	17742915	Escusa
Portugal	2154815	Aldeia_freiras
Portugal	21541215	Chao_forca
Portugal	21541315	Marmeleiro
Portugal	2156214	Botão
Portugal	21567815	Ponte de Perrães
Portugal	2156815	Mogofores
Portugal	21568315	Seixo
Portugal	2554114	Pisão
Portugal	2554214	Cachoeiras
Portugal	2554314	Casal das Antas
Portugal	2554414	Arrouquelas
Portugal	2554514	Casais do Vidigão
Portugal	2554614	Rio Maior
Portugal	2554714	Valada
Portugal	2554914	Agroal
Portugal	25541515	Casal_aboboreiras
Portugal	25542115	Azoia
Portugal	25542715	Alenquer
Portugal	2555115	Fervenca
Portugal	2555215	Malasia
Portugal	2555315	Rolica
Portugal	2555415	Vimeiro
Portugal	2556414	Redinha
Portugal	2556514	Ponte de Assamaça

Portugal	25561114	Almagreira
Portugal	25561214	Pombal-sul
Portugal	25561314	Azóia
Portugal	25564215	Colmeias
Portugal	25564515	Anobra
Portugal	2634114	Vale das Barrocas
Portugal	26341114	Cerejeira
Portugal	26341815	Casal_rei
Portugal	2636515	Ereira
Portugal	3411315	Alferce
Portugal	3412214	Alegrete
Portugal	3412215	Cabroeira de Baixo
Portugal	3412315	Ribeira da Fadagosa
Portugal	3414114	Ponte Velha
Portugal	34141915	Machoquinho
Portugal	3417215	Fervença
Portugal	3417715	Febros
Portugal	34671814	Tâmega 2 (Veral)
Portugal	34671914	Tâmega 1 (Veral)
Portugal	34682215	Retorta
Portugal	4117215	Torto 2
Portugal	4217115	Roios
Portugal	4217314	Róios (Qtª do Vale da Cal)
Portugal	4217615	Viduedo
Portugal	4467615	Vale de Moinhos (V4)
Portugal	4467715	Azibo (Azi 1)
Portugal	4467814	Azibo 2 (Foz do Azibo)
Portugal	4467815	Sabor (Sab4)
Portugal	44671414	Sabor 4 (Meirinhos)
Portugal	44671514	Sabor 3 (Ponte do Sabor)
Portugal	44671614	Sabor 2 (Felgar)
Portugal	44671714	Sabor 1 (Foz do Azibo)
South Africa	Was1	Was Goedspruit
South Africa	Was2	Was Goedspruit downstream
South Africa	MoiR	Mooi river
South Africa	MoiD	Mooi downstream
South Africa	M1	Mooi Source
South Africa	WFSA	Rietsphruit River site 2
South Africa	MoiDam	Wonder Fontein

South Africa	Moi2	Schoenspruit River
South Africa	Moi1	Mooi Dam
South Africa	Vaal1	Mooi River
South Africa	Vaal2	R507
South Africa	Roihass	Mooi River
South Africa	Reits	Vaal River Schoenansdrift
South Africa	Reits2	Vaal River Parys
South Africa	Shoen	Roihasskraal River
South Africa	R507	Bamboesspruit River
South Africa	Bambo	Rietsphruit River
Sweden	SW684	Hamrangean, Upstream Hamrangefjrden
Sweden	SW874	ƒlgngsan, Furuvik
Sweden	SW875	Forsmarksan, Johannisfors
Sweden	SW876	Hagaan, Lurbo
Sweden	SW877	Tmnaran
Sweden	SW878	Stromaran, Hillebola
Sweden	SW879	Penningbyan, Kvarnberget
Sweden	SW880	Jrsostrommen
Sweden	SW881	Muskan, North-West of Ogesta
Sweden	SW883	Husbyan, Finsta
Sweden	SW887	Skeboan, South of Gropen
Sweden	SW888	Brostrommen, Hârnackalund
Trinidad	T1	Trinidad Arouca River tributary
Trinidad	T2	Trinidad Arouca River tributary
Trinidad	T3	Trinidad Arouca River
Trinidad	T4	Trinidad Arouca River
Trinidad	T5	Trinidad Arouca River
Trinidad	T6	Trinidad Arouca River
Trinidad	T7	Trinidad Arima River
Trinidad	T8	Trinidad Arima River
Trinidad	T9	Trinidad Arima River
Trinidad	T10	Trinidad Plain stream
Trinidad	T11	Trinidad Quara River
Trinidad	T12	Trinidad Valencia River
Trinidad	T14	Trinidad River back water
Trinidad	T15	Trinidad River

Trinidad	T16	Aripo Tributary
Trinidad	T17	Aripo River
Trinidad	T18	Aripo River
Trinidad	T19	Cumaca River
UK	UK640	Sweatford Water, Fordingbridge
UK	UK641	Tadnoll Brook, Old Knowle
UK	UK643	Tadnoll Brook, Crossways
UK	UK644	Barkham Brook, Arborfield
UK	UK646	Cuddington Brook, Cuddington
UK	UK647	Pill River, Blue Anchor
UK	UK648	Cliff Brook, Crowton
UK	UK674	Clun, Marlow
UK	UK675	Llynfi, Glasbury
UK	UK676	Onny, Plowden Woods
UK	UK677	Monnow, at Monmouth Cap
UK	UK679	Onny, Stokesay
UK	UK680	Rhymney, Bedwas
UK	UK681	Sirhowy, Ynysddu
UK	UK682	Dean, Handforth
UK	UK683	Cole, Small Heath
UK	UK889	Hyde Brook, Bishops Cleeve
UK	UK890	Arrowe Brook, Moreton
UK	UK891	Wettenhall Brook, Wettenhall
UK	UK892	Tame, Stockport
UK	UK893	Darwen, Cann Bridge
UK	SK2	Bere Stream at Bere heath
UK	SK3	River Bourne at Idmiston
UK	SK5	Bristol Avon at Great Summerford
UK	SK6	Bristol Avon at Lacock Abbey
UK	SK7	Cam Brook at Carlingcott
UK	SK8	Cam Brook at Abbotsbury
UK	SK9	River Cerne at Cowden
UK	SK1	RiverChew at Copton Dando
UK	SK11	River Achew at Publow
UK	SK12	River Ebble at Odstock
UK	SK14	River Frome at Frampton
UK	SK15	River Frome at Lewell Mill
UK	SK16	River Frome at Lower Brockhampton
UK	SK17	River Frome at Maiden Newton
UK	SK18	River Frome at Moreton
UK	SK19	River Frome at Notton
UK	SK2	Hillfarrance Brook at Hillfarrance
UK	SK21	River Itchen at Brambridge House
UK	SK22	River itchen at Chiland
UK	SK23	River Itchenat Winchester

UK	SK24	River Kennet at Lockeridge
UK	SK25	River Loddon at old basin
UK	SK26	River Loddon at Twyford
UK	SK27	River Loddo at Wildmoor
UK	SK30	River Piddle at Affpuddle
UK	SK31	River Piddle at Hyde
UK	SK32	River Rye at East Newton
UK	SK33	River Salisbury Avon at Middle Woodsford
UK	SK34	River Salisbury Avon at Netheravon
UK	SK35	River Salisbury Avon at Upavon
UK	SK36	River Salisbury Avon at woodgreen
UK	SK37	River Surrey whitewater at Risely
UK	SK38	River Teidi at Altyblata
UK	SK40	Waterson Stream at Druce
UK	SK42	River Wylye at Codford Saint Mary
UK	Sk44	Tweed where crossed by the A68
UK	Sk45	Pool near Broughton in Furness
UK	Sk46	River Irt at Holmrook
UK	Sk47	River Nidd at Pateley Bridge
UK	Sk48	River Rye at Nunnington
UK	Sk49	River Hull (West Beck) at Wansford Bridge
UK	Sk50	River Spey near Garmouth
UK	MAC10609	Mouse Water
UK	MAC20609	Mouse Water
UK	MAC30609	Mouse water
UK	MAC40609	South Medwin River
UK	MAC50609	2 South Medwin Newholm Bridge
UK	MAC60609	3 South Medwin
UK	MAC70609	4 South Medwin furthest d/s
UK	MAC80609	Urigill River Na Luirgean
UK	MAC90609	1 Knockan Burn
UK	MAC10609	2 Knockan Burn
UK	MAC11609	3 Knockan Burn
UK	MAC12609	4 Knockan Burn
UK	MAC13609	1 Croispol Burn u/s of loch c. 400m
UK	MAC14609	2 Croispol Burn d/s
UK	MAC15709	Siabost stream Lewis
UK	MAC16709	Moven stream Lewis
UK	MAC17709	Berneray Boraf stream
UK	MAC18709	North Uist Grogary stream outflow
UK	MAC19709	North Uist Lealthann stream
UK	MAC20709	North Uist Machair Robach stream
UK	MAC21709	South Uist Stilligarry stream
UK	MAC22709	South Uist Lon Mur stream
UK	MAC23709	South Uist Loch Olaidh Meadhanach outflow

UK	MAC24709	South Uist Druidibeg outflow stream
UK	MAC25709	South Uist Bornish stream
UK	MAC26709	Oban River Lonnan
UK	MAC27709	Oban River Lonnan u/s
UK	YK001	Fornah Gill (tributary of River Ribble)
UK	YK002	Inflow (minor stream) to Malham Tarn
UK	YK003	Outflow of Malham tarn
UK	YK004	Gordale Beck Malham
UK	YK005	River Aire, Calton
UK	YK006	River Aire, upstream of Gargrave
UK	YK007	Kilnsey stream (Wharfe tributary)
UK	YK008	Bainbridge stream (near Hawes)
UK	YK009	River Bain Raydale
UK	YK010	River Ure at Wensley
UK	A2	RIVER WICK
UK	A3	Alltan Fearna
UK	A4	BURN OF LATHERONWHEEL
UK	A5	BERRIEDALE WATER
UK	A6	ABHAINN NA FRITHE
UK	A7	SCOTTARIE BURN
UK	A9	Balnagown/Strathrory
UK	A10	STRATHRORY RIVER
UK	A11	Red Burn
UK	A12	Allt na Feithe Buidhe
UK	A15	Unnamed
UK	A45	RIVER WICK
UK	A46	REISGILL BURN
UK	A47	Lewis:Unnamed
UK	A48	Unnamed
UK	A49	DORBACK BURN
UK	A50	Skye:Allt Dubh
UK	A51	An Garbh-allt
UK	A52	Abhainn Mhor
UK	A53	Colonsay:Unnamed
UK	A54	Eye Water
UK	A55	Burdiehouse Burn
UK	A56	Unnamed
UK	A57	Unnamed
UK	A58	Balcreuchan Burn
UK	A61	ALLT MOR
UK	A62	Unnamed
UK	A64	Unnamed
UK	A66	The Uair
UK	A67	CNOCGLAS WATER
UK	B1	Foul Burn

UK	B3	BREAMISH
UK	B4	ALN
UK	B5	UNSWAY BURN
UK	B6	COQUET
UK	B7	COQUET
UK	B8	WANSBECK
UK	B9	HOW BURN
UK	B10	Unnamed
UK	B11	KING WATER
UK	B57	BOLLIN
UK	B58	Dean
UK	B59	DEAN
UK	B60	RYTON
UK	B61	TUXFORD BECK
UK	B62	DERWENT
UK	B63	ROTHER
UK	B64	MAUN
UK	B65	MANIFOLD
UK	B66	MAUN
UK	B119	RIVACRE BROOK
UK	B120	WEAVER
UK	B121	MEDEN
UK	B122	MEDEN
UK	B123	MAUN
UK	B125	WEAVER
UK	B126	WEAVER
UK	B127	CHURNET
UK	B128	AMBER
UK	B129	EREWASH
UK	B242	FOSS
UK	B244	YARROW
UK	B245	IDLE
UK	B246	NEW DYKE
UK	B247	IDLE
UK	B248	WYE
UK	B249	FORD BROOK
UK	B251	Tarff Water
UK	B255	YARROW
UK	B256	HERTFORD
UK	C1	TRENT
UK	C3	BRANT
UK	C4	TERRIG
UK	C5	FODDER DIKE
UK	C6	WITHAM
UK	C7	ANWICK

UK	C8	OLD RIVER SLEA
UK	C9	WITHAM
UK	C10	Polser Brook
UK	C72	NORTH BROOK
UK	C73	WENSUM
UK	C74	ROTHLEY BROOK
UK	C75	UN-NAMED
UK	C76	WELL CREEK
UK	C77	TIFFEY
UK	C78	TWENTY FOOT RIVER
UK	C79	TIFFEY
UK	C80	BURTON BROOK
UK	C81	WATTON BROOK
UK	C116	WEY
UK	C117	WINGHAM
UK	C118	BLACKWATER
UK	C119	RHODEN STREAM TRIBUTARY
UK	C120	RHODEN STREAM TRIBUTARY
UK	C121	Hammer Stream
UK	C122	GROM
UK	C123	Unnamed
UK	C124	PARK WATER
UK	C125	ROTHER
UK	C36	NENE
UK	C37	GREAT OUSE
UK	C38	HIZ
UK	C39	BRENT
UK	C310	KENNET
UK	C311	GREAT STOUR
UK	C312	TEST
UK	C313	SLEA
UK	C314	NENE
UK	C315	NENE
UK	D1	ELWY
UK	D3	ELWY
UK	D4	ELWY
UK	D5	ALED
UK	D6	SEIONT
UK	D7	CONWY
UK	D8	DEE
UK	D9	Unnamed
UK	D10	UN-NAMED
UK	D11	SLEAP BROOK
UK	D34	AFON BRAN
UK	D35	UN-NAMED

UK	D36	UN-NAMED
UK	D37	LEADON
UK	D38	HONDDU
UK	D39	USK
UK	D40	USK
UK	D41	USK
UK	D42	USK
UK	D43	USK
UK	D66	CALE
UK	D67	BRAY
UK	D68	Mole
UK	D69	Unnamed
UK	D70	Unnamed
UK	D71	TAW
UK	D72	STURCOMBE
UK	D73	TORRIDGE
UK	D74	TRIB. OF TORRID
UK	D75	HUNTACOTT WATER
UK	D168	ALLEN
UK	D169	AVON
UK	D170	STOUR
UK	D171	TRIB. OF CREEDY
UK	D172	TORRIDGE
UK	D34	EBBLE
UK	D341	AVON
UK	D342	OTTER
UK	D343	MOORS RIVER
UK	D348	AVON
UK	E1	GLENSHESK
UK	E2	DERVOCK
UK	E3	ROE
UK	E4	AGHADOWEY
UK	E6	ROE
UK	E7	AGIVEY
UK	E11	BRAID
UK	E12	BURNDENNET
UK	E44	QUIGGERY
UK	E45	RAVERNET
UK	E46	QUIGGERY
UK	E47	BLACKWATER (NORTHERN IRELAND)
UK	E49	TYNAN RIVER
UK	E50	COLEBROOK
UK	E51	MONEYCARRAGH
UK	E52	CARRIGS
UK	E53	FINN

UK	E83	ERNE
UK	E84	RAVERNET
UK	E85	LAGAN
UK	E87	BALLYNAHINCH
UK	E89	CUSHER
UK	E90	GLASSWATER
UK	E91	MANYBURNS
UK	E157	ERNE
UK	E158	BLACKWATER (NORTHERN IRELAND)
UK	E159	RHONE
UK	E160	TALL
UK	E180	JERRETTSPASS
UK	E181	LACKEY
UK	E182	UPPER BANN
UK	E184	SILLEES
UK	E185	TEMPO
UK	E187	UN-NAMED
UK	E188	B MALLARD
UK	E189	NEWRY
UK	E191	SCREENAGH
UK	E192	FINN
UK	E74	BALLINDERRY
UK	E136	LAGAN
UK	F1	Ireland
UK	F2	Ireland
UK	F3	Ireland
UK	F4	Ireland
UK	F5	Ireland
UK	F8	Ireland
UK	F9	Ireland
UK	F10	Ireland
UK	F11	Ireland
UK	F12	Ireland
UK	F15	Ireland
UK	F16	Ireland
UK	F17	Ireland
UK	F18	Ireland
UK	F19	Ireland
UK	F20	Ireland
UK	F21	Ireland
UK	F26	Ireland
UK	F27	Ireland
UK	F30	Ireland
UK	F31	Ireland
UK	F33	Ireland

UK	F36	Ireland
UK	F40	Ireland
USA	FLOR11	Rainbow springs Florida
USA	FLOP11	Pk Hole , rainbow spring run
USA	FLO3S11	Florida 3 Sisters Crystal River
USA	FLOKS11	Florida 3 Sisters Crystal River
USA	SR21	Blue Springs
USA	SR22	Ichetucknee 1
USA	SR23	Ichetucknee 2
USA	SR24	Ichetucknee 3
USA	SR25	Santa Fe River
USA	SR26	Manatee Springs
USA	SR31	Silver Glen
USA	SR32	Silver River 2
USA	SR33	Silver River 3
USA	SR 34	Juniper Creek
USA	SR 35	Fern Hammock
USA	SR36	De Leon
USA	SR37	Alexander Springs I
USA	SR 38	Alexander Springs II
USA	SR 39	Juniper Springs II
USA	SR 310	Silver River 1
USA	SR 41	Wacissa I
USA	SR42	Wacissa II
USA	SR43	Wacissa III
USA	SR44	Wacissa IV
USA	SR 45	Wakulla Springs I
USA	SR 46	Wakulla Springs II
USA	SR 47	St Marks River
Zambia	Mule506	Mulembo
Zambia	Mula306	Mulaushi
Zambia	Muso306	Musola
Zambia	Mula406	Mulaushi
Zambia	Muso506	Musola
Zambia	ChiL106	Chilengwa na Lese
Zambia	KasR106	Kasanka
Zambia	KasR606	Kasanka
Zambia	KasR706	Kasanka
Zambia	LuwR106	Luwombwa
Zambia	Chit106	Chitikilo
Zambia	Muso308	Musola
Zambia	LuwR108	Luwombwa
Zambia	LuwR208	Luwombwa
Zambia	LuwR308	Luwombwa
Zambia	LuwB108	Luwombwa Backwater

Zambia	LusR108	Lusenga
Zambia	Chit108	Chitikilo
Zambia	Njel108	Njelele
Zambia	KasR108	Kasanka
Zambia	Sb108	Lukulu
Zambia	Sb208	Lukulu
Zambia	Sb308	Lukulu
Zambia	Sb408	Lukulu
Zambia	Sb508	Lukulu
Zambia	Sb608	Lukulu
Zambia	Sb708	Lukulu
Zambia	Sb808	Lukulu
Zambia	Sb908	Lukulu
Zambia	Sb1008	Lukulu
Zambia	Sb1108	Lukulu
Zambia	Sb1208	Lukulu
Zambia	Sb1308	Lukulu
Zambia	Sb1408	Lukulu
Zambia	Sb1508	Lukulu
Zambia	Sb1608	Lukulu
Zambia	Sb1708	Lukulu
Zambia	Sb1808	Lukulu
Zambia	Sb1908	Lukulu
Zambia	Sb2008	Lukulu
Zambia	Sb2108	Lukulu
Zambia	ChiD108	Chiunaponde Dambo
Zambia	WakL108	Wakawaka
Zambia	ChiL108	Chilengwa na Lese
Zambia	Muso408	Musola
Zambia	KapaL108	Kapempa
Zambia	Mula608	Mulaushi
Zambia	Mule408	Mulembo
Zambia	Mule109	Mulembo
Zambia	Mula109	Mulaushi
Zambia	Mula209	Mulaushi
Zambia	Mula309	Mulaushi
Zambia	KasR109	Kasanka
Zambia	KasL109	Kasanka
Zambia	KasR209	Kasanka
Zambia	KasR309	Kasanka
Zambia	KasR409	Kasanka
Zambia	Kabu109	Kasanka Backwater
Zambia	Chit109	Chitikilo
Zambia	Njel109	Njelele
Zambia	Mula409	Mulkaushi

Zambia	Muso109	Musola
Zambia	Muso209	Musola
Zambia	Muso309	Musola
Zambia	KasR509	Kasanka
Zambia	Mule209	Mulembo
Zambia	Mula509	Mulaushi
Zambia	Mule309	Mulembo
Zambia	Mule509	Mulembo
Zambia	Luli109	Lulimala
Zambia	Kaom109	Kaombe
Zambia	Mufu109	Mufuishe
Zambia	Luku109	Lukulu
Zambia	Chim109	Chitikilo
Zambia	Muso409	Musola
South Africa	Moo109	Mooi
South Africa	Moo2	Mooi
South Africa	Moo3	Mooi
South Africa	Moo4	Mooi

Appendix 3. Species list across different tropical and subtropical countries.

Species	Florida	Trinidad	Argentina	Brazil	Zambia	South Africa
Alternanthera philoxeroides	+	+				
Azolla filliculoides			+			
Bacopa monierri	+				+	+
Brachiaria subquadripara			+	+		
Ceratophyllum demersum			+	+		
Colocasia esculenta	+	+				
Commelina schomburgkiana			+	+		
Cyperus alopecuroides					+	+
Cyperus difformis	+	+			+	+
Cyperus digitatus					+	+
Cyperus erythrorhizos	+	+				
Echinochloa crus-galli	+		+			
Echinodorus grandiflorus			+	+		
Eichhornia azurea			+	+		+
Eichornia crassipes	+		+	+		
Eleocharis atropurpurea	+				+	
Hydrocotyle bonariensis					+	+
Hydrocotyle ranunculoides			+	+		
Hydrocotyle umbellata	+	+				

Leersia hexandra					+	+
Lemna minor	+	+	+	+		
Limnobium laevigatum	+			+		
Ludwigia leptocarpa		+	+	+	+	+
Ludwigia palustris					+	+
Ludwigia peploides	+		+	+		
Myriophyllum aquaticum						+
Myriophyllum spicatum	+					
Nasturtium officinale	+		+		+	+
Nuphar luteum	+	+				
Nymphaea amazonum			+	+		
Panicum repens	+	+			+	+
Paspalum repens	+	+	+	+		
Persicaria attenuata					+	+
Persicaria hydropiper					+	+
Persicaria lapathifolia					+	+
Persicaria senegalensis					+	+
Phragmites australis					+	+
Pistia stratiotes			+	+		
Polygonum punctatum	+		+			
Pontederia cordata	+		+			
Potamogeton nodusus					+	+
Potamogeton pusillus	+				+	+

Potamogeton schweinfurthii					+	+
Salvinia minima	+	+	+	+		
Stuckenia pectinata	+				+	+
Typha domingensis					+	+
Typha latifolia	+		+			+
Veronica anagallis-aquatica					+	+
Zannichellia palustris	+		+			

Appendix 4. Species list across different tropical and subtropical countries.

Species	British Isles	Denmark	France	Portugal	Germany	Greece	Italy	Latvia	Portugal	Sweden
Alisma plantago-aquatica	+	+						+		+
Apium nodiflorum	+		+			+				
Azolla filiculoides			+	+					+	
Berula erecta		+	+					+		
Butomus umbellatus	+							+		+
Callitriche hamulata	+			+				+		
Callitriche obtusangula	+		+	+				+		
Callitriche stagnalis	+				+					
Carex rostrata	+							+		
Catabrosa aquatica			+					+		
Ceratophullum demersum			+	+	+					
Eleogiton fluitans	+			+						
Elodea canadensis	+	+	+	+	+	+		+		+
Equisetum fluviatile		+						+		+
Glyceria maxima	+	+								+
Hippurus vulgaris	+	+	+					+		+
Iris pseudacorus	+	+	+					+		+
Lemna minor	+	+	+	+	+			+	+ +	+
Myriophyllum		+		+						+

alterniflorum										
Myriophyllum spicatum	+		+	+	+				+	
Myriophyllum verticillatum			+	+						
Nasturtium officinale	+	+	+	+		+	+		+	
Nuphar lutea	+		+		+			+		+
Persicaria amphibia	+				+					+
Phragmites australis					+		+	+		+
Potamogeton alpinus								+		+
Potamogeton berchtoldii	+		+		+					
Potamogeton crispus	+	+	+		+			+		
Potamogeton gramineus	+							+		+
Potamogeton lucens	+		+							+
Potamogeton natans	+	+	+					+		
Potamogeton nodosus	+			+						
Potamogeton perfoliatus	+		+							
Potamogeton polygonifolius	+	+		+						+
Potamogeton praelongus	+							+		
Potamogeton pusillus	+		+	+						

									+	
Ranunculus peltatus			+					+		
Ranunculus aquatilis		+						+		
Ranunculus circinatus	+		+							
Ranunculus flammula	+									+
Ranununculus fluitans	+		+							
Ranunculus penicillatus	+		+							
Rorippa amphibia	+							+		
Rumex hydrolapathum	+	+						+		
Sagittaria sagittifolia	+				+			+		
Schoenoplectus lacustris	+							+		+
Sparganium emersum	+		+		+			+		+
Sparganium erectum	+	+	+	+	+			+		+
Spirodela polyrrhiza	+		+					+		
Stuckenia pectinata	+		+	+	+				+	
Typha latifolia	+	+						+		+
Veronica anagalis-aquatica	+	+				+	+	+		+
Veronica beccabunga	+		+							
Zanichellia palustris	+		+							

Appendix 5. Model tests.

1. Marginal Functional Group

a) Number of marginal species

```
Generalized linear mixed model fit by the Laplace approximation
Formula: No.marg.sp ~ Width.cat + Alkalinity.cat + (1 | Country)
   Data: data2
   AIC   BIC logLik deviance
 302.2 326.3 -144.1    288.2
Random effects:
 Groups  Name        Variance Std.Dev.
 Country (Intercept) 0.43026  0.65595
Number of obs: 233, groups: Country, 10
```

```
Fixed effects:
                Estimate Std. Error z value Pr(>|z|)
(Intercept)       1.5707     0.2715   5.784 7.28e-09 ***
Width.cat2       -0.4626     0.1497  -3.090 0.002000 **
Width.cat3       -0.5326     0.1610  -3.308 0.000940 ***
Alkalinity.cat2  -0.3275     0.1200  -2.728 0.006366 **
Alkalinity.cat3  -0.5587     0.1616  -3.458 0.000545 ***
Alkalinity.cat4  -0.7968     0.1967  -4.051 5.09e-05 ***
---
```

Model selection tests:

```
anova(margs1.mix2,margs1.mix3,test="Chisq") #to test the significance of the alkalinity factor

Models:

margs1.mix3: No.marg.sp ~ Width.cat + (1 | Country)

margs1.mix2: No.marg.sp ~ Width.cat + Alkalinity.cat + (1 | Country)

            Df    AIC    BIC  logLik  Chisq Chi Df Pr(>Chisq)

margs1.mix3  4 316.01 329.81 -154.00

margs1.mix2  7 302.17 326.32 -144.08 19.842      3   0.000183 ***
```

```
anova(margs1.mix2,margs1.mix4,test="Chisq") #to test the significance of the width factor

Models:

margs1.mix4: No.marg.sp ~ Alkalinity.cat + (1 | Country)

margs1.mix2: No.marg.sp ~ Width.cat + Alkalinity.cat + (1 | Country)

            Df    AIC    BIC  logLik  Chisq Chi Df Pr(>Chisq)

margs1.mix4  5 309.13 326.38 -149.56

margs1.mix2  7 302.17 326.32 -144.08 10.961      2   0.004167 **
```

a) Number of emergent species

```
Generalized linear mixed model fit by the Laplace approximation

Formula: No.emergent.sp ~ Width.cat + (1 | Country)

   Data: data2

   AIC   BIC logLik deviance

 338.7 352.5 -165.4    330.7

Random effects:

 Groups  Name        Variance Std.Dev.

 Country (Intercept) 0.045332 0.21291

Number of obs: 233, groups: Country, 10
```

```
Fixed effects:

            Estimate Std. Error z value Pr(>|z|)

(Intercept)   1.6134     0.1383  11.662  < 2e-16 ***

Width.cat2   -0.3813     0.1295  -2.945  0.00323 **

Width.cat3   -0.3535     0.1335  -2.648  0.00809 **

---

Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
See R script for model selection procedure – significance of width category :

Models:

emergs1.mix5: No.emergent.sp ~ 1 + (1 | Country)

emergs1.mix4: No.emergent.sp ~ Width.cat + (1 | Country)

                                    Df    AIC    BIC  logLik  Chisq Chi Df Pr(>Chisq)

emergs1.mix5  2 343.02 349.92 -169.51

emergs1.mix4  4 338.71 352.51 -165.35 8.3111      2    0.01568 *
```

3. Floating Functional Group

a) Number of Species

```
Generalized linear mixed model fit by the Laplace approximation

Formula: No.free.floating.sp ~ Velocity.cat + (1 | Country)

   Data: data2

   AIC   BIC logLik deviance

 291.9 305.7   -142    283.9

Random effects:

 Groups  Name        Variance Std.Dev.

 Country (Intercept) 1.8716   1.3681

Number of obs: 233, groups: Country, 10
```

```
Fixed effects:
             Estimate Std. Error z value Pr(>|z|)
(Intercept)    -1.2628     0.4783  -2.640  0.00828 **
Velocity.cat2   0.2567     0.1738   1.477  0.13969
Velocity.cat3  -0.5801     0.3286  -1.766  0.07748 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
Significance of velocity variable:
Data: data2
Models:
float1.mix5: No.free.floating.sp ~ 1 + (1 | Country)
float1.mix4: No.free.floating.sp ~ Velocity.cat + (1 | Country)
            Df    AIC    BIC  logLik  Chisq Chi Df Pr(>Chisq)
float1.mix5  2 295.63 302.53 -145.81
float1.mix4  4 291.90 305.71 -141.95 7.7281      2    0.02098 *
```

4. Floating Rooted Functional Group

a) Number of Species

```
Generalized linear mixed model fit by the Laplace approximation
Formula: No.floating.rooted.sp ~ Velocity.cat + Alkalinity.cat + (1 |      Country)
   Data: data2
   AIC   BIC logLik deviance
 229.2 253.4 -107.6    215.2
```

```
Random effects:
 Groups  Name        Variance Std.Dev.
 Country (Intercept) 1.0195   1.0097
Number of obs: 233, groups: Country, 10
```

```
Fixed effects:
                Estimate Std. Error z value Pr(>|z|)
(Intercept)     -0.37944    0.40401  -0.939  0.34764
Velocity.cat2   -0.01292    0.19567  -0.066  0.94736
Velocity.cat3   -0.94424    0.40966  -2.305  0.02117 *
Alkalinity.cat2 -0.36419    0.23392  -1.557  0.11950
Alkalinity.cat3 -0.84292    0.32250  -2.614  0.00896 **
Alkalinity.cat4 -0.67789    0.35120  -1.930  0.05358 .
```

```
Significance of velocity:
#Models:
#root1.mix4: No.floating.rooted.sp ~ Alkalinity.cat + (1 | Country)
#root1.mix3: No.floating.rooted.sp ~ Velocity.cat + Alkalinity.cat + (1 | Country)
#                  Df    AIC    BIC  logLik  Chisq Chi Df Pr(>Chisq)
#root1.mix4  5 232.38 249.63 -111.19
#root1.mix3  7 229.22 253.38 -107.61 7.1572      2    0.02791 *

Significance of alkalinity (close to 0.05 so best to keep it in):
            Df    AIC    BIC  logLik  Chisq Chi Df Pr(>Chisq)
#root1.mix4b  4 230.78 244.58 -111.39
#root1.mix3   7 229.22 253.38 -107.61 7.5587      3    0.05607
```

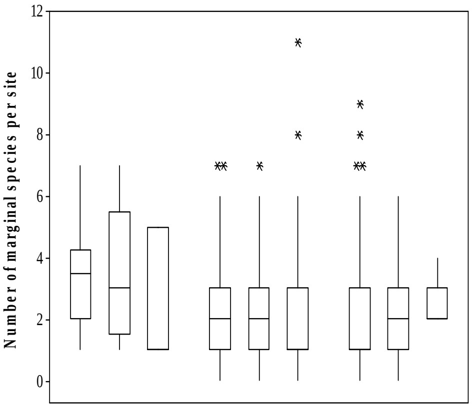
5. Submerged Functional Group

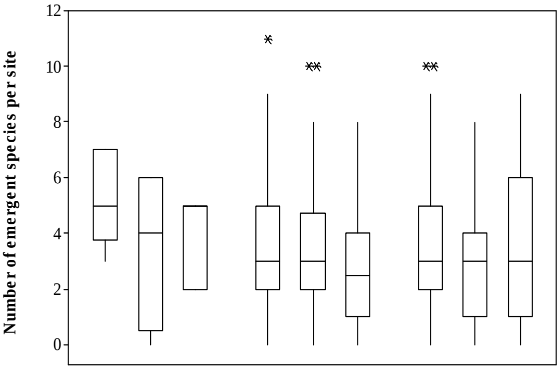
a) Number of submerged species

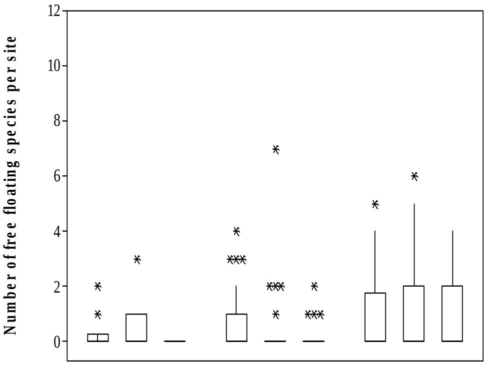
```
Generalized linear mixed model fit by the Laplace approximation

Formula: No.submerged.sp ~ Shading.cat + (1 | Country)

   Data: data2

   AIC   BIC logLik deviance

 325.6 339.4 -158.8    317.6

Random effects:

 Groups  Name        Variance Std.Dev.

 Country (Intercept) 1.3228   1.1501

Number of obs: 233, groups: Country, 10
```

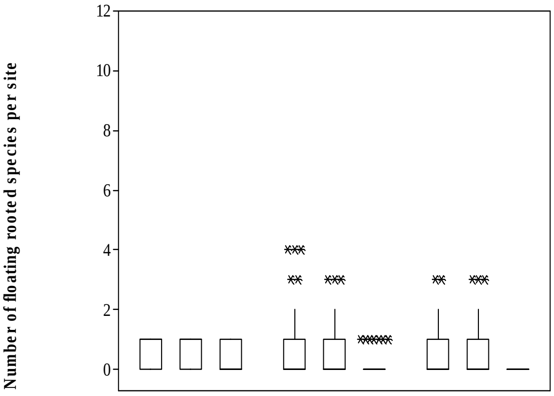
```
Fixed effects:

             Estimate Std. Error z value Pr(>|z|)

(Intercept)   -0.5008     0.3917  -1.278  0.20108

Shading.cat2  -0.1586     0.1428  -1.111  0.26659

Shading.cat3  -0.9548     0.3202  -2.982  0.00286 **

---

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Significance of the shading cat term:

#Models:

#sub1.mix5: No.submerged.sp ~ 1 + (1 | Country)

#sub1.mix4: No.submerged.sp ~ Shading.cat + (1 | Country)

#           Df    AIC    BIC  logLik Chisq Chi Df Pr(>Chisq)

#sub1.mix5  2 333.00 339.90 -164.50

#sub1.mix4  4 325.57 339.37 -158.78 11.434      2   0.003289
```

Appendix 6. Boxplots of macrophyte functional groups (number of species) as a function of stream flow and width.



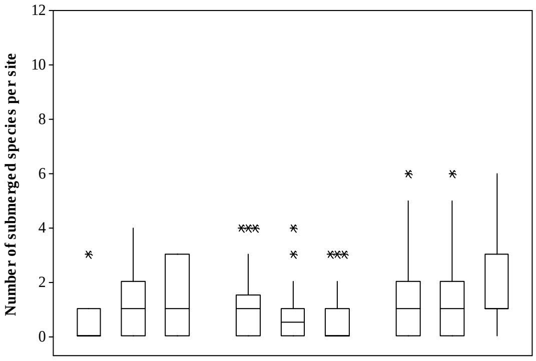






Flow Slow moderate fast Slow moderate fast Slow moderate fast

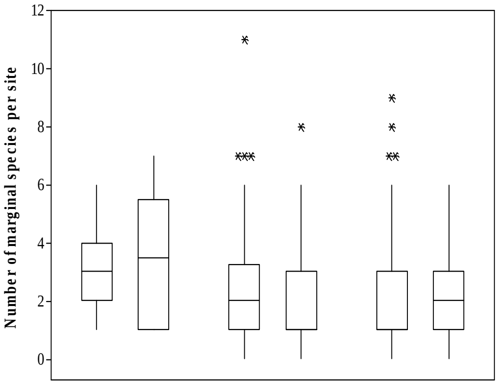
Width <10m >10m <100m

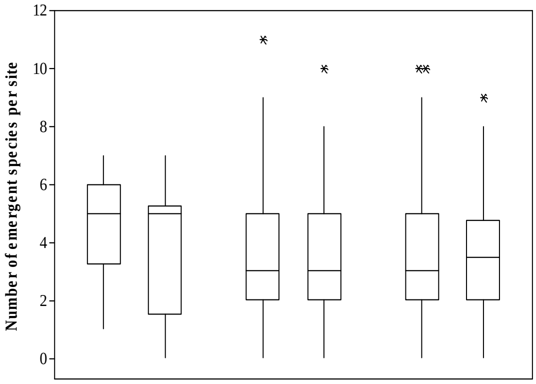


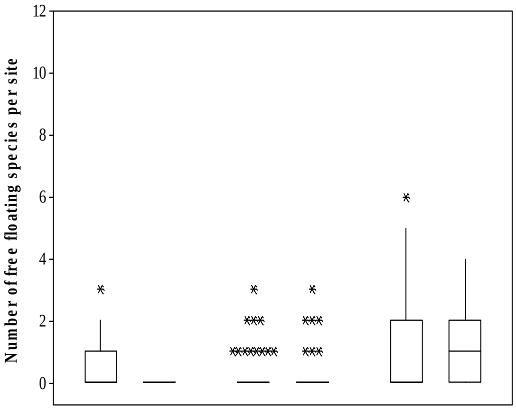
Flow Slow moderate fast Slow moderate fast Slow moderate fast

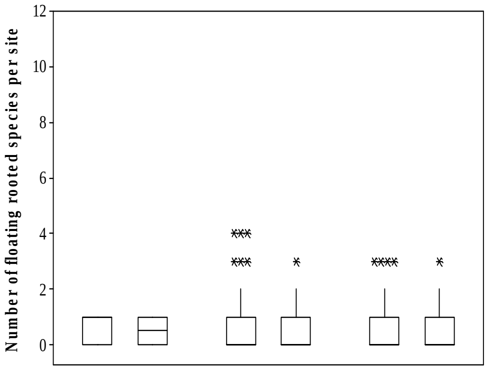
Width <10m >10m <100m

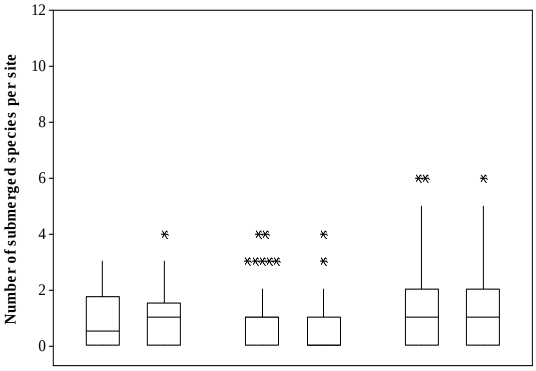
Appendix 7. Boxplot of macrophyte functional groups (number of species= as a function of stream cover and width.











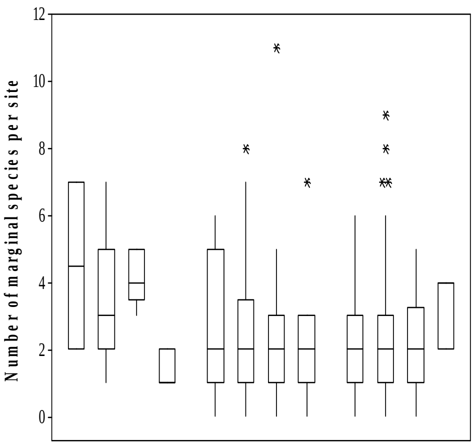
Cover none moderate none moderate none moderate

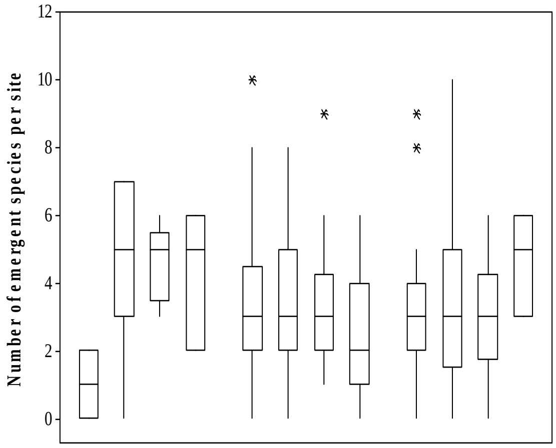
Width <10m >10m <100m

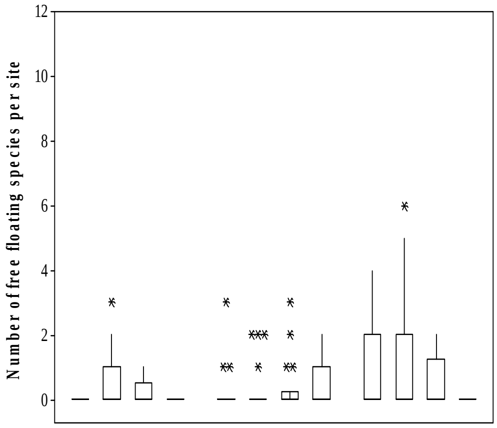
Cover none moderate none moderate none moderate

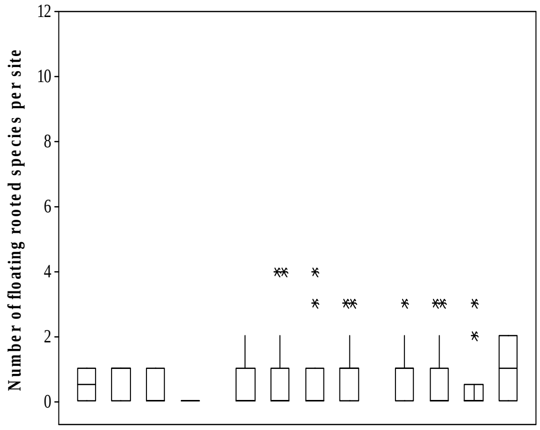
Width <10m >10m <100m

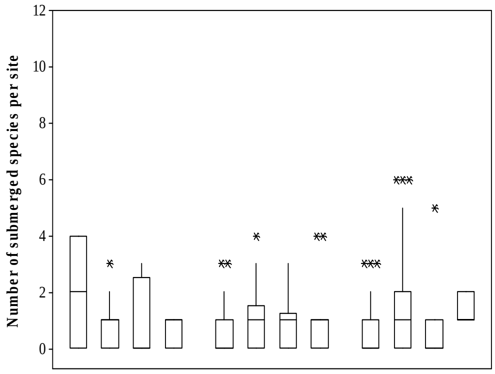
Appendix 8. Boxplots of macrophyte functional groups (number of species) as a function of alkalinity (1, marginal, 2 moderate, 3 hard, 4 very hard) and width.











Alkalinity 1 2 3 4 1 2 3 4 1 2 3 4

Width <10m >10m <100m

Alkalinity 1 2 3 4 1 2 3 4 1 2 3 4

Width <10m >10m <100m