

Environmental drivers of aquatic macrophyte communities in southern tropical African rivers: Zambia as a case study

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Abstract

The first-ever extensive macrophyte survey of Zambian rivers and associated floodplain waterbodies, conducted during 2006 – 2012, collected 271 samples from 228 sites, mainly located in five freshwater ecoregions of the world primarily represented in Zambia. The results supported the hypothesis that variation in macrophyte community structure (measured as species composition and diversity) in southern tropical African river systems, using Zambia as a case study area, is driven primarily by geographical variation in water physico-chemical conditions. In total 335 macrophyte taxa were recorded, and a chronological cumulative species records curve for the dataset showed no sign of asymptoting: clearly many additional macrophyte species remain to be found in Zambian rivers. Emergent macrophytes were predominant (236 taxa), together with 26 floating and 73 submerged taxa. Several species were rare in a regional or international context, including two IUCN Red Data List species: *Aponogeton rehmanii* and *Nymphaea divaricata*. Ordination and classification analysis of the data found little evidence for temporal change in vegetation, at repeatedly-sampled sites, but strong evidence for the existence of seven groups of samples from geographically-varied study sites. These supported differing sets of vegetation (with eight species assemblages present in the sample-groups) and showed substantial inter-group differences in both macrophyte alpha-diversity, and geographically-varying physico-chemical parameters. The evidence suggested that the main environmental drivers of macrophyte community composition and diversity were altitude, stream order, shade, pH, alkalinity, NO₃-N, and underwater light availability, while PO₄-P showed slightly lower, but still significant variation between sample-groups.

Key words: river plants, Africa, macrophyte diversity, tropical river ecology

Highlights

- The first macrophyte survey of Zambian rivers was undertaken during 2006 – 2012
- In total 335 macrophyte taxa were found from 228 sites sampled
- 236 emergent taxa were recorded, together with 26 floating and 73 submerged taxa
- Rare species found included *Aponogeton rehmanii* and *Nymphaea divaricata*
- Seven primary environmental drivers of plant community structure were identified

1. Introduction

Macrophytes form an important component of the freshwater biodiversity of the Afrotropical biogeographic region, which includes southern tropical African rivers. Chambers et al. (2008) stated that the minimum figure for macrophyte diversity in this region is 614 species (64% of these endemic to the Afrotropics), belonging to 196 genera. The high biodiversity of tropical river systems minimally-affected by human impact is much less studied than that of similar-status rivers in temperate regions, despite the fact that these tropical ecosystems are of major conservation importance, forming a unique and rich component of global freshwater biodiversity (e.g. Murphy et al., 2003; Thomaz et al., 2004; Dallas & Mosepele, 2007; Takahashi, 2009; Taylor et al., 2014a, b). The drivers of tropical river ecosystem functioning, and in particular of riverine macrophyte community dynamics, remain poorly known, though factors such as high stream network density and strong intra- and inter-annual variability in precipitation and discharge are thought to contribute to the high biodiversity of such systems in the Tropics (Wantzen & Junk, 2000; Chessman et al., 2006; Varandas Martins et al., 2013). Maintaining habitat integrity in near-pristine African rivers is vital for conservation of the ecological and genetic diversity of tropical freshwater ecosystems, and the varied ecosystem services that they provide (Seidl & Moraes, 2000; Hoeinghaus et al., 2009). However, all too often basic knowledge of the ecology of tropical rivers remains severely deficient.

Macrophytes commonly play a major role in the ecosystem functioning of tropical river systems (Denny, 1985; Mitchell et al., 1990; Murphy et al., 2003; de Sousa et al., 2011; Varandas Martins et al., 2013). In addition, in all low-income sub-Saharan countries, river systems are often critically important to the livelihoods of rural populations (e.g. von der Heyden, 2004). Given their rich biodiversity they also provide substantial prospects for income-generation through ecotourism (e.g. Salum, 2009). Consequently, in human terms, as well as for ecological reasons, there is a clear and present need for improved knowledge of the freshwater ecology of tropical river systems.

This study, in large part, was based on results obtained during fieldwork undertaken during 2010 – 2012 for development and testing of the Southern African River Assessment Scheme (SAFRASS), which aimed to produce a pilot river-quality biomonitoring scheme appropriate to river systems in tropical southern Africa (Kennedy et al., 2012a, 2012b, 2014; Lowe et al., 2013; Gibbins et al., 2013). The data were supplemented by information from previous survey work, undertaken by the authors during 2006 – 2009, on Zambian rivers and associated waterbodies, including riverine floodplain lagoons, backwaters (including oxbows and distributaries) and dambos (floodplain seasonal standing waterbodies).

The study provides the first-ever extensive assessment of the ecology of the riverine vegetation of Zambia, though earlier studies have examined smaller subsets of the country's rivers and associated wetland waterbodies (e.g. Chabwela & Siwale, 1986; Sinkala et al., 2002; Mumba & Thompson, 2005; Lang et al., 2008; Murphy et al., 2009). There is also a steadily-improving taxonomic coverage of aquatic plant species present in Zambia, through the ongoing Flora Zambesiaca project, sequentially published, on a family basis since 1960, by the Royal Botanic Gardens, Kew (e.g. Martins, 2009).

The study tested the hypothesis that variation in macrophyte community structure (species composition and diversity) in southern tropical African river systems is driven primarily by geographical variation in water physico-chemical conditions. The work also examined differences in riverine conditions prevalent in five Freshwater Ecoregions of the World (FEOWs: Abell et al., 2008), which are primarily represented in Zambia.

Zambia provides an ideal location for a case study to test this hypothesis because of its wide range of both natural and human-associated variability in riverine conditions. In total, the country contains geographical parts of nine FEOWs (four of which are, however, only of very limited extent within Zambia, being mainly located in adjoining countries), and also straddles the major watershed of southern Africa: the Congo/Zambezi watershed. FEOWs represented in Zambia which contain rivers lying in the Congo Basin, and flowing to the Atlantic Ocean, are Bangweulu-Mweru, Lake Tanganyika, and Upper Lualaba. Lake Rukwa is a closed endorheic basin mainly in Tanzania but with a small part of its area located in north eastern Zambia. All other Zambian FEOWs (Upper Zambezi Floodplain, Zambezian Headwaters, Middle Zambezi-Luangwa, Kafue Flats, Lower Zambezi) lie in the Zambezi Basin, flowing to the Indian Ocean (Fig. 1).

Human impacts range from almost nothing in near-pristine upland streams, to high levels of pollution and other anthropogenic influences, for example in parts of the country affected by copper-mining activities, urban developments, and, increasingly, intensive irrigated agriculture. Finally, whilst it is the 30th largest country by land area, Zambia, with a population of 15 million in 2014, had a low population density at just 17 people per square kilometre, but with the bulk of the population (some 44%) concentrated in two relatively small areas: the capital city Lusaka, and the Copperbelt mining area to the north of Lusaka. Hence, human population density is currently extremely low across much of rural Zambia. However, Zambia has one of the fastest human population growth rates in the world, with its population projected to increase by almost 1000% by 2100 (United Nations, 2011), which will certainly increase anthropogenic pressure on river ecosystems.

2. Methods

Within each FEOW, river sites representing a range of stream orders and flow conditions were sampled. In two FEOWs some floodplain standing waterbodies with high connectivity to the river channel were also sampled. Macrophyte surveys at each site followed the guidelines of the international standard EN 14184, incorporating emergent vegetation due to its importance in Zambian rivers. Where present, macroalgae (charophytes) and aquatic bryophytes were also included.

Macrophyte surveys (with collection of supporting physico-chemical data) were conducted during 2006 – 2012 with 271 samples being collected from 228 sites, mainly located in the five FEOWs which are primarily present in Zambia. These are Upper Zambezi Floodplain (UZF: 13 samples), Bangweulu-Mweru (BM: 151 samples), Zambezi Headwaters (ZH: 31 samples), Middle Zambezi-Luangwa (MZL: 55 samples), and Kafue Flats (KF: 18 samples) (Fig. 1). Only one sample was taken from the small areas of four other FEOWs, otherwise mostly located in neighbouring countries, which are also represented in Zambia: this sample (from a river in the Lower Zambezi ecoregion), was included with samples from the adjoining ecoregion (MZL) for analytical purposes.

In total 27 sites, all located in the MZL and BM ecoregions, with the majority being from rivers and associated waterbodies in the latter, were repeat-sampled (up to five times during the survey period of 2006 - 2012) in order to assess the potential importance of temporal change affecting the aquatic vegetation.

The survey protocol required a standard 100 m stretch of waterbody to be sampled at five random points within the stretch. All macrophyte species present were recorded per sampling point, and frequency (as %F per stretch) was calculated for each species, as number of hits out of five maximum possible (thus giving scores in the range 20 – 100 %F). Visual records for emergent and floating species were supplemented by the use of a rope-thrown grapnel (from bank or boat as appropriate) to access submerged species. The high risk of attack by dangerous animals (crocodile and hippopotamus) largely precluded entry into the water for sampling purposes, except in small shallow clear-water streams, or where (rarely) armed guards were available to provide protection.

Samples were retained as herbarium-sheet specimens for subsequent identification. Identification was a major issue in Zambia at the time of this project, as no appropriate identification guides for aquatic vegetation pre-existed for the country. Consequently, identification was carried out using aquatic and wetland plant identification resources available for other parts of southern Africa and tropical Asia (primarily Cook, 1996, 2004), as well as guides to identification of riverine macrophytes in Zambian rivers, produced as outputs from the SAFRASS project (Murphy et al., 2009; Kennedy & Murphy, 2012). Taxonomic literature (primarily *Flora Zambesiaca*: Exell & Wild, 1960 et seq.) was also utilised, but again this was incomplete at the time of the study, with some major aquatic families not yet covered (notably Cyperaceae), although coverage is good for others, e.g. Aponogetonaceae (Martins, 2009). An inevitable consequence was that identification was not always possible to species level, though the authors are confident that taxonomic separation of specimens into individually-distinct taxa was carried out. Nomenclature followed *Flora Zambesiaca* (Exell & Wild, 1960 et seq.), cross-checked against The Plant List (www.theplantlist.org).

Environmental data collected in the field for each sampling point (equipment malfunction prevented some data from being collected at some sample locations) were geospatial coordinates and altitude (using a Garmin Etrex hand-held GPS); visually-assessed flow class (0 = static; 1 = slow flow: “pool”; 2 = moderate

flow: “glide”; 3 = fast flow: “riffle” or white water showing); waterbody type: river (215 samples), backwater (11), dambo (14), or lagoon (31); shade category (1 = unshaded to 3 = heavy shade); and stream order (taken from an ArcGIS-generated regional stream network, derived from a digital elevation model). Total dissolved solids (TDS: mg L⁻¹), electrical conductivity (μS cm⁻¹), and pH were measured in situ, using a multi-function meter. Underwater light attenuation (as absorption coefficient: k m⁻¹) was measured in situ using data collected by a SKYE photosynthetically-active radiation (PAR) sensor system. Water samples were collected, and stored in sets of 60 mL LDPE bottles and 10 mL glass sample vials, as appropriate, for subsequent laboratory determination of gran alkalinity, orthophosphate (PO₄-P), and nitrate (NO₃-N), using standard procedures (MAFF, 1986; APHA, 1998; Neal, 2001).

Variables were assessed for normality using Ryan–Joiner testing and transformed if necessary by taking the square root or natural logarithm. Direct gradient analysis of the data was undertaken using Canonical Correspondence Analysis (CCA: ter Braak, 1986), with species data constrained by physico-chemical variables (transformed where appropriate to ensure normality). The analysis was complemented by the use of TWINSpan classification (Hill, 1979) to identify species assemblages and sample-groups present. Unconstrained Detrended Correspondence Analysis was used to examine temporal variation in plant community at 27 sites which underwent repeat sampling, with a minimum of two and a maximum of five samples collected from these sites during 2006 – 2012. Sampling intervals ranged from less than a year (i.e. wet to dry season sampling within a given year), up to a maximum of seven years separating first and last sample collected.

One-way ANOVA, with Tukey’s post-hoc multiple comparisons test, was used to compare between means of sample-groups, and FEOWs, for environmental and vegetation variables that were normally distributed (with or without transformation, as necessary). Kruskal–Wallis non-parametric testing was used to undertake equivalent comparisons between sample-groups for non-normal variables. All outcomes were considered significant at $p < 0.05$.

3. Results

Full datasets for macrophyte records, sample-site locations and environmental data are provided as supplementary files, published online alongside the electronic version of this article.

3.1. Macrophyte vegetation of Zambian river systems

In total 335 macrophyte taxa were recorded, of which 224 were identified to species level, 48 to genus, and the remaining 63 only to family level. The majority of those in the third category were Poaceae, for which, in many cases, flowering specimens were not present to permit full identification, and Cyperaceae, for which no taxonomic guide for Zambian species has, to date, been published.

In the absence of any preceding extensive studies of Zambian river vegetation, an important initial question was to what extent this survey succeeded in finding a reasonable proportion of the riverine flora of Zambia. The answer (Fig. 2) would seem to be that many species remain to be found, since the chronological cumulative species records curve, for samples collected during 2006 – 2012, shows no sign of reaching a plateau.

In terms of life-form (defined by position of the majority of a plant’s photosynthetic tissue relative to the water surface: Chambers et al., 2008), emergent taxa were predominant, with 236 taxa recorded, together with 26 floating, and 73 submerged taxa. This pattern was fairly consistent across the ecoregions of Zambia with only minor variation between FEOWs in terms of the life-forms of species found in samples from each ecoregion (Fig. 3), though sites in the BM ecoregion had the highest proportion of submerged species.

Of the ten commonest species, nine were emergent. The African reed *Phragmites mauritianus* (recorded in 185 samples out of the total 271) was the commonest species encountered, and was frequently dominant at individual sites. The remaining eight common emergents were *Panicum repens* (in 134 samples), *Panicum subalbidum* (60), *Cyperus alopecuroides* (54), *Persicaria attenuata* (53), *Commelina diffusa* (52), *Ludwigia adscendens* (48), *Persicaria decipiens* (41) and *Floscopa glomerata* (40). The commonest floating species was the water lily *Nymphaea nouchali* var. *caerulea*, occurring third in the list, in

100 samples. The commonest submerged species, *Potamogeton schweinfurthii*, was at number 13 in the list, found in 33 samples. Cyperaceae and Poaceae were the best-represented families in the dataset, with 72 taxa of the former present (39 identified to species, and a further 18 to genus). The Poaceae comprised 60 taxa (28 identified to species, and a further five to genus). Other individual families with high numbers of species recorded from sample sites included Hydrocharitaceae (11 species identified, plus two at genus level), Eriocaulaceae (10 species), Lentibulariaceae (nine species, plus a further three identified to genus), and Potamogetonaceae (nine species).

The species occurrence data followed a classic “reverse J” distribution, with a lengthy tail of 135 taxa occurring with just a single record in the dataset. The taxa recorded only to family or genus level made up the bulk of these single records. A number of families of limited worldwide distribution were found, including several IUCN Red List species: a good example being Aponogetonaceae, two species of which were present in the dataset. *Aponogeton desertorum* was quite widespread, occurring in 17 samples, in rivers in three ecoregions (BM, MZL and ZH). *Aponogeton rehmanii* (an IUCN Red List “Least Concern” category species: Ghogue, 2010) was much less common, found in only 4 samples, but again was widely scattered, occurring in rivers and a dambo located in three ecoregions (BM, ZH, MZL). Other species rare in an international context were also found, an example being two new records for *Nymphaea divaricata*, also classed as an IUCN Red List species (“Data Deficiency” category: Juffe, 2010), which was found in samples from the Mansha and Katete Rivers (both in the BM ecoregion).

Comparing macrophyte diversity between FEOWs, it was apparent (Table 1) that there were substantial differences in both alpha-diversity (S: number of taxa per sample, including taxa identified to species, genus and family), and gamma-diversity (total number of taxa recorded per FEOW). Although the trend in S between ecoregions was not significant (Table 1), UZF had the highest, and KF the lowest diversity. Raw values for gamma-diversity, as shown in Table 1, suggest that BM had the highest total diversity, and (as for alpha-diversity) KF the lowest. However, the picture changes when these values are corrected for sampling effort (as gamma-diversity/ number of samples collected per FEOW). On this basis UZF (which had the lowest number of samples, n = 13) had the highest gamma-diversity, mirroring the alpha-diversity results for that ecoregion, and suggesting that an increased sampling effort in that ecoregion would be highly likely to yield a substantial number of additional species records.

3.2. Temporal change

Results of the DCA analysis of temporal vegetation change, over repeat-sampling periods at individual sites from <1 - 7 years, showed very little evidence for any substantial vegetation change over time at the sites where repeated sampling was undertaken, and there was no evidence for any consistent pattern or direction of change from the DCA ordination plot. Only two sites had shifts over time of >2 standard deviations of species turnover on Axis 1 or 2 of the ordination, suggesting a more substantial change in floristic composition of the vegetation at these sites. Musola 05 (sampled in 2006 and again in 2012) is a seasonally-filled lagoon in the floodplain of the Musola stream, located in Kasanka National Park in the BM ecoregion. The only other site showing substantial change over time (in this case across a four-year period) was Kasanka River 03 (also in Kasanka National Park). In both 2009 and 2012 samples from this latter site were dominated by *Ceratophyllum demersum*, but emergent diversity was higher in 2012 than in 2009.

3.3. Geographical variation

Only two variables (Table 1), flow class and underwater light absorption coefficient, showed an absence of significant inter-ecoregion differences, with the low standard errors for the mean values also suggesting only limited variation in these two environmental variables, across the sample sites within each ecoregion.

Altitude showed significant differences: most BM rivers lie on the high north Zambia plateau, with hilly terrain, and hence the mean altitude of sample sites in that FEOW is considerably higher than for most other ecoregions (although not the similarly high-altitude ZH ecoregion). There was a significant inter-ecoregion trend in mean stream order, with the low value for BM again reflecting the terrain: many samples were located on small upland streams high in the catchments of the ecoregion. BM sites tended to exhibit much

lower conductivity, TDS and pH than in other ecoregions, and also had low underwater light absorption coefficients (though the data for k are incomplete, and as noted above, the trend was not significant). There was a strong trend in alkalinity across the five ecoregions, with BM samples (within the Congo Basin) being significantly (and substantially) lower than in rivers in three of the remaining ecoregions (MZL, KF, ZH), all lying within the Zambezi Basin. KF sites on average had alkalinity approximately an order of magnitude higher than BM samples.

Shade showed significant variation between ecoregions, though the trend was fairly weak (see Table 1). UZF sites tended to be little-shaded, whilst BM and ZH sites were more likely to be shaded by woodland or overhanging bankside vegetation. Finally, there was quite substantial, and significant, variation in nutrient status between rivers occurring in the five ecoregions. Once again BM sites tended to differ from the other ecoregions, with much lower $\text{NO}_3\text{-N}$ values than elsewhere, and $\text{PO}_4\text{-P}$ also low (though not as low as in UZF samples).

3.4. Environmental drivers of macrophyte community structure

Significant outcomes (Axis 1: F-ratio 4.027, $p = 0.002$; all canonical axes: F-ratio 2.301, $p = 0.002$) for Monte Carlo testing of the CCA ordination analysis of the species x samples dataset (constrained by six environmental variables for which full data existed) provided evidence that the observed distribution of samples in relation to environment gradients on the ordination plot was non-random. The ordination diagram, with full ordination statistics, is supplied as a supplementary file, published online alongside the electronic version of this article. The ordination outcome suggested that the strongest explanatory predictors of macrophyte community, for the variables included in the analysis, were altitude, stream order and flow, with conductivity showing a weaker gradient through the dataset, and pH and shade being of lesser importance.

TWINSPAN classification of the same dataset (but based only on the 272 taxa identified at least to genus level, i.e. excluding taxa identified only to family) identified seven end-groups of samples, labelled Groups A - G, produced with eigenvalues in the range 0.358 - 0.481, suggesting reasonable separation of groups based on the macrophyte species composition of their component samples. Fig. 4 depicts the FEOWs and habitat types represented in the sample-groups. Table 2 shows the outcome of statistical comparisons of environmental variables and macrophyte alpha-diversity between the seven sample-groups. This exercise suggested that the seven most-strongly significant environmental drivers of macrophyte community structure (specifically, measured as taxonomic composition and diversity of TWINSPAN sample-groups) in Zambian streams and associated waterbodies were altitude, stream order, shade, pH, alkalinity, $\text{NO}_3\text{-N}$, and underwater light availability, while $\text{PO}_4\text{-P}$ showed slightly lower (though still significant) variation between sample-groups. TDS also showed significant variation between the three sample groups for which data were available, but on this evidence flow did not appear to be a major driver of plant community.

Group A ($n = 23$ samples; eigenvalue for group formation: 0.374) was characterised by the presence (at a minimum abundance of 20 %F per sample) of four indicator species (*Mimulus gracilis*, *Schoenoplectus brachyceras*: now accepted as a synonym of *S. corymbosus*, *Ledermanniella tenax* and *Hydrostachys polymorpha*), and supported the highest macrophyte alpha-diversity of any sample-group. This widely-distributed group comprised samples from all FEOWs except KF, and was mainly made up of river sites, together with a single dambo (Figs. 4a and 4b). Table 2 shows that these samples were from a fairly high-altitude set of sites, with the highest mean flow, and generally intermediate stream order. They were the most heavily-shaded sites of all the sample-groups. They were mainly fast flowing, moderate-size hill streams and rivers, often flowing through wooded terrain, and tending to support plants adapted to fast flow (e.g. *Hydrostachys polymorpha*, and Podostemaceae such as *Ledermanniella tenax*). The data for k suggest generally clear water, together with low alkalinity and conductivity (no TDS data were available for samples in this group). In terms of nutrient status, samples from this group tended towards the low end of the mesotrophic category (following the trophic bands commonly used in UK freshwater trophic status assessment protocols: Vollenweider & Kerekes, 1981).

Group B ($n = 20$ samples; eigenvalue for group formation: 0.358) had five indicator species. Four characterised the sample-group by their presence alone, i.e. at a minimum occurrence of 20 %F at the site

(*Lagarosiphon ilicifolius*, *Ceratophyllum demersum*, *Azolla filiculoides* and *Potamogeton schweinfurthii*), and a fifth was an indicator species if present at moderate abundance, minimum 40 %F at the site (*Panicum subalbidum*). Macrophyte diversity again was high, though with a mean value of S lower than in Group A (and Group F) sites. The group principally comprised moderately-fast flowing river sites, all from the main channel of large rivers (Luangwa, Zambezi and Kafue Rivers), together with a single lagoon in the floodplain of the Luangwa River (Fig. 4b). All sites were in low-lying river valleys, located exclusively in two ecoregions, ZF and MZL (Fig. 4a), with the lowest mean altitude of any group, and correspondingly high stream order (Table 1). Sites usually had little or no shade. Alkalinity, conductivity, TDS and pH all tended to be quite high, towards the top end of the range for Zambian rivers. Water transparency, indicated by fairly low values for k, was quite high. The mean phosphate value for the sample-group suggests moderately-high mesotrophic status, but nitrate values were the highest of any group for which NO₃-N data were available, suggesting that these sites probably showed some degree of nutrient enrichment, tending towards meso-eutrophic conditions. For example, the only lagoon in this group (Hide Lagoon at Flatdogs Camp, an ecotourism lodge in the South Luangwa valley), receives “grey” water from the camp’s sewerage system, while the river sites tend to receive additional nutrients from farmland drainage, as well as effluents derived from the towns in their catchments.

Group C (n = 112 samples; eigenvalue for group formation: 0.358) was the biggest sample-group. It contained samples from all five major ecoregions, and had all four habitat types represented in the group, though river sites predominated (Figs. 4a and 4b). Indicator species were the presence of two emergents, *Panicum repens* and *Persicaria attenuata*. Macrophyte alpha-diversity showed moderate values at sites in the group, and the small standard error for the mean value of S (Table 2) suggests that most samples had intermediate values. Given the size of the group it is likely that the vegetation which it supports represents the commonest macrophyte community present in Zambian rivers and associated waterbodies, and the values for environmental variables reflect an absence of extreme conditions, with intermediate mean values being seen for altitude, flow, shade and pH. Group C sites, however, showed quite high stream order values (indicating that the group included a fairly high proportion of medium to large river sites), and also had high conductivity and alkalinity (the latter being significantly higher than for groups A and F). Water clarity was quite low, but was still indicative of clear water in absolute terms. Mean values for NO₃-N and PO₄-P were the second highest across the sample-groups, suggesting a degree of nutrient enrichment, probably tending towards an average meso-eutrophic status.

Group D (n = 39 samples; eigenvalue for group formation: 0.432) had no indicator species, but TWINSpan identified three species as preferentials characterising the vegetation supported by samples in this group: *Ottelia verdickii* (at “presence” abundance, a minimum of 20 %F), and two other species when present at high abundance (minimum 60 %F), namely *Panicum repens* and *Phragmites mauritianus*. Fig. 4 shows that this group contained samples from four ecoregions, but was heavily dominated by BM samples, and it had the best-balanced mix of samples from all four habitat types (though river samples still formed the biggest subset within Group D). Samples were mostly from quite high altitude sites (reflecting the predominance of samples from rivers on the north Zambian plateau, within BM), whilst the group had the lowest mean stream order, and second lowest mean flow, of any sample-group containing rivers. The group primarily consists of small, fairly slow-flowing high plateau streams. Shade conditions were similar to Group C. Conductivity, TDS and pH values were low to intermediate compared with other sample-groups, but mean alkalinity was third highest of the groups for which data were available. Water clarity, as indicated by mean k value, was intermediate compared with other groups. Average phosphate values were the highest of any sample-group, but nitrate was much lower than in Groups B or C. The results for these two parameters together suggested that a nutrient status towards the upper end of mesotrophic, or possibly mildly-eutrophic conditions, characterised Group D sites.

Group E (n = 16; eigenvalue for group formation: 0.432). Indicated by the presence of *Ottelia exserta*, the samples making up this small group were all from BM, with a fairly even mix of river and lagoon sites. Macrophyte diversity was low, but a number of rare species occurred. All but one of the group’s samples came from a fairly restricted area (hence the minimal standard error for mean altitude: most samples were effectively at the same altitude), in the upland inland delta of the Lukulu River, as it enters the Bangweulu

Swamp on the north Zambian plateau, in the vicinity of Shoebill Camp. Mean flow class and stream order had low mean values, reflecting the high proportion of lagoon samples in the group. All sites were unshaded. Although no alkalinity data were available, samples from this group had high pH (usually around pH 8.0), suggesting alkaline conditions (the catchment lies partially on limestone rock). No TDS data were available, but conductivity was the lowest of any sample-group. Water clarity was also low compared with other sample-groups, but still represented clear-water conditions in absolute terms. Nutrient availability was low for both phosphate and nitrate, suggesting conditions that were generally very oligotrophic. This sample-group supports a fairly unusual type of macrophyte vegetation, occurring in base-rich but nutrient-poor conditions, characterising a habitat probably found, in Zambia, only in upland slow-flowing rivers (and their associated highly-connected riverine lagoons), and containing a number of species which occur rarely, or not at all, elsewhere in the dataset (e.g. *Aldrovanda vesiculosa*, *Ottelia exserta*, *Ottelia cylindrica*, *Najas horrida*).

Group F (n = 57; eigenvalue for group formation: 0.481) was the second largest sample-group, with samples from all five FEOWs represented, but comprising only rivers and closely-connected backwater and lagoon sites (dambo sites were absent) (Figs. 4a and 4b). TWINSpan found no indicators, but identified a diverse group of preferential species characterising the vegetation of samples making up this Group (mostly at “present” status, i.e. a minimum 20 %F occurrence in the sample). These were *Floscopa glomerata*, *Fuirena umbellata*, *Nymphaea nouchali* var. *caerulea*, *Osmunda regalis*, *Panicum parvifolium*, *Persicaria decipiens*, *Thelypteris confluens* and *Spirodela polyrhiza*. In addition *Phragmites mauritianus*, at moderate abundance (a minimum of 40 %F) was a ninth preferential species. After Group C this is the second commonest macrophyte vegetation type found in Zambian rivers and closely-associated waterbodies. It has a higher macrophyte diversity than the Group C vegetation type, and not dissimilar to that of Group A (though with a very different flora). It occurs at the highest mean altitude of any of the sample-groups, with a high mean flow class and quite low stream order, and comprises primarily, though not exclusively, samples from upland streams and rivers, and their high-connectivity associated waterbodies. Like Group A, many samples in this Group were from streams running through woodland habitats, so shade score was quite high. Alkalinity, pH and conductivity were all low, reflecting the upland nature of the sample-group. The nutrient status of this sample-group was mesotrophic, and water clarity was very high.

Group G (n = 3; eigenvalue for group formation: 0.481). This very small group only comprised lagoons. Because of its small size, and absence of data for several variables, it is unwise to draw conclusions about the vegetation and environmental characteristics of Group G, but a few words are in order. Indicator species for the group was *Spirodela polyrhiza*, and the samples of this group showed a tendency towards dominance by free-floating species (notably *Azolla nilotica* and *Pistia stratiotes*, as well as *S. polyrhiza*). Diversity was very low. Altitude was intermediate but with a high standard error, because two sites were from the low-altitude Luangwa valley, and the third from up on the north Zambian plateau. Values of pH were also intermediate but these lagoons had the highest conductivity of any sample-group. All three sites were quite heavily shaded by surrounding trees, and surface mats of free-floating macrophytes would undoubtedly also severely reduce underwater light availability. One of the pools (Mushroom Lagoon in the Luangwa floodplain) was very muddy, with very low water clarity: visibly in use as a wallow by large animals, such as hippopotamus and elephant, with much resulting resuspension of sediment on a daily basis.

TWINSpan classification of species (and taxa identified to genus level) in terms of samples in which they occurred, produced eight assemblages (labelled I – VIII: Fig. 5) which showed varying degrees of separation. Assemblages I and II had a division eigenvalue of only 0.193, suggesting that the taxa forming these two assemblages showed a high degree of overlap between the samples in which species of the two assemblages occurred. Separation was better for the remaining assemblages, at 0.580 for the division eigenvalue producing assemblages III and IV, 0.407 for V and VI, and 0.316 for assemblages VII and VIII. Examination of the assemblage-membership of the species characteristic (as indicators or preferentials) of the seven sample-groups reflected this varying degree of assemblage separation. Species representing some assemblages were quite closely associated with a single sample-group, whilst other sample-groups were indicated by species from > 1 assemblage. Group A is a good example of the latter case, being characterised by two species from assemblage II (*Hydrostachys polymorpha*, *Ledermanniella tenax*) and two from assemblage V (*Mimulus gracilis*, *Schoenoplectus brachyceras* (= *S. corymbosus*)). On the other hand, the

characteristic species for Group B were mostly from assemblage I (*Azolla filiculoides*, *Lagarosiphon ilicifolius*, *Potamogeton schweinfurthii*), plus one from assemblage IV (*Ceratophyllum demersum*). For Group C, one indicator was from assemblage II (*Persicaria attenuata*) and the other from assemblage VIII (*Panicum repens*). Group D was also characterised by *Panicum repens* from assemblage VIII (though at a different abundance), as well as the assemblage II species *Phragmites mauritanus* and assemblage VI *Ottelia verdickii*. Group E samples had only one indicator, *Ottelia exserta*, from assemblage VIII. The lengthy list of preferential species characterising Group F was dominated by species from assemblage VII (*Fuirena umbellata*, *Osmunda regalis*, *Panicum parvifolium*, *Thelypteris confluens* and *Spirodela polyrhiza*), together with *Persicaria decipiens* (assemblage IV), *Floscopa glomerata* (assemblage V), and *Nymphaea nouchali* var. *caerulea* (assemblage VIII). Finally, Group G had as its indicator *Spirodela polyrhiza* (assemblage VII).

The proportions of individual life-forms within the taxa making up the eight assemblages identified by the TWINSpan classification are shown in Fig. 5. As expected, given their predominance in the dataset as a whole, every assemblage was dominated by emergents, particularly so in assemblages IV, V and VII. Floating plants were completely absent from assemblage III, where submerged species characteristic of faster-flowing streams formed a high proportion of the assemblage (e.g. *Bolbitis heudelottii*, *Ledermanniella tenax*, *Tristicha trifaria*, *Eriocaulon teusczii*). In contrast, floating species formed a substantial proportion of the flora in assemblage II, with 3 of the 11 species present in this small assemblage belonging to this life form (*Trapa natans*, *Wolfiella arrhiza*, *Salvinia molesta*). As well as being of importance in assemblage III, submerged species formed >30% of the total flora in assemblages VI (*Lobelia erinus*, *Najas horrida*, *Ottelia verdickii*, *Ottelia muricata*) and VIII (with 15 submerged species, including three *Ottelia* species (*Ottelia luapulana*, *O. exserta*, *O. ulvifolia*) and five *Utricularia* species (*Utricularia benjaminiana*, *U. foliosa*, *U. inflexa*, *U. stellaris*, plus a fifth identified only to genus level).

4. Discussion

The evidence produced by this study suggests that variation in macrophyte community in Zambian river systems does not seem to be driven, to any major extent, by temporal variation in environmental conditions. This clearly leaves geographical variation in physico-chemical factors as the more likely candidate driver of aquatic macrophyte community composition and diversity in Zambian river systems. There appears to be good evidence to support this suggestion when looking in detail at the results of the exercise to compare environmental variables between the rivers (and their associated waterbodies) sampled in five ecoregions of Zambia (Table 1).

Of the physico-chemical drivers of macrophyte community structure examined in this study, those most likely to be influenced by human activities are probably nutrient status and flow, though relatively few of the samples in this survey came from river stretches strongly-affected by river regulation (examples of those that did are a number of MZL and KF samples, respectively downstream of the Kariba Dam on the Zambezi, and Itezhi-Tezhi and Kafue Gorge Dams on the Kafue River). The snapshot results for qualitatively-assessed flow class gained from this survey may however be misleading. Longer-term historical data (Kennedy et al., 2012) for flow regime in gauged rivers in Zambia suggest that BM (in the Congo Basin) was notably different from the other FEOWs. In this ecoregion Q95 and Q50 curves (flows exceeded 95% and 50% of the time, respectively) indicated substantially greater flows under low to moderate flow conditions than elsewhere in Zambia, and even under flood conditions (Q5 values: flows exceeded 5% of the time) flows tend to be greater than in other ecoregions (Kennedy et al., 2012). In this context it is interesting to note (see Fig. 3) that the BM ecoregion contained the highest proportion of submerged species, which may well (at least in part) be associated with the different flow conditions seen in this ecoregion's river systems, compared to the rest of the country. Overall we consider it quite likely that flow regime may be of greater importance in driving macrophyte community structure than was suggested by the data analyses presented here, and further work is needed to examine this issue in more detail.

The main source of nutrient enrichment in Zambian rivers is from the increasing prevalence of commercial large-scale irrigated agriculture (primarily sugar cane, coffee, cereals, and a range of other crops), all using large quantities of fertiliser, though this is concentrated in the KF and MZL ecoregions, and much less common elsewhere. Alongside these diffuse sources are point sources of effluent pollution,

derived from the major urban areas of Zambia (e.g. Obrdlik, 1987), which lie in these same two FEOs, as does the main Copperbelt area of mining for metals. Heavy metal pollution (Norrgrén et al., 2000; Pettersson & Ingri, 2001) was not assessed in this study, and is a third potential source of impact on macrophyte communities, though, as noted above, it tends to affect only rivers in the areas which also experience major human impacts from nutrient enrichment, whether urban or agriculture-derived. Again, further research is clearly needed in this context to determine the impacts of metal pollution on Zambian river macrophyte communities.

With the exception of South Africa (most of which, however, lies outwith the Tropics), where a moderate research effort relating to riverine plant ecology has been made (e.g. O’Keeffe, 1986; Meek et al., 2013) there is remarkably little information in the literature against which our work can be compared in the context of the vegetation ecology of southern tropical African river systems. A rare example is the survey of river, and associated riverine wetland, vegetation carried out by Gichuki et al. (1994) in the Lower Sondu Miriu wetlands (0°18’S; 34°46’ E) which lie at the mouth of the Sondu Miriu River, where it enters the Winam Gulf of Lake Victoria in Kenya. This study found that the vegetation of the river and its adjoining riverine wetland habitat was, as in Zambian rivers, dominated by emergent species (23 taxa recorded), with eight floating species and six submerged species also present. Several species were found both in Zambian rivers and in this small Kenyan river system, including *Cyperus papyrus*, *Vossia cuspidata*, *Typha domingensis*, *Potamogeton schweinfurthii*, *Ceratophyllum demersum*, *Najas horrida*, *Trapa natans*, *Azolla nilotica* and *Pistia stratiotes*. However, on present evidence, 51% of the plants listed for the Sondu Miriu River do not occur in Zambian rivers. In contrast, examination of the survey data from a recent, as yet unpublished study (T. Davidson, University College London, pers. comm.) of the macrophytes of the Okavango River system (19°S; 23° E) in Botswana, much closer to Zambia than the Kenyan site, indicates that 47 of 58 species recorded there were also present in Zambian rivers, leaving just 20% which occurred only in the Botswanan system (e.g. *Typha capensis*, *Brasenia schreberi*, *Utricularia reflexa*). These outcomes highlight the likely differences in ecology within southern tropical African river vegetation, and emphasise the strong need for additional research in this area.

5. Conclusions

Our work not only constitutes the first-ever extensive survey of riverine macrophyte communities in Zambia, but also appears to be the first such study to be undertaken, at national scale, anywhere in southern tropical Africa. The outcome provided evidence to support the hypothesis that macrophyte community composition and diversity can be quantitatively related to seven geographically-varying physico-chemical drivers influencing Zambian river systems. However, macrophyte community structure seems to be relatively little-influenced by temporal change in Zambian river systems (in strong contrast to the situation relating to drivers of freshwater biodiversity in some tropical river systems elsewhere in the Southern Hemisphere: Thomaz et al., 2009; Davidson et al., 2012; Varandas Martins et al., 2013).

The results also indicated the presence of a very rich macrophyte biodiversity in Zambian river systems, supporting some 55% of the currently-recognised total Afrotropical macrophyte flora (Chambers et al., 2008), within seven recognisable riverine macrophyte communities.

From the data collected in this study it is not possible to separate the relative importance of human versus natural factors influencing the drivers of Zambian macrophyte vegetation structure, and further work is needed in this context. However the study represents a substantial advance in knowledge of the macrophyte ecology of Zambian rivers, and makes a contribution to assessment of the status of rare aquatic plant species for southern tropical Africa. It also provides a baseline for future work, particularly in the context of likely impacts from climate change, and human population increase, upon tropical rivers and the ecosystem services that they provide, and points the way for extension of this work to rivers in neighbouring southern tropical African countries. These, in turn, are all important factors in aiding the development of appropriate and sustainable river monitoring and conservation programmes in southern Africa.

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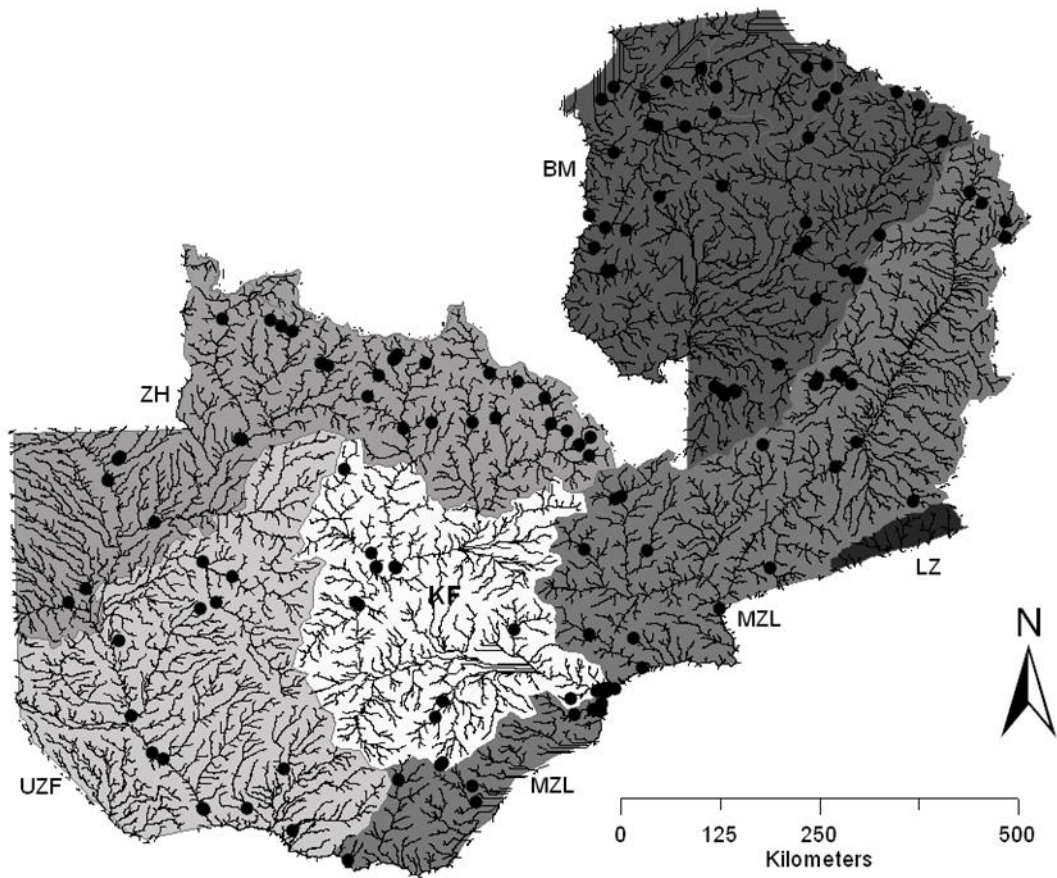


Fig. 1. Sampling site locations, river systems, and within-country boundaries of Freshwater Ecoregions of the World (FEOWs) sampled in Zambia. BM: Bangweulu-Mweru; KF: Kafue Flats; LZ: Lower Zambezi; MZL: Middle Zambezi-Luangwa; UZF: Upper Zambezi Floodplain; ZH: Zambezian Headwaters.

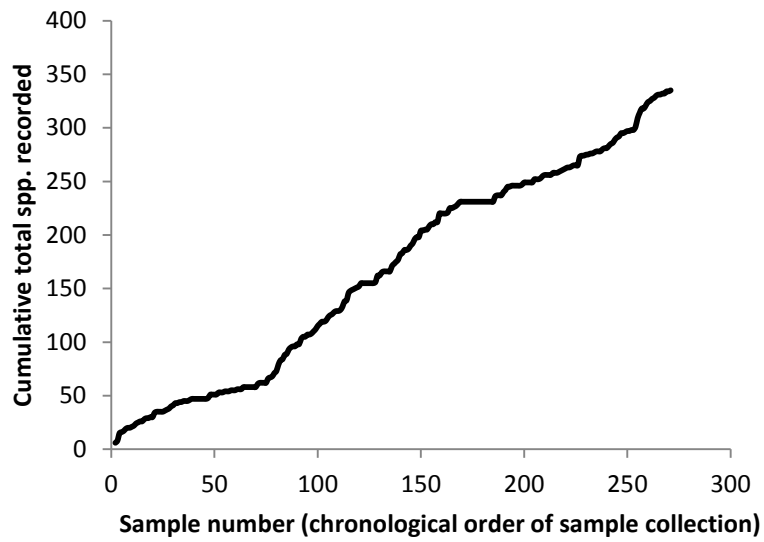


Fig. 2. Cumulative sequential total macrophyte taxa records for 271 samples collected from 228 sites on Zambian rivers and associated water bodies during 2006 – 2012.

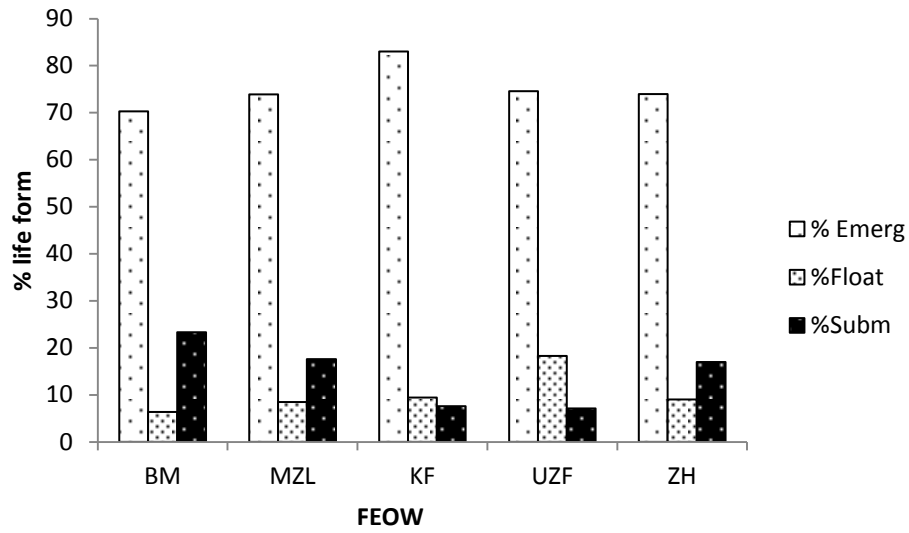
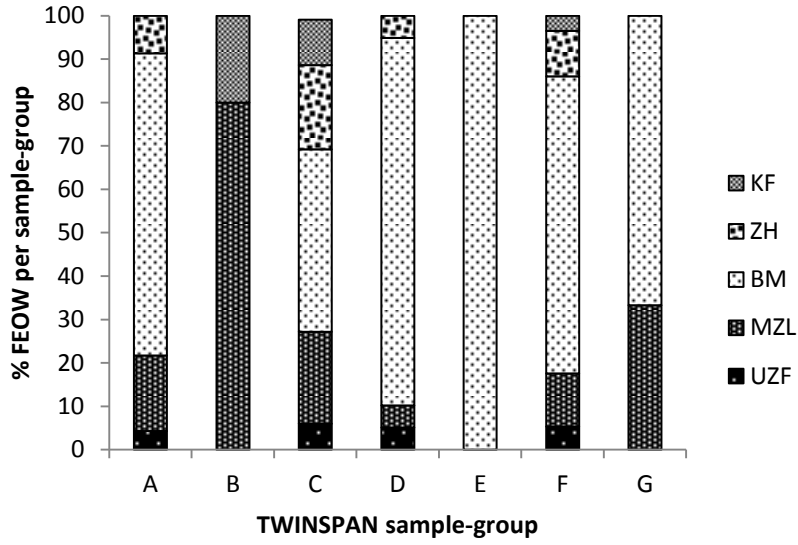
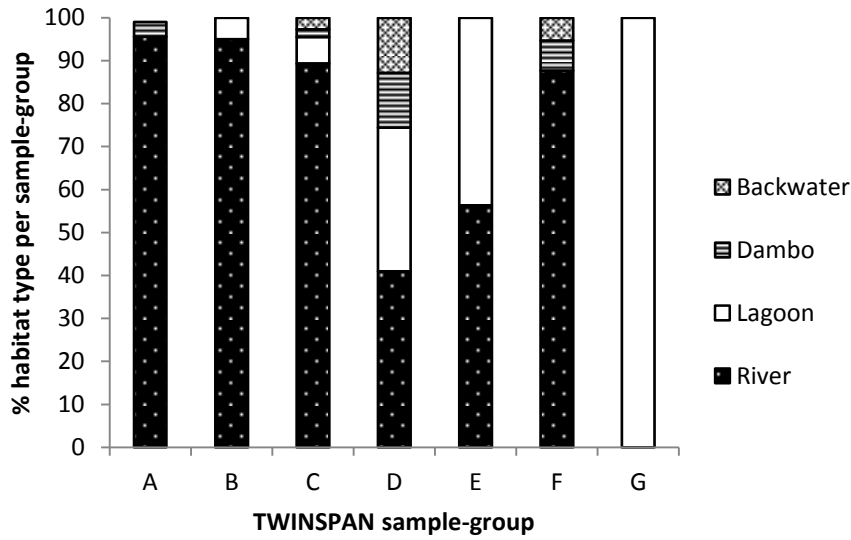


Fig. 3. Proportion of species in each of three life forms (Emerg: emergent; Float: floating; Subm: submerged; Chambers et al., 2008) occurring in each of the five freshwater ecoregions primarily represented in Zambia (see Fig. 1 caption for abbreviations).



(a)



(b)

Fig. 4 (a). Freshwater ecoregions of the world (FEOWs: see Fig. 1 caption for abbreviations) represented in TWINSpan sample-groups; (b) Habitat types represented in TWINSpan sample-groups.

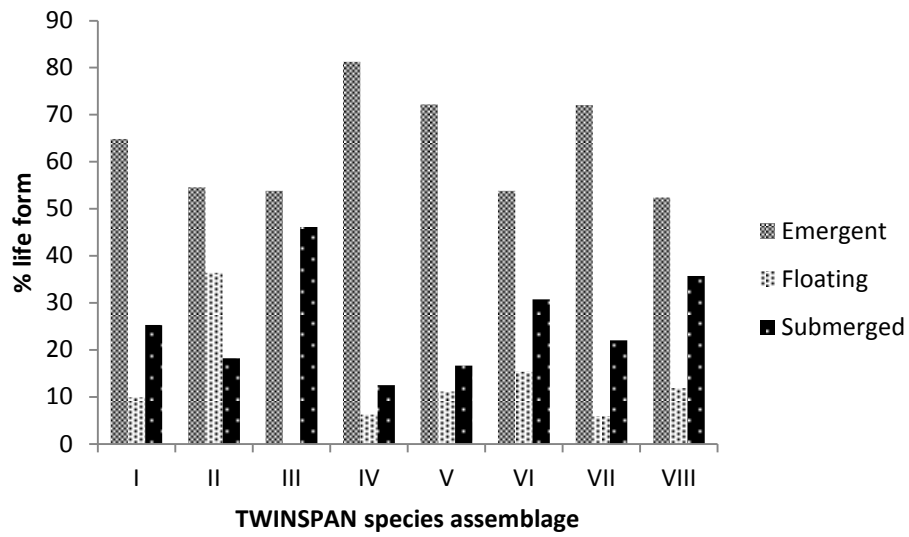


Fig. 5. Life-forms represented in individually-distinguished taxa, comparing eight assemblages identified by TWINSpan classification.

	Freshwater Ecoregion					p	Signif.
	BM	UZF	MZL	ZH	KF		
Altitude (m)	1207.2 ± 8.37	1019.5 ± 17.7	740.0 ± 51.8	1201.4 ± 18.9	924.1 ± 75.9	<0.001	***
Flow class	1.5 ± 0.1	1.8 ± 0.2	1.8 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	>0.05	n.s.
Stream order	2.9 ± 0.2	5.5 ± 0.5	5.4 ± 0.3	4.8 ± 0.2	5.7 ± 0.3	<0.001	***
Shade class	1.6 ± 0.1	1.1 ± 0.1	1.4 ± 0.1	1.7 ± 0.1	1.3 ± 0.1	0.028	*
pH	7.3 ± 0.1	7.5 ± 0.2	7.8 ± 0.1	7.6 ± 0.1	8.2 ± 0.1	<0.001	***
EC (µS cm ⁻¹)	58.4 ^d ± 7.4	77.8 ^{c,d} ± 11.4	179.3 ^{b,c} ± 25.1	235.1 ^{a,b} ± 29.3	260.5 ^a ± 34.1	<0.001	***
Alkalinity (µEq L ⁻¹)	338.0 ^c ± 41.1	777.4 ^{b,c} ± 104.1	1345.8 ^{a,b} ± 216.9	2196.6 ^a ± 270.8	2228.1 ^a ± 272.6	<0.001	***
PO ₄ -P (mg L ⁻¹)	0.016 ^b ± 0.002	0.006 ^a ± 0.001	0.017 ^b ± 0.003	0.020 ^c ± 0.005	0.021 ^c ± 0.003	<0.001	***
NO ₃ -N (mg L ⁻¹)	0.029 ± 0.005	0.190 ± 0.056	0.380 ± 0.050	0.330 ± 0.039	0.460 ± 0.086	<0.001	***
k (m ⁻¹)	2.37 ± 0.21	n.d.	8.05 ± 3.40	n.d.	1.76 ± 0.33	>0.05	n.s.
TDS (mg L ⁻¹)	0.02 ± 0.003	0.04 ± 0.007	0.11 ± 0.019	0.18 ± 0.033	0.18 ± 0.028	<0.001	***
S: alpha-diversity (all taxa)	8.8 ± 0.6	11.1 ± 1.4	9.2 ± 0.6	8.2 ± 0.7	6.6 ± 0.9	>0.05	n.s.
Total species recorded (gamma-diversity)	236	71	142	100	53	-	-

Table 1. Means ± standard errors (s.e.) and significance of differences for outcomes of one-way ANOVA (flow class; conductivity: EC; alkalinity; orthophosphate: PO₄-P) and Kruskal–Wallis tests (all other variables), for environmental variables and macrophyte alpha-diversity, compared between sample-sets occurring in five major ecoregions of Zambia. For significant outcomes (ANOVA only: post-hoc mean separation was not carried out for non-parametric test outcomes) mean values that are not significantly different, per variable, (Tukey mean separation test, P >0.05) share a superscript letter in common. n.d. = no data. Significance: * p < 0.05; ** p < 0.01, *** p < 0.001, n.s = not significant (p > 0.05).

	TWINSPAN Sample-group							p	Signif.
	A	B	C	D	E	F	G		
Altitude (m)	1181.5 ± 25.9	490.8 ± 55.9	1039.1 ± 26.2	1155.3 ± 18.9	1160.8 ± 0.8	1258.7 ± 24.4	972.3 ± 186.3	<0.001	***
Flow class	2.1 ± 0.2	1.5 ± 0.1	1.7 ± 0.1	0.9 ± 0.2	0.8 ± 0.2	2.0 ± 0.1	0	>0.05	n.s.
Stream order	4.1 ± 0.3	7.85 ± 0.5	4.4 ± 0.2	1.7 ± 0.3	2.8 ± 0.6	3.6 ± 0.2	0	<0.001	***
Shade class	1.8 ± 0.1	1.1 ± 0.1	1.5 ± 0.1	1.3 ± 0.1	1.0 ± 0	1.7 ± 0.1	2.0 ± 0.5	<0.001	***
pH	7.6 ± 0.2	7.9 ± 0.1	7.7 ± 0.1	7.3 ± 0.1	8.1 ± 0.1	7.1 ± 0.1	7.5 ± 0.2	<0.001	***
EC (µS cm ⁻¹)	54.3 ^a ± 9.9	160.1 ^c ± 21.3	168.0 ^c ± 17.4	106.4 ^b ± 24.6	52.7 ^a ± 5.9	61.4 ^{a,b} ± 13.0	186.3 ^c ± 35.1	<0.001	***
Alkalinity (µEq L ⁻¹)	651.5 ^b ± 159.1	1104.4 ^{a,b} ± 89.7	1773.2 ^a ± 188.2	1055.2 ^{a,b} ± 294.7	n.d.	657.2 ^b ± 156.7	n.d.	<0.001	***
PO ₄ -P (mg L ⁻¹)	0.011 ^{a,b} ± 0.002	0.016 ^{a,b} ± 0.003	0.019 ^{a,b} ± 0.002	0.027 ^a ± 0.005	0.004 ± 0	0.013 ^b ± 0.003	n.d.	0.002	**
NO ₃ -N (mg L ⁻¹)	0.063 ± 0.021	0.428 ± 0.061	0.292 ± 0.035	0.076 ± 0.029	0.003 ± 0	0.109 ± 0.025	n.d.	<0.001	***
k (m ⁻¹)	2.9 ± 0.4	2.4 ± 0.9	3.0 ± 1.0	2.8 ± 0.6	3.1 ± 0.6	1.5 ± 0.3	18.4 ± 12.6	<0.001	***
TDS (mg L ⁻¹)	n.d.	0.09 ± 0.01	0.15 ± 0.02	0.05 ± 0.01	n.d.	0.07 ± 0.02	n.d.	<0.05	*
S: alpha-diversity (all taxa)	10.2 ± 1.3	9.6 ± 1.0	8.4 ± 0.5	8.6 ± 0.7	5.7 ± 0.6	10.1 ± 0.7	4.3 ± 0.7	<0.01	**

Table 2. Means ± standard errors (s.e.) and significance of differences for outcomes of one-way ANOVA (flow class; conductivity: EC; alkalinity; orthophosphate: PO₄-P) and Kruskal–Wallis tests (all other variables), for environmental variables and macrophyte alpha-diversity, compared between sample-groups (A – G) delineated by TWINSPAN classification. For further information see caption to Table 1.