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The Zambian Macrophyte Trophic Ranking scheme, ZMTR: a new biomonitoring protocol to assess the trophic status of tropical southern African rivers

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Abstract

The Zambian Macrophyte Trophic Ranking system (ZMTR) is a new bioassessment scheme to indicate the trophic status of tropical southern African river systems. It was developed using a dataset of 218 samples of macrophytes and water chemistry, collected during 2009-2012, from river sites located in five world freshwater ecoregions primarily represented in Zambia. A typology based on these ecoregions, and three stream order categories, was used to determine soluble reactive phosphate (SRP) reference conditions. Zambian Trophic Ranking Scores ($ZTRS_{sp}$) were calculated for 156 species, using direct allocation from SRP data for 80 species, in samples for which sufficient available SRP data existed. An indirect quantitative procedure, based upon occurrence of species in six sample-groups, of differing mean SRP status, produced by TWINSPAN classification, allocated provisional $ZTRS_{sp}$ values for the remaining 76 species. Additional data for nitrate, pH, alkalinity and conductivity were used to help assess the trophic preferences of macrophyte species showing differing $ZTRS_{sp}$ values. $ZMTR_{sample}$ values were calculated as the mean $ZTRS_{sp}$ score of species present per sample. ZMTR indicated trophic status reasonably accurately for 83.1% of Zambian samples, and for all samples within a test dataset from Botswanan rivers. Examples of application of the methodology, and its potential for hindcasting river trophic status are provided. The scheme currently underestimates highly-enriched conditions, and, to a lesser extent, overestimates the trophic status of some very low-nutrient rivers, but at this pilot stage of development it generally predicts the trophic status of tropical southern African river systems quite well.

Key words

Biomonitoring, Aquatic macrophytes, Eutrophication, Tropical Africa, Southern African River Assessment Scheme

Highlights

- A new plant-based biomonitoring scheme for tropical African rivers is described
- Trophic ranking scores ($ZTRS_{sp}$) are given for 156 Zambian macrophyte species
- ZMTR indicated trophic status reasonably accurately for 83.1% of Zambian samples
- An independent test dataset from Botswana also showed a positive test outcome
- Examples show the ability of ZMTR to indicate current and hindcast trophic status

1. Introduction

Freshwater biomonitoring uses organisms that live in freshwater systems as indicators of ecosystem health (or "biointegrity": Norris and Hawkins, 2000), and also potentially of specific ecosystem characteristics which provide an indication of biointegrity status, such as nutrient conditions (e.g., Holmes et al., 1999; Hartman et al., 2010).

The maintenance of good quality, clean rivers, supporting high-quality biodiversity, is universally recognised as a vital element of societal wellbeing. The successful development and implementation of inexpensive but effective biomonitoring schemes to assess river biointegrity is crucial to improving human and environmental welfare in all developing countries, including those in tropical Africa. However, despite their obvious low-cost advantages for water quality monitoring in low-income tropical countries, to date river biomonitoring schemes have been developed for only a few tropical African countries (e.g., South Africa, Zimbabwe, Namibia and Tanzania), and are usually based on the use of benthic invertebrates, rather than macrophytes, as indicator organisms (e.g., Dickens and Graham, 2002; Palmer and Taylor, 2004; Bere and Nyamupingidza, 2014; Kaaya et al., 2015). A major reason for this state of affairs lies with the general dearth of baseline information about the freshwater biota and environmental conditions occurring in tropical African rivers, and their associated waterbodies, which is obviously needed as a prerequisite for bioassessment scheme development.

It is in Europe (or to be more precise, in European Union (EU) nations) that a major effort has been made to develop macrophyte-based river biomonitoring protocols, which are currently in routine use for river biomonitoring, after macrophytes were recognised as biological quality elements for the implementation of the European Water Framework Directive (Birk and Willby (2010). Two examples of the many different macrophyte-based national river bioassessment schemes in use in EU countries include LEAFPACS in the UK (WFD-UKTAG, 2014) and TIM (Trophic Index of Macrophytes) in Germany (Schneider and Melzer, 2003). The national protocols vary quite considerably in detail, but tend to utilise a similar basic approach, and are usually reference-condition based schemes (e.g., Ferreira et al., 2002; Pardo et al., 2012). In addition to development and implementation of these biomonitoring protocols, much work in Europe has been done on comparison, critical assessment, and intercalibration of macrophyte-based river bioassessment schemes using a range of metrics (e.g., Birk et al., 2006; Aguiar et al., 2014).

The Southern African River Assessment Scheme, SAFRASS, developed during 2010-2014, following preliminary work in Zambia from 2006 onwards, aimed to produce a pilot set of river-quality biomonitoring protocols for use in tropical southern Africa (Kennedy et al., 2012a, 2012b, 2014, 2015; Lowe et al., 2013). SAFRASS uses three biotic indicator groups (benthic diatoms, benthic macroinvertebrates, and macrophytes) that variously respond to changes in river conditions over time-scales from weeks to years (e.g., Smith et al., 1999; Harding et al., 2005; Schneider, 2007; Dallas et al., 2010; US EPA, 2012; Moore and Murphy, 2015). Zambian rivers, and their closely-associated floodplain waterbodies, were selected as the target systems for this study because of the naturally-wide range of ecological conditions occurring in the country. There is also a widely-varying scale of impacts from human-associated activities, across the country, including nutrient enrichment, pollution by heavy metals and other toxins, flow changes, catchment degradation, sedimentation, and impacts of invasive aquatic weeds (e.g., Kennedy et al., 2012a, 2012b, 2014, 2015).

No river biomonitoring protocols existed for Zambia prior to development of the pilot SAFRASS procedures. Water chemistry testing had been conducted at a few sites over past years by government institutions but data are both extremely limited, and spatially and temporally sporadic. The combination of lack of capacity to monitor water resource quality, and the potential for increased impacts on these resources, as well as likely impacts upon the people who rely on them, made the need for development of appropriate freshwater biomonitoring tools in Zambia particularly pressing.

Here we outline the basic features of the SAFRASS monitoring approach, and describe in detail its macrophyte-based biomonitoring protocol, the Zambian Macrophyte Trophic Ranking

(ZMTR) system. This was developed using a subset of the data (utilising vascular macrophytes only: lower plants are not currently included in the scheme), collected from the first-ever extensive survey, during 2006 – 2012, of Zambian rivers and associated high-connectivity floodplain standing waters (Kennedy et al., 2015). The SAFRASS approach aimed to bring together, modify as necessary, and recalibrate appropriate features of similar schemes developed for use in non-tropical parts of the world. In particular we made use of (in the case of the SAFRASS macrophyte element, ZMTR) the UK Macrophyte Mean Trophic Ranking system (MTR: Holmes et al., 1999), and the Swedish Environmental Quality Criteria for Lakes and Rivers (Swedish EPA, 2000), as well as relevant baseline aspects of the South African Scoring System (SASS: Dickens and Graham, 2002). The work reported here was, in large part, based on results obtained during fieldwork undertaken during 2010 – 2012 for development and testing of SAFRASS. The data were supplemented by information from previous and subsequent surveys of Zambian rivers and closely-associated waterbodies, including riverine floodplain lakes, backwaters and dambos (seasonal standing waterbodies), undertaken by the authors during 2006 - 2012. A further test dataset was obtained for riverine sites independently surveyed during 2006 – 2007 in Botswana (Mackay et al., 2011; Davidson et al., 2012). The outcomes reported here update and replace previously-published provisional findings for the ZMTR protocol (Kennedy et al., 2012a, 2012b, 2014).

2. Material and methods

Macrophyte surveys (with collection of supporting physico-chemical data) were conducted during 2006-2012, with 271 samples being collected from 228 sites in Zambia, located on rivers and closely-associated (high connectivity) floodplain waterbodies, including riverine lakes, backwaters and dambos (seasonal standing waterbodies). From this dataset a subset of 218 samples collected during 2009-2012 was primarily used for the purposes of this study. Surveys followed the guidelines of the international standard EN 14184 (European Committee for Standardization, 2003), including emergent vegetation due to its importance in Zambian rivers (Dallas et al., 2010). The survey protocol (as outlined in Kennedy et al. (2015) and briefly summarised here) required a standard 100 m stretch of waterbody to be sampled at five random points within the stretch. All macrophyte species present within the waterbody were recorded per sampling point, and frequency (as %F per stretch) was calculated as a measure of abundance for each species, based on number of hits out of 5 maximum possible. Records for emergent and floating species were supplemented by the use of a standard macrophyte-sampling grapnel (attached to a 5 m long cord, and thrown from bank or boat as appropriate) to collect submerged species, which were generally less conspicuous compared to life forms which projected above or resided at the water surface. The high risk of attack by dangerous animals (particularly crocodile and hippopotamus) largely precluded entry into the water for plant sampling purposes, except in small shallow clear-water streams, or where (rarely) armed guards were available to provide protection.

Where feasible, plant samples were retained as herbarium-sheet specimens for subsequent identification. This was a major issue in Zambia at the time of the project, as no appropriate identification guides for aquatic vegetation pre-existed for the country. Consequently, identification was carried out using other aquatic and wetland plant identification and distribution source material, currently available for other parts of southern Africa and tropical Asia (e.g. Cook, 1996, 2004; Gerber et al., 2004; Weyl et al., 2016), as well as guides to identification of riverine macrophytes in Zambian rivers, produced as outputs from the SAFRASS project (e.g., Kennedy and Murphy, 2012). Taxonomic literature was also utilised, primarily Flora Zambesiaca (Exell and Wild, 1960 et seq.: (http://apps.kew.org/efloras/search.do;jsessionid=703937FE97D2FF27F59F5EA53BE81871)), but again this was incomplete at the time of the study, with some major aquatic plant families not yet covered by Flora Zambesiaca (notably Cyperaceae), although coverage is good for others, e.g., Aponogetonaceae (Martins, 2009). Our records were also cross-checked against species occurrence records given in the Flora of Zambia and Flora of Zimbabwe websites (www.zambiaflora.com;

www.zimbabweflora.co.zw). Species nomenclature follows The Plant List (www.theplantlist.org). Only taxa fully identified to species level (or, where relevant, to infra-species level: e.g., *Nymphaea nouchali* var. *caerulea*) were included in this study. Taxa which were recorded only to genus or family level in the survey data reported by Kennedy et al. (2015) were not used here.

A smaller independent dataset on macrophytes and water chemistry was additionally made available to us for comparative test purposes, from work undertaken by a separate study team, conducted during 2006 - 2007 in Botswana (for full details of methodology see Mackay et al. (2011) and Davidson et al. (2012)). This comprised data from 21 riverine sites within the Okavango Delta (centred on 18.8°S, 22.5°E; located approximately 200 km south of Zambia), which was used as a test dataset for the pilot ZMTR scheme.

At the Zambian sites a range of site physico-chemical variables was assessed (listed in full, with results given in detail, in Kennedy et al. (2015) and online supplementary files associated with that paper). Environmental data collected in the field and used in this study included geospatial coordinates and altitude (using a Garmin Etrex hand-held GPS); and stream order (taken from an ArcGIS-generated regional stream network, derived from a digital elevation model). Electrical conductivity (EC: µS cm^{-1}), and pH were measured in situ, using a Schott Handylab 264 multi-function meter. Water samples were collected, and stored, as appropriate, in sets of 60 mL LDPE bottles and 10 mL glass sample vials, then transported in a coolbox for subsequent laboratory determination of alkalinity (by Gran titration (Neal, 2001): µEq L^{-1}); and, after filtration, of soluble reactive phosphate (SRP: PO_4-P: µg L^{-1}), and nitrate (NO_3-N: mg L^{-1}) following standard procedures (MAFF, 1986; APHA, 1998), to respective limits of detection of 1 µg L^{-1} PO_4-P, and 0.005 mg L^{-1} NO_3-N.

A few samples from Zambia and all of those from Botswana (see Section 3.1 below) were analysed using unfiltered samples, giving data for total phosphate (TP). Total phosphate (TP (mgL^{-1})), was analysed, within three weeks of sample collection, using an air segmented flow analyser (Bran & Luebbe AA3) after persulphate digestion (McKay et al., 2011). There is evidence that SRP may represent from <5% to >90% of TP (e.g., Ernstberger et al., 2004), but as a general rule of thumb, using empirical evidence from the literature (e.g., Tarapchak and Rubitschun, 1981; Jeppesen et al., 2000) TP is usually about an order of magnitude greater than SRP concentration. In consequence we divided TP values from unfiltered samples by 10 to achieve a rough equivalence with the SRP data held for the bulk of the Zambian sites.

Full datasets for Zambian sampling site locations, macrophyte occurrences and physico-chemical information, for the data used in this study, are provided as supplementary files alongside the online version of Kennedy et al. (2015).

TWINSPAN classification (Hill, 1979) was used to identify species assemblages and sample-groups present within the dataset (Kennedy et al., 2015). One-way ANOVA and Tukey's multiple comparisons test were used to compare between means of TWINSPAN sample-groups, for values of SRP (a priori Ryan-Joiner testing showed this variable to be normally distributed). Outcomes were considered significant at p <0.05. The same test routines were used to examine differences in other water chemistry variables, as appropriate.

2.2. Development of the ZMTR metric

2.2.1. Direct derivation of $ZTRS_{sp}$ values from SRP data

For those species which occurred in at least five samples, and for which SRP data were also available, a direct calculation of $ZTRS_{sp}$ values was undertaken. The mean value for SRP in samples supporting the species was calculated, and Mean Trophic Score (MTS: allocated as shown in Box 1 on the basis of the trophic banding system developed by Vollenweider and Kerekes (1981)), was used to assign the $ZTRS_{sp}$ value (equivalent to calculated MTS) for each species. This direct method was used to calculate mean $ZTRS_{sp}$ values, on a 1 – 5 scale from average MTS score, for each species at sample-sites where it occurred, for 80 species out of the total of 156 included in the scheme. Species which occurred across the full range of samples, showing tolerance of trophic status conditions ranging from oligotrophic to eutrophic were designated as "ubiquitous" species (U).

Box 1. Mean Trophic Score (MTS), allocated on the basis of the trophic banding system developed by Vollenweider and Kerekes (1981).

Mean Trophic Score (MTS)	SRP (µg L^{-1})	Trophic Status
1	<7.5	oligotrophic (including <2.5: ultraoligotrophic)
2	7.5 – 12.9	oligo-mesotrophic
3	13.0 - 18.9	mesotrophic
4	19.0 - 25.0	meso-eutrophic
5	>25.0	eutrophic (including > 80.0: hypertrophic)

2.2.2. Indirect derivation of $ZTRS_{sp}$ values via TWINPAN classification of species

For the remaining species, occurring in sets of samples for which no or insufficient SRP data were available to permit direct derivation of $ZTRS_{sp}$ values, an indirect method was utilised to provide an estimation of $ZTRS_{sp}$ values. The starting point was a TWINSPAN ordered samples x species matrix, developed by classification of a dataset of 225 macrophyte taxa x 271 samples collected from Zambian rivers and associated floodplain waterbodies during 2006 – 2012 (TWINSPAN outcomes are reported in detail by Kennedy et al. (2015)). The classification exercise identified 7 end sample-groups. One of these was too small (at *n* = 3 samples) to be utilised in a meaningful way in the ZMTR metric development process, and so was not used. The remaining six groups (labelled A - F) varied in size between *n* = 16 to *n* = 112 samples (see Kennedy et al. (2015) for more details).

The Mean Trophic Score (MTS) appropriate to each sample group was first allocated, using all available SRP data for samples comprising each group, on the basis of the trophic banding system described in Section 2.2.1., above. Values of mean SRP (µg L^{-1}) ± standard error (with ANOVA mean separation test outcomes shown: means sharing a letter in common are not significantly different), and Mean Trophic Scores (MTS_{A-F}) allocated for the six TWINSPAN sample groups on this basis are shown in Box 2 (listed in ascending order of nutrient status). The analysis showed significant differences in mean SRP values between some, but not all, sample-groups (ANOVA: p=0.002; with subsequent mean separation using Tukey post hoc test procedure: see Kennedy et al. (2015) for full details).

Box 2. Values of mean SRP (µg L^{-1}) ± standard error (with ANOVA mean separation test outcomes shown: means sharing a letter in common are not significantly different), and Mean Trophic Scores (MTS_{A-F}) allocated for six TWINSPAN sample groups.

TWINSPAN sample group	Mean SRP (µg L^{-1}) ± standard error	MTS
Group E:	4.0^{c} ± 0.0	1
Group A:	$11.0^{a,b}$ ± 2.0	2
Group F:	13.0^{b} ± 3.0	3
Group B:	$16.0^{a,b}$ ± 3.0	3
Group C:	$19.0^{a,b}$ ± 2.0	4
Group D:	27.0^{a} ± 5.0	5

The % occurrence of each species in every sample making up each of the six sample-groups was calculated, by working through the TWINSPAN output classification table, species by species. For example, for *Potamogeton nodosus*, the outcome was as shown in Box 3.

Box3. Example of calculation of % occurrence (OCC_{A-F}) of species in samples comprising each of six TWINSPAN sample-groups, for *Potamogeton nodosus.*

TWINSPAN sample-group:	E	A	F	B	C	D
Number of samples in group (*n*)	16	23	57	20	112	39
Number of samples containing records of *P. nodosus* in group	1	1	1	0	1	5
% occurrence (OCC_{A-F}) of *P. nodosus* in samples making up group	6.2	4.3	1.8	0	0.9	12.8

The Zambian Trophic Ranking Score for each species ($ZTRS_{sp}$), taking account of relative occurrence of the species in samples with differing mean trophic status, was then calculated as:

$$ZTRS_{sp} = [(MTS_AOCC_A)+(MTS_BOCC_B)+(MTS_COCC_C)+(MTS_DOCC_D)+(MTS_EOCC_E)+ (MTS_FOCC_F)]/\sum(OCC_{A-F})$$

For the example of *P. nodosus* this produced a $ZTRS_{sp}$ value of 3.4, which was rounded to the nearest whole number, giving a mean value of 3, suggesting that the species is most commonly associated with mesotrophic conditions. This approach was used to calculate $ZTRS_{sp}$ for 76 species in the dataset, occurring in >1 sample. A note was made of species whose calculated $ZTRS_{sp}$ value was based on <5 records in the dataset. Species with only a single record in the dataset were excluded from the protocol and were not used in the final calculation of $ZMTR_{sample}$ scores. As for species directly allocated $ZTRS_{sp}$ values, species which occurred across the full range of samples, showing tolerance of trophic status conditions ranging from oligotrophic to eutrophic were designated as "ubiquitous" species (U).

2.2.3. Water chemistry associated with $ZTRS_{sp}$ values

In order to assess whether $ZTRS_{sp}$ values, calculated directly or indirectly for species present in the dataset, were associated with variation in water chemistry parameters other than SRP, an exercise was undertaken to calculate the mean values for nitrate, alkalinity, pH and electrical conductivity measured at samples supporting each of the target species. Differences in means of these variables for samples supporting species in each of four MTS classes (meso-eutrophic and eutrophic sets were combined owing to the relatively small number of species showing $ZTRS_{sp}$ values of 4 or 5) were assessed using one-way ANOVA tests, with Tukey's post hoc test used for mean separation for significant ANOVA outcomes, following assessment of datasets for normality using Ryan-Joiner testing, and transformation to normalise data where necessary.

2.2.4. Calculation of $ZMTR_{sample}$ scores for samples from sites in Zambia and Botswana

The final stage of calculating $ZMTR_{sample}$ scores from $ZTRS_{sp}$ data involved the substitution of $ZTRS_{sp}$ values for 156 species in place of their abundance values, in the species x samples Excel datafile holding the full dataset of taxa identified to species level, for the Zambian survey samples used in this exercise. $ZMTR_{sample}$ was then calculated as the mean of $ZTRS_{sp}$ values for species present in each sample.

A similar exercise was also carried out for the independent test data set of 21 sample-sites from Botswana, to examine how the ZMTR system performed for riverine sites located elsewhere in tropical southern Africa. Out of 49 species fully identified to species level in this dataset, three quarters (75.5%) also occurred in Zambian river systems, with allocated $ZTRS_{sp}$ scores, and this was considered sufficient to run a small test exercise utilising the pilot bioassessment scheme.

3. Results

3.1. $ZTRS_{sp}$ values and interpretation of ZMTR

Table 1 lists the $ZTRS_{sp}$ values allocated to 156 species, and used to calculate $ZMTR_{sample}$ values for samples in the Zambian and Botswanan datasets. Interpretation of the range of $ZMTR_{sample}$ values calculated for the Zambian samples, in relation to SRP gave the results shown in Box 4.

Box. 4. Interpretation of the range of $ZMTR_{sample}$ values in relation to SRP values and TSB.

$ZMTR_{sample}$ range	Approx. range of SRP (µg L^{-1})	Trophic Status Band (TSB)	
<1.5	<7.5	1	oligotrophic
1.5 – 2.4	7.6 – 13.0	2	oligo-mesotrophic
2.5 – 3.4	13.1 – 19.0	3	mesotrophic
3.5 – 4.4	19.1 – 25.0	4	meso-eutrophic
>4.4	>25.0	5	eutrophic

A system like this contains a large element of variability, and hidden noise, and there was only a weak (though significant: R = 0.425; p <0.001***) correlation between individual sample values of snapshot SRP concentration and $ZMTR_{sample}$ data. However the approach is certainly not designed to predict actual SRP concentration, at a given point in space and time, but rather to give an indication of the trophic status of a given system integrated over a longer period of time. To this end it is of interest to examine how well or poorly the method estimates the trophic band of a sample, as measured by its SRP concentration. This exercise was first undertaken for all the Zambian samples for which SRP data were available, by examining the trophic status for each sample as indicated by its $ZMTR_{sample}$ score, and comparing that with the trophic status of the sample suggested by its SRP value, with the outcomes shown in Box 5.

Box 5. Percentage of samples for which calculated $ZMTR_{sample}$ score underestimates, correctly estimates, or overestimates SRP-derived trophic status.

Outcome	Calculated $ZMTR_{sample}$ (% of samples)
Underestimates SRP-derived Trophic Status by >1 Band	13.8
Underestimates SRP- derived Trophic Status by 1 Band	8.2
Correctly estimates SRP- derived Trophic Status Band	39.2
Overestimates SRP-derived Trophic Status by 1 Band	35.7
Overestimates SRP-derived Trophic Status by >1 Band	3.1

The system gave inaccurate results (out by >1 trophic band) in 16.9% of outcomes, was moderately successful (out by 1 trophic band) in 43.9% of outcomes, and gave accurate results in 39.2% of the outcomes, always assuming that snapshot SRP concentrations are themselves a reasonable indication of actual trophic status of a site. The ZMTR system performed least well in

indicating highly enriched conditions (eutrophic or hypertrophic), consistently underestimating such trophic status by one or more trophic bands, but in total 83.1% of samples indicated SRP-derived trophic status accurately, or reasonably so, using this pilot ZMTR scheme.

For the independent test dataset from Botswana the outcome of a similar exercise showed that no sample trophic status was inaccurately predicted by the ZMTR metric (i.e. none out by >1 trophic band), 80.9% were reasonably accurately predicted (out by 1 trophic band), and 19.1% were correctly predicted, assuming that the estimates of SRP made from TP data for these sample-sites provided a reasonable approximation of actual trophic status. In this case the pilot ZMTR scheme indicated SRP-derived trophic status accurately, or reasonably so, for all the sample-sites tested.

3.2. Typology of the ZMTR approach

Table 2 shows the trophic conditions characterising Zambian rivers and associated waterbodies, as indicated by SRP snapshot data, and ZMTR macrophyte-based bioassessment values, calculated for the 15 categories making up a typology of five freshwater ecoregions (BM: Bangweulu-Mweru; UZF: Upper Zambezi Floodplain; MZL: Middle Zambezi-Luangwa; ZH: Zambezian Headwaters; KF: Kafue Flats) x three stream order classes (Abell et al., 2008). The values in Table 2 are:

(i) SRP (PO_4-P: µg L^{-1}): mean, range and category reference value (calculated as the mean of the five lowest values per category);

(ii) $ZMTR_{sample}$ (mean and range);

(iii) EP_P: Enriched Proportion based on SRP concentration. EP_P is defined as the proportion of samples in each typology category showing high enrichment based on SRP concentration, namely samples showing a value for SRP which would place them in a trophic band at least one band higher than the mean trophic band indicated by mean SRP for the typology category. For example, a sample with SRP of 11.0 µg L^{-1} (indicating oligo-mesotrophic conditions), within a typology category showing a mean SRP value of 7.0 µg L^{-1} (oligotrophic) would be tallied as an EP_P sample.

(iv) EP_{ZMTR}: Enriched Proportion based on $ZMTR_{sample}$ score. EP_{ZMTR} is defined as the proportion of samples in each typology category showing high enrichment, based on $ZMTR_{sample}$ score, namely samples showing an increase to at least the next highest trophic status band above the mean trophic status band as indicated by mean $ZMTR_{sample}$ value for the typology category. For example, a sample scoring 2.3 (indicating oligo-mesotrophic conditions), within a typology category showing a mean $ZMTR_{sample}$ score of 1.4 (oligotrophic), would be tallied as an EP_{ZMTR} sample.

Missing or insufficient data left a number of gaps in completing the typology information, but the general picture is reasonably clear for those categories that could be filled in. The average SRP value for the whole dataset was 11.8 µg L^{-1}, corresponding to oligo-mesotrophic status. Most categories within the typology showed on average oligotrophic to meso-eutrophic status, based on snapshot SRP values. BM lagoons and small UZF streams had the lowest mean trophic status at oligotrophic (with a notably small range of SRP values). Only two samples showed extremely high (hypertrophic) values for SRP. These were a small ZH stream site (119.0 µg L^{-1}) and a sample from a large MZL stream (148.0 µg L^{-1}). Both samples also showed high nitrate concentrations (respectively 0.817 and 0.564 mg L^{-1}, compared with the average for the dataset of 0.210 mg L^{-1}), suggesting that local pollution was affecting these sites.

For those categories where sufficient data were available to permit calculation of reference SRP values these were, with one exception (KF large streams: oligo-mesotrophic), always within the oligotrophic band, suggesting that relatively-unimpacted reference sites, measured by P-status, can be found throughout Zambia.

$ZMTR_{sample}$ means and ranges reflect the predominance of low to mid-range nutrient conditions prevailing across the sample sites, with mean values in all cases being in the oligo-mesotrophic to mesotrophic bands. In the UZF ecoregion no sample was found with $ZMTR_{sample}$

scores indicative of anything greater than oligo-mesotrophic conditions, which agrees quite well with the outcome based on SRP ranges. BM sites also had $ZMTR_{sample}$ scores indicating low nutrient conditions, mesotrophic at best in this ecoregion, which also largely concurs with the results based on SRP data, though there were a few higher trophic outcomes (up to eutrophic) which the ZMTR approach failed to predict adequately. In the remaining three ecoregions the ZMTR approach indicated a wider range of trophic status (up to meso-eutrophic for both ZH and KF large streams), but again failed to give adequate indication of samples which the SRP data suggested were eutrophic.

Finally, an exercise was conducted to examine the prevalence of enriched samples from sites in each typology category, using both SRP values and $ZMTR_{sample}$ values. These enriched proportion (EP_P and EP_{ZMTR}) values compare individual sample values against the means for each typology category. The results suggest that evidence of moderate to high enrichment could be detected in most typology categories, using both indicators. Though SRP data were more likely to reveal such sites, the bioassessment procedure found evidence of enrichment in more than half the typology categories for which EP_{ZMTR} data were available.

3.3. Water chemistry associated with $ZTRS_{sp}$ values

Clear and significant water chemistry trends (in terms of nitrate, conductivity and alkalinity, but not pH) were seen for the sets of species occurring in Zambian sample-groups making up each of the four categories of trophic status (oligotrophic, oligo-mesotrophic, mesotrophic and meso-eutrophic/eutrophic). For each of the three variables showing significant outcomes, the trend closely followed the rising trend of SRP status between the groups (Fig. 1). For all three variables, samples supporting species characterised as occurring preferentially in oligotrophic conditions had significantly lower alkalinity, nitrate and conductivity than species typical of higher trophic status sites.

3.4. Temporal change: ZMTR and hindcasting potential

A potentially useful way to examine the value, or otherwise, of the pilot biomonitoring protocol is to look at sites for which repeated samples of macrophytes and SRP were available over time, in order to see if SRP and $ZMTR_{sample}$ values followed similar or different temporal trends at these sites.

3.4.1. Examples of temporal change indicated using the ZMTR scheme

Two contrasting examples are considered here (one of an unenriched, unpolluted stream, and the other of a river which suffers nutrient pollution), for streams from which repeat samples were taken over extended time periods (at least four months), in order to assess the ability of the bioassessment scheme to indicate changes in trophic status over time.

The Coso River at Musamfushi (12.45088°S; 31.29500°E; 1420 m above sea level (a.s.l.) at the sampling point) flows through the Mutinondo Wilderness area of northern Zambia. It is a small (stream order 3), medium-flow, low-nutrient, clear-water stream, which was sampled in the dry and wet seasons of 2010, then again in the dry season of 2011. The water chemistry is that of a typical north Zambian mountain stream, with a mean alkalinity over the three samples of 332.6 µEq L^{-1}, mean pH of 7.11, and low values for nitrate (averaging 0.050 mg L^{-1}) and conductivity (mean 19 µS cm^{-1}). SRP values for the 3 samples taken from this site were: July 2010 2.0 µg L^{-1}, November 2010 5.0 µg L^{-1}, and July 2011 4.0 µg L^{-1}, placing the site consistently in the oligotrophic band (TSB 1). The relevant SRP_{ref} value for the typology category to which this site belongs (MZL1-3) is 4.8 µg L^{-1} (see Table 2), so all samples showed no or only very slight enrichment at the site. The chemical data clearly show the consistently low trophic status of this stream. $ZMTR_{sample}$ scores for the site slightly overestimated its trophic status as oligo-mesotrophic, but also did this consistently over the three samples, with scores of 2.1, 2.1 and 2.0, all placing the stream in TSB 2 over the sampling period.

The second example is the Chongwe River, sampled at the road bridge (15.32306°S; 28.70251°E) to the east of Chongwe town, in July and November 2010. This is a moderate-size (stream order 5), medium-altitude (1048 m a.s.l. at the sampling point), fast-flowing tributary of the Zambezi. It is a hardwater, high pH stream with high conductivity, alkalinity and nitrate (mean values: pH 8.19, alkalinity 3030.1 µEq L^{-1}, conductivity 411.8 µS cm^{-1}, nitrate 0.155 mg L^{-1}). It is polluted by urban waste water from the city of Lusaka, draining to the river via the Ngwerere Stream, upstream of the sampling point (Obrdlik, 1987). In a small impoundment of the river, located between the sampling site and the Ngwerere Stream entry point, Obrdlik (1987) found an elevated chlorophyll$_a$ concentration averaging 11 mg m^{-3}, and a mean standing crop of green filamentous algal periphyton of 28.3 mg m^{-2}, both being characteristic of moderately nutrient-enriched conditions.

SRP values for the two samples taken from this site were 12.0 and 26.0 µg L^{-1}, respectively in July (dry season) and November (wet season) 2010, suggesting conditions in the mesotrophic to low eutrophic bands. The relevant SRP_{ref} value for the typology category to which this site belongs (MZL≥4) is 5.0 µg L^{-1} (see Table 2). A moderate enrichment ratio of 2.4 in July 2010 was found, and a higher ratio of 5.2 in November 2010, when compared to the mean SRP value for the category. However, neither sample fell into the 17.6% of samples in the MZL≥4 category which were classified (Table 2) as showing high enrichment (on EP_P data). In this case $ZMTR_{sample}$ scores for the site showed consistency, with both being 2.4 (at the top end of the oligo-mesotrophic range), but certainly in one case underestimated the enriched trophic status of the river.

3.4.2. Hindcasting using ZMTR

Relatively few sites in the dataset were repeat-sampled on numerous occasions over the sampling period but one example is a site on the Musola Stream in Kasanka National Park (12.5917°S; 30.2519°E; altitude at sampling point 1196 m a.s.l.) for which macrophyte samples were available from 2006 and 2008 (preceding the dataset primarily used for this study), then also in 2009, 2010 and 2012 (Kennedy et al., 2015). SRP values, available only for 2010 and 2012, at 1.0 and 2.0 µgL^{-1} respectively, placed this site firmly in the low oligotrophic band. As in many other cases for such low trophic-status sites, $ZMTR_{sample}$ slightly overestimated nutrient status with values of 1.8 and 1.6 calculated for these two samples, indicating oligo-mesotrophic conditions. However when $ZMTR_{sample}$ values for the preceding years are examined there is no suggestion of any trend, with values of 1.9, 1.7 and 2.0 being calculated respectively for 2006, 2008 and 2009, suggesting little hindcast change in trophic status, always in the oligo-mesotrophic band. This is consistent with the evidence showing little change in other water chemistry parameters which were measured over the seven-year sampling period. Conductivity was always low, in the range 20 - 73 µS cm^{-1}; pH was circumneutral (6.7 - 7.5), alkalinity in the range 323.3 - 747.0 µEq L^{-1}; and nitrate was also low (0.005 - 0.047 mg L^{-1}), all consistent with conditions typical of an unpolluted high-altitude plateau stream in northern Zambia.

4. Discussion

Only three studies are known to us which have, to date, applied macrophyte-based bioassessment protocols to tropical or subtropical freshwater systems. In Kenya, Achieng' et al. (2014) used the Plant Index of Biotic Integrity (PIBI: Rothrock et al., 2008) to assess the ecological health of the small palustrine/ riverine King'wal wetland (almost on the Equator), in the Lake Victoria Basin, concluding that the vegetation metrics used could effectively delineate different levels of anthropogenic disturbances affecting the wetland area. In subtropical Brazil, Pereira et al. (2012) carried out a small study to assess the potential of macrophytes as bioindicators of water quality in shallow lakes located in the State of Rio Grande do Sul (at 32° 04'S). A third, larger, study, also in

subtropical Brazil, is that of Umetsu et al. (2015) who examined the potential of using macrophytes to assess riverine ecological integrity in the Itanhaém River basin (which lies just south of the Tropic of Capricorn, in São Paulo State). Vegetation and environmental data were collected in 2013 from 137 sites within this 950 km^2 river basin, and used to construct a multimetric index of biointegrity, which the authors considered to show good discriminatory efficiency between undisturbed and degraded river sites. None of these studies attempted specifically to predict the trophic status of their target sites.

The ZMTR scheme hence appears to be the first large-scale attempt to develop a macrophyte-based biomonitoring scheme, utilizing reference conditions, to assess the biointegrity of tropical rivers. In its current form the scheme predicts reasonably well the trophic status of the tropical African streams, rivers and floodplain waterbodies, in Zambia and Botswana, where it has been applied to date. Considering that values for $ZTRS_{sp}$ were unavailable for one quarter of the species occurring at the Botswana test sites (because these species were not found in Zambia, or had insufficient data to permit calculation of the metric) this seems an encouraging result.

Due to the current relatively good ecological condition of many of Zambia's water bodies (Kennedy et al., 2015), the timing of the SAFRASS project ensured the development of bioassessment protocols that are proactive, establishing with confidence baseline reference conditions (e.g., Dallas, 2002) for river water chemistry variables of primary interest (e.g., nutrient status). This contrasts with biomonitoring protocols in more economically-developed temperate countries that were generally developed as a reaction to long-standing, widespread water quality impacts, which made establishment of reference conditions for many river types difficult, or a matter of conjecture (e.g., Ferreira et al., 2002; Pardo et al, 2012).

Given that most of the survey samples used in the development of the pilot scheme were from mid-range trophic status sites, it is unsurprising that $ZMTR_{sample}$ scores calculated in this study were predominantly in the oligotrophic to mesotrophic bands. It is clear that at this stage of its development ZMTR tends to underestimate the status of highly-enriched streams and waterbodies, and, to a lesser extent, overestimate the status of very low-nutrient sites. In order to rectify this situation future additional sampling effort should prioritise low-nutrient and high-nutrient sites. Given the lack of evidence for asymptoting of the cumulative species records from the survey sites (Kennedy et al., 2015) there is a strong likelihood that a survey effort along these lines will find additional species, so far unrecorded, which may show tolerance of low- or high-nutrient status, and which would hence help fill out the gaps at each end of the $ZMTR_{sp}$ range. In addition, future inclusion in the protocol of lower taxonomic groups of macrophytes may well prove useful in this context. Bryophytes and charophytes mainly (though not entirely) tend to be indicators of oligotrophic conditions (e.g. Lang and Murphy, 2011), while several orders of filamentous algae, such as Cladophorales and Zygnematales, have long been known as strong indicators of eutrophic or hypertrophic conditions in rivers (e.g., Whitton, 1970; Obrdlik, 1987; Dokulil, 2003).

The phosphate data available for this study usually comprised single, snapshot, measurements of SRP for each sample. These are clearly unlikely to represent the actual mean value of SRP for each site, over longer periods of time (although repeat samples were taken over time from some sites, and examples of these are discussed further in Section 3.4 above). This problem was compounded by the need to arbitrarily convert TP to SRP values for some samples, which undoubtedly adds to the noise within the phosphate datasets. However the two datasets used in Zambia and Botswana, were, in 2015, the only extensive phosphate data in existence for the river systems targeted in this study, and so represented the only possible set of values that could be used for this exercise. It is known that Zambian river systems can exhibit strong inter- and intra-annual variability in terms of hydroclimatically-driven discharge characteristics (e.g. Kennedy et al. 2012b); hence any future monitoring would ideally include regular chemical sampling over one or more hydrological years to give a clearer understanding of temporal variation in patterns and mean values of SRP and other water chemistry parameters.

A further issue is that of ubiquitous species (species which occur across all trophic categories from oligotrophic to eutrophic). These were quite prevalent within the bioassessment scheme (34 species out of the total of 156: see Table 1). Whilst these species were still allocated a calculated $ZTRS_{sp}$ value, clearly this will usually (but not always: see, for example, *Ottelia ulvifolia* and *Bolbitis heudelotii*: Table 1) represent only a score close to the middle of the range of trophic conditions in which these ubiquitous species occurred. Hence these species are likely to be relatively weak indicators of actual trophic status. However because these plants tended to be common species, occurring at numerous sites, they were retained in the calculation procedure for $ZMTR_{sample}$: with the rider that caution should be exercised if a calculated ZMTR value is based wholly or mainly upon the presence of ubiquitous species, since in this situation the uncertainty of the outcome prediction of nutrient status will be increased (Demars and Edwards, 2009).

Species identification issues also need to be addressed in further developing the bioassessment scheme. In Table 1, species are noted for which correct identification is doubtful because these plants are not listed in Flora of Zambia, or Flora of Zimbabwe, nor given as recorded for either country in Flora Zambesiaca (however several families of aquatic macrophytes have yet to be covered by Flora Zambesiaca), although they are included in Cook (2004) as macrophyte species recorded from southern Africa. Problems of species identification in this study were highlighted in Section 2, above, and the species marked as doubtful in Table 1 are those for which we have least confidence in correct identification. Some of these are probably indeed misidentifications, but herbarium material was either not collected, or is inadequate to prove the case either way. An example is the tiny duckweed species *Wolffia arrhiza*, which is possibly a misidentification of *Wolffia globosa* (recorded by Flora of Zimbabwe).

Nevertheless, given the severe lack of previous survey data for Zambian river plants the possibility remains that some of these are actually new records for Zambia. To take one example, *Hydrocotyle bonariensis* is not recorded by either the Flora of Zambia or Flora of Zimbabwe, but is listed by Flora Zambesiaca from coastal regions of Mozambique. The plant can and does occur inland in Africa, with the GBIF database (http://www.gbif.org/species), for example showing a record from an inland site near Kruger National Park in the northern part of South Africa, and another inland location in Namibia. In South America it commonly occurs well inland (in addition to coastal areas), with the GBIF database (see above) holding multiple records in, for example, the catchment of the Rio Paraná in Brazil and Argentina. Its occurrence in inland Zambian river systems cannot therefore be ruled out as completely improbable.

In order to further improve and apply the ZMTR scheme there is clearly a need for additional SRP data to be collected from sites supporting species with $ZTRS_{sp}$ scores at present calculated only by the indirect approach, so that provisional scores can be checked and modified as necessary. It would also be of value to find and record appropriate data from sites supporting those species which were found, in our survey, only at a single site in Zambian rivers, in order to add them to the existing scheme; and possibly also in future (as discussed above) to include lower plants (filamentous algae, charophytes and bryophytes) in the protocol, though the evidence to data suggests that these plants are not common in Zambian rivers (Kennedy et al., 2015).

Finally, the test application of the ZMTR scheme to river sites in Botswana provides an initial indication of its potential for use in southern tropical African rivers outwith Zambia. Clearly a large sampling effort will be necessary in neighbouring countries to amass the requisite information needed to add their river systems into the scheme, including data for additional species (such as *Typha capensis*, found in the Okavango sites from Botswana, but not present in the Zambian dataset) required to permit their inclusion in the macrophyte protocols of the SAFRASS scheme.

5. Conclusions

The results of this study suggest that evidence of moderate to high enrichment could be detected in most of the Zambian typology categories, using both biological and chemical indicators. Though SRP data were more likely to reveal such sites, the bioassessment procedure found evidence

of enrichment in about half the typology categories for which EP_{ZMTR} data were available, suggesting that bioassessment can add both value and subtlety to river quality monitoring systems.

The jury must remain out on whether this new pilot macrophyte-based methodology is likely to become a useful biomonitoring approach for assessing the biointegrity, and specifically, trophic status, of southern tropical African rivers. There are encouraging indications that the scheme might be further developed into a fully-working system, with potential for application across a broader spectrum of rivers in southern tropical Africa, but at this point the protocol remains at development stage, in need of substantial further work. This is not unexpected given the relatively small research effort that has so far gone into ZMTR development, within the SAFRASS protocols, compared with the orders-of-magnitude greater research effort devoted to development of macrophyte-based river bioassessment procedures in Europe. Nevertheless we feel that the pilot ZMTR scheme, as currently developed for Zambian river bioassessment, has at least made a useful start in this context.

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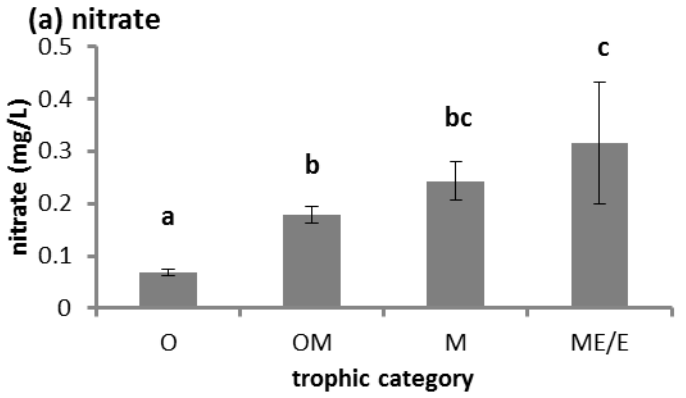
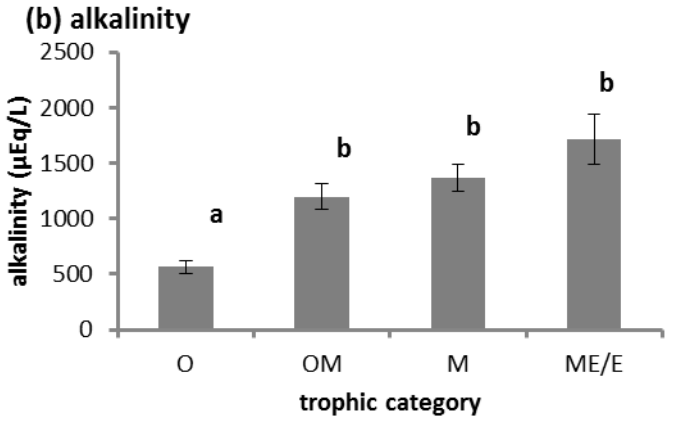
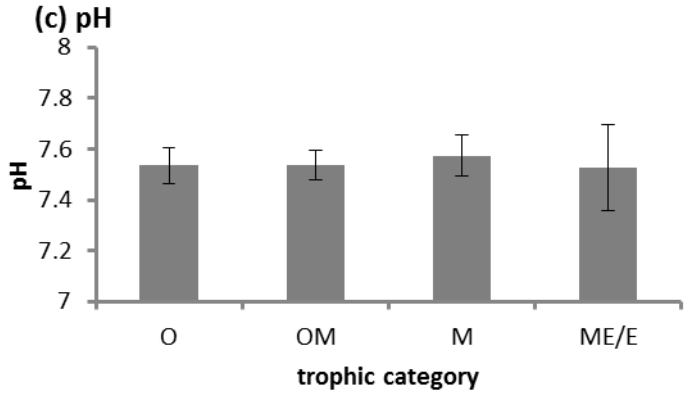

(a) nitrate
nitrate (mg/L)
0.5
0.4
0.3
0.2
0.1
0
a
b
bc
c
O
OM
M
ME/E
trophic category


(b) alkalinity
alkalinity (µEq/L)
2500
2000
1500
1000
500
0
a
b
b
b
O
OM
M
ME/E
trophic category


(c) pH
pH
8
7.8
7.6
7.4
7.2
7
O
OM
M
ME/E
trophic category

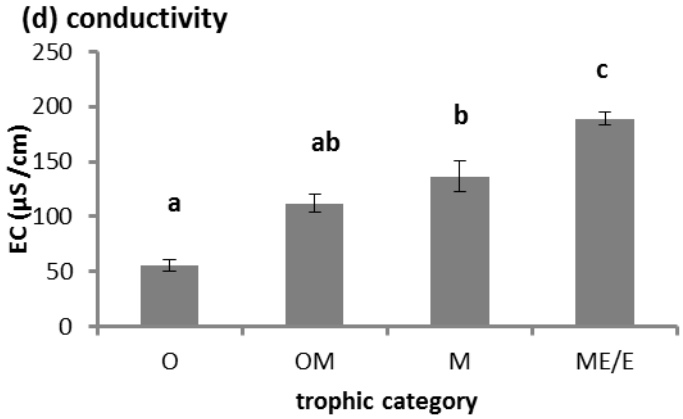

(d) conductivity
EC (µS/cm)
250
200
150
100
50
0
a
ab
b
c
O
OM
M
ME/E
trophic category

Fig. 1. Means and standard errors for four water chemistry variables (a) nitrate (NO_3-N); (b) alkalinity (c) pH; (d) electrical conductivity (EC)) recorded at Zambian riverine sample-sites supporting macrophyte species with differing trophic preferences. O: oligotrophic; OM: oligo-mesotrophic; M: mesotrophic; ME: meso-eutrophic; E: eutrophic. ANOVA (undertaken on square-root transformed data for nitrate, conductivity and alkalinity, which were non-normal according to preliminary Ryan-Joiner testing) showed no significance ($p>0.05$) for pH, and outcomes significant at $p<0.001$ for nitrate, conductivity and alkalinity. For these three variables means sharing a letter in common are not significantly different from each other ($p>0.05$: Tukey's post-hoc test).

Table 1. $ZTRS_{sp}$ scores for macrophyte species occurring in >1 sample within 2009 – 2012 Zambia rivers dataset. $ZTRS_{sp}$ score: 1: associated with very low nutrient conditions (oligotrophic); 2: associated with low nutrient conditions (oligo-mesotrophic); 3: associated with intermediate nutrient conditions (mesotrophic); 4: associated with moderately high nutrient conditions (meso-eutrophic); 5: associated with high nutrient conditions (eutrophic). Scores given in brackets are provisional values derived from TWINSPAN community-based procedure described in text (Section 2.2.2).

Notes: (i) * = score based on <5 species records; U = ubiquitous species, occurring across trophic categories from oligotrophic to eutrophic: assessments based wholly or largely on the presence of these species are likely to show reduced confidence outcomes: see text (Sections 2.2.2 and 4) for more detail

(ii) species listed † are doubtful identifications: see text for detail

(iii) life form: S = submerged, F = floating-leaved (free-floating or rooted floating-leaved), E = emergent (following Chambers et al., 2008)

(iv). authorities and synonyms (where appropriate) for species names follow The Plant List (www.theplantlist.org), except for some infraspecies not listed by this source.

	Genus	Species	Authority	Synonym	Life form	Species code	$ZTRS_{sp}$
1	*Aeolanthus*	cf. *abyssinicus*	Hochst.		E	Ael aby	(3*)
2	*Aeschynomene*	*fluitans*	Peter		E	Aes flu	3 U
3	*Alternanthera*	*sessilis*	(L.) R.Br. ex DC.		E	Alt ses	2
4	*Aponogeton*	*desertorum*	Zeyh. ex Spreng.		F	Apo des	1
5	*Aponogeton*	*rehmannii*	Oliv.	*Aponogeton junceus* Lehm. ex Schltdl. subsp. *rehmannii* (Oliv.) Oberm.	F	Apo reh	(3*)
6	*Azolla*	*filiculoides*	Lam.		F	Azo fil	3
7	*Azolla*	*nilotica*	Mett.		F	Azo nil	(5*)
8	*Azolla*	*pinnata*	R. Br.		F	Azo pin	(3*)
9	*Bacopa*	*floribunda*	(R.Br.) Wettst.		S	Bac flo	(2 U)
10	*Bacopa*	*monnieri*†	(L.) Wettst.		S	Bac mon	(3*)
11	*Bolbitis*	*heudelotii*	(Bory ex Fée) Alston		S	Bol heu	1 U
12	*Bolboschoenus*	*glaucus*	(Lam.) S.G. Sm.		E	Bol gla	(4*)
13	*Caldesia*	*reniformis*	(D.Don) Makino		E	Cal ren	(4*)
14	*Ceratophyllum*	*demersum*	L.		S	Cer dem	3
15	*Ceratophyllum*	cf. *muricatum*†	Cham.	*Ceratophyllum submersum* subsp. *muricatum* (Cham.) Wilmot-Dear	S	Cer mur	(3*)
16	*Cladium*	*mariscus*	(L.) Pohl		E	Cla mar	(2*)
17	*Commelina*	*diffusa*	Burm.f.		E	Com dif	2
18	*Commelina*	*fluviatilis*	Brenan		E	Com flu	(3*)
19	*Crinum*	*macowanii*	Baker		E	Cri mac	2 U
20	*Cyperus*	*alopecuroides*	Rottb.		E	Cyp alo	2
21	*Cyperus*	*articulatus*	L.		E	Cyp art	2
22	*Cyperus*	*difformis*	L.		E	Cyp dif	2 U
23	*Cyperus*	*digitatus*	Roxb.		E	Cyp dig	2
24	*Cyperus*	*involucratus*	Rottb.		E	Cyp inv	4
25	*Cyperus*	*longus*	L.	*Cyperus rotundus* L.	E	Cyp lon	2
26	*Cyperus*	*papyrus*	L.		E	Cyp pap	1
27	*Cyperus*	*pectinatus*	Vahl		E	Cyp pec	2 U

28	*Cyperus*	*procerus*	Rottb.		E	Cyp pro	2
29	*Echinochloa*	cf. *crus-galli*†	(L.) P. Beauv.		E	Ech cru	(2*)
30	*Echinochloa*	*jubata*	Stapf		E	Ech jub	2 U
31	*Echinochloa*	*stagnina*	(Retz.) P. Beauv.		E	Ech sta	1
32	*Eichhornia*	*crassipes*	(Mart.) Solms		F	Eic cra	3
33	*Eichhornia*	*natans*	(P.Beauv.) Solms	*Eichhornia diversifolia* (Vahl) Urb.	F	Eic nat	2 U
34	*Elatine*	*triandra*	Schkuhr		S	Ela tri	(4*)
35	*Eleocharis*	cf. *acutangula*	(Roxb.) Schult.		E	Ele acu	(3*)
36	*Eleocharis*	*atropurpurea*	(Retz.) J. Presl & C.Presl		E	Ele atr	(3*)
37	*Eleocharis*	*dulcis*	(Burm.f.) Trin. ex Hensch.		E	Ele dul	1
38	*Eleocharis*	cf. *naumanniana*†	Boeckeler		E	Ele nau	(3*)
39	*Eleocharis*	cf. *mutata*†	(L.) Roem. & Schult.		E	Ele mut	(3*)
40	*Equisetum*	*ramosissimum*	Desf.		E	Equ ram	(3*)
41	*Eriocaulon*	*abyssinicum*	Hochst.	*Eriocaulon subulatum* N.E.Br.	S	Eri aby	(3*)
42	*Eriocaulon*	*africanum*	Hochst.		S	Eri afr	(3*)
43	*Eriocaulon*	cf. *dregei*†	Hochst.		S	Eri dre	1
44	*Eriocaulon*	*teusczii*	Engl. & Ruhland		S	Eri teu	1
45	*Floscopa*	*glomerata*	(Willd. ex Schult. & Schult.f.) Hassk.		E	Flo glo	1
46	*Fuirena*	*umbellata*	Rottb.		E	Fui umb	1 U
47	*Grammatotheca*	*bergiana*†	(Cham.) C. Presl		E	Gra ber	(4*)
48	*Hydrocotyle*	cf. *bonariensis*†	Comm. ex Lam.		E	Hyd bon	2 U
49	*Hydrocotyle*	*ranunculoides*	L.f.		S	Hyd ran	(3*)
50	*Hydrocotyle*	*sibthorpioides*	Lam.		E	Hyd sib	(3*)
51	*Hydrostachys*	*polymorpha*	Klotzsch		S	Hyd pol	1
52	*Hygrophila*	*linearis*	Burkill		E	Hyg lin	(3*)
53	*Hygrophila*	*prunelloides*	Heine		E	Hyg pru	2.9
54	*Hygrophila*	*schulli*	M.R. Almeida & S.M. Almeida	*Hygrophila auriculata* (Schumach.) Heine	E	Hyg sch	(2*)
55	*Impatiens*	*hydrogetonoides*	Launert		E	Imp hyd	(3*)
56	*Ipomoea*	*aquatica*	Forssk.		E	Ipo aqu	1

57	*Ipomoea*	*fistulosa*	Mart. ex Choisy	*Ipomoea carnea* subsp. *fistulosa* (Martius ex Choisy) D. F. Austin	E	Ipo fis	(3*)
58	*Isolepis*	cf. *prolifera*	(Rottb.) R.Br.		E	Iso pro	(3*)
59	*Juncus*	*effusus*	L.		E	Jun eff	2 U
60	*Juncus*	*oxycarpus*	E.Mey. ex Kunth		E	Jun oxy	(4*)
61	*Lagarosiphon*	*ilicifolius*	Oberm.		S	Lag ili	2
62	*Laurembergia*	*repens*	(L.) P.J. Bergius		E	Lau rep	(4*)
63	*Ledermanniella*	*tenax*	(C.H. Wright) C. Cusset		S	Led ten	3
64	*Leersia*	*hexandra*	Sw.		E	Lee hex	2
65	*Limnobium*	*laevigatum*	(Humb. & Bonpl. ex Willd.) Heine		F	Lim lae	(3*)
66	*Limnophila*	*bangweolensis*	(R.E. Fr.) Verdc.		E	Lim ban	1 U
67	*Limnophila*	*ceratophylloides*	(Hiern) Skan		E	Lim cer	2 U
68	*Limnophyton*	*angolense*	Buchenau		E	Lim ang	(2 U)
69	*Limosella*	*australis*	R.Br.		S	Lim aus	(3*)
70	*Linzia*	*glabra*	Steetz	*Vernonia glabra* (Steetz) Vatke	E	Lin gla	(3*)
71	*Lobelia*	*erinus*	L.		S	Lob eri	(4*)
72	*Ludwigia*	*abyssinica*	A. Rich.		E	Lud aby	5 U
73	*Ludwigia*	*adscendens*	(L.) H. Hara		E	Lud ads	2
74	*Ludwigia*	*erecta*	(L.) H. Hara		E	Lud ere	3 U
75	*Ludwigia*	*octovalvis*	(Jacq.) P.H. Raven		E	Lud oct	(4*)
76	*Ludwigia*	*palustris*	(L.) Elliott		E	Lud pal	2 U
77	*Ludwigia*	*senegalensis*	(DC.) Troch.		E	Lud sen	2
78	*Lythrum*	*hyssopifolia*†	L.		E	Lyt hys	(3*)
79	*Mentha*	*aquatica*	L.		E	Men aqu	(3*)
80	*Mimulus*	cf. *gracilis*	R.Br.		E	Mim gra	1
81	*Myriophyllum*	*spicatum*	L.		S	Myr spi	(3*)
82	*Najas*	*horrida*	A. Braun ex Magnus		S	Naj hor	2
83	*Nymphaea*	*divaricata*	Hutch.		F	Nym div	(2*)
84	*Nymphaea*	*lotus*	L.		F	Nym lot	2 U
85	*Nymphaea*	*nouchali* var. *caerulea*	(Savigny) Verdc.		F	Nym noc	2 U

86	*Nymphoides*	*indica* subsp. *occidentalis*	(L.) Kuntze, A. Raynal		F	Nym ind	1
87	*Oryza*	*barthii*	A. Chev.		E	Ory bar	(2*)
88	*Osmunda*	*regalis*	L.		E	Osm reg	1
89	*Ottelia*	*cylindrica*	(T.C.E.Fr.) Dandy		S	Ott cyl	(1*)
90	*Ottelia*	*exserta*	(Ridl.) Dandy		S	Ott exs	(1 U)
91	*Ottelia*	*fischeri*	(Gürke) Dandy		S	Ott fis	(3*)
92	*Ottelia*	*muricata*	(C.H. Wright) Dandy		S	Ott mur	(4*)
93	*Ottelia*	*ulvifolia*	(Planch.) Walp.		S	Ott ulv	1 U
94	*Ottelia*	*verdickii*	Gürke ex De Wild.		S	Ott ver	1
95	*Oxycaryum*	*cubense*	(Poepp. & Kunth) Palla		E	Oxy cub	(3*)
96	*Panicum*	*parvifolium*	Lam.		E	Pan par	2
97	*Panicum*	*repens*	L.		E	Pan rep	2
98	*Panicum*	*subalbidum*	Kunth		E	Pan sub	2
99	*Paspalum*	*distichum*	L.		E	Pas dis	2
100	*Paspalum*	*scrobiculatum*	L.		E	Pas scr	(4*)
101	*Pennisetum*	*glaucocladum*	Stapf & C.E. Hubb. ex Stent & J.M. Rattray	*Pennisetum macrourum* Trin.	E	Pen gla	4 U
102	*Pennisetum*	cf. *natalense*†	Stapf	*Pennisetum macrourum* Trin.	E	Pen nat	(3*)
103	*Persicaria*	cf. *amphibia*†	(L.) Delarbre		F	Per amp	1
104	*Persicaria*	*attenuata*	(R. Br.) Soják		E	Per att	3
105	*Persicaria*	*decipiens*	(R.Br.) K.L. Wilson		E	Per dec	2
106	*Persicaria*	*glomerata*	(Dammer) S. Ortiz & Paiva		E	Per glo	(3*)
107	*Persicaria*	cf. *hydropiper*†	(L.) Delarbre		E	Per hyd	2 U
108	*Persicaria*	*lapathifolia*	(L.) Delarbre		E	Per lap	1
109	*Persicaria*	*limbata*	(Meisn.) H. Hara		E	Per lim	(3*)
110	*Persicaria*	cf. *meisneriana*†	(Cham. & Schltdl.) M. Gómez		E	Per mei	2 U
111	*Persicaria*	*nogueirae*	S. Ortiz & Paiva		E	Per nog	(2*)

112	*Persicaria*	*senegalensis*	(Meisn.) Soják		E	Per sen	3 U
113	*Phragmites*	*mauritianus*	Kunth.		E	Phr mau	2
114	*Pistia*	*stratiotes*	L.		F	Pis str	(3*)
115	*Potamogeton*	*crispus*	L.		S	Pot cri	(4*)
116	*Potamogeton*	*nodosus*	Poir.		S	Pot nod	(3 U)
117	*Potamogeton*	*octandrus*	Poir.		S	Pot oct	2 U
118	*Potamogeton*	*pusillus*	L.		S	Pot pus	2
119	*Potamogeton*	*richardii*	Solms		S	Pot ric	(4*)
120	*Potamogeton*	*schweinfurthii*	A. Benn.		S	Pot sch	2
121	*Potamogeton*	cf. *trichoides*	Cham. & Schltdl.		S	Pot tri	(3*)
122	*Pycreus*	*mundii*	Nees		E	Pyc mun	2
123	*Pycreus*	*unioloides*	(R.Br.) Urb.		E	Pyc uni	3 U
124	*Ranunculus*	*multifidus*	Forssk.		E	Ran mul	(4*)
125	*Rhynchospora*	*corymbosa*	(L.) Britton		E	Rhy cor	5 U
126	*Rotala*	*fluitans*	Pohnert		S	Rot flu	1
127	*Rotala*	*myriophylloides*	Welw. ex Hiern		S	Rot myr	(4*)
128	*Sacciolepis*	*indica*	(L.) Chase		E	Sac ind	(2*)
129	*Salvinia*	*molesta*	D.S. Mitch.	*Salvinia adnata* Desv.	F	Sal mol	3 U
130	*Schoenoplectus*	*confusus*	(N.E.Br.) Lye		E	Sch con	(3*)
131	*Schoenoplectus*	*corymbosus*	(Roth ex Roem. & Schult.) J. Raynal	*Schoenoplectus brachyceras* (Hochst. ex A.Rich.) Lye	*E*	Sch cor	2
132	*Schoenoplectus*	cf. *decipiens*†	(Nees) J. Raynal		E	Sch dec	(4*)
133	*Schoenoplectus*	cf. *triqueter*†	(L.) Palla		E	Sch tri	(3)
134	*Scleria*	cf. *greigiifolia*	(Ridl.) C.B. Clarke		E	Scl gre	2
135	*Sium*	*repandum*	Welw. ex Hiern	*Berula repanda* (Welw. ex Hiern) Spalik & S.R.Downie	E	Siu rep	(3*)
136	*Sphaerothylax*	*algiformis*	Bisch. ex C. Krauss		S	Sph alg	2 U
137	*Spirodela*	*polyrrhiza*	(L.) Schleid.		F	Spi pol	(4)
138	*Stuckenia*	*pectinata*	(L.) Börner		S	Stu pec	3 U
139	*Thelypteris*	*confluens*	(Thunb.) C.V. Morton		E	The con	2
140	*Thelypteris*	*interrupta*	(Willd.) K. Iwats.	*Cyclosorus interruptus* (Willd.) H. Itô	E	The int	1
141	*Torenia*	*thouarsii*	(Cham. & Schltdl.)		E	Tor tho	1

			Kuntze				
142	*Trapa*	*natans*	L.		F	Tra nat	4 U
143	*Tristicha*	*trifaria*	(Bory ex Willd.) Spreng.		S	Tri tri	2
144	*Typha*	*domingensis*	Pers.		E	Typ dom	2
145	*Utricularia*	*foliosa*	L.		S	Utr fol	1
146	*Utricularia*	*gibba*	L.		S	Utr gib	(2*)
147	*Utricularia*	*inflexa*	Forssk.		S	Utr inf	(4*)
148	*Utricularia*	*stellaris*	L.f.		S	Utr ste	(4)
149	*Vallisneria*	*spiralis*	L.		S	Val spi	3 U
150	*Vossia*	*cuspidata*	(Roxb.) Griff.		E	Vos cus	(3)
151	*Websteria*	*confervoides*	(Poir.) S.S. Hooper	*Eleocharis confervoides* (Poir.) Steud.	E	Web con	(4*)
152	*Wiesneria*	*schweinfurthii*	Hook.f.		E	Wie sch	(2*)
153	*Wolffia*	cf. *arrhiza*†	(L.) Horkel ex Wimm.		F	Wol arr	(3*)
154	*Xyris*	cf. *anceps*†	Lam.		E	Xyr anc	(4*)
155	*Xyris*	*gerrardii*	N.E.Br.		E	Xyr ger	(3*)
156	*Xyris*	*rehmannii*	L.A. Nilsson		E	Xyr reh	(3*)

Table 2. Typology showing characterisation of trophic conditions indicated by soluble reactive phosphate (SRP) snapshot data, and ZMTR macrophyte-based bioassessment of samples, for which both SRP and ZMTR values were available, from streams and associated floodplain waterbodies in five ecoregions and three stream-order categories in Zambia. Freshwater ecoregion (after Abell et al., 2008): BM: Bangweulu-Mweru; UZF: Upper Zambezi Floodplain; MZL: Middle Zambezi-Luangwa; ZH: Zambezian Headwaters; KF: Kafue Flats. Stream order: 0 = static floodplain waterbodies; 1 – 3 = small streams; ≥ 4 = larger streams. Reference values for SRP (SRP_{ref} : µg L^{-1}) are the mean of 5 lowest values for SRP in each typology category; i.d. = insufficient data, <5 samples in category. Trophic band categories: O: oligotrophic; OM: oligo-mesotrophic; M: mesotrophic; ME: meso-eutrophic; E: eutrophic. $EP_{P:}$ proportion of samples in each typology category showing high enrichment based on SRP concentration, namely samples showing a value for SRP which would place them in a trophic band at least one band higher than the mean trophic band indicated by mean SRP for the typology category. EP_{ZTMR}: proportion of samples in each typology category showing high enrichment based on $ZMTR_{sample}$ score, namely samples showing an increase to at least the next highest trophic status band above the mean trophic status band as indicated by mean $ZMTR_{sample}$ value for the typology category.

Freshwater Ecoregion	Stream order	No. of samples (n)	SRP mean ($\mu g\ L^{-1}$) (trophic band category)	SRP range ($\mu g\ L^{-1}$)	SRP_{ref} ($\mu g\ L^{-1}$)	$ZMTR_{sample}$ mean (trophic band category)	$ZMTR_{sample}$ range	EP_P samples (%)	EP_{ZMTR} samples (%)
BM	0	23	4.5 (O)	1.0- 9.4	2.0	1.9 (OM)	1.3 - 2.4	4.3	0
	1 - 3	17	17.0 (M)	1.0 - 66.7	4.8	2.1 (OM)	1.6 - 2.6	29.4	5.9
	≥ 4	46	7.2 (O)	1.0 - 39.3	1.8	1.9 (OM)	1.4 - 2.6	27.5	2.2
UZF	0	0	-	-	-	-	-		
	1 - 3	3	5.0 (O)	2.0 - 10.0	i.d.	2.0 (OM)	2.0 - 2.1	33.3	0
	≥ 4	10	6.4 (O)	1.0 - 16.0	2.4	2.0 (OM)	1.3 --2.3	40.0	0
MZL	0	0	-	-	-	-	-		
	1 - 3	13	11.0 (OM)	2.0 - 29.0	4.8	2.3 (OM)	1.7 – 3.4	38.5	38.5
	≥ 4	34	19.7 (ME)	2.0 - 148.0	5.0	2.3 (OM)	1.5 - 3.1	17.6	23.5
ZH	0	0	-	-	-	-	-		
	1 - 3	3	50.0 (E)	8.0 – 119.0	i.d.	3.0 (M)	2.5 - 3.4	33.3	0
	≥ 4	29	13.4 (M)	2.0 - 44.0	5.0	2.4 (OM)	1.5 - 4.0	17.2	51.7
KF	0	0	-	-	-	-	-		
	1 - 3	2	21.0 (ME)	10.0 - 32.0	i.d.	2.2 (OM)	2.1 - 2.3	50.0	0
	≥ 4	16	21.2 (ME)	7.0 - 50.0	8.8	2.4 (OM)	1.7 - 4.0	18.7	25.0