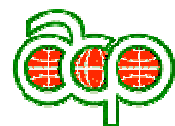


SAFRASS: SOUTHERN AFRICAN RIVER ASSESSMENT SCHEME

**WP4: Review of existing biomonitoring methodologies
and appropriateness for adaptation to river quality
assessment protocols for use in southern tropical Africa**

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Table of Contents

1	Introduction.....	1
1.1	Introduction to biomonitoring	1
1.1.1	The importance of bioassessment and biomonitoring	1
1.1.2	Site selection	2
1.1.3	Monitoring timing and frequency.....	3
2	Existing biomonitoring protocols.....	4
2.1	Diatoms.....	4
2.1.1	Biotic indices based on diatoms	6
2.2	Macroinvertebrates	9
2.2.1	Biotic indices based on aquatic macroinvertebrates.....	10
2.2.2	Using biotic indices and interpreting bioassessment data	11
2.3	Macrophytes	15
2.3.1	Macrophyte monitoring and classification schemes	18
2.3.2	Development of reference based macrophyte monitoring methodologies.....	18
2.4	Habitat integrity.....	20
3	Conclusions and recommendations	21
3.1	Diatoms: recommendations for sampling within the SAFRASS project.....	21
3.1.1	Substrate selection for diatom-based water quality monitoring	21
	Preferred substratum	21
	Alternative substrata (in order of preference)	21
3.1.2	Sampling protocol for diatom-based water quality monitoring	21
	Solid substrata	21
	Sampling from emergent aquatic macrophytes	22
	Sampling from submerged aquatic macrophytes	22
3.1.3	Preservation of diatom material and labelling samples	22
3.2	Macroinvertebrates: recommendations for sampling within the SAFRASS project	22
3.2.1	Sampling protocol for macroinvertebrate-based water quality monitoring	23
	Stones	23
	Vegetation.....	23
	Gravel, sand and mud	24
	Hand picking and visual observation	24
	Sample processing	24
3.2.2	Interpretation of macroinvertebrate data.....	24
3.3	Macrophytes: recommendations for sampling within the SAFRASS project	24
3.4	Habitat integrity: recommendations for sampling within the SAFRASS project.....	26
4	References	26

1 INTRODUCTION

Effective river biomonitoring procedures have been successfully developed, and are currently being implemented in all countries of the European Union, as well as, for example, in the United States, Australia and Canada. Partially-completed river biomonitoring schemes (e.g. using riparian vegetation, macroinvertebrates and diatoms: Dickens & Graham 2002, DWAF 2008) are currently under development or implementation, in South Africa, Namibia (Taylor & Palmer 2002) and Botswana (Dallas 2009), and have been tested in Swaziland and Zimbabwe on a smaller scale. Successful development, and implementation, of an inexpensive but effective biomonitoring scheme to assess river health is crucial to improving human and environmental welfare. The maintenance of good quality, clean rivers, supporting high-quality biodiversity, is universally recognised as a vital element of societal wellbeing.

The Southern African River Assessment Scheme (SAFRASS) project aims to establish a capacity-building research framework to promote river health and biodiversity in tropical southern Africa. This document reviews information on existing biomonitoring methodologies and evaluates its appropriateness for adaptation to river quality assessment protocols for use in southern tropical Africa. It compares river biomonitoring procedures worldwide, their effectiveness, and their suitability (or otherwise) for modification for use in southern tropical Africa. The activities undertaken involved literature and on-line searches, reviews and synthesis of findings. Discussions with organizations in Europe and Africa involved in river water quality assessment were undertaken where feasible.

The biotic components included in the SAFRASS project are diatoms, macroinvertebrates and macrophytes. An additional component that influences general characterisation of a site is that of habitat integrity, both instream and riparian. Existing river health monitoring schemes that focus on these components are discussed in the following sections.

1.1 Introduction to biomonitoring

Biological monitoring (biomonitoring) is the use of biological responses to assess changes in the environment. Biomonitoring is based on the concepts of biological integrity and use is made of biological indicators and indices (e.g. diatoms, macroinvertebrates, fish, riparian vegetation), as well as indices for assessing instream and riparian habitats. Monitoring programmes may be at screening-level, which operates on a low sampling frequency and low resolution of sites selected to ensure that adequate coverage is given to all types of rivers in the area. Monitoring may also be more focused and aimed at site-specific impacts or conditions. The design of a monitoring programme refers to “what (indicators) needs to be monitored where (site selection), how (monitoring protocols and procedures) and when (frequency)” in order to meet the objectives of the programme (DWAF 2008).

1.1.1 *The importance of bioassessment and biomonitoring*

Bioassessment is the process of determining if human activity has altered the biological properties of an ecosystem. It is acclaimed to be a more sensitive and reliable measure of

environmental conditions than either physical or chemical measurements. Bioassessment provides a time- and constituent- integrated assessment of the ecological or biological integrity of the system under consideration. **Biomonitoring** is defined as the systematic use of biological responses to evaluate changes in the environment with the intent to use this information in a quality-control programme. One or several components of the biota may be used in bioassessment, including diatoms, macroinvertebrates, fish and vegetation (e.g. macrophytes).

Traditionally, physico-chemical monitoring formed the backbone of most water quality monitoring programmes. Limitations identified with this type of monitoring include:

- the assessment is limited to the period of sample collection, therefore pulsed releases of effluents may be missed,
- the assessment is limited to the physical and chemical analyses performed and, since the number of constituents that could be present is vast, and routine analyses are usually limited, potentially toxic compounds may be missed,
- the sensitivity of chemical analytical methods when measuring very low concentrations of pollutants may be inadequate, particularly for substances that are characteristically present in these low concentrations but which are persistent and tend to accumulate in the environment,
- the cost of a full spectrum of chemical analyses is high, and
- synergistic (magnifying) and antagonistic (reducing) effects are difficult to establish, e.g. pH significantly alters the toxicity of trace metals.

Biota, however, because they are dependent on the medium in which they live, in this case the water body, are sensitive to all alterations to the water body by, for example, pollution or habitat alteration, and this alteration will be reflected in the biotic assemblage. Biota therefore act as indicators of the overall ecological condition of the aquatic system, by acting as continuous monitors of the water they inhabit, thereby enabling long-term analysis of both regular and intermittent discharges, variable concentrations of pollutants, single and multiple pollutants, and synergistic or antagonistic effects (Dallas 2009). Biota, however, whilst indicating that a water body is impacted, do not provide insight into the cause of the problem. For this reason, bioassessment, which produces biological data, and physico-chemical monitoring, which produces physical and chemical data, should really be viewed as complementary.

1.1.2 Site selection

Selection of sites for biomonitoring is an important process and adequate time and effort should be assigned to this task to ensure that sites are optimal. Two types of sites are generally included namely: **reference** and **monitoring sites**; and comparison is often made between reference sites or conditions and monitoring sites. The number of sites is generally determined by the homogeneity of the area being monitored and the variety of potential anthropogenic impacts on river health. From a practical perspective however, it is often financial and logistical constraints that influence the number of sites selected, in addition to the objectives of the biomonitoring

programme. The location of biomonitoring sites depends on several factors. The following questions will assist with site location:

- What is the extent of the area to be monitored?
- How homogenous is the area to be monitored in terms of natural characteristics (i.e. geology, natural vegetation, gradient, climate, etc.)?
- Are there sites which represent the reference or natural condition, within the area to be monitored?
- How homogenous is the area to be monitored in terms of anthropogenic modifications and impacts (e.g. land uses, water quality impacts, physical modifications, etc.)?
- What are the key anthropogenic activities that need to be monitored and where are they occurring?
- What existing monitoring sites are present in the area (e.g. hydrological or water quality).

Generally the same factors are used to guide the selection of these sites although key differences do exist. These are described below:

- Reference sites are selected to represent the natural (or as near to natural as possible) condition, i.e. minimally impacted or disturbed. They are used to define the best physical habitat, water quality and biological parameters. Reference sites may be used to generate a "reference condition", which is the expected condition for a particular biotic component for a specific river type. It acts as a benchmark with which a monitoring site is compared.
- Monitoring sites, selected to monitor integrity or health, are commonly those sites identified as important in assessing the condition of a river reach. Sites may range from those showing little impact to those experiencing a large impact with respect to water quality or habitat degradation.

1.1.3 Monitoring timing and frequency

The optimum sampling frequency will vary for the different biotic indices, for example aquatic macroinvertebrates, which have a relatively short life span, will be sampled more frequently than fish, which have a longer life expectancy. Baseline monitoring is the assessment and characterization of existing conditions to provide a standard, or "baseline," against which future change is measured. The baseline must be distinguished from the reference, which typically would be the natural or unimpacted condition of the system. The baseline may represent the reference condition if the site is not influenced by anthropogenic impacts. It may also be important to determine whether the baseline is stable (stationary) or changing in a particular direction. For the reference situation this would represent natural variability, and it may be within a year, i.e. seasonally, and between years. Understanding natural variability is important as this can influence the determination of whether an observed effect is within the expected normal natural variability, OR whether the effect is the result of an anthropogenic impact on the system. Standard monitoring

refers to the monitoring at sites selected to assess the condition of the site (river reach), and may range from point-source monitoring to “basin” monitoring.

2 EXISTING BIOMONITORING PROTOCOLS

Existing river health monitoring schemes can provide the skeleton for application to rivers of the target region (for example diatom-based and macroinvertebrate-based quality indices developed for temperate European rivers have been applied to South African rivers: Ollis et al 2006; Taylor et al. 2007), but required substantial modification and recalibration for use in this region. Further modification and recalibration is likely for the tropical regions of Africa, because of the constraints imposed by the likely differences in riverine flora and fauna.

Monitoring schemes based on the use of biological indicators of aquatic ecosystem integrity should in general aim to meet six criteria (Norris & Hawkins 2000, Murphy et al. 2002). Effective methods will:

- quantify and simplify complex ecological phenomena;
- provide easily interpretable outputs;
- respond predictably to damage caused by humans, while being insensitive to natural spatial/ temporal variation;
- relate to an appropriate scale;
- relate to management goals;
- be scientifically defensible.

2.1 Diatoms

No single group of organisms is best suited for detecting the diversity of environmental perturbations associated with human activities (Kelly 2002). If the maintenance of ecosystem integrity is the aim of environmental management of a river system, the need to monitor the status of different taxonomic groups is vital. Harding et al. (2005) list the following reasons as to why diatoms are useful for monitoring aquatic environments as:

- they collectively show a broad range of tolerance along a gradient of aquatic productivity, and with individual species having specific water chemistry requirements;
- they have one of the shortest generation times of all biological indicators (~2 weeks). They reproduce and respond rapidly to environmental change and provide early warnings of both pollution increases and habitat restoration;
- they are sensitive to change in nutrient concentrations, supply rates and silica/phosphate ratios. Each taxon has a specific optimum and tolerance for nutrients such as phosphate and nitrogen, usually quantifiable to high degree of certainty. Moreover, whereas the use of historical water chemistry data are constrained by the level of analytical sophistication prevailing at the time, the associations of diatoms with water quality remain unchanged;

- their assemblages are typically species-rich – augmenting the information gained from a diversity of ecological tolerances. Moreover, the large number of taxa provides redundancies of information and important internal checks in datasets, increasing the confidence of environmental inferences;
- they respond rapidly to eutrophication. Because diatoms are primarily photoautotrophic organisms, their growth response is directly affected by changes in prevailing nutrient concentrations and light availability;
- their rapid immigration rates and the lack of physical dispersal barriers ensure there is little lag-time between perturbation and response;
- diatom frustules, the siliceous walls of the individual cells, demonstrate a lasting permanence in sediments, such that sediment cores provide details of changes in the quality of the overlying water for as far back as one is able to search. This attribute alone has significant and far-reaching relevance for the determination of reference conditions, not only climatic but also the condition of the system prior to intrusion from cultural development;
- the taxonomy of diatoms is comprehensively documented. Species identifications are largely based on frustule morphology – an attribute readily identifiable with modern light microscopy techniques, and not dependent on electron microscopic techniques as is commonly misconceived;
- they can be found on substrata in streambeds even when dry, so they can be sampled at most times of the year and still accurately reflect recent or prevailing conditions;

Additionally the use of diatoms is supported by:

- their ease of collection, preparation for observation, and storage (small sample volumes, no desiccation risk) for reference purposes;
- the considerable amount of tried and tested ecologically-associative information already available, both nationally and world-wide;
- their suitability for diversity analysis;
- the availability of the OMNIDIA interpretive software package.

Although diatom taxonomy is currently in a state of flux, this should pose no unsolvable problems for the application of diatom indices, as the taxonomy of diatoms is generally well documented (Krammer & Lange-Bertalot 1986-91) and full lists of synonyms are available in the aforementioned identification volumes and works, such as that of Kellogg & Kellogg (2002) and in the electronic database OMNIDIA (Lecointe et al. 1993).

Criticism of diatom-based techniques has been expressed regarding the difficulty involved in accurate species identification necessary for the effective use of diatom indices. Descy & Coste (1991), however, are of the opinion that species identification problems can be solved by editing complex identification keys to allow for accurate identification of a limited number of taxa. Such a guide has been developed for French inland waters (Prygiel et al. 2000). This guide provides a means for identification of all the diatom taxa used in the Biological Diatom Index (BDI) of Lenoir & Coste (1996) developed for use in national river quality monitoring networks in France. Kelly

(2000) developed a similar guide for the identification of common benthic diatoms in Great Britain and Taylor et al. (2007) for South Africa. Taxonomic difficulties may also be avoided by using a simplified diatom index such as the Generic Diatom Index (GDI) of Coste & Ayphassorho (1991). The GDI allows for the determination of water quality at a particular site, based on the identification of diatoms to the genus level. GDI index has been found comparable to indices such as the Specific Pollution sensitivity Index (SPI; CEMAGREF 1982), which is based on a large number of taxa (Kelly et al. 1995, Kwandrans et al. 1998). An advantage of using diatom-based methods is the very rapid field methodology. This is however offset by more labour intensive laboratory procedures.

2.1.1 Biotic indices based on diatoms

Within the last decade diatom indices have gained considerable popularity throughout the world as a tool to provide an integrated reflection of water quality, which can form the basis of management decisions regarding rivers and streams (Table 1). The vast majority of the development and testing of diatom indices has been carried out in French drainage basins. The fact that these French diatom indices have been tested on the scale of territory as large and as typologically diversified as France, enabled the more general application on the European continent (Prygiel & Coste, 1999). The design of software programmes such as OMNIDIA for the calculation of diatom indices has also facilitated the use of diatom based bio-monitoring methods (Lecointe et al. 1993). A variety of diatom indices have been adopted and tested by many European countries including Finland (Eloranta & Andersson 1998) and Poland (Kwandrans et al. 1998). European and British diatom indices were derived, applied and tested in temperate regions, and there is little information regarding their application in the tropics and subtropics (Wu & Kow 2002). Thus the need exists for the evaluation of these indices before they can be routinely applied in warmer climates. Jüttner et al. (2003) found that the TDI index of Kelly & Whitton (1995), developed to demonstrate trophic levels in British inland waters, showed consistent responses in TDI scores between Europe and the Himalayas. This has many important implications for research into the use of diatom indices in southern Africa. If European indices such as the SPI can be used in their current state or slightly modified, this will negate the need for highly detailed research into the ecological tolerances and distribution of diatom species encountered in southern Africa.

The majority of the indices used are based on the weighted average equation of Zelinka & Marvan (1961) and have the basic form:

$$index = \frac{\sum_{j=1}^n a_j s_j v_j}{\sum_{j=1}^n a_j v_j}$$

where a_j = abundance (proportion) of species j in sample, v_j = indicator value and s_j = pollution sensitivity of species j . The performance of the indices depends on the values given to the constants s and v for each taxon and the values of the index ranges from 1 to an upper limit equal to the highest value of s . Diatom indices differ in the number of species used (Table 1.1) and in the values of s and v which have been attributed after compiling the data from literature and from ordinations (Prygiel & Coste, 1993).

Table 1: Comparison of biotic indices based on diatoms

Biotic Index	Abbreviation	Biotores sampled¹ (in order of preference)	Sampling equipment	Taxonomic level²	No of taxa used	Identification protocol	Final index range	Current usage
Specific Pollution sensitivity Index	SPI	SIC, S, V	Small brush, size not stipulated	G+S+Var	2035	Lab-based	0–20	EU, France, South Africa
Generic Diatom Index	GDI	SIC, S, V	Small brush, size not stipulated	G	174	Lab-based	0–5	EU
Leclercq & Maquet	LMI	SIC, S, V	Small brush, size not stipulated	S	403	Lab-based	0–c.40	France
Commission for Economical Community index	CEC	SIC, S, V	Small brush, size not stipulated	S	208	Lab-based	0-20 based on 2-way table	EU
Artoise-Picardie Diatom Index	ADPI	SIC, S, V	Small brush, size not stipulated	S	41G + 91S	Lab-based	0-20	France
Biological diatom Index	CBS	SIC, S, V	Small brush, size not stipulated)	G+S	209	Lab-based	0-20	EU, France
Eutrophication/Pollution Index	CBI	SIC, S, V	Small brush, size not stipulated	G+S	93	Lab-based	0-20	Italy
Sládeček	DSFI	SIC, S, V	Small brush, size not	S	323	Lab-based	0-20	Czech Republic
Diatom Assemblage Index of Organic Water Pollution	DAIpo	SIC, S, V	Small brush, size not stipulated	S	87	Lab-based	0-20	Japan

Biotic Index	Abbreviation	Biotores sampled¹ (in order of preference)	Sampling equipment	Taxonomic level²	No of taxa used	Identification protocol	Final index range	Current usage
Trophic Diatom Index (Germany)	TDI	SIC, S, V	Small brush, size not stipulated	S	105	Lab-based	0-20	Germany
Trophic Diatom Index (UK)	TDI - UK	SIC, S, V	Small brush, size not stipulated	G+S	86	Lab-based	0–100	UK

¹ SIC = stones-in-current (riffles); S = stones (in- & out-of-current); V = vegetation; GSM = gravel, sand and mud

² Var = Variety; S = species; G = genus

Diatom indices function in the following manner: In a sample from a body of water with a particular level of a water quality determinant, diatom taxa with their optimum close to that level will be most abundant. Therefore an estimate of the level of that determinant in the sample can be made from the average of the optima of all the taxa in that sample, each weighted by its abundance. This means that a taxon that is found frequently in a sample has more influence on the result than one that is rare. A further refinement is the provision of an 'indicator value' that is included to give greater weight to those taxa which are good indicators of particular environmental conditions. In practice, use of diatom indices involves making a list of the taxa present in a sample, along with a measure of their abundance. The index is expressed as the mean of the optima of the taxa in the sample, weighted by the abundance of each taxon. The indicator value acts to further increase the influence of certain species (Kelly, 1998).

2.2 Macroinvertebrates

Aquatic macroinvertebrates form a major component of the biota of aquatic ecosystems and are associated with one or other aquatic habitat such as stony beds; marginal and instream vegetation; floating vegetation; gravel, sand and mud. They are mostly primary (feeding on plant material) and secondary (feeding on planktonic or benthic organisms) consumers near the base of the food chain and are therefore essential elements in the functioning of aquatic ecosystems. Macroinvertebrates are largely dependent on the aquatic environment in which they live, and are sensitive to factors such as water quality, water quantity (environmental flows), and habitat and food availability.

There is general consensus that macroinvertebrates are amongst the most sensitive components of aquatic ecosystems and they have been widely used in bioassessment. Briefly, as summarised by Rosenberg & Resh (1993):

- Macroinvertebrates are ubiquitous and diverse, and are therefore affected by a variety of disturbances in many different types of aquatic habitats.
- Sensitivity to stress varies with species and the large number of species within an assemblage offers a spectrum of responses to environmental stresses.
- In their aquatic phase, macroinvertebrates are largely non-mobile and are thus representative of the location being sampled, which allows effective spatial analyses of disturbance.
- They have relatively long life cycles compared to other groups (e.g. planktonic organisms), which allows elucidation of temporal changes caused by disturbances.

One **limitation**, however, of using macroinvertebrates in bioassessment is their heterogeneous (patchy) distribution that results in spatial and temporal variability in macroinvertebrate assemblages (e.g. Dallas 2004a, b). If patchiness is very high, then accurate interpretation of biomonitoring data is difficult. Similarly, if the distribution of macroinvertebrates varies with time of year, e.g. season, then data interpretation is further complicated. To overcome the influence of the spatial patchiness, bioassessment is often undertaken within a spatial framework such that natural heterogeneity is accounted for. The development of a river typology forms one work

package of the SAFRASS project. Further, to address the potential temporal variation, sampling is to be undertaken in two seasons, the wet and the dry seasons.

2.2.1 Biotic indices based on aquatic macroinvertebrates

Biological community data can be summarised and presented as simple, numeric or categorised indices. Ollis et al. (2006) provide a detailed overview of the biotic indices used worldwide. These indices allow the results of ecological assessments to be communicated in a way that is understandable to natural resource managers, decision-makers, politicians and the general public (Ollis et al. 2006). Three basic types of indices can be generated: diversity indices, comparison (similarity or dissimilarity) indices and biotic indices. Of these, biotic indices are the most widely used. With biotic indices, each taxon from a particular group of organisms is assigned a sensitivity weighting based on the tolerance or sensitivity to particular pollutants. The scores of all the individual taxa sampled at a site are summed and/or averaged to provide a value by which the integrity of the biotic community at the site can be gauged. Some biotic indices include abundance estimates in the scoring system.

The Saprobien or Saprobic System, which stems from the research work of Kolkwitz and Marsson in German rivers in the early 1900's, is generally considered to be the first biological scoring system for the assessment of water quality in river ecosystems (Ollis et al. 2006). Indices based on the Saprobien System are determined by the presence and absence of specific indicator species from a number of different groups and trophic levels (mainly bacteria, algae, protozoans and rotifers, but including some benthic invertebrates and fish) for which the tolerances to organic pollution have been established (Herricks & Cairns 1982, Metcalfe 1989, Reynoldson & Metcalfe-Smith 1992, Metcalfe-Smith 1994). Most modern biotic indices, on the other hand, are based on the presence and pollution-tolerances of the community of organisms sampled from a particular group (such as the benthic macroinvertebrates) (Ollis et al. 2006).

According to Ollis et al. (2006) in recent years, with limited time and resources available for ecological assessments, there has been a great emphasis on community-level rapid bioassessment techniques and the use of biotic indices, particularly in the field of aquatic ecosystem assessment (e.g. Chessman & McEvoy 1998, Barbour et al. 1999, Norris & Thoms 1999, Brown 2001, Dallas 2002, Metzeling et al. 2003). Rapid bioassessment techniques, which usually involve qualitative (or semi-quantitative) sampling with few or no replicates and limited taxonomic resolution, have been developed to cost-effectively highlight problem areas where follow-on and more intensive, quantitative ecological and chemical studies need to be undertaken (Chessman 1995, Chutter 1995, Resh et al. 1995). Several authors caution, however, that rapid assessment techniques should not be seen as a replacement for more traditional quantitative studies and detailed biological surveys, but rather as a precursor to these.

Numerous biotic indices have been developed for the assessment of river ecosystems that are based on aquatic macroinvertebrates (Ollis et al. 2006). A number of these have been described by Washington (1984), Metcalfe (1989), Metcalfe-Smith (1994), Resh & Jackson (1993) and Dallas (1995). Ollis et al. (2006) provide a comprehensive, updated comparative description of the more important or widely used indices and bioassessment methods, listed in chronological order. They

also provide a comparative summary of the biotic indices, which has been modified in Table 2 by including more recent indices developed in the southern African region. For each biotic index, a description is given of the habitats or biotopes sampled, the sampling equipment used, the sampling protocol followed, the level of taxonomic identification, whether identifications are laboratory- or field-based, the range of the final index value, and its current usage.

2.2.2 Using biotic indices and interpreting bioassessment data

The primary aim of any biomonitoring programme is to identify areas or sites where anthropogenic activities are negatively impacting upon an aquatic ecosystem so that management action may be taken to curtail this impact. From the macroinvertebrate perspective, there are essentially two approaches to the interpretation of bioassessment data, namely the multimetric and multivariate approaches. Both approaches generate numerical values, which use one or more components of the biota to provide a measure of the biological condition of a site. One of the advantages of numerical values such as multimetric or biotic indices is that they formalise what any good biologist, familiar with local biota, knows about the biological condition; and they communicate biological condition to managers, thus providing a scientific basis for management decisions that affect aquatic resources (Dallas 2009).

The multimetric approach involves the integration of a number of structural and functional attributes of macroinvertebrate communities, known as metrics, into a composite index (Ollis et al. 2006). A metric is a measurable component of a biological system with an empirical change in value along a gradient of human disturbance. An example of a metric is the "Number of macroinvertebrate taxa". Typically, an Index of Biotic Integrity (IBI) is formed by combining at least 7 metrics from one biological assemblage (e.g., plants, macroinvertebrates). Each metric is assigned a score of 1, 3, or 5 according to how it responds to human disturbances, with 1 representing highly impacted (poor condition), 3 representing moderately impacted (intermediate condition) and 5 representing minimally impacted (good condition). Most multimetric indices are based on the Index of Biotic Integrity (Karr 1981, Karr et al. 1986), initially developed for riverine fish communities. The macroinvertebrate Rapid Bioassessment Protocols for streams and rivers developed by the USEPA (Plafkin et al. 1989, Barbour et al. 1999), which are used widely throughout the USA, are based on the multimetric approach to bioassessment Ollis et al. 2006). Multimetric indices are constructed and interpreted in an ecoregional or bioregional framework, such that natural spatial variation is taken into account (Dallas 2004a, Ollis et al. 2006).

The multivariate approach is based on the association between macroinvertebrate communities and the environmental attributes of sampling sites. A small number of site-specific environmental features are used to predict the macroinvertebrate fauna to be expected in the absence of major environmental stress. Predictions of the expected taxa can be undertaken at a species or family level, or at the level of a biotic index, with Scores, Number of Taxa and Average Score per Taxon (ASPT) predicted. This approach is exemplified by the River InVertebrate Prediction And Classification System (RIVPACS) developed for lotic systems in the United Kingdom (Wright et al. 1984, 1989; Furse et al. 1984; Moss et al. 1987), where it has since 1990 been used in five-yearly nation-wide bioassessments of river water quality (Wright 1995, Wright et al. 1998a, Hemsley-Flint 2000) (Ollis et al. 2006).

Table 2: Comparison of biotic indices based on aquatic macroinvertebrates (modified from Ollis et al. 2006)

Biotic Index	Abbreviation	Biotopes sampled¹	Sampling equipment²	Sampling protocol³	Taxonomic level⁴	Identification protocol	Final index range	Current usage
Average Chandler Biotic Score	Avg. CBS	SIC	Hand-net (1000 µm)	SQ, 5 min	G+S	Not stipulated	0–100	USA
BalkaN Biotic Index	BNBI	All, combined	Benthos net	Q	F+sF+G	Lab-based	0–5	Serbia
Beck's Biotic Index	Beck's BI	All, combined	Not stipulated	NQ	S	Lab-based	0–c.40	None
Belgian Biotic Index	BBI	All, combined	Hand-net (300–500 µm)	NQ, 3/5 min	F+G	Lab-based	0–10	Belgium and surrounding countries
Biological Monitoring Working Party Score System	BMWP	All, combined	Hand-net (900 µm)	NQ/SQ, 3 min	F	Field-based	0–c.200 (Score) 0–10 (ASPT)	UK, Finland, Sweden
Chandler's Biotic Score	CBS	SIC	Hand-net (1000 µm)	SQ, 5 min	G+S	Not stipulated	0–∞	USA
Chutter's Biotic Index	CBI	SIC	Hand-net / Surber (290 µm)	Q	F+G+S	Not stipulated	0–10	None
Danish Stream Fauna Index	DSFI	All, combined	Hand-net (500 µm)	SQ, 12 samples	F+G	Lab-based	0–7	Denmark, Sweden
Extended Biotic Index / Expanded TBI	EBI	All, combined	Hand-net	NQ, 10 min	F+G+S	Lab-based	0–15	Italy (modified)
Family-level Biotic Index	FBI	SIC	Hand-net	Q, 100 organisms	F	Field-based	0–10	USA, Chile
Florida Index	FI	All, combined	Hand-net	NQ, 10 min	G+S	Lab-based	0–40	Florida (USA)
Hilsenhoff's Biotic Index	HBI	SIC	Hand-net	Q, >100 organisms	G+S	Lab-based	0–10	USA

Biotic Index	Abbreviation	Biotores sampled¹	Sampling equipment²	Sampling protocol³	Taxonomic level⁴	Identification protocol	Final index range	Current usage
Iberian BMWP	IBMWP / BMWP'	Lotic + Lentic, combined/separate	Hand-net	NQ	F	Field-based	0–c.200 (Score) 0–10 (ASPT)	Spain, Italy
Indice Biologique Global Normalisé	IBGN	8 pre-defined habitats, separate	Surber + Hand-net (500 µm)	NQ/SQ	F	Lab-based	0–20	France
Indice Biotico Esteso	IBE	All, combined	Hand-net	NQ, 10 min	F	Lab-based	0–15	Italy
Indice Biotique	IB	Lotic + Lentic, separate	Surber + Grab	SQ	F+G+S	Lab-based	0–10	None
Macroinvertebrate Community Index	MCI	SIC	Hand-net / Surber	NQ	G	Lab-based	0–200	New Zealand
Namibian Scoring System, Version 2	NASS	S+V+GSM, separate	Hand-net (1000 µm)	NQ/SQ	F	Field-based	0–c.250 (Score) 0–15 (ASPT)	Namibia
Okavango Scoring System, Version 1	OKASS	V, separated to Lotic and Lentic	Hand-net (1000 µm)	NQ/SQ	F	Field-based	0–c.125 (Score) 0–7 (ASPT) 0–c.25 (number of Taxa)	Botswana
Quantitative MCI	QMCI	SIC	Surber	Q	G	Lab-based	0–10	New Zealand
Rivers of Vaud Index	RIVAUD	SIC	Hand-net	SQ	F+G	Not stipulated	0–10	None
Rivers of Vaud Index, 1995 Version	RIVAUD 95	SIC	Hand-net	SQ	F+G	Not stipulated	0–20	Western Switzerland
Semi-Quantitative MCI	SQMCI	SIC	Hand-net	SQ	G	Lab-based	0–10	New Zealand
South African Scoring System, Version 4	SASS4	S+V+GSM, combined	Hand-net (1000 µm)	NQ/SQ	F	Field-based	0–c.250 (Score) 0–15 (ASPT)	None
South African Scoring System, Version 5	SASS5	S+V+GSM, separate	Hand-net (1000 µm)	NQ/SQ	F	Field-based	0–c.250 (Score) 0–15 (ASPT)	Southern Africa

Biotic Index	Abbreviation	Biotores sampled¹	Sampling equipment²	Sampling protocol³	Taxonomic level⁴	Identification protocol	Final index range	Current usage
Stream Invertebrate Grade Number – Average Level Biotic Index	SIGNAL	6 pre-defined habitats, separate	Hand-net (250 µm) + Grab	NQ, 100 orgs.	F	Lab-based	0–10	Australia
Stream Invertebrate Grade Number – Average Level Weighted Biotic Index	SIGNAL-W	6 pre-defined habitats, separate	Hand-net (250 µm) + Grab	SQ, 100 orgs.	F	Lab-based	0–10	Australia
Trent Biotic Index	TBI	All, combined	Hand-net	NQ, 10 min	F+G+S	Lab-based	0–10	None

¹ SIC = stones-in-current (riffles); S = stones (in- & out-of-current); V = vegetation; GSM = gravel, sand and mud

² Mesh size in brackets, where known; hand-net also known as a kick-net, sweep-net, dip-net or pond-net

³ Q = quantitative; SQ = semi-quantitative; NQ = non-quantitative (qualitative)

⁴ S = species; G = genus; F = family; sF = sub-family

In Australia, the development and use of a RIVPACS-type approach to the biomonitoring of river ecosystems has been advocated within their National River Health Programme, as part of the component based on aquatic macroinvertebrates known as the AUStralian RIVer Assessment Scheme (AusRivAS) (Uys et al. 1996, Smith et al. 1999). Fundamental to AusRivAS are predictive models, based on the British RIVPACS models (Wright 1995).

A modification of this method, which has been developed for use in South Africa (Dallas and Day 2007, Dallas 2007a), makes use of a biotic index, SASS, whereby each macroinvertebrate taxon is pre-assigned a sensitivity weighting based on its tolerance to water quality impairment and general river or wetland condition or health. The index is applied within a spatial framework that takes into account potential natural variation in macroinvertebrate assemblages that respond to geographic and or habitat differences. Monitoring data is interpreted relative to a derived reference condition that takes into account the natural variation at a suite of reference sites, normally established via classification and ordination techniques.

2.3 Macrophytes

There are several advantages of using aquatic macrophytes in freshwater monitoring (Murphy et al 2002; Birks et al 2007); which include:

(1). Submerged and floating macrophytes are an integral component of the littoral ecosystem in many lakes:

- Macrophyte vegetation provides bioarchitecture, cover, feeding and breeding sites and primary production for many other biota: information relevant to whole-ecosystem biointegrity is therefore gained by monitoring the plants.

(2). Macrophytes are relatively long lived organisms (months to years), and have very limited motility (usually limited to propagule movement); they can therefore:

- Provide an integrated representation of longer term environmental conditions, thus providing a longer-term prospect for integrating the impacts of environmental change or other factors affecting river and lake ecosystem biointegrity; the main types of pressure, to which river vegetation responds at the site-scale, are: hydrological, structural, chemical and mechanical.

(3). Macrophytes are relatively cheap and easy to sample in rivers

- They are usually restricted to river margins in large and fast-flowing channels;
- They are relatively easy to identify (though some groups require specialist assistance to reach species-level identification), and there is usually no need for laboratory analysis.

A list of sampling methods which have been used to varying degrees in the monitoring of macrophytes in standing waters, but which are also generally applicable to rivers is provided in Table 3.

Table 3: Technical details of sampling methods commonly used in the biomonitoring of standing waters (adapted from Murphy et al. 2002)

Method	Organisms Sampled	Depth	Substrate	Comment	Usefulness	Reference
Diving	Benthic Invertebrates / Macrophytes	Deep	Hard Bottom/Stones, Rocks	Scraping or Coring. Care must be taken that the diver does not kick up substrate while swimming	-	-
Gerking's Sampler	Vegetation Fauna	Shallow	Vegetation	Square frame, wood or copper. Placed over vegetation and plants cut with glass clippers.	Not necessarily representative of full vegetation community as sampling directed towards collection of fauna	Gerking, S.D. (1957). A method of sampling the littoral macrofauna and its application, <i>Ecology</i> . 38: 219 - 226.
Quadrat Frame Sampler	Macrophytes	Shallow/Deep	Soft Bottom	Plants may be removed by hand or by various apparatus. It is important to ensure that the roots are also sampled.	Useful for tall plants and small root systems.	Forsberg, C. (1959). Quantitative sampling of sub-aquatic vegetation. <i>Oikos</i> , 10: 233 - 240.
Scoop	Macrophytes	Deep	Soft/Hard Bottom	Diver operated	Important for root systems.	Nygaard, G. (1958). On the productivity of bottom vegetation in Lake Grane Langso. <i>Verh. Intern. Verein. Limnol.</i> 13: 144 – 155
Ekman Dredge	Macrophytes	Shallow/Deep	Soft Bottom	-	Important for small root system.	Welch, P.S. (1948). <i>Limnological Methods</i> . McGraw-Hill, New York, Toronto, London, 382 pp.
Petersen Dredge	Macrophytes	Shallow/Deep	Hard Bottom	-	Poor Sampling	Welch, P.S. (1948). <i>Limnological Methods</i> . McGraw-Hill, New York, Toronto, London, 382 pp.

Method	Organisms Sampled	Depth	Substrate	Comment	Usefulness	Reference
Petersen Dredge, Modified	Macrophytes	Shallow/Deep	Hard Bottom	-	Good Sampling, improvement on simple Petersen Dredge.	Potzger, E. and van Engel, A. (1942). Study of the rooted aquatic vegetation of Weber Lake, Vilas County, Wisconsin. <i>Trans. Wisconsin Acad. Sci.</i> 34: 149 - 166.
Cylindrical Sampler	Macrophytes	Shallow/Deep	Soft Bottom	-	Useful for upright plants and small root systems.	Grontved, J. (1957). A sampler for underwater macrovegetation in shallow waters. <i>J. Cons. Int. Explor. Mer.</i> 22: 293 - 297.
Pronged Grab	Macrophytes	Shallow/Deep	Soft Bottom	-	Very useful for luxuriant vegetation, but will only work for roots which are located on soft bottoms.	Bernatowicz, S. (1960). Metody badania roslinnosci naczyniowej w jeziorach (The methods of investigations of vascular plants in lakes: <i>Roczn. Nauk Roln.</i> 77 (B1): 61 - 78.
Leaf Index	Macrophytes	Shallow/Deep	Soft/Hard Bottom	Mean area may be calculated using a planimetry or by weighing cut-outs of tracings or contact prints of a sample leaf. The Punch method may also be used, it is based on the weight number and known area of discs punched from a random heap of weighed leaves.	Difficult technique to apply to small plants with photosynthetic stems.	Watson, D.J. and Watson, M.A. (1953). Comparative physiological studies on the growth of field crops. III. The effect of infection with beet yellows and beet mosaic viruses on the growth and yeild of the sugerbeet root crop. <i>Ann. Appl. Biol.</i> 40: 1 - 37.
Point Quadrat	Macrophytes	Shallow/Deep	Soft/Hard Bottom	-		Warren Wilson, J. (1959). Analysis of the distribution of foliage area in grassland. In: Ivens, J.D. (ed.). <i>The Measurement of Grassland Productivity</i> , 51 - 61 pp. University of Nottingham. Butterworths Scientific Publications, London.
Rake	Macrophytes	Shallow	Hard/Soft Bottom	Generally three to four replicates are taken	Limited size of rake may result in a - non-representative sample of macrophytes being taken.	

(4). Several schemes have been developed, both in Europe (predominantly in the UK) and elsewhere (though largely restricted to the northern United States, Australia, and sub-tropical to temperate South America), which make use of macrophyte community composition, or other metrics, to monitor standing water biointegrity (see Murphy et al. 2002 for a comprehensive list)

2.3.1 Macrophyte monitoring and classification schemes

Historically classification schemes for macrophytes have been less well developed than those for macroinvertebrates, and especially so with regards to the development of schemes for rivers and to the development of reference-based systems (Murphy et al. 2003). Classification schemes do exist for macrophytes in rivers and lakes of the UK: the Mean Trophic Rank (MTR) scheme in rivers (Environment Agency 1999); and the Trophic Ranking Score (TRS) system in lakes (Palmer et al. 1992). Neither scheme is reference-based, so neither meets the relatively stringent requirements of the European Union Water Framework Directive (WFD: 2000/60/EC) (Murphy et al. 2003). Briefly, the MTR was developed to assess pressures on and degradation to river systems based on the occurrence and abundance of submerged and riparian indicator macrophytes species to derive a single index to describe the trophic status of a site. However, whilst useful for indicating point source pollution (e.g. sewage works) the success of the methodology has been questioned in headwater streams, where total number of indicator species is low (Thiebaut et al. 2002). With reference to the situation in South African savanna systems, Khomo et al (2009) found that fifth order hillslope streams were far more complex and heterogeneous in terms of both geomorphology and vegetation communities than were first order streams, which had more homogeneous geomorphology and vegetation communities. Intermediate order streams had intermediate levels of complexity.

However, attempts have been made to modify the MTR methodology (e.g. in Poland: Szoszkiewicz et al 2002), and may represent the first stage in the development of monitoring schemes in many countries. In other examples modified MTR methodologies or similar have been adopted into national multi-biota biomonitoring schemes (e.g. Swedish Environmental Quality Criteria: Swedish EPA 2000). Numerous examples also exist, generally from a European perspective, of river macrophyte biological monitoring indexes developed on a basis of species trophic scores (e.g. Haury et al 2006; Schneider 2007).

While there is good understanding of the impacts that eutrophication has on species assemblages and biomass on river vegetation in Africa, and evidence of research on the problems (e.g. De Villiers 2007), there is little evidence for detailed research outside of South Africa, or of the development of macrophyte-based classification and biomonitoring methodologies outwith that region.

2.3.2 Development of reference based macrophyte monitoring methodologies

As yet there are still no standardised methodologies (across Europe) for macrophyte monitoring (Lansdown and Bosanquet 2010), though examples exist across a number of European member states for reference based macrophyte monitoring methodologies. Within Northern Europe these include examples given in Table 4 (and reviewed fully by Birk et al 2007).

In terms of trans-boundary intercalibration of methods (strictly within the context of European lowland streams), the British MTR and the French Indice Biologique Macrophytique en Rivière (IBMR) methods were found to be highly related (much more so than the German Reference Index (RI), and Dutch Macrophyte Score methodologies). MTR also related well to Ellenberg_N values, representing potential to use this common metric for intercalibration of the methodologies (or methodologies partially based upon it) across a wider geographical range (Birk et al 2006).

MTR has been superseded by LEAFPACS (Willby 2006; UK-TAG 2008) for implementation by environmental agencies within the UK responsible for implantation of the WFD. MTR and LEAFPACS are essentially similar in the use of comprehensive surveying along a standard stretch of river which will record both Indicator and non-indicator species. Whilst MTR scores were derived from expert judgement and literature, LEAFPACS scores were derived from statistical analysis of a large data-set collected on 100, 500 or 1,000 m long sections of rivers (Lansdown and Bosanquet 2010), and is therefore reference based, modelling reference communities using abiotic parameters and allowing a site-specific quality appraisal. In contrast, the remaining methods detailed in Table 4 assess the macrophyte communities present against stream type-specific reference conditions (Birk et al 2007).

Table 4 National macrophyte assessment methods within selected EU states (adapted from Birks et al 2003)

Country	Scheme	Reference
Austria	Austrian Index for Macrophytes in Rivers (AIM Rivers)	BMLFUW (2006); Pall & Moser (2006)
Belgium (Flanders)	MAFWAT (Macrofyten Waterlopen)	Leyssen et al. (2005)
Belgium (Wallonia)	Indice Biologique Macrophytique en Rivière (IBMR)	NF T90-395: 2003; Galoux (2007)
France	Indice Biologique Macrophytique en Rivière (IBMR)	NF T90-395:2003
Germany	Reference Index (RI)	Meilinger et al. (2005); Schaumburg et al. (2006)
Great Britain	LEAFPACS	Willby et al. (2006); UK-TAG (2008)
Netherlands	Maatlatten	Molen & Pot (2007)
Poland	Macrophyte Index for Rivers (MIR)	Szozzkiewicz et al. (2006)

All of the methods Listed in Table 4 conform to the EN 14184:2003 international standard which lays down requirements for the procedures applied to acquire data on macrophyte composition and

abundance. Representative river stretches are visually surveyed by wading, diving or boating, using rake, grapnel, aqua-scope where necessary. Representative sites span about 100 metres of river length, but may be more extensive in case of large river surveys.

All the methodologies in Table 4 take higher plants down to species level, and aim to take other groups such as bryophytes, charophytes, spermatophytes and algae down to genus level (though sometimes group level is considered acceptable). However, only the Belgian (Flanders) MAFWAT methodology includes emergent bank vegetation in the assessment (although others record species present), and the way in which the ecological quality class and pressures assess differ between all methodologies. Most of the methodologies recommend that the river cannot be assessed where macrophytes are absent. However, some recommend further investigation, whilst the Belgian MAFWAT methodology states that assessment may be based on riparian vegetation only.

2.4 Habitat integrity

Habitat assessment has become an important component in evaluating the ecological integrity of river ecosystems internationally, with habitat assessment on a larger spatial scale, in particular, being used to an increasingly greater extent (Ollis *et al.* 2006). Examples of broad-scale habitat assessments currently in use include the Qualitative Habitat Evaluation Index (QHEI) in Ohio and a number of similar habitat assessment systems across North America (Rankin 1995), and the River Habitat Survey (RHS) in the United Kingdom (Raven *et al.* 1998, 2000) and similar habitat assessment methods in European countries including Austria, Germany and Switzerland (Muhar & Jungwirth 1998). Broad-scale habitat assessment systems used in Australia include a method developed for the assessment of the environmental condition of rivers in the state of Victoria (Mitchell 1990, cited by Ladson & White 2000) and a rapid technique for assessing the physical and environmental condition of rivers in the state of Queensland (Anderson 1993, cited by Ladson & White 2000; Jackson & Anderson 1994), while in South Africa the Index of Habitat Integrity (IHI) is used (Kleynhans 1996, Dallas 2005, Graham and Louw 2009, Kleynhans *et al.* 2009).

The South African Index of Habitat Integrity (IHI, Kleynhans 1996), which includes both riparian and instream habitat, may be applied on a site basis (Dallas 2005). The IHI aims to assess the number and severity of anthropogenic perturbations on a river and the damage they potentially inflict on the habitat integrity of the system. These disturbances include abiotic factors, such as water abstraction, weirs, dams, pollution and dumping of rubble, and biotic factors, such as the presence of alien plants and aquatic animals which modify habitat. The emphasis in the site-based assessment is placed on the field-based site assessment, supplemented, where possible, with information gleaned from other sources such as catchment study reports, Integrated Strategic Plans, Ecological Studies (which may include aerial video material for the river), land cover, together with local knowledge. It should be noted that any site-based assessment will lack longitudinal continuity and therefore may not adequately reflect the habitat integrity of the river. Aspects considered in the assessment comprise those instream and riparian zone perturbations regarded as primary causes of degradation of a river ecosystem.

3 CONCLUSIONS AND RECOMMENDATIONS

3.1 Diatoms: recommendations for sampling within the SAFRASS project

Samples will be collected in such a way that any one of the indices, or groups of indices discussed above could be applied and tested. However, the autecological indices developed in France show perhaps the most potential for testing and adaptation. In particular the SPI has a very large species base and may thus prove to be a very useful index for demonstrating impact on water quality. The biggest challenge however will be to modify these indices to include tropical and subtropical species and also to allow for the correct inferences regarding reference condition.

3.1.1 Substrate selection for diatom-based water quality monitoring

Preferred substratum

Cobbles and small boulders (rocks) are the preferred substratum for monitoring diatoms in the riverine environment, and almost all diatom indices throughout the world can be applied to the community (i.e. the epilithon) that is found on this substratum. The most important reasons for this choice of substratum can be summarised as follows:

- Cobbles and small boulders are generally widely available (riffles, cobble beds, benches and shelves), throughout the length of a river from headwaters to lowland stretches, and throughout the year.
- The type of stone sampled can usually be discounted when assessing the flora at a particular site.
- The performance of major diatom-based indices on this substratum is well understood.
- The ecology of the epilithon is better known than any other group.

Alternative substrata (in order of preference)

- Man made objects (bricks, pieces of concrete, bridge supports, canal walls etc.).
- Emergent macrophytes, such as *Typha* spp. or *Phragmites* spp.
- Submerged macrophytes, such as *Potamogeton* spp, *Ceratophyllum* spp. etc. may be used as an alternative substratum.

3.1.2 Sampling protocol for diatom-based water quality monitoring

Solid substrata

- Five to ten cobbles, boulders, pebbles or other substrata of similar proportions should be collected from a reach of at least 10 m in the river or stream.
- Gently rinse the substrata in the stream and carefully place in a sampling tray on the river bank, together with about 50 ml of stream water.
- Diatoms should be removed by vigorously scrubbing the upper surface of the substratum with a small brush (e.g. clean toothbrush) to dislodge the diatom community. Some

diatomists prefer to scrape the substrata with a knife or a spoon as these implements are easier to clean and reduce the possibility of contamination between sites.

- Only the upper side (the side most exposed to flowing water) of boulders should be scrubbed to avoid contamination with sediment that might be present on the undersides of the cobbles.
- The resulting diatom suspension is then poured into a labeled wide-mouth plastic sample bottle of 100ml capacity or greater.
- Care should be taken to avoid equipment contamination between sites by rinsing both the toothbrush and the plastic tray in the river both before and after taking the diatom sample.

Sampling from emergent aquatic macrophytes

- The emergent macrophyte stem is cut with a knife above the water line.
- This procedure needs to be repeated until five stems have been collected.
- Scrubbing and removal of the diatom communities can then proceed in a similar fashion to that described above for solid substrata.

Sampling from submerged aquatic macrophytes

- Select replicates from five different plants growing in the main flow of the river.
- Each replicate, consisting of a single stem plus associated branches of the plant from the lowest healthy leaves to the tip, should be placed in a plastic bag together with 50 ml of stream water. Diatoms should be visible as a brown film associated with the macrophytes.
- The plants should be shaken vigorously and squeezed in the plastic bag and the resulting brown suspension poured into a sample bottle.

3.1.3 Preservation of diatom material and labelling samples

- Fresh diatom samples should be stored in the following manner:
- In a refrigerator if the period of storage is to be less 24 hours.
- If the samples are not going to be analysed immediately the samples should be fixed with ethanol to reach a final concentration of 20% by volume.

The above sections outline the sampling protocol and preservation of diatom samples. Laboratory preparation of the samples, enumeration and data generation will follow those methods outlined by Taylor et al. (2005).

3.2 Macroinvertebrates: recommendations for sampling within the SAFRASS project

An important consideration in the selection of an appropriate biomonitoring protocol for aquatic macroinvertebrates is the long term success of its implementation in the target country. Factors that need to be considered include local capacity, financial constraints, and logistical considerations. For these reasons it is often the simpler approaches that have the greatest chance for long term success. While many of the biomonitoring protocols and biotic indices would be suitable and could potentially be modified for use in SAFRASS, it is recommended that a rapid bioassessment protocol be adopted. Given the development of three related protocols within the

southern African region, namely SASS, NASS and OKASS, it is further recommended that these form the basis for the development of a biomonitoring protocol and biotic index for aquatic macroinvertebrates in Zambia. The advantages of SASS (NASS/OKASS) include:

- Its rapidity and relatively easy for use in the field,
- Its relative cost effectiveness,
- Its ability to be undertaken by technicians who have received training,
- Its non-destructive sampling protocol,
- Its ability to enable a quick assessment of the improvement or regression in water quality at a site through repetitive sampling, and
- Its relatively simple data output, which consists of two scores easily interpreted by water resource managers.

Of key importance in the modification of the SASS into ZASS (Zambian Scoring System) is the validation of the sensitivity weightings currently assigned to macroinvertebrate taxa, which are mostly at the taxonomic level of family. It is also likely that families not currently in SASS/NASS or OKASS, and which occur in the more tropical regions, will need to be included in the ZASS. The sampling protocol to be adopted is as per Dickens and Graham (2002) as described below. During the development and validation phase, all macroinvertebrate samples will be collected and identified beyond the family level.

3.2.1 Sampling protocol for macroinvertebrate-based water quality monitoring

Ideally all three biotopes, namely stones (in and out of current), vegetation (in and out of current) and gravel/sand/mud, should be available for sampling at a site. However, in reality this is unlikely to be the case since many mountain stream sites do not have vegetation, while many lowland sites do not have stones. By sampling each biotope separately, variation due to differences in the availability of biotopes may be taken into consideration when interpreting biomonitoring data (Dallas 2007b). The recommended sampling protocol is as follows:

Stones

- Kick stones in current (SIC) and bedrock for 2 minutes if stones are loose and a maximum of 5 minutes if stones are immovable. Note that the above times refer to actual kicking time, and not to time spent crossing the river.

(Where the river reach is non-wadeable or too dangerous to safely sample, an alternative method will be used such as repeated drag sample throws from the bank or boat for same time periods.)

- Kick stones out of current (SOOC) and bedrock for 1 minute.

Samples collected both in and out of current are combined into a single Stones (S) biotope sample.

Vegetation

- A total length of approximately two meters of vegetation must be sampled, spread over one or more locations, especially where different kinds of marginal vegetation are present (e.g. reeds plus grasses) in different flow velocities, and aquatic vegetation for a 1m² area.

- Samples collected in and out of current and aquatic vegetation are combined into a single Vegetation (Veg) biotope sample.

Gravel, sand and mud

- Stir and sweep gravel, sand, mud (GSM) (both in and out of current) for 1 minute total.
- Samples collected in and out of current are combined into a single GSM biotope sample.

Hand picking and visual observation

- Assess the site for 1 minute and record any new taxa in the biotope where it was found by circling estimated abundance on score sheet.

Sample processing

For each of the 3 major biotopes (Stones, Veg, GSM), tip net contents into tray, remove leaves and twigs, score for 15 minutes per biotope but stop if no new taxa seen after 5 minutes. Estimate abundances as follows: 1 = 1, A = 2-10, B = 10-100, C = 100-1000, D = >1000.

For the purposes of SAFRASS, collect each sample and preserve in 70% alcohol for subsequent laboratory processing and identification.

3.2.2 Interpretation of macroinvertebrate data

The approach for data interpretation will be determined and refined once the river typology and sampling of aquatic macroinvertebrates has been completed. The likely spatial and temporal variation at reference sites will be taken into consideration when developing the data interpretation guidelines. The feasibility of developing predictive models along the lines of RIVPACS will be explored during the analysis phase, but it is likely that a simpler alternative will be developed for this pilot study.

3.3 Macrophytes: recommendations for sampling within the SAFRASS project

There is a dearth of information readily available concerning either macrophyte species present or their distribution within Zambia, with a recent report by Murphy et al. (2008) being apparently one of the first (albeit very preliminary and limited in geographical coverage) field identification guides available. Unsurprisingly given this situation, there is also no evidence for any systematic surveys of aquatic macrophytes in the country. Whilst there is some water chemistry data available (bi-yearly water quality surveys conducted by Department of Water Affairs; DWA), this has not been in conjunction with any biological surveys.

It is recommended that macrophyte surveys should follow the guidelines laid down in the international standard EN 14184:2003, and which is common to all of the methodologies detailed in Table 4:

'Visual survey of representative river stretch (record of macrophyte taxa and estimation of abundance) by wading, diving or boating, using rake, grapnel, aqua-scope where necessary'.

However, safety is of paramount importance: conditions and safety issues in tropical Africa differ greatly from those in Western Europe. Therefore, diving will be avoided and wading will also be avoided in all but small streams. For larger rivers grapnel surveys will only be conducted from a stable area of riverbank or from boats. Aqua-scopes are not considered appropriate due to the often very turbid nature of the rivers and safety issues outlined earlier.

Emergent vegetation has been found from initial surveys often to be an important component of Zambian stream vegetation (Murphy et al 2008) and will therefore be recorded and included in surveys and subsequent analyses. A constrained ordination analysis and classification of species data by TWINSpan (Lang et al 2008) showed:

- a group dominated by rooted floating-leaved species with submerged species absent, and characterised by slower flowing turbid waters with a lower pH;
- a group dominated by mixed submerged, floating and emergent species characterised by generally faster flowing, clearer water, with generally circumneutral to base pH.

There is a very apparent lack of macrophyte data and associated water quality data available for Zambia, and hence a lack of knowledge relating to the individual species as indicators of river trophic status. Therefore it will not be possible at this stage to produce a reference based monitoring methodology along the lines of those included in Table 4. Nor will it be possible to produce a methodology along the lines of the MTR, which indicates trophic status but is not reference based. However, as the SAFRASS project is a capacity building exercise its primary aim is to provide the basis for potential development of such a scheme at a later time. The methodology will therefore follow the guidelines given in Table 5 (and pertinent to the guidelines laid down in international standard EN 14184:2003).

Table 5 Summary of macrophyte sampling protocol

Survey Procedure	Details
Length of Survey Site	100m
Survey Months	May - July (High flow); October – November (low flow)
Surveyed River Compartment	Channel and banks
Recorded Channel Vegetation	All floating and submerged in channel, plus emergents present at time of sampling
Recorded Taxonomic groups (and preferred level of ID)	Bryophytes (Genus) Charophytes (Genus) Pteridophytes (Species) Spermatophytes (Species) Filamentous algae (Family)
Method of Sample	Grapnel/rake survey from boat and or bank at five equal intervals along 100m reach for submerged species; Visual assessment of emergents and non-rooted floating species along entire reach centred on each grapnel survey. Any floating fragments of normally rooted species, not otherwise recorded, noted separately.
Abundance scoring	Frequency score based on grapnel surveys.

The methodology will follow the approach of building a countrywide database of macrophyte species data, and of exploring relationships between macrophyte occurrence and abundance, and water physico-chemical factors using constrained ordination techniques. Community differentiation in samples and occurrence of indicator species within communities will be investigated using TWINSPLAN clustering.

3.4 Habitat integrity: recommendations for sampling within the SAFRASS project

Protocols for the assessment of habitat integrity vary in their level of complexity. For the purposes of the SAFRASS project it is recommended that an intermediate level be adopted as described in Dallas (2005) and outlined in Appendix A.

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Appendix A. Index of habitat integrity (from Dallas 2005)

The severity of each impact is assessed, using scores as a measure of impact (Table A1). The assessor must assign a confidence level (high, medium or low) to each criterion based on his/her knowledge of the site and catchment. High confidence would be based on the assessor having a thorough knowledge and understanding of the site and area of at least 5 kilometres upstream. Low confidence would be based on the assessor having knowledge based on the site visit only and some supplementary information (e.g. land cover). Whilst it is near impossible to remove all subjectivity involved in making Index of Habitat assessments, descriptions of each criterion are provided to assist with the assessment (Table A2).

Table A1. Summary of the scoring procedures to determine the Index of Habitat Integrity.

Impact Class	Description	Score
None	No discernible impact or the modification is located in such a way that it has no impact on habitat quality, diversity, size and variability.	0
Small	The modification is limited to very few localities and the impact on habitat quality, diversity, size and variability is limited.	1 - 5
Moderate	The modifications are present at a small number of localities and the impact on habitat quality, diversity, size and variability are fairly limited.	6 - 10
Large	The modification is generally present with a clearly detrimental impact on habitat quality, diversity, size and variability. Large areas are, however, not affected.	11 - 15
Serious	The modification is frequently present and the habitat quality, diversity, size and variability in almost the whole of the defined area are affected. Only small areas are not influenced.	16 - 20
Critical	The modification is present overall with a high intensity. The habitat quality, diversity, size and variability in almost the whole of the defined section are influenced detrimentally.	21 - 25

Weightings and calculation of instream and riparian status

Once a score has been allocated to an impact, it is moderated by a weighting system, devised by Kleynhans (1996). Assignment of weights is based on the relative threat of the impact to the habitat integrity of the riverine ecosystem. The total score for each impact is equal to the assigned score multiplied by the weight of that impact (Table A3). Based on the relative weights of the criteria, the impacts of each criterion are estimated as follows: Rating for the criterion /maximum value (25) x the weight (percent). Example: for a criterion which receives a rating of 10 in the assessment, with a weighting of 14, the impact score is calculated as follows: $10/25 \times 14 = 5.6$

The estimated impacts of all criteria calculated in this way are summed, expressed as a percentage and subtracted from 100 to arrive at a present status score for the instream and riparian components, respectively. The Index of Habitat Integrity scores (%) for the instream and riparian zone components are then used to place these two components into a specific class. These classes are indicated in Table A4.

Table A2. Descriptions of criteria used in the IHI assessment (Kleynhans 1996).

Criterion	Description
Water abstraction	Direct abstraction from within the specified river/river reach as well as upstream (including tributaries) must be considered (excludes indirect abstraction by for example exotic vegetation). The presence of any of the following can be used as an indication of abstraction: cultivated lands, water pumps, canals, pipelines, cities, towns, settlements, mines, impoundments, weirs, industries. Water abstraction has a direct impact on habitat type, abundance and size; is implicated in flow, bed, channel and water quality characteristics; and riparian vegetation may be influenced by a decrease in water quantity.
Extent of inundation	Destruction of instream habitat (e.g. riffle, rapid) and riparian zone habitat through submerging with water by, for example, construction of an in-channel impoundment such as a dam or weir. Leads to a reduction in habitat available to aquatic fauna and may obstruct movement of aquatic fauna; influences water quality and sediment transport.
Water quality	The following aspects should be considered; untreated sewage, urban and industrial runoff, agricultural runoff, mining effluent, effects of impoundments. Ranking may be based on direct measurements or indirectly via observation of agricultural activities, human settlements and industrial activities in the area. Water quality is aggravated by a decrease in the volume of water during low or no flow conditions.
Flow modification	This relates to the consequence of abstraction or regulation by impoundments. Changes in temporal and spatial characteristics of flow such as an increase in duration of low flow season can have an impact on habitat attributes, resulting in low availability of certain habitat types or water at the start of the breeding, flowering or growing season.
Bed modification	This is regarded as the result of increased input of sediment from the catchment or a decrease in the ability of the river to transport sediment. The effect is a reduction in the quality of habitat for biota. Indirect indications of sedimentation are stream bank and catchment erosion. Purposeful alteration of the stream bed, e.g. the removal of rapids for navigation is also included. Extensive algal growth is also considered to be bed medication.
Channel modification	This may be the result of a change in flow which alters channel characteristics causing a change in instream and riparian habitat. Purposeful channel modification to improve drainage is also included.
Presence of exotic aquatic fauna	The disturbance of the stream bottom during exotic fish feeding may influence, for example, the water quality and lead to increased turbidity. This leads to a change in habitat quality.
Presence of exotic macrophytes	Exotic macrophytes may alter habitat by obstruction of flow and may influence water quality. Consider the extent of infestation over instream area by exotic macrophytes, the species involved and its invasive abilities.
Solid waste disposal	The amount and type of waste present in and on the banks of a river (e.g. litter, building rubble) is an obvious indicator of external influences on stream and a general indication of the misuse and mismanagement of the river.
Decrease of indigenous vegetation from the riparian zone	This refers to physical removal of indigenous vegetation for farming, firewood and overgrazing. Impairment of the riparian buffer zone may lead to movement of sediment and other catchment runoff products (e.g. nutrients) into the river.
Exotic vegetation encroachment	This excludes natural vegetation due to vigorous growth, causing bank instability and decreasing the buffering function of the riparian zone. Encroachment of exotic vegetation leads to changes in the quality and proportion of natural allochthonous organic matter input and diversity of the riparian zone habitat is reduced.
Bank erosion	A decrease in bank stability will cause sedimentation and possible collapse of the river bank resulting in a loss or modification of both instream and riparian habitats. Increased erosion can be the result of natural vegetation removal, overgrazing or encroachment of exotic vegetation.

Table A3. Instream and riparian criteria used to develop the Index of Habitat Integrity. Each criterion is weighted (Kleynhans 1996).

Instream Criteria	Wgt	Riparian Zone Criteria	Wgt
Water abstraction	14	Water abstraction	13
Extent of inundation	10	Extent of inundation	11
Water quality	14	Water quality	13
Flow modification	7	Flow modification	7
Bed modification	13		
Channel modification	13	Channel modification	12
Presence of exotic macrophytes	9		
Presence of exotic fauna	8		
Solid waste disposal	6		
		Decrease of indigenous vegetation from the riparian zone	13
		Exotic vegetation encroachment	12
		Bank erosion	14

Table A4. Habitat Integrity classes (from Kleynhans 1999).

Class	Description	Score (% Of Total)
A	Unmodified, natural.	90 - 100
B	Largely natural with few modifications. A small change in natural habitats and biota may have taken place, but the assumption is that ecosystem functioning is essentially unchanged.	80 – 89
C	Moderately modified. A loss or change in natural habitat and biota has occurred, but basic ecosystem functioning appears predominately unchanged.	60 - 79
D	Largely modified. A loss of natural habitat and biota and a reduction in basic ecosystem functioning is assumed to have occurred.	40 - 59
E	Seriously modified. The loss of natural habitat, biota and ecosystem functioning is extensive.	20 - 39
F	Modifications have reached a critical level and there has been an almost complete loss of natural habitat and biota. In the worst cases, the basic ecosystem functioning has been destroyed.	0 - 19