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The South African Scoring System (SASS) Version 5 Rapid Bioassessment Method for Rivers

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Summary
The assessment of biota in rivers is a widely recognized means of determining the condition or 'health' of rivers. Benthic macroinvertebrates, in particular, are recognized as valuable organisms for bioassessments, due largely to their visibility to the naked eye, ease of identification, rapid life cycle often based on the seasons and their largely sedentary habits. Numerous bioassessment techniques have been developed over the last three decades, varying in complexity and region of implementation. South Africa has an exemplary history in this field, culminating in the refinement of invertebrate and other techniques and their application in a National River Health Programme. The method presented here is a refinement of the highly successful SASS (South African Scoring System) method developed by Chutter (1994), which forms the backbone of this programme. This paper takes the method to a level where it can, and has been, accredited to ISO standards. The principal changes made include the tighter definition of the technique and the sampling and analytical methods, as well as the introduction of quality control procedures. Some changes have also been made to the list of invertebrates used in this method
Field trials were conducted to test the variability of the method. Of the various indices available to the method, the ASPT is the most consistent over all biotopes (lowest CV%). On the other hand, of the biotopes examined the Gravel/Sand/Mud (GSM) combination is the most variable with respect to the SASS Score and number of taxa encountered. The spatial variability on a reach of river with similar water quality characteristics was found to be statistically negligible. However, one generally finds that statistically significant differences occur between the SASS Scores and the number of taxa counted by different operators. The ASPT, on the other hand, is a more consistent and repeatable measure of river health assessment and, within a given reach of river and considering all biotopes, the differences in results produced by different operators were statistically negligible. The results highlight the need for appropriate competency-based training and consistent application of the method.

Keywords: bioassessment, aquatic invertebrates, biotic index, method, health, water quality, rivers, South Africa

Introduction

The world has recently seen the proliferation of techniques used for the rapid bioassessment of rivers, many of which are reviewed in books such as those by Rosenberg and Resh (1993) and Metcalf-Smith (1994). These techniques are used for the assessment of general river condition or health as influenced by a variety of factors but principally water quality. Many of these methods have been promoted by regulatory authorities who see bioassessment data as valuable for the management of aquatic resources. Spearheading this approach in its level of sophistication has probably been the RIVPACS method developed by Wright *et al.* (1984), which has been implemented in the United Kingdom and in Australia after some adaptation.

South Africa has also experienced a surge of support for this type of river assessment, expanding from rather hesitant beginnings nearly three decades ago when Chutter (1972) developed a Biotic Index. This index was never widely used (Chutter 1998) as it

was excessively labour-intensive. In the 1990s Chutter set out to develop an index that would be faster and easier, basing it on the BMWP method developed earlier in the UK (described in Walley and Hawkes, 1996). His index, called SASS (South African Scoring System), evolved through several iterations of refinement, receiving the input of a large group of practitioners via a *SASS Forum* hosted by the South African Water Research Commission. This culminated in the publication of the method, in brief, in 1994 and more fully in 1998 (Chutter 1994, 1998). Right from the first version that appeared as a draft even before 1994, the method was enthusiastically embraced by practitioners around the country as it was found to be fast and cost-effective. Over recent years the method has become the standard for the rapid bioassessment of rivers in South and, indeed, southern Africa. It now forms the backbone of the National River Health Programme (Uys *et al*. 1996) and is increasingly being included in the determination of the Ecological Reserve as required by the South African National Water Act (1998). SASS has been recommended for the determination of the flow requirements of rivers (O'Keeffe and Dickens 2000) and has also been used for many impact assessments, such as reported in Dickens and Graham (1998), but also in others that remain unpublished. The method is being used by institutions such as Cape Metro Council, Umgeni Water, Umlaas Irrigation Board, Mpumalanga Parks Board, the Department of Water Affairs and Forestry, CSIR and many others including forestry companies and heavy industry. The method has undergone extensive testing, in particular by Dallas (1995, 1997, 2000a, 2000b, 2001), who investigated its performance in a variety of circumstances.

Over time, the inevitable deficiencies in the SASS version 4 method have come to light. These include the following:

1. the documented method was not sufficiently prescriptive, resulting in excessive variability in the way the method was being practiced, with the results all being presented under the name of SASS,
2. the method initially allowed for samples from all biotopes (biological habitat types) to be combined into a single assessment tray, resulting in a loss of resolution (but greater cost saving). Where biotope samples were kept separate, this was not done in any consistent way,
3. there was general dissatisfaction with the way that the cased Trichoptera were dealt with, where no identification was necessary. According to de Moor *et al.* (1997) identification, to family level at least, was essential to prevent erroneous results due to the variable appearance of Leptocerid cases.
4. some invertebrate taxa that had been excluded from the original method warranted inclusion and the tolerance values of some groups required re-assessment.

Because of these deficiencies, a revision to the SASS version 4 method was necessary, and is the subject of this paper. This revision was based on the requirements of ISO 17025 (ISO 1999) and has been successfully accredited in the authors' laboratory. Note that many additional requirements of ISO accreditation apply to the specific laboratory, and include issues such as traceability of results, training, etc. These issues have not been included in this paper.

The procedure itself has undergone significant change, most of which was done with the participation and approval of a large working group of 27 people, convened in late 2000, which contributed suggestions to rectify the deficiencies noted above. The most controversial aspect was whether to combine samples from similar substrate biotopes (e.g. all stone, all vegetation) or to combine samples from similar hydraulic biotopes (e.g. all in-current samples). The final method reported below is the culmination of all of this group's input.

Scope

SASS is suitable for the assessment of river water quality and river health.
Further to statements by Roux (1997), biomonitoring (including SASS) may be used to:

1. Assess the ecological state of aquatic ecosystems

2. Assess the spatial and temporal trends in ecological state
3. Assess emerging problems
4. Set objectives for rivers
5. Assess the impact of developments
6. Predict changes in the ecosystems due to developments
7. Contribute to the determination of the Ecological Reserve (National Water Act 1998).

Interference

The method is designed for low/moderate flow hydrology and is not applicable in wetlands, impoundments, estuaries and other lentic habitats. It has also not been sufficiently tested in ephemeral rivers and so should be used with caution. The method works best when the diversity of biotopes is wide and includes riffles or rapids, but it also produces valuable results from poor habitats. It is necessary to interpret the data in relation to habitat quality, availability and diversity and ultimately also in relation to the ecoregion from which it comes. It is also necessary to interpret the data in relation to the season of collection, as some natural variation will occur during the course of the year and between years.

Hazards

1. Close contact with polluted and pathogen-infected water and the dangers of drowning demand that care be taken with health issues.
2. Water-proof boots, arm-length gloves, waders and life jackets should be available for protection when sampling.
3. A life-jacket should be used when using waders in deep water, but this judgement is left to the operator. It is important that the operator has the ability to swim.
4. Caution must be exercised in deep, soft mud and in rivers with dangerous animals, especially snakes and crocodiles.
5. It is recommended that sampling immediately upstream of strongly flowing drains or culverts should be avoided.
6. A threat exists where boots become lodged between rocks, especially in strong currents (a knife should be carried and used to cut away a boot if stuck).
7. When preserving samples, formalin and ethanol may be used. The operator should be acquainted with the dangers and treatments associated with each of these chemicals, which are dangerous and should be handled with care.
8. Sampling should not be performed in rivers with flows high enough that they are likely to endanger the operator, or other adverse conditions. It is the prerogative of operator to desist from sample collection if he/she considers conditions in the river or surroundings to be unsafe.

Sample collection

A sample should not be collected when a river is in flood, because the collection will not be a true representation of the biota at the site. Judgment of this is left to the operator, but a description of river flow must always be indicated on the SASS form. Various biotopes, as defined below, are a combination and simplification of those defined by Dallas (2000a) after extensive consultation with users. These are sampled using the specified net in different ways. *NB: It is important that a sample is collected over a wide area to ensure that the full variability of the biotope is sampled. Where possible, for example, a stones-in-current sample should be collected across the width of the river and from the top to the bottom of the riffle. Neighbouring riffles should also be sampled if they look different. Avoid pools or backwaters adjacent to, but <u>not connected</u> to, the river as these would contain water unrelated to the river itself.* It is important that the procedures described below are adhered to. SASS does not set out to provide a full inventory of *all* inhabitants of a river, so increased sampling time, for example, would result in an inflated *and erroneous* score. Where the sample effort is exceeded during a

survey (for example during a Reserve assessment), this must be noted on the SASS score sheet and in the relevant database. Comparisons of this data with regular SASS data should be done with caution and should, where possible, be limited to comparisons between ASPT results, as these are less affected by sample effort.

1. **Stone (S) biotopes:**

   Stones in current (SIC), bedrock, or any solid object in current

   Stones in current are free/loose stones (pebbles and cobbles) of 2-25 cm average size situated where the movement of water prevents the settling out of fine silt. Bedrock includes boulders greater than 25 cm, large sheets of rock, waterfalls and chutes. The net should be placed close to but downstream of the stones to be kicked, in a position where the current will carry the dislodged biota into the net. Where possible the stones should be kicked or turned over against each other to dislodge the invertebrates. Stones, in particular bedrock, may also be rubbed with the hands or boots. Hold the net sufficiently far from the feet as to avoid picking up coarse sediments, which will sink to the bottom faster than the dislodged invertebrates. The kicking of stones should continue for *approximately two minutes*. If the stones are embedded or difficult to move, especially bedrock, then sampling may continue for up to a *maximum of five minutes*, which must be recorded. Note that the above times refer to actual kicking time, and not to time spent crossing the river.

   Stones out of current (SOOC), bedrock or any solid object out of current

   SOOC are moveable stones (pebbles and cobbles averaging 2-25 cm) out of any perceptible current such that fine sediments settle on their upper surfaces. Bedrock includes boulders greater than 25 cm and large sheets of rock.
   Stones, bedrock or other solid objects should be sampled for *approximately one minute* by kicking, turning or scraping them with the hands and/or feet, whilst continuously sweeping the net through the disturbed area.

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

2. **Vegetation (Veg) biotopes:**

   Marginal vegetation

   This is vegetation hanging into or growing at the edge of the stream, often emergent, both in current (MVegIC) and out of current (MVegOOC).
   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. The net is pushed vigorously into the vegetation moving backwards and forwards through the same area. Particular care should be taken in strong currents to catch dislodged invertebrates. To avoid the tendency to collect organisms from above the water surface, the net should be kept below the surface. It is useful to record the dominant plant species, as this will give insight into the type of biotope present.

   Aquatic vegetation

   This is vegetation not confined to the river banks, is largely submerged and includes filamentous algae and the roots of floating aquatics such as water hyacinth. In order to dislodge and collect invertebrates, the net is pushed repeatedly against and through the vegetation under the water over an area of *approximately one square meter*.

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

3. **Gravel, Sand & Mud (GSM) biotopes:**

Samples are collected by ranging between all of the following three biotopes (where available) in a variety of water currents for *approximately one minute total*;

Gravel

Gravel is made up of small stones <2 cm in size. The gravel should be stirred by shuffling or scraping with the feet, whilst continuously sweeping the net over the disturbed area to catch dislodged biota.

Sand

Sand grains are < 2 mm diameter. The sand should be stirred by shuffling the feet, whilst continuously sweeping the net over the disturbed area to catch dislodged biota. A few seconds should be allowed for large sediments to settle before sweeping the net, otherwise excessive sediment will be collected. Collection effort should concentrate on slow flowing or still water.

Mud

Mud, silt and clay are particles < 0.06 mm diameter. Mud should be stirred by shuffling with the feet whilst sweeping the net over the disturbed area to catch dislodged biota, but avoiding collecting mud into the net.

Samples collected in and out of current are combined into a single *Gravel, Sand & Mud (GSM)* biotope sample.

4. Hand-picking and visual observation:

During sampling, approximately 1 minute of 'hand-picking' for specimens that may have been missed by the sampling procedure must be carried out. Commonly, this will also involve noting (not necessarily collecting) snails and fast moving pond skaters. In very thick mud, lumps of substrate should be dug out and broken into pieces with the hands, looking for burrowing organisms. Extra taxa thus recorded should be added to the SASS sheet under the biotope with which they are most clearly associated (e.g. pond skaters swimming over sand would go into the GSM biotope).

Sample preparation

Once collection is complete, each of the above samples should be washed down to the bottom of the net (repeatedly, until the water passing through the net runs clear), then carefully tipped into separate trays by inverting the net. The net should be flushed out with water to ensure that biota do not remain in it. The net should be checked and all remaining specimens taken out, using forceps, and placed into the tray. Sufficient clean water is then added to the tray to immerse the sample. It is important to have a clean sample with all or most of the fine sediment removed. Before the identification of organisms can begin, the larger obstructing leaves, twigs and other loose debris and stones can be removed from the tray. They should be shaken in the water and checked for clinging biota before being removed. It is useful to retain this debris when out of the water, as clinging organisms will frequently emerge after a time and can then be recorded.

It is recommended that three separate trays be used, one for each of the biotope samples. It is advantageous to allow samples to stand while other biotopes are being examined, as many invertebrates emerge from the debris after a time.

Preservation

Although samples should be examined in the field, they may be preserved for laboratory identification, long term storage or for auditing of the samples. Preservation is done in different ways depending on the needs of the situation.

Cold preservation: On occasions it may be difficult to analyse the sample on-site, in which case it may be brought back *alive* to the laboratory. This should only be done

when circumstances in the field are not conducive to on-site analysis. The sample must be drained of *all* its free water and placed immediately into a ~2 litre clean lidded bucket and into a cold-box containing cold ice-packs. On returning to the laboratory the sample can be refrigerated for up to 72 hours at just above freezing point. It should be removed from the refrigerator 30 minutes prior to analysis, tipped into a tray with clean water and allowed to warm up to room temperature, whereupon the organisms should become active again. With the aid of a light the sample can then be analysed as normal, with the normal 15 minute time constraint.

Chemical preservation: Wet samples may be preserved for later checking in jars/buckets to which approximately 10% formalin (40% formaldehyde) is added, or individual organisms may be picked out and put into vials containing 70 % ethanol. Preserved samples are best labelled with a pencil on a slip of water-resistant paper inserted with the sample. *Note that chemical preservation is intended for further investigating the contents of a sample or for the long-term collection of samples. A result derived from a chemically preserved sample should not be called a SASS result.*

Apparatus

The minimum amount of equipment necessary is: a soft 1mm mesh net on a 30 cm square frame on a stout handle, white or cream flat-bottomed tray/s (approximately 'A3' or 30x45 cm size and 10 cm deep), forceps, soft plastic wide-mouth pipettes, timer, magnifying lens, a pair of boots or appropriate foot protection and several SASS scoring sheets, or a data logger, or other means of capturing data.

The following articles may also be useful: waders, life jacket, field identification books, towel, antiseptic soap, clean water (for drinking and adding to samples), funnel, gloves, sample collection bottles and pill vials, pH meter, DO meter, conductivity meter, table and chair, two-way radio, grass-slasher and spade. Specially prepared chemistry and microbiology sample bottles are useful for collecting water samples.

Reagents

Concentrated (40%) industrial or analytical grade formaldehyde and 70% industrial grade ethanol.

Preparation of standards

No standards are used for this analysis

Analytical Quality Control

Quality Control (QC) for this method comprises three possible aspects, each of which contributes to overall quality control:

1. Intra-laboratory Proficiency Testing.
2. External auditing of samples.
3. Inter-laboratory Proficiency Testing.

Depending on what the data is to be used for, a given laboratory may or may not choose to follow these procedures. *(Note that any quality control procedure requires that action be taken in response to the QC procedure, to correct and improve performance).* QC should also, where possible, be quantitatively measured. All data produced in the absence of QC procedures, or produced by an individual who has failed to meat QC standards, should be treated with circumspection.

1. Intra-laboratory Proficiency Testing.

This procedure is suitable for larger institutions where two or more persons are using SASS. This internal assessment has two aspects:

- At an appropriate frequency, possibly six monthly but depending on the needs of the organisation, a second operator will re-sample a river site sampled by the first operator, not more than 48 hours after the original sample was collected and on the condition that no weather changes of a nature that could have altered the SASS results (e.g. floods) have subsequently taken place. This site must also be without threat of exposure to alterations in water quality, such as may happen below a wastewater works.
- At an appropriate frequency, possibly six monthly but depending on the needs of the organisation, a skilled SASS operator will accompany a junior operator into the field to several sites. He/she will monitor the collection procedure for compliance with the method. A quantitative audit of the above proficiency testing is encouraged.

The samples used in both the above procedures should be preserved and returned to the laboratory. Discrepancies should be investigated.

2. External auditing of samples

All samples collected in the field for routine analysis should be returned to the laboratory, after the addition of just sufficient formalin to immobilise the invertebrates. On arrival in the laboratory, an independent person selects a percentage of the samples, covering a range of water quality conditions, for external audit.

The selected audit samples should be concentrated by removing most of their water and placing them into smaller bottles. They should then be comprehensively preserved by adding approximately 10% formalin (40% formaldehyde). The samples are then forwarded, together with the SASS score sheet, to an external auditor for re-analysis. If the samples are to be subjected to excessive disturbance during transportation, the liquid should be drained from them. Allowance should be made for the fact that the original analysis was done on a live sample in the field, whereas the audit will be done on a preserved sample. It is unnecessary for the auditor to investigate the sample exhaustively, but only until satisfied that the submitted score sheet is a fair reflection of the sample. Missing and additional taxa detected by the auditor should be recorded and a quantitative audit carried out. Queries on identification and erroneously-recorded taxa should be followed up.

3. Inter-laboratory Proficiency Testing Scheme.

A Proficiency Testing Scheme (PTS) has been initiated as part of the National River Health Programme. Essentially, it entails passing a single preserved sample, either in formalin or cast in resin, from laboratory to laboratory, with data sheets fed back to a single coordinator. From the returned data sheets, a “model” score sheet is compiled, against which individual sheets are compared. Performance is assessed quantitatively and information fed back to participants. Whilst this is not an accurate assessment of a person’s capacity to perform SASS, as it does not include field work, it does provide a measure of the person’s identification skills using dead organisms. A more comprehensive Inter-laboratory PTS can be carried out by conducting field assessments, which would thus include all aspects of SASS sampling and analysis.

Analytical Procedure

Organisms as listed on the SASS 5 scoring sheet (Table 1) are identified to family level. Each taxon has been assigned a ‘quality’ score, based on its susceptibility or resistance to pollution and perturbations. The lowest scores are assigned to the taxa that are resistant and the highest score to those susceptible to pollution. Viewing and identification is done for a maximum of 15 minutes per biotope but, if no new taxon is seen for approximately 5 minutes, the operation should be stopped. A lens may be used at this stage to check on characteristic features and should always be available. Taxa seen are ticked off under the appropriate biotope heading before combining the three columns into a single total column. Where organisms are too young to allow accurate

identification, the presence of these should be noted in the Comment box on the sheet. The abundance of organisms within each taxon is roughly estimated (i.e. a single individual is recorded as '1', from 2 to 10 is allocated an 'A', from 10 to 100 a 'B', from 100 to 1000 a 'C' and > 1000 a 'D'). Upon completion of sample identification and scoring the sample is either returned to the river or preserved for returning to the laboratory.

Additional information required on the SASS sheet (Table 1) must be captured, e.g. site code and description, signs of disturbance etc. This information is valuable during later interpretation of the results. A useful evaluation of the diversity of the biotopes, and thus their of ability to support a diverse invertebrate population at the site, is gained by rating them, with a value of 1 for a biotope of limited diversity, up to 5 for a biotope with wide diversity. For example, a SIC biotope of limited diversity would be one with low numbers of stones embedded in the substrate, with no undersurfaces available, occurring in a current of uniform but slow velocity. A SIC of wide diversity would have a range of stone sizes, all of them loose and easy to move, with accessible undersurfaces, occurring in a range of flow velocities. Marginal vegetation of low diversity could be just the bases of *Phragmites* reeds, while high diversity could comprise a mixture of reeds, sedges and fine grasses.

Table 1. The SASS Version 5 score sheet.
(insert table)

SASS Version 5 Score Sheet

Version date: Sept 2005

Date (dd:mm:yr):			(dd.ddddd)	**Biotopes Sampled (tick & rate)**	**Rating (1 - 5)** / **Time (min)**
RHP Site Code:		**Grid reference (dd mm ss.s) Lat:**	S	**Stones In Current (SIC)**	
Collector/Sampler:		**Long:**	E	**Stones Out Of Current (SOOC)**	
River:		**Datum (WGS84/Cape):**		**Bedrock**	
Level 1 Ecoregion:		**Altitude (m):**		**Aquatic Veg**	
Quaternary Catchment:		**Zonation:**		**MargVeg In Current**	
	Temp (°C):	**Routine or Project? (circle one)**	**Flow:**	**MargVeg Out Of Current**	
Site Description:	**pH:**	**Project Name:**	**Clarity (cm):**	**Gravel**	
	DO (mg/L):	**Thukela survey @ Mandini**	**Turbidity:**	**Sand**	
	Cond (mS/m):		**Colour:**	**Mud**	
	Riparian Disturbance:	Limited - some subsistence farming		**Hand picking/Visual observation**	
	Instream Disturbance:	None			

Taxon	QV	S	Veg	GSM	TOT
PORIFERA (Sponge)	5				
COELENTERATA (Cnidaria)	1				
TURBELLARIA (Flatworms)	3				
ANNELIDA					
Oligochaeta (Earthworms)	1				
Hirudinea (Leeches)	3				
CRUSTACEA					
Amphipoda (Scuds)	13				
Potamonautidae* (Crabs)	3				
Atyidae (Freshwater Shrimps)	8				
Palaemonidae (Freshwater Prawns)	10				
HYDRACARINA (Mites)	8				
PLECOPTERA (Stoneflies)					
Notonemouridae	14				
Perlidae	12				
EPHEMEROPTERA (Mayflies)					
Baetidae 1sp	4				
Baetidae 2 sp	6				
Baetidae > 2 sp	12				
Caenidae (Squaregills/Cainflies)	6				
Ephemeridae	15				
Heptageniidae (Flatheaded mayflies)	13				
Leptophlebiidae (Prongills)	9				
Oligoneuridae (Brushlegged mayflies)	15				
Polymitarcyidae (Pale Burrowers)	10				
Prosopistomatidae (Water specs)	15				
Teloganodidae SWC (Spiny Crawlers)	12				
Tricorythidae (Stout Crawlers)	9				
ODONATA (Dragonflies & Damselflies)					
Calopterygidae ST,T (Demoiselles)	10				
Chlorocyphidae (Jewels)	10				
Synlestidae (Chlorolestidae)(Sylphs)	8				
Coenagrionidae (Sprites and blues)	4				
Lestidae (Emerald Damselflies/Spreadwings)	8				
Platycnemidae (Stream Damselflies)	10				
Protoneuridae (Threadwings)	8				
Aeshnidae (Hawkers & Emperors)	8				
Corduliidae (Cruisers)	8				
Gomphidae (Clubtails)	6				
Libellulidae (Darters/Skimmers)	4				
LEPIDOPTERA (Aquatic Caterpillars/Moths)					
Crambidae (Pyralidae)	12				

Taxon	QV	S	Veg	GSM	TOT
HEMIPTERA (Bugs)					
Belostomatidae* (Giant water bugs)	3				
Corixidae* (Water boatmen)	3				
Gerridae* (Pond skaters/Water striders)	5				
Hydrometridae* (Water measurers)	6				
Naucoridae* (Creeping water bugs)	7				
Nepidae* (Water scorpions)	3				
Notonectidae* (Backswimmers)	3				
Pleidae* (Pygmy backswimmers)	4				
Veliidae/M...veliidae* (Ripple bugs)	5				
MEGALOPTERA (Fishflies, Dobsonflies & Alderflies)					
Corydalidae (Fishflies & Dobsonflies)	8				
Sialidae (Alderflies)	6				
TRICHOPTERA (Caddisflies)					
Dipseudopsidae	10				
Ecnomidae	8				
Hydropsychidae 1 sp	4				
Hydropsychidae 2 sp	6				
Hydropsychidae > 2 sp	12				
Philopotamidae	10				
Polycentropodidae	12				
Psychomyiidae/Xiphocentronidae	8				
Cased caddis:					
Barbarochthonidae SWC	13				
Calamoceratidae ST	11				
Glossosomatidae SWC	11				
Hydroptilidae	6				
Hydrosalpingidae SWC	15				
Lepidostomatidae	10				
Leptoceridae	6				
Petrothrincidae SWC	11				
Pisuliidae	10				
Sericostomatidae SWC	13				
COLEOPTERA (Beetles)					
Dytiscidae/Noteridae* (Diving beetles)	5				
Elmidae/Dryopidae* (Riffle beetles)	8				
Gyrinidae* (Whirligig beetles)	5				
Haliplidae* (Crawling water beetles)	5				
Helodidae (Marsh beetles)	12				
Hydraenidae* (Minute moss beetles)	8				
Hydrophilidae* (Water scavenger beetles)	5				
Limnichidae (Marsh-Loving Beetles)	10				
Psephenidae (Water Pennies)	10				

Taxon	QV	S	Veg	GSM	TOT
DIPTERA (Flies)					
Athericidae (Snipe flies)	10				
Blepharoceridae (Mountain midges)	15				
Ceratopogonidae (Biting midges)	5				
Chironomidae (Midges)	2				
Culicidae* (Mosquitoes)	1				
Dixidae* (Dixid midge)	10				
Empididae (Dance flies)	6				
Ephydridae (Shore flies)	3				
Muscidae (House flies, Stable flies)	1				
Psychodidae (Moth flies)	1				
Simuliidae (Blackflies)	5				
Syrphidae* (Rat tailed maggots)	1				
Tabanidae (Horse flies)	5				
Tipulidae (Crane flies)	5				
GASTROPODA (Snails)					
Ancylidae (Limpets)	6				
Bulininae*	3				
Hydrobiidae*	3				
Lymnaeidae* (Pond snails)	3				
Physidae* (Pouch snails)	3				
Planorbinae* (Orb snails)	3				
Thiaridae* (=Melanidae)	3				
Viviparidae* ST	5				
PELECYPODA (Bivalves)					
Corbiculidae (Clams)	5				
Sphaeriidae (Pill clams)	3				
Unionidae (Perly mussels)	6				
SASS Score					
No. of Taxa					
ASPT					
Other biota:					

Comments/Observations:

Procedure:

Kick SIC & bedrock for 2 mins, max. 5 mins. Kick SOOC & bedrock for 1 min. Sweep marginal vegetation (IC & OOC) for 2m total and aquatic veg 1m^2. Stir & sweep gravel, sand, mud for 1 min total. * = airbreathers

Hand picking & visual observation for 1 min - record in biotope where found (by circling estimated abundance on score sheet). Score for 15 mins/biotope but stop if no new taxa seen after 5 mins.

Estimate abundances: 1 = 1, A = 2-10, B = 10-100, C = 100-1000, D = >1000 S = Stone, rock & solid objects; Veg = All vegetation; GSM = Gravel, sand, mud SWC = South Western Cape, T = Tropical, ST = Sub-tropical

Rate each biotope sampled: 1=very poor (i.e. limited diversity), 5=highly suitable (i.e. wide diversity) Rate turbidity: V low, Low, Medium, High, Very High

Rate flows: Zero, trickle, low, medium, high, flood Rate colour: transparent, tea brown, light brown, dark brown, light green, dark green, yellow, red, grey, milky white, black

Calculation of Results

The calculation of results is done by totalling the scores of each taxon recorded in the Total column (= SASS Score), counting the number of taxa found (= No. Taxa) and dividing the former by the latter (= ASPT – Average Score per Taxon). While separate results may be calculated for each biotope and used in various investigations, only the result calculated from the Total column will represent the SASS5 result for a site. *Do not try to combine the scores from the three biotopes by adding the Score, number of taxa and ASPT and dividing the total by three.*

Note: When adding the number of taxa:

1. Baetidae, (any number of species) must be counted as only ONE taxon.
2. Hydropsychidae, (any number of species) must be counted as only ONE taxon.
3. For those taxa which require the number of types/species to be recorded, namely Baetidae and Hydropsychidae, the Total column should reflect the total number of types/species found at that site, without duplication, e.g. species A may appear in the SIC and Veg biotopes but will only be considered as a single species in the Total column assessment. Similarly, a single Species B in the SIC and a single Species C in the Veg must be considered as two species in the Total column assessment.

Interpretation of data

SASS data is meaningful only when assessed together with the various factors that may influence the scores. Most important of these are measures of habitat quantity, quality and diversity. A method of habitat assessment developed by McMillan (1998) is available, although, at the time of writing, this method is still under development. Other methods include those by Roux *et al.* (1994) and Plafkin *et al.* (1989) but these are less suited to invertebrate assessments than to fish. The principle to be aware of is that, where habitat diversity is poor, there will be less biotic diversity and consequently a lower SASS Score. ASPT will be less affected (Dallas 1997, Chutter 1998), because the few organisms present may have the appropriate sensitivity. The ASPT score may be depressed where, for example, a sand bed river in pristine condition may produce a low ASPT, as it will be occupied by hardy, adaptable taxa. Chutter (1998) also points out that ASPT is a more reliable measure of the health of good quality rivers [as opposed to poor quality rivers] than SASS Score is.

Ancillary analyses can also be useful for the interpretation of SASS data. Most common are measures of temperature, dissolved oxygen, conductivity and pH. All these are only as good as the method and instruments used. It is advised that, if used, quality control procedures should be implemented.

As yet, there is no definitive reference which provides objective guidance on the interpretation of SASS data. An investigator must always assess the data produced with intelligence and an open mind. A sound knowledge of aquatic ecology and biology will assist in interpreting the numerous combinations of biotic and abiotic situations that are found in the environment. It is important to look widely in order to discover the reasons for some scores – the scores themselves may be accurate enough, but the cause behind an inexplicable result may be elusive. In order to gain background information on the use of SASS in research, several references may be useful, including: Roux (1993), Chutter (1998), Dallas (1995, 1997, 2000a,b), Dickens and Graham (1998).

At the time of writing, a procedure for the determination of the Water Quality Reserve (DWAF, unpublished) is in draft form and contains boundaries for SASS data which define Natural, Good, Fair and Poor rivers. Chutter (1998) and Dallas (1997) also presented boundaries of this type.

Note that taxa marked with an * on the score sheet are air-breathers – which information may be used as an indication of the prevalence of taxa relying on air for oxygen.

Sources of Error

1. The SASS5 method should be used only under the conditions for which it is designed. It is not accurate in wetland, lentic or estuarine conditions or in rivers recently subjected to heavy flooding and should be used with caution in ephemeral rivers.
2. Habitat variability and high flows may lead to the incorrect interpretation of results.
3. This is an *in situ* type of analysis and needs to be performed in daylight and in reasonable weather conditions. Analysis of the sample in the laboratory should only be done if the sample is alive. Chemically preserved samples may produce different results. Results from preserved samples should be compared to field data with circumspection and should not be called SASS results.
4. Invertebrate biota are *not* spread uniformly across a river, even within a single biotope. For this reason, the sample must be collected across the full range of the biotope available. If this is not done error may be introduced.
5. The amount of sampling effort is important and so the method attempts to regulate this by stipulating the duration and/or area/length of sample collection. Nevertheless, different people will collect different samples under the same conditions, so it is important that every effort be made to ensure that sampling effort is as constant as possible. For this reason the guidelines for sample collection must be strictly adhered to. Where sampling effort is deliberately exceeded (for example during more detailed river surveys) then this must be indicated and that result compared with regular SASS data with circumspection. It is also important to note that 'sampling time' refers to actual collection time, not including time walking between sections of the river.
6. The same applies to the analysis of the sample, which is also subject to the skill and effort put in by the operator. Every effort must be made to keep this factor as constant as possible. Times must be adhered to. Remember also that two pairs of eyes used would require a shorter analysis time.
7. Misidentification of taxa is always a possibility and should be minimized through adequate training in SASS techniques. Participation in a Proficiency Testing Scheme is designed to assist in overcoming this problem. *(Note: The SASS system does not cover ALL possible aquatic invertebrate taxa. The user is encouraged to send unfamiliar specimens to experts for identification).*

Variability and error associated with the SASS5 method

Two key questions, related to the inherent variability associated with the SASS5 technique, were identified as the following:

1. are there significant differences in SASS results between sites on the same reach of river?, and
2. are there significant differences in SASS results between operators on the same reach of river?

For the purposes of statistical testing, these questions could be expressed in terms of the null hypothesis as:

1. H_0: there are no significant differences (in SASS results) between sites sampled on the same reach of river
   and;
2. H_0: there are no significant differences (in SASS results) between operators on the same reach of river (for any site sampled)

To test these questions, a SASS5 "field-day" was organised in late 2001. Six relatively "competent" SASS operators were assembled for the investigation, their experience ranging from those who had been actively performing SASS from its earliest conception (SASS1) to those recently trained in the method (SASS5). Prior to sampling, all six were provided with refresher-training on the finer points of the new SASS5 method (as compared to previous versions of SASS), particularly with respect to the separation of biotopes and the timing and extent of sampling required within these biotopes.

A relatively "pristine" reach of the Karkloof River, just above its confluence with the uMngeni, in kwaZulu-Natal, South Africa (29º 26' 23"S; 30º 25' 58"E) was chosen for the trial. By local standards, this site is traditionally characterised by high SASS Scores and is known to have a good range and extent of available biotopes. As an indication of the quality of the river at this site the average of twenty-nine SASS4 surveys, collected over the previous nine years, was an ASPT of 7.3. It was assumed that this particular reach of river had uniform water quality flowing through it for the duration of the exercise.

During this exercise some 100m of river reach was surveyed. This reach was broken up into three discrete sampling areas or sites, each with a good representation of biotopes. This gave all six operators relatively easy access to undisturbed (un-sampled) biotopes. Sampling was conducted sequentially from the lowest sites (down-river) up to the highest sites, so as to avoid errors associated with invertebrate drift. During sampling an independent observer signaled the start and end of all timed samplings within the Stones biotope, although operators were allowed to add extra time at their discretion if they felt the biotope had not been adequately sampled (eg non-sampling time spent ranging across the site). Generally, the SASS5 protocol for sampling and analysis was strictly adhered to for each of the three sites within the study area/river reach. The SASS5 data sheets were then collated and statistically analysed. Summary statistics for each of the respective SASS indices in respective biotopes are presented in Table 2.

Table 2: Summary statistics for each of the SASS indices in respective biotopes

Biotope	SASS Index	n	mean	median	minimum	maximum	std.dev	CV %
All Biotopes Combined	Score	18	183	180.50	128	275	37.65	20.60
	No. Taxa	18	27.1	26.50	17	39	5.63	20.78
	ASPT	18	6.76	6.75	6	8	0.44	6.49
Stones	Score	18	135	136.00	74	213	31.26	23.21
	No. Taxa	18	18.3	18.00	9	30	4.52	24.74
	ASPT	18	7.43	7.32	6	9	0.77	10.40
GSM	Score	18	96.2	93.00	51	175	35.30	36.69
	No. Taxa	18	14.7	13.50	8	26	4.79	32.66
	ASPT	18	6.51	6.38	5	8	0.76	11.74
Vegetation	Score	18	78.6	80.00	33	133	20.89	26.59
	No. Taxa	18	12.2	12.50	4	19	3.15	25.81
	ASPT	18	6.5	6.55	5	8	0.77	11.86

Results of the first objective, i.e. testing whether there are significant differences (in SASS results) between sites sampled, are presented in Table 3 (Note: data collected by all operators were pooled together for each site).

Table 3: Summary results and statistics for SASS indices over 3 sites sampled (by six SASS operators) on the lower Karkloof River (KZN)

Biotope	SASS Indices	Site 1	Site 2	Site 3	mean	std. dev.
All Biotopes Combined	Score	177.00	185.33	185.83	182.72	4.96
	No. Taxa	26.00	27.67	27.67	27.11	0.96
	ASPT	6.89	6.72	6.67	6.76	0.12
	Score	130.50	139.33	134.17	134.67	4.44

Stones	No. Taxa	16.83	19.50	18.50	18.28	1.35
	ASPT	7.87	7.17	7.24	7.43	0.38
	Score	92.67	106.50	89.50	96.22	9.04
GSM	No. Taxa	14.17	15.83	14.00	14.67	1.01
	ASPT	6.54	6.70	6.29	6.51	0.21
	Score	66.67	83.67	85.33	78.56	10.33
Vegetation	No. Taxa	11.17	12.50	13.00	12.22	0.95
	ASPT	6.28	6.69	6.53	6.50	0.20

Analysis of variance (ANOVA) of these results indicated *no statistically significant difference between sites* ($p>0.05$), as measured by any SASS index. Or, in other words, sites monitored on the Karkloof River were similar to each other as characterised by respective SASS indices (low spatial variability on this particular reach). The implication of this was that any variation in the data, arising from subsequent analyses, could primarily be ascribed to 'operator differences' rather than to differences in the characteristics (available biotopes, etc.) of the river reach.

Results of the investigation to answer the second question, i.e. "are there significant differences in SASS (results) between operators on the same reach of river?" are summarised in Table 4 and Figures 1 & 2. Two forms of analysis of variance, parametric and non-parametric, were used to answer different aspects of this question.

Table 4: Summary results of analysis of variance (by ANOVA and the Kruskal-Wallis non-parametric method), and corresponding statistical significance, for respective SASS indices tested against SASS operators on the lower Karkloof River (KZN) (Sig = Significant at $p<0.05$, NS = $p>0.05$)

Biotope	SASS Indices	ANOVA p-levels	ANOVA Significance	Kruskal-Wallis p-levels	Kruskal-Wallis Significance
All biotopes	Score	0.013	Sig	0.20	NS
Combined	No. Taxa	0.010	Sig	0.08	NS
	ASPT	0.352	NS	0.20	NS
	Score	0.032	Sig	0.08	NS
Stones	No. Taxa	0.019	Sig	0.03	Sig
	ASPT	0.052	NS	0.08	NS
	Score	0.003	Sig	0.20	NS
GSM	No. Taxa	0.004	Sig	0.20	NS
	ASPT	0.314	NS	0.21	NS
	Score	0.066	NS	0.20	NS
Vegetation	No. Taxa	0.033	Sig	0.03	Sig
	ASPT	0.717	NS	0.85	NS

A Kruskal-Wallis test (Siegal 1988) of the *ranks* of SASS indices (particularly SASS Score and ASPT) indicated that the order of SASS results did not differ significantly between operators ($P>0.05$). However, because of the very disparate scores by two operators, the means of the SASS results (Scores and Number of Taxa) differed significantly among operators in all biotopes combined, Stones and GSM (Table 4). The exception was ASPT over all biotopes, where the difference in mean score among operators was not significant ($P>0.05$). The low scores affecting the sample means could be related to the low sampling-effort (technique) of one particular individual.

The interpretation of this is that, for a given river (of assumed uniform water quality and biotope availability), different SASS operators will (in this case with the exception of scores derived for the vegetation biotope) return different SASS Scores and numbers of taxa and hence measures of river health (e.g. Figure 2). However, the ASPT appears to be a more consistent and repeatable measure of river health and was always statistically non-significant between all operators across all biotopes (Table 4 and Figure 2). SASS

Scores for the vegetation biotope were also not statistically significant between different operators.

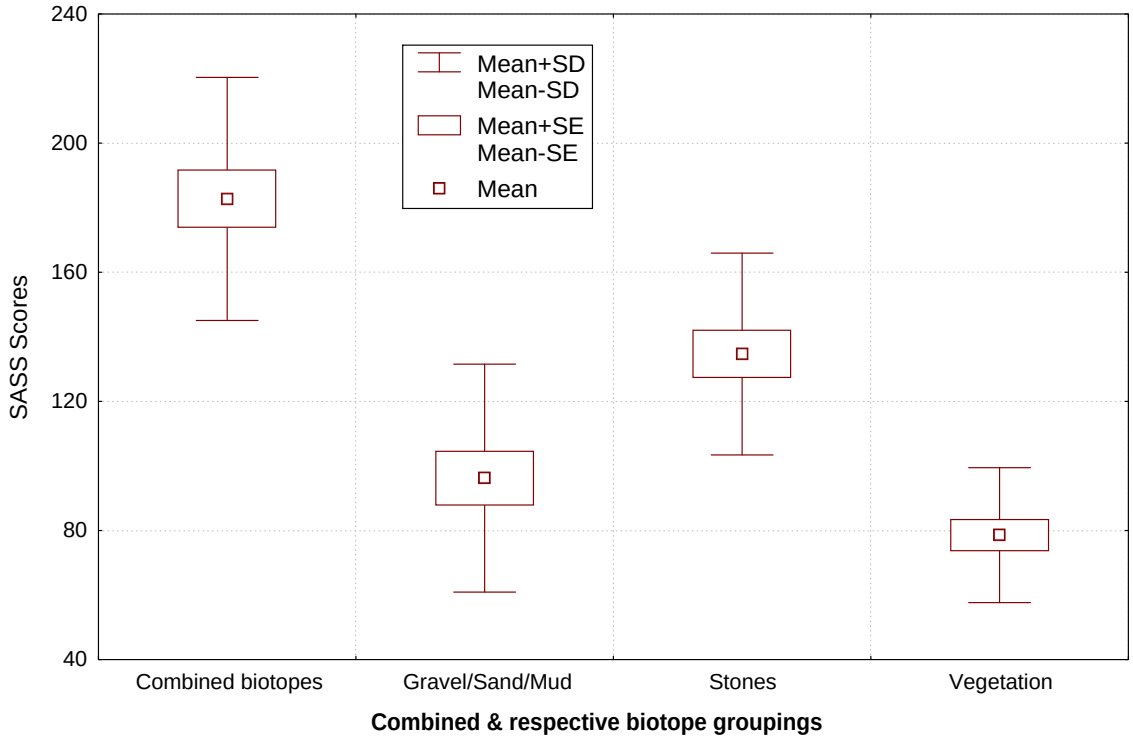

Figure 1: SASS Scores for respective biotopes collected by six SASS operators on the lower Karkloof River (KZN)

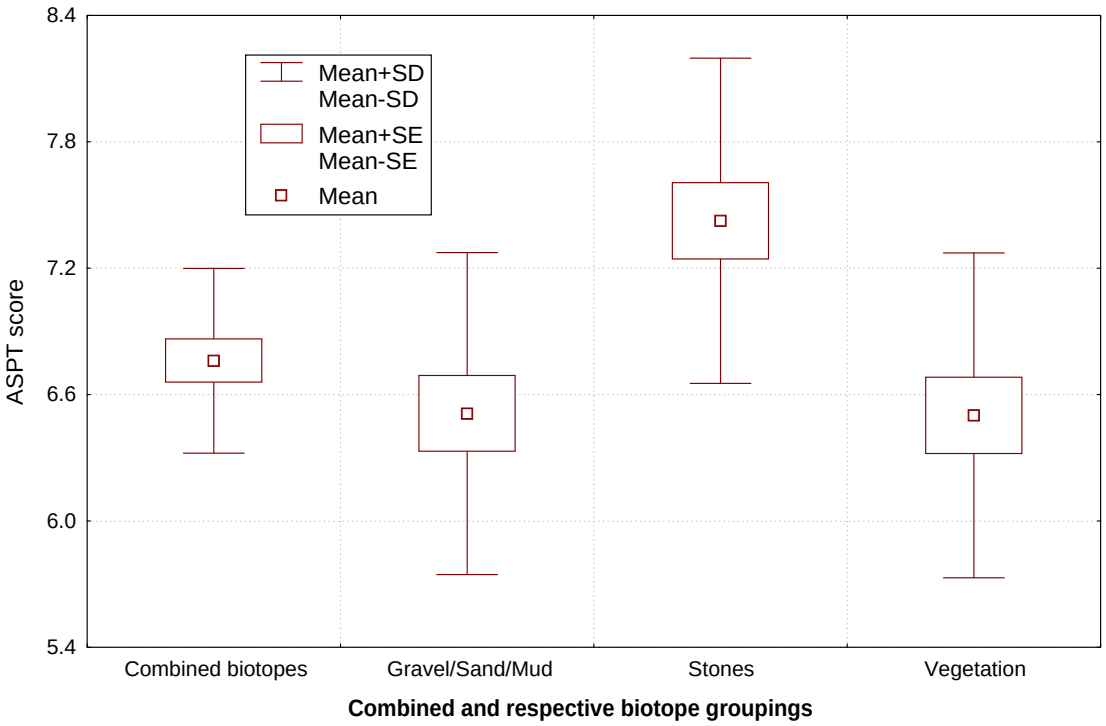

Figure 2: SASS ASPT scores for respective biotopes collected by six SASS operators on the lower Karkloof River (KZN)

Examination of the coefficients of variation for respective SASS indices (Table 2) gives a useful indication as to which indices are the most consistent and in which biotopes they are so. Clearly, ASPT is the most consistent (lowest CV%) over all biotopes. On the other hand, the Gravel/Sand/Mud (GSM) biotope combination is the most variable with respect to the SASS Score and number of taxa encountered. This highlights the need for consistency in the application of the SASS5 method, particularly in the area of GSM sampling, as it is here that most variability in SASS results is likely to arise.

It appears that the SASS5 method may be applied to a river, with assumed uniform water quality and suitably available biotopes, to produce consistent results, i.e. the variation between sites sampled on a reach of river with similar characteristics is statistically negligible. The implication of this is that any variation in results obtained from such a river could be ascribed to operator differences, rather than to differences in the characteristics (available biotopes, water quality, etc.) of the river.

On the other hand, there are generally statistically significant differences between the results of SASS Scores and the number of taxa counted by different operators. In other words, different SASS operators will (with the exception of scores derived for the vegetation biotope) return different SASS Scores and numbers of taxa and hence measures of river health. However ASPT (and SASS Scores derived from the vegetation biotope) appears to be a more consistent and repeatable measure of river health and was always statistically non-significant between all operators across all biotopes.

In terms of the SASS indices, the Gravel/Sand/Mud biotope is the most variable biotope sampled.

Two issues require further explanation as they are likely to arouse some discussion, firstly the combination of biotopes used in the method and, secondly, the avoidance of the use of abundance ratings as part of the score.

1. The combination of biotopes into groups, as done in this method, was necessary for practical reasons, principally to improve the speed of assessment. The combination could have been done differently, for instance by combining hydraulic groups (e.g. all 'in-current' biotopes) instead of substrate groups (e.g. all stone), as was done. Since different aquatic organisms have different substrate and hydraulic requirements, differences in these components may translate into different SASS results (Dallas 2001, Dallas in press).

2. The effect of weighting SASS Scores on the basis of rank abundance values was examined (Dallas in press). Results indicate that the inclusion of rank abundances did not alter the assessment of disturbance appreciably.

Conclusions

The SASS method developed by Chutter (1994, 1998) has been extended by defining the SASS5 method in greater detail so as to minimize variability. While this has had the negative impact of increasing the time in the field, as compared to SASS4 which allowed the combining of all biotopes into a single sample tray, it is generally agreed that the greater accuracy thus achieved makes this acceptable. Changes to the list of taxa have also been made, resulting in a need for greater expertise, but, again, the benefits make this acceptable. SASS5 has already been used in the field in draft form since May 2001 and has been reported to produce more meaningful and less variable results.

Included in this paper is an assessment of the error inherent in the method, and the error induced by the operator. Results presented suggest that the latter is the greater concern, which supports the need for good training and tight adherence to the written method. The investigation also supports conclusions by others including Chutter (1998) and Dallas (2000b) that ASPT is a less variable measure than SASS Score or the Number or

Taxa and should be the common SASS index of choice. However, in some instances, particularly in polluted water, the SASS Score becomes more meaningful (Chutter 1998).

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