

# **Do SASS5 scores vary with season in the South African highveld? A case study on the Skeerpoort River, North West Province, South Africa**

HE Fourie<sup>1\*</sup>, C Thirion<sup>2</sup> and CW Weldon<sup>1</sup>

*1. Department of Zoology and Entomology, University of Pretoria, Private Bag X20, Hatfield 0083, South Africa*

*2. Department of Water Affairs: Resource Quality Services, Private Bag X313, Pretoria 0001, South Africa*

*\* Corresponding author, e-mail: hermina.fourie@zoology.up.ac.za*

## **Abstract**

Macroinvertebrate assemblages can vary with season as a result of changing environmental conditions and phenology of individual species. The objective of this study was to determine whether results from the South African Scoring System version 5 (SASS5) are affected by season. The standard SASS5 protocol was used to sample three sites on the Skeerpoort River, South Africa, in autumn, winter and spring 2013, and summer 2014. At each site, stream morphology was characterised and physicochemical variables were measured. Two-way analysis of variance (ANOVA) was used to compare SASS indices in relation to site and season, and a one-way ANOVA was used to test the effect of season using both current and historical data. Macroinvertebrate assemblage similarity of sites was determined using non-metric multidimensional scaling ordination, followed by analysis of similarity. Whilst macroinvertebrate assemblages differed between seasons and sites, there was no seasonal variation in the values of SASS indices, but they did differ between sites. SASS5 appears to be robust to seasonal variation in macroinvertebrate assemblage and seems suitable for use throughout the year in a perennial river draining the South African highveld region.

**Keywords:** Aquatic macroinvertebrates, bioassessment, seasonal variation, SASS5, water quality

## **Introduction**

A range of methods, including biotic and abiotic indicators, are available to measure the effects of human activities on aquatic environments. Macroinvertebrates, i.e. those that do not

pass through a 500 µm mesh, can be particularly useful bioindicators for assessing the quality of aquatic environments (Hodkinson and Jackson 2005). The life cycles of many macroinvertebrate taxa have unique ecological requirements and changes in habitat and environment may cause them to migrate to other habitats (Carchini et al. 2005, Balzan 2012). A range of biotic indices have been developed around the world based on the utility of macroinvertebrates as bioindicators. Since the implementation of the Clean Water Act in 1972 in the United States, bioassessment methods have been designed to evaluate aquatic resource conditions (Davies and Jackson 2006). These authors proposed a descriptive model, the Biological Condition Gradient, which is used to describe how ecological features change when introduced to stressors. Other aquatic biomonitoring methods include the Biological Monitoring Working Party Score System in the United Kingdom (Hawkes 1998) and SIGNAL (Stream Invertebrate Grade Number – Average Level) in Australia (Chessman 2001). In South Africa, an empirical biotic index was developed by Chutter (1972), which summarised expected deviation in observed assemblages of organisms found in organically polluted rivers. In the 1990s, however, Chutter (1998) used BMWP as a foundation for the development of an easier and more cost-effective method for South Africa, called the South African Scoring System (SASS) (Dickens and Graham 2002). The key aspects of SASS are that taxa are identified, mostly to family level, in the field and each taxon has been allocated a quality value indicative of its sensitivity to pollution and disturbance. Based on the composition of the invertebrate assemblage in a river sample, the number of taxa is recorded and the SASS score and Average Score per Taxon (ASPT) are calculated (Dickens and Graham 2002). Over time, SASS has been further developed to overcome deficiencies identified by researchers and SASS practitioners. The current method, SASS version 5 (SASS5), has become the backbone of the South African River Health Programme and many institutions now use it to monitor water quality and river health.

Aquatic invertebrates utilise the variety of habitats available within rivers with different taxa found in very diverse or specific patches within a river (Malmqvist 2002). Environmental factors such as water flow, dissolved oxygen and habitat availability have a strong influence on macroinvertebrate assemblages (Hawkins et al. 1997). Aquatic invertebrates, like all ectotherms, are also very sensitive to temperature, which affects their growth and development. It is therefore reasonable to expect that seasonal changes in water flow, dissolved oxygen, habitat availability and temperature will have a dramatic effect on the distribution, abundance and life cycles of aquatic insects (Dallas 2004). For this reason, when

a bioassessment is made using SASS5 it is not only necessary to take into account the habitat quality and diversity of biotopes, but it is also necessary to interpret the data in relation to the prevailing season (Dickens and Graham 2002). Data accounting for season, when interpreting SASS5 metrics, are scarce and include only a few bioclimatic regions. Dallas (1997) found that SASS scores in rivers in the Western Cape were highest in spring and autumn. Similarly, differences in number of taxa and ASPT were recorded between seasons in the Western Cape (Dallas 2004). However, in Mpumalanga Province, when using the SASS4 method the SASS score, number of taxa, and ASPT were not significantly different between seasons (Dallas 2004). By contrast, SASS4 data from the coastal lowlands of KwaZulu Natal showed temporal variation (Vos et al. 2002).

To date, no reports have been published on the seasonal variability of SASS5 data from the highveld region of South Africa. The aim of this study was to determine the effect of season on macroinvertebrate assemblages in a relatively untransformed perennial stream in the highveld region, and its consequences for the principal metrics of SASS5: total SASS score, number of taxa, and Average Score per Taxon (ASPT). Sampling along the Skeerpoort River was used to ascertain whether any detected differences were due to season rather than to random variation at an individual site. Based on the results of earlier studies using the SASS4 method (e.g. Dallas 2004; Vos et al. 2002), it was predicted that SASS5 metrics would vary between seasons due to the associated changes in environmental conditions and aquatic invertebrate phenology.

## **Materials and methods**

### ***Study area***

Aquatic invertebrates were sampled in the relatively unpolluted second order perennial Skeerpoort River, a tributary of the Magalies River in the Limpopo catchment, North West Province, South Africa (Figure 1). The Skeerpoort originates in the rocky highveld grasslands of the Cradle of Humankind World Heritage Site. The geology of the region is dominated by dolomitic rock and the climate is characterised by wet, warm summers and very dry, cold winters (River Health Programme 2005). Sampling occurred at three sites, A, B and C, approximately 15, 13 and 11 km downstream from the source at altitudes of 1252, 1264 and 1288 masl, respectively. The sites were easily accessible, had few human settlements nearby and no known sewage outlets along the sampled stretch. These were important considerations

because the study aimed to determine the impact of season, rather than of disturbances, on the SASS5 metrics. The river channel morphology at all sites included mixed bedrock and alluvial deposits, but was dominated by cobbles (Tables 1 and 2). At Site C water is pumped from the river into a canal for domestic use. At Site B water is extracted from the river to fill ponds in which trout are farmed. Site A is located downstream of a provincial road that crosses the river.

Each site was sampled in the first weeks of April, July and October 2013 and January 2014. Each site was divided into three 50 m long plots. Plots at each site were sampled from downstream to upstream over three successive days in each month to provide replication at each site. Water flow was moderate throughout the study. Heavy rainfall did not occur in the weeks prior to any of the sampling periods (Table 2).

### ***Physicochemical water quality***

A multiparameter water quality tester (PCSTestr 35, Eutech Instruments, Oakton<sup>®</sup>, Singapore) was used to determine water temperature, pH and conductivity. Before SASS sampling, a water sample of 250 ml was taken with a plastic beaker in the main channel at the upstream end of the plot. Measurements were taken at approximately the same time of the day for each site. Water clarity was measured with a clarity tube (Ground Truth, South-Africa, KwaZulu-Natal) (Kilroy and Biggs 2002) filled with water from the main channel. A clarity tube reading is the depth in cm that light penetrates into the water. The tube was held perpendicular to the sun and, by moving a black disk in it with a magnet, the clarity of the water was measured at the point at which the disk was no longer visible.

### ***SASS5 sampling***

The standard SASS5 (South African Scoring System, Version 5 bioassessment method (Dickens and Graham 2002) was used to sample aquatic macroinvertebrates in three biotopes: stones; vegetation; and gravel, sand and mud. All sampling was performed by an accredited SASS5 practitioner (HEF).

### ***Data analyses***

Total SASS score, number of taxa present and Average Score per Taxon (ASPT) were calculated. The three SASS5 metrics were analysed using two-way analyses of variance (ANOVA) to test the effect of season and site. Post-hoc multiple comparisons were performed using Fisher's least significant difference (LSD) tests. Data did not violate any

ANOVA assumptions. ANOVA models were performed using IBM SPSS Statistics 21 (IBM Corporation 2012).

In addition, historical SASS5 data for the Skeerpoort River at Site B collected in 2002, 2003, 2004 and 2007 were extracted from the Rivers Database <http://www.dwa.gov.za/iwqs/rhp/database.html>, maintained by the South African Department of Water Affairs. To assess the effect of season on the three SASS metrics across multiple years, a further one-way ANOVA using data from 2013/14 and the Rivers Database was performed.

Taxon-by-site abundance data from 2013/14 were used to perform a non-metric multi-dimensional scaling (nMDS) ordination to assess the effect of season and site on aquatic macroinvertebrate assemblages, using the 'vegan' library in R version 2.15.2 (R Foundation for Statistical Computing). For the taxon-by-site abundance matrix abundance categories assigned to each taxon during sampling were converted into numerical scores: 0 = 0, A = 1, B = 5, C = 50, D = 500. These values were selected because they represented the mid-value of each category. A dissimilarity matrix was generated automatically from the taxon-by-site abundance matrix using the Bray-Curtis dissimilarity index and reduced to two dimensions with the 'metaMDS' procedure. Using 'rankindex' the Bray-Curtis index was determined as the best similarity index for this purpose because it resulted in the highest rank-order similarity with gradient separation. Using the 'vegdist' procedure, analysis of similarity was performed to determine whether there was a significant effect of season or site on macroinvertebrate assemblage. Where there was a significant difference between seasons or sites, ellipses representing the 95% confidence interval of the centroid for each season or site were plotted on the two-dimensional ordination.

The effect of season and site on physicochemical water quality variables collected during 2013/14 such as temperature, pH, salinity, conductivity and clarity was determined with two-way ANOVA and post-hoc multiple comparisons (Fisher's LSD). A series of Pearson's rank correlations was performed to assess the relationship between physicochemical parameters and the SASS5 indices.

## Results

### *Effect of season on SASS indices*

Analysis of the SASS scores revealed no significant difference between seasons ( $F_{3,24} = 0.64$ ,  $P = 0.596$ ), but there was a significant difference between sites ( $F_{2,24} = 6.45$ ,  $P = 0.006$ ). Site C had the lowest SASS score in all seasons (Figure 2a). The interaction of season and site had no significant effect on the SASS score ( $F_{6,24} = 0.83$ ,  $P = 0.561$ ). The number of taxa was not significantly affected by season ( $F_{3,24} = 0.55$ ,  $P = 0.656$ ). Site, however, did have a significant effect on the number of taxa ( $F_{2,24} = 12.86$ ,  $P = 0.000$ ), with Site B having the highest number of taxa (Figure 2b). The interaction between season and site had no significant effect on the number of taxa ( $F_{6,24} = 1.17$ ,  $P = 0.354$ ). The ASPT was not significantly affected by season ( $F_{3,24} = 0.75$ ,  $P = 0.535$ ). There was, however, a significant difference in ASPT between sites, where sites A and B differed significantly from each other (Figure 2c,  $F_{2,24} = 2.23$ ,  $P = 0.129$ ). The interaction between season and site did not significantly affect ASPT ( $F_{6,24} = 0.88$ ,  $P = 0.524$ ).

### *Combined historical and recent data*

Analysis of the combined historical and 2013/2014 data revealed that season had no significant effect on SASS score ( $F_{3,16} = 0.70$ ,  $P = 0.564$ ), number of taxa ( $F_{3,16} = 0.73$ ,  $P = 0.545$ ), or ASPT ( $F_{3,16} = 0.10$ ,  $P = 0.956$ ). Repeated sampling of the Skeerpoort River between 2002-2013/14 using the SASS5 method provided values for the SASS score, number of taxa, and ASPT that were consistent across seasons (Figure 3).

### *Macroinvertebrate assemblage*

The macroinvertebrate assemblages of the Skeerpoort River appeared to be structured by season and site, where some taxa were more often found at some sites or seasons than at others (Figure 4). This visual pattern was confirmed with ANOSIM, which indicated that macroinvertebrate community composition varied significantly between seasons (Global  $R = 0.335$ ,  $P = 0.001$ ), and sites (Global  $R = 0.172$ ,  $P = 0.004$ ). The stress of the nMDS ordination was 0.241. The stress value reveals the degree of correspondence between the data points, where a stress value of zero indicates perfect fit and a value close to one no correspondence (Kingston and Rosel 2004). The abundance of Dixidae was generally similar in winter and spring, whereas the presence of Elmidae was characteristic of spring and summer. Autumn was characterised by high abundances of Lestidae and Naucoridae. The macroinvertebrate

assemblages of Sites B and C were least similar, with Site A seeming to be intermediate between the other two sites (Figure 4). The families Aeshnidae, Corduliidae, Dytiscidae, Notonectidae and Physidae were characteristic of Site B. At Sites A and B Coenagrionidae, Turbellaria, Planorbidae, Hydracarina and Hydroptilidae were more abundant than at Site C. The relative similarity of assemblages at Sites A and C may have been driven by a higher abundance of Veliidae. Sites B and C were also characterised by low hydropsychid species abundance.

### *Physicochemical parameters*

Temperature varied significantly between seasons ( $F_{3,24} = 366.25$ ,  $P < 0.001$ ) and sites ( $F_{2,24} = 106.90$ ,  $P < 0.001$ ), as well as with the interaction of season and site ( $F_{6,24} = 2.68$ ,  $P = 0.039$ ). Water temperature was consistently lowest in winter and highest in spring and summer, with Site C always being warmer than the other sites (Table 3). The pH differed significantly with season ( $F_{3,24} = 10.207$ ,  $P < 0.001$ ) and site ( $F_{2,24} = 39.00$ ,  $P < 0.001$ ). There was also a significant effect of the interaction of season and site on pH ( $F_{6,24} = 2.61$ ,  $P = 0.043$ ). In general, pH was highest at the furthest upstream site, Site C, and in summer, autumn and winter pH was significantly lower than in spring (Table 3). Conductivity differed significantly with season ( $F_{3,24} = 16.78$ ,  $P < 0.001$ ) and site ( $F_{2,24} = 17.82$ ,  $P < 0.001$ ), but the interaction of season and site did not differ significantly ( $F_{6,24} = 0.58$ ,  $P = 0.744$ ). Post-hoc analyses revealed that conductivity in spring was significantly higher than in autumn, winter and summer, and was significantly higher at Sites A and B than at Site C. Season ( $F_{3,24} = 56.20$ ,  $P < 0.001$ ) and site ( $F_{2,24} = 6.45$ ,  $P = 0.006$ ) had a significant effect on clarity. Water clarity in autumn was significantly lower than in the other seasons, and Site A, the furthest downstream site, had the lowest clarity.

The results of Pearson's rank correlation did not reveal strong associations between most physicochemical parameters and the three SASS5 metrics (Table 4). A significant negative correlation was found between pH and ASPT in spring, as well as between temperature and SASS score in summer (Table 4). When data from the seasons were combined, a strong positive relationship was detected between number of taxa and salinity, as well as between total SASS score and salinity (Table 4).

## Discussion

The key SASS5 metrics did not vary by season on the Skeerpoort River. The lack of seasonal variation in total SASS score, number of taxa and ASPT may be explained by the generally similar water level throughout the year, as it is fed by groundwater from dolomitic eyes (springs) (Witthüser and Holland 2008) and no major rainfall or flooding events occurred during the sampling period. There was, however, variation in total SASS score, number of taxa and ASPT between sites. Site B had the highest number of taxa and SASS score but slightly, although significantly, lower ASPT values when compared to Sites A and C. This suggests that Site B had a larger number of taxa that are less sensitive to water pollution than those found at the other two sites. When taxa with low quality values are recorded it does not necessarily indicate that a site is polluted, because aquatic invertebrates are dependent on specific habitats (Dias-Silva et al. 2010). Some of these taxa include Hirudinae, Notonectidae, Nepidae and Physidae, which have a quality value of three and which did not occur at Site A and C. The large number of taxa at Site B with both high and low quality values may be explained by a high diversity of biotopes including aquatic vegetation which favours Notonectids and Nepids (Dias-Silva et al. 2010). Families such as the Veliidae and Gerridae were more commonly recorded at Sites A and C, which had more pool habitats compared to Site B (Dias-Silva et al. 2010) (Table 1).

The lack of seasonal effect on SASS indices in the current study is generally similar to that of earlier reports from similar bioclimatic regions. Gratwicke (1998) studied the effect of season on indices from the SASS4 method in two rivers in Zimbabwe and established that SASS scores changed with season in polluted streams due to the change in water quality between seasons. His SASS scores were highest at the end of the rainy season and lowest during the dry season. The results for relatively unpolluted streams studied by Gratwicke, however, were comparable with those of the current study where no significant differences were found for ASPT between seasons. Dallas (2004) studied two regions in South Africa and found no significant seasonal variability in Mpumalanga, a summer rainfall area, but significant seasonal variability for ASPT and number of taxa in the Western Cape, a winter rainfall area. Similarly, in the cool, temperate climate of the United Kingdom, a study of 268 sites in unpolluted streams indicated that ASPT changed with season (Armitage et al. 1983); higher ASPT values were found in spring and autumn, which revealed better water quality compared to summer. It is therefore important to consider not only the health of the river when sampling, but also the region in which the river is located, because the occurrence of



macroinvertebrate families depends on habitat availability, climate and regional differences in taxa (Dias-Silva et al. 2010).

Although season did not affect the total SASS5 score or ASPT, differences in macroinvertebrate assemblages were recorded between seasons and sites. Development, emergence and feeding represent some of the biological aspects of invertebrates that are influenced by season (Dallas 1997), while factors such as shade (Quinn et al. 1997), habitat availability and food resources influence macroinvertebrate assemblages at sites (Lenat and Crawford 1994). Dixidae were characteristic of samples taken in winter and spring, but invertebrate families such as Hydroptilidae, Ceratopogonidae, Physidae, Planorbidae and Sphaeriidae also occurred in much larger numbers during these seasons, with estimated abundances of 10-100 (B, following standard SASS5 methods). An increase in invertebrate abundances from October to March, during and after the rainy season, may suggest that autochthonous resources (sessile organisms attached to submerged surfaces in aquatic ecosystems) are of more importance to the invertebrates as a food resource than allochthonous (leaf-fall) energy resources (Lenat and Crawford 1994). The availability of these energy resources and dietary shifts (e.g. see Rosi-Marshall and Wallace 2002) may be drivers for the differences in macroinvertebrate assemblages between sites that were seen in the current study. Sites A and C, which had lower vegetation diversity compared to site B, had more Potamonautids and Veliids than Site B, whereas Coenagrionidae and Hydroptilidae were uncommon in these sites compared to Site B. Sites A and C are shallower, more rocky and potentially have fewer autochthonous resources, which favours the Potamonautidae, while allochthonous energy resources and a diversity of vegetation types favours the families Coenagrionidae and Hydroptilidae (Lenat and Crawford 1994).

In addition to seasonal variability and biotope availability, physico-chemical variables such as temperature, pH, conductivity and clarity can also influence water quality and thus the aquatic ecosystem. Most physico-chemical parameters, especially conductivity and pH, change due to the hydrological functioning of the catchment (Hill and Neal 1997) or due to the geological formations of an area (Davies et al. 1986). Factors such as water flow (Schälchli 1992), landscape, local environment and climate also contribute to the effect of physico-chemical parameters on aquatic life forms (McEvoy and Goonan 2003) and possibly thus explain the differences in temperature, conductivity and clarity between seasons and sites.

Changes in the number of taxa and the SASS score were identified between sites but not between seasons either when using the 2013/14 data alone or combined with historical data. Differences in the macroinvertebrate assemblage were found between seasons, which did not support our hypothesis. It appears, however, that SASS5 is robust to seasonal variations in the macroinvertebrate assemblage which is independent of changes in water quality. Consequently, SASS5 seems suitable for use throughout the year in rivers draining the highveld region. A positive correlation was detected between small changes in salinity and two of the SASS5 metrics, number of taxa and total SASS score, which suggests that these SASS5 metrics may represent sensitive measures for natural variations in salinity or saline pollution in freshwater streams. Various studies that used SASS4 demonstrated seasonal variability of SASS indices, but that was dependent on geographic region. It is therefore important that further studies be conducted to determine the effect of the geographic region of a catchment and seasonal variability in the physicochemical and biotic environment on indices derived from using the SASS5 method.

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**Tables:**

**Table 1:** Characterisation of sampling in the Skeerpoort River by catchment condition, land-use and abiotic and biotic environment in April (autumn), July (winter), and October (spring) 2013, and January (summer) 2014. Substrate abundance ratings: 0 = absent; 1 = rare; 2 = sparse; 3 = common; 4 = abundant; 5 = entire

<b>Character</b>	<b>Site A</b>	<b>Site B</b>	<b>Site C</b>			
Coordinates	25°50'9.2" S, 27°47'0.3" E	25°50'32.5" S, 27°47'0.3" E	25°51'23.8" S, 27°46'57.1" E			
Land use and channel condition (Distance from sampled stretch; m)	Road and bridge (5 m) Irrigation (20 m)	Weir (5 m) Trout farm (20 m)	Wildlife and cattle Weir (5 m) Water canal (10 m)			
Canopy cover	Partially open	Partially open	Closed			
Coarse woody debris impact	No impact	Limited impact	Extensive impact			
Water flow	Moderate	Moderate	Moderate			
Riffles	Abundant	Abundant	Abundant			
Pools	Abundant	Common	Abundant			
Bank height-Active channel (m):						
Left	1-3	1-3	>3			
Right	>3	1-3	>3			
Deep-water depth (m)	1	1	2			
Shallow-water depth (m)	0.3	0.3	0.3			
Active channel width (m)	2-5	2-5	2-5			
Water surface width (m)	2-5	2-5	2-5			
<b>Substrate</b>	<b>Bed</b>	<b>Bank</b>	<b>Bed</b>	<b>Bank</b>	<b>Bed</b>	<b>Bank</b>
Bedrock	1	4	1	0	1	0
Boulders	2	1	2	0	2	0
Cobble	4	0	4	0	4	1
Pebble	3	2	3	1	4	1
Gravel	4	1	4	0	3	0
Sand	3	1	4	1	3	1
Silt/mud/clay	1	0	2	0	1	0

**Table 2:** Weather records from near the Skeerpoort River in April (autumn), July (winter), and October (spring) 2013, and January (summer) 2014. Mean air temperatures calculated using hourly records over the three days of each sampling period, records were obtained from an automatic weather station at Hartebeeshoek Radio Astronomy Observatory. Rainfall data from the trout farm located close to Site B.

Weather variable	Autumn	Winter	Spring	Summer
Mean ( $\pm$ 1 S.E.) daily air temperature ( $^{\circ}$ C)				
Minimum	5.63 $\pm$ 0.75	5.07 $\pm$ 0.81	8.77 $\pm$ 0.47	15.27 $\pm$ 0.34
Average	13.81 $\pm$ 0.02	10.74 $\pm$ 0.02	19.29 $\pm$ 0.01	21.09 $\pm$ 0.01
Maximum	22.60 $\pm$ 1.62	17.47 $\pm$ 1.57	29.60 $\pm$ 0.46	29.43 $\pm$ 0.49
Rainfall (mm) in week prior to sampling	15	0	0	2

**Table 3:** Mean ( $\pm 1$  S.E.) values of physicochemical parameters in the Skeerpoort River in April (autumn), July (winter), and October (spring) 2013, and January (summer) 2014

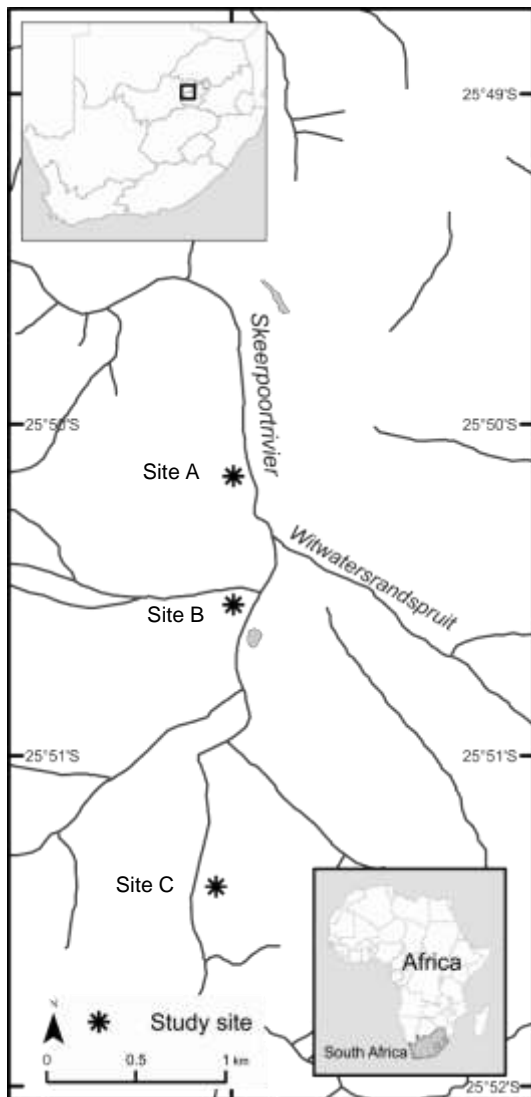
Physicochemical parameter	Site	Season			
		Autumn	Winter	Spring	Summer
Temperature ( $^{\circ}$ C)	A	16.50 $\pm$ 0.40	11.7 $\pm$ 0.24	19.89 $\pm$ 0.09	20.40 $\pm$ 0.29
	B	18.77 $\pm$ 0.39	13.00 $\pm$ 0.21	21.17 $\pm$ 0.28	20.57 $\pm$ 0.33
	C	20.03 $\pm$ 0.18	15.63 $\pm$ 0.54	24.07 $\pm$ 0.42	22.77 $\pm$ 0.46
pH	A	8.35 $\pm$ 0.04	8.49 $\pm$ 0.03	8.54 $\pm$ 0.02	8.19 $\pm$ 0.17
	B	8.45 $\pm$ 0.04	8.42 $\pm$ 0.03	8.62 $\pm$ 0.01	8.53 $\pm$ 0.05
	C	8.73 $\pm$ 0.04	8.67 $\pm$ 0.01	8.96 $\pm$ 0.03	8.66 $\pm$ 0.01
Conductivity ( $\mu$ S/m)	A	327.00 $\pm$ 1.00	320.67 $\pm$ 2.33	303.33 $\pm$ 4.37	325.33 $\pm$ 8.51
	B	327.33 $\pm$ 0.67	320.00 $\pm$ 3.06	309.67 $\pm$ 3.18	319.67 $\pm$ 3.53
	C	315.33 $\pm$ 1.20	306.00 $\pm$ 5.03	295.33 $\pm$ 0.88	306.67 $\pm$ 0.88
Clarity (cm)	A	86.67 $\pm$ 2.33	100.00 $\pm$ 0.00	97.33 $\pm$ 2.67	100.00 $\pm$ 0.00
	B	93.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00
	C	93.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00

**Table 4:** Correlation of physicochemical parameters and SASS5 indices recorded from the Skeerpoort River in April (autumn), July (winter), and October (spring) 2013, and January (summer) 2014. Significant correlations at 0.05-level in bold type

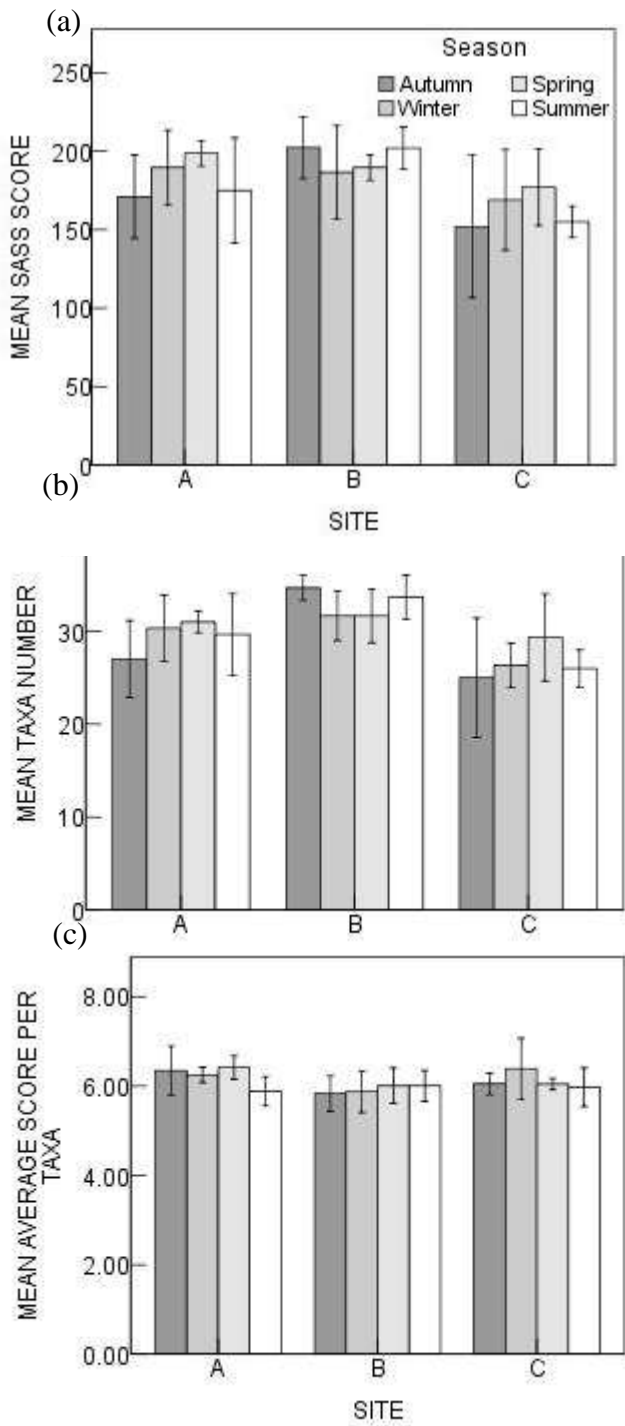
Variable	Temperature (°C)	pH	Conductivity (µS/m)	Clarity (cm)
<b>Summer</b>				
SASS score	<b>-0.701</b>	-0.248	0.293	0.000
Number of Taxa	-0.661	-0.354	0.361	0.000
ASPT	-0.298	0.184	-0.150	0.000
<b>Autumn</b>				
SASS score	-0.206	-0.244	0.325	0.230
Number of Taxa	-0.126	-0.248	0.316	0.320
ASPT	-0.285	-0.167	-0.043	-0.259
<b>Winter</b>				
SASS score	-0.367	-0.536	0.577	0.000
Number of Taxa	-0.468	0.641	0.650	0.000
ASPT	0.200	0.151	0.008	0.000
<b>Spring</b>				
SASS score	-0.644	-0.636	0.335	-0.413
Number of Taxa	-0.311	-0.067	0.193	-0.207
ASPT	-0.467	<b>-0.683</b>	0.250	-0.137
<b>Overall</b>				
SASS score	-0.226	-0.298	0.153	0.088
Number of Taxa	-0.101	-0.294	0.218	0.028
ASPT	-0.251	-0.029	-0.100	0.064



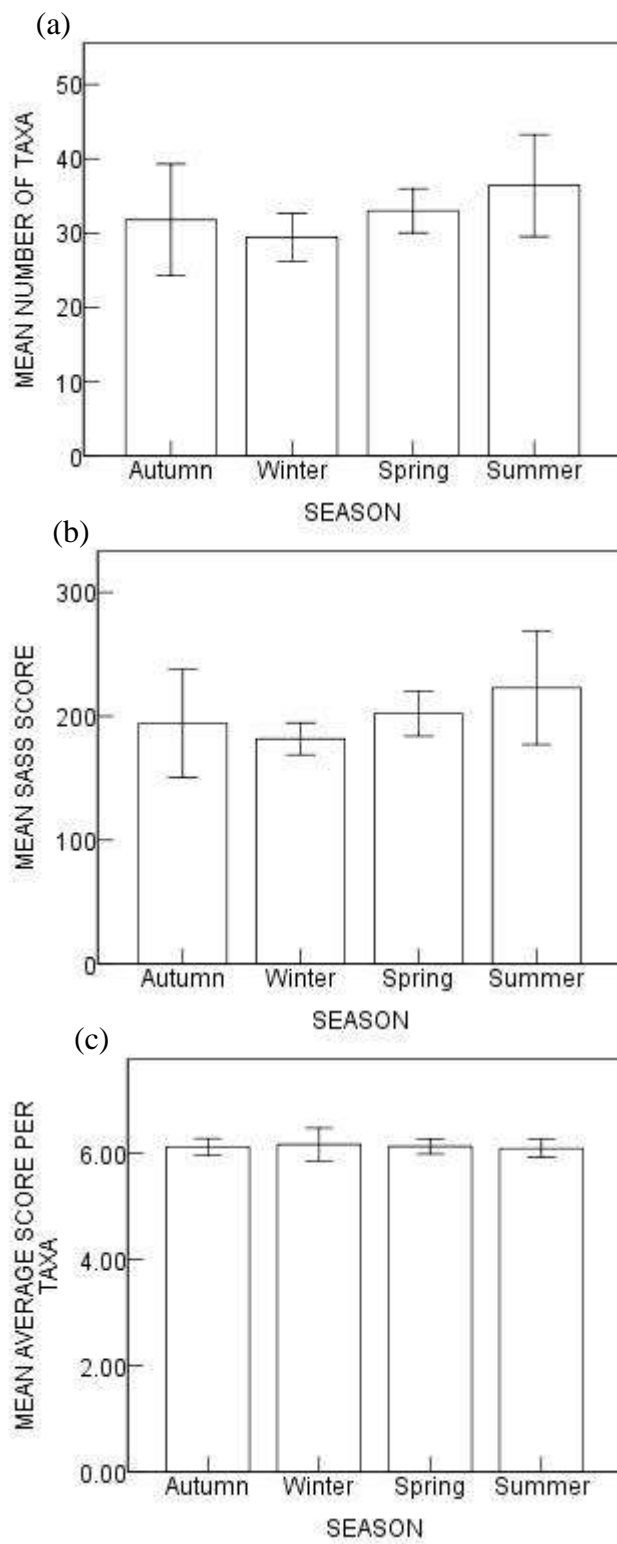
**Figures:**



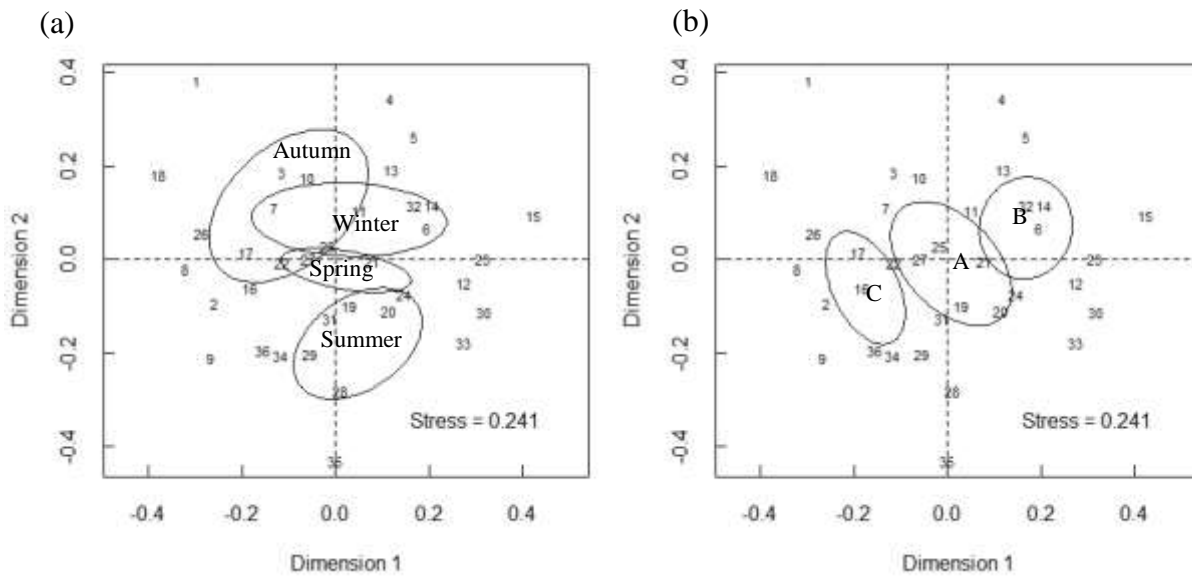
**Figure 1:** Map of the Skeerpoort River, North West Province, South Africa, indicating the location of Site A, B and C. Sites were sampled in April (autumn), July (winter), and October (spring) 2013, and January (summer) 2014.



**Figure 2:** Mean ( $\pm$ SE) (a) SASS score, (b) number of taxa and (c) average score per taxon (ASPT) for Sites A, B and C in the Skeerpoort River. These sites were sampled seasonally from April 2013 to January 2014.



**Figure 3:** Mean ( $\pm$ SE) SASS score (a), number of taxa (b) and average score per taxa (ASPT) (c) from historical and 2013/2014 data for all seasons.



**Figure 4:** Non-metric multidimensional scaling ordination of aquatic invertebrate community assemblage for all seasons (a), autumn (1-9), winter (10-18), spring (19-27) and summer (28-36); and sites (b) A (1-3, 10-12, 19-21 and 28-30), B(4-6, 13-15, 22-24 and 31-33) and C (7-9, 16-19, 25-27 and 34-36). Ellipses represent the 95% confidence interval of the centroid for each season or site, respectively.