# On the use of diatom-based biological monitoring Part 1: A comparison of the response of diversity and aut-ecological diatom indices to water quality variables in the Marico-Molopo River catchment

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# Abstract

Two main approaches have been followed in using diatoms as bio-indicators in the past few decades namely species diversity indices and aut-ecological indices. This study, based on 102 water quality and epilithic diatom samples from the Crocodile Groot-Marico catchment in South Africa, evaluated both types of indices by establishing how well they reflect changes in water quality. It was found that less of the variation in diversity indices could be attributed to changes in water quality variables than was the case for the aut-ecological indices. Furthermore it was found that species diversity indices tend to be higher at intermediate levels of pollution, rather than at low levels of pollution.

Keywords: diatoms; Bacillariophyceae; bioindicators; species diversity indices; water quality; aut-ecological indices

# Introduction

Southern Africa is a subcontinent notorious for its unpredictable rainfall. South Africa is a semi-arid country, and the decline in the quality of available water is one of the biggest problems currently facing the country (Davies and Day, 1998). For this reason the integrated management of water resources has enjoyed high priority in the National Water Act as well as in research and management actions by both government and non-government organisations.

Walmsley et al. (2000) state that indicators are an ideal means by which progress towards integrated water resource management can be monitored, in that they provide a summary of conditions, rather like temperature and blood pressure are used to measure human health. Using the same analogy, it is important to be able to distinguish between indicators which can truly be linked to health and are not just features of the system which do not have relevance to the question posed. For the aforementioned reason it is important to be able to test indicators quantitatively in order to assess how closely they can be linked to the health of aquatic ecosystems, in the case of water resource management, and/or water quality. Diatoms have been shown to have narrow tolerance ranges for many environmental variables and respond rapidly to environmental change, making them ideal indicators (Reid et al., 1995).

Several kinds of indicators have been proposed over the years to evaluate ecosystem health. Two main groups of indicators using diatom community data will be discussed in this paper namely diversity indices and aut-ecological indices.

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Species diversity/species richness indicators

Ecosystem stability can be defined as the ability of a system to recover to an equilibrium state after disturbance, or simply the persistence of the system (May, 1976). The diversity-stability hypothesis asserts that species vary in their traits and that in a highly diverse (species rich) system, there will be some species than can compensate for the loss of others should disturbance occur in such a system (Pimm, 1984; Elton, 1958). Thus, species rich systems are more likely to be considered stable. Another common view is that this theory predicts a decrease of diversity as pollution increases. The pollution intolerant species decline in abundance and the pollution tolerant species can grow rapidly without competition for space, nutrients, or other resources. This results in community abundance patterns of heavy dominance and fewer species (Van Dam, 1982).

It is on the basis of the two abovementioned hypotheses that species diversity indices enjoy widespread use in ecology and, more specifically, aquatic ecology. Diversity indices are related to community structure and are not specific to any type of contamination. Species diversity indices consist mainly of three measures namely: species richness (related to the number of species), the evenness (how evenly the individuals are distributed between the species) and a combined measure called the diversity index such as the Shannon Diversity Index (Shannon and Weaver, 1949).

Species diversity indices based on benthic diatom assemblages are regularly used in the study of water resources. In such instances these indices are mainly used to determine the impact of certain actions and pollutants on aquatic systems (Cunningham et al., 2003; Gómez 1999; Gracia-Criado et al., 1999). An alternative to species diversity/species richness indicators are aut-ecological indices.

# **Aut-ecological Indices**

Aut-ecological indices use the relative abundance of species

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in assemblages, their ecological preferences, sensitivities, or tolerances to infer environmental conditions in an ecosystem (Stoermer and Smol, 1999). Put in another way, aut-ecological indices make use of the niche requirements and habitat preferences of the individual species or higher taxonomic groupings. In such indices long-term data gathered about the tolerances of a species are used to compile an index which can, in turn, be used to deduce environmental conditions from the species composition by taking into account the specific tolerances of the species in the community surveyed. These indices can be constructed to measure specific pollutants or general environmental conditions. A number of diatom-based aut-ecological indices are based on the weighted average equation of Zelinka and 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:

 $a_i$  = abundance (proportion) of species j in sample

 $v_i =$  indicator value

 $s_{i}$  = pollution sensitivity of species j

The method mainly utilises the distribution of species along a water quality gradient in terms of sensitivity to pollution as well as broadness of the species distribution along the water quality gradient. This equation is used in many diatom-based indices including the Descy's Index or DES (Descy, 1979), the Generic Diatom Index or GDI (Coste and Ayphassorho, 1991), the Specific Pollution sensitivity Index or SPI (Coste in Cemagref, 1982), the Biological Diatom Index or BDI (Lenoir and Coste, 1996), the Artois-Picardie Diatom Index or APDI (Prygiel et al., 1996), Sládeček's index or SLA (Sládeček, 1986), the Eutrophication/Pollution Index or EPI (Dell'Uomo, 1996), Rott's Index or ROT (Rott, 1991), Leclercq and Maquet's Index or LMI (Leclercq and Maquet, 1987), etc. Such indices mainly vary in terms of species included in the calculation and the tolerances assigned to such species.

Diatom indices constructed by this approach have been tested with success in South Africa (Taylor et al., 2007; De la Rey et al., 2004) and in many countries in Europe and the rest of the world (Sabater, 2000; Kelly, 1998; Reavie et al., 1995; Zeeb et al., 1994; Hall and Smol, 1992).

#### Rationale

This research paper consists of two parts. This paper (Part 1) tests the performance of two approaches to the use of diatoms as bio-indicators namely diversity indices and aut-ecological indices. The second part of this paper (Part 2) (De la Rey, 2008) compares the performance of diatom-based indices with that of a macro-invertebrate index (SASS 5) in terms their ability to indicate changes in water quality variables.

The purpose of Part 1 is to compare diversity indices and aut-ecological indices as measures of aquatic ecosystem health by comparing their response to water quality variables. This will enable an informed decision as to which of the two approaches will be most appropriate when using diatoms as bio-indicators in river and stream ecosystems in South Africa.

Since water quality is one of the main environmental factors affecting the ecology in rivers and streams, it can be used as a measure of the applicability of indicators for integrated water resource management. Diatoms are appropriate for the purpose of this study as they provide interpretable indications of specific changes in water quality (McCormack and Cairns, 1994).

### Materials and methods

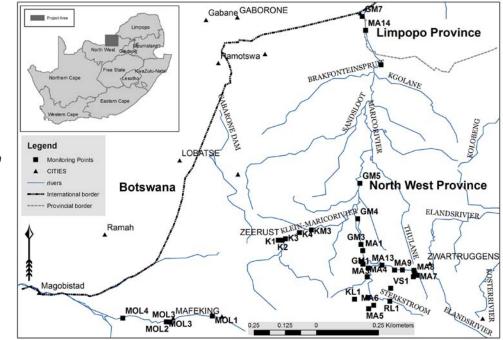
#### **Sampling localities**

Thirty three sites were identified in the Groot Marico and Molopo River systems for the study (Fig. 1). The identified sites form part of the River Health Program of the North West Province, South Africa. Samples were collected in April, June, September and November 2005 at the sites, as water levels permitted (at times certain samples could not be collected due to low water levels), resulting in a total of 102 separate data points with diatom data and same-day water quality data. These sites were chosen to represent a wide range of water quality (see water quality summary in Table 1) as well as the wide range of diatom index scores recorded (SPI: 1.3-19.7 out of a possible 20).

The Marico River originates south of Groot Marico town and feeds into the Limpopo River in the north. The origin of the Marico River falls within the Groot Marico dolomitic aquifer compartment. The two primary sources of this river are the Grootfontein dolomitic eye, that gives rise to Kaaloog-se-loop and an eye on the farm Renosterhoek 343JP that feeds the Rietspruit. Secondary sources include Draaifontein tributary that is of a mixed dolomitic nature and the Sterkstroom tributary that is fed by springs of a non-dolomitic nature. Other tributaries of the Marico River include the Klein Marico River. This tributary is seasonal and is thus not a major flow contributor to the Marico River. The 'Molemane-se-Loop' is of a dolomitic nature originating in the Molemane Nature Reserve. Major dams in this sub-catchment include the Marico-Bosveld Dam in the upper catchment and the Molatedi Dam further downstream. The upper reaches of this sub-catchment are not densely populated (RHP, 2005).

The Molopo River originates east of Mafikeng and feeds into the Vaal River in the south west. The origin of the Molopo River falls within the Groot Marico dolomitic aquifer compartment. The primary source of this river is the Molopo dolomitic eye. Major dams in this sub-catchment include Cooke's Lake, Montshioa Dam, Lotlamoreng, Modimolo Dam (Setumo) and the main dam in the system is the Disaneng Dam close to the Botswana Border.

TABLE 1 Descriptive statistics of water quality variables					
Descriptive s	Mean	Median	Min	Max	Standard deviation
Ca (mg/ℓ)	32.18	30.06	1.49	82.25	23.78
Cl (mg/l)	14.98	4.99	2.00	145.57	24.12
F (mg/ℓ)	0.18	0.15	0.05	0.61	0.14
K (mg/ℓ)	2.17	0.73	0.15	11.28	2.91
Mg (mg/ℓ)	24.27	18.52	1.02	65.79	18.52
$NH_4(mg/\ell)$	0.68	0.02	0.02	16.78	2.43
NO <sub>3</sub> +NO <sub>2</sub>	0.38	0.09	0.04	7.52	1.02
Na (mg/l)	11.91	3.45	1.00	109.31	18.05
$PO_4 (mg/\ell)$	0.21	0.02	0.01	4.90	0.70
$SO_4(mg/\ell)$	15.18	5.70	2.00	113.27	20.22
Si (mg/ℓ)	6.26	5.94	1.27	11.20	2.16
pН	8.16	8.23	7.31	8.66	0.30
DO (mg/l)	6.55	6.94	1.81	11.48	2.49
Temperature (°C)	17.98	18.60	7.70	29.52	4.18
Turbidity (NTU)	12.49	6.77	0.00	75.10	14.34
EC (mS/m)	39.21	31.10	2.66	113.70	28.57
TAL (mg/ℓ)	168.73	146.83	4.00	497.00	121.00



Marico and Molopo River systems (North West Province, South Africa) showing the location of the study area

Figure 1

The Groot

From the results in the study, it is clear that the water quality deteriorates downstream, especially as it flows through the towns of Groot Marico, Zeerust and Mafikeng. Downstream water quality impacts may also occur from impoundments (dams and weirs) as well as from agricultural activities.

#### Indices

Diatoms were collected, prepared and enumerated according to the protocol as set out in Taylor et al. (2005). Only epilithic diatoms were sampled for the study due to the fact that this is the community of preference for the most diatom indices as well as being the community that yields the best coefficients when multiple regressions are performed with water quality variables (Hodgkiss and Law, 1985). Diatom identification was carried out according to the nomenclature of Krammer and Lange Bertalot (1986-1991) and the SPI, BDI, SPI was chosen as it has the broadest species base; BDI showed the best overall correlation to water quality variables in studies performed recently on the Vaal River (Taylor et al., 2007).

Species diversity index, species evenness and number of species were calculated using the OMNIDIA software package (Lecointe et al., 1993). For the biodiversity indicator (species diversity, species evenness and number of species) calculations, the same numbers of cells counted for the aut-ecological index calculation were used (400 cells per slide). It is a well-known fact that there is a relationship between diversity indices and sample size (Seber, 1986; Lewins and Joanes, 1984). Since the same numbers of cells were counted on each slide, the data are comparable between samples as well as between indices.

### Water quality

Instream water quality measurements for pH, dissolved oxygen (mg/ $\ell$ ), electrical conductivity (mS/m) as well as temperature (°C) were done at each locality by means of an YSI 556 MPS Multimeter. Water samples were taken concomitantly with biological samples at every site.

The water sample was preserved by means of an HgCl<sub>2</sub>

ampoule broken into each sample and delivered to Resource Quality Services (Department of Water Affairs and Forestry, Roodeplaat) for analysis. The following water quality variables formed part of the analysis:

Calcium (mg/ $\ell$  Ca), chloride (mg/ $\ell$  Cl), fluoride (mg/ $\ell$  F), potassium (mg/ $\ell$  K), magnesium (mg/ $\ell$  Mg), ammonia (mg/ $\ell$  NH<sub>4</sub>-N), nitrate and nitrite (mg/ $\ell$  NO<sub>3</sub>+NO<sub>2</sub>), sodium (mg/ $\ell$  Na), ortho-phosphate (mg/ $\ell$  PO<sub>4</sub>-P), sulphate (mg/ $\ell$  SO<sub>4</sub>), silica (mg/ $\ell$  Si), total alkalinity (mg/ $\ell$  TAL) and turbidity (NTU, nephelometric turbidity units).

#### Statistical analysis

Multiple regressions and correlation analysis were performed using the STATISTICA software package (Release 7, Stat Soft. Inc., United States of America), while principle component analysis (PCA) was performed using CANOCO for Windows (Version 4.51, Biometris-Plant Research International, The Netherlands). Before analysis, all the data were standardised by subtracting the sample mean and dividing the result by the sample standard deviation (Hair et al., 1998). This was done to ensure that all the variables could contribute equally to the regressions and correlations by removing the scale factor. For the regressions the forward stepwise method was chosen to eliminate variables that do not contribute to the regression. For the purpose of the multiple regressions the Electrical Conductivity and Total Alkalinity were left out of the analysis as these variables contributed to multi-colinearity in the data, due to high correlation with other water quality variables (e.g. Ca, Cl, F, K, Mg, Na, SO<sub>4</sub>). The R<sup>2</sup> value was used, instead of the R value, as it is a stricter measure of the predictability of multiple regression.

#### Results

#### **Diversity indices**

Table 2 represents the results for the multiple regression analysis for water quality and diatom species diversity. The  $R^2$  for the regression was 0.215. This shows that approximately 21.5% of

the variation in the diatom species diversity could be attributed to the measured water quality variables. The beta values in the table are the regression coefficients while the p-values indicate whether a specific variable contributed statistically significant to the regression. Water quality variables that contributed significantly (p<0.05) to the regression were fluoride and the pH of the water. The fact that changes in fluoride and pH concentrations influence diatom community structures have been noted by several authors (Joy and Balakrishnan, 1990; Ares et al., 1983; Lewin, 1962).

TABLE 2Regression summary for Shannon speciesdiversity with water quality (italicised valuessignificant at p<0.05)						
N=102		R <sup>2</sup> = 0.215				
	Beta Std. err. of Beta p-level					
Intercept			<0.001			
F (mg/ℓ)	0.385	0.131	0.004			
pН	-0.347	0.109	0.002			
$SO_4(mg/\ell)$	0.175	0.125	0.164			
DO (mg/l)	0.101	0.100	0.313			

The results for the regression performed to explain species evenness with water quality are provided in Table 3. The  $R^2$  value for prediction significance of water quality for this variable was only slightly lower than for the species diversity at 20%. Once again the significant contributors to the regression were pH and fluoride.

TABLE 3Regression summary for Pielou speciesevenness with water quality (italicised valuessignificant at p<0.05)						
N=102	R <sup>2</sup> = 0.201					
	Beta Std. err. of Beta p-level					
Intercept			<0.001			
F(mg/l)	0.430	0.104	<0.001			
pН	-0.211 0.105 0.048					
$PO_4 (mg/\ell)$	0.183	0.093	0.051			

The regression performed for the number of diatom species and water quality (Table 4) indicates that considerably more of the variation in the values of this index can be explained by the measured water quality variables. Just over 32% of the variation in the number of species encountered in samples could be attributed to water quality variables and Ca, Mg, Si, and K were the significant contributors in the regression model.

Archibald (1971) stated, in his studies on South African diatom species diversity, that clean water samples displayed diatom diversity scores between that of moderately enriched and polluted samples. This result was supported by findings of Van Dam (1982) by demonstrating that the highest number of diatom species occurred in moorland pools which were moderately affected by acidification as opposed to the low diversity found in very acidic pools. That diversity tends to be high at 'medium water quality' and lower at either 'good' or 'poor' water quality suggests a quadratic relationship of species diversity to disturbance may be more appropriate than a linear one. To test whether this tendency is also followed in the current data set, a quadratic or 2<sup>nd</sup> degree polynomial regression was fitted on the data. This

	Inncant	species with water quality (italicised values significant at p<0.05)					
N=102		R <sup>2</sup> = 0.321					
	Beta	Std. err. of Beta	p-level				
Intercept			<0.001				
рН	-0.239	0.123	0.056				
F (mg/l)	0.151	0.219	0.494				
Na (mg/ℓ)	-1.462	0.845	0.087				
Temperature (°C)	-0.090	0.102	0.379				
Ca (mg/ℓ)	-1.072	0.270	<0.001				
Mg (mg/ℓ)	0.802	0.310	0.011				
Si (mg/ℓ)	0.319	0.149	0.036				
$SO_4 (mg/\ell)$	0.251	0.174	0.153				
K (mg/ℓ)	0.592	0.287	0.042				
Turbidity (NTU)	-0.198	0.115	0.089				
Cl (mg/ℓ)	0.740	0.735	0.317				

was done by the use of, for example Ca as well as  $(Ca)^2$  (a quadratic term), as terms in the regression with species diversity indices (see e.g. Hair et al., 1998 or Neter et al., 1985). If the results are better than those for the linear regression it would show that the relationship is therefore rather quadratic than linear. This observation would indicate that the use of diatom species diversity in a strictly linear fashion as tool to evaluate water quality is not optimal.

Tables 5, 6 and 7 present the  $2^{nd}$  degree polynomial regressions that were performed, for species diversity, species evenness and number of species, respectively, with water quality.

As can be seen from the  $R^2$  values for the regression, which includes  $2^{nd}$  degree polynomial terms of water quality variables, much more of the variation in the diversity data was explained than with the linear regressions. The  $R^2$  for species diversity increased from 21.5% to 54.5% and the  $R^2$  for the species evenness increased from 20% to 40% when including the  $2^{nd}$  degree polynomial terms. The number of species showed the least amount of improvement in the  $R^2$  that increased from 32% to 43%.

# Aut-ecological/biotic indices

The regression summary for the biotic indices BDI and SPI is presented in Table 8 and Table 9. For both these indices water quality contributed to about 80% of the variation in the data. For the SPI the significant contributors were Na, Si, pH,  $PO_4$ , Ca, Cl and  $SO_4$ . The significant water quality contributors to the BDI were much the same as for the SPI with the addition of  $NO_3+NO_2$ , Mg and F. When 2<sup>nd</sup> degree polynomial regressions were fitted to the BDI and SPI, the R<sup>2</sup> value for the regressions changed marginally from near 80% to 84% (not shown). The increase in the R<sup>2</sup> value is probably due to a minor degree of "overfitting" due to the addition of variables to the model in the case of the 2<sup>nd</sup> degree polynomial regression (Hair et al., 1998). This change in R<sup>2</sup> value is small when compared to the changes encountered in the diversity related regressions (see above).

As indices are usually applied in a linear fashion (high value indicating good environmental condition, low values indicating poor environmental conditions), a linear response of index scores to water quality variables is a desirable attribute

TABLE 5 Polynominal regression summary for Shannon species diversity with water quality (italicised values significant at p<0.05)					
(Italicised values	$R^2 = 0.546$				
	Beta	Std. err. of Beta	p-level		
Intercept			<0.001		
F*F (mg/l)	0.658	0.167	<0.001		
Ca*Ca (mg/ℓ)	0.375	0.255	0.146		
Temperature (°C)	0.223	0.101	0.030		
Ca (mg/ℓ)	-0.947	0.377	0.014		
F (mg/ℓ)	-0.253	0.229	0.273		
Si (mg/ℓ)	0.103	0.166	0.537		
DO*DO (mg/l)	0.007	0.097	0.945		
$PO_4 (mg/\ell)$	0.720	0.552	0.196		
K*K (mg/l)	-1.773	0.447	<0.001		
Mg (mg/ℓ)	0.255	0.532	0.633		
Mg*Mg (mg/ℓ)	-0.378	0.265	0.158		
$SO_4*SO_4(mg/\ell)$	-1.075	0.290	<0.001		
$SO_4 (mg/\ell)$	1.648	0.499	0.001		
Turbidity*Turbidity (NTU)	0.670	0.219	0.003		
Turbidity (NTU)	-0.601	0.193	0.003		
DO (mg/l)	0.210	0.119	0.080		
K (mg/ℓ)	0.916	0.388	0.021		
$PO_4*PO_4(mg/\ell)$	-0.141	0.524	0.789		
$NH_4*NH_4(mg/\ell)$	0.878	0.371	0.020		
$NH_4(mg/\ell)$	-1.014	0.547	0.068		
Cl*Cl (mg/l)	0.391	0.217	0.075		

TABLE 6 Polynomial regression summary for Pielou species evenness with water quality (italicised values significant at p<0.05)				
N=102	R <sup>2</sup> = 0.404			
	Beta	Std. err. of Beta	p-level	
Intercept			<0.001	
Si*Si (mg/ℓ)	0.416	0.143	0.005	
Ca*Ca (mg/ℓ)	0.212	0.098	0.034	
F*F (mg/l)	0.532	0.138	<0.001	
Temperature (°C)	0.185	0.087	0.036	
DO* DO (mg/l)	-0.086	0.085	0.312	
$PO_4 (mg/\ell)$	0.771	0.225	0.001	
K*K (mg/l)	-0.737	0.223	0.001	
Turbidity*Turbidity (NTU)	0.257	0.118	0.032	
$NH_4(mg/\ell)$	-0.604	0.382	0.117	
$NH_4*NH_4(mg/\ell)$	0.298	0.270	0.272	

of such indices. Since the linear regressions of SPI and BDI with water quality variables are high ( $\pm$  80%) and little is gained from the addition of polynomial terms, it is suggested that the linear relationship of SPI and BDI with a water quality gradient is sufficient for use of the index in a linear fashion in river systems.

TABLE 7				
Polynomial regression summary for the number of				
diatom species with water quality (italicised values				
significant at p<0.05) N=102 R <sup>2</sup> = 0.430				
N=102				
	Beta	Std. err. of Beta	p-level	
Intercept			<0.001	
F*F (mg/l)	0.341	0.153	0.029	
pH	-0.193	0.161	0.233	
K*K (mg/l)	-0.497	0.364	0.176	
Ca*Ca (mg/ℓ)	0.710	0.262	0.008	
Ca (mg/ℓ)	-1.778	0.386	<0.001	
Mg (mg/ℓ)	1.946	0.482	<0.001	
Mg*Mg (mg/ℓ)	-0.638	0.281	0.026	
Si*Si (mg/ℓ)	-0.211	0.181	0.246	
Na (mg/ℓ)	-1.153	0.413	0.006	
Si (mg/ℓ)	0.242	0.172	0.163	
$PO_4^*PO_4(mg/\ell)$	0.106	0.209	0.614	
K (mg/ℓ)	0.831	0.414	0.048	
Cl*Cl (mg/l)	0.492	0.308	0.115	
Temperature (°C)	-0.148	0.100	0.142	
pH*pH	-0.135	0.109	0.220	
DO (mg/l)	-0.113	0.107	0.295	

TABLE 8
Regression summary for the SPI with water quality
(italicised values significant at p<0.05)

N=102		R <sup>2</sup> = 0.796				
	Beta	Std. err. of Beta	p-level			
Intercept			1.000			
Na (mg/ℓ)	-1.978	0.524	<0.001			
Si (mg/ℓ)	-0.297	0.093	0.002			
pН	0.305	0.068	<0.001			
NO <sub>3</sub> +NO <sub>2</sub>	0.249	0.150	0.101			
K (mg/ℓ)	-0.231	0.175	0.191			
$PO_4 (mg/\ell)$	0.368	0.173	0.036			
$\mathrm{NH}_4(\mathrm{mg}/\ell)$	-0.054	0.171	0.752			
Ca (mg/l)	-0.344	0.172	0.049			
Cl (mg/ℓ)	1.108	0.449	0.015			
$SO_4 (mg/\ell)$	-0.195	0.097	0.048			
F (mg/ℓ)	0.186	0.137	0.179			
Mg (mg/ℓ)	0.229	0.190	0.231			

# Principle component analysis (PCA)

A principle component analysis was performed to visually represent the response of the two types of indicators with water quality variables. The results of this analysis are shown in Fig. 2 and Table 10. From the figure we can see that the main drivers in the water quality in the catchment are Na and Cl (correlates with the first ordination axis). The BDI and SPI indices (representing the aut-ecological indices) are strongly affected by the main drivers of the water quality in the catchment and show a strong negative response to increasing salt loadings (Na and Cl). The length of the vectors representing the various indices in Fig. 2

TABLE 9Regression summary for the BDI with water quality (italicised values significant at p<0.05)					
N=102	R <sup>2</sup> = 0.810				
	Beta	Std. err. of Beta	p-level		
Intercept			1.000		
Na (mg/ℓ)	-2.032	0.458	<0.001		
Si (mg/ℓ)	-0.287	0.080	0.001		
$NO_3 + NO_2 (mg/\ell)$	0.305	0.083	<0.001		
K (mg/ℓ)	-0.265	0.152	0.084		
pH (mg/ℓ)	0.178	0.064	0.006		
Ca (mg/ℓ)	-0.571	0.152	<0.001		
Cl (mg/ℓ)	1.159	0.387	0.004		
$PO_4$ -P (mg/ $\ell$ )	0.305	0.096	0.002		
Mg (mg/ℓ)	0.360	0.178	0.046		
F (mg/ℓ)	0.229	0.113	0.047		
$SO_4 (mg/\ell)$	-0.174	0.089	0.054		

also indicates a much stronger effect of water quality variables on the BDI and SPI than on the diversity index measures (as the vectors of the former are longer than those of the latter).

Diversity and evenness as shown in Fig. 2 seem to increase in the same direction as most of the water quality variables. This might be caused by the fact that diversity seems to be higher at sites with intermediate values for the water quality parameters measured in the study.

The number of species (Fig. 2) was associated with increased oxygen in the water and negatively associated with the chemical variables that denote possible organic loading of the water system ( $NH_4$ ,  $PO_4$ ,  $NO_2+NO_3$ ). However, the relationship of the number of species to water quality variables was low, with  $R^{2^*}$ s of 0.320 and 0.430 (Tables 4 and 7).

# Discussion

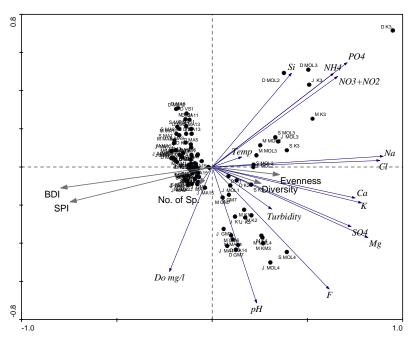
From the results it is clear that both types of diatom-based indicators (diversity and aut-ecological) used were significantly influenced by water quality variables. From the three different diversity indices calculated, the number of species showed the strongest response to changes in water quality variables, although this relationship is fairly weak (Fig. 2). Of all the indicators tested in the study, the species diversity and the species evenness showed the weakest response to water quality variables (Tables 2 and 3).

From the 2<sup>nd</sup> degree polynomial multiple regressions, it can be seen that the highest species diversity and evenness as well as number of species occur in moderately impacted water as suggested by Archibald (1971) and Van Dam (1982). A high degree of dominance may therefore be expected at both clean water and polluted water sites. It would seem logical to conclude that moderately impacted water can harbor species that can be dominant in either good or polluted water, as observed by Van Dam (1982).

Due to the fact that the species evenness does not exhibit a strong linear association with water quality, it would be logical to conclude that, in the case of diatoms, a high level of dominance in the population (as would be represented by a low evenness index in this study) cannot be equated to polluted or less favorable conditions. It would be more consistent with the data to expect that diatoms have well defined niches and that taxa best suited to water quality conditions at a specific point in time will become dominant. This is borne out by Cholnoky (1960) who stated that: "...it should be pointed out that changes in one or other of the factors which have been discussed here [pH, salinity and nutrient concentrations] need not necessarily bring about the death of one or other of the algal species so long as the changes remain within the limits occurring in nature. On the contrary, these changes will inhibit the multiplication of some of the species originally present, and encourage that of others, so that primarily the association i.e. the percentage composition and not the flora as such, will be changed" (own italics).

This would also suggest that specific diatom taxa will be dominant in certain (and most) water quality conditions. Representatives of many species are always present in low numbers in the population and can become dominant when water quality is suitable.

The data are also consistent with postulations from Kelly (1998) who discussed the concept that diatoms are 'subcos-



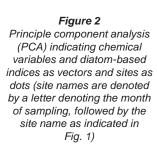


TABLE 10 Results for PCA performed for sites in the Marico and Molopo Rivers (Biological and habitat indices were used as supplementary data in the ordination)					
Axes	1	2	3	4	Total variance
Species-environment correlations	0.803	0.378	0.331	0.143	
Cumulative percentage variance:					
of species data	40.6	57.8	70.5	78.8	
of species-environment relation	81.1	88.7	93	93.6	
Sum of all canonical eigenvalues					0.323

mopolitan', i.e. they occur anywhere in the world if certain environmental conditions are fulfilled. This concept suggests that geographical location is not the determining factor in the distribution of diatom species and the composition of communities, but it is rather the specific environmental variables at a specific site that determine this distribution. Finlay (2005) also states that it is now clear that distribution patterns of protists are quite different from those of macroscopic organisms - e.g. the recent discovery of the ubiquity-biogeography transition, where organisms smaller than about 1 mm occur worldwide wherever their required habitats are realised. However, some diatoms may be more susceptible to desiccation etc. and thus may not be so easily distributed. This would appear to be the case when (possibly) endemic diatoms such as Achnanthes standerii Cholnoky are found en masse in certain rivers and streams around South Africa but have never been reported from outside our borders. Whether diatoms such as these are in fact true endemics or if their distribution is simply governed by the factors such as local geology and climate, which may not be found elsewhere, remains a topic for further investigation.

In comparison to the diversity indices, the BDI and SPI as representatives of aut-ecological indices displayed a significantly better relationship with measured water quality variables as shown by the multiple regression results. It is also important to note that the relationship of the BDI and SPI with water quality did not increase significantly when the quadratic functions were added. The implication of this would be that the aut-ecological indices may be applied in a linear fashion in contrast to diversity indices.

The abovementioned results indicate that aut-ecological indices based on diatoms are more useful in biomonitoring programs of rivers and streams than diversity indices; a point strongly supported by the high  $R^2$  values of the linear multiple regressions for SPI and BDI in Table 8 and 9.

# Conclusions

The current study and the results presented in the different sections of the current study warrant the following conclusions:

- Diversity measures based on the abundance of diatoms appear to show a relationship to water quality variables although that relationship is not linear
- The results from the linear and 2<sup>nd</sup> degree polynomial regressions show that diatom species diversity (especially as reflected by the Shannon Species Diversity Index used in this study) tends to be higher in moderately impacted water
- Due to the highly significant relationship of aut-ecological diatom indices with water quality, these indices are deemed more relevant and reliable for use in rivers and streams to inform decision making in integrated water resource management.

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