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The impact of drought in the Karoo - revisiting diatoms as water quality indicators in the upper reaches of the Great Fish River, Eastern Cape, South Africa

M. Holmes[a,*], E.E. Campbell[a], M. de Wit[b], J.C. Taylor[c,d]

[a] Department of Botany, Nelson Mandela University, Nelson Mandela Bay, South Africa
[b] Africa Earth Observatory Network (AEON)- Earth Stewardship Science Research Institute (ESSRI) Nelson Mandela University, Nelson Mandela Bay, South Africa
[c] Unit for Environmental Science and Management, North-West University, Potchefstroom, South Africa
[d] South African Institute for Aquatic Biodiversity (SAIAB), P Bag 1015, Grahamstown 6140, South Africa

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A B S T R A C T

This paper examines the efficacy of diatoms as bioindicators of water quality in the upper reaches of the Great Fish River following a period of reduced flow from drought conditions. Of the five sites, three were sampled three times in an 18 month period while the remaining two were sampled once. As a comparison, one headwater site within Mountain Zebra National Park, situated in the same district, was sampled. A total of 166 diatom taxa belonging to 29 genera were identified. Dominant taxa for the Great Fish River were identified as *Amphora pediculus; Epithemia sorex; Nitzschia frustulum; Navicula veneta* and *Craticula buderi.* These species, indicative of high nutrient concentrations and moderately saline to brackish conditions can tolerate low flow conditions. The Generic Diatom Index (GDI), the Specific Pollution sensitivity Index (SPI) the Biological Diatom Index (BDI) and the% Pollution Tolerant Valves (%PTV) – part of the UK Trophic Diatom Index (TDI) were used for interpretation of the results. The indices showed that this river is in poor condition. By comparison the headwater site in the Mountain Zebra National Park had *Cocconeis placentula* var. *euglypta, Achnanthidium minutissimum* and *Cocconeis placentula* var. *lineata* as dominant species and, using the same indices, the river was classified as in moderate to good condition with respect to water quality. When compared to a previous study (2010 – 2012), these sites showed a shift in dominant species indicating a change in water flow conditions and quality.

1. Introduction

Water resources in the semi-arid Karoo are under pressure as they are elsewhere in South Africa (Taylor et al., 2007a, 2007c; Glazewski and Esterhuyse, 2016). The Karoo is prone to extended periods of drought and this, together with human consumption, has placed strain on the freshwater systems in the area. The multi-year drought resulted in a much lower underground water table causing many boreholes to run dry. The knock-on effect has seen many farmers battling to provide water for their livestock and pastures. Towns in the Karoo, such as Graaff Reinet, has been facing severe water shortages for the last seven years (OCHA, 2021). The Great Fish River is an economically and ecologically important river with its source in the mountains of the Karoo, on the eastern side of the Nardou Peak in the Agtersneeuberg. This river flows for 692 km before discharging into the Indian Ocean between Port Alfred and East London (South African History Online, 2020). The upper reaches of the Great Fish River rely solely on spring water that is recharged by rain and snow melt. While this area saw higher rainfall in previous decades, the current drought has affected large parts of the 30 800 km^2 catchment for more than five years (Du Toit, 2019). The drought has resulted in little recharge of these upper reaches and certain parts of this section of the river have become intermittently dry (A. Olivier, personal communication, 2 January 2020). In places the river has been dry for more than three years. Agricultural practices have also transformed not only the river channel but the drainage areas that feed it. Many of the farm pastures extend right up to the edge of the river leaving little or no buffer zone (Fig. 1). This allows for fertilisers, herbicides, and pesticides used on the lands to drain directly into the river (Taylor et al., 2005b).

An additional threat to the water in the area is the prospect of hydraulic fracturing (fracking) for shale gas. In the Karoo, rivers cannot be seen as separate from groundwater sources as many become subterranean flows in dry periods (Hobbs et al., 2016; Glazewski and Esterhuyse, 2016). Many of the contaminants from fracking related

* Corresponding author at: PO Box 713, Cradock, 5880, South Africa.
E-mail addresses: info@karoocats.org (M. Holmes), Eileen.Campbell@mandela.ac.za (E.E. Campbell), Jonathan.Taylor@nwu.ac.za (J.C. Taylor).

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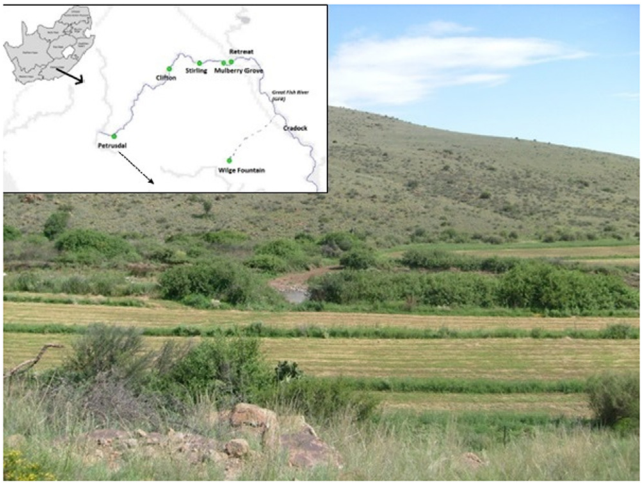

Fig. 1. Great Fish River pools near source surrounded by pastures extending up to the riverbank.

activities contain chemicals that are not included in standard water chemical analysis (DWAF, 1993, 1996; Mamba et al., 2008). This makes biomonitoring of water bodies important in all freshwater sources in the Karoo. Biomonitoring makes use of fish, aquatic invertebrates, aquatic macrophytes and periphyton (including diatoms) to determine deviations from natural conditions (Uys et al., 1996; Kleynhans, 1999; Van der Molen, 2000). The latter is best suited as a method of monitoring the Great Fish River because they respond rapidly to the intermittent flow patterns of this Karoo rivers (Holmes and Taylor, 2015).

Diatoms are a diverse group of single-celled algae in the Bacillariophyta that occur in most aquatic habitats (Patrick, 1977; Round et al., 1990). Species have distinctively ornamented silica cell walls that allow for morphological identification. They respond rapidly to changes in their environment (Harding et al., 2005) and, as many of their environmental requirements are known, diatoms can be used to indicate water quality (Schoeman, 1979; Van Dam et al., 1994; Bate et al., 2002; Taylor et al., 2005). Diatoms were originally proposed as a bioindicators and the development of the first biotic indices in 1901 (Harding and Taylor, 2011). This was improved upon by the Shannon Wiener Index which measured the diversity in a freshwater stream (Shannon and Weaver, 1948) and then in 1961 by Zelinka and Marvan. The latter developed a weighted formula which for the basis for many biotic indices in use today (Harding and Taylor, 2011).

A previous study of this 80 km stretch of the Great Fish River (Holmes and Taylor, 2015) showed that the condition of the river, using diatoms and diatom indices, was not significantly correlated with water chemistry measurements. Standard chemical analysis of river water (such as NO_3^--N, NO_2^--N, NH_4^+-N, PO_4^{3-}-P, SO_4, and $CaCO_3$) alone could not explain the presence of frustule deformities that were recorded (Walsh and Wepener, 2009; Holmes and Taylor, 2015). Szczepocka et al. (2014) provided evidence that macronutrient monitoring does not include the 'ecological memory' of waters nor take into account the synergistic or antagonistic effects of nutrients measured (Dalu et al., 2016). They propose that biomonitoring provides a more holistic picture of water condition, as compared to information obtained from once-off measurements of physical and chemical water variables that does not take temporal variation into account (Taylor, 2004; Bere and Tundisi, 2011; Álvarez et al., 2012; Szczepocka et al., 2014; Dalu et al., 2016).

Water quality assessment using diatoms has been used more frequently in Africa in recent years with varying success (Bate et al., 2004; De la Rey et al., 2004; Taylor, 2004; Taylor et al., 2007a, 2007c; De la Rey et al., 2008; Walsh and Wepener, 2009; Harding and Taylor, 2011; Bere et al., 2014; Holmes and Taylor, 2015; Mangadze et al., 2015; Dalu et al., 2016). The increased information gained from these studies of ecological tolerances and preferences of African diatom species, should make the South African Diatom Index (SADI) which is incorporated into the Sensitivity Pollution Index (in Omnidia), or SPI, more applicable. This study compares the results from four different indices which have different inclusivity criteria. The more precise SPI/IPS (CEMAGREF, 1982; Tan et al., 2017) uses over 2000 taxa when taking salinity, eutrophication and organic pollution into account while the Biological Diatom Index (BDI/IBD; Lenoir and Coste, 1996) is an indicator for nitrates, phosphates, pH, EC, dissolved oxygen and biological oxygen demand (Besse-Lototskaya et al., 2011). The latter takes 146 'abnormal forms' into account (Holmes, 2015). The Generic Diatom Index (GDI; Coste and Ayphassorho, 1991) uses 174 taxa identified to genus level. The Percentage Pollution Tolerant Valves (%PTV) as part of the UK Trophic Diatom Index (TDI: Kelly and Whitton, 1995), reflects the percentage of the diatom species tolerant of organic pollution.

The aim of this study was to determine the diatom community composition in the same stretch of the Great Fish River as previously studied (Holmes and Taylor, 2015), and to determine if long term change has occurred and if these changes were a reflection of changing water chemistry, anthropogenic influences or droughts.

2. Materials and methods

Five sampling sites used by Holmes and Taylor (2015) were resampled as part of the AEON Karoo Shale Gas Baseline Project (Fig. 2).

Topography in the in the Cradock district of the Nama Karoo Biome is varied (Mucina and Rutherford, 2006). Most of the area's plains have low shrub vegetation with grasses, but the drainage lines have more trees, some of which are alien. Summers are hot (>40 °C daily maximum taken in the sun) and winters are generally cold (as low as −10 °C), dry and windy, with occasional snow on the higher lying areas (personal observation, unpublished data). A severe drought has been affecting large parts of the eastern Karoo since 2015 (Krause, 2021). Rainfall is highly localised, and some parts of the study area received more rain than others – the higher mountain areas received particularly low rainfall (Fig. 3). Snowfall during this period was also low. The original sampling (Holmes and Taylor, 2015) commenced in 2010 when rainfall levels were only slightly higher than in the period during which sampling was done for this study. During the study of Holmes and Taylor (2015), rainfall increased over the study period (2010 to 2012). During the subsequent study however (sampled in 2016–2017), samples were taken during a consistently dry period. The annual average rainfall, under non-drought conditions, for Clifton and Stirling (Fig. 2) is 350 mm while at Petrusdal (Fig. 2) it is 450 mm (A. Olivier, personal communication, 2 January 2020).

For comparison, the high mountain site at the source of the Wilge River in the Mountain Zebra National Park (at 1 435 m.a.s.l.) was sampled. The source of the Wilge River is in the Bankberg Mountains and has been protected from agricultural influences since approximately 1964 when the farms Rooiplaat and Doornhoek were proclaimed as part of the Mountain Zebra National Park. The source is in the southern part of the park that is underdeveloped and where there is almost no road infrastructure (SANParks, 2016). The high-altitude sampling site on the Great Fish River, near its sources, is Petrusdal at 1 581 m.a.s.l. The Clifton and Stirling sites are at an altitude of 1129 and 1039 m.a.s.l. respectively. The lower altitude sites of Mulberry and Retreat are at 993 and 991 m.a.s.l. respectively.

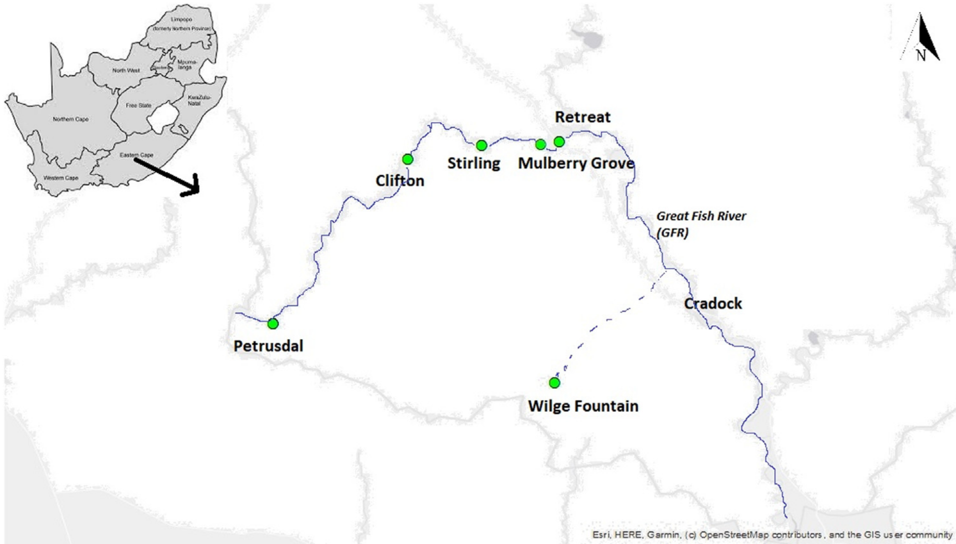

Fig. 2. The locality of five sampling sites measured along the Great Fish River as well as one at the source of the Wilge River in Mountain Zebra National Park.

2.1. Field sampling

As part of a larger project undertaken to obtain a baseline dataset for diatom community composition and water quality prior to the possible commencement of fracking, three sampling trips were done. Two of these were seasonal – summer (January 2016) and winter (June 2017) with an additional summer sampling trip (February 2018). All five Great Fish River sites were sampled in the first summer. Subsequently, Mulberry Grove was sold, and permission was not received from the new owner for continued monitoring while the headwater site, Petrusdal, had extremely low flow after the first sampling and was excluded from further sampling. Mulberry Grove has subsequently dried up (2017 onwards) as a result of the drought. The Retreat study site has regular cattle activity along the river's edge. This was not the case in the study of Holmes and Taylor (2015). There have been no other major changes that could have affected the riverine area at the other sites, apart from possible additional abstraction. Samples from the Wilge River (Mountain Zebra National Park) were collected in April 2016 (autumn) and October 2016 (spring).

Physical water quality variables were measured at the time of diatom sampling. A Hanna HI98129 hand-held multimeter was used to measure water pH, electrical conductivity and temperature. Dissolved oxygen was measured using a Hanna HI98393 dissolved oxygen metre. Samples for water chemistry were taken together with diatom samples in 2016 and 2017. Water samples were processed by InnoVenton Laboratory in Port Elizabeth where samples were preserved with nitric acid for further testing after anion analysis. Anions were analysed using a Metrohm IC 761 Compact with external 5-point calibration. The cations analysed using the Inductively Coupled Plasma Mass Spectrometer method (ICP-MS) with a detection standard by Sci-Ba Laboratories. Diatom samples were taken by scrubbing cobbles (100–256 mm particles) from the middle section of the river as well as from near the bank. All samples were checked for presence of chloroplasts (live cells) within 24 h of collection before preservation with ethanol.

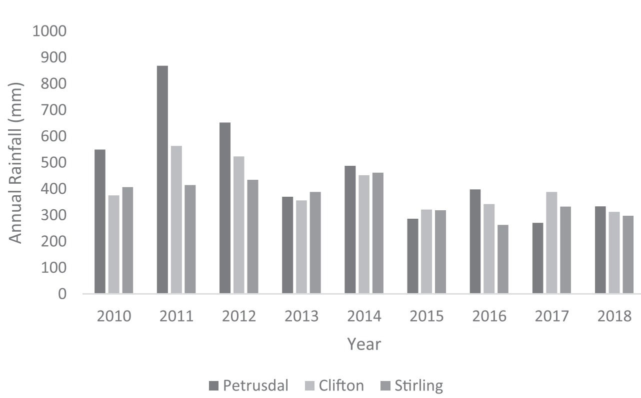

Fig. 3. Annual rainfall at the three of the Great Fish River sites between 2010 and 2017.

2.2. Laboratory analysis

The diatom frustules were cleaned of organic matter using the potassium permanganate and hydrochloric acid method after which they were mounted on slides using Pleurax (Taylor et al., 2005, 2007b). Slides were viewed using a Nokia E100 phase contrast microscope using an Olympus 100 × 1.35 N.A. phase contrast objective or a Nikon 100 × 1.25 phase contrast objective. Frustule measurements were done using the software IC Measure (The Imaging Software Company). Micrographs of cells were taken with a 5 Mpixel $1^1/_4$ inch CMOS camera using the software IC Measure.

Identification guides used were Krammer and Lange Bertalot (1987, 1999a, 1999b), Lange-Bertalot, 1996, Krammer (1997, 2000), Prygiel and Coste (2000), Taylor et al. (2007d), Lavoie and Hamilton, 2008 as well as Bey and Ector (2013) as well as the Diatoms of North America (2018) and Academy of Natural Sciences (2011) databases. A total of 400 valves per slide were enumerated for species as well as the number of deformed frustules. Each whole valve was counted, and broken valves (>2/3rd size) were only included in the count if they could be positively identified.

2.3. Statistical analysis

Omnidia 6 (Lecointe et al., 1993; 1999; 2016) was used in the calculation of the diatom indices. The ecological indicator values of Van Dam et al. (1994) within Omnidia was used for interpretation. To allow for comparison with Holmes and Taylor (2015), the same indices were used in this study: the Specific Pollution sensitivity Index (SPI); the Generic Diatom Index (GDI); the Biological Diatom Index (BDI) and the Percentage Pollution Tolerant Valves (%PTV) designed to reflect organic pollution (part of the UK Trophic Diatom Index of Kelly and Whitton 1995). The first three indices are scored between 0 and 20 with the lower values indicating pollution or eutrophication.

They are calculated using the weighted average of Zelinka and Marvan (1961). Any%PTV count over 20% indicates organic pollution (Kelly and Whitton, 1995). Eloranta and Soininen (2002) applied a scale (for the GDI, SPI and BDI) so that poor water quality had a score of <6 and high-quality waters had a score of >17. Good quality ranged from 14 to 17, moderate was taken to be from 10 to 14 and poor water quality was indicated by a score between 6 and 10. Tests for normality (Shapiro Wilks), Pearsons Correlation and Spearman Rank Order Correlation analysis were performed using Statistica v13.3 (TIBCO, 2020).

3. Results

3.1. Diatom community composition

A total of 166 taxa in 29 genera were identified from the samples collected. Of these, 9 taxa could not be identified to genus level. When rare species were removed (<1% abundance), 89 taxa remained. Dominant diatom species were taken to be those at greater than 5% relative abundance (Table 1).

When the nutrient spectrum (Van Dam et al., 1994) is assigned to each dominant species, the majority fall into the eutrophic category except for *Cocconeis placentula* which falls into the meso- to eutrophic range. The species *Achnanthidium minutissimum* and *Craticula buderi* recorded fall in the category of those with a wide nutrient tolerance range.

When the dominant species (Table 1) are compared to those reported in Holmes and Taylor (2015), several species are no longer dominant: *Diatoma vulgaris* Bory, *Fragilaria biceps* (Kützing) Lange-Bertalot, *Nitzschia linearis* (Agardh) W.M.Smith, *Nitzschia paleacea* (Grunow) Grunow in Van Heurck, *Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot and *Rhopolodia gibba* (Ehrenberg) O. Müller. Combining both the relative abundance data reported here, for the GFR, with that reported in Holmes and Taylor (2015) the river dominants (2010 – 2017) show an increase in *Amphora pediculus, Epithemia sorex* and *Navicula veneta* for all sites and the disappearance of *Cocconeis placentula* var. *euglypta, Diatoma vulgaris* and *Planothidium lanceolatum* from all sites (Table 2). A dramatic increase is seen for *Craticula buderi* at the headwater site (from 0.5 to 60.5%) and a decrease at the site below the dairy (Retreat, from 18.5 to 2.9%, Table 2). A decrease in the abundance of *Nitzschia frustulum, Nitzschia paleacea, Rhopolodia gibba* and *Ulnaria biceps* is seen (Table 2). There were 94 taxa listed in Holmes and Taylor (2015) that were not recorded again but 41 diatom taxa were recorded in the 2016–2017 period that were not listed before.

Table 1
Dominant diatom species (>5%) recorded in the waters of the Great Fish River (GFR) and Mountain Zebra National Park (MZNP). Species authorities are provided in Appendix 1.

	Sites	Species	Relative abundance%
1	When all sites and samples combined (GFR & MZNP)	*Amphora pediculus* (Kützing) Grunow	10.6
		Epithemia sorex Kützing	9.7
		Nitzschia frustulum (Kützing) Grunow	7.0
		Cocconeis placentula var. *euglypta* (Ehrenberg) Grunow	6.0
		Craticula buderi (Hustedt) Lange-Bertalot	5.4
2	GFR sites for 2016–2017	*Amphora pediculus*	12.3
		Epithemia sorex	10.6
		Nitzschia frustulum	8.3
		Craticula buderi	6.4
		Navicula veneta Kützing	5.4
3	Wilger River MZNP	*Cocconeis placentula* var. *euglypta*	37.7
		Achnanthidium minutissimum (Kützing) Czarnecki	16.3
		Cocconeis placentula var. *lineata* (Ehrenberg) Van Heurck	7.4

Table 2
Relative abundance of dominant diatom species for each site in the Great Fish River found in current study (2016–2017) and compared to relative abundance data from 2010 to 2012 in Holmes and Taylor (2015).

Species	Site	2010	2017
Amphora pediculus	Clifton	11.9	12.1
	Stirling	1.2	14.5
	Mulberry Grove	0.7	3.2
	Retreat	6.8	17.3
Cocconeis placentula var.*euglypta*	Petrusdal	11.6	0
Craticula buderi	Petrusdal	0.5	60.5
	Retreat	18.5	2.9
Diatoma vulgaris	Clifton	9.1	0
	Stirling	7.2	0
Epithemia sorex	Petrusdal	2.3	0
	Clifton	0	16.6
	Stirling	0.8	20.7
	Mulberry Grove	2.8	4.5
Navicula veneta	Clifton	1.4	6.6
	Stirling	2.1	7
	Mulberry Grove	2.1	12.4
	Retreat	1.3	1.6
Nitzschia frustulum	Clifton	12.5	6.7
	Stirling	19.9	6.3
	Mulberry Grove	19.4	13.4
	Retreat	19.4	13
Nitzschia linearis	Clifton	5.4	0.7
Nitzschia paleacea	Petrusdal	6.6	2.9
Planothidium lanceolatum	Petrusdal	8	0
Rhopolodia gibba	Stirling	15.3	4.5
	Mulberry Grove	27.8	0.7
Ulnaria biceps	Clifton	9	0
	Stirling	7.5	2.1
Deformed cells	Petrusdal	3.2	2
	Clifton	3.1	1.1
	Stirling	4.1	2.4
	Mulberry Grove	4	0.7
	Retreat	2.9	2.4

3.2. Diatom deformities

The average percentage of deformed valves measured in the 2016–2017 period was 1.7% and the average for all sites was lower than previously reported (Table 2; Holmes and Taylor, 2015) in the 2010–2012 study.

3.3. Water chemistry variables

The pH of the water at the Wilge River and the Petrusdal and Stirling sites of the Great Fish River was on average above 8 (Table 3). The electrical conductivity of the water at Retreat was the highest (1001 μS/cm) and the lowest was recorded at Petrusdal (212 μS/cm; Table 3). The lower readings for the Wilge River (MZNP) followed the trend of a headwater site (251 - 300 μS/cm). Holmes and Taylor (2015) reported nitrate and nitrite values (from 2010 to 2012) which have been combined in the present study to oxidized nitrogen to facilitate comparison with the values presented in Table 3. Sites Petrusdal and Mulberry Grove had a drop in concentration (mean values; 2.3 to 0.04 mg/L and 0.3 to 0.04 mg/L respectively) with Retreat retaining similar levels. The remaining two sites saw a substantial increase, from the values in 2010–2012, (mean values); 0.3

Table 3
The mean (ranges given) for measured water chemistry variables of the waters of the Great Fish River and Wilge River sampling sites for 2016 - 2017. MZNP = Mountain Zebra National Park.

Site	Petrusdal	Clifton	Stirling	Mulberry Grove	Retreat	Wilge MZNP
Elevation m	1581	1129	1039	993	991	1435
pH	8.07	7.55 (7.40 - 7.84)	8.56 (8.02 - 9.10)	7.97	7.37 (7.03 - 7.86)	8.37 (8.04 - 8.69)
EC (μS/cm)	212	787 (721 - 819)	712 (621 - 872)	500	1001 (858 - 1148)	276 (251 - 300)
Dissolved oxygen mg/L	5.91	6.5 (4.0 - 8.3)	9.3 (7.5 - 11.4)	9.69	6 (4.5 - 6.9)	8 (7.8 - 8.3)
Water Temperature (^{0}C)	22	22.6 (12.7 - 27.6)	23.3 (14.9 - 29.1)	31	19.2 (10.7 - 23.7)	21 (18.5- 23.6)
Oxidized nitrogen (NO_x^-) mg/L	0.04	1.7 (0.04 - 3.2)	0.7 (0.03 - 2.2)	0.04	8.5 (7.7 - 9.5)	0.3 (0.04 - 0.5)
Orthophosphate (PO_4^-) mg/L	0.5	2 (0.5 - 5)	2 (0.5 - 5)	0.5	2 (0.5 - 5)	0.5
Sulphate (SO_4) mg/L	12.2	37 (22.7 - 61.5)	36.5 (5 - 59.2)	28.5	42.7 (41.2 - 45.2)	14.6 (13.2 - 16.1)

to 1.7 mg/L for Clifton and 0.3 to 1.7 mg/L for Stirling (Table 3). Phosphate readings (mean values) increased from 2010/2 to 2016/7 for Clifton (0.6 to 2 mg/L) and Stirling (0.5 to 2 mg/L) with the other three sites remaining at similar values (Holmes and Taylor, 2015; Table 3). Sulphate levels (mean values) decreased substantially when compared to 2010/2 (Holmes and Taylor, 2015) for Petrusdal (from 27 to 12.2 mg/L) and Mulberry Grove (45.7 to 28.5 mg/L) while Stirling showed a slight decrease (43.3 to 36.5 mg/L) (Table 3). However, concentrations (mean values) for Clifton increased from 29 to 37 mg/L and from 38.2 to 42.7 mg/L for Retreat (Holmes and Taylor, 2015; Table 3).

3.4. Diatom indices

The diatom indices (Generic Diatom Index, the Specific Pollution sensitivity Index, and the Biological Diatom Index) were normalised to a five-point scale ranging from bad, through poor, moderate, and good to high quality (Table 4; Eloranta and Soininen, 2002). The%PTV is a percentage and is interpreted as such. The mean values of the percentage of species inclusion in the calculation of the indices (Table 4) below were:%PTV was 88.3% ($\pm$3.8 SE); SPI was 95.5% ($\pm$2.0 SE); BDI was 87.9% ($\pm$3.8 SE) and GDI was 97% ($\pm$1.1 SE).

Overall, the Great Fish River scores ranged from bad to moderate (Table 4) while the Wilge River (MZNP) was of moderate to good quality (Table 4). The differences between the score of the SPI and BDI (t = 0.39; df = 24; p = 0.35) and GDI (t = 0.41; df = 24; p = 0.35) as well as the BDI and GDI (t = 0.71; df = 24; p = 0.24) were not statistically significant. The Wilge River site did not show much of a difference in the three indices. Surprisingly Petrusdal, as the headwater site, scored a bad classification (Ecological Category F to E). This contrasts with the previous classification of poor (Ecological Category D towards E) for the 2010 – 2012 study. Clifton remained a poor site while Stirling changed from poor/bad to moderate (Ecological category C towards D). Retreat improved from bad to poor in the current study.

3.5. Correlation of diatom indices and water variables

Not all the water variables were parametric, therefore Spearmans Rank Order Correlation was used for correlation between environmental variables and the diatom indices as well as the environmental variables and diatom species. Water variables and diatom indices were log transformed. The only significant correlation ($p < 0.05$) between any of the dominant species (Table 2) and the water chemistry presented in Table 3 was between *Amphora pediculus* and electrical conductivity (r_s = 0.55). There were no significant correlations ($p < 0.05$) between the diatom indices (Table 4) and any of the water chemistry variables in Table 3.

4. Discussion

4.1. Community composition

Rare taxa (<1%) provide no significant ecological information when calculating diatom indices (Lavoie et al., 2009b). Once these were removed, approximately half of the taxa remained. Out of the remaining 89 taxa, the seven taxa which are dominant (>5%) constitute 47% of the cells counted.

Once again, the majority of the species identified in this section of the GFR are cosmopolitan and known to be pollution tolerant. The conclusion that can be drawn from this is that whole stretch of this river is subject to agricultural and/or other unidentified contamination. However, some of the species have been found, within the same hemisphere, to have a varied range of ecological preferences which sometimes differs depending on locality (Van Dam et al., 1994; Bate et al., 2002).

Table 4
Diatom index score and class for each site during the period 2016–2017.%PTV = Percentage of pollution tolerant valves; SPI = Specific Pollution sensitivity Index; BDI = Biological Diatom Index and GDI = Generic Diatom Index. Classes assigned using the values of Eloranta and Soininen (2002).

Sites	Season	%PTV	SPI	BDI	GDI	Class
Petrusdal	Summer 2016	35.8	4.5	2.6	5.0	Bad
Clifton	Summer 2016	13.5	12.4	10.6	13.2	Moderate
	Summer 2017	36.0	7.8	9.5	7.2	Poor
	Winter 2017	47.9	8.8	7.9	7.6	Poor
Stirling	Summer 2016	20.2	9.1	9.8	10.5	Poor
	Summer 2017	23.7	9.2	10.1	8.3	Poor
	Winter 2017	6.1	11.0	10.1	11.3	Moderate
Mulberry Grove	Summer 2016	53.1	6.9	8.1	6.2	Poor
Retreat	Summer 2016	54.2	7.1	8.8	4.7	Poor to Bad
	Summer 2017	54.7	6.9	7.6	4.2	Poor to Bad
	Winter 2017	26.4	9.0	10.6	7.6	Poor
Wilge River	Autumn 2016	1.2	13.7	14.7	13.6	Moderate
	Spring 2016	7.4	13.8	15.4	14.2	Moderate to Good

Some of the common pollution resistant diatoms include *Nitzschia palea* (Kützing) W.Smith and *Navicula veneta. Amphora pediculus* (a dominant species, Table 2) is known to prefer high nutrient levels in hard water but low organic pollution level (Kelly et al., 1995). The previous GFR study found *A. pediculus* dominant in waters with higher electrical conductivity (367 to 1219 μs/cm) and water temperature (Holmes and Taylor, 2015). This result confirms the correlation with electrical conductivity. However, although there was no statistical difference between the means for EC, for Clifton, Stirling and Retreat, between the 2010−2012 data and the current project, the ranges of values are narrower as a result of the low flow and lack of flooding events. This could be a factor in the change of the dominant species.

Craticula buderi can survive in a wide range of conditions but is usually found in hard waters or those with elevated EC. Dominant at the headwater site (Table 2; EC 212 μS/cm), previously it was only dominant at the most polluted site (mean EC 1028 μS/cm) (Holmes and Taylor, 2015). Van Dam et al. (1994) suggest this taxon prefers a moderate to higher EC which is contrary to the present study but in line with the previous one. *Nitzschia frustulum* and *Nitzschia inconspicua* Grunow are able to tolerate changes in flow (osmotic pressure) in brackish water with a high EC (Archibald, 1971 in South Africa) and a nutrient and high organic load (Urrea-Clos and Sabater, 2012 in Spain; Kelly et al., 2001 in the UK; Van Dam et al., 1994 in Europe). *Nitzschia frustulum*, with a moderate oxygen requirement (Van Dam et al., 1994) has been linked to medium to high levels of sulphate and EC (Bate et al., 2004 in South Africa), high phosphate (Slate and Stevenson, 2007 in the USA) higher nitrate levels (Ndiritu et al., 2006 in Kenya).

Achnanthidium minutissimum, with only 2.4% total abundance, occurred in six of the 13 samples and > 5% in two of them (MZNP only). This species occurs in a broad range of conditions and does is not considered a reliable indicator species. This may be in part due to the fact that this small-celled species complex is difficult to identify correctly. The species complex consists of 18 different varieties all with different ecological preferences (Rimet and Bouchez, 2012). Known as a pioneer species, *A. minutissimum* was found in warmer low altitude sites with higher nitrate levels in Zimbabwe (Bere et al., 2013) while being moderately pollution tolerant in Brazil (Bere and Tundisi, 2011; Kelly et al., 2001) and being found it in large quantities in the clean waters of the Jukskei-Crocodile River (Schoeman, 1976). Requiring a high oxygen level (Van Dam et al., 1994), it was dominant in low flow conditions in Algeria (Nehar et al., 2015) while others found it to prefer high velocities (Kelly 2002; Stenger-Kovács et al., 2013; B-Béres et al., 2016). The Wilge River, in the MZNP, has mostly low flow with high velocity water only during flooding events which have been rare since 2012.

The *Cocconeis* species dominant in this study have not shown the same ecological preferences worldwide. The nominate variety *Cocconeis placentula*, an adnate species (allowing for firm attachment to a substrate), was found in a variety of flow conditions (Gallo et al., 2015 in Italy), sensitive to organic pollution (Szczepocka and Szulc, 2009 in Poland) in less polluted or unpolluted conditions (Wu, 1999 in Taiwan; Salomoni et al., 2006), high flow conditions (Martínez de Fabricius et al., 2003 in Argentina), *meso* to eutrophic or hypereutrophic conditions (Korhonen et al., 2013 in Finland; Vilbaste and Truu, 2003 in Estonia), clean water with high Ca^{2+}in Zimbabwe (Mangadze et al., 2015), clean water in India (Amutha and Muralidharan, 2017), in a lower EC in South Africa (Taylor et al., 2007c) and at agriculturally impacted sites in Canada (Lavoie et al., 2004, Lavoie et al., 2009). This wide range of tolerances, some in the same hemisphere, makes it difficult to correctly categorise this species. *Cocconeis placentula* is also morphologically variable and part of a species complex containing a number of varieties. The SPI and GDI scores sensitivity for the nominate variety range from 3.5 to 4 while the TDI sensitivity value is 3. As *Cocconeis* species are resistant to grazing, more so than *Epithemia* and *Rhopolodia*, it could influence the abundance result in favour of the former (Müller, 1999; Kelly et al., 2001). *Cocconeis placentula* var. *euglypta* is suggested as a pioneer species in a small, low velocity high altitude mountain stream (Gomà et al., 2005 in the Pyrenees), tolerating high UV intensity (Müller, 1999), preferring limestone geology (Rimet and Bouchez, 2012) and tolerant of organically bound nitrogen conditions (Van Dam et al., 1994). The SPI and GDI sensitivity scores suggest it tolerates moderate pollution and eutrophication. A dominant species for the site Petrusdal during 2010−12 (headwater site, Holmes and Taylor, 2015), it was not found in the single sample collected during 2016/7. This could be attributed to the lack of high velocity flow in the current drought conditions (personal observation) and less grazing pressure. Found at higher elevations (Triest et al., 2009 in Africa), in crystalline geology (Rimet and Bouchez, 2012) *Cocconeis placentula* var. *lineata* is more sensitive to organic pollution (Szczepocka and Szulc, 2009 in Poland) and at a lower EC (Beltrami, 2010 in Italy) but higher nitrate levels (Ndiritu et al., 2006 in Kenya) than the above variety. The SPI and GDI rate this species moderate eutrophic. Krammer and Lange-Bertalot (1999) found both varieties together in the same habits.

The overall percentage of deformed cells encountered in the present study was lower (1.8%) when compared to the previous study of the GFR study (in 2010 to 2012; Holmes and Taylor, 2015) which was 3.5%. A contributing factor to the reduction in deformed cell percentage could be the fact that Petrusdal, with high percentage deformities in 2010 − 2012 (Holmes and Taylor, 2015), was only sampled once during this period. If the deformities in this river system are influenced by herbicide pollution, the reduction of rainfall (Fig. 3) would reduce the toxicity load and allow for a recovery from the higher deformities percentage when runoff is reduced (Davis et al., 2012; Wood et al., 2019). Several of the species encountered (>7.5% abundance) are tolerant of herbicide pollution: *Cocconeis placentula, Achnanthidium minutissimum, Nitzschia inconspicua, Nitzschia paleacea* (Wood et al., 2019). Species that are listed as sensitive to herbicide pollution are *Fragilaria capucina* var.*vaucheriae* (Kützing) Lange-Bertalot and *Planothidium lanceolatum.* Two species that have shown contrasting results are *Sellaphora nigri* (De Notaris) C.E. Wetzel & Ector (syn. *Eolima minima* (Grunow) Lange-Bertalot in Moser & al.) and *Nitzschia palea. Epithemia sorex,* has been found in sites with high herbicide contamination (Wood et al., 2019) but to be sensitive to elevated nutrient conditions (Kelly et al., 2001).

4.2. Diatom based indices

Studies that have found the diatom indices developed in the northern hemisphere not to be directly applicable in South Africa (Dalu et al., 2016; Bate et al., 2002). The present study shows that all 3 diatom indices used have followed the same pattern with their classification which is borne out by the result of the%PTV. The inclusion rate for two of the two indices often used in South Africa was >95% for the SPI and GDI while for the%PTV and the BDI it was just below 90%. Although there is a high inclusion rate for the SPI and GDI, the environmental tolerances used in the calculation of these indices may vary in Southern African conditions. This is discussed in the community composition section. According to Dalu et al. (2016) and Bate et al. (2002) these differences have resulted in inconsistent results when applying the European diatom indices in South Africa. Nehar et al. (2015) and Chaib and Tison-Roseberry (2012) found the BDI did not work well in Algeria while Bere (2016) found the indices to be useful in Zimbabwe. In this study, the BDI had the lowest inclusion rate at 87.9%. As it is necessary to adjust indices to specific ecoregions, the robustness of the SADI can only be improved with continued analysis. The lack of correlation between diatom species as well as diatom indices and the environmental variables is not uncommon. There are many dynamic interactions, which cannot be quantified, between the physical, chemical and biological processes which affect the diatom community composition.

An attempt was made to understand why the score for the headwater site Petrusdal was low. This site shows the ecological status as bad. This mountainous site is subject to a small amount of animal excreta with pasture lands not irrigated for many years. The only farm above the site does irrigate or grow crops on the old lands but only when spring fed water is available, which during the drought period has been infrequent. Historical herbicide and pesticide application is a possibility should it have long term persistence in the environment. As is common along this and many other rivers in the Eastern Cape, there is a large stand of grey poplars (*Populus* × *canescens*), planted decades ago, above the site. While the allelopathic effects of different *Populus* species has been documented on winter season crops (Singh et al., 2001) and green alder seedlings (Jobidon and Thibault, 1982), no research could be found on grey poplars. Catalán et al. (2013) found white poplars (*Populus alba*) to have fairly strong allelopathic chemicals in their bark (flavonoids and terpenoids) with branches and leaves exhibiting inhibitory properties on the germination and growth of surrounding plants (Chen et al., 2009). This could impact the diatom communities downstream when bark and leaf litter enters the water. However, the landowner of the Clifton site regularly cuts down the grey poplars to head height, to allow game animals to browse the leaves, and has found that as soon as the canopy is opened, several different plant species will grow between the still living poplars (personal communication). This should not be the case if grey poplars had strong allelopathic properties. However, any possible allelopathic properties could only impact aquatic vegetation and not terrestrial plants.

5. Conclusion and recommendations

There was a marked decrease in the rainfall for the area from 2011 to 2017 (Fig. 3). The annual rainfall for Petrusdal, which is responsible for the recharge of the spring waters downstream, steadily fell from 868 mm in 2011 to 270.5 mm in 2017. There was decreasing snowfall on the Agtersneeuberg Mountains in the winter months over the same period (personal observation). The region has been subject to drought since 2012 and, although drought is part of the normal climatic cycle in the area, it could be exacerbated by climate change. This is borne out by the fact that one of the sites, Mulberry Grove, no longer has any flow above ground while Clifton only has pools remaining in the riverbed with almost no above ground flow (end 2017). The flow at Petrusdal was reduced to a low flow by 2017. The poor ecological status assigned by all 3 indices used is indicative of strong human and agricultural pressure with abstraction exceeding re-charge resulting in the loss of ecological integrity.

A surprising result was that a headwater site in the Wilge River in the Mountain Zebra National Park only scored a moderate ecological status after no agricultural influence for approximately 50 years. Although wildlife is defecating and urinating in the water and in the surrounding drainage basin, the volumes of wild animals compared to a commercial farm, are low and are unlikely to have much of an effect on the water bodies. This suggests that it is the local geology influencing the water chemistry and thereby the diatom species composition. (Holmes, 2022). These influences would arise mostly from the anions and cations and would be linked to a specific rock type.

As can be seen from the above, some of the ecological preferences in Southern Africa for the cosmopolitan species will need to be adjusted within the index calculations to suit local conditions. This information will improve the ecological dataset for diatoms in South African conditions making the SADI more robust for diatom-based water quality indication within South Africa. With the possibility of fracking in the karoo possible in the future, it is important to develop robust and reliable bioindicators for this area.

The possibility of allelopathy should also be investigated to ensure that the ecological scores are not negatively influenced by this or similar factors. Future research of this river could include ecotoxicology tests to determine if there is persistent herbicide and pesticide contamination causing the low scores in a water resource commonly considered as ‘clean water’ by the surrounding community.

This present study provides valuable insight into the long-variation in diatom community composition linked to ecological change (drought conditions). The information from this study can be incorporated into the River Health Programme for improved management of the upper section of the GFR.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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