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Appearance of new taxa: invertebrates, phytoplankton and bacteria in an alkaline, saline, meteorite crater lake, South Africa

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With 5 figures and 2 tables

Abstract: Data generated during 12 field trips in 2006 were used to investigate possible changes in the biota communities of Lake Tswaing after a borehole was sunk in 1988/89 near the centre of the crater lake. The Tswaing meteorite crater lake is a small (0.07 km^2), shallow (maximum depth 2.1 m), alkaline (surface water pH varied from 9.6 to 10.3), meromictic lake with a Secchi disk transparency of between 6 and 12 cm. The lake had a distinct surface layer of cyanobacteria (*Arthrospira fusiformis*), located above a layer of purple sulphur bacteria, that was located, in turn, above a layer of green sulphur bacteria. This is the first report on the presence of sulphur bacteria in the lake. The absence of submerged and emergent aquatic macrophytes in the lake limits habitat diversity for attached diatoms in the littoral regions. Both the numbers of families and the density of the benthic invertebrates were very low. The dominant invertebrate groups present were Nematodes, Muscidae and Hydrophilidae. Seven macroinvertebrate families were recorded, five of which are new to Lake Tswaing. In addition, the periphytic ciliate *Blepharisma* sp. was also observed for the first time within the land/water contact zone of Lake Tswaing. Benthic diatoms were scarce and the dominant species present was *Gyrosigma rautenbachiae*, while *Nitzschia quadrangula* was the dominant planktonic diatom, previously not recorded in the main basin of the lake. The diatom species *Nitzschia communis*, although in low numbers, was also not reported in previous studies of Lake Tswaing.

Key words: Bacterial plate distribution, benthic diatoms, macroinvertebrates, *Nodularia spumigena*.

Introduction

Although there are numerous volcanic crater lakes in East Africa, the Tswaing meteorite crater lake, located near the city of Pretoria in South Africa, is the only southern African example of a lake occupying a meteorite crater (Partridge et al. 1991). The gradient of salinity between the surface (30–80) and bottom water (280–310) in Lake Tswaing varies seasonally, demonstrating the hypersaline nature of the lake water (Ashton & Schoeman 1983, 1985). In most saline lakes, intensive biological productivity is restricted to a shallow mixing zone at the surface of the lake. The photic zone in most of these lakes is less than 50 cm deep because of the density of planktonic organisms in their surface waters (Hecky & Kilham 1973). Additionally, the seasonal changes in salinity in these lakes may also have important implications for the survival of algae

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and invertebrates, and can result in low biotic diversity (Wehr & Sheath 2003). The floristic and faunistic surveys conducted on alkaline saline lakes in tropical Africa over the last 7 decades indicate that the biological diversity and community structure of these lakes are governed primarily by salinity and the degree of environmental stability (Verschuren et al. 1999). The ion content of these inland saline lakes is influenced by Na^+, K^+, Ca^{2+}, and Mg^{2+}, and the major anions are typically Cl^-, SO_4^{2-}, HCO_3^- and CO_3^{2-}. These systems are usually well buffered and neutral to alkaline with a pH between 7.5–10.0 (Day 1993).

The dominant phytoplankton species associated with some of these lakes are the small coccoid cyanobacteria *Synechococcus bacillaris* (Lake Sonachi, Kenya) and the filamentous cyanobacteria *Arthrospira fusiformis* and *Anabaenopsis abijatae* (Lake Chitu, Bogoria, Nakura and Elmenteita which are situated in the Kenyan Rift Valley) (Vareschi 1982, Kebede 1997, Verschuren et al. 1999, Ballot et al. 2004). Benthic invertebrates related with these lakes are the Chronomid larvae *Kiefferules disparilis* and *Microtendipes* sp., as well as the salt tolerant Hemiptera and Coleoptera families (Verschuren et al. 1999, 2004), while zooplankton of these East African lakes are dominated by Rotifera, Copepoda and Cladocera (Green 1993). However, in a previous study on Lake Tswaing by Ashton & Schoeman (1983) they reported the total absence of zooplankton and macrophytes and the occurrence of only nematodes and brine flies (Ephydridae).

The work by Ashton & Schoeman (1983) thirty years ago provided details of the morphometric, physical and chemical features of Lake Tswaing. The scope of this study was not to determine if any changes had occurred in the physical and chemical features of Lake Tswaing, but rather to focus on potential changes in the composition of the phytoplankton and invertebrate communities after human interference by drilling a new borehole near the centre of the crater in 1988/89 which provides inflow with a different chemical quality. In the period between the study by Ashton & Schoeman (1983) and the present study, the Lake Tswaing crater has received very variable annual rainfall, with at least four years of well above average regional annual rainfall. The aim of this study was to determine the impact of human intervention in terms of species richness and dominance in Lake Tswaing.

Fig. 1. Oblique aerial view of Lake Tswaing within its crater, viewed from the north-east. The peninsula with the inflowing artesian spring is visible on the north-west shore of the lake.

Material and methods

The study area and background

Lake Tswaing is located at 25° 24″ 30′ S and 28° 04″ 59′ E, some 60 km north-northwest of the city of Pretoria in the Gauteng Province of South Africa (Fig. 1). The lake was formed by the flooding of a crater created by the high-velocity impact of a large meteorite some 220,000 years ago (Reimold et al. 1992). The lake is small (surface area: 0.07 km^2), shallow (maximum depth = 2.10 m) and is located at an altitude of approximately 1,045 metres a.s.l. (Ashton 1999).

The annual rainfall for the region varies between 400 and 750 mm and it is received mainly in the form of thunderstorms during the summer months (Tyson 1987).

From 1912 until 1956 the crater floor was exploited as a source of soda brines, but this venture became economically impractical and the diggings were abandoned during the late 1950s (Levin 1991). During the 1920s, exploratory boreholes were drilled into the crater floor and the cores revealed beds of organic clays intercalated with layers of brine and crystals of trona, halite and gaylussite to a depth of 80 m. One borehole struck a perennial source of groundwater at a depth of approximately 150 m below the current lake surface. Since the elevation of this bore outflow is approximately 10 m below the water table of the surrounding countryside, it now acts as an artesian spring that created a small permanent lake, Lake Tswaing (Ashton & Schoeman 1983). In 1988/89 a borehole was sunk near the centre of the crater to investigate the origin of the crater, and the results of the ensuing investigations confirmed that the crater originated from a meteorite impact (Reimold et al. 1992). During our study in 2006, field trips were conducted once a month to collect samples of water and aquatic biota at the 8 sampling sites shown in Fig. 2.

Phytoplankton sampling

Sampling frequencies of phytoplankton were every four weeks, while most of the samples were analyzed within one week after collection. Samples of phytoplankton were collected at sites 1, 2, 3 and 4 (Fig. 2) using a syringe sampler modified from the design of Baker et al. (1985). Water column samples were collected at 25 cm intervals from the surface to bottom. These samples were combined to form a single composite sample

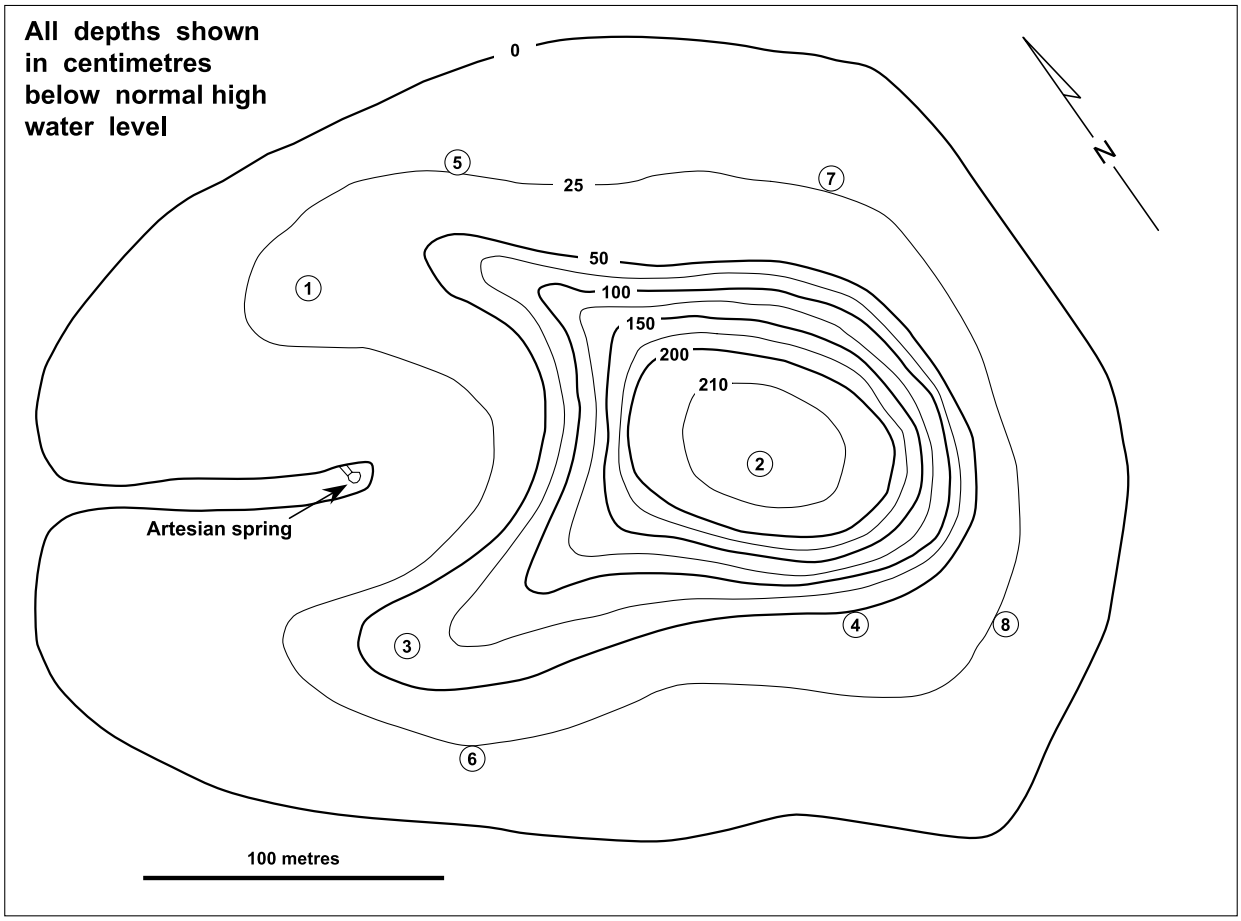

Fig. 2. Bathymetric map of Lake Tswaing showing the shape of the lake basin and the location of the artesian spring at the end of the peninsula, and the position of eight sampling sites. Depth contours are indicated in centimetres below the high-water mark (Diagram redrawn from Ashton 1999).

for each sampling site. The phytoplankton samples were fixed with formaldehyde (final concentration 1 % v/v) because acidic Lugol's solution was unsuitable for preservation of the highly alkaline samples. Sub-samples of 100 ml volume were concentrated 10-fold by centrifugation of the sample at 400 rpm and transferring 3 ml to vials for cell enumeration. Phytoplankton cells were counted at 1250× magnification, under an Olympus inverted binocular compound microscope with phase contrast using the strip-count method (American Public Health Association 1989) and identified according to Wehr & Sheath (2003) and Van Vuuren et al. (2006). The percentage of total phytoplankton volume was calculated for each genus present in the sample. The sediment samples for benthic diatoms were collected with a Willner sampler and mud samples were stored in the dark until preparation in the laboratory. The sediment was cleared of organic matter in a potassium dichromate and sulphuric acid solution and the cleared material was rinsed, diluted, and mounted in Pleurax medium for microscopic examination. Diatom species were identified and counted, placing a drop of non-drying immersion oil on top of the cover slip just prior to microscope examination at 1250× magnification. The biovolumes of the more abundant taxa were estimated by measuring cell dimensions of at least 20 individuals and using the closest geometric formulae (Willen 1976). Species identifications were performed according to Patrick & Reimer (1975), Wehr & Sheath (2003) and Taylor et al. (2007). The total number of taxa and their frequency were recorded after careful examination for at least 15 minutes and after not finding additional taxa. The total number of dominant planktonic taxa and their frequency during the study (n = 12 months) were categorised according to Hörnström (1999), where 1 = ≤ 500, 2 = 501–5,000, 3 = 5,001–25,000 and 4 = 25,001–100,000 cells l^{-1} Benthic diatom numbers were express in cells cm^{-2}.

Bacterial sampling

Bacterial cells were collected at sites 1, 2, 3 and 4 (Fig. 2) using a syringe sampler modified from the design of Baker et al. (1985), which can simultaneously collect water samples at 5 cm intervals. The numbers of bacterial cells in each sample were determined as described by Porter & Feig (1980). After fixation in 2 ml of the water with formalin (final concentration, 2 %) the cells were stained with 4,6-diamidino-2-phenylindole, collected on a precoloured (irgalan black) 0.2 µm pore size Nuclepore filter, and counted by using an epifluorescence microscope. Identification of the different microbial taxa in the two different layers of pink and green photosynthetic bacteria was done according to Blankenship et al. (1995).

Invertebrate sampling

Macroinvertebrates were collected in the littoral zone at sampling stations 5, 6, 7 and 8 each month during our 12 month study, using a vertical net (63 µm mesh size) haul from the bottom to the water surface. Zooplankton was sampled with a vertical net, while care was taken to sample from all depths of sampling sites 1–4. This was done in order to get a random sampling and avoid possible complications due to different vertical migration patterns. The macroinvertebrate samples were immediately preserved in 70 % ethanol and later washed using 75 µm mesh sieves to remove fine detritus particles. The invertebrates were sorted, counted and identified to family level, according to Merrit & Cummins (1996) and Thorp & Covich (2001), by randomly sub-sampling one-tenth of the total sample under an illuminated dissection microscope at 20× magnification. Sorting continued until at least 100 individuals had been counted, or the entire sample was sorted. Invertebrates within the substrate were collected with a 5 cm diameter corer that was driven into the substrate to an approximate depth of 10 cm. The material within the corer (approximately 200 g) was removed by hand and the above counting procedures were followed. Invertebrate diversity was calculated using the Shannon's diversity index (Shannon & Weaver 1949).

Protozoa sampling

Protozoa were determined and quantified using the live counting technique at 400× or 1,250× magnification with a light microscope, and identification was based on the quantitative protargol (QPS) method of Montagnes & Lynn (1987a, b) and Skibbe (1994). The Berger-Parker dominance index (Berger & Parker 1970) was used to measure the evenness or dominance of phytoplankton and macroinvertebrates at each site.

Chlorophyll and physico-chemical analysis

The chlorophyll-*a* content of phytoplankton samples was determined photospectrometrically at 647 and 664 nm and calculated according to Porra et al. (1989). Bacteriochlorophyll-*a* and *c* was determined after centrifugation of water samples and cold extraction of the pellet (99.5 % v/v acetone, 4 °C, 24 h). Absorbance was measured at 772 nm for BChl-*a* and 662 nm for BChl-*c*, with a BioRad SmartSpec™ spectrophotometer (BioRad, USA). Pigment concentrations were calculated from the equations given by Steenbergen & Korthals (1982). Water transparency was estimated with a 20 cm diameter Secchi disc painted in black and white quadrants. Vertical profiles (intervals of 25 cm from the surface to the bottom of sampling sites 1–4) of selected lake characteristics (pH, electrical conductivity and temperature) were measured directly in the lake with a Hach™sension 156 portable multiparameter probe (Loveland, USA).

Due to the high solute content, all samples were diluted with deionized distilled water, to give a final dilution ratio of 1:10, 1:100 and 1:1000, before analysis. A 100 ml aliquot of each of the remaining preserved samples was sonicated for 2 min to disintegrate cellular material and then analyzed for total nitrogen and phosphorus. All chemical analyses were performed using published methods (American Public Health Association (A. P.H. A.) 1992). The salinity of each water sample was calculated as the sum of the major ionic constituents (Hutchinson 1957) while the total dissolved solids (TDS) content was determined gravimetrically after evaporating a Whatman GF/C filtered sample to dryness at 105 °C. Wind velocity was measured in the field with a Weather Monitor 2 (Hayward, CA 94545 USA).

Data analyses

All of the results were recorded on standard Excel spreadsheets for data processing and statistical analysis was performed using Systat 7.01 (SPSS Inc. 1997). Statistical differences were analyzed by calculating the Pearson correlation and a *t* test using the Sigma Plot (Jandel Scientific) program. Values of $p \leq 0.05$ were regarded as significant. Correlations of *r* near zero were regarded as unrelated.

Results

Physico-chemical conditions

On every sampling visit (n = 12), the water of Lake Tswaing was characterized by low water transparency (Secchi depths always less than 0.12 m), alkaline pH values (always above 9.2) and moderate water temperatures (midday surface water temperatures were always above 16 °C). In addition, the total average phosphorus concentration in the surface water was relatively low (< 0.2 mg l^{-1}), contrasting with the high total nitrogen concentration (11 mg l^{-1}). In the summer, sulphate concentrations tended to decrease slightly (7,800 mg l^{-1} to 7,510 mg l^{-1}) possibly due to sulphate reducing activity associated with organic matter decomposition at the higher temperature. The higher average water temperature (31.5 °C) in summer also stimulated microbial production of CO_2 and consequently lowered the pH from 10.3 to 9.2. Ammonium was the main form of inorganic nitrogen in the water, with high average concentrations (1.8 mg l^{-1}) in the warmer months. In Lake Tswaing, nutrient concentrations (total N = 12 mg l^{-1}; total P ≈ 0.2 mg l^{-1}) of the inflowing spring water were high throughout the 12 months of sampling.

In Lake Tswaing, electrical conductivity values and the concentrations of major cations and anions increased with increasing depth; TDS concentrations increased from 32,545 mg l^{-1} in the inflowing spring water to 95,170 mg l^{-1} at a depth of 2 m. The dominance order for cations and anions was Na >> Mg > K > Ca :: Cl >> SO_4 > CO_3/HCO_3, respectively. Water samples from depths greater than 0.8 m were blackish in colour and smelled strongly of H_2S. Examination of bathymetry data revealed that Lake Tswaing consists of two distinct depth zones: an upper, shallow zone (0–0.5 m) within the original gently sloping basin overlies a smaller deep zone (0.5–2.1 m), created by the abandoned salt diggings (Fig. 2; Ashton 1999).

Phytoplankton community

The highest average surface water (0–0.15 m) concentration of chlorophyll-*a* in December 2006 was 781 µg l^{-1} and this correlated positively (r = 0.9876; $p \leq 0.05$) with the high abundance of *Arthrospira fusiformis* (Vorochinin) Komárek (approximately 85,000 cells l^{-1}) observed during this sampling period (Fig. 3). The numerical abundance of *Arthrospira fusiformis* (as cells l^{-1}, Fig. 4) was always very high in the upper 5 cm of the water column (especially in the bloom forming summer months), while other species of cyanobacteria (*Nodularia spumigena* Martens ex Bornet et Flahault, *Anabaena spiroides* Bory ex Bornet et Flahault and *Oscillatoria* Vaucher ex Gomont spp.) were present in very low numbers (microscopically verified < 500 cells l^{-1}) and correlated negatively (r = –0.8951; $p \leq 0.03$) with the average high total nitrogen concentration of 11 mg l^{-1}. These species were present in the shallow north-western and south-eastern portion of the lake within the benthic mats of algae but were absent in the surface water column of the main basin of the lake.

The dominant species of benthic diatoms recorded over the 12 month study period in Lake Tswaing were *Gyrosigma rautenbachiae* Cholnoky, *Nitzschia pusilla* Kützing (Berger and Parker index = 0.348; 0.302)

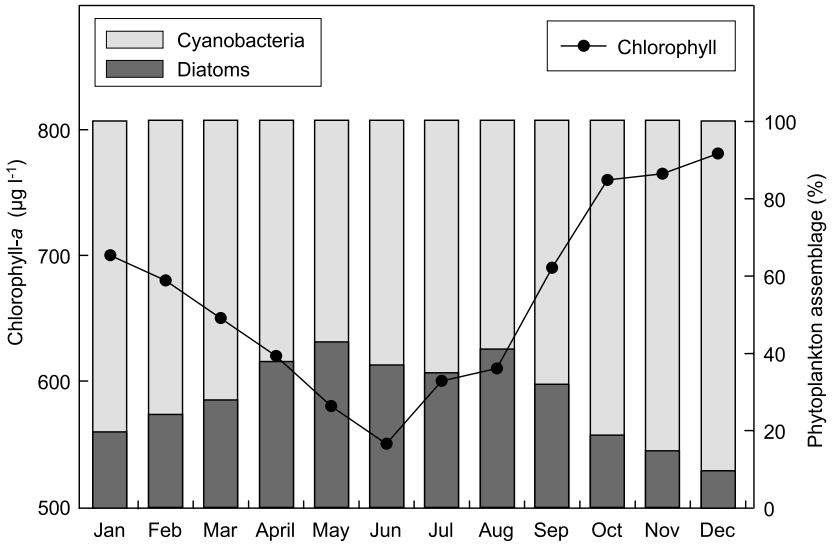

Fig. 3. Comparison between phytoplankton assemblage (%) and chlorophyll *a* concentration during the study over a period of 12 months.

and in much lower numbers *Surrirella ovalis* Brebisson (< 250 cells cm^{-2} in the sediment, recorded at each sampling trip). The phytoplankton occupying the upper portion of the mixolimnion also contained low numbers (< 750 cells l^{-1} in the water column) of the diatom *Nitzschia quadrangula*. The latter was the most abundant at site 2 and was not previously recorded in this area. The diatom species *Nitzschia communis* Rabenhorst, although present in low numbers (> 100 cells l^{-1} in the water column) throughout the study (n = 12 months) were also not recorded in previous studies on Lake Tswaing (Table 1).

During morning calm (windless) periods, large numbers of *Arthrospira fusiformis* filaments accumulated in the upper portion of the mixolimnion and their cell numbers declined sharply below a depth of 15 cm. The maximum abundance of *Arthrospira fusiformis* filaments (85,000 cells l^{-1}) were recorded in December at a maximum temperature of 34 °C while the lowest number of filaments were recorded in July at a water temperature of 16.1 °C. A strong correlation exists ($r = 0.9861$; $p \leq 0.05$) between the high average water temperature (31.5 °C) during the sampling dates November and December 2006 and the high average abundance of *Arthrospira fusiformis* filaments (75,000 cells l^{-1}) during this period of time. Due to the sheltering effect provided by the relatively high crater rim and small surface area of the lake, extensive wind mixing

Table 1. Comparison of the average phytoplankton numbers at the four sampling sites in Lake Tswaing from January 2006 to December 2006 (n = 12). The numbers (1–4) represent the maximum frequencies of the phytoplankton taxa: where 1 = ≤ 500, 2 = 501–5,000, 3 = 5,001–25,000 and 4 = 25,001–100,000 cells l^{-1}.

Taxonomic group	Sampling sites			
	1	2	3	4
Cyanophyceae				
Arthrospira fusiformis	4	3	4	4
Nodularia spumigena,	1	0	1	0
Anabaena spiroides	1	0	1	0
Oscillatoria spp.	2	0	1	1
Bacillariophyceae				
Gyrosigma rautenbachiae	2	3	3	2
Nitzschia pusilla	1	1	3	2
Surrirella ovalis	0	0	1	1
Nitzschia quadrangula	1	2	1	0
Nitzschia communis	1	0	1	0

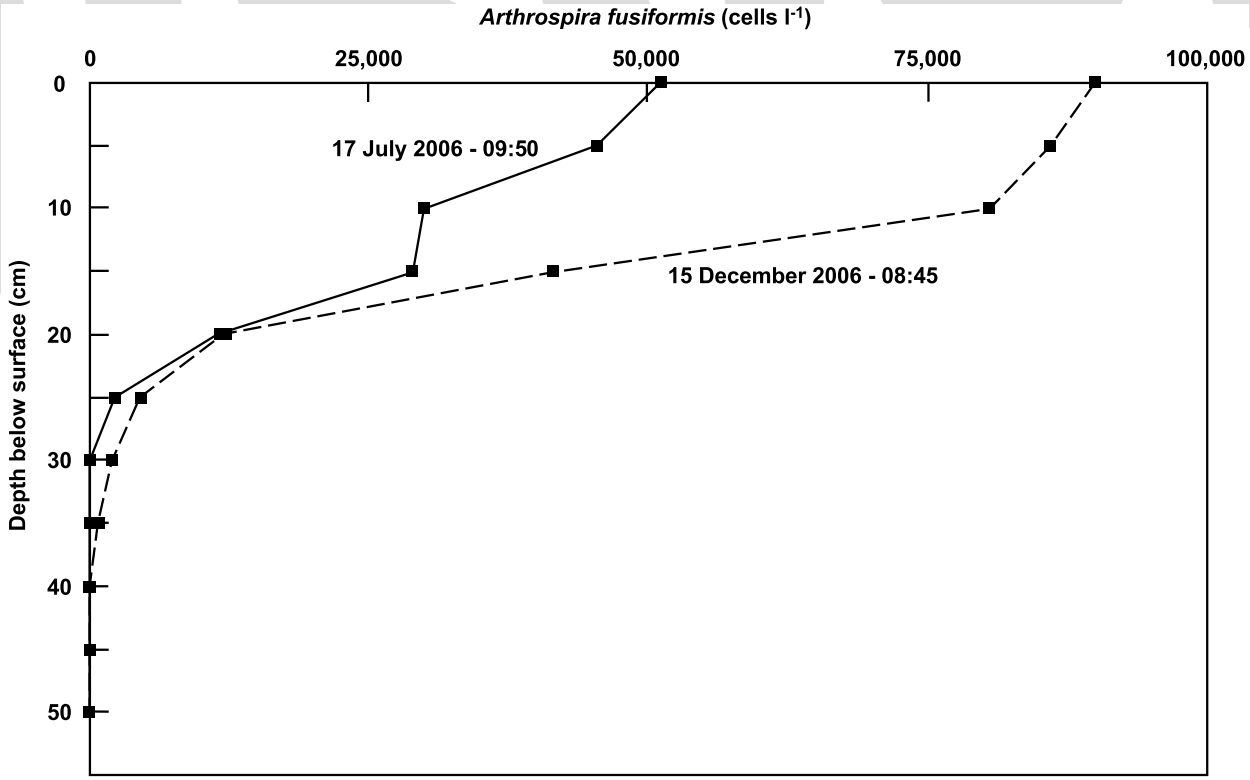

Fig. 4. Vertical profiles showing the variation in the numbers of *Arthrospira fusiformis* (as cells per litre) in Lake Tswaing during the winter (July 2006) and summer (December 2006) months.

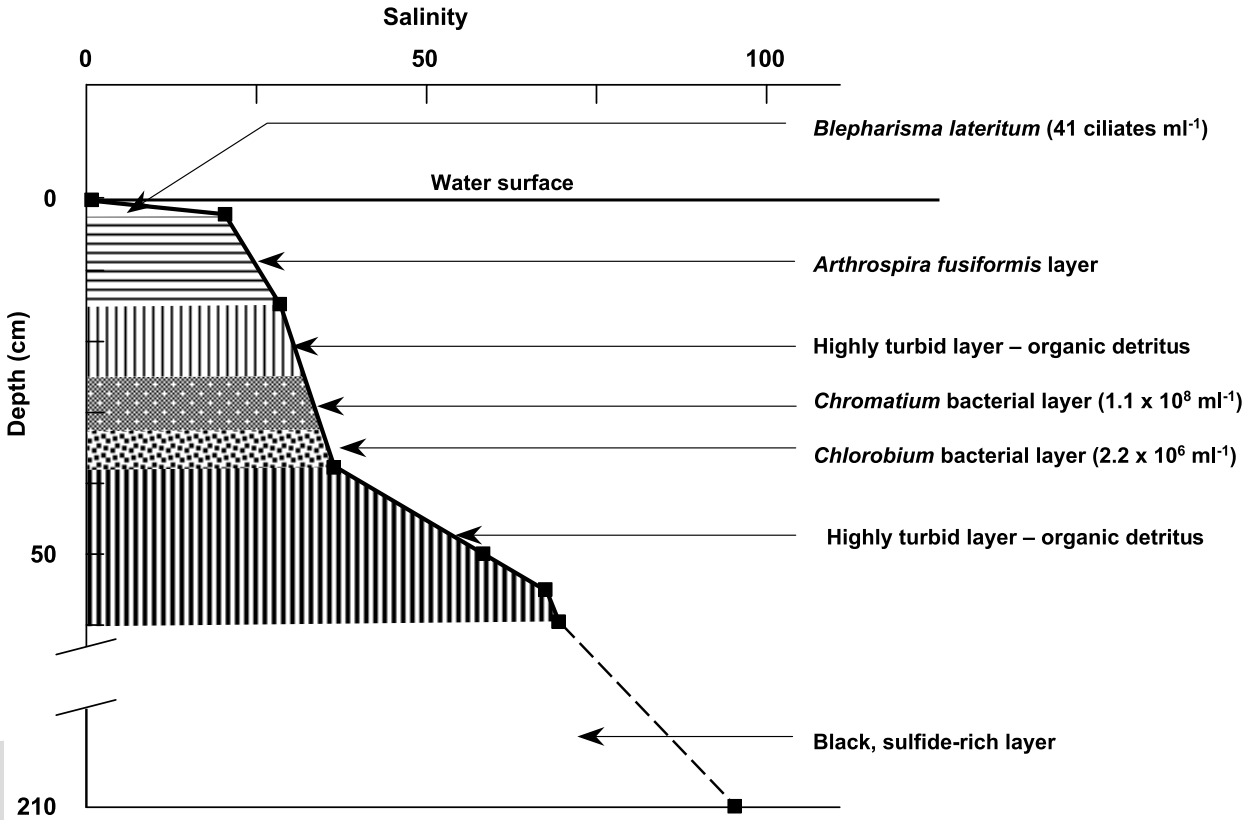

Fig. 5. Schematic vertical profile of the water column in Lake Tswaing to show the positions of the phytoplankton layer and the two bacterial layers, in relation to the prevailing salinity at each point. (Vertical profile shortened to highlight the details of the complex structure of the shallow, surface layers).

of the water is usually inhibited (Ashton & Schoeman 1983). The average wind speeds measured during our 12 month study – at a height of 1 m above the lake's water surface – were 1.51 m s^{-1} for February, March, April, May and June and 3.57 m s^{-1} in July, August, September, October, November and December. The wind direction was predominantly north-east. Furthermore, visual observations of Lake Tswaing during our 12 month sampling period indicated no sign of the existence of aquatic macrophytes within the lake.

Bacterial community

During all our sampling trips over the period of 12 months dense layers of purple and green sulphur bacteria were observed within Lake Tswaing. The layer of purple sulphur bacteria (*Chromatium* sp.), was approximately 7 cm in depth and was visible directly under the planktonic layer of *Arthrospira fusiformis* in the upper water column, reaching a maximum cell density of 1.1×10^8 ml^{-1} (BChl-*a* = 1,339 µg l^{-1}) in the summer months of November and December. A layer of green sulphur bacteria (*Chlorobium* sp.), approximately 3 cm in depth and with a cell density of 2.2×10^6 ml^{-1} (BChl-*c* = 868 µg l^{-1}) was located immediately below the layer of purple sulphur bacteria. The highest cell density of green sulphur bacteria (5.1×10^6 ml^{-1}) was recorded in April 2006, and correlated positively ($r = 0.8672$; $p \leq 0.04$) with the highest Secchi disc reading (0.12 m) measured during this study. The average BChl-*a* and -*c* values measured during our 12 sampling trips were for purple sulphur bacteria (*Chromatium* sp.) – BChl-*a* =1,124 µg l^{-1} and green sulphur bacteria (*Chlorobium* sp.) – BChl-*c* = 681 µg l^{-1} (Fig. 5).

Invertebrate community, protozoa and substrate conditions

The distribution of free-living protozoa occur in the upper 1.5 cm of the surface water of the land/water contact zone of sampling sites 1, 3 and 4, within the dense suspension of *Arthrospira fusiformis* – but were absent in the main basin at sampling site 2. The only protozoa species observed was the periphytic ciliate *Blepharisma lateritium* (Kahl 1930–1935) which is primarily a bacterivorous species (Kalff 2002). Av-

Table 2. Average abundance of macroinvertebrate families at four sampling sites in Lake Tswaing from January 2006 to December 2006 (n = 12). Diversity indices for each sampling site are indicated at the bottom of the table.

Macroinvertebrate families	Sampling sites			
	5	6	7	8
Nematoda	17	49	9	11
Coleoptera				
Hydrophilidae	9	3	1	2
Diptera				
Ceratopogonidae	7	5	3	1
Ephydridae	7	9	5	2
Muscidae	11	5	1	2
Syrphidae	3	1	0	1
Tipulidae	6y	3	4	3
Total no. of Families	7	7	6	7
Shannon Index (*H*)	2.49	2.25	2.18	2.67

erage numerical abundance of ciliates in the hyperneuston (species that live just underneath the water surface) increased from 27 ciliates ml^{-1} in the winter months of May and July to 41 ciliates ml^{-1} during the summer months November and December. The higher ciliate numbers during November and December correlated significantly (r = 0.9916; $p \leq 0.05$) with the high average water temperature (31.5 °C) of these two months. This periphytic ciliate (*Blepharisma lateritium*) was not previously recorded by Ashton & Schoeman (1983) in Lake Tswaing, while the zooplankton Rotifera, Copepoda and Cladocera were totally absent in the Lake Tswaing during our 12 month sampling.

A crystalline and efflorescent crust probably caused by the evaporation of interstitial brines drawn to the mud surface was observed. This crust showed a dominance of trona (80 %) over halite (20 %) (Ashton & Schoeman 1983). The sediments at most of the sites sampled for benthic invertebrates consisted predominantly of dark organic clays and silt and on average, almost 65 % of the sediment mass in a sample consisted of particles < 300 µm in diameter.

The benthic invertebrate population was characterized by a low diversity of invertebrate families and very low abundances of individual species that correlated negatively (r = –0.9651; $p \leq 0.04$) with the small clay and silt particles (< 300 µm in diameter) size observed at al 4 sampling sites (Table 2). A total of 7 invertebrate families were recorded from Lake Tswaing with the average number of individuals per sample usually < 40 per sampling site over the period of 12 months. Nematodes recorded from Lake Tswaing were of meiofauna size ranging from 40 µm to 200 µm (Strayer, 1985). Nematodes were the dominant invertebrates (Berger and Parker index = 0.477), followed by the Dipterans (Muscidae, Ceratopogonidae, Ephydridae, Tipulidae and Syrphidae) and Coleoptera (Hydrophilidae) (Table 2). The maximum average numerical abundance of nematodes (n = 66 m^{-2}) were observed at sites 5 and 6 that had extensive benthic algal mats. However, the highest diversity of macroinvertebrates (H' = 2.67) was observed at sampling site 8.

Discussion

Phytoplankton community

Alkaline, saline lakes have a restricted fauna and flora composition (Talling & Talling 1965, Hecky 1971) and intensive biological productivity is normally restricted to a shallow mixing zone close to the surface of the lake. In lakes, the first subtle shift from freshwater to saline species in both the phytoplankton composition and total algal biomass produced (per unit of total phosphorus) tends to occur when salinity values rise to about 1 (approximately 1,600 µS cm^{-1}) (Bierhuizen & Prepas 1985). However, in the zone between 1 and 2, increasing salinity begins to exert distinct effects on the species composition of aquatic biota. The species richness of both plants and animals declines with increasing salinity. The largest changes occur under conditions of very low salinity (< 0.01) and little change in species richness occurs at salinities above 50 (Hammer et al. 1990).

No submerged or emergent macrophytes occur in the saline waters of Lake Tswaing and it appears that the salinity levels are too high to sustain aquatic macrophyte communities. The exclusion of aquatic macrophytes from Lake Tswaing reduced the diversity of habitat for diatoms and macroinvertebrates in the littoral zone. Only low numbers (< 500 cells cm^{-2} in the sediment) of benthic diatoms were recorded in the sediment, while the highest numbers of individuals were recorded in the benthic algal mats at sampling site 1. However, the low numbers of *Nitzschia quadrangula* observed at sampling site 2 of Lake Tswaing in this study, was previously only recorded from the inflowing saline spring (Schoeman & Ashton 1982).

There are two plausible explanations for the presence of *Nitzschia quadrangula* in the open water of the Lake Tswaing. Firstly, although *Nitzschia quadrangula* is classified as benthic, Hecky (1971) noted that they are abundant in the phytoplankton of lakes where bloom populations of *Arthrospira fusiformis* occur,

suggesting that there may be specific ranges of conditions that allow these species to change from a benthic to a planktonic mode of existence. Hecky & Kilham (1973) also observed that the hydrodynamic requirements which allow *Arthrospira fusiformis* to dominate the phytoplankton in stratified, meromictic lakes could also favour a planktonic existence for *Nitzschia quadrangula* and that there may be a nutritional link between these diatoms and *Arthrospira fusiformis* (Tuite 1981). A second possible explanation is that the water chemistry of Lake Tswaing may have changed as a result of the inflow of groundwater from the borehole that was sunk in 1988 to confirm the origin of the crater (Reimold et al. 1992). Earlier studies by Schoeman & Ashton (1982) confirmed the presence of *Nitzschia quadrangula* in groundwater samples drawn from boreholes in the area around Lake Tswaing, though the chemistry of these groundwaters was quite different from that of the artesian spring flowing into Lake Tswaing.

During our study, *Arthrospira fusiformis* was the dominant cyanobacterium present in Lake Tswaing and accounted for approximately 98 % of the total cyanobacterial biomass. Although Ashton & Schoeman (1983) reported this species thirty years ago in Lake Tswaing, little information is given on the reason for the dominance of this species in Lake Tswaing. This dominance is possibly attributable to the ability of *Arthrospira fusiformis* to tolerate a wide range of salinities (Kebede 1997). Besides salinity, low water transparency (Maximum Secchi depth = 0.12 m, indicating a very shallow euphotic zone) is another factor that might also influence the dominance of *Arthrospira* in Lake Tswaing, since *Arthrospira* cells contain gas vacuoles (Oberholster et al. 2006) that enable them to control their vertical location in the water column. *Arthrospira* cells are therefore favoured by the high turbidity levels observed in the surface waters of Lake Tswaing. High nutrient loads and water temperature are also important environmental factors that could influence the cyanobacterial blooms as observed in this study (Ballot et al. 2004; Oberholster et al. 2004). Laboratory studies on cultures of *Arthrospira* have shown that the organism has an optimal temperature in the range of 35–38 °C (Vonshak 1997). In Lake Tswaing, the midday surface waters temperature varied from 34 °C in summer to 16.1 °C in winter – the summer temperatures are close to the optimal temperature range for *Arthrospira*. High nutrient concentrations (total N = 12 mg l^{-1}; total P ≈ 0.2 mg l^{-1}) in the inflowing spring water create conditions favourable for the growth of bacteria and cyanobacteria.

The water budget of Lake Tswaing is governed by evaporation, precipitation and the inflow of the artesian spring; the absence of a surface outflow promotes the progressive concentration of nutrients and dissolved salts over time (Ashton 1999). The effects of this concentration were seen as an increase in the TDS content of water samples taken at progressively greater depths in the lake. In December 2006, the TDS of the surface waters (35,000 – 40,000 mg l^{-1}) increased steadily to 95,170 mg l^{-1} at a depth of 2 m (Fig. 5). The detection of the cyanobacterial species *Nodularia spumigena* in Lake Tswaing, where the water was dominated by the ion composition of $Cl >> SO_4$ during our study, was in contrast with the situation reported for other saline lakes (e.g. Lake Corangamite in Australia and Lake Manito in Canada) where *Nodularia spumigena* was the dominant species present at an ion composition of $Cl >> CO_3/HCO_3$ (Hammer 1984, Hammer et al. 1990). Although we did not analyse this species for cyanotoxin it is known to produce the cyanobacterial toxin nodularin (Sivonen & Jones 1999). Moreover, the ion composition in the East African alkaline saline lakes are exclusively dominated by Na^+ and CO_3/HCO_3, while saline pans of South Africa are dominated by Na^+ and Cl^- ions (Seaman et al. 1991).

Bacterial community

The main factors that determine the growth of purple sulphur bacteria in lakes are the availability of suitable light regimes, low dissolved oxygen concentrations, and the presence of adequate concentrations of sulphide (Guerrero 1985). Many previous studies on lakes (e.g. Parkin & Brock 1980) showed that photosynthesis by sulphur bacteria is directly related to the percentage of surface irradiance available for the bacteria. Our observations in this study agreed with the observations by Pfennig (1989) that green sulphur bacteria have exceptionally efficient chlorosomes, allowing them to grow photosynthetically deeper in the water column where they benefit from higher concentrations of H_2S at the chemocline. The phenomenon that purple sulphur bacteria dominate the near-surface water has also been observed in studies by Gorlenko et al. (1983). The earlier studies of Lake Tswaing by Ashton & Schoeman (1985) reported the presence of purple sulphur bacteria, but no traces of green sulphur bacteria were found in the water column.

Invertebrates and protozoa

Zooplankton was reported to be abundant at a salinity of 1.8 in an eutrophic brackish water lake, and absent

from another at a slightly higher salinity of 3–4 (Moss 1994). The absence of zooplankton in Lake Tswaing was possibly due to the high conductivity (> 4,000 µS cm^{-1}) measured throughout our 12-month study. However, a study conducted by Green (1993) on zooplankton in 38 East African lakes showed that rotifers species diversity declines at a conductivity of 3,000 µS cm^{-1}, and falls to 2 or 3 species above 3,000 µS cm^{-1}. Similar reduction trends were observed in the Copepoda and Cladocera. Interestingly, the number of protozoa in Lake Tswaing was much greater than expected – given the high salinities recorded in this lake. However, the presence of numerous ciliates in the surface waters of Lake Tswaing (Fig. 5) resemble strongly the observations by Finlay et al. (1987) on lakes Simbi and Nakuru. The unexpected occurrence of the bacteriovorous ciliate *Blepharisma lateritium* in the land/water contact zone of Lake Tswaing, but absent in the main basin (sampling site 2) of the lake, may possibly be due to abiotic water factors, such as higher content of organic matter (algal mats) and concentrations of nutrients (presence of purple and green sulphur bacteria) in the littoral zone (Mieczan & Radwan 2005).

In our study, the benthic macroinvertebrates were dominated numerically by three groups (i.e. Nematodes, Muscidae and Hydrophilidae) which, in combination, contributed up to 85 % of the total number of invertebrates captured at the different sampling stations. Both the numbers of taxa and the density of the benthic macroinvertebrates remained very low throughout our study, possibly due to the prevailing high salinity levels and the associated physical conditions. We suspect that the fine-grained inorganic sediment present in Lake Tswaing creates an unfavourable environment for most invertebrate taxonomic groups. This is due to the fact that the fine-grained sediment would likely hinder feeding and respiration processes, particularly in the Orders Odonata and Trichoptera (Beadle 1981, Minshall 1984). Although Tudorancea et al. (1989) observed in their study that most benthic invertebrates were associated with medium and fine grained sand which occurred in the littoral zones of seven rift lakes and three crater lakes in the Ethiopian Rift Valley. A possible explanation for the abundance of nematodes at sites 5 and 6 are that these organisms feed on both primary decomposers, such as bacteria and fungi, as well as primary producers, such as diatoms, in the benthic algal mats of the shallow peripheral region (Nicholas 1984).

Interestingly, the present study has revealed that the restricted species composition of the fauna and flora in Lake Tswaing displays several differences from the fauna and flora recorded for the alkaline saline lakes of East Africa. The most striking faunal members of the alkaline saline lakes of East Africa are the rotifer *Brachionus plicatilis* Muller and the copepod *Paradiaptomus africanus* Daday, with Corixid Hemiptera and chironomid Diptera being the most common insects. Whereas, flamingos *Phoenicopterus ruber* Pallas, *Phoenicopterus minor* Geoffrey and the cichlid fish *Tilapia grahami* Boulenger are the dominant vertebrate fauna (Hecky & Kilham 1973). In Lake Tswaing, where the dominant invertebrate species consist of the free-living protozoa (*Blepharisma lateritium*) and nematodes; aquatic vertebrates are absent, and waterfowl are only occasional visitors to Lake Tswaing. Moreover, the cyanobacteria *Nodularia spumigena* that was reported in Lake Tswaing in this study is absent in other alkaline saline lakes of East Africa.

Although outside the scope of this study, it is worth mentioning that the 2006 study has highlighted the fact that Lake Tswaing has undergone several important changes since the physical and chemical features of the crater lake were first studied in 1978–1980 (Ashton & Schoeman 1983, 1985, Ashton 1999). In addition, a new borehole was sunk in the crater floor (Partridge et al. 1991) and the activities associated with the drilling contributed a large quantity of sediment that entered the deeper portion of Lake Tswaing (Fig. 2) and reduced its depth from an original 2.85 m (Ashton 1999) to its present value of 2.1 m. It is clear from the data generated in this study that the new borehole now provides inflows that have a different chemical quality to that reported by Ashton & Schoeman (1983, 1985) and Ashton (1999). In particular, the inflowing waters have a higher salinity (32.5 in 2006, compared to 3.5 in 1978–1980), and the ionic dominance ratio has altered from Na >> Ca > K >> Mg :: Cl >> CO_3/HCO_3 >> SO_4 (Ashton 1999) to Na >> Mg > K > Ca :: Cl >> SO_4 > CO_3/HCO_3 (this study). These changes in TDS and chemical composition suggest very strongly that the water flowing into lake Tswaing is now drawn from a different geological source, possibly at greater depth than the original borehole.

Conclusion

The species comprising the diatom flora that were present in Lake Tswaing during 2006 are different from the species that were recorded during the earlier 1978–1980 study. Another noticeable difference during our 2006 study was the presence of two distinct bacterial layers – a purple sulphur bacterial layer over-

lying a green sulphur bacterial layer (Fig. 5), compared to the single bacterial layer of purple sulphur bacteria that was recorded by Ashton & Schoeman (1983, 1985) and Ashton (1999), and which remained present throughout their entire 28-month duration study in 1978–1980. This presence of a second bacterial layer could be due to the changed salinity values and altered water chemistry that were recorded in our 2006 study. Studies on meromictic saline lakes elsewhere (e.g. Hammer 1984) have revealed that several lakes possess two distinct bacterial layers, usually of purple sulphur bacteria overlying green sulphur bacteria, similar to those recorded in this study.

The 2006 study also revealed that the species composition of phytoplankton and bacteria in Lake Tswaing have changed, apparently in response to the altered physical conditions (shallower depth) and chemical composition of the inflowing waters. In addition, the earlier studies (Ashton & Schoeman 1983, 1985, Ashton 1999) noted the almost complete absence of invertebrates from Lake Tswaing, while the present study revealed that more families of macroinvertebrates are present, and in greater numbers, than recorded earlier.

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