Oscillatoria simplicissima: An autecological study

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Abstract

Oscillatoria simplicissima is a blue-green alga that was first observed in the Vaal River during 1984. It replaced *Microcystis aeruginosa* as the dominant blue-green algal species and became dominant after an increase in the organic phosphorus supply in the Loch Vaal catchment area to form algal blooms of great intensity. Although this blue-green alga is presumably non-toxic, a study over a seven-year period shows that it is one of the most important bloom-forming blue-green algal species in the Vaal River, interfering with recreational activities as well as water purification. During this study, environmental variables influencing the development of blue-green algae in a natural environment were studied in order to provide background information for growth studies of this group under controlled conditions. This paper gives an overview of an autecological study done on *O. simplicissima* in the Vaal River at Balkfontein, as well as the isolation and growth requirements of this blue-green alga under controlled conditions.

Introduction

Freshwater is South Africa's most valuable natural resource. Rivers in South Africa are under constant pressure of pollution from agricultural, mining, industrial and domestic uses. Pollution leads to the presence of high concentrations of organic and inorganic compounds, which enhance algal blooms and concomitantly decrease water quality.

The Vaal River is one of South Africa's largest rivers, supplying water to highly populated and industrialised areas. Demands on the Vaal River system for water are exceptionally high and the river and its tributaries are the only water bodies into which effluents from the Johannesburg, Vereeniging and Sasolburg regions can be discharged (Braune and Rogers, 1987). The Vaal River has been described as a nutrient enriched (eutrophic) river system (Pieterse, 1986a; Janse van Vuuren, 1996; 2001 and Roos, 1991). Eutrophication leads to changes in the phytoplankton composition, often shifting the dominance towards blue-green algae. Algal blooms occurring in the Vaal River result in the production of unpleasant odours and tastes and a general decline in water quality that can, in some cases, be aggravated by the production of toxins. These problems are costly to alleviate, may pose health risks to humans and animals alike and in some areas may contribute to the shortage of clean fresh water (Cooke and Carlson, 1989).

O. simplicissima Gomont was first identified in the Vaal River during 1984 (Pieterse and Steynberg, 1993). Since then extensive blooms of *O. simplicissima* were observed in the Loch Vaal (Pieterse and Steynberg, 1993), part of the Vaal River Barrage subcatchment area where self shading conditions may favour gas vacuolated blue-green algae such as *Microcystis aeruginosa* Kützing and *O. simplicissima* that are able to float to the upper, better-illuminated, part of the water column where they can utilise the available light. In the Loch Vaal catchment area, *O. simplicissima* replaced *Microcystis aeruginosa* as the dominant blue-green algal species after an increase in inorganic phosphorus supply, which indicates that growth of *O. simplicissima* had previously been limited by the available inorganic phosphorus concentration (Pieterse and Steynberg, 1993). Swanepoel (1999) also found that during times of excess nitrogen, high concentrations of phosphorus induced algal blooms in the Loch Vaal.

Although *O. simplicissima* is presumably non-toxic, the magnitude of its blooms causes many problems in the Vaal River during summer seasons. The blooms are responsible for musty odours and colour changes in the water that discourage recreational activities, which have economic implications for the holiday resorts in the area. Soapy scums with methyl-isoborneol (MIB) that cause bad odours and tastes in final purified water as well as the clogging of filters in purification plants by *O. simplicissima* filaments, are impairing the utilisation of water from the river and adding to the high cost of water purification (Krüger, 1999).

With the estimated increase in South Africa's population, sustainable resource management is vital to enhanced economic stability in Southern Africa. In order to manage the Vaal River as a sustainable resource, it is essential to investigate the occurrence of, and factors leading to, blooms of nuisance species closely.

Due to the fairly recent appearance of *O. simplicissima* very little was known about the physiology and growth requirements of this blue-green alga. Because of its increasing disruptions of activities in the Vaal River, Pieterse and Steynberg (1993) recommended that *O. simplicissima* be isolated and cultured in an artificial growth medium and its growth requirements be determined under controlled conditions - hence the decision to do an autecological study on *O. simplicissima*.

The aims of this study were, therefore, to identify environmental variables influencing *O. simplicissima* in a natural (riverine) environment, to isolate *O. simplicissima* from samples taken from the Vaal River at Balkfontein and to investigate the growth requirements of this blue-green alga in controlled growth conditions.

Study area, material and methods

Study area

The study area falls within the middle Vaal River region (between the Vaal Barrage and Bloemhof Dam). A sampling locality was selected at Sedibeng Water at Balkfontein (± 20 km by road from Bothaville) because it is one of the important localities where water is withdrawn from the Vaal River for purification purposes. Massive

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blooms of *O. simplicissima* were also observed at the chemical dosing point of this purification plant. The sampling locality is situated near the pumping station of the purification plant (27°23' 45" S, 26°30' 30" E). The width of the river at the sampling locality is about 77 m and the average depth is about 4 m (Pieterse, 1986b). Sedibeng Water supplies water to the Welkom, Virginia and Bothaville areas (DWAF, 1986), as well as to the Leeudoringstad-Wolmaransstad-Makwassie area. Irrigation farmers along the river and on the Vaalharts Government Water Scheme are also supplied with water from this point.

Material and methods

Sampling

Surface water samples, containing mixed algal assemblages, were taken every two weeks from 1991 to 1997 at the Balkfontein locality. Two-liter aliquots of these water samples were filtered through a 10 μm Nybolt net in order to concentrate the algal cells for identification purposes. A 100 mℓ aliquot of the same sample was used for quantitative phytoplankton analysis. Both the net and the 100 mℓ samples were fixed with two percent formaldehyde (final concentration).

Algal counts and environmental variables

All algae present were identified to species level with a light microscope and texts such as Geitler (1932), Huber-Pestalozzi (1950; 1955; 1961; 1962a,b,c), Prescott (1951), Bourelly (1966; 1968; 1970), Prescott et al. (1981; 1982), Förster (1982), Komárek and Fott (1983), Starmach (1985) and Komárek and Anagnostidis (1986; 1999).

After identification with the light microscope, each sample was shaken in order to suspend the algae uniformly throughout the water. Gas vacuoles of blue-green algae were pressure-deflated in a special container using a mechanical hammer (see Walsby, 1977). Equal volumes of river water, usually 0.5 to 5.0 mℓ (depending on the density of the algae), were then pipetted into two identical sedimentation tubes. The sedimentation tubes were filled with distilled water and covered with circular glass cover slips. The sedimentation tubes were left for a period of at least two days in a desiccator (filled with distilled water in the bottom section), in order to allow the algal cells to settle and to avoid evaporation of water in the sedimentation tubes. Settling times of one centimetre length per day of the sedimentation tubes were allowed. Algal cells were counted in duplicate by means of the technique described by Utermöhl (1931; 1958) and modified by Lund et al. (1958).

Data on physical and chemical environmental variables were obtained from the Department of Water Affairs and Forestry, as well as from Sedibeng Water. Data measured at the onset (when chlorophyll-*a* levels increased) of an *O. simplicissima* bloom in the Loch Vaal and in the Vaal River at Stilfontein were obtained from Rand Water and Midvaal Water Company respectively. Dissolved inorganic nitrogen (DIN) was calculated as the sum of NH_4-N, NO_3-N and NO_2-N.

Isolation and growth requirements

A water sample taken from the Vaal River at Balkfontein that contained a mixed assemblage of green algae or Chlorophyta (*Chlorella* Beyerinck species, *Ankistrodesmus* Corda species, *Monoraphidium* Kom.-Legn. species and *Scenedesmus* Meyer species), Bacillariophyta (*Cyclotella* Kützing species) and Cyanophyta (*O. simplicissima*), was used to isolate *O. simplicissima*. The following methods were employed:

An aliquot sample of 100 mℓ was initially mixed with 100 mℓ GBG 11-medium (Krüger, 1978), and incubated at 18°C at a light intensity of 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (conditions in culture room) to obtain a mixed culture.

The omission of silicate-silicon from the GBG 11-medium eliminated *Cyclotella* species. Repeated subculturing and the use of actidione (50 $mg \cdot \ell^{-1}$) eliminated *Ankistrodesmus* species, *Monoraphidium* species and *Scenedesmus* species from the mixed culture. The resultant cultures were, however, still contaminated with *Chlorella* species. Different methods such as standard plating methods (Stainer et al., 1971), washing by centrifugation and the dilution method of Belcher and Swale (1982) were used in an attempt to isolate *O. simplicissima* without any success.

Allen and Stainer (1968) showed that many mesophilic blue-green algae, both filamentous and unicellular, could be isolated from mixed algal populations by temperature selection. In a general medium, which at 25°C supports growth of both blue-green algae and many eukaryotic algae, the development of eukaryotic algae was almost completely suppressed by incubation at 35°C. According to Allen and Stainer (1968), 25°C is the optimum growth temperature for most *Chlorella* species and temperatures above 30°C are lethal for these green algae. Mixed cultures of *Chlorella* species and *O. simplicissima* were, therefore, incubated in a water bath at 35°C at a light intensity of 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Although no increase in the chlorophyll-*a* concentration was measured, *O. simplicissima* filaments were still visible in suspension after 5d. The cultures were then incubated at 28 ºC and 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$. After 9d, increased growth of *O. simplicissima* was observed in the medium, and it was microscopically established that a unialgal culture of *O. simplicissima* had been obtained.

In order to determine the growth requirements of *O. simplicissima* under controlled conditions, GBG 11-growth medium (without $Na_2SiO_3.9H_2O$) was used as the initial medium with which to conduct growth. The effect of a range of different nutrient concentrations was tested to find the specific concentration necessary for optimum growth of *O. simplicissima*. One millilitre from a stock solution of *O. simplicissima* in the exponential phase was inoculated in 100 mℓ culture medium in a 250 mℓ Erlenmeyer flask and initially incubated at 28ºC and 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Three replicate cultures were used. Turbidity and chlorophyll-*a* concentration of filaments in suspension were used to determine the growth of *O. simplicissima*. Turbidity, measured with a Klett-Summerson photoelectric colorimeter equipped with a 540 nm (green) filter, was used for the initial experiments when the effect of different temperatures and light intensity was tested. Turbidity measurements are a fast and non-destructive method to determine growth and the risk of contamination are minimal (Krüger, 1978). However, filaments of *O. simplicissima* formed clumps causing the turbidity readings to be unreliable. It was therefore decided to use chlorophyll-*a* concentration (Sartory, 1982) as a measure of growth.

The nutrients tested are shown in Table 1. Na_2CO_3 as well as $NaHCO_3$ were tested to determine which carbon source is more suitable to obtain optimum growth for *O. simplicissima*. $NaNO_3$, urea and NH_4Cl were tested as potential nitrogen sources. Micronutrients were not tested separately, but the effect of different concentrations of a mixture of micronutrients (GBG 11-growth medium) on the growth of *O. simplicissima* was investigated.

Maximum yields, expressed by chlorophyll-*a* concentration and growth rate, were used to determine the concentration of a specific nutrient at which optimum growth occurred.

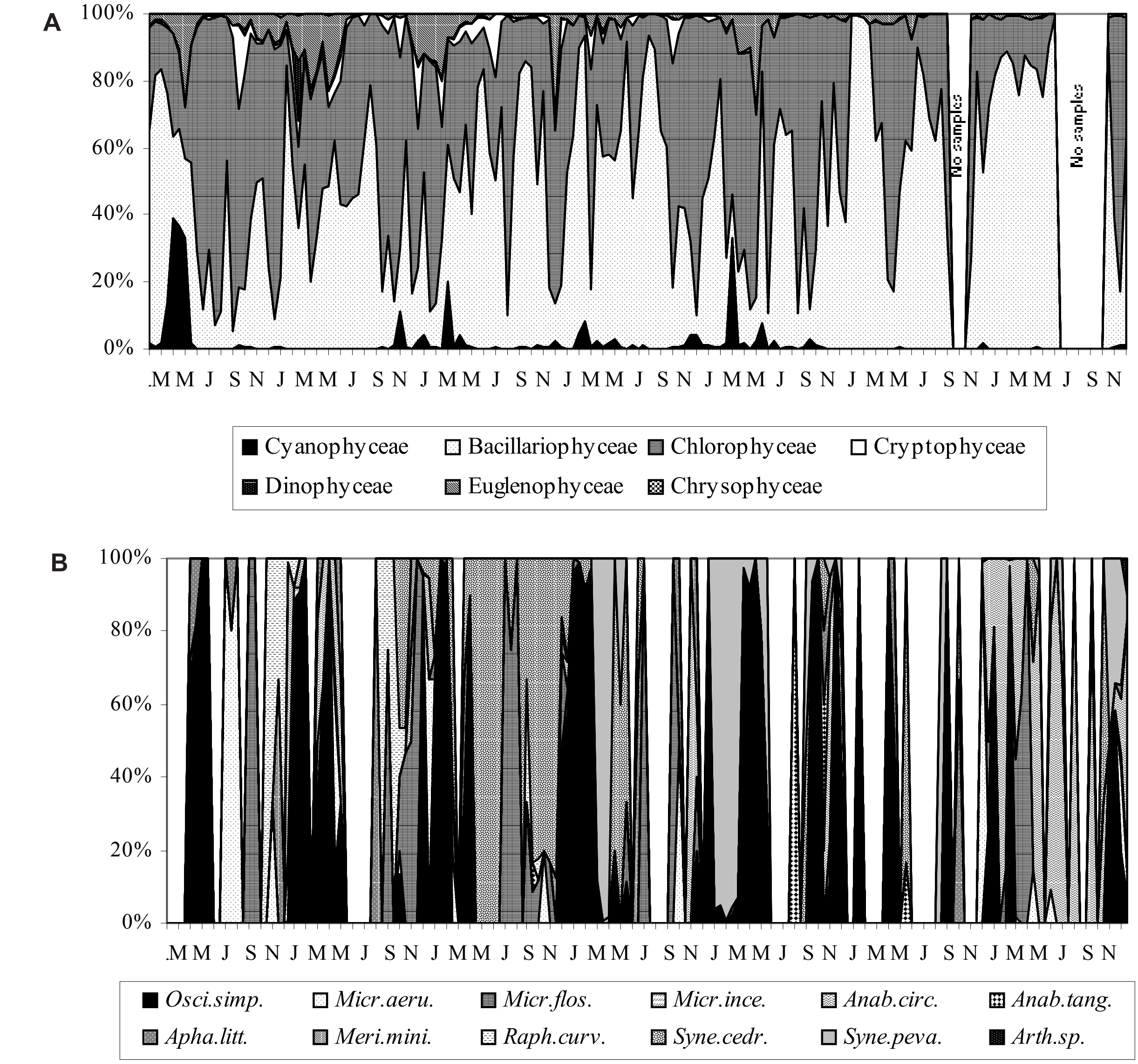

Figure 1

***A** - Percentage composition of different algal groups in the Vaal River from 1991 to 1997.*

***B** - Percentage composition of blue-green algal species identified in the Vaal River from 1991 to 1997. (*Osci.simp. = Oscillatoria simplicissima, Micr.aeru. = Microcystis aeruginosa, Micr.flos. = Microcystis flos-aquae, Micr.ince. = Microcystis incerta, Anab.circ. = Anabaena circinalis, Anab.tang. = Anabaenopsis tanganyikae, Apha.litt. = Aphanocapsa littoralis, Meri.mini. = Merismopedia minima, Raph.curv. = Raphidiopsis curvata, Syne.cedr. = Synechococcus cedrorum, Arth.sp. = Arthrospira *sp.)*

Results and discussion

Blooms of algal species are the result of environmental conditions favourable for growth. Before isolating and growing *O. simplicissima* in culture conditions, it was considered important to study environmental variables influencing this species under natural (riverine) conditions as knowledge of these growth requirements was used as a point of reference for culture experiments.

Results from the environmental study are presented in Figs. 1 and 2. The percentage composition of different algal groups is shown in Fig. 1A. Although the blue-green algae seemingly represent only a small proportion of all algal groups in the Vaal River, they are probably one of the most important algal groups, taking into account their potential to be problematic (whether toxin-producing, taste and odour-forming, filter-clogging, scum-forming or discouraging recreational activities). Figure 1B shows the percentage composition of all blue-green algal species identified during the 7-year study period. It is clear that *O. simplicissima* can be regarded as the most important bloom-forming blue-green algal species in the Vaal River.

Figure 2A shows that *O. simplicissima* reached maximum concentrations during May 1991 (5 300 filaments per mℓ), while the rest of 1991 and 1992 was characterised by low *O. simplicissima* abundances. During these periods diatoms and green algae succeeded each other as the dominants (compare Fig. 1A). From 1993 to 1995 *O. simplicissima* again became more prominent in the river. Other periods during which *O. simplicissima* reached high concentrations include May 1995 (1 100 filaments per mℓ), March 1995 (850 filaments per mℓ) and February 1994 (700 filaments per mℓ). During peaks in *O. simplicissima* biomass, chlorophyll-*a* concentrations ranged between 15 and 60 $\mu g \cdot \ell^{-1}$. It was found in Natal, that tastes and odours from blue-green algae occur at relatively low

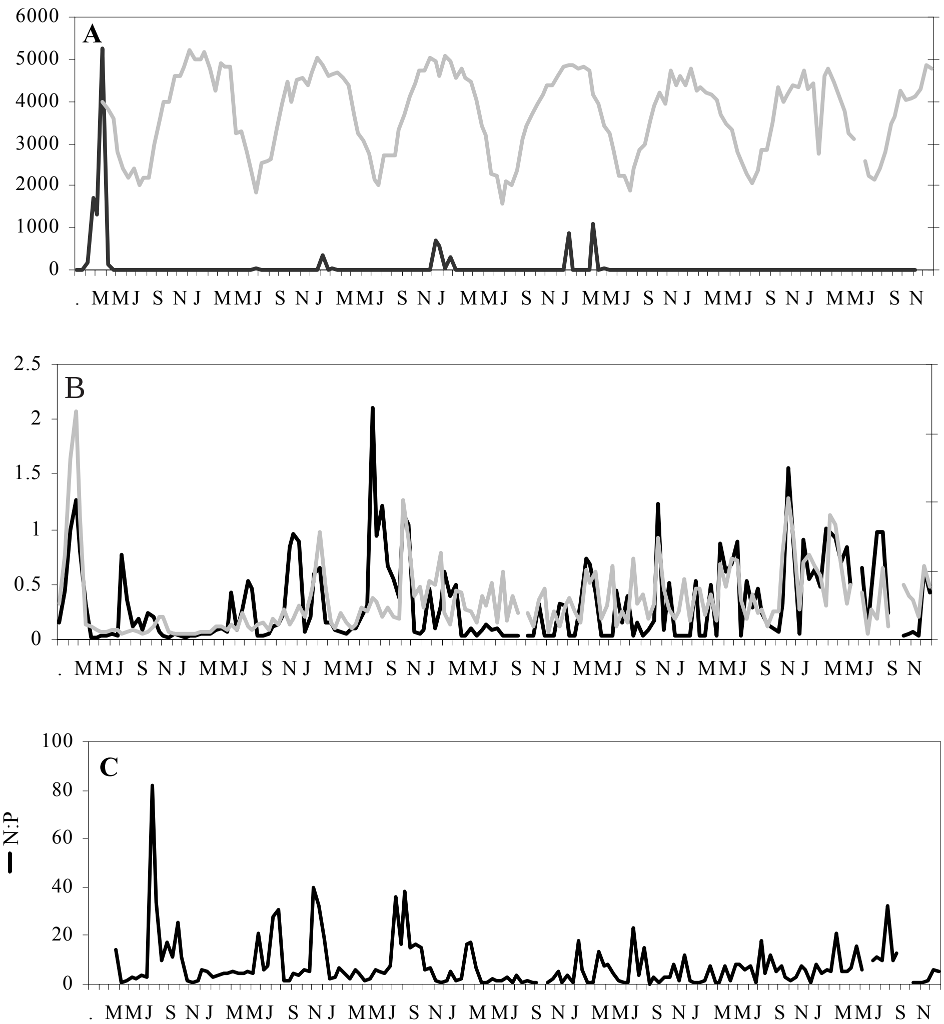

Figure 2
***A** - Variation in temperature and* Oscillatoria simplicissima *concentration.*
***B** - Variation in dissolved inorganic nitrogen (DIN) and ortho-phosphate (PO_4-P) concentration.*
***C** - Variation in N:P ratio in the Vaal River at Balkfontein from 1991 to 1997.*
*(*Osci.simp. = Oscillatoria simplicissima*)*

concentrations of chlorophyll-*a* (5 to 10 $\mu g \cdot \ell^{-1}$; DWAF, 1993a). Guidelines by DWAF (1993b) for water used for recreational purposes indicate that at a mean chlorophyll-*a* concentration of more than 30 $\mu g \cdot \ell^{-1}$, severe algal blooms (scums), as well as other symptoms of eutrophication occur and recreational users may experience skin irritations from water contact and gastroenteritis if blue-green algae-laden water is ingested. Chlorophyll-*a* concentrations in the Vaal River often exceed 30 $\mu g \cdot \ell^{-1}$ (average of 46.5 $\mu g \cdot \ell^{-1}$ for the period 1992 to 1997; Janse van Vuuren, 2001), thereby indicating the potential that problems may be expected in the Vaal River, especially during blue-green algal blooms. The whole of 1996 and 1997 was characterised by low concentrations (less than 10 filaments per mℓ) of *O. simplicissima.* During this period centric diatom species were dominant in the river and they out-competed Cyanophyceae species (Fig. 1A). High diatom concentrations may also increase the costs of water purification (e.g. the filamentous diatom *Aulacoseira granulata* clogs sand filters).

Temperature seems to play an important role in the selection of *O. simplicissima.* Fig. 2A shows that high concentrations of *O. simplicissima* were usually recorded during from middle to late summer periods. Temperature ranges during *O. simplicissima* blooms in the Vaal River generally varied between 15 and 25°C. Findings of this study are in agreement with observations made on field data measured in the Loch Vaal and in the Vaal River at Stilfontein. In both localities water temperature varied between 21 and 24°C during the development of *O. simplicissima* blooms (data obtained from Rand Water and Midvaal Water Co.).

The results of the growth study showed that *O. simplicissima* did not grow at 20 and 38°C (Fig. 3). Maximum growth rate of *O. simplicissima* was at 28°C, but there was no significant difference between the maximum yields at 24, 28 and 32°C. The high maximum yield of *O. simplicissima* measured at 24°C in artificial conditions corresponds well with field data.

In assessing physical factors and the response of phytoplankton thereto, it is important to realise that conditions at the time of sampling are not necessarily those which brought about the particular assemblage of algae being collected. As stated by Allen and Koonce (1973; cited in Sommer, 1989) "today's assemblage is not the product of today's environment, but is rather yesterday's assemblage altered by a factor determined by yesterday's environment". It was also stated by Vollenweider (1953) that it is the conditions which cause an algal bloom (or were present during the development of the bloom) that are important, not the conditions during the peak of the bloom. Figure 2B shows the dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (PO_4-P) concentrations over the study period. It is clear from Fig. 2B that both DIN and PO_4-P concentrations were usually low during peaks in *O. simplicissima* biomass, probably as a result of utilisation of these important nutrients. If the DIN and PO_4-P concentrations prior to *O. simplicissima* blooms are, however, taken into account, it is clear that peaks in nutrient concentrations were experienced prior to blooms of *O. simplicissima*. Peaks in DIN and PO_4-P concentrations during February 1991 may have resulted in the observed peak in *O. simplicissima* biomass during May the same year. In a similar way there is a possibility that peaks in nutrient concentrations during October 1993 and April 1995 may have resulted in peaks in *O. simplicissima* biomass during February 1994 and May 1995, respectively. Janse van Vuuren (2001) that showed lag-periods of approximately four to eight weeks exist between high nutrient concentrations and the response of phytoplankton thereto.

Janse van Vuuren (1996; 2001) observed that blooms of *O. simplicissima* developed in the Vaal River during periods of low turbidity (usually less than 70 NTU). The high turbidity of Vaal River water (maximum of 640 NTU; Janse van Vuuren, 2001) results in low light intensities and low light penetration in the river water. *O. simplicissima* was isolated from this turbid water and may have adapted to the low light intensities. The pH values in the natural waters at the time varied between 7 and 9, ideal conditions for the growth of *O. simplicissima* in artificial conditions (Venter, 2000).

The results of growth studies showed that maximum yields were high for *O. simplicissima* grown at 10 to 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ but low for *O. simplicissima* grown at 5 and 25 to 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 4). Growth rates of *O. simplicissima* grown at 10, 15 and 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were the highest and *O. simplicissima* filaments grown at 15 to 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ increasingly clumped together with an increase in light intensity. Filaments grown at 25 to 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were observed to be bleached. Chlorophyll-*a* florescence data analysed with the JIP-test showed that high light intensities (20 $\mu mol \cdot m^{-2} \cdot s^{-1}$) cause damage to photosystem II of *O. simplicissima* (Venter, 2000).

Maximum yield and growth rate of *O. simplicissima* grown in 5.0 $mg \cdot \ell^{-1}$ carbon of Na_2CO_3 were higher than those of *O. simplicissima* grown in 5.0 $mg \cdot \ell^{-1}$ carbon of $NaHCO_3$. Carbon concentrations higher than 5.0 $mg \cdot \ell^{-1}$ of both carbon sources were tested, but due to heavy precipitation that occurred after the medium was autoclaved, *O. simplicissima* did not grow. Using aeration with CO_2 enriched air instead of inorganic carbon can eliminate precipitation at high carbon concentration. Krüger (1978) obtained a high growth rate of *Microcystis aeruginosa* by aeration of the medium with CO_2 (0.52%) enriched air. Pieterse (1968) also observed a higher growth rate in *Oscillatoria raoi* when the medium was aerated with 0.7% CO_2.

The inorganic N:P ratio is often described in literature to be an important factor associated with the occurrence of blue-green algae (Schindler, 1977; Barica et al., 1980; Smith, 1982; Harris, 1986). It was often illustrated that low N:P ratios are beneficial to the growth of blue-green algae (Kalff and Knoechel, 1978; Romo and Miracle, 1993). During the present study N:P ratios were usually less than 10 during peaks in *O. simplicissima* biomass (Fig. 2C). This was confirmed by the results of the growth studies where optimum growth occurred at a N:P ratio of 5.53 achieved at a NO_3-N concentration of 45.16 $mg \cdot \ell^{-1}$ and a PO_4-P concentration of 8.16 $mg \cdot \ell^{-1}$ (Fig. 5). It was also determined that *O. simplicissima* can utilise urea and ammonia as a nitrogen source, but had a higher growth rate and maximum yield when grown in a growth medium

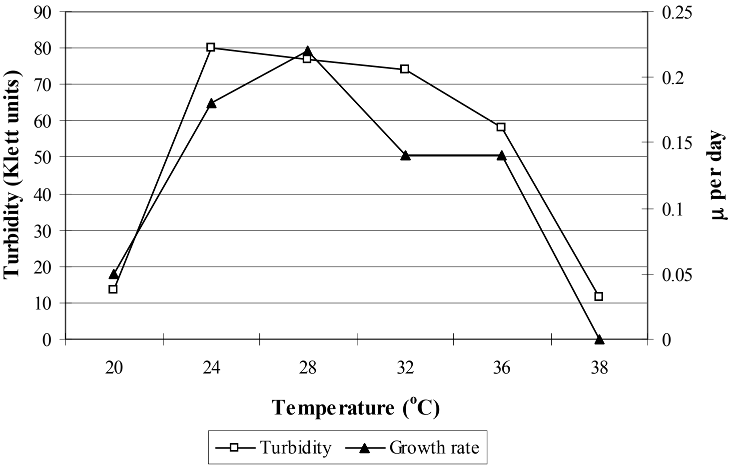

Figure 3
Maximum yields and growth rates of Oscillatoria simplicissima *incubated at 20, 24, 28, 32, 36 and 38°C and a light intensity of 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$.*

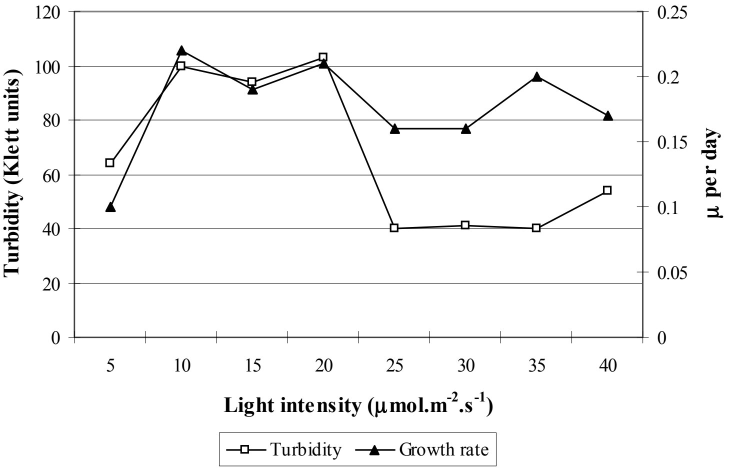

Figure 4
Maximum yields and growth rates of Oscillatoria simplicissima *incubated at light intensities of 5, 10, 15, 20, 25, 30, 35 and 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 28°C.*

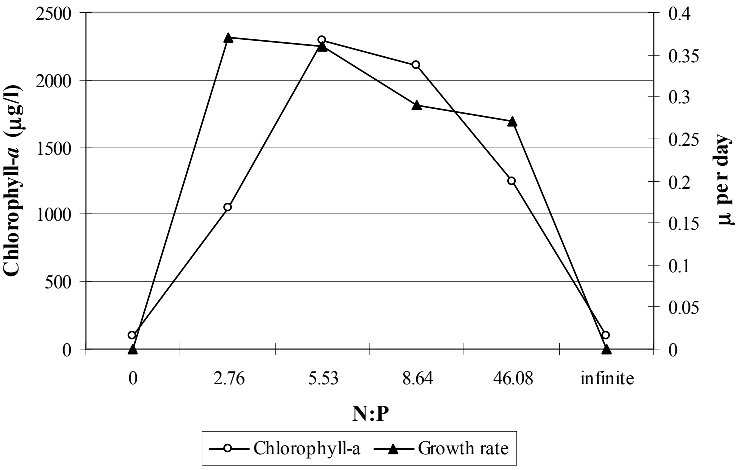

Figure 5
The effect of different N:P ratios on the growth of Oscillatoria simplicissima *incubated at 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 28°C*

with NO_3-N. Nitrate is mostly used as a nitrogen source in culture mediums and can be supplied in high concentrations without an adverse decline in the pH of the culture medium during growth, which is characteristic when ammonium salts are used as a nitrogen source (Fogg et al., 1973). According to Wetzel (1983) blue-green algae have high growth rates with NH_4-N as the nitrogen source but NH_4OH that forms at high pH values is toxic to algae and can inhibit their growth.

EDTA or citric acid can be used as a chelating agent but *O. simplicissima* grown in growth medium with 12 mg·ℓ^{-1} citric acid had the highest maximum chlorophyll-*a* concentration and the highest growth rate.

O. simplicissima needs vitamins and micronutrients (B, Mn, Zn, Mo, Co and Cu) in low concentrations for optimum growth (Venter, 2000). According to Provasoli and Carlucci (1974) Cyanophyceae only needs vitamin B_{12}. The vitamins in the vitamin mixture needed by *O. simplicissima* were not tested separately and therefore Cyanocobalamin (B_{12}), Biotin and Thiamine-HCl will be included in the growth medium of *O. simplicissima*. It is however, necessary to investigate the possibility that *O. simplicissima* may only need vitamin B_{12} for optimum growth.

Riverine studies showed that blue-green algae often respond to changes in nutrient conditions with a lag phase, in other words peaks in nutrient concentrations precedes peaks in blue-green algal biomass. This emphasise the fact that it is conditions during the development of a bloom that should be taken into consideration and not those during the peak of the bloom. The magnesium (12 to 24 mg·ℓ^{-1}) and calcium (31 to 89 mg·ℓ^{-1}) ion concentrations were higher in the Loch Vaal and Vaal River than the concentrations necessary for optimum growth in artificial conditions. The iron (0.28 to 0.9 mg·ℓ^{-1}), orthophosphate (0.7 to 0.12 mg·ℓ^{-1}) and nitrogen (1.6 to 3.9 mg·ℓ^{-1}) concentrations in the natural waters were much lower than the concentrations needed for optimum growth in artificial conditions, and could have been growth limiting. However, a continuous supply of nutrients in natural waters can maintain high growth rates.

The results of the inorganic growth requirements of *O. simplicissima* grown in artificial conditions are given in Table 1.

TABLE 1
The inorganic growth requirements of *O. simplicissima* grown in artificial conditions at 28ºC and 10 µmol·m^{-2}·s^{-1}

	Ion concentration	Concentration of salt
Macro-nutrients:		
1) $NaNO_3$	N = 45.16 mg·ℓ^{-1}	270.0 mg·ℓ^{-1}
2) $K_2HPO_4.3H_2O$	P = 8.16 mg·ℓ^{-1}	60.0 mg·ℓ^{-1}
3) $MgSO_4.7H_2O$	Mg^{2+} = 7.5 mg·ℓ^{-1}	77.0 mg·ℓ^{-1}
4) $CaCl_2.2H_2O$	Ca^{2+} = 10 mg·ℓ^{-1}	36.5 mg·ℓ^{-1}
5) Na_2CO_3	C = 5 mg·ℓ^{-1}	44.2 mg·ℓ^{-1}
6) Citric acid		12.0 mg·ℓ^{-1}
7) $FeSO_4.7H_2O$	Fe^{2+} = 2 mg·ℓ^{-1}	10.0 mg·ℓ^{-1}
Micronutrients:		
i) H_3BO_3	B = 0.128 mg·ℓ^{-1}	1.43 mg·ℓ^{-1}
ii) $MnCl_2.4H_2O$	Mn = 0.159 mg·ℓ^{-1}	0.57 mg·ℓ^{-1}
iii) $ZnSO_4.7H_2O$	Zn = 0.025 mg·ℓ^{-1}	0.111 mg·ℓ^{-1}
iv) $NaMoO_4.5H_2O$	Mo = 0.07 mg·ℓ^{-1}	0.2 mg·ℓ^{-1}
v) $Co(NO_3)_2.6H_2O$	Co = 0.004 mg·ℓ^{-1}	0.02 mg·ℓ^{-1}
vi) $CuSO_4.5H_2O$	Cu = 0.01 mg·ℓ^{-1}	0.04 mg·ℓ^{-1}
Vitamin mixture:		
a) Cyanocobalamin (B_{12})		33.0 µg·ℓ^{-1}
b) Thiamine-HCl		33.0 µg·ℓ^{-1}
c) Biotin		330.0 µg·ℓ^{-1}

Conclusions

O. simplicissima is, from an ecological point of view, one of the most important blue-green algal species present in the Vaal River. Results of growth studies correspond well with results of environmental variables observed during field studies. Environmental variables favourable for the growth of *O. simplicissima* in a natural environment include temperatures of 15 to 25°C compared to temperatures of 24 to 32°C in artificial conditions. In a natural environment, as well as in artificial conditions, growth of *O. simplicissima* is favoured by an N:P ratio of less than 10.

Growth of *O. simplicissima* under controlled conditions is favoured by low light conditions (10 to 20 µmol·m^{-2}·s^{-1}). This observation confirms indications of possible adaptation of *O. simplicissima* to low light intensities in the turbid water of the Vaal River.

The magnesium (12 to 24 mg·ℓ^{-1}) and calcium (31 to 89 mg·ℓ^{-1}) ion concentrations during the development of an *O. simplicissima* bloom were higher in the Loch Vaal and Vaal River than the concentrations necessary for optimum growth in artificial conditions. The iron (0.28 to 0.9 mg·ℓ^{-1}), PO_4-P (0.7 to 0.12 mg·ℓ^{-1}) and NO_3-N (1.6 to 3.9 mg·ℓ^{-1}) concentrations in natural waters were much lower than the concentrations needed for optimum growth in artificial conditions and could have been growth limiting. However, a continuous supply of nutrients in natural waters can maintain high growth rates.

The inorganic growth requirements that were experimentally determined were used to compile a growth medium for *O. simplicissima*, called the experimentally modified medium (EM; shown in Table 1).

Acknowledgements

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