The factors which influence algal growth in the P.K. le Roux impoundment, Orange River, South Africa.

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One must learn
By doing the thing; for though you
think you know it
You have no certainty, until you try.
Sophocles,
Trachiniae.

Summary

In South Africa, much of the work on algal limitation in man-made lakes has centered on nutrients rather than the possible effects of physical factors such as turbidity. In order to determine which is the most important factor in algal limitation in the turbid Orange River system, a comprehensive study of spatial and seasonal changes of the following factors was undertaken in the P.K. le Roux impoundment: trace elements, silicon, nitrate, phosphate, total phosphate, phytoplankton standing stock, total suspended solids, secchi disc transparency, light attenuation and the spectral quality of the light. None of the chemical factors appeared to be in short supply but the shallowness of the euphotic zone (0.8 m) had two effects. Firstly, the quantity of light in the upper 1.5 metres was extremely small due to the rapid attenuation of the light by silt. Secondly, the quality of the light was poor due to the selective screening of the shorter wavelengths of light.

Once light limitation had been implicated as an important factor in the control of phytoplankton stocks, a series of column experiments were used to demonstrate that increased circulation rate, and therefore a higher light dosage, would elevate the algal standing stocks without the addition of nutrients. It was demonstrated that light, as a result of the negative effects of turbidity, was the principal cause of low algal standing stocks within the impoundment, and not nutrients as has previously been suggested by other workers.

Part I

The P.K. le Roux impoundment - a holistic assessment

Introduction

One of the first published works on the Orange River project (Van Zinderen Bakker *et al.*, 1972) stated that suspended silt was a major factor limiting primary production in the Hendrik Verwoerd impoundment. Stegmann (1975) attributed the regulation of biological activity in the impoundment to turbidity while Walmsley (1978) has emphasised the capability of suspended silt to decrease the depth of the euphotic zone in Lindleypoort Dam, another turbid impoundment. Similarly Walmsley and Bruwer (1980) have demonstrated that of 92 South African impoundments studied, 70 had a secchi disc depth of less than 1.5 metres. It is therefore unreasonable that so much emphasis has been placed on nutrients, rather than turbidity, as being a major factor causing algal limitation in South African freshwater bodies. With respect to the Orange River system, more particularly the major reservoirs, Toerien *et al*. (1975) considered nitrate to be the most limiting nutrient. The work of Stegmann (1975) on the Verwoerd impoundment did not support this conclusion and suggested that phosphate was in short supply.

In an attempt to clarify the above and shift emphasis away from nutrient orientated studies and towards the possible effects of turbidity on phytoplankton, Selkirk (in press) used natural phytoplankton populations from the P.K. le Roux impoundment to determine the factors which limit growth. Ambient nitrate (300 $\mu g\ \ell^{-1}$) and phosphate (20 $\mu g\ \ell^{-1}$) levels were adequate to sustain growth at low light flux (16 $\mu E\ m^{-2}\ s^{-1}$) and increase algal yield at high light flux (400 $\mu E\ m^{-2}\ s^{-1}$). However in experiments where photoperiod was altered, cells which received less than 6 hours light (100 $\mu E\ m^{-2}\ s^{-1}$) showed no growth (senescence). On the basis of these results it was suggested that the turbidity of the water in the P.K. le Roux impoundment, coupled with the large mixed depth (25 m) creates a light climate which severely limits algal growth. Although the simple experimental design of this work demonstrated that the rapid attenuation of light due to suspended silt, coupled with a low euphotic to mixed depth ratio (Talling, 1971), was a probable cause of the low algal stocks in the impoundment, it is possible that not all factors which could limit algal growth had been considered.

In an attempt to evaluate the relative roles of nutrient chemistry and light in the regulation of phytoplankton populations within the impoundment, two different

approaches were used. In the first instance, surveys were used to monitor changes in nitrate and phosphate levels throughout an annual cycle. Concurrent with this analysis, a number of trace elements and silicon concentrations were measured. Factors related to the physical environment (light and temperature) were also monitored to determine whether an interaction between chemical and physical factors caused algal limitation, or whether one of the two groups of factors dominated.

Trends in the survey data showed that light was probably the single most important factor in algal limitation. To demonstrate whether this was a valid hypothesis, in situ column experiments were used to determine the effect that increased circulation, in the epilimnion, would have on algal growth. This increase in circulation rate was the only way in which the light dosage received by algal cells could be increased in the turbid waters of the impoundment.

Study site description

The P.K. le Roux impoundment (Fig 1), 40 km downstream of the Hendrik Verwoerd dam wall, is 75 km long. These two dams form the major impoundments on the Orange River, which rises in the mountains of Lesotho. Eighty percent of the catchment (88500 km^2) is above the

Verwoerd Dam (Stegmann, 1975; Davies, 1978). The local catchment is an arid to semi-arid region (less than 300 mm precipitation p.a.) with river flow restricted to the late summer (Davies, 1978; Keulder, 1979). For this reason, the discharge pattern of the upper reservoir has a profound influence on the P.K. le Roux impoundment.

Excessive erosion in the upper catchment results in massive silt loads (3102 metric tonnes per annum) being transported during the wet season (Keulder, 1979). The eroded material, derived from Drakensberg basalt, the Molteno and upper and lower Beaufort series, and Dolerite inclusions, influences the chemical and physical properties of the water (Stegmann, 1974 & 1975; Roosenboom, 1978; Keulder, 1979). Although the turbidity of the water is a result of erosion of the Beaufort and Molteno series shales, other geological formations are responsible for inputs of biologically important chemical elements.

The P.K. le Roux impoundment, being downstream of the Verwoerd Dam has two major advantages over other turbid reservoirs. Firstly the water level can be compensated for by the Verwoerd dam discharge pattern, and the initial proposal, to maintain the level to within 1 - 2 m of full supply level (Olivier, undated), would be biologically advantageous. Secondly, the upper reservoir acts as a silt trap and could be used to increase the

Table 1. The mean surface temperature (1 m) for each of the 90 station surveys.

Date of survey	Season	Temperature		
		Mean	Standard deviation	Number
01/04/81-02/04/81	Autumn*	19.8	0.5	5
22/06/81-24/06/81	Winter	12.7	0.6	90
05/10/81-07/10/81	Spring	13.8	0.8	90
17/01/82-19/01/82	Summer	21.6	0.9	90
01/04/82-03/04/82	Autumn	20.4	0.6	90

* Temperatures from Institute for Freshwater Studies records.

water clarity of the lower system (Olivier, undated). Although both the above objectives would have been biologically advantageous, they have of late, been negated due to increased throughflow in the system because of increased hydro-electric power demand during the study period (C. du Toit, pers.comm., Dept. of Water Affairs).

Materials and methods

The seasonal and spatial changes of the chemical and physical factors related to phytoplankton were monitored at 90 randomly selected stations throughout the impoundment (Fig 1). Stations were allocated at the start of the study by means of a grid-square with boundaries at the full supply level of the reservoir. The average station density was 1 station per 1.53 X 10^6 m^2.

Sampling for each survey was undertaken at biologically important, rather than time-related intervals (Table 1) so as to increase the usefulness of the information collected. Winter samples were taken at the mid-point of the destratified period when algal stocks were lowest. Summer samples were collected when surface temperatures were highest and the stratified layer most stable. The spring sampling was used to establish post-winter

Table 2. The coefficient of variation of the analyses used during this study. All results are based on 10 or more replicates. The coefficient of variation is calculated for the mid-point of the concentration range of each of the different determinations. Details of methods are given in the text.

Analysis	Coefficient of variation (%)
Chlorophyll *a*	7.4
Nitrate-Nitrogen	7.8
Phosphate-phosphorus (SRP) (Hexanol extraction)	2.5
Phosphate-phosphorus (SRP) (Without extraction)	2.0
Total phosphorus	5.0
Soluble reactive Silicon	4.0
Total suspended solids	10.0
Trace elements	
Fe	1.0
Mn	1.0
Mg	1.0
Cu	1.0
Ca	2.0
Na	1.0
K	4.0

nutrient concentrations immediately before an increase in phytoplankton numbers. To this end, sampling took place prior to temperatures in the upper 10 m reaching 14°C, as previous experience has shown that after this temperature is attained algal stocks increase rapidly. Due to the unpredictable timing of overturn, which can occur any time between April and August, the autumn survey took place as soon as temperatures dropped to, or below, 20°C.

Methods for nutrient analysis were tested at the start of the study to determine how reproducible and accurate they were. Those selected are the methods which were most reliable. The level of precision for each of the procedures is given in Table 2. As much of the biological activity occurs in surface waters all results refer to samples from the upper 1.5 metres of the water column. Throughout the study, all chemical analyses were carried out on samples which were preserved immediately after collection with mercuric chloride. In view of the number of samples collected, analyses took up to 14 days to complete.

Soluble reactive phosphate (SRP) concentrations were determined from GF/C filtered water samples and analysed by a hexanol extraction method (Mackereth *et al*., 1978). Total phosphate was determined by reduction of bound phosphorus with potassium persulphate and sulphuric acid at positive pressure (Eisenriech *et al*., 1975; Mackereth *et al*., 1978). After reduction, samples were membrane filtered (0.45 μm pore size) following addition of 1 mℓ of 6.5 N ammonium chloride to eliminate a colloidal suspension from the final colour reagent. Analysis for phosphate was by a modified non-extractive method (Allanson, in manuscript).

Nitrate, measured as NO_3-N and uncorrected for nitrite, was determined using a cadmium-copper reduction method (Strickland and Parsons, 1968). Soluble reactive silicon was determined by the method of Mackereth *et al*., (1978) from GF/C filtered samples. Samples for trace element analysis were analysed as described by Stainton *et al*. (1974) on a Varian atomic absorption spectrophotometer. Samples were not filtered prior to analysis.

Total suspended solids were measured gravimetrically after collection on pre-ashed GF/C filters, and drying for 24 hours at 60°C. Total inorganic material was determined after ignition at 500°C.

Chlorophyll *a* analyses incorporated recommendations made at the symposium on the measurement of photosynthetic pigments (Rai, 1980). Integral surface samples were collected with a 1.5 m long tube. Samples (250 mℓ) were filtered through a GF/C filter. Filters were folded and cut into 5 mm strips, placed in 95% methanol, and allowed to stand in sealed test tubes for 1½ to 2 hours. After infiltration, samples were boiled for two minutes, cooled, and centrifuged. Absorbance was measured at 665 and 750 nm on a Shimadzu double beam spectrophotometer. The amount of chlorophyll a present was calculated from the equation given by Marker et al. (1980). No correction was made for phaeophytin as the level of phaeopigments was negligible when monitored at intervals throughout the study.

Secchi disc transparency was measured using a 0.2 m diameter white disc. Readings were recorded to the nearest 0.01 m. As a more sensitive measure of transparency, attenuation coefficients for photosynthetic active radiation (PAR) (Kirk, 1977) were determined at each station using a Lambda LI-185 Quantum flux meter. Quantum flux was measured at the surface and at three fixed depths in the water column with a cosine corrected sensor. The relative penetration of different spectral components was measured monthly using a quantum sensor in conjunction with Schott RG1 (red), VG9 (green) and BG12 (blue) filters.

Temperature-depth profiles were recorded in the lower basin of the reservoir (1; Fig 1) using a Yellow Springs Instrument thermistor probe. Readings are accurate to 0.5°C. Surface temperatures (1 m) for 90 station surveys

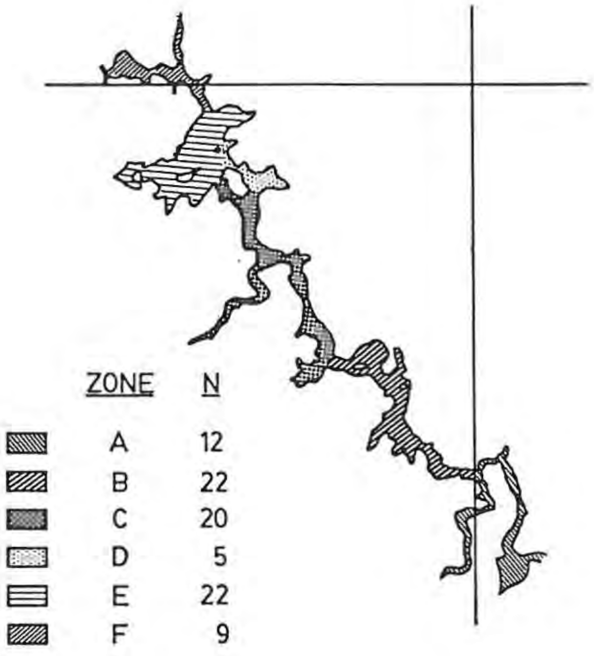

Figure 2. The six zones into which the impoundment was sub-divided to increase the sensitivity of statistical analysis. The number of stations in each zone is also given.

Table 3. The measured mean depth of all stations included in the each of the six zones for the four surveys winter 1981 to autumn 1982.

Zone	Depth		
	Mean	Standard deviation	Number
A	13.2	8.7	48
B	21.7	7.1	88
C	22.8	11.7	80
D	11.9	8.6	20
E	28.8	12.1	88
F	33.9	15.6	36
Grand mean for all six zones.	23.2	12.4	360

were measured with a mercury in glass thermometer (0.1% accuracy).

In all cases results are reported in the text as the mean and one standard deviation. In order to increase the sensitivity of statistical analysis, the impoundment was divided into 6 zones in an attempt to reduce the within zone variance. This sub-division of the habitat is particularly important as horizontal (spatial) variation within the impoundment was large (cf. Table 6 and 8).

Each zone was defined according to its locality (Fig 2) and measured mean depth (Table 3). This type of sub-division is independent of both biological and climatological factors, and allowed the zones, with the exception of D, to follow the bathymetric gradient of the impoundment. This is demonstrated by the increase in the measured mean depth of each zone (Table 3).

It could be argued that this type of zonal division is insensitive to some biological events in those zones where major rivers occur (zone A - Seekoei River and zone C - Hondeblaf River; Fig 1 and Fig 2). However the creation of two further zones would have led to an ungainly data-base. Further sub-division would only have decreased the within zone variance during the autumn

1981 survey when Microcystis aeruginosa populations formed extensive surface scums in the two rivers in question.

Results and discussion

The physico-chemical environment of the impoundment

The conductivity in surface waters was constant throughout the study period (152.7 ± 5.3 µS; n=12) but slight differences were detected in bottom waters where the conductivity was higher. The pH of the reservoir was alkaline within the range 7.6 to 8.1 (mean of 7.90; n=12). The water column was generally well oxygenated, but for a short period in autumn 1981 oxygen concentrations in the hypolimnion dropped to as low as 3 mg ℓ^{-1}. The concentration of carbon, as measured by calcium carbonate, is high (15 mg ℓ^{-1}; Allanson, pers.comm.).

Thermal properties of the impoundment

During summer and autumn 1981 the epilimnion deepened with time and reached a maximum depth of 25 m in late April (Fig 3). Water temperatures started to decline in May and the water column was homothermal by the middle of June 1981. The lowest temperatures recorded during the destratified period were 9.2°C. During the latter

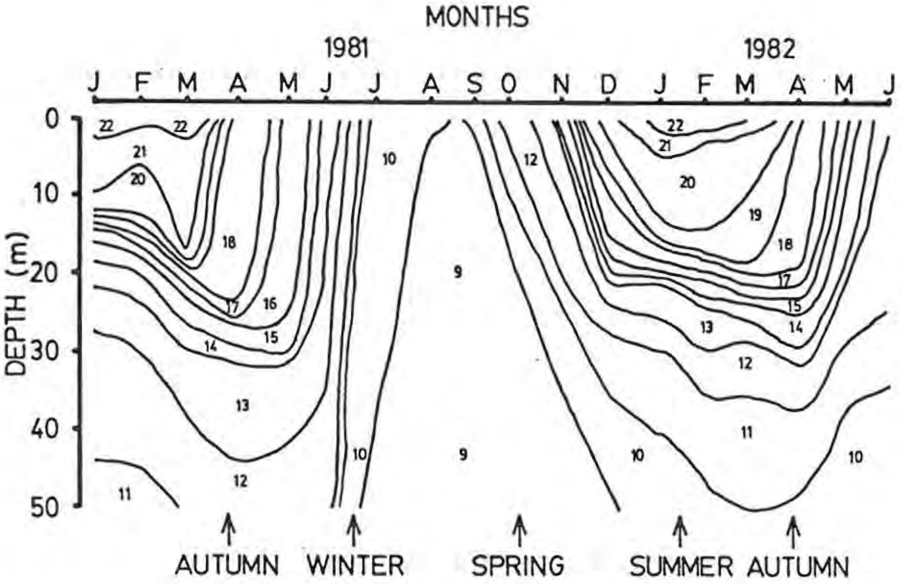

Figure 3. The isotherms at station 1 for the duration of the study period, the arrows indicate the days on which the 90 station surveys took place.

Table 4. The mean concentration (mg ℓ^{-1}) of trace elements in unfiltered samples from surface waters for the whole impoundment during the period winter 1981 to autumn 1982.

Trace element	Concentration		
	Mean	Standard deviation	Number
Mg	7.5	2.0	348
Ca	5.7	2.7	348
Fe	2.2	0.5	347
K	1.8	0.3	348
Na	$<3.0\text{X}10^{-2}$	-	90
Cu	$<3.0\text{X}10^{-1}$	-	348
Mn	$<3.0\text{X}10^{-1}$	-	348
Zn	$<3.0\text{X}10^{-1}$	-	348

part of September surface warming created a weak thermocline which strengthened in October. The rate of temperature change across the metalimnion increased into the summer and was highest during January 1982. The water column remained relatively stable until late March, when surface cooling took place and the rate of temperature change in the metalimnion decreased. The stability of the water column continued to weaken until June when the metalimnion was ill-defined. By the end of July 1982 the water column was completely homothermal.

The marked stratification of the water column during the summer months means that algae are circulated through large distances and volumes. The rate of this circulation is probably low as the wind speed is small (3.1 ± 0.8 m s^{-1}; n=52), wind fetch short (less than 5000 m) and the thermocline deep (20 - 30 m). The above have the effect of reducing wind induced stirring and favoring convective mixing. This may be particularly important as the diel air/water temperature differential can be large at night. For the purpose of this study, the thermocline has been taken as the boundary of the mixed layer.

Trace elements

No seasonal or spatial differences were noted for any of

Table 5. The mean concentrations ($\mu g \ \ell^{-1}$) of soluble reactive silicon in the impoundment during summer 1981/1982.

Survey	Silicon		
	Mean	Standard deviation	Number
Spring	299	82	30
Summer	361	42	30
Autumn	330	41	30

the micro-nutrients monitored during the study period (Table 4). The dominance of Mg^{++} over Ca^{++} is in keeping with the geology of the catchment (Keulder, 1979). The known association of trace elements with suspended particles (Gibbs, 1973) probably explains the high Fe^{++} concentrations in the unfiltered samples, and may also account for the lower concentrations of Mg, Ca and K when compared with results from other studies in the Orange River (Stegmann, 1975; Keulder, 1979).

Silicon

Although the measured silicon in the water was much lower than concentrations measured in European lakes (Paasche, 1980) and African lakes (Talling and Talling, 1965), it cannot be considered limiting as large quantities of silicon are known to adhere to the suspended sediments. While there was no significant difference in seasonal or spatial silicon concentrations between Spring 1981 and Autumn 1982 (Table 5) analyses indicated that, in unfiltered samples, up to 18 mg ℓ^{-1} silicon was present. This suggests that the principal clay types of the Orange River, illite and monomorillonite, have a high silicon content, part of which is readily soluble under the physico-chemical conditions in the Orange River.

Table 6. The spatial and temporal changes in the principal nutrients, nitrate and phosphate, and chlorophyll a within the impoundment during 90 station surveys,giving the mean and on standard deviation. The summary statistics for the whole impoundment and the annual zonal means are given.

Parameter	Survey	Zone												Whole lake n = 90	
		A n = 12		B n = 22		C n = 20		D n = 5		E n = 22		F n = 9			
		x	s	x	s	x	s	x	s	x	s	x	s	x	s
Nitrate	Autumn 1981	477	119	464	74	306	108	292	45	336	42	287	35	372	110
	Winter 1981	242	47	228	72	557	301	370	36	442	100	453	49	385	201
	Spring 1981	567	176	997	810	687	346	532	88	611	128	824	354	734	464
	Summer 1982	447	38	490	153	589	201	910	384	626	184	629	60	577	201
	Autumn 1982	445	82	495	42	491	32	480	29	484	35	470	34	482	47
	Annual mean	436	148	535	444	526	235	517	272	500	155	533	241		
Phosphate	Autumn 1981	57.8	12.9	50.8	8.0	27.5	7.1	26.6	3.2	24.2	3.6	23.0	3.6	35.9	15.6
	Winter 1981	32.9	6.9	28.3	7.3	32.0	5.0	30.4	2.8	30.9	6.6	32.8	2.2	30.9	6.1
	Spring 1981	28.4	3.7	29.8	3.7	27.3	5.3	30.8	1.1	25.8	4.4	30.1	4.2	28.2	4.5
	Summer 1982	36.1	2.8	34.0	1.9	34.0	1.5	34.6	1.8	34.1	1.2	33.5	1.5	34.3	1.9
	Autumn 1982	31.8	2.0	30.7	0.6	31.0	1.0	32.2	1.0	31.8	1.4	31.7	0.8	31.4	1.3
	Annual mean	37.4	12.5	34.7	9.7	30.4	5.2	30.9	3.3	29.3	5.5	30.2	4.7		
Chlorophyll	Autumn 1981	5.3	5.8	3.8	2.7	9.0	11.1	13.5	11.5	2.5	0.9	2.7	1.1	5.3	6.9
	Winter 1981	1.0	0.4	0.7	0.2	0.6	0.4	0.8	0.3	0.5	0.2	0.5	0.2	0.7	0.3
	Spring 1981	7.5	7.0	2.1	0.8	1.3	0.4	1.8	0.5	2.5	0.9	2.0	1.0	2.7	3.2
	Summer 1982	2.5	1.1	1.8	0.4	1.7	0.4	1.3	0.5	1.4	0.4	1.4	0.3	1.7	0.6
	Autumn 1982	3.8	1.7	2.5	0.6	2.5	0.6	2.2	0.3	2.0	0.4	1.6	0.1	2.4	1.0
	Annual mean	4.0	4.6	2.2	1.7	3.0	5.8	3.9	6.8	1.8	1.0	1.6	1.0		

Nitrate

This present study gives little supportative evidence for nitrate limitation in the P.K. le Roux impoundment, especially as the lowest nitrate levels (372 ± 110 $\mu g\ \ell^{-1}$; n=90) recorded in the impoundment during the study were in autumn 1981 when phytoplankton was at the highest levels recorded (Table 6; Fig 4). These nitrate concentrations gradually increased throughout the study to reach a maximum of 734 ± 464 $\mu g\ \ell^{-1}$ during spring 1981. This spring nitrate concentration represents the initial concentration of nitrate prior to the summer increase in phytoplankton. As phytoplankton populations built up throughout the growing season, nitrate concentrations where gradually depleted to 482 ± 47 $\mu g\ \ell^{-1}$ (n=90) by the autumn 1982 survey.

The seasonal trend shown for the whole lake was also visible in zones C - F, however in zones A and B, the lowest nitrate concentrations were recorded during the winter 1981 survey when levels dropped to 228 $\mu g\ \ell^{-1}$ (Table 6). The cause of these lower concentrations in the upper reaches of the reservoir is unknown. During spring 1981 the influence of the upper impoundment was evident, as areas of high nitrate concentrations (997 ± 810 $\mu g\ \ell^{-1}$) were located in zone B (Fig 4). On subsequent investigation it was determined that high volume discharges from the silt gates at the Verwoerd

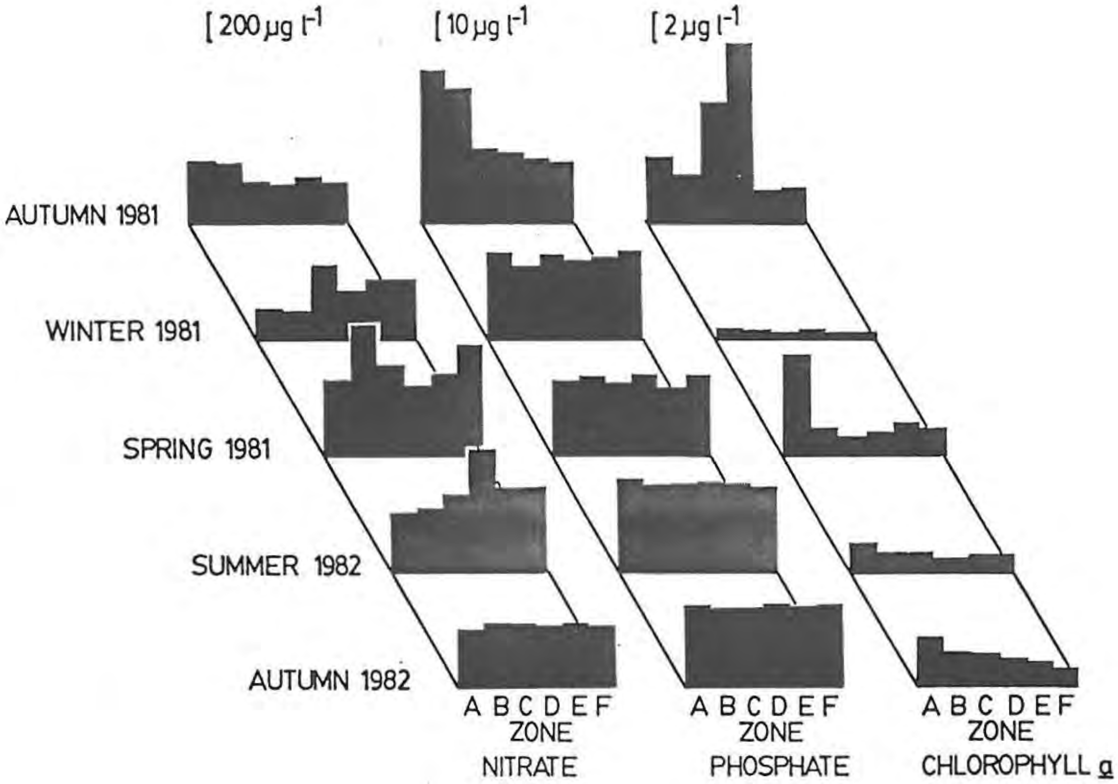

Figure 4. The spatial and temporal changes in the principal nutrients, nitrate and phosphate, and chlorophyll a within the impoundment during 90 station surveys.

Dam took place during the preceeding 96 hours prior the the survey (H.Smit pers.comm., Dept. of Water Affairs). It is therefore suggested that this pulse of water, moving through the P.K. le Roux impoundment, was the cause of these abnormally high nitrate levels. It is also possible that these inputs of nutrient-rich waters from the hypolimnion of the upper impoundment may serve to maintain the ambient nitrate concentrations in the the P.K. le Roux impoundment.

This buffering effect may explain why nitrate levels in the P.K. le Roux impoundment show so little fluctuation in zones A and B during summer and autumn 1981/1982 (474 ± 96 μg ℓ^{-1}), when compared with the wider variation of nitrate concentrations in zones C - F, during the same period (Table 6). The slightly lower annual mean of nitrate in zone A, when compared with the rest of the impoundment, is probably the result of the elevated phytoplankton population in this zone (see page 18).

Phosphate

i) Soluble reactive phosphate

With the exception of the autumn 1981 survey, there was no significant zonal variation in phosphate concentrations, nor was there any seasonal variation (Table 6; Fig 4). The stability of the system is a marked deviation from the norm for northern temperate water bodies (Hutchinson, 1957; Nalewajko & Lean, 1980) and seasonal patterns observed in other South African impoundments (Stegmann, 1975; Walmsley, 1976; Toerien & Walmsley, 1978; Walmsley *et al*., 1978a, 1978b; Archibald & Warwick, 1980; Archibald *et al*., 1980; Butty *et al*., 1980)where a decline inphosphate (SRP) normally follows the spring increase in phytoplankton populations.

During autumn 1981 the distribution of phosphate concentrations within the impoundment can be divided into two groups. In zones A and B, the phosphate concentrations were higher than at any other time during the study period (Fig 4). In the remainder of the reservoir (zones C - F) the phosphate levels were lowest during autumn 1981, particularly in the more transparent waters of zones E and F. The causes of the higher concentrations in the upper reaches of the reservoir during autumn 1981 are difficult to explain, but may result from inputs of water from the Verwoerd impoundment. The lower concentrations in the remainder of the impoundment were probably the result of increased phytoplankton activity brought about by the increased water clarity (see Fig 5) in these zones.

The constancy of phosphate concentration within the

Table 7. The mean concentration ($\mu g\ \ell^{-1}$) of total phosphate in the impoundment during summer 1981/1982.

Survey	Total phosphate		
	Mean	Standard deviation	Number
Spring	79	12	30
Summer	67	11	30
Autumn	71	11	30

impoundment for the remainder of the study period (Table 6; Fig 4), may lend tangible support to the views expressed by others (Van Zinderen Bakker et al., 1972; Grobbelaar, 1979) that silt may act as an active site for phosphate exchange, the direction of the equilibrium being dependent on the physico-chemical conditions which prevail at the time. Golterman et al. (1969) showed that re-suspended sediment can act as a source of phosphate for algal growth. More recently Viner (1982) has demonstrated that the suspended silt is a major source of phosphate in a turbid, Papua New Guinean river, and that the phoshate associated with the silt can be more important than the dissolved portion, under certain conditions. It is therefore possible that silt, of the type found in the Orange River (Keulder, 1979), could act in a similar manner.

If the above assumption is correct, then in turbid water-bodies, as found on the Orange River, an equilbrium could exist between the water and silt particles. Under such conditions, it is then improbable that phosphate could limit algal growth.

ii) Total phosphate

The total phosphate (TP) concentrations in surface waters were low (Table 7) with little seasonal variation. For the most part, total phosphate was uniform throughout the impoundment however higher concentrations (90 ± 15 µg ℓ^{-1}; n=13) were recorded in zone A. The highest values were recorded in the shallow, upper reaches of the Seekoei River during each of the three surveys (110 ± 10 µg ℓ^{-1}). Associated with these higher total phosphate levels was an elevated chlorophyll a (see following section). The low ratio of TP to SRP (75 µg ℓ^{-1} TP/32 µg ℓ^{-1} SRP = 2.34) indicates that biological activity in surface waters is limited, especially as the available phosphate (SRP) is high and is not being transferred to phytoplankton and there is a large pool of phosphate associated with the silt.

Chlorophyll a

Phytoplankton standing stock, measured as chlorophyll a, showed seasonal differences which were not only influenced by physical factors, such as light and temperature, but also affected by hydrological events in the reservoir (see page 25). As would be expected chlorophyll a concentrations were lowest during the winter 1981 survey (0.7 ± 0,3 µg ℓ^{-1}; n=90) but no clearly defined peak was evident during the growing season (Table 6; Fig 4). The mean chlorophyll a concentration for the period spring 1981 to autumn 1982 was 2.3 ± 2.0 µg ℓ^{-1} (n=270). However a closer inspection of the data shows that the greatest variation within the system occurred during the summer in zone A (4.6 ± 4.6 $\mu g\ \ell^{-1}$; n=36) while the remainder of the system (zones B - F) exhibits a high degree of stability (1.9 ± 0.7 $\mu g\ \ell^{-1}$; n=234).

These results are in contrast to the higher chlorophyll a concentrations recorded during the autumn 1981 survey (5.3 ± 6.9 $\mu g\ \ell^{-1}$; n= 90; Table 6) when the transparency of the water increased two-fold (Secchi disc was 0.27 ± 0.16 m; n=90; Table 8). During this survey large concentrations of Microcystis aeruginosa were found in zones C and D, and these tended to elevate zonal chlorophyll a levels and increase the within zone variance. In the shallower areas of the reservoir, particularly in the Hondeblaf River and 4 of the 5 stations in zone D, the M. aeruginosa populations formed dense surface scums and elevated chlorophyll a concentrations above 25 $\mu g\ \ell^{-1}$. The highest concentration of chlorophyll a (51 $\mu g\ \ell^{-1}$) was recorded in the Hondeblaf River.

An important factor in all surveys after autumn 1981 was that zone A always had a higher chlorophyll a concentration than the rest of the impoundment (Fig 4). The large variance about the mean in this zone was due to the patchy distribution of phytoplankton (Table 6). An example of this was the occurrence of a large population of Pandorina sp in the Seekoei River during

Table 8. The spatial and temporal changes in the total suspended solids, secchi disc transparency and attenuation coefficient within the impoundment during 90 station surveys giving the mean and one standard deviation. The summary statistics for the whole impoundment and the annual zonal means are given.

		Zone													
Parameter	Survey	A n = 12		B n = 22		C n = 20		D n = 5		E n = 22		F n = 9		Whole Lake n = 90	
		x	s	x	s	x	s	x	s	x	s	x	s	x	s
Total suspended solids	Autumn 1981	111	21	88	21	43	13	27	7	18	9	7	3	52	39
	Winter 1981	148	13	108	19	80	17	51	6	50	12	42	10	83	38
	Spring 1981	103	21	88	10	76	13	59	4	59	5	58	4	76	30
	Summer 1982	97	24	74	6	63	8	52	2	50	6	47	2	65	19
	Autumn 1982	82	19	59	6	52	6	46	2	39	4	40	1	53	16
	Annual mean	108	29	83	21	63	18	46	12	43	16	39	18		
Attenuation coefficient	Autumn 1981	11.75	3.48	9.12	1.23	5.77	1.28	4.10	0.77	3.62	0.41	2.90	0.22	6.48	3.39*
	Winter 1981	12.18	1.89	10.27	0.95	7.63	0.87	5.70	0.64	5.36	0.47	3.66	2.63	7.82	3.01
	Spring 1981	8.83	1.34	9.44	0.80	7.67	0.61	7.14	0.31	6.89	0.54	6.58	0.16	7.92	1.30
	Summer 1982	7.60	1.16	7.07	0.48	7.00	0.21	6.66	0.30	6.39	0.43	6.25	0.24	6.86	0.68
	Autumn 1982	8.73	1.74	6.90	0.53	6.31	0.15	6.16	0.11	6.41	0.52	5.60	0.12	6.70	1.10
	Annual mean	9.84	2.73	8.56	1.58	6.88	1.05	5.95	1.16	5.74	1.26	5.00	1.88		
Secchi disc	Autumm 1981	0.10	0.03	0.13	0.02	0.23	0.08	0.35	0.10	0.40	0.06	0.55	0.07	0.27	0.16
	Winter 1981	0.13	0.03	0.14	0.01	0.17	0.02	0.25	0.03	0.22	0.02	0.27	0.04	0.18	0.05
	Spring 1981	0.14	0.02	0.15	0.01	0.16	0.01	0.16	0.01	0.17	0.01	0.19	0.02	0.16	0.02
	Summer 1982	0.14	0.02	0.16	0.01	0.17	0.01	0.16	0.02	0.17	0.01	0.19	0.02	0.16	0.02
	Autumn 1982	0.13	0.02	0.15	0.01	0.15	0.01	0.17	0.01	0.18	0.01	0.23	0.01	0.17	0.03
	Annual mean	0.13	0.03	0.14	0.02	0.18	0.05	0.22	0.09	0.23	0.09	0.28	0.15		

* Data for autumn 1981 calculated from the equation k = 1.039 +1.013 (Secchi^{-1}); n=360, r=0.86.

spring 1982 when chlorophyll a concentrations reached 19 $\mu g\ \ell^{-1}$.

This trend of increased chlorophyll a concentrations in zone A is reinforced by the fact that when all available data (autumn 1981 - autumn 1982; Table 6) are ranked, then zonal means decrease with distance downstream. The only tied rank (C and D) could be expected, as zone C, by providing water via the narrow entrance channel, would have some influence over zone D. However it must be stated that the magnitude of changes in the lower reservoir (zones B - F) is small when compared to the differences between zones A and B. This trend is not evident in the autumn 1981 survey, probably as a result of the masking effect of *M. aeruginosa*.

The light climate of the impoundment

i) Secchi disc transparency and total suspended solids.

During all the seasonal surveys the highest suspended solids, and the associated lower transparencies, were recorded in zone A (Table 8; Fig 5). These high turbidities can be expected in this region of the reservoir as it is subject to the discharge pattern through the Verwoerd Dam. The lowest suspended solid concentrations, and highest transparencies, were

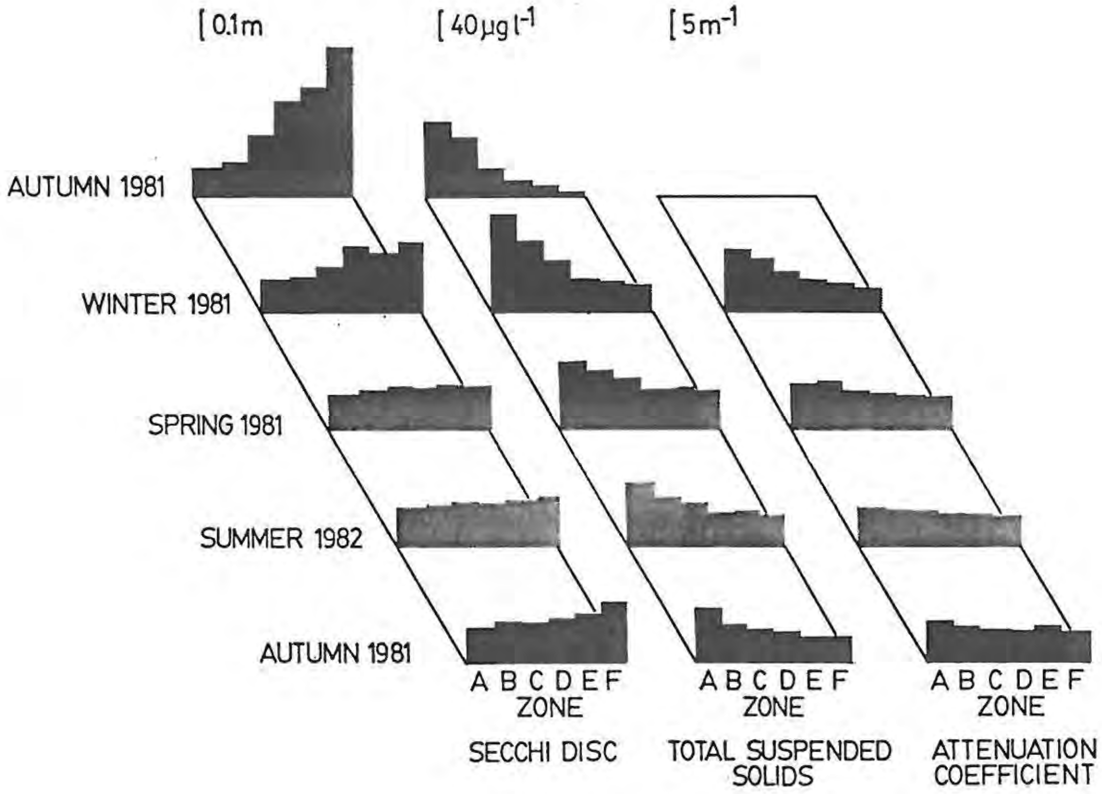

Figure 5. The spatial and temporal changes in total suspended solids, secchi disc transparency and attenuation coefficient within the impoundment during 90 station surveys.

recorded in zone F. The transparency of the water increased with distance downstream as a result of the deposition of some of the silt load in the intermediate zones (B - D), however the rate of deposition was markedly affected by the hydrological regime of the upper reservoir.

During the study period, two different flow regimes were maintained, due to different management strategies at both the Verwoerd and P.K. le Roux Dams. In autumn and winter 1981 there was high volume discharge from Verwoerd Dam but the retention time of the P.K. le Roux impoundment was long (326 days). This favoured an increase in transparency with distance downstream (Table 8; Fig 5), but the greatest change was observed at the entrance to Rolfontein (zone E; Fig 1 and 3), where the combined effect of increased channel width and depth (decreased flow) resulted in deposition of some of the silt load.

The second management strategy (summer and autumn 1982) was to utilize water from both reservoirs for the generation of hydro-electric power. The rapid discharge from both Dams had the effect of increasing the throughflow in the lower reservoir. The retention time of water in P.K. le Roux dropped (237 days) and the distribution of the suspended solids in the impoundment was more even (Table 8; Fig 5). The water transparency

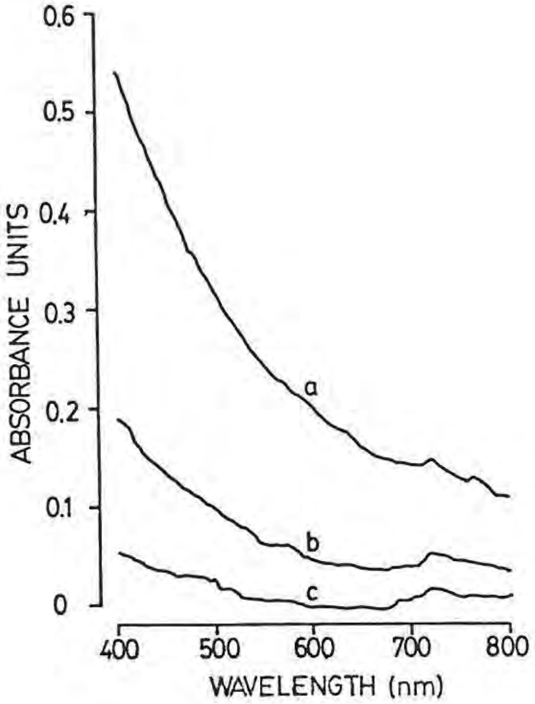

Figure 6. The relative absorbance of light by unfiltered (a), GF/C (b) and membrane (0.45 µm) filtered water (c) from station 1.

decreased markedly and the within zone variation was reduced for both total suspended solids (59 ± 19 mg ℓ^{-1}; n=180) and secchi disc transparency (0.17 ± 0.03 m; n=180) (cf. autumn and winter 1981, Table 8).

ii) Attenuation of light

A consequence of the high turbidity is that the incident light is rapidly attenuated within the water by the back-scattering effect of silt particles. This background attenuation of light is related to both the size (Burt, 1956) and abundance of silt particles in the water column. It is therefore not surprising that a high degree of correlation exists between the total suspended solids (TSS) and the attenuation coefficient of photosynthetically active radiation (K_{PAR}) (TSS = $13,5K_{PAR} + 29,9$; r=0,86; n=357; p 0,001; see Fig 5, Table 8).

Most of the available light (99 % of surface flux) is attenuated within the first metre of the water column by the high attenuance of silt. This can be demonstrated by the fact that other factors known to contribute to light attenuation in freshwaters do not make any significant contribution to this attenuation. Algae do not contribute as chlorophyll a concentrations are normally well below the 10 $\mu g\ \ell^{-1}$ level set by Kirk (1977).

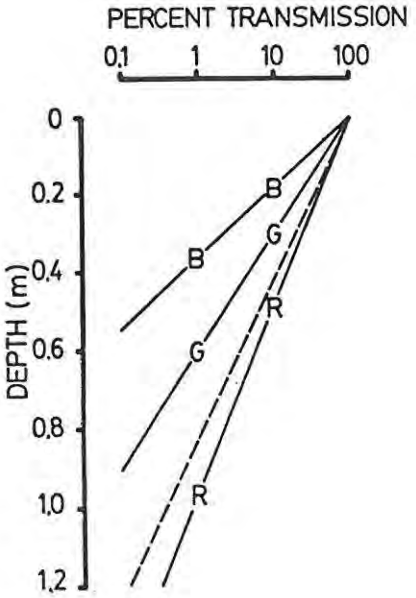

Figure 7. The mean attenuation profiles of blue (B), green (G) and red (R) light during the study period (July 1981 to June 1982) at station 1 (Fig 1). The average attenuation of PAR is represented by the dashed line.

Table 9. Changes in the attenuation coefficients of PAR, red, green and blue light at station 1 for the duration of the study.

	Attenuation coefficient "k" (m^{-1})			
Date	White	Red	Green	Blue
01/07/81	5.17	4.49	7.11	11.86
01/10/81	6.28	5.24	9.39	14.16
04/11/81	5.86	4.60	6.52	13.55
09/12/81	5.64	4.50	7.53	7.95
10/01/82	6.11	5.45	8.80	15.10
10/02/82	5.71	5.21	8.21	13.85
06/03/82	5.70	5.06	9.20	13.01
08/04/82	5.65	4.99	7.89	16.78
07/05/82	5.12	4.24	6.58	12.23
10/06/82	4.78	4.33	6.29	11.18
08/07/82	5.01	4.35	7.09	12.35

Furthermore the water has no coloured compounds in solution which could add to light attenuation, as filtered water from the impoundment has similar optical properties to distilled water (Fig 6).

Similarly it can be seen that not only is the euphotic zone extremely shallow when compared with the mean depth of the impoundment (23.3 m; Noble & Hemens, 1978), but the quality of the light is severely affected. Blue light (430 nm) is most rapidly attenuated while red light (630 nm) penetrates furthest (Fig 7; Table 9). The rapid attenuation of blue light is related to the size dependent interference of particles (Ellis, 1936; Burt, 1956) within the euphotic zone.

Conclusions

It would appear that there is little evidence for nutrient limitation of phytoplankton. Those trace elements monitored (Table 4) were present in sufficient quantities to be able to sustain, and even promote, growth. Although the list of elements monitored is not exhaustive, other preliminary studies have indicated that many other elements are present in trace amounts. Both vanadium and molybdenum, previously implicated as possible limiting nutrients (Stegmann, 1975), were detected using EDAX (Energy dispersive X-ray analysis).

Silicon, although present in lower concentrations than those measured in other South African impoundments (see Walmsley & Butty, 1980), maintains a background concentration of 300 µg ℓ^{-1} of soluble reactive silicon (Table 5). However, a large pool of silicon must adhere to the silt for concentrations of up to 18 mg ℓ^{-1} to be measured. It is therefore unlikely that silicon could be limiting algal growth.

Nitrate and phosphate are normally considered to be the major growth limiting nutrients in freshwaters (McCarthy, 1980; Nalewajko & Lean, 1980), but neither give any indication of being limiting in P.K. le Roux (Table 6; Fig 4). The depletion of nitrate to lower levels in autumn 1981 (372 ± 100 µg ℓ^{-1}; n=90) than in autumn 1982 (482 ± 47 µg ℓ^{-1}; n=90) is probably a result of the increased transparency and higher algal populations in 1981. However a decline of nitrate to 372 ug ℓ^{-1} does not suggest nutrient limitation, especially as the lowest concentration recorded was 228 µg ℓ^{-1}.

The apparent capacity for phosphate concentrations to be buffered by an equilibrium between the silt and the water means that phosphate cannot be considered a limiting factor. The stability of phosphate concentrations, is however dependent on the presence of silt, so major increases in water transparency could have a detrimental effect and cause phosphate limitation of algae.

It has previously been demonstrated (Selkirk, in press) that at ambient nutrient concentrations, phytoplankton increase from 10^3 to 10^8 cells $m\ell^{-1}$ after exposure to a constant light flux (90 $\mu E\ m^{-2}\ s^{-1}$). This increase in cell density was accompanied by a ten-fold increase in chlorophyll a concentration in spite of no other nutrients being added. Therefore in the absence of any chemical limitation, low phytoplankton standing stock in the impoundment must be limited by the unfavorable light climate. Light limitation can be brought about by a combination of any of the following factors.

1. The euphotic zone is shallow (c. 0.8 m; Fig 7) and the quantity of light available to the algae is rapidly reduced to below optimal flux by the attenuation due to silt particles.
2. The quality of light is adversely affected by the selective adsorption of wavelengths in the blue region of the spectrum (Fig 7; Table 9).
3. The mixed depth of the impoundment (Fig 3), in combination with the shallow euphotic depth, further acts to limit algal growth. When the lake is stratified, the epilimnion extends to 20 - 25 m and during destratification the maximum depth can exceed 50 m (maximum depth 105 m).

As an example of the possible effect of the large path of circulation in the mixed layer, zones E and F had a z_{eu}/z_m ratio of 0.03 (z_{eu}=0.8, Fig 7; z_m=25 m, Fig 3). However in zone A where the water depth, with the exception of two stations adjacent to zone B, is shallow (7.5 m), the z_{eu}/z_m ratio was 0.07 in spite of higher turbidity and the resultant decrease in light penetration (z_{eu}=0.5, Table 8). This means that twice as much light is available to algae in zone A than in the relatively more transparent waters of zones E and F given a similar circulation rate. This assumption could easily be realised as water entering zone A, from the Verwoerd impoundment, creates extensive turbulence. The increased frequency of algae in the euphotic zone probably accounts for the elevated chlorophyll a concentrations in zone A, compared with the rest of the impoundment (Fig 4). The conclusions drawn from these results are concurrent with the thinking of Verduin (1982) where he suggests that a low z_{eu}/z_m ratio is a major factor to be considered in light limited systems.

The controlling influence of light is demonstrated by comparision of results from autumn 1981 and 1982 (Table 6 and 8). The Secchi disc was twice as great in autumn 1981 (0.27 ± 0.16; n=90) compared with autumn 1982 (0.17 ± 0.03; n=90). The effect of this decrease in light penetration (increased turbidity) was that autumn chlorophyll a concentrations were halved between the surveys in question (Table 6). This demonstrates the extremely sensitive nature of the system to small changes of the light climate.

Part II

In situ column experiments to assess the effect of increased circulation (light) on algal growth.

Introduction

Part I suggests that two factors, which act singly or in combination, limit algal growth. These are:

1. The limited depth of the euphotic zone due to light attenuation by suspended silt.
2. The mixing pattern of the reservoir due to the marked stratification, and therefore the circulation pattern, of the upper 25 metres of the water column during the growing season (see Fig 3) which could cause light limitation.

In order to understand the manner in which these constraints affect phytoplankton, a measure of circulation rate within the upper mixed layer is required. Although many techniques have been developed to determine circulation rates and patterns, they are not readily applicable the P.K. le Roux impoundment. The turbidity of the water, adsorption by silt and volume effects, make dye-stuff determinations (Drew and Smith, 1969; Church, 1974) difficult. Drogue monitoring (George, 1981; Myers *et al*., 1982) was not practical during the study, as unfortunately neither the equipment nor the man-power was available.

In view of these difficulties associated with determining circulation rates in the upper mixed layer, it was not possible to simulate an approximate light climate which could be experienced by algal cells, therefore an indirect approach was used to determine the role of circulation and/or turbidity in algal limitation. Selkirk (in press) has demonstrated that algal cell concentrations increased from 10^3 to 10^8 cells $m\ell^{-1}$ in 168 hours, given a constant light flux of 90 $\mu E\ m^{-2}\ s^{-1}$ (equivalent to 5 % of surface flux). This increase in cell number was associated with a ten-fold increase in chlorophyll a concentration. This response to increased light dosage must, however, be tested *in situ* before it can be conclusively shown that light, as a result of the magnitude of the mixing pattern, is the cause of algal limitation.

The use of columns (Goldman, 1962) provides an ideal opportunity to study *in situ* algal populations under natural environmental conditions. An artificial increase in the circulation rate allows the response of phytoplankton populations to be monitored against that of a control. If indeed the small euphotic to mixed depth ratio is the cause of algal limitation, increased circulation could be expected to decrease or overcome

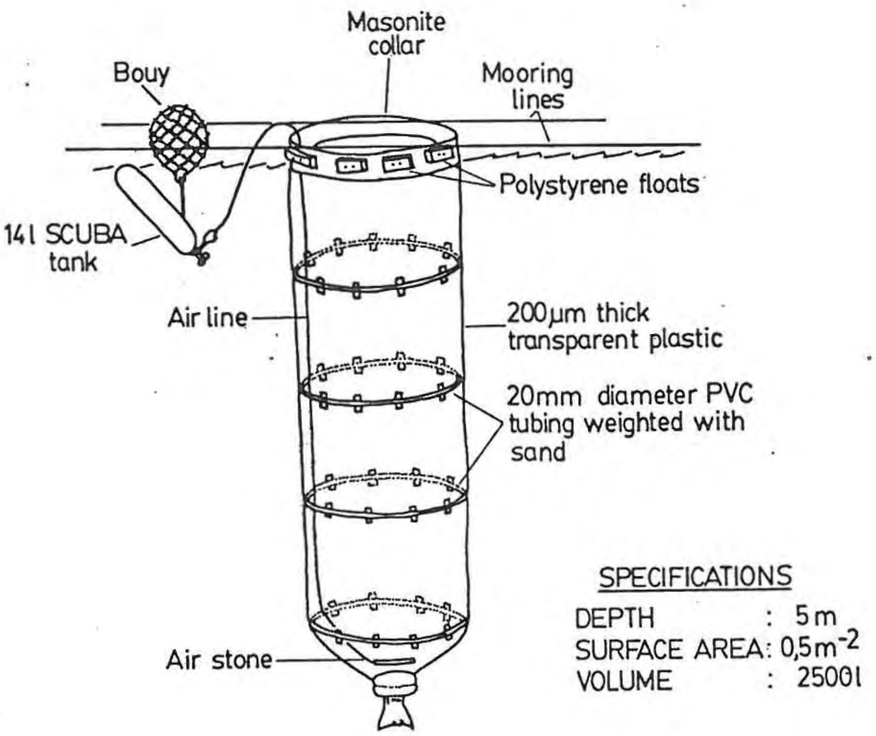

Figure 8. An annotated sketch of the specifications and materials of plastic enclosures used in column experiments.

Figure 9. The columns in position at the experimental site in the first basin of the impoundment. The different controls and treatments are shown.

this light block, with a resultant increase in algal standing stock.

Materials and methods

Columns (Fig. 8) were deployed in ten metres of water in a sheltered bay near the research station (2; Fig 1). A mooring harness was secured to opposite shores (Fig 9) and columns attached prior to filling with water from 1 metre depth. A high speed centrifugal pump (9 $\ell\ s^{-1}$) was used to fill the columns. Zooplankton were partially excluded by filtration through a 60 - 150 µm aperture zooplankton net.

Initial experiments were designed to test the effect of enclosure on algal populations. The lake control system, a collar without a plastic liner, was compared with a column control to determine enclosure effects. The responses of all other treatments were tested against this column control. As zooplankton were to be partially excluded from all treatments, the effect of exclusion was also tested (zooplankton control). A second objective of this study was to establish whether increased nitrate and phosphate concentrations would increase algal growth. Enrichment was achieved by a ten-fold addition of the appropriate salt (KNO_3 and K_2HPO_4).

Once the effect of enclosure had been established, that of increased circulation and the resultant increase in light dosage was assessed. An air-lift pump was used to circulate water (Fig 8), but could only be operated during daylight to allow time to re-charge SCUBA tanks used as air supply. The volume of air bubbled through each column per day was equal to twice the total volume of the column. The transfer of water by air had the effect of doubling the circulation rate in these columns and the assumption was made that algal cells in turn received a higher light dosage as cells in circulated treatments were present in the euphotic zone more frequently than cells in uncirculated columns.

In all experiments columns were monitored from the time of deployment. However, three days were allowed for phytoplankton to adapt to the new light climate. This acclimation period ensured that all samples, for chlorophyll a, which were used in statistical analyses were collected from a community in which all individuals were in a similar physiological state. This was particularly relevant to the phytoplankton in circulated treatments. One limitation of the experimental design was that periphyton on the plastic of columns increased after 10 days. For this reason, replication, rather than extended monitoring, was favoured in all experiments.

Columns were sampled daily (at midday) and the concentrations of nitrate, phosphate and chlorophyll a determined. Soluble reactive phosphate was determined by a modified method of Mackereth et al. (1978) (Allanson, in manuscript) after floculation of fine suspensoids with 1 mℓ of 6.5 N ammonium chloride and membrane filtration. It should be noted that membrane filtration resulted in a 25 % reduction of the measured soluble reactive phosphate when compared with results from Part I where water samples were GF/C filtered. Nitrate and chlorophyll a were determined by the same methods as used in Part I.

To increase the sensitivity of results obtained from chlorophyll a measurements, two different samples were collected from each column.

1. An integrated tube sample (1.5 m) measured the amount of chlorophyll a in the euphotic zone (Surface chlorophyll a).
2. An integrated tube sample (5 m) measured the chlorophyll a in the whole column (Total chlorophyll a).

This allowed the amount of chlorophyll a in the aphotic zone of the columns (i.e. water below 1.5 m) to be calculated by subtraction.

Experiments were designed to allow two-way analysis of variance tests (without replication) to be performed on data using Model I anova (Sokal and Rolhf, 1973). This model makes the assumption that no interaction exists between treatments* and days. In the first instance all controls and treatments were tested to determine whether significant differences between treatments were present and results are presented in Anova tables. Secondly, as internal tests between treatments had been decided upon at the start of experiments, a priori tests for significance (Sokal and Rohlf, 1973) were used to test differences between individual treatments. Tests were carried out in the following order:

1. Lake control vs. column control
2. Column control vs. other treatments.

To increase the effectiveness of the data display for column experiments, box and whisker diagrams (McNiel, 1977; as modified by McGill et al. 1978) were used. The major advantage of this type of display, especially where the number of samples is small, is that the plots show the absolute variance and distribution of the population sampled. Furthermore, five properties of the original data set are displayed, namely the extremes of the range, the 25 % and 75 % quartiles and the median. The inclusion of each of these points allows for more accurate intepretation of trends in the data (e.g. skewness or normality).

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* The word "treatment" is used in preference to "column" in the text as the latter refers to a component of the Anova table. The word "column" is used only if the context is qualified.

Results

The effects of enclosure and nutrient enrichment

i) Daily variation in chemical concentrations

In the lake and column control treatments phosphate concentrations fluctuated between 17 and 22 $\mu g\ \ell^{-1}$. Nitrate concentrations varied over a 70 $\mu g\ \ell^{-1}$ range between 480 and 550 $\mu g\ \ell^{-1}$. The exclusion of zooplankton had little or no observed effect during the study period.

In enriched treatments nitrate and phosphate concentrations declined to 25 % of the initial inoculum after 24 hours. The exponential decline in concentrations continued until the 6th day when nitrate and phosphate concentrations in enriched columns were equal to the concentrations in other treatments. The uptake rate of nitrate and phosphate was 2.7 and 2.5 pg s^{-1} respectively. This similarity in the rate of uptake of both nutrients suggests that an external factor, other than phytoplankton, acts to absorb both nutrients. If phytoplankton were responsible for this rapid assimilation, it would be expected that both nutrients would be utilized at a rate equivalent to their intercellular ratios (16:1). This was not the case however, and the significance of this finding will be

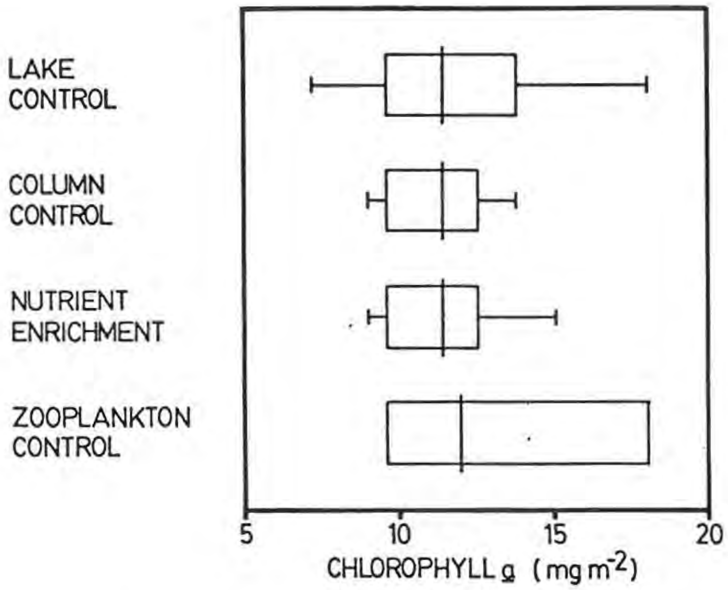

Figure 10. Modified box and whisker diagrams of the response of chlorophyll a standing stocks in control and nutrient enriched treatments (n=7 in all treatments).

Table 10. Analysis of variance table for control and nutrient enrichment treatments in column experiments conducted from 27/02/1982 to 05/03/1982.

Source of variation	Degrees of freedom df	Sums of squares SS	Mean squares MS	F ratio
Between days	6	140.0	23.3	6.6***
Between columns	3	15.3	5.1	1.4n.s
Discrepancies	18	63.8	3.5	
Total	27	219.1		

n.s = not significant
*** = p<0.001

dealt with in the discussion (page 41).

ii) Changes in chlorophyll a standing stocks

Box and whisker diagrams show that there is little difference between the medians, and the lower quartiles in each of the treatments (Fig. 10). The increased range of chlorophyll a in the lake control can be attributed to the free movement of phytoplankton in the surrounding water mass and probably accounts for the significant difference between days ($F_{(6,18)}=6.6$; $p<0.001$; Table 10). These variations can be attributed to different weather conditions which prevail during a 10 day experiment, and are expected in dynamic biological systems, but need not be of concern here as the main question is directed to the "between treatment" effects.

The analysis of variance table (Table 10) shows that there is no significant difference in the chlorophyll a standing stock between the different treatments in the experiment ($F_{(3,18)}=1.4$; $p>0.05$). A priori tests between the lake control and the column control showed that enclosure had no significant effect on algal standing stocks ($F_{(1,18)}=0.04$; $p>0.05$). The response of algal standing stocks in the column and the zooplankton controls were not significantly different ($F_{(1,18)}=3.24$; $p>0.05$) although the median chlorophyll a concentration was slightly higher and more variable in the zooplankton control (Fig 10). The co-incidence of the range and the quartiles in the zooplankton control suggest that zooplankton which remained in the water could, by exerting some grazing pressure, increase variation within the enclosed system.

The ten-fold addition of nitrate and phosphate, and the subsequent rapid assimilation of both, did not significantly increase the chlorophyll a concentration in the whole column ($F_{(1,18)}=1.85\times10^{-5}$; $p>0.05$). This gives support to the suggestion that nutrient additions are being utilized by an external, non-biological agent. The similarity of the column control and nutrient enriched treatments are easily seen from the box and whisker diagrams (Fig 10).

The effect of increased circulation

i) Daily variation in chemical concentrations

Phosphate concentrations in circulated treatments (19.2 ± 1.3 $\mu g\ \ell^{-1}$; n=27) were not significantly different to those recorded in the lake control (21.2 ± 1.1 $\mu g\ \ell^{-1}$; n=8). Nitrate concentrations were similar in both the lake and column controls but were depressed by approximately 50 $\mu g\ \ell^{-1}$ in the circulated treatments. As these results are atypical when compared

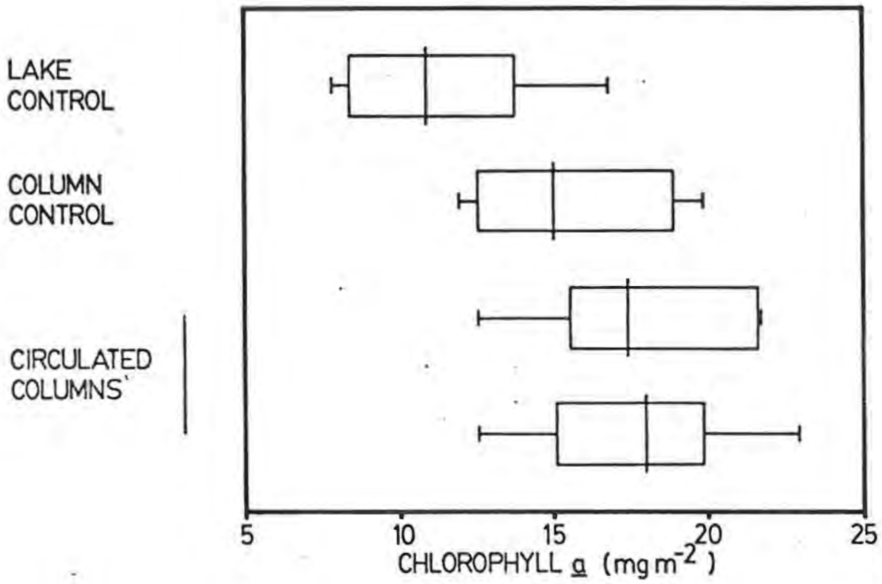

Figure 11. Modified box and whisker diagrams of the response of chlorophyll a in control and circulated treatments for the whole column (n=7 in all treatments).

Table 11. Analysis of variance table of total chlorophyll a for control and circulated treatments in column experiments conducted from 22/03/1982 to 30/03/1982.

Source of variation	Degrees of freedom df	Sums of squares SS	Mean squares MS	F ratio
Between days	6	166.8	27.8	6.1**
Between columns	3	178.9	59.6	13.1***
Discrepancies	18	81.9	4.5	
Total	27	427.6		

** = $p<0.01$
*** = $p<0.001$

with nutrient levels in circulated treatments in previous experiments, it is possible that lower initial concentrations (75 $\mu g\ \ell^{-1}$ less than ambient) were the cause of this discrepancy. In spite of this, the lower nitrate concentration (460 $\mu g\ \ell^{-1}$) is not considered to limit phytoplankton growth.

ii) Changes in total chlorophyll a standing stocks

Box and whisker diagrams (Fig 11) suggest that there was a different response in each of the three treatments, this was confirmed by analysis of variance (Table 11; $F_{(3,18)}=13.1$; $p<0.001$). The degree of overlap of the lower and upper quartiles (Fig 11), particularly between the column control and circulated treatments, suggests that there was little difference between enclosed treatments in spite of the higher median chlorophyll a standing stock in circulated columns.

A priori tests between the lake control and the enclosed treatments showed that the response was significantly different ($F_{(1,18)}=34.2$; $p<0.001$). This contrasts with results from the previous section (Table 10) where there was no difference between the lake control and other treatments. A possible cause of this difference was that two different weather conditions prevailed during each experiment. In this experiment (22/03/1982 to

30/03/1982) a period of calm weather allowed chlorophyll a standing stocks to increase in surface waters, while evening winds effectively distributed the algae at the surface, thereby diluting chlorophyll a concentrations in the lake control but not in columns. This is further demonstrated by the small significant difference in the chlorophyll a integral between column control and circulated treatments ($F_{(1,18)}=5.6$; $0.025<p<0.05$). In previous experiments (27/02/1982 to 05/03/1982) windy conditions prevailed and prevented elevation of surface chlorophyll a concentrations.

As it is possible that the integral of chlorophyll a for the whole column was influenced by this build-up in surface chlorophyll a, it was necessary to break down analyses into two components.

a) The standing stock of chlorophyll a in the upper 1.5 metres (Surface chlorophyll a).

b) The standing stock of chlorophyll a in the remainder of the column (Bottom chlorophyll a).

This division was undertaken to increase the sensitivity of analyses.

a) Changes in surface chlorophyll a

Standing stocks of surface chlorophyll a in different treatments were not significantly different from each other ($F_{(3,18)}=2.1$; $p>0.05$; Table 12). *A priori* tests

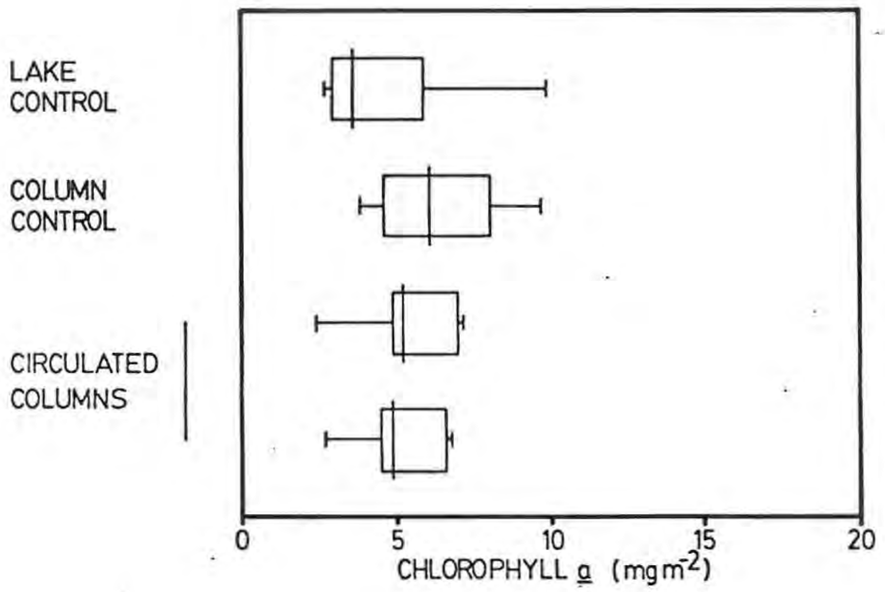

Figure 12. Modified box and whisker diagrams of the response of surface chlorophyll a standing stocks in control and circulated treatments for the upper 1.5 metres (n=7 in all treatments).

Table 12. Analysis of variance table for control and circulated columns to show the effect of each treatment on surface chlorophyll a standing stocks.

Source of variation	Degrees of freedom df	Sums of squares SS	Mean squares MS	F ratio
Between days	6	59.9	10.0	5.3^{**}
Between columns	3	11.8	3.9	$2.1^{n.s}$
Discrepancies	18	33.6	1.9	
Total	27	105.4		

n.s = not significant
** = $p<0.01$

between the lake and column control treatments showed that the column control had a significantly higher chlorophyll a standing stock ($F_{(1,18)}=5.3$; $0.025<p<0.05$ and Fig 12). However, when the response of chlorophyll a in circulated treatments is compared with that of the control columns, there was a small decrease in the chlorophyll a standing stock in circulated treatments which was just significant ($F_{(1,18)}=4.3$; $p=0.05$).

In the lake and column controls the distribution of the measured chlorophyll a standing stocks are skewed towards the higher values (Fig 12). Circulated treatments showed the opposite trend, and chlorophyll a standing stocks were skewed towards the lower end of the range (Fig 12). The increased variation in the system, being divided between two different sets of treatments, has decreased the sensitivity of the analysis, but the downward extension of the range in circulated treatments is important, the significance of which will be dealt with in the discussion (page 42).

b) Changes in bottom chlorophyll a

The marked difference between circulated and control treatments is evident from Figure 13. Analysis of variance (Table 13) shows a highly significant difference between treatments ($F_{(3,18)}=20.2$; $p<0.001$). A

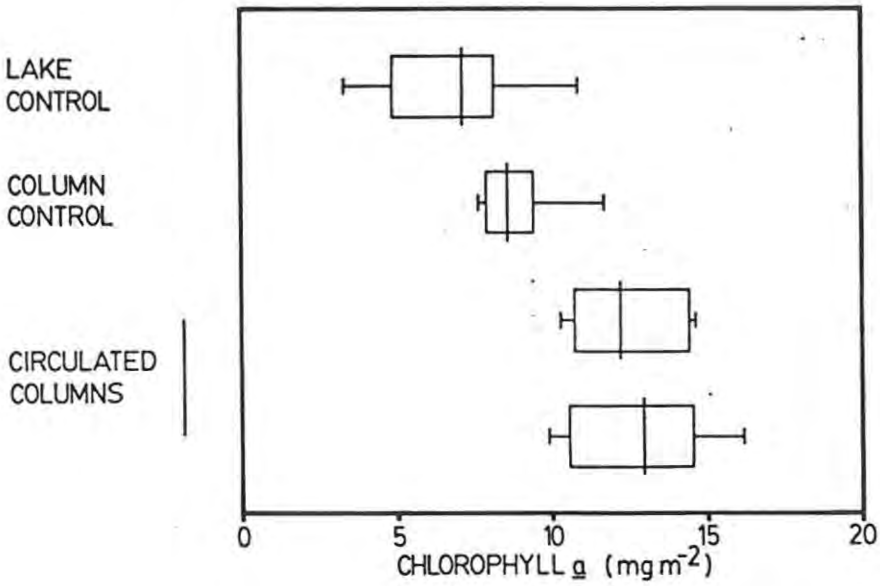

Figure 13. Modified box and whisker diagrams of the response of bottom chlorophyll a standing stocks in control and circulated treatments for the bottom 3.5 metres of water(n=7 in all treatments).

Table 13. Analysis of variance table for control and circulated columns to show the effect treatments on bottom chlorophyll a standing stocks.

Source of variation	Degrees of freedom df	Sums of squares SS	Mean squares MS	F ratio
Between days	6	47.6	7.9	2.8*
Between columns	3	173.1	57.7	20.2***
Discrepancies	18	51.3	2.8	
Total	27	271.9		

* = $p<0.05$
*** = $p<0.001$

priori tests showed a small significant difference in the chlorophyll a standing stocks of the lake and column controls ($F_{(1,18)}=5.8$; $0.025<p<0.05$). More important however is the highly significant difference between the chlorophyll a standing stock in column control and circulated treatments ($F_{(1,18)}=22.2$; $p<0.001$).

The marked elevation of chlorophyll a in circulated treatments shows the true effect of increased mixing, and therefore increased light dosage, on the algal population is thus demonstrated: algal cells from the whole column are able to participate more actively in photosynthesis because algal cells from the bottom waters of columns are being brought to the surface, and receive a light dose, the effect of which allows photosynthesis to continue in spite of algae moving out of the light zone. This has the effect that chlorophyll a concentrations are uniform with depth, therefore allowing the chlorophyll a standing stock to increase.

Discussion

The positive and negative effects of the use of enclosures have been widely discussed (Goldman, 1962; Schindler et al., 1971; Walmsley, 1976; Twinch and Breen, 1978a). However, in the light of the circumstances mentioned in the introduction, the use of columns is probably the most suitable in situ method to accurately determine the factor which limits phytoplankton in the P.K. le Roux impoundment. Experiments were of sufficiently short duration to overcome problems associated with periphyton build-up encountered by Walmsley (1976) and Twinch and Breen (1978a,b; 1980a). Similarly no significant warming or increase in transparency was observed during experiments in spite of this being a problem in similar studies (Goldman, 1962; Twinch and Breen, 1980a).

The inclusion of both a lake and a column control treatment, as suggested by Goldman (1962), has allowed calibration for different treatments, despite observed differences between the first control experiments (Fig 10) and the second set (Fig 11) being large. While there is little doubt that longer term experiments, such as those of Walmsley (1976) and Twinch and Breen (1978a,b; 1980a,b) buffer weather effects, the negative effects of the short duration of experiments in this work are more than compensated for by the use of replication.

Nitrate and phosphate enrichment have yielded results which are different to findings of other workers, particularly as nitrate has been considered a limiting factor in the Orange River (Toerien et al., 1975). The rapid return of both nutrients to ambient concentrations after enrichment, and the equivalence in uptake rate, would suggest that both nutrients are in high demand. The question is, "Where are they utilized?"

Similar adsorption of phosphate has been reported by Walmsley (1976) in Midmar dam in experiments over a longer time period (20 days), while Twinch and Breen (1978b) also noted the rapid return of water in columns to ambient phosphate levels after enrichment. Similar uptake of phosphorus has been reported elsewhere (Weatherley and Nicholls, 1955; Smith, 1969; Schindler et al., 1971). In many cases the sediments of lakes have been implicated in this process (Mortimer, 1941; Holden, 1961; Kamp-Nielsen, 1974; Twinch and Breen, 1980b). However in this study water in columns was isolated from the sediments. This suggests that the suspended silt acts in the same manner as the bottom sediments and can act as a sink for both nitrate and phosphate.

It is therefore no longer necessary to talk about nutrient limitation in a turbid system such as the P.K. le Roux impoundment. Nutrients are probably available in ample supply from the silt, and will be released during periods of high demand. Similarly, nutrients would be adsorbed when concentrations are high. This explains the consistency of seasonal phosphate concentrations during the 90 station surveys discussed in the Part I.

On the basis of the small significant difference between the chlorophyll a standing stock in the control columns and the circulated treatments in total (Fig 11) and surface chlorophyll a (Fig 12) it would probably have been justifiable to conclude that increased circulation had no effect on algal standing stock. However the small decrease in surface standing stock of chlorophyll a in circulated treatments (Fig 12) is important. This decrease has major physiological implications and could mean that an inhibitory factor (light) has been removed. The decrease in chlorophyll a concentration suggests that the algae have changed from a shade to light-adapted phase of growth in response to increased light dosage brought about by increased circulation. The downward extension of the range of chlorophyll a standing stocks in the circulated treatments (Fig 12) adds credibility to this statement especially when comparision is made with the results from non-circulated columns (Fig 10 - 12) where the tendency was for chlorophyll a concentrations to increase.

This decrease in chlorophyll a is associated with a physiological adaptation of phytoplankton to reduce the risk of light inhibition (Falkowski and Owens, 1980). Similar declines in chlorophyll a with increased light dosage (and flux) have been reported by Kok (1956) and Brown and Richardson (1968). The changes which took place in circulated treatments infer that the light climate within columns has become more favourable for phytoplankton growth.

Finenko (1978) has stated that "an increase in algae in the entire water-mixing layer is only possible when the production of organic matter in the euphotic zone quantitatively exceeds respiration of algae present in the entire water column subject to mixing". By considering only the bottom chlorophyll a in circulated treatments it can be seen that Finenko's (1978) criteria have been fulfilled, but this is not the case in the control columns. In circulated treatments increased surface production has occurred in the euphotic zone with sufficient frequency to reduce the respiration effects of the "dark" water. The absence of a similar increase in chlorophyll a in both control systems means that the "dark" period is so large that it overrides all benefits gained by algal cells during short, and infrequent, light exposure in uncirculated treatments.

In the open water the magnitude of the mixing pattern can be so large that algal cells may only infrequently receive a light dose. This would have the effect that algae which cannot remain in the euphotic zone undergo little change in chlorophyll a and growth, while algal cells which remain in the surface waters are subjected to a favorable light dosage and grow rapidly. Still, calm days mean that algae show large increases in standing stock, while on windy days chlorophyll a concentrations are diluted as cells are mixed into the water column. Under such conditions it is not suprising that motile Chlorophyta (Volvox sp, Pandorina sp) and bouyant Cyanophyta (M. aeruginosa, Anabaena spp) dominate the phytoplankton commumity during summer.

The manner in which the shallow euphotic zone, the depth of the thermocline and the mixing pattern of the reservoir limit algal growth has been demonstrated by a series of preliminary algal bioassay experiments. Algae collected from epilimnetic water (7.5 m) showed a rapid response to incubation at 80 $\mu E\ m^{-2}\ s^{-1}$ (13:$\overline{11}$ hour light cycle). However algae collected in the hypolimnion (30 m) showed no response to increased light until 96 hours after collection (Selkirk, unpublished data). This series of experiments serves to demonstrate that unless algal cells can periodically receive a light dose, they will die. Therefore, under natural conditions, phytoplankton are not subject to any major nutrient limitation but are severely limited by light limitation imposed by the effects of turbidity.

Concluding remarks

In this study, both the survey and the field experiments have shown that algal growth in the reservoir is light limited by the high suspensoid concentrations. The shallow depth of the euphotic zone (<1 m), coupled with the depth of the seasonal thermocline (25 m) imposes this light limitation because the rate of wind-driven circulation is slow. As a result algal cells are infrequently in the euphotic zone to receive a light dose and the negative effects of respiration dominate.

Light limitation may, however, not be a negative effect in view of the suggested ion exchange properties of the suspended silt. The silt is materially involved in the maintainance of the nitrate and phosphate equilibria such that the N/P ratio of 15:1 is maintained in spite of external loading and algal growth. This offers, in part, an explanation for Walmsley and Bruwer's (1980) suggestion that turbid impoundments should be able to tolerate increased phosphate loading without increased algal growth. This finding is of economic importance, particularly as the Orange River impoundments account for some 45 % of South Africa's impounded water.

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