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Nutrient Limitation of Phytoplankton in the Upper Swan River Estuary, Western Australia

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Abstract. During 1993–94 the phytoplankton community in the upper Swan River estuary had a peak chlorophyll *a* concentration of 57 mg m^{-3} during early summer (December 1993) and a second peak of 35 mg m^{-3} during late autumn (May 1994). Mid summer was characterized by low cell densities and low chlorophyll *a* concentrations. The potential of the phytoplankton community for nutrient limitation was assessed with dilution bioassays given nutrient mixes deficient in one of the following: nitrogen, phosphate, silicate, iron, trace metals, chelators, or vitamins. During the mid-summer period of low phytoplankton abundance, nitrogen was the nutrient with the greatest potential to limit algal biomass. During mid summer, ambient N:P ratios tended to be near unity and bioassays indicated that the available pool of N was up to 20 times more limiting to biomass development than was available P. Also during mid summer, bioassay treatments given no nitrogen and control treatments given no nutrients showed little growth, reaching chlorophyll *a* concentrations ~1/30th of those given a full suite of nutrients. Chlorophyll *a* concentrations in the bioassay control treatments given no nutrients were correlated ($r^2 = 0{\cdot}74$) with measured surface nitrate concentrations; this suggested that nitrate inputs may be a major factor controlling phytoplankton biomass in this ecosystem. The correlation between surface nitrate concentration and rainfall ($r^2 = 0{\cdot}69$) further suggests that rainfall may be the most important mechanism supplying nitrate to the surface waters of this estuary.

Introduction

The Swan River is one of the major riverine systems in western Australia. It is approximately 50 km long and drains a catchment area of approximately 190000 km^2. The Swan River catchment area contains substantial sheep and cattle farming, wheat cultivation and a majority of the >1 million inhabitants of the city of Perth. The catchment area is becoming increasingly urbanized and in 1994 there was the first recorded toxic cyanobacterial bloom in a major Swan River tributary (Hosja, unpublished observation).

A number of factors influence phytoplankton growth rate, biomass and species composition. In turbid, nutrient-rich estuaries phytoplankton growth rate may be controlled by the availability of light (Cole and Cloern 1984) and temperature. Nutrient availability is often invoked as a mechanism to limit phytoplankton standing stock. The effects of low nutrient availability, however, remain conten-tious. Evidence, primarily from oligotrophic ecosystems in which phytoplankton growth and nutrient recycling are highly coupled, suggests that cells can be near their maximum growth rates and therefore not nutrient-limited even at very low ambient concentrations (Goldman *et al.* 1979; McCarthy and Goldman 1979; Harris 1986). However, bloom events clearly demonstrate the capacity for phytoplankton growth to become uncoupled from nutrient regeneration, thus increasing the likelihood that nutrient limitation will occur. Nutrient limitation is hypothesized to be highly variable in time and space (Sterner 1994), possibly acting as an intermediate disturbance (Jacobsen and Simonsen 1993) and thus influencing phytoplankton species succession, yet very few studies have assessed nutrient limitation on short time-scales (Elser *et al.* 1990).

Several techniques are available for measuring nutrient limitation of the phytoplankton community (Laws 1993). We used bioassays of the pre-existing endemic phytoplankton community after they were diluted and a selection of nutrients added. Bioassay control treatments, which were diluted but received no additional nutrients, measured the potential for phytoplankton growth by using either the existing external nutrient pool or the existing internal nutrient pool. For macronutrients where the capacity for internal storage is small, e.g. carbon, nitrogen, and sometimes phosphorus, the ability of cells to grow by using internal reserves is limited and the external nutrient pool is usually the most important nutrient source. Conversely, the internal nutrient pool may provide sufficient micronutrients to allow many divisions of an algal cell and its descendants. Therefore, conventional bioassays may fail to detect nutrient limitation if the external concentrations of a micronutrient have become limiting while internal reserves are still high. Dilution bioassays also provide a superior tool relative to perturbation bioassays because they permit the assessment of growth at ambient nutrient concentrations (Sterner 1994). Finally, bioassays using the multiple 'all but one' nutrient-addition technique can unambiguously determine the nutrient with the greatest potential to limit biomass (Laws 1993).

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Management efforts to control algal blooms in the Swan River are largely focused on reducing nutrient inputs from the catchment. In order to assist this management effort we attempted to define the extent to which phytoplankton in the Swan River were nutrient-limited. Specifically we assessed whether nutrient limitation was likely to occur and which nutrient was most likely to limit biomass. We tested for potential limitation by nitrogen, phosphate, silicate, trace metals, chelators and vitamins.

Materials and Methods

Site Description

The primary study site was adjacent to Ron Courtney Island, 32 km upstream along the main river channel from Fremantle Harbour (31°55·3′S,115°56·3′W) (Fig. 1). During summer, marine waters intrude up the river past the study site. The mean vertical tidal range is only 0.4 m at Fremantle, and horizontal water movement is further restricted by a number of sills (Spencer 1956). Anoxia and high nutrient concentrations frequently occur in the bottom waters during summer (Jack 1987). The primary study site was selected so that it would be subject to the seasonal change in salinity and be in an area where summer phytoplankton blooms had previously occurred.

Collection of Chemical, Physical and Biological Data

Water samples for chemical analysis were centrifuged at ~20000 *g* for 1 h and the supernatant was then removed for analysis by standard methods (Anon. 1989). Temperature, salinity, dissolved oxygen, pH, and turbidity were measured at 0.5-m intervals in the water column with a Model 3800 Yellow Springs instrument. Samples for chlorophyll *a* (chl*a*) were also collected at 0.5-m intervals, filtered through GF/C (Whatman) filters, and analysed by spectrophotometry in 1992–93 (Anon. 1989) or by high-performance liquid chromatography (HPLC) in 1993–94 (modified from Wright *et al.* 1991). The overall difference between the techniques used for measuring chl*a* is expected to be small (Sartory 1985). Light penetration into the water column was measured as photosynthetically active radiation (PAR, ~400–700 nm) with a Licor 185B meter (4π collector) and a 30-cm-diameter Secchi disk. Rainfall was measured nearby at the Perth Airport and incoming PAR (2π collector) at the Marmion Marine Laboratory some 30 km north-west of the sampling site. Data were collected from October 1992 to September 1994, but the suite of parameters measured and the frequency of measurements varied.

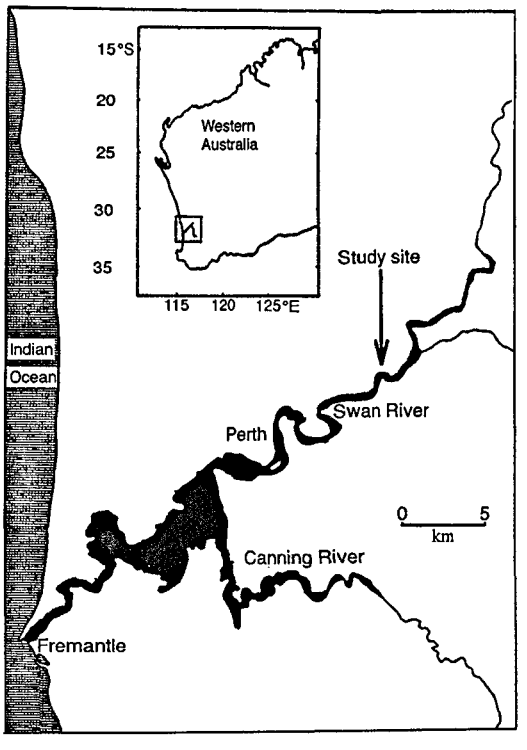

Fig. 1. Location of study site on the Swan River, adjacent to Ron Courtney Island (arrow).

Bioassays

Bioassays were conducted on 25 occasions between October 1993 and October 1994. Treatment cultures and controls were incubated (in triplicate) at temperatures of 17 ± 1°C (winter) and 23 ± 1°C (summer) at irradiances of 200 μmol photons m^{-2} s^{-1} ('cool white' fluorescence bulbs) on a day:night cycle adjusted to track the ambient photoperiod. In Perth this ranged from 14:10 L:D (summer) to 12:12 L:D (winter). Cultures were grown in 50-mL borosilicate test-tubes with Teflon-lined caps. Treatment cultures were given nutrients (Table 1) according to the recipe of Harrison *et al.* (1980). Final concentrations of the major nutrients added to the treatment cultures were considered high enough to saturate initial growth rates. Trace metals, a chelator and vitamins were also added to achieve final concentrations considered to be near the ideal chelator:metal ratio and saturating (Harrison *et al.* 1980). Bioassays were conducted by the deletion of a single nutrient type from the full complement (Ryther and Dunstan 1971). For example, to test for nitrogen limitation, all nutrients except NO_3^- would be added to the bioassay medium. If this medium produced good growth and high biomass, then nitrogen was not considered limiting to algal growth or biomass in the river on the day the sample was taken. Algae were inoculated into the bioassay cultures by the addition of 1 mL of unfiltered Swan River water to 40 mL of 0·45-μm-filtered Swan River water. At intervals of approximately 24 h (occasionally 48 h), the fluorescence *in vivo* of each culture was measured by inserting the whole

Table 1. Nutrients and approximate initial concentrations in bioassay experiments

Nutrient	Compound	Concentration (μM)
Nitrogen	$NaNO_3$	549
Phosphate	$Na_2HPO_4 \cdot 7H_2O$	21·8
Silicate	$Na_2SiO_3 \cdot 9H_2O$	105·6
Iron	$FeCl_3 \cdot 6H_2O$	6·56
& chelator	$(CH_2N(CH_2COOH)CH_2COONa)_2 \cdot 2H_2O$	6·56
Trace metals	$MnSO_4 \cdot H_2O$	2·42
	$CoSO_4 \cdot 7H_2O$	0·0569
	$ZnSO_4 \cdot 7H_2O$	0·254
	$NaMoO_4 \cdot 2H_2O$	0·520
	$NiCl_2 \cdot 6H_2O$	0·050
	$NaSeO_3$	0·010
& chelator	$(CH_2N(CH_2COOH)CH_2COONa)_2 \cdot 2H_2O$	8·30
Vitamins	B_1 (thiamine HCl)	0·297
	B_{12} (cyanocobalamin)	0·0015
	B_6 (biotin)	0·0041

test-tube into a Turner Designs Model 10 fluorometer. The fluorometer was calibrated to give ~1 unit of fluorescence *in vivo* per 1 μg chl*a* L^{-1}. When all or most of the cultures showed signs of senescence (end of exponential growth), 5-mL subsamples were preserved with Lugol's solution for later phytoplankton identification and other 5-mL subsamples were filtered through a GF/C filter for the fluorometric determination *in vitro* of chl*a* (Parsons *et al.* 1984). The potential errors in estimating chl*a* from mixtures of primarily chl*a* and chlorophyll *c* with spectrophotometric, fluorometric *in vitro*, and fluorometric *in vivo* techniques are expected to be up to 1%, 6% and 50% respectively (Lorenzen and Jeffrey 1980).

Where necessary, data were transformed to achieve normality and homoscedasticity prior to statistical analyses.

Results

General Results: Physical, Chemical and Biological

At the study site (Fig. 1), the 6-m-deep water column was isohaline in July during mid winter (Figs 2*a* and 2*b*). Periods of low salinity during winter coincide with periods of high rainfall (Fig. 2*a*) and high river flow (Fig. 2*c*). About mid October 1993, the top and bottom salinity measurements diverged, indicative of salinity-driven stratification (Figs 2*a* and 2*b*). During the next three months the site became increasingly saline (Fig. 2*a*). On the basis of measurements made during 1979–82 just north of the mouth of the Swan River (Johannes *et al.* 1994), the source water for the intruding salt wedge could be expected to have a salinity of ~36, low nitrate concentrations (~0.5 μM NO_3^-), and relatively high PO_4^{3-} concentrations (~1 μM). By the end of March (late summer), surface salinities increased to ~27 and bottom salinities to more than 30. Between September 1993 and the end of June 1994, salinity stratification was maintained except for a brief period in March 1994 (Fig. 2*b*).

Water temperature was lowest (~12°C) during mid winter, rising nearly 15°C to mid summer highs exceeding 26°C (Fig. 2*d*). Surface and bottom temperatures were always similar, indicating little or no effect of temperature on stratification.

The average daily insolation increased about 240% between mid winter and mid summer because of increases in both daylength and daytime maximum irradiance (Fig. 3*a*). Light attenuation in the water column decreased substantially between August 1993 and March 1994 (Fig. 3*b*). On the basis of the combination of increasing insolation and decreasing water-column light attenuation, we calculate an increase in irradiance at 1 m from 1·1 to 9·6 mol photons m^{-2} day^{-1}, or an 860% increase between August 1993 and January 1994.

In the surface waters, nitrate and ammonium concentrations were quite variable (Fig. 4*a*). During most of the summer period, concentrations of both nitrate and

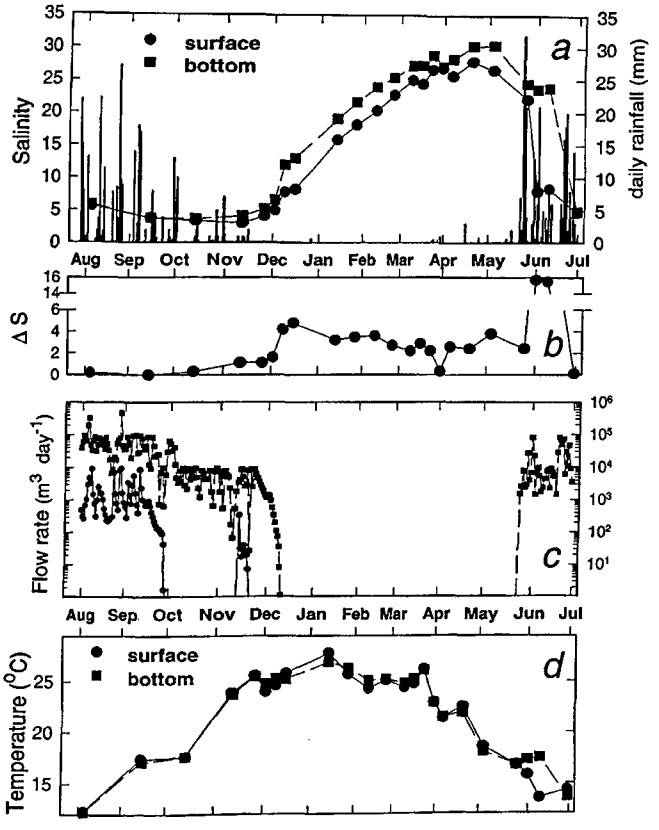

Fig. 2. Physical and biological parameters measured at Ron Courtney Island between August 1993 and July 1994: *(a)* salinity measured at surface and bottom, and rainfall at Perth Airport; *(b)* difference between surface and bottom salinities (*S*); *(c)* flow rates for Swan River tributaries (●) Mooranoppin Creek and (■) Ellen Brook; (*d*) temperature at surface and bottom.

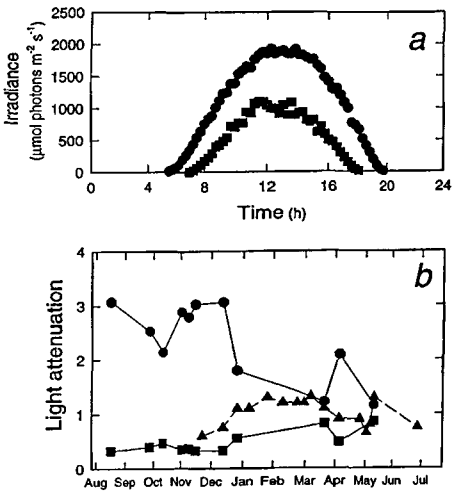

Fig. 3. *(a)* Daily irradiance measured on roof of Marmion Laboratory (squares are average values for August 1993, circles are average values for January 1994); *(b)* measures of light penetration between August 1993 and July 1994: (▲) Secchi disk depth, (●) *k* (value of negative slope from the regression of ln irradiance versus depth), and (■) k^{-1}.

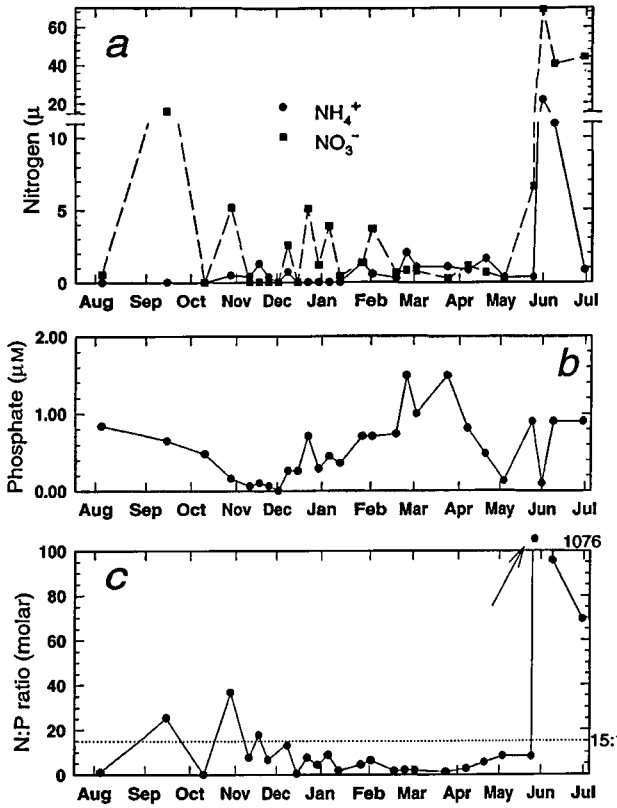

Fig. 4. Surface nutrient concentrations at Ron Courtney Island between August 1993 and July 1994: *(a)* nitrate and ammonium; *(b)* phosphate; *(c)* DIN:P ratio (molar) (dotted line is 15:1 ratio).

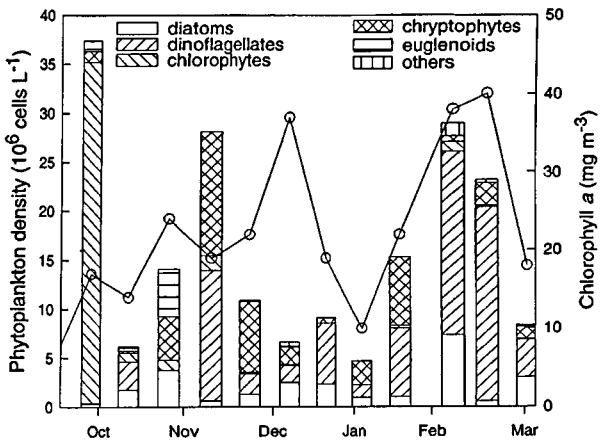

Fig. 5. Changes in the phytoplankton community composition for the summer of 1992–93, and (○) chlorophyll *a* concentrations for surface samples from Ron Courtney Island.

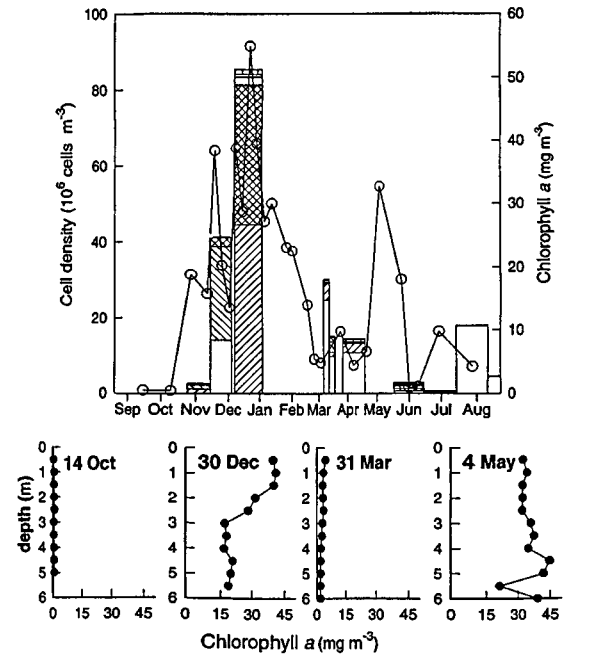

Fig. 6. Phytoplankton community composition between September 1993 and August 1994. Legend for phytoplankton is the same as in Fig. 5. Chlorophyll *a* biomass is plotted versus month in upper panel and versus depth in lower panels.

ammonium were low. Nitrate was often the dominant form of nitrogen during the sporadic increases in surface dissolved inorganic nitrogen (DIN). Concentrations of nitrate were always greater than NH_4^+ during these high [DIN] periods. With the onset of autumn rains, surface nitrate concentration rose to ~70 μM. Throughout the annual cycle, phosphate concentrations were variable, ranging from the detection limit to 1·6 μM (Fig. 4*b*). High phosphate concentrations were measured in the surface waters throughout most of the summer period. At this time the molar DIN:P ratio (i.e. $[NO_3^- + NH_4^+]:[PO_4^{3-}]$) was approximately unity (Fig. 4*c*). The molar DIN:P ratio stayed well below 15:1 from the end of December 1993 to the end of May 1994 (Fig. 4*c*).

Phytoplankton dynamics in 1992–93 were characterized by a chlorophyte bloom early in the spring (Fig. 5). Following the chlorophyte bloom in 1992, a mixed population of cryptophytes and dinoflagellates peaked during December. Throughout the rest of the summer, phytoplankton biomass was relatively low until an autumn bloom dominated by dinoflagellates. An early spring chlorophyte bloom was not detected in 1993, but otherwise the seasonal biomass cycles in 1992–93 and 1993–94 were similar (Figs 5 and 6). The early summer bloom of 1993–94 was a mixture of cryptophytes and dinoflagellates (Fig. 6), with standing biomass reaching more than 50 μg chl*a* L^{-1} near the end of December. The net increase in biomass from October 1993 to December 1994 was equivalent to a net growth rate of 0·049 day^{-1}, or a doubling time of about 14

days. Following the December 1993 bloom, the 1993–94 summer phytoplankton community was dominated by diatoms. During both 1992–93 and 1993–94, there were large late summer or autumn blooms. Particularly during 1992–93, the periods of high chl*a* standing stock were often dominated by motile, flagellated phytoplankton (phytoflagellates).

The vertical distribution of chl*a* was homogeneous during the October 1993 and March 1994 periods of low biomass (Fig. 6). The late summer bloom during May 1994 was also distributed evenly throughout the water column, whereas the December 1993 bloom had a large near-surface biomass peak.

Bioassays

The rate of biomass change and the point in time when maximum biomass had been obtained (when cultures reached stationary phase) were determined from measures of fluorescence *in vivo*. For example, the water sample collected on 10 March 1994 was diluted 40:1 to an initial fluorescence of 1 (Fig. 7*a*). During five days of incubation the biomass fluorescence increased to over 100 (Fig. 7*a*) in those treatments given all nutrients (ALL; see Table 1) or all nutrients except phosphate ($-PO_4$). Those samples given no nutrients (CONTROL2) or all nutrients except nitrogen ($-NO_3^-$) increased their biomass fluorescence to only ~10 over the same period. In the example shown, maximum biomass was reached and the cultures were filtered after a five-day incubation, and the chl*a* concentrations were determined from acetone-extracted samples. Cultures given all nutrients (ALL) produced about 100 μg chl*a* L^{-1} (Fig. 7*b*, data for all 11 treatments). This is similar to those treatments given all nutrients except one of the following: phosphate ($-PO_4$), silicate (–Si), iron (–Fe), trace metals and chelators (–TM), vitamins (–VITS). Hence, none of these nutrients could be considered to limit the potential phytoplankton biomass at this sampling time. Rather, the lack of N resulted in a low biomass identical to that of the control given no nutrients.

Water from near the bottom of the water column was also bioassayed because these waters were often high in nutrients relative to the surface. On 10 March 1994, the bottom water controls developed about twice as much biomass as did surface waters (Fig. 7*b*). Over an annual cycle, the bottom waters bioassayed produced an average of 2·9 times more chl*a* biomass than did the surface waters. The bioassays indicated that the potential for the bottom waters to produce more chl*a* biomass was most extreme during December 1993, when they produced 7·7 ± 6·6 (mean ± 1 s.d., $n = 5$) times more than did the surface waters.

In all bioassays, those treatments given no NO_3^- or, less frequently, those given no PO_4^{3-} most resembled controls given no nutrients. For this reason and because catchment management strategies are focused on limiting N and P inputs, we primarily considered the severity and frequency of N and P limitation. To compare the degree of N versus P limitation, we calculated the ratio of maximum biomass from cultures without added P relative to those without added N. During summer the samples incubated without P produced up to 20 times more biomass than did those incubated without N (Fig. 7*c*). Over the entire annual cycle, N limitation was an average of 4·4 times more severe than was P limitation. Over the 1993–94 annual cycle, 25 bioassays were conducted; 20 showed some degree of N limitation and five a low degree of P limitation. The phytoplankton community was generally N-limited throughout the summer and did not shift from potential N limitation to P limitation until after the autumn–winter rains arrived.

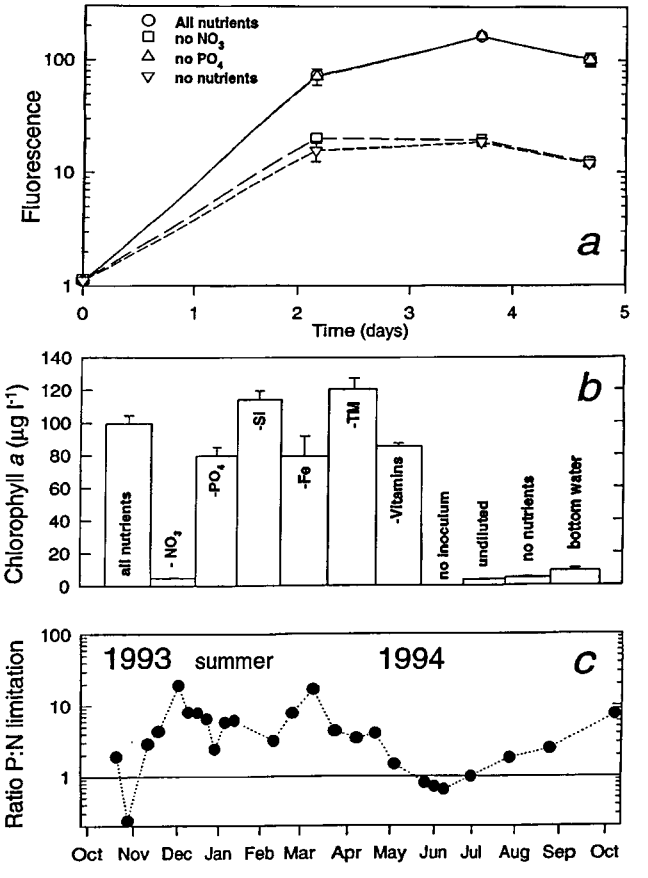

Fig. 7. Bioassay results: *(a)* representative changes in fluorescence in a bioassay sample versus time (error bars are ±1 s.d.); *(b)* chlorophyll *a* biomass of bioassay subsamples from example in panel *(a)* but for all treatments (error bars are +1 s.d.); *(c)* relative amount of N or P limitation (values are ratios of chl*a* biomass in treatments given no P to chl*a* biomass in treatments given no N (–P:–N); see Materials and Methods).

The overall degree of nutrient limitation was measured by comparing the chl*a* biomass in those bioassay treatments with the lowest biomass when exponential growth ceased (always either $-NO_3^-$ or $-PO_4$ treatments) with the chl*a* biomass in treatments with all (ALL) nutrients (Fig. 8*a*). This comparison indicated that lack of N or P constrained potential biomass by a factor of up to 45 times. A period of high nutrient limitation lasted more than 200 days, extending from 20 October 1993 to 25 May 1994. Some variability in the degree of limitation within this period (Fig. 8*a*) was probably due to variation in species composition, degree of senescence, temperature, irradiance, and minor nutrient inputs. Over the annual cycle the average biomass in all bioassay treatments given all nutrients was 228 μg chl*a* L^{-1} (Fig. 8*b*), but this was divided into two distinct sections. During summer the fully enriched bioassay cultures produced less than 230 μg chl*a* L^{-1} but after May 1994 reached 630 μg chl*a* L^{-1} (Fig. 8*b*). The bioassay cultures given no nutrients or no N showed the same pattern. During the summer period of greater nutrient stress, maximum biomass in the controls given no nutrients averaged 14 μg chl*a* L^{-1} (Fig. 8*b*), but this increased to more than 200 μg chl*a* L^{-1} after May 1994. Thus, during June–August 1994 there seems little potential for nutrients to have limited phytoplankton biomass in the field, because nutrient concentrations were high and ambient chl*a* concentrations never approached 200 μg L^{-1}. Over the entire annual cycle, biomass in the control cultures given no nutrients was correlated (r^2 = 0·74) with surface nitrate concentrations measured at the time of sample collection (Fig. 8*c*). The factor limiting the maximum biomass achieved in bioassay treatments given all nutrients was not determined. Microscopic examination of a few bioassay cultures at the cessation of exponential growth indicated that they were dominated by diatoms, particularly *Skeletonema costatum*.

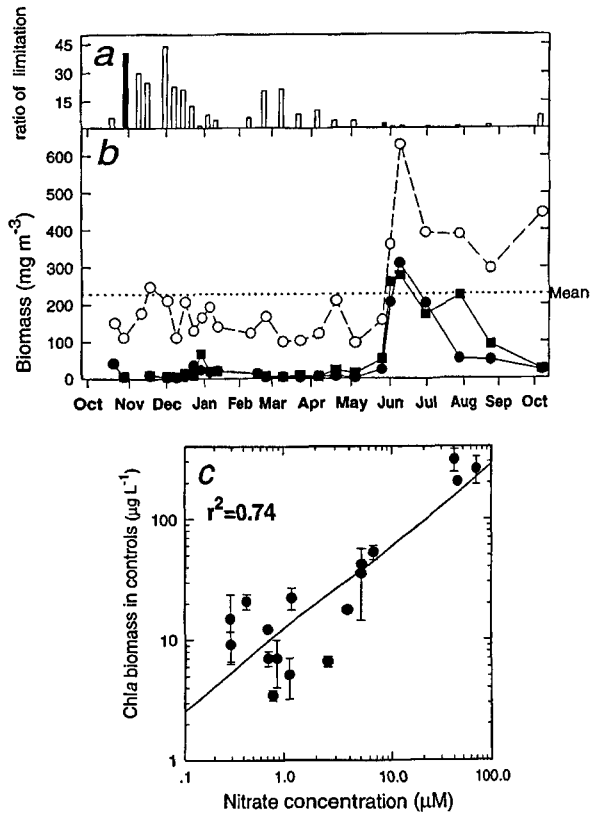

Fig. 8. Bioassay results: *(a)* ratio of chl*a* biomass in treatments given all nutrients to that in treatments given either (solid bar) no P or (open bar) no N; *(b)* chl*a* biomass in treatments given (○) all nutrients, (●) no nutrients or (■) no N; *(c)* log–log relationship between chl*a* biomass in bioassay control cultures given no nutrients and the concentration of NO_3^- at the time of sampling.

In most bioassay trials, treatment effects on growth rates measured during exponential phase were negligible. Only two exceptions were noted, on 21 and 28 October 1993, when the bioassay treatments given no PO_4^{3-} grew more slowly (*t*-test, $P \leq 0.01$) at only 0.16 doublings day^{-1} compared with ~0.52 doublings day^{-1} for the treatments given no NO_3^- and other treatments (except controls, which were similar to $-PO_4^{3-}$ treatments).

Discussion

The lower reaches of the Swan River, like most estuaries, exhibit significant temporal variation in salinity (Hodgkin 1987). In contrast to other estuaries that may have semi-diurnal, diurnal or fortnightly periodicities in salinity variation, the Swan River cycle is annual because of the low tidal amplitude and the strongly seasonal rainfall in south-western Australia. The absence of detectable freshwater flow during summer creates a situation where marine waters intrude well upstream. At the present study site, the seasonal ranges in temperature and salinity were 12°C and 26 during 1993–94. The present data indicate that the water column was salinity-stratified during summer, with slightly fresher water at the surface. The deeper waters of the Swan River estuary are frequently anoxic (Hodgkin 1987) and often have high concentrations of NH_4^+ and PO_4^{3-} (Jack 1987).

During 1993–94 at 1 m, irradiance varied 860% seasonally. The combination of increased insolation with increasing water clarity during spring would allow the compensation depth for net photosynthesis by phyto-plankton to increase markedly. The onset of a significant increase in phytoplankton biomass at the study site coincided with increased stability of the water column (salinity stratification), with increased water-column temperatures and increased irradiance. This situation appears analogous to that observed in most temperate waters, where these factors are often associated with a spring bloom (Parsons and Takahashi 1973).

The net growth rate of 0·049 day^{-1} calculated from the temporal changes in chl*a* biomass measured at the Ron Courtney Island site during the build-up to the early summer bloom in December 1993 was 3% of the growth rates measured in the bioassay experiments. This difference may reflect the low average irradiance received by phytoplankton *in situ* relative to that in the bioassays, but it may also indicate high rates of loss *in situ* due to grazing, sinking or advection.

Concentrations of dissolved inorganic nitrogen at the surface were frequently very low during summer (this study), quite similar to those in the nearshore marine waters from which they are mostly derived (Johannes *et al.* 1994). Concentrations of PO_4^{3-} were relatively high during mid summer (this study), also similar to those of the nearshore marine waters (Johannes *et al.* 1994), with N:P ratios approaching unity. The build up of PO_4^{3-} and the low N:P ratios in the surface waters during mid summer in the Swan River could have several possible causes: *(1)* the replacement of riverine water with marine waters of low N:P ratios, *(2)* a high rate of N loss from the system (preferential N burial or denitrification), *(3)* the relatively more rapid recycling of PO_4^{3-} into the surface waters, and *(4)* other nutrient sources with low N:P ratios. There are insufficient data to select the cause of the low N:P ratios. However, from mid December 1993 to April 1994, phosphate concentrations rose steadily, nitrogen concentrations remained low, and chlorophyll *a* concentrations fell. Therefore, between December 1993 and April 1994 phosphate resupply to the upper portion of the water column appeared to exceed demand and was uncoupled from nitrogen resupply.

It is difficult to measure nutrient limitation. Bioassays are imperfect tools that cannot completely replicate the *in situ* light and temperature fields; they restrict processes that recycle nutrients and frequently contaminate samples with trace metals. We therefore interpret our bioassay results conservatively and generally consider only the largest differences and most consistent patterns. The results indicate a protracted period of low concentrations of DIN, low N:P ratios, and bioassays showing N limitation of biomass. These results are quite consistent with the model of Harris (1986), in which nutrients regulate biomass even when growth rates are near maximal. In the present bioassay experiments, in which the lack of nitrogen frequently limited biomass, there was no obvious case of variation in growth rate associated with low DIN concentrations. Growth rates were similar in treatments given all nutrients or no NO_3^- and in controls given no nutrients. The only exceptions to this were two samples collected in late October 1993 that were P-limited and, in treatments not given P, grew more slowly than those receiving P additions. Thus, our results are consistent both with the results of Sterner (1994), which demonstrated significantly lower growth rates in a P-limited ecosystem, and with those of McCarthy and Goldman (1979), which demonstrated near-maximum growth rates in the marine environment with very low DIN concentrations. Hence, ecological models of phytoplankton dynamics may need to include nutrient limitation of biomass but not growth rate for N-limited ecosystems, or nutrient limitation of both growth rate and biomass in P-limited ecosystems.

The nutrients required for the development of the phytoplankton bloom (standing crop ~30–50 μg chl*a* L^{-1}) observed during December 1993 and May 1994 were not measured at the surface. Given the relatively shallow depth of the Swan River and the vertical stratification, anoxia and high nutrient concentrations (primarily phosphate and ammonium) near the bottom (Jack 1987), internal resupply would require vertical transport mechanisms to move the nutrients upwards into the euphotic zone. Wind mixing, convective overturn, and vertical migration by motile phytoplankton species are all possible mechanisms, but we have insufficient data to ascertain their relative importance. The dominance of summer blooms in the Swan River by motile species, particularly marine dinoflagellates, has been noted before (John 1987) and may reflect the ability of these species to vertically migrate between nutrient-rich bottom waters and the near-surface waters with higher average irradiance (e.g. Eppley *et al.* 1977). The December 1993 bloom was concentrated in the upper 2–3 m of water, whereas the autumn bloom of May 1994 was more evenly distributed throughout the 6-m water column. Whether the relatively deep autumn bloom was fuelled by nutrients mixed upwards during the convective overturn observed in April 1994, by the vertical migration capability of the species involved, or by increasing light penetration was not resolved. Conversely, there was a strong degree of association between maximum biomass in the bioassay controls and the nitrate concentration at the surface, suggesting that NO_3^- was the primary N source for algal growth. There was also a significant correlation ($r^2 = 0{\cdot}69$) between rainfall and surface nitrate (Fig. 9). For this analysis, rainfall measured at the nearby Perth airport was summed over the seven days preceding each sampling date for surface nitrate measurements. We interpret these relationships to suggest that nitrate may be supplied primarily to the euphotic zone by groundwater flow associated with rain events (Mallin *et al.* 1993). Rainfall can act as a direct deposited source of nitrogen (Likens *et al.* 1972; Axler *et al.* 1993) or cause nitrogen to enter the river and estuary in response to overland and groundwater flow (Caraco *et al.* 1992).

Rainfall within catchments is known to inject nutrients into estuaries that, following a lag, stimulate primary production (Rudek *et al.* 1991; Mallin *et al.* 1991, 1993).

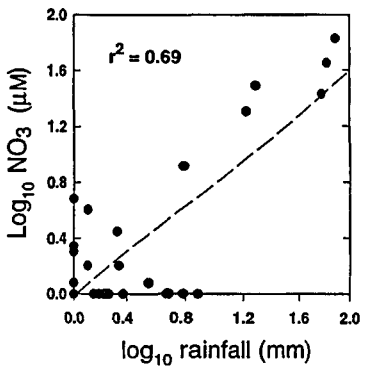

Fig. 9. Relationship between NO_3^- concentration measured at the surface at the Ron Courtney Island site and weekly rainfall at the Perth Airport; data were transformed by $\log_{10}$(datum + 1).

Nitrate concentrations of 70 μM measured in the Swan River after high rainfall during May–June 1994 presumably had a similar potential. We consider this relationship to be important for two major reasons: sampling strategies need to be designed to respond to rainfall or other events in order to document the true primary production of an estuary, and years of low runoff could be expected to encourage cyanobacterial growth due to low N inputs and lower-than-normal N:P nutrient ratios. In the case of the Swan River and other similar aquatic ecosystems, the timing of the rainfall events may have a significant impact on the annual primary production and biomass concentrations because event-driven supplies of nitrogen to the euphotic zone may result in a more rapid increase in algal production during the summer than during the winter.

In conclusion, during most of the 1993–94 annual cycle the potential for nitrogen to limit phytoplankton biomass was much greater than that for any other tested nutrient. The residual pool of unused PO_4^{3-} that existed in the warm surface layer during summer is of concern because of its potential to facilitate the development of cyanobacterial populations (McQueen and Lean 1987). Mechanisms that may control the resupply of nutrients to the euphotic zone and influence phytoplankton dynamics require additional research in the Swan River ecosystem.

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