J. Phycol. **37,** 16–29 (2001)

NUTRIENT LIMITATION OF PHYTOPLANKTON IN A SEASONALLY OPEN BAR-BUILT ESTUARY: WILSON INLET, WESTERN AUSTRALIA[1]

Luke Twomey[2]

School of Environmental Biology, Curtin University of Technology, G.P.O. Box U1987, Perth 6845, Western Australia

and

Peter Thompson

School of Aquaculture, University of Tasmania, P.O. Box 1214, Launceston 7250, Tasmania, Australia

The potential for nutrient limitation to affect the phytoplankton community was assessed from 1997 to 1998 in Wilson Inlet, Western Australia. Samples of the endemic phytoplankton community were assayed by giving them nutrient mixes deficient in one of the following: nitrogen, phosphate, silicate, iron, trace metals, or vitamins. When ranked from those treatments with the most potential to limit phytoplankton biomass to those with less potential, the bioassay results suggested that N>P>>Si>Fe, and trace metals and vitamins were never potentially limiting. In summer and autumn, the bioassay data suggested that nitrogen and phosphorus were the nutrients with greatest potential to constrain phytoplankton biomass. Though the concentrations of soluble nutrients were high in winter, bioassays indicated that the phytoplankton community was potentially nutrient limited. Physical conditions such as high flow rate, greater turbidity, lower temperature, and greater light attenuation contributed to lower phytoplankton growth rates during winter. The bioassay data indicated that the phytoplankton biomass was least likely to be constrained by dissolved inorganic nutrients in spring than any other time of the year. In spring the endemic phytoplankton community responded to salinity-stratification induced sediment-nutrient release with a marked increase of biomass. Nitrogen was the nutrient with greatest potential to limit the phytoplankton biomass during spring. The bioassay data conflicted with the dissolved inorganic nitrogen to phosphorus molar ratios, which suggested that P was more likely to limit the phytoplankton biomass during spring. The discrepancy between the two data sets was probably caused by inaccuracies in measuring the concentration of dissolved inorganic phosphorus in spring. Therefore, the results from the current study suggest that the Redfield paradigm, based on the ratio of dissolved inorganic nutrient concentration, provided an inadequate description of phytoplankton nutrient limitation in Wilson Inlet.

Key index words: **bar-built estuary; bioassay; eutrophication; nutrient limitation; phytoplankton; Wilson Inlet**

Abbreviations: **DIN, dissolved inorganic nitrogen; DIP, dissolved inorganic phosphorus; DO, dissolved oxygen**

Many aquatic ecosystems throughout the world have been effected by eutrophication, which occurs when inorganic nutrient supplies exceed phytoplankton growth demands (e.g. Fisher et al. 1988, McComb and Lukatelich 1995). Eutrophic systems often display nuisance algal blooms, which can lead to the degradation of water quality and the loss of habitat for biota. Subsequently, the recreational and commercial value of afflicted aquatic ecosystems can be severely affected. To restore the water quality of eutrophic ecosystems to an acceptable level, it is necessary to identify the growth-limiting nutrients to develop nutrient input constraints (Paerl and Bowles 1987).

Although it is generally accepted that primary production in marine systems is nitrogen (N) limited (Redfield 1958) and that freshwater systems may be phosphorus (P) limited (cf. Elser et al. 1990), research indicates that the opposite may occur depending on the nutrient characteristics of the particular system (Callender and Hammond 1982, D'Elia et al. 1985). In estuaries, studies of phytoplankton nutrient limitation are often complicated by transitions between N and P limitation, which may occur on both spatial and temporal scales. Seasonal switching from P to N limitation may occur in estuarine basins when marine water, which is generally N limited but P sufficient, mixes with fresh water having relatively greater N and lower P concentration (D'Elia et al. 1985). Under these circumstances, some spatial variability of nutrient limitation will probably occur, depending on the size of the estuary and the proximity of the phytoplankton community to nutrient sources such as river mouths, deep anoxic layers, or the ocean entrance.

Typically, dissolved inorganic nutrient ratios and/or nutrient enrichment bioassays have been used to identify the nutrients with the greatest potential to limit phytoplankton growth. Small-scale bioassays often have a limited application in natural ecosystems

[1]Received 9 February 2000. Accepted 5 October 2000.

[2]Author for correspondence and present address: University of North Carolina at Chapel Hill, Institute of Marine Sciences, 3431 Arendell St., Morehead City, NC 28557. E-mail twomey@email.unc.edu.

due to inherent problems associated with isolating a phytoplankton sample from nutrient sources and grazers, difficulties in simulating the *in situ* light and temperature fields, and container contamination (Hecky and Kilham 1988, Rudek et al. 1991, Sterner 1994). However, when supplemented with water quality data, bioassays can provide valuable information on potential nutrient limitation of the phytoplankton community biomass, with results that indicate benefits of such techniques far outweigh the limitations (see review by Hecky and Kilham 1988).

Despite numerous successful studies using nutrient enrichment bioassays in both fresh water (Storch and Dietrich 1979, Lin and Schelske 1981, Suttle and Harrison 1988, Chang et al. 1991, Siep 1994, Sterner 1994) and marine systems (Smayda 1974, Laws and Bannister 1980, Paerl et al. 1990), their application in estuaries has largely been limited to ecosystems in North America (D'Elia et al. 1985, Malone et al. 1988, Rudek et al. 1991, Pennock and Sharp 1994). In Australia, many estuaries in the southwestern region have demonstrated the potential to become nutrient enriched. For example, recently a $65 million environmental engineering project was completed on the Peel-Harvey Estuary to improve marine flushing and rid it of the nuisance cyanobacterium, *Nodularia spumigena* (Hosja and Deeley 1994). Similarly, in a study of nutrient limitation in the eutrophic Swan River Estuary, Thompson and Hosja (1996) used nutrient dilution bioassays to provide evidence that the summer phytoplankton bloom was most likely to be limited by nitrogen. In this example, the bioassay proved a useful tool in assisting resource managers to focus on nutrient reduction schemes that could potentially decrease the likelihood of phytoplankton blooms (Thompson et al. 1997).

In the current study we apply nutrient deletion bioassays over a 1-year period to the phytoplankton community of Wilson Inlet, an oligotrophic estuary in summer, autumn, and winter that has developed eutrophic symptoms in spring. The bioassay data were supplemented with *in situ* measurements of physical and chemical variables to provide an indication of the nutrient status of the estuary and of the potential for the phytoplankton to become nutrient limited. Bioassays were conducted to determine whether seasonal changes in the nutrient characteristics of the system, which might not be detected by routine nutrient analysis, were important in determining growth and succession of the phytoplankton community. Considering that the treatments that showed most potential for nutrient limitation were N and P and that current catchment management schemes have focused on the reduction of these nutrients into the system, the primary focus of this research was concerned with the occurrence and extent of N and P limitation.

MATERIALS AND METHODS

Site description. Wilson Inlet lies on the southern coast of Western Australia between latitudes 34° 56′ and 35° 06′ south and longitudes 117° 18′ and 117° 30′ east (Fig. 1). The estuary has an area of 48 km^2, a volume at mean sea level of approximately 85 $\times$ 10^6 m^3, and an average depth of 1.78 m (Humphries et al. 1982, Lukatelich et al. 1987).

Wilson Inlet fits the definition of a bar-built estuary (Prichard 1967) with a free opening to the Southern Ocean for 3–5 months of the year when river flow is high. In the warm and dry summer months, a sandbar usually closes the estuary mouth (Humphries et al. 1982). The climate is Mediterranean, with peak rainfall occurring in winter and a dry period of less than 4 months (Lenanton 1974, Lukatelich et al. 1986, Hodgkin and Clark 1988). The Wilson Inlet catchment has an area of ca. 2379 km^2 (Lenanton 1974), which feeds two main rivers, the Denmark River and the Hay River, and three smaller rivers, the Sleeman River, Cuppup Creek, and Little River. Nutrient concentration is greater in the southern catchment rivers where rainfall is higher (Hodgkin and Clark 1988). The southern catchment areas have been heavily cleared for agriculture, and the sandy soils have a low absorptive capacity for nutrients (Lukatelich et al. 1986, Hodgkin and Clark 1988). This means that during periods of high rainfall, there is a significant amount of nitrogen and phosphorus introduced into the system from fertilizer runoff.

In recent years Wilson Inlet has displayed a number of symptoms associated with excessive nutrient enrichment. The major symptom has been the excessive growth of the seagrass *Ruppia megacarpa* Mason, which in the past has reached nui-

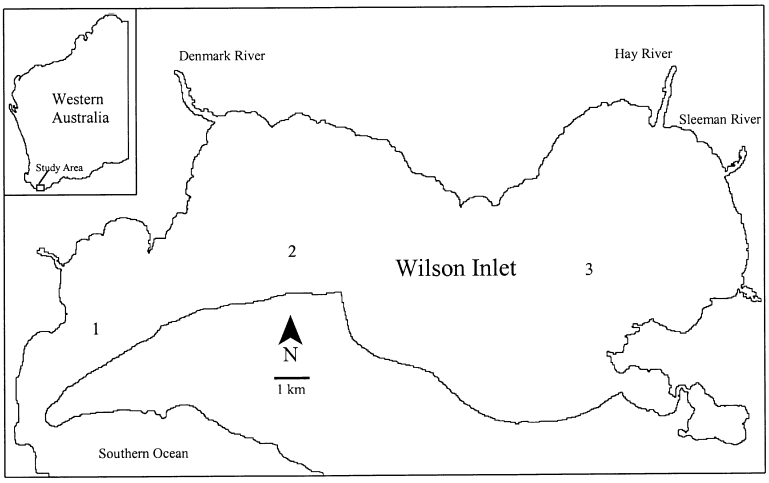

FIG. 1. Map of Wilson Inlet in Western Australia showing the three sampling sites.

sance proportions. In previous years, *Ruppia* has constituted more than 90% of plant biomass in the estuary (up to 14,089 tonnes per month), often impeding boats and rotting in the shallows in late summer (Lukatelich et al. 1987). More recently, mild spring phytoplankton blooms have become more frequent, raising fears that the system is slowly becoming eutrophic (Hosja and Deeley 1994). For this reason three study sites were chosen: site 1 located in the western basin nearest the ocean, site 2 in the mid-estuary, and site 3 in the eastern basin proximal to the Hay and Sleeman Rivers (Fig. 1).

Collection of physical, chemical, and biological data. Data were collected at each of the three sampling sites from January 1997 to November 1998. Water quality sampling of physical, chemical, and biological parameters was performed on a weekly basis, except during winter (June, July, and August) when sampling was conducted every 2 weeks. Temperature, salinity, and dissolved oxygen (DO) concentrations were measured with a Hydrolab H20 multiprobe (Hydrolab Corporation, Austin, TX) at 0.5-m intervals from the surface to the bottom of the water column. Light attenuation was measured with a 30 cm diameter Secchi disk and as photosynthetically active radiation (~400–700 nm) with a Licor (Lincoln, NE) 185B meter (4π collector). Water samples for chemical analyses were collected from the surface and bottom using a nondestructive submersible pump assembly. Inspection of the collected samples showed no evidence of cell damage associated with pump collection. Samples collected for dissolved nutrient analyses were immediately filtered through 0.45-μm cellulose nitrate membrane filters (Whatman). Samples were stored below 4° C and analyzed by the Australian Environmental Laboratories. Samples for chl *a* analysis were collected at the surface and bottom, filtered through glass fiber filters (Whatman, GF/C), extracted with 90% acetone, and analyzed by spectrophotometry (Parsons et al. 1984). Because of the large numbers of samples collected on each field trip, nutrient and chl *a* analysis was conducted without replication. Daily rainfall data for Wilson Inlet was recorded at the nearby Denmark Post Office.

Bioassays. Between October 1997 and December 1998, 46 bioassays were conducted on the endemic phytoplankton community in Wilson Inlet. An integrated water column sample was collected at each of the three sampling sites by mixing 1 L of water from 0.5-m intervals from the surface to the bottom. Within 24 h of collection, 40 mL of integrated sample was incubated, in triplicate, within 50-mL borosilicate culture tubes with Teflon-lined caps. Cultures were incubated at an irradiance of 200 μmol photons·m^{-2}·s^{-1} (cool white fluorescent bulbs) with a light:dark ratio adjusted to track the ambient photoperiod (between 14:10 summer and 12:12 winter). Temperature was adjusted to the mean temperature of the three sampling sites measured at the time of sample collection.

Each treatment culture was prepared by the addition of nutrients based on the recipe of Harrison et al. (1980) at a concentration considered high enough to saturate the initial growth rates (Table 1). Each treatment culture was prepared by omitting one of the nutrients from the full compliment. For example, to test for phosphorus limitation, all the nutrients were added to the medium except PO_4^-. If the treatment produced good growth and high biomass, then phosphorus was not considered limiting on that particular day. A control with no added nutrients and a treatment with all added nutrients were prepared to enable a comparison of each treatment biomass at the end of incubation. At intervals of 24 h the *in vivo* fluorescence of each culture was measured by inserting the whole culture tube into a Turner Designs model 10 fluorometer. At the end of exponential growth, typically 4–5 days, a 30-mL subsample was filtered through a glass fiber filter (Whatman, GF/C) and the chl *a* concentration of each culture was estimated by the *in vitro* fluorometric method (Parsons et al. 1984). The bioassay incubation times were sufficiently short to reduce significant bottle effects such as large shifts in the nutrition of the phytoplankton, species successional changes, or growth of bacteria on the sides of culture tubes (Vincent et al. 1984). All bioassay treatments and controls were cultured in triplicate. Where necessary data were transformed to achieve normality and homoscedasticity before statistical analysis.

TABLE 1. Nutrients and the approximate initial concentrations added to bioassay treatments.

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 and chelator	$FeCl_3 \cdot 6H_2O$	6.56
	$(CH_2N(CH_2COOH)$ $CH_2COONa)_2 \cdot 2H_2O$	6.56
Trace metals and chelator	$MnSO_4 \cdot H_2O$	2.42
	$CoSO_4 \cdot 7H_2O$	0.0569
	$ZnSO_4 \cdot 7H_2O$	0.254
	$NaMoO_4 \cdot 2H_2O$	0.52
	$NiCl_2 \cdot 6H_2O$	0.5
	$NaSeO_3$	0.01
	$(CH_2N(CH_2COOH)$ $CH_2COONa)_2 \cdot 2H_2O$	8.3
Vitamins	B_1 (thiamine HCl)	0.297
	B_{12} (cyanocobalamin)	0.0015
	B_6 (biotin)	0.0041

RESULTS

Physical data. The sandbar at the mouth of the estuary was closed to the ocean from 23 February to 18 August 1997 and from 19 January to 10 August 1998. Rainfall was seasonal, with minimum values recorded in summer and maximum values recorded in winter in both 1997 and 1998 (Fig. 2, A–C). The salinity concentration also displayed a distinct seasonal pattern with maximum concentrations observed during autumn (34.6 ppt) and minimum concentrations recorded in spring (8.8 ppt). From March to June 1997 and February to May 1998 salinity was vertically and horizontally homogenous with means of ~24 and ~26 ppt in the respective years and a difference of less than 2 ppt across the entire inlet. Periods of low salinity in Wilson Inlet during winter were associated with concurrent high rainfall (Fig. 3). Surface and bottom salinity concentration diverged in winter as less dense fresh water flowed over the more dense salt water to produce a distinct halocline (Fig. 2, A–C). At the shallow site 3 (~3 m), the halocline lasted for a period of approximately 1 month during winter before mixing established homogenous conditions (Fig. 2C). In spring, short-lived periods of stratification were observed on a number of occasions. At site 2 in the relatively deeper basin (~5 m), a halocline was observed from August 1997 to January 1998 and from August 1998 to the end of the study period in 1998 (Fig. 2B). Bottom salinity fluctuated during stratification from a minimum of ~17 ppt to a maximum of ~34 ppt. When the bar was open during winter and spring, tidal intrusion of marine water was noticeable with the rapid divergence of surface and bottom salinity concentration, particularly at site 1 (Fig. 2A). The minimum salinity concentrations recorded during September throughout the inlet were associated with

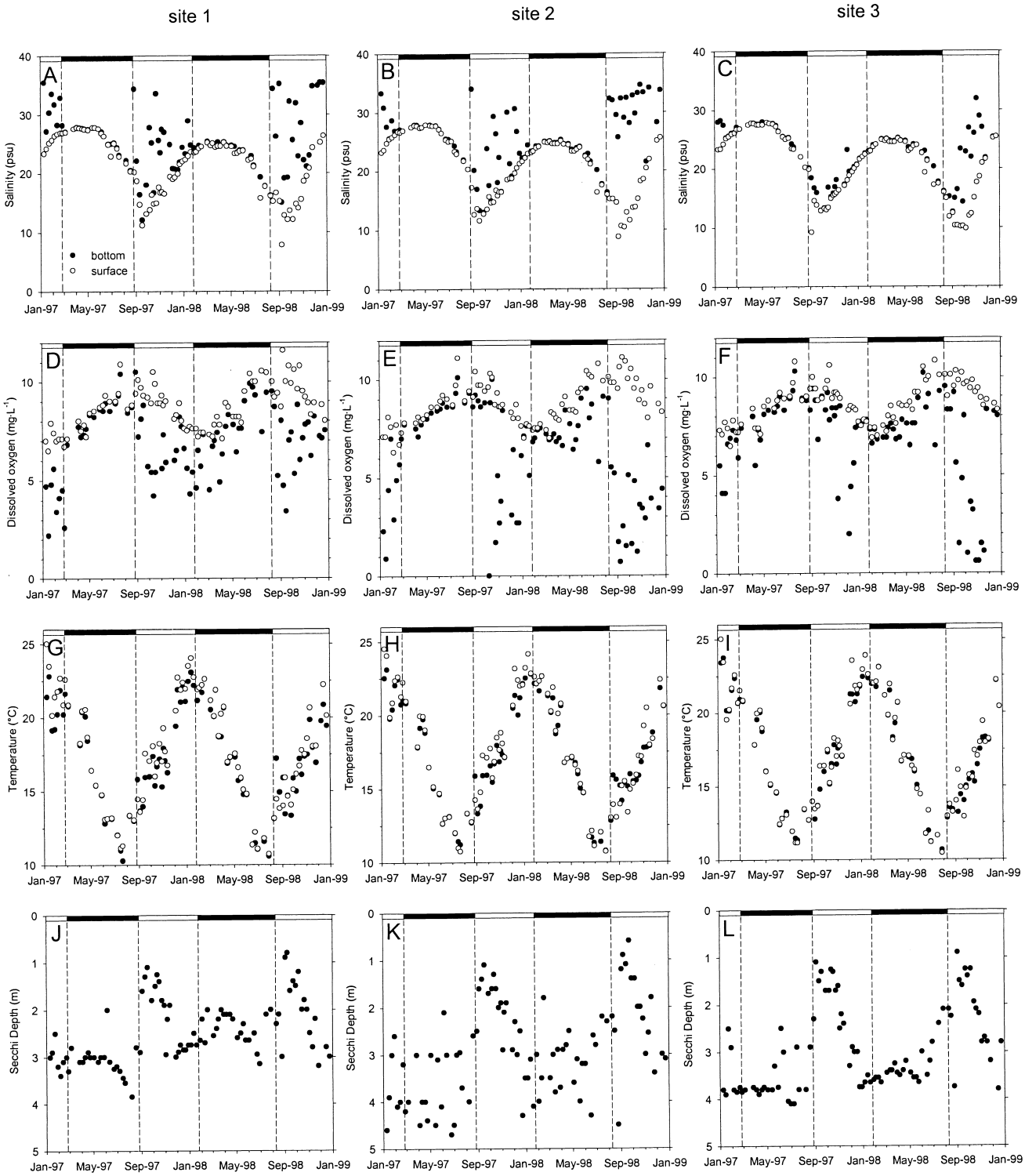

FIG. 2. Seasonal pattern of physical parameters measured at the three sampling sites in Wilson Inlet between 1997 and 1998 at the surface (○) and bottom (●) of the water column: (A–C) salinity concentration, (D–F) dissolved oxygen concentration, (G–I) temperature, (J–L) secchi depth. Vertical lines represent the status of the sandbar: closed (■) or open (□).

high flow from the tributaries. The annual water column salinity concentration was significantly greater at sites 1 and 2 than at site 3 (analysis of variance [ANOVA], $P < 0.001$).

Surface DO concentrations were high and spatially homogenous throughout the entire study period (Fig. 2, D–F). The DO concentration in bottom water at each site exhibited a strong seasonal pattern. Highly

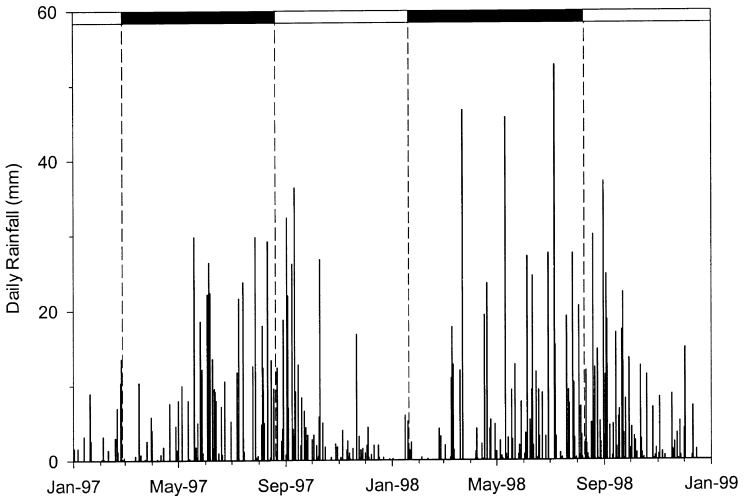

FIG. 3. Daily rainfall measured at Denmark Post Office between January 1997 and January 1998. Vertical lines represent the status of the sandbar: closed (■) or open (□).

oxygenated water was present at the bottom from late summer to winter (7.1–11 mg·L^{-1}), whereas during spring and early summer the concentration was often very low. Anoxia was associated with the halocline as demonstrated by the strong negative correlation between bottom salinity and DO at site 2 during spring and summer ($r = -0.631$, $n = 34$, $P < 0.01$) (Fig. 2E). The mean annual DO concentration was significantly higher at site 1 than at site 3, whereas the mean DO concentration at site 2 was significantly lower than the other sites (ANOVA, $P < 0.001$, $n = 84$).

The water temperature followed a seasonal pattern with lowest values recorded in mid-winter (10.7° C) and highest observations in mid-summer (24.2° C) (Fig. 2, G–I). There was little temperature difference horizontally between sites or vertically through the water column; however, a thermocline was observed in spring with a maximum difference between the surface and bottom layers of less than 3° C.

The secchi disk was usually visible at the bottom of each site from January to July with a maximum recorded at the deepest site 2 (4.7 m). Fisher's least significant difference test determined that secchi depth was significantly lower in spring than any other season during 1997 and 1998 ($\alpha = 0.05$, $n = 27$). This was reflected by the minimum secchi values of ~1.5 m recorded during September/October in both years (Fig. 2, J–L), which indicated that light attenuation was greatest during spring. At site 2, secchi depth and rainfall were negatively correlated ($r = -0.61$, $n = 25$, $P < 0.01$) (Fig. 2K). Light attenuation through the water column at site 2 measured as photosynthetically active radiation further demonstrated the seasonal reduction of light penetration from summer to spring (Fig. 4).

Seasonal nutrient availability. There were considerable differences in the seasonal concentration of both dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP). The concentration of nitrate (NO_3^-) ranged from the detection limit of 0.33 μmol·L^{-1} during the warmer drier months of summer and autumn to a maximum of 10 μmol·L^{-1} during periods of greatest rainfall and highest river discharge in the winter and spring (Fig. 5, A–C). The ammonium concentration (NH_4^+) ranged from the detection limit of 0.33 μmol·L^{-1} during summer–autumn to a maximum of 85.7 μmol·L^{-1} during the spring (Fig. 5, D–F). The maximum phosphate (PO_4^{-3}) concentration was also observed in the anoxic bottom water during spring (0.87 μmol·L^{-1}) (Fig. 5, G–I). When DO concentration was less than 4 ppm in the bottom water, there was a negative correlation with log transformed ammonium ($r = -0.688$, $P < 0.05$) and log transformed phosphate ($r = -0.603$, $P < 0.05$). During the rest of the year the phosphate concentration was low and mostly less than the detection limit of 0.1 μmol·L^{-1}. With the concentration of N and P below the limit of detection for most of summer and au-

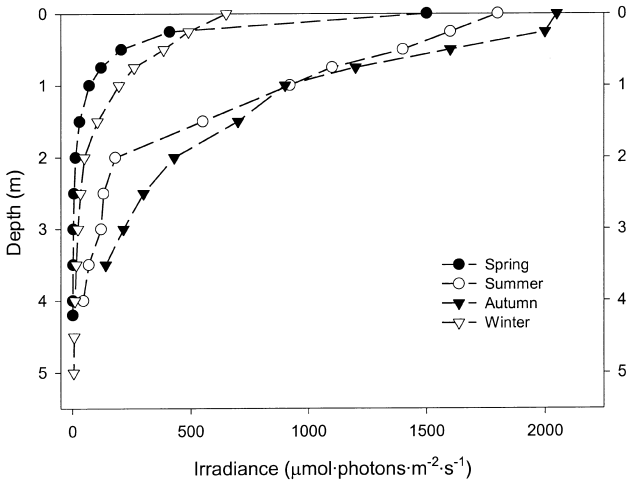

FIG. 4. Seasonal examples of light attenuation with depth through the water column at site 2. (○) Summer, measured on 10 December 1997. (▼) Autumn, measured on 4 March 1998. (▽) Winter, measured on 28 July 1998. (●) Spring, measured on 15 September 1998.

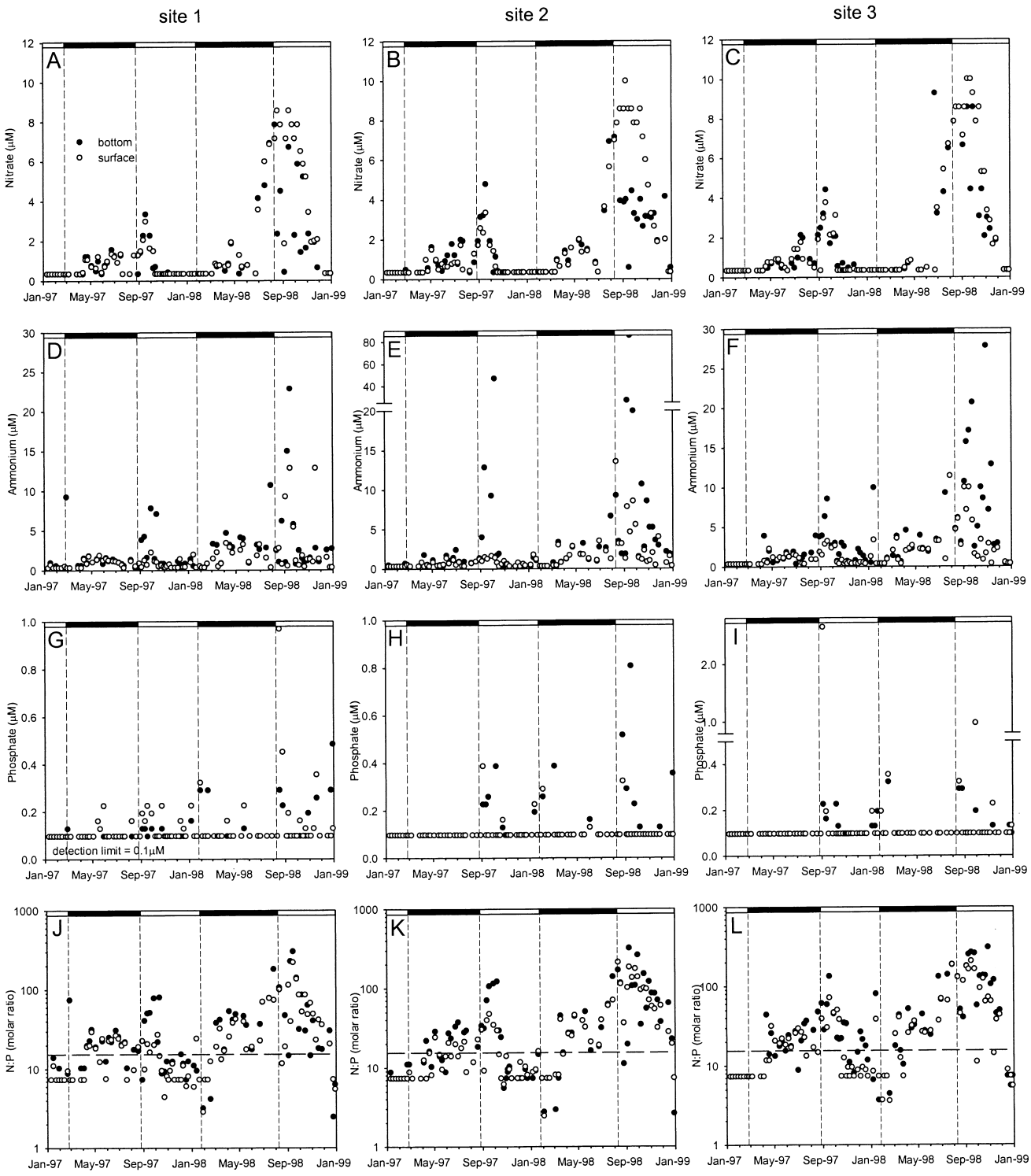

FIG. 5. Seasonal cycle of dissolved inorganic nutrient concentrations at the three sampling sites measured at the surface (○) and bottom (●) of the water column: (A–C) nitrate concentration, (D–F) ammonium concentration, (G–I) phosphate concentration, (J–L) N:P molar ratio. Vertical lines represent the status of the sandbar: closed (■) or open (□).

tumn, the molar DIN:DIP ratio could not be calculated accurately during these periods (Fig. 5, J–L). The DIN:DIP molar ratio was greatest in spring; however, it periodically approached unity when the phosphate concentration was relatively great. There was no significant difference in the mean annual ammonium, nitrate+nitrite, or phosphate concentrations between sites (Kruskal-Wallace ANOVA, $P > 0.05$).

Chlorophyll a. Chl *a* concentration was greatest during spring and was low throughout the rest of the year (Fig. 6). Site 1, nearest the ocean, displayed a similar pattern in 1997 and 1998 with low chl *a* concentration during the summer, autumn, and winter and a maximum concentration observed in spring of 18 and 20 μg chl $a \cdot L^{-1}$, respectively (Fig. 6A). In the middle of the estuary, at site 2, the highest chl *a* concentrations of the three study sites were observed in the spring of 1997 and 1998 with a maximum of 31 and 47 μg chl $a \cdot L^{-1}$, respectively (Fig. 6B). In the eastern basin, at site 3, the chl *a* concentrations were lower than the other sites with a maximum spring value of 12 μg chl $a \cdot L^{-1}$ in 1997. In 1998 there was no spring bloom observed at site 3 where chl *a* concentrations were less than 5 μg chl $a \cdot L^{-1}$ throughout the spring (Fig. 6C).

Bioassays. The daily *in vivo* fluorescence was recorded for each bioassay culture to provide an estimate of the rate of biomass change and a determination of when the maximum biomass had been attained. The daily *in vivo* fluorescence also provided a method to compare the growth response of each treatment culture with the growth response of the control and that of the treatment with all nutrients added. The bioassay conducted from the water sample collected at site 2 on the 15 September 1998 is used here as an example (Fig. 7A). On the first day of incubation, the initial fluorescence of each treatment and the control was approximately 3 fluorescence units (FU). On day 4 the treatments with all nutrients and those treatments missing silicon, iron, trace metals, and vitamins each recorded a maximum biomass of more than 40 FU. Conversely, the control and the treatments with no N and no P remained constant with a biomass of less than 4 FU. On day 4 the bioassay was terminated and all cultures were filtered and the chl *a* concentration was determined for each (Fig. 7B). Performing one-way ANOVA tested differences between the means of each treatment, controls, and treatments with all nutrients added (ALLs). After 4 days of incubation, the chl *a* concentration of the treatment with all nutrients added was approximately 150 μg chl $a \cdot L^{-1}$, which was not significantly different from the treatments given all nutrients, except silica, iron and chelators, trace metals and chelators, and vitamins. Therefore, on 29 October 1997, the bioassay data suggest that these nutrients were not likely to limit the phytoplankton biomass of Wilson Inlet at site 2. However, there was strong evidence of nitrogen and phosphorus limitation. After 4 days of incubation there had been no significant increase in biomass of the treatment given all nutrients but N and P. Both the control and -N treatments finished the bioassay with a chl *a* concentration similar to that observed in the inlet at the time of sampling (~4 μg chl $a \cdot L^{-1}$), which was significantly lower than the other treatments (~150 μg chl $a \cdot L^{-1}$). These results suggest insufficient DIN in the natural system at the time of testing for phytoplankton to bloom.

When the mean final biomass of the treatment with all nutrients added was significantly greater than the control, three levels of potential nutrient limitation were determined: 1) severe limitation, when a treatment biomass showed no significant difference from the control; 2) moderate limitation, when a treatment biomass was greater than the control but less than the treatment with all nutrients; and 3) no limitation, when a treatment biomass was the same as the treatment with all nutrients added. Of the 46 bioassays conducted at three sites between 1997 and 1998, nitrogen was the most potentially limiting nutrient, with 40 incidences of severe nitrogen limitation and 6 of moderate nitrogen limitation. Phosphate was the next most potentially limiting nutrient, displaying severe limitation 29 times, moderate limitation 9 times, and no P limitation 8 times. The other treatments never severely limited phytoplankton biomass; however, iron was moderately limiting 15 times, as was silicon on five occasions. Treatments with trace metals and vitamins were potentially moderately limiting only once.

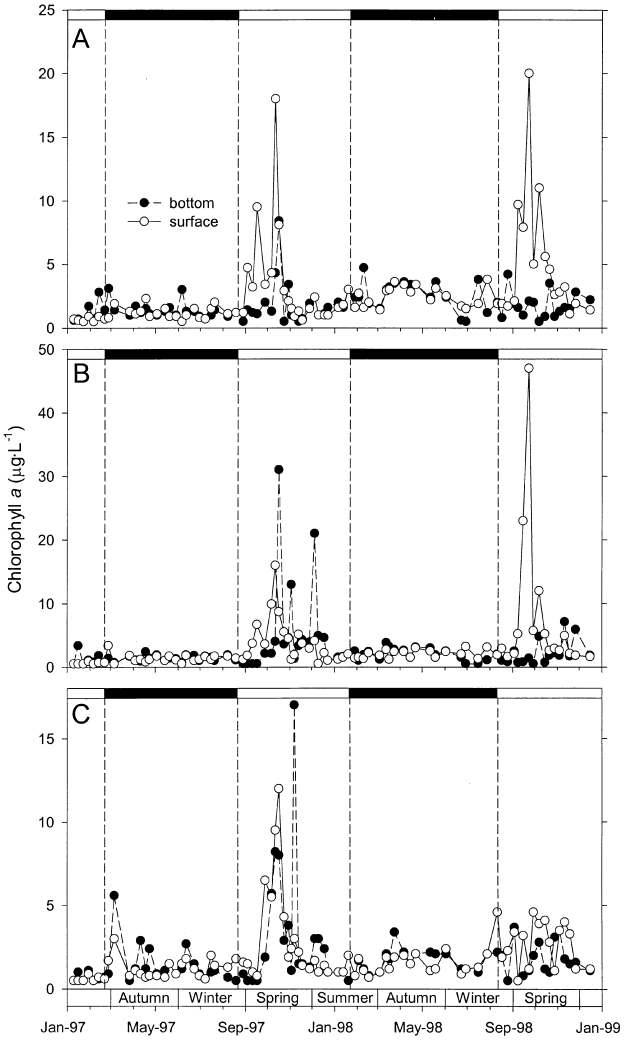

FIG. 6. Seasonal pattern of chl *a* concentration at the surface (○) and bottom (●) of the water column: (A) site 1, (B) site 2, (C) site 3. Vertical lines represent the status of the sandbar: closed (■) or open (□).

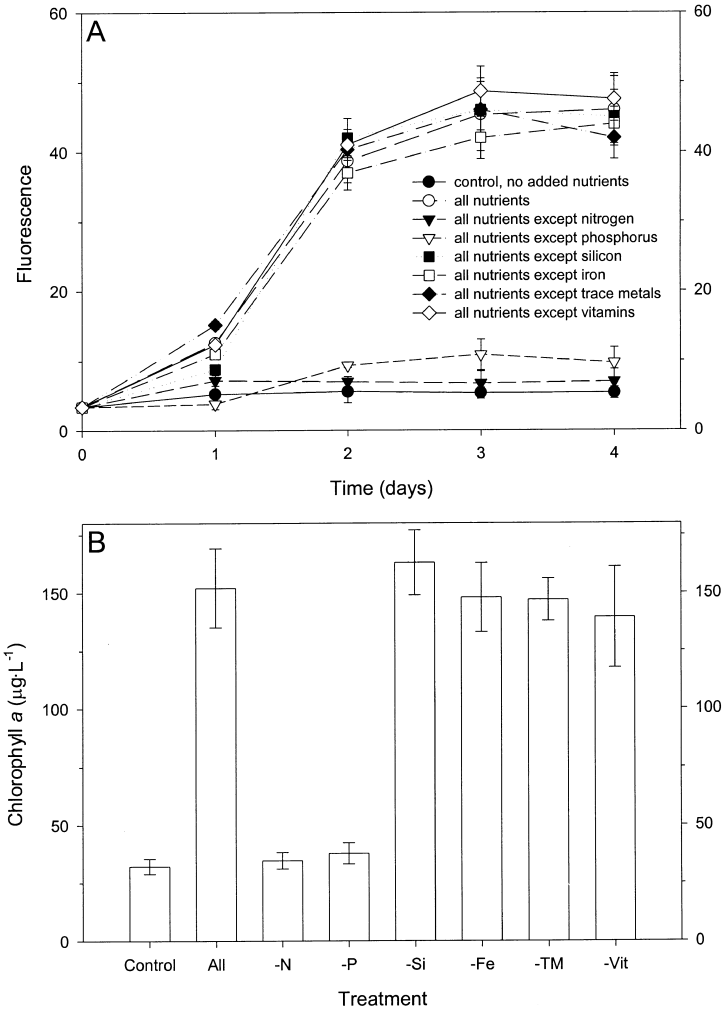

FIG. 7. Bioassay results for samples collected on the 29 October 1997 at site 2. (A) The change in fluorescence vs. incubation time. (B) Chl *a* concentration of bioassay subsamples after 8 days of incubation (error bars are ± 1 SD). TM, trace metals; Vit, vitamins.

A temporal pattern of nutrient limitation in the phytoplankton community of Wilson Inlet was evident through comparison of the chl *a* biomass determined on the final day of incubation in treatments with all nutrients, no added nutrients, no added nitrogen, and no added phosphorus (Fig. 8). During summer, autumn, and winter (December–August), the biomass of the control and the treatment without N were somewhat constrained, never approaching the maximum potential biomass of the treatment with all nutrients. The maximum biomass of each treatment was greatest in spring (September–November). A state of low nutrient limitation was observed once throughout the investigation in spring 1998 when all treatments, including the control with no added nutrients, had a similar chl *a* biomass at the end of incubation. This suggests that for a short period during spring there were sufficient ambient nutrients within the water column to support a modest biomass increase provided favorable physical conditions.

To assess the potential of N versus P to limit the phytoplankton biomass, the ratio of maximum biomass from bioassay treatments with all nutrients except P (−P) to the bioassay treatment with all nutrients except N (−N) was compared (Fig. 9A). When the ratio was greater than 1, N was more potentially limiting than P; when the ratio was less than 1, P was considered most likely to limit biomass; and when the ratio was at unity, N and P limitation was equitable. The degree of N versus P limitation demonstrated a seasonal pattern over the annual cycle, with N limitation more pronounced during summer and autumn (December–May) than during winter and spring (June–November). During summer and autumn the bioassay treatments with all nutrients except P had a combined average of approximately five times more biomass than treatments without added N. However, there was evidence of considerable spatial variability during summer and autumn with sites 1 and 2, demonstrating a similar pattern in the degree of N or P limitation that was frequently uncoupled with site 3. The potential for P to be more limiting than N was only evident at all sites once on 16 June 1998. From August to November 1998 N and P were approximately equal in their potential to limit phytoplankton biomass.

To assess the overall degree of nutrient limitation, a comparison of chl *a* in the treatment with the lowest

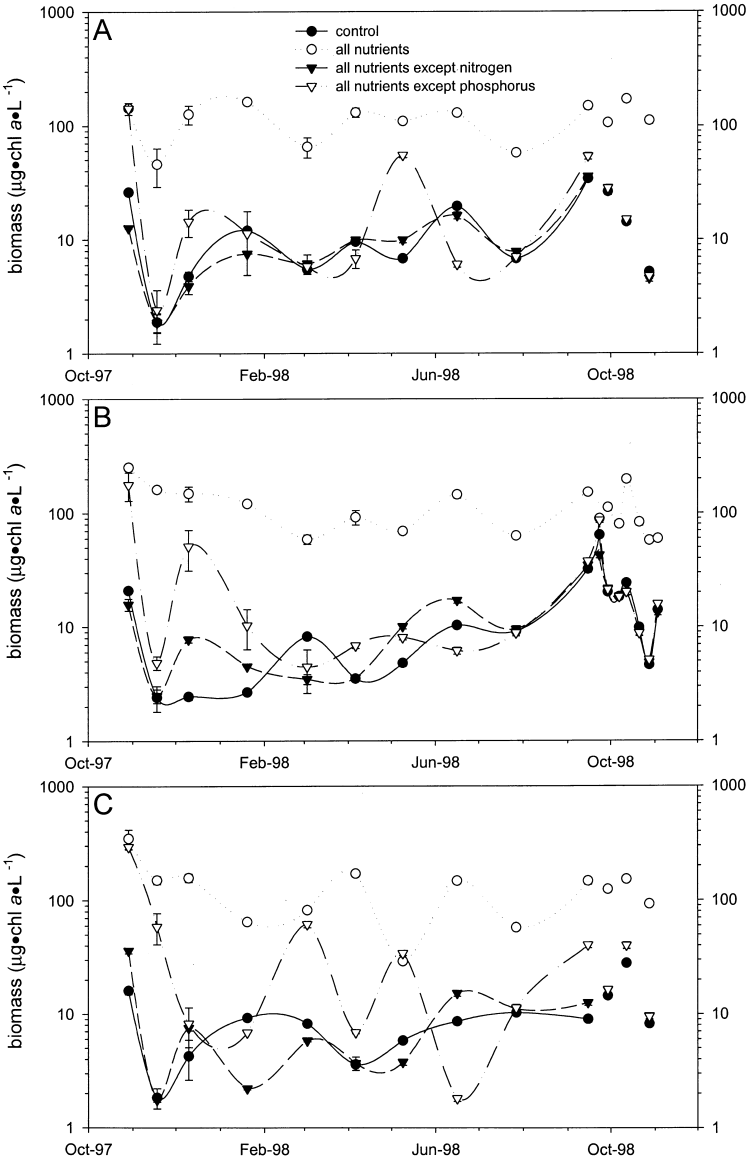

FIG. 8. Comparison of chl *a* biomass at the end of incubation in treatments given no nutrients, all nutrients, all nutrients except nitrogen, and all nutrients except phosphorus. (A) site 1, (B) site 2, (C) site 3 (error bars are ± SD).

biomass at the end of the bioassay (either no N or no P) and the chl *a* in the treatment with all nutrients was made (Fig. 9B). The comparison indicated that lack of N or P constrained the biomass by a factor of up to 32 times at site 1, 66 times at site 2, and 85 times at site 3. Biomass was most highly constrained by lack of N or P in summer, autumn, and winter, whereas during spring the water column did not appear very nutrient limited.

DISCUSSION

The nutrient addition bioassays in the current study differ from traditional approaches where the direct effects of adding one or more nutrients are evaluated by positive changes in phytoplankton community growth. The major advantage of the "all but one" technique is that it enables comprehensive evaluation of a large suite of nutrients, which clearly identify the nutrients with the greatest potential to limit phytoplankton biomass (Laws and Bannister 1980). The technique can unambiguously determine whether or not there is the potential for colimitation by comparing each treatment culture with treatments containing all nutrients (Lin and Schelske 1981, Maestrini et al. 1984). Lin and Schelske (1981) used the all but one technique to demonstrate that the combination of several limiting nutrients may have a seasonally variable effect on the phytoplankton community composition.

Nutrient limitation bioassays have been successfully used in estuaries to demonstrate seasonal variation of nutrient limitation. In eutrophic estuaries throughout the world, a common pattern of seasonal nutrient limitation has emerged. Typically, when rainfall is low and tributary flow is low, many estuaries are potentially N limited, whereas during high flow periods P limitation has been more commonly observed. This

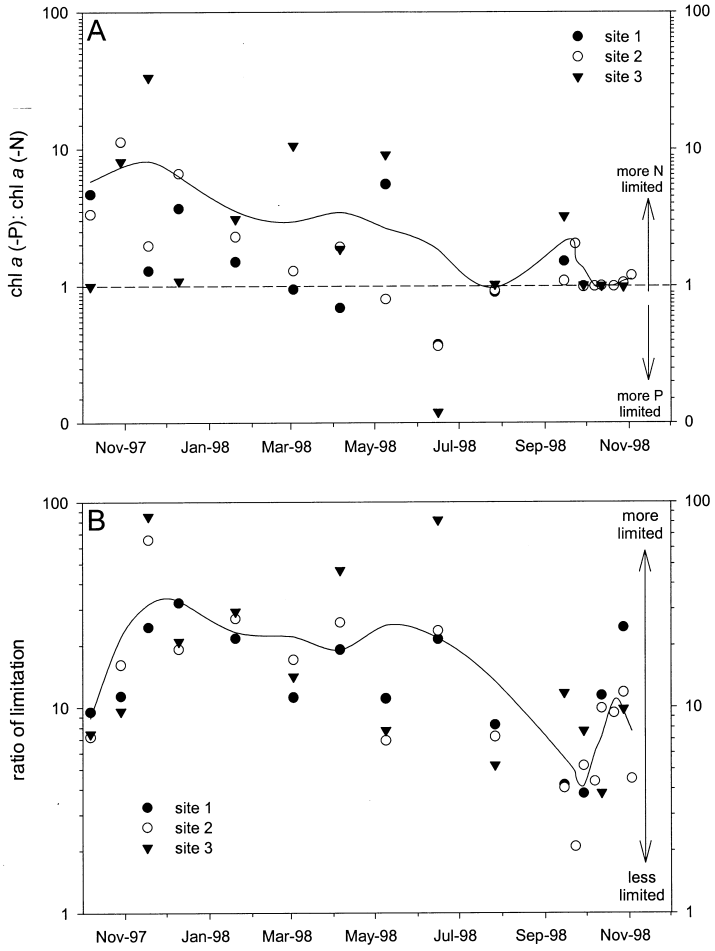

FIG. 9. (A) Potential for nutrient limitation. The ratio of chl *a* in treatments given all nutrients vs. the chl *a* biomass in the treatments given either all nutrients except N or all nutrients except P. See Results for details. A higher order polynomial least-squares regression is fitted to the data and a reference line is shown at 1.0 (B) Assessment of N or P limitation. The ratio of chl *a* in treatments given all nutrients except P vs. the chl *a* biomass in the treatments given all nutrients except N. A higher order polynomial least-squares regression is fitted to the data and a reference line is shown at 1.0.

pattern has been recorded in numerous systems, including the Swan River Estuary, Western Australia (Thompson and Hosja 1996); the lower Neuse River Estuary, NC, United States (Rudek et al. 1991); the Delaware Estuary, DE, United States (Pennock and Sharp 1994); Chesapeake Bay, VA, United States (Fisher et al. 1992); and in the Pauxtent River Estuary, MD, United States (D'Elia et al. 1985). The seasonal pattern of nutrient limitation in Wilson Inlet was somewhat different in that during summer, autumn, and winter N and P were critically limiting, whereas during spring only N appeared to limit phytoplankton biomass. This was perhaps a reflection of the trophic status of Wilson Inlet, which suggests that the system is predominantly oligotrophic, displaying high water column productivity for only a relatively short period during spring.

Summer and autumn. During summer and autumn, the water column was horizontally and vertically homogenous with low light attenuation (Figs. 2, J–L, and 3), marine salinity (Fig. 2, A–C), and relatively high temperature (Fig. 2, G–I), which in such a shallow system produced a physical environment that was suitable for rapid phytoplankton growth (Reynolds 1984). However, phytoplankton biomass was low throughout the estuary during summer and autumn, probably restricted by the low concentration of dissolved inorganic nutrients. The bioassay results support these observations and indicate that during the summer the ecosystem was heavily constrained by the absence of nutrients (Fig. 8A) and critically limited by the availability of both N and P (Fig. 8, B and C).

The dissolved inorganic nutrient concentration was considerably less in summer and autumn relative to the rest of the year (Fig. 5). The closed state of the estuary during these seasons reduced the input of nutrients from coastal marine waters, whereas nutrient loading from the tributaries was minimal due to low rainfall and runoff during the dry season. The low and consistent phytoplankton biomass recorded throughout the estuary during summer and autumn (Fig. 6) was probably maintained by internal nutrient processes, such as recycling and remineralization (Jensen et al. 1990), or from small nutrient inputs such as ground water, wastewater, or small episodic rainfall events (Thompson and Hosja 1996).

The chemical and biological dynamics of Wilson Inlet during summer and autumn were inconsistent with those of eutrophic estuaries where the phytoplankton biomass and nutrient concentrations are generally very high (Fisher et al. 1992, Pennock and

Sharp 1994, Thompson and Hosja 1996). In eutrophic estuaries, summer and autumn biomass is often fuelled by high soluble nutrient concentrations, largely derived from the sediments during periods of horizontal salinity stratification and anoxia (Thompson and Hosja 1996). In Wilson Inlet, the summer and autumn nutrient concentrations were more characteristic of an oligotrophic ecosystem where limited nutrient supply restricts phytoplankton primary production. The low DIN and DIP concentrations combined with our bioassay results that suggest that both N and P are potentially limiting to phytoplankton biomass during summer and autumn are evidence that the water column was nutrient poor with limited resupply of nutrients from potential sources.

Winter. A common feature of temperate estuaries is the decline of phytoplankton community biomass in winter, which is typically controlled by decreasing temperature and solar radiation as day length decreases and winter meteorological conditions prevail (Cloern 1987, Pennock and Sharp 1994). The increased light attenuation during winter (Fig. 4), which was probably induced by the steady influx of humic substances and suspended particulate material from the tributaries combined with short days and overcast skies, was a likely cause of low phytoplankton growth and biomass accumulation in Wilson Inlet. Though the concentration of nitrate was elevated as rainfall and tributary flow increased during winter, there was no evidence to suggest that the influx of DIN stimulated the winter growth of phytoplankton. During winter both the DIN:DIP ratio and the bioassay data suggest that during winter DIP was more likely to limit biomass than DIN (Figs. 5, J–L, and 8). A switch from potential N limitation to P limitation could be suggested as a mechanism responsible for the increase in the proportion of diatoms during winter. However, the observed successional changes were more likely due to the sudden presence of epiphytic and periphytic diatoms that had become detached and dislodged with the increase of tributary flow. The low biomass during winter, reflected by chl *a* concentration (Fig. 6), indicated that the phytoplankton community was severely retarded, probably caused by a combination of P limitation and the growth limiting physical environment.

Spring. The open status of the sandbar was instrumental in the changes observed in the physical and chemical environment during spring. When the bar is first opened in Wilson Inlet there is an initial period where marine water cannot penetrate into the estuary until the high tributary flow decreases to a threshold level of 35 $m^3 \cdot s^{-1}$ (Marshall 1993). Below this threshold, a saltwater wedge will propagate into the estuary driven by the tides. The degree of marine water penetration depends on the tidal range and total stream flow (Marshall 1993). In the current study the intrusion of marine water was first evident in spring when it flowed into the estuary underneath the less dense fresh water in the week after the bar had opened (Fig. 2, A–C). This resulted in horizontal salinity stratification, which progressively extended from the western basin into the eastern basin during spring. Associated with horizontal salinity stratification was the rapid divergence of DO concentration in the bottom waters to values of less than 2 $mg \cdot L^{-1}$ (Fig. 2, D–F). The strong negative correlation between DO concentration when less than 4 $mg \cdot L^{-1}$ and dissolved inorganic ammonium and phosphate concentration suggest that nutrients were then released from the sediments into the water column. The response of the phytoplankton was remarkably similar in both 1997 and 1998, with the bloom occurring 2–3 weeks after the minimum DO concentration in both years. Thus, under these conditions it could be suggested that the spring phytoplankton bloom was fueled by the sediment release of nutrients, particularly phosphate. On the basis of high DIN and DIP concentrations, it was likely that provided the phytoplankton community was not limited by other nutrients or some physical constraint, there was enough bioavailable nutrients during spring to achieve the maximum phytoplankton biomass potential. These findings were supported by the bioassay data, which identify spring as the period when biomass was least likely to be constrained by the concentration of soluble nutrients as indicated by the low ratio of nutrient limitation (Figs. 8 and 9).

In attempting to determine which nutrient, N or P, had the most potential to limit the phytoplankton biomass in spring, our analysis of the bioassay data and the dissolved inorganic nutrient data provided conflicting evidence. During the spring bloom in both 1997 and 1998, the DIN:DIP molar ratio in the surface and bottom waters was typically more than 50:1, much higher than the optimum Redfield ratio for phytoplankton of 16:1 (Redfield 1958). This suggests that during the bloom there was an excess of N in the water column and that if nutrient limitation did occur we would expect P to limit the phytoplankton biomass. Conversely, phytoplankton biomass in bioassay treatments without P compared with treatments without N (Fig. 8) indicates that during spring in 1997, N was more likely to limit phytoplankton biomass than P, whereas in 1998 the value was approximately at unity, an indication that N and P have a similar potential to limit phytoplankton biomass. These bioassay data provide clear and unambiguous evidence that the spring phytoplankton bloom had enough DIP to reach its maximum potential.

When the DIN:DIP ratios are observed on a finer temporal scale, they appear to agree more closely with the bioassay data. For example, in the week before the bloom at site 2 in 1997, the concentration of ammonia was $\sim$47 $\mu mol \cdot L^{-1}$, nitrate was undetectable, and phosphate was $\sim$0.4 $\mu mol \cdot L^{-1}$, producing an DIN:DIP molar ratio of $\sim$120:1 (Fig. 5K). In the week after the bloom, ammonia and nitrate were undetectable, whereas phosphate was $\sim$1.0 μL^{-1}, giving an DIN:DIP molar ratio of less than 10:1. Similarly, in 1998 the DIN:DIP molar ratio was 110:1 in the week before the bloom and 35:1 in the week after the bloom. These data indicate that the

DIN:DIP molar ratio had decreased during the bloom because N had become relatively less available than P. Though this evidence suggests N utilization, the DIN:DIP ratios remained high, which also suggest potential P limitation of phytoplankton biomass. Although DIN:DIP molar ratios are often used to infer potential nutrient limitation of phytoplankton growth (Smith 1982, Krom et al. 1991), in some ecosystems phytoplankton have such a high affinity for nutrients that if nutrient limitation did occur, it would occur at concentrations not detectable by commonly used analytical methods (Hecky and Kilham 1988). When nutrient concentrations are high, their ratios may provide a useful indication of potential for nutrient limitation. However, when the concentrations are below detection limits, dissolved inorganic nutrient ratios cannot provide a reliable interpretation of phytoplankton nutrient limitation with the common analytical methods presently in use (Hecky and Kilham 1988). The results from this investigation suggest that the use of dissolved inorganic nutrients to infer nutrient limitation of phytoplankton growth in estuaries should be viewed cautiously and a conservative approach should be taken when interpreting the results. Although dissolved inorganic nutrient ratios may provide a useful measure of potential nutrient limitation in eutrophic systems, when nutrient concentrations are low, enrichment bioassays are a more powerful tool, which can account for variation in nutrient flux relative to phytoplankton demand.

The question remains if the phytoplankton community was not limited by the supply of P, why was an accumulation of P in the water column not seen during the spring bloom? It was likely that either the concentration of DIP was mostly undetected or that the phytoplankton community was using another source of P. In ecosystems where nutrients are at very low concentrations, the rate of recycling can determine the availability of N or P (Graneli et al. 1990). In many shallow estuarine systems, the flux of N and P from the sediments under salinity stratification and anoxic conditions may rapidly elevate the water column concentration (Pomeroy et al. 1972, Callender and Hammond 1982, D'Elia et al. 1985). The rate of nutrient efflux from the sediments may be regulated by numerous physical, chemical, and biological factors (see review by Bostrom and Pettersson 1982). In shallow estuaries, the presence of microphytobenthos can influence the spatial and temporal efflux of nutrients from the sediments through localized oxygenation via photosynthesis during the day, whereas during the night the surface sediments may become anoxic through an increase of respiration, and subsequently P may be released (Revsbech et al. 1983, Carlton and Wetzel 1988). Considering that Wilson Inlet is relatively shallow (mean depth 1.78 m), diel fluctuation of nutrient efflux from the sediments should not be discounted as a likely scenario during spring when anoxic conditions were prevalent. If this situation did exist, it is quite possible that the daily sampling regime (usually between 0900 and 1400) had consistently missed the periods when the concentration of NH_4^+ and PO_4^{-3} was highest, at night.

Considering that the phytoplankton community was generally high during the spring when anoxia was prevalent, by the time we had sampled the water column for nutrients, most N and P may have already been assimilated by the phytoplankton. Parslow et al. (1984) suggested that some phytoplankton have the ability to rapidly increase the specific uptake rates of P after a starvation period and that after the depletion of external phosphate may continue to divide for a period of greater than 48 h and undergo three cell divisions. Taking this into account, if the flux of P in Wilson Inlet was dynamic, occurring in temporally short bursts, then the previously P starved phytoplankton could rapidly remove P from the water column and potentially increase the biomass by up to eight times before more P was needed.

It could also be possible that the phytoplankton community in Wilson Inlet was accessing P in forms other than dissolved inorganic phosphate. In some systems phytoplankton can extract the nutrients bound to small particulate material via the enzyme alkaline phosphatase when the dissolved nutrient forms have been depleted (Smith and Kalff 1981). Other researchers suggest that algae may extract up to 80% of their P from organic compounds excreted by bacterioplankton (Currie 1984). Although the question of where the phytoplankton obtain sufficient P to support the spring phytoplankton bloom in Wilson Inlet remains open, our present understanding of spring phytoplankton bloom dynamics suggests that N rather than P is more important in potentially limiting the phytoplankton biomass during spring.

Spatial variation of nutrient limitation. The bioassay data indicate that nutrient limitation of the phytoplankton community was largely spatially homogeneous; however, the severity of nutrient limitation was frequently variable between sites. The maximum potential for phytoplankton biomass (growth of ALLs in Fig. 8) was similar for each of the three sampling sites throughout the study. This suggests that if each site was provided with a similar physical and chemical environment, the phytoplankton biomass would achieve comparable maxima. Given that either N or P and frequently both nutrients were typically limiting in Wilson Inlet, evidence of spatial heterogeneity in the control treatments with no added nutrients suggests that the concentrations of N and P available for phytoplankton production were variable between sites. However, the bioassay treatments with no added N had comparable biomass potential between sites, indicating that degree of N limitation in Wilson Inlet was similar in space and time. It appears then that the response of phytoplankton to P limitation, which had an inconsistent spatial pattern with P frequently nonlimiting at one particular site while limiting at the others, was largely responsible for the spatial heterogeneity of the resident phytoplankton community biomass.

In recent years the literature has tended to over-

look spatial variability of nutrient limitation in estuarine environments; however, some studies demonstrate considerable inconsistencies between sites (Harrison et al. 1990, Fisher et al. 1992). Studies of spatial heterogeneity in aquatic ecosystems have generally hypothesized that phytoplankton community disequilibrium stems from patchy nutrient supply, which is regulated by the variable physical and chemical environment (Hecky and Kilham 1988, Rudek et al. 1991, Fisher et al. 1992, Sterner 1994, Pinckney et al. 1997). Elser and Kimmel (1985) suggested that longitudinal differences in water quality might arise from riverine inflow, whereas others have implied that nutrient point sources, tidal mixing with marine waters, and biological interactions are responsible (Kivi et al. 1993, Sterner 1994). The proximity of sites to areas of sediment nutrient recycling (which can vary depending on sediment types, organic loading, water depth, and mixing) may also induce phytoplankton community patchiness (Sterner 1994, Pinckney et al. 1997). In Wilson Inlet it was likely that spatial variability in the severity of phytoplankton nutrient limitation was due to patchiness in the supply of dissolved inorganic nutrients, which were probably supplied through processes such as riverine input, ground water supply, sediment recycling, remineralization, and point sources.

Managing spring nutrient regeneration. The bioassay data suggest that Wilson Inlet would be oligotrophic throughout the year, if during spring there was no anoxic event. Without the prolonged periods of anoxia in spring, the pool of nutrients in the sediments would probably remain out of reach for water column production, and thus phytoplankton blooms would be unlikely. Ecological management of the spring phytoplankton bloom should therefore be poised toward controlling the processes that lead to the flux of nutrients in Wilson Inlet.

The sediments are obviously not a permanent nutrient sink in Wilson Inlet, and thus it is likely that most of the organic matter that accumulates there during the year is recycled back into the water column during spring. Under stratified conditions during spring the microbial decomposition of the accumulated supply of carbon would exert an enormous biological oxygen demand on the saline bottom layer, thereby causing rapid deoxygenation. The nutrient complexes formerly associated with the decomposing organic material would then provide a large supply of regenerated nutrients, later used by the phytoplankton community to bloom. A potential management option would be to remove the organic material before its accumulation at the sediments and before the system becomes stratified, which would thereby reduce the biological oxygen demand and subsequent anoxia. Decomposing seagrass biomass provides a large proportion of the labile organic material available at the sediments during spring (Lukatelich et al. 1987). The removal of seagrass biomass at the end of the growing season would provide one method of reducing the accumulation of organic material at the sediments.

The major factor influencing the nutrient dynamics in Wilson Inlet was the opening of the sandbar in late winter. The influx of marine water after the bar had opened led to the rapid development of salinity stratification, and subsequently this event was closely associated with the anoxic release of sediment nutrients. A potential management option to control the flux of nutrients from the sediments would be to destratify the water column after the bar has been opened. This would effectively ensure enough oxygen at the sediment–water interface to supply the high biological oxygen demand from the microbial decomposition of organic material and thereby restrict breakdown of nutrient complexes and nutrient flux from the sediments.

Finally, perhaps the most effective management option is to reduce nutrient import to the inlet and increase nutrient export. Although the control of nutrient imports is a slow process, which requires the implementation of integrated catchment management schemes, it would ensure that fewer nutrients would be available for recycling during spring. The export of nutrients may be achieved through harvesting biomass, dredging sediments, and improving flushing to the sea.

The assistance from the Water and Rivers Commission of Western Australia in providing the water quality data for this study is gratefully acknowledged. In particular we thank Geoff Bastyan for his field assistance, Linda Kalnajais for the collation of the physical and chemical data, and Wasele Hosja and Sarah Grigo for phytoplankton taxonomy and enumeration. We also thank the CSIRO Marmion Marine Laboratories for the provision of laboratory equipment. Supported by the National Eutrophication Management Program (NEMP) and the Water and Rivers Commission of Western Australia.

Bostrom, B. & Pettersson, K. 1982. Phosphorus release from lake sediments in laboratory experiments. *Hydrobiologia.* 92:415–29.

Callender, E. & Hammond, D. E. 1982. Nutrient exchanges across the sediment-water interface in the Potomac River estuary. *Est. Coast. Shelf Sci.* 15:395–413.

Carlton, R. G. & Wetzel, R. G. 1988. Phosphorus flux from lake sediments: effects of epipelic algal oxygen production. *Limnol. Oceanogr.* 33:562–70.

Chang, C. C. Y., Kuwabara, J. S. & Pasilis, S. P. 1991. Phosphate and Iron limitation of phytoplankton biomass in Lake Tahoe. *Can. J. Fish. Aquat. Sci.* 49:1206–15.

Cloern, J. E. 1987. Turbidity as a control on phytoplankton biomass and productivity in estuaries. *Cont. Shelf Res.* 7:1367–81.

Currie, D. J. 1984. The relative importance of bacterioplankton in phosphorus uptake in freshwater. *Limnol. Oceanogr.* 29:311–21.

D'Elia, C. F., Sanders, J. G. & Boynton, W. R. 1985. Nutrient enrichment studies in a coastal plain estuary: phytoplankton growth in large-scale, continuous cultures. *Can. J. Fish. Aquat. Sci.* 43:397–415.

Elser, J. J. & Kimmel, B. L. 1985. Nutrient availability for phytoplankton production in a multiple-impoundment series. *Can. J. Fish. Aquat. Sci.* 42:1359–70.

Elser, J. J., Marzolf, E. R. & Goldman, C. R. 1990. Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: a review and critique of experimental enrichments. *Can. J. Fish. Aquat. Sci.* 47:1468–77.

Fisher, T. R., Harding, L. W., Stanley, D. W. & Ward, L. G. 1988. Phytoplankton, nutrients, and turbidity in the Chesapeake, Delaware, and Hudson estuaries. *Est. Coast. Shelf Sci.* 27:61–93.

Fisher, T. R., Peele, E. R., Ammerman, J. W. & Harding, L. R. 1992.

Nutrient limitation of phytoplankton in Chesapeake Bay. *Mar. Ecol. Prog. Ser.* 82:51–63.

Graneli, E., Wallstrom, K., Larsson, U., Graneli, W. & Elmgren, R. 1990. Nutrient limitation of primary productivity in the Baltic Sea area. *Ambio* 19:142–51.

Harrison, P. J., Hu, M. H., Yang, Y. P. & Lu, X. 1990. Phosphate limitation in estuarine and coastal waters of China. *J. Exp. Mar. Biol. Ecol.* 140:79–87.

Harrison, P. J., Waters, R. E. & Taylor, F. J. R. 1980. A broad spectrum artificial seawater medium for coastal and open ocean phytoplankton. *J. Phycol.* 16:28–35.

Hecky, R. E. & Kilham, P. 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: a review of recent evidence on the effects of enrichment. *Limnol. Oceanogr.* 33: 796–822.

Hodgkin, E. P. & Clark, R. 1988. *Estuaries and Coastal Lagoons of the South Western Australia: Wilson, Irwin and Parry Inlets, the Estuaries of the Denmark Shire.* Environmental Protection Authority, Perth, Western Australia. Estuarine Studies Series. Number 3.

Hosja, W. & Deeley, D. 1994. *Harmful Phytoplankton Surveillance in Western Australia.* Waterways Commission, Perth. .

Humphries, R. B., Robertson, J. G. M. & Robertson, F. E. 1982. *A Resource Inventory and Management Information System for Wilson Inlet, Western Australia.* Dept. Conservation & Environment, Perth, Western Australia. Bulletin. 132.

Jensen, M. H., Lomstein, E. & Sørensen, J. 1990. Benthic NH_4^+ and NO_3^- flux following sedimentation of a spring phytoplankton bloom in Aarhus Bight, Denmark. *Limnol. Oceanogr.* 61:87–96.

Kivi, K., Kaitala, S., Kuosa, H., Kuparinen, J., Leskinen, E., Lignell, R., Marcussen, B. & Tamminen, T. 1993. Nutrient limitation and grazing control of the Baltic plankton community during annual succession. *Limnol. Oceanogr.* 38:893–905.

Krom, M. D., Kress, N. & Brenner, S. 1991. Phosphorus limitation of primary productivity in the eastern Mediterranean Sea. *Limnol. Oceanogr.* 36:424–32.

Laws, E. A. & Bannister, T. T. 1980. Nutrient- and light-limited growth of *Thalassiosira fluviatilis* in continuous culture, with implications for phytoplankton growth in the ocean. *Limnol. Oceanogr.* 25:457–73.

Lenanton, R. C. J. 1974. *Wilson Inlet: A Seasonally Closed Western Australian South Coast Estuary.* Dept. Fisheries & Fauna, Perth, Western Australia. Report no. 14.

Lin, C. K. & Schelske, C. L. 1981. Seasonal variation of potential nutrient limitation to chlorophyll production in Southern Lake Huron. *Can. J. Fish. Aquat. Sci.* 38:1–9.

Lukatelich, R. J., Schofield, N. J. & McComb, A. J. 1986. *The Nutrient Status of Wilson Inlet (1984–1985).* Dept. Conservation & Environment, Perth, Western Australia. Report no. 9.

Lukatelich, R. J., Schofield, N. J. & McComb, A. J., 1987. Nutrient loading and marcophyte growth in Wilson Inlet, a barbuilt southwestern Australian estuary. *Est. Coast. Shelf Sci.* 24: 141–65.

Maestrini, S. Y., Bonin, D. J. & Droop, M. R. 1984. Phytoplankton as indicators of sea water quality: bioassay approaches and protocols. *In* Shubert, L. E. [Ed.] *Algae as Ecological Indicators.* Academic Press, New York, pp. 71–131.

Malone, T. C., Crocker, L. H., Pike, S. E. & Wendler, B. W. 1988. Influences of river flow on the dynamics of phytoplankton production in a partially stratified estuary. *Mar. Ecol. Prog. Ser.* 48:235–49.

Marshall, J. 1993. *Physical Processes of Wilson Inlet.* Coastal and Hydraulic Engineering Laboratory, Centre for Water Research, University of Western Australia, Nedlands, Western Australia. Report.

McComb, A. J. & Lukatelich, R. J. 1995. The Peel-Harvey estuarine system, Western Australia. *In* McComb, A. J. [Ed.] *Eutrophic Shallow Estuaries and Lagoons.* CRC Press, London, pp. 5–17.

Paerl, H. W. & Bowles, N. D. 1987. Dilution bioassays: their application to assessments of nutrient limitation in hypereutrophic waters. *Hydrobiologia* 146:265–73.

Paerl, H. W., Rudek, J. & Mallin, M. A. 1990. Stimulation of phytoplankton production in coastal waters by natural rainfall inputs: nutritional and trophic implications. *Mar. Biol.* 107: 247–54.

Parslow, J. S., Harrison, P. J. & Thompson, P. A. 1984. Saturated uptake kinetics: transient response of the marine diatom *Thalassiosira pseudonana* to ammonium, nitrate, silicate or phosphate starvation. *Mar. Biol.* 83:51–9.

Parsons, T. R., Maita, Y. & Lalli, C. M. 1984. *A Manual of Chemical and Biological Methods for Seawater Analysis.* Pergamon Press, New York, 173 pp.

Pennock, J. R. & Sharp, J. H. 1994. Temporal alteration between light- and nutrient-limitation of phytoplankton production in a coastal plain estuary. *Mar. Ecol. Prog. Ser.* 111:275–88.

Pinckney, J. L., Millie, D. F., Vinyard, B. T. & Paerl, H. W. 1997. Environmental controls of phytoplankton bloom dynamics in the Neuse River estuary, North Carolina, U.S.A. *Can. J. Fish. Aquat. Sci.* 54:2491–501.

Pomeroy, L. R., Shenton, L. R., Jones, R. D. H. & Riemhold, R. J. 1972. Nutrient fluxes in estuaries. *In* Likens, G. E. [Ed.] *Nutrients and Eutrophication.* American Society of Limnology and Oceanography. Special Symposium. Lawrence, Kansas.

Prichard, D.W. 1967. What is an estuary: Physical viewpoint. *In* Lauff, G. H. [Ed.] *Estuaries.* American Association of Science, Washington, DC, pp. 3–5.

Redfield, A. C. 1958. The biological control of chemical factors in the environment. *Am. Sci.* 46:205–22.

Revsbech, N. P., Jørgensen, B. B., Blackburn, T. H. & Cohen, Y. 1983. Microelectrode studies of the photosynthesis, and O_2, H_2S and pH profiles of a microbial mat. *Limnol. Oceanogr.* 28:1062–74.

Reynolds, C. S. 1984. *The Ecology of Freshwater Phytoplankton.* Cambridge University Press, New York, 384 pp.

Rudek, J., Paerl, H. W., Mallin, M. A. & Bates, P. W. 1991. Seasonal and hydrological control of phytoplankton nutrient limitation in the lower Neuse River Estuary, North Carolina. *Mar. Ecol. Prog. Ser.* 75:133–42.

Siep, K. L. 1994. Phosphorus and nitrogen limitation of algal biomass across trophic gradients. *Aquat. Sci.* 56:16–28.

Smayda, T. 1974. Bioassay of the growth potential of the surface water of lower Narragansett Bay over an annual cycle using the diatom *Thalassiosira pseudonana. Limnol. Oceanogr.* 19:889–901.

Smith, R. E. H. & Kalff, J. 1981. The effect of phosphorus limitation on algal growth rates: evidence from alkaline phosphate. *Can. J. Fish. Aquat. Sci.* 38:1421–7.

Smith, V. H. 1982. The nitrogen and phosphorus dependence of algal biomass in lakes: an empirical and theoretical analysis. *Limnol. Oceanogr.* 27:1101–12.

Sterner, R. W. 1994. Seasonal and spatial patterns in macro- and micronutrient limitation in Joe Pool Lake, Texas. *Limnol. Oceanogr.* 39:535–50.

Storch, T. A. & Dietrich, G. A. 1979. Seasonal cycling of algal nutrient limitation in Chautauqua Lake, New York. *J. Phycol.* 15: 399–405.

Suttle, C. A. & Harrison, P. J. 1988. Ammonium and phosphate uptake rates, N:P supply ratios, and evidence for N and P limitation in some oligotrophic lakes. *Limnol. Oceanogr.* 33:186–202.

Thompson, P. A. & Hosja, W. 1996. Nutrient limitation of phytoplankton in the upper Swan River Estuary, Western Australia. *Aust. J. Mar. Freshw. Res.* 47:659–67.

Thompson, P., Adeney, J. & Gerritse, R. 1997. Phytoplankton of the Swan River: research results 1993 to 1996 and management implications. *In* Davis, J. R. [Ed.] *Managing Algal Blooms: Outcomes from CSIRO's Multi-divisional Blue-Green Algal Program.* CSIRO Land and Water, Canberra, pp. 1–14.

Vincent, W. F., Wurtsbaugh, W., Vincent, C. L. & Richardson, P. J. 1984. Seasonal dynamics of nutrient limitation in a tropical high-altitude lake (Lake Titicaca, Peru-Bolivia): application of physiological bioassays. *Limnol. Oceanogr.* 29:540–52.