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Regulation of phytoplankton primary production along a hypernutrified estuary

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ABSTRACT: The influence of nutrients on algal biomass and primary production was investigated in the estuary of the River Colne, a turbid hypernutrified estuary on the east coast of England. Different approaches were used to examine nutrient regulation of both algal biomass formation and algal primary production in the Colne Estuary. Stoichiometric nutrient ratios indicated that silicate was always potentially limiting to biomass relative to nitrogen and phosphorus, and there were only small numbers of diatoms present in the phytoplankton community. Addition of silicate stimulated diatom growth. At the freshwater end of the estuary, low N:P ratios resulting from the input of effluent from sewage treatment works with high P levels created a potential for N limitation of algal biomass production. The N:P ratios at the seaward end of the estuary were much higher, suggesting greater potential for P limitation of algal biomass formation. This is the reverse of the usual assumption for P limitation in freshwater and N limitation in seawater. Nutrient depletion experiments corroborated the indication of greater N limitation of phytoplankton in the upper estuary. Ammonium rather than nitrate was used first by the algae despite being at much lower concentrations than nitrate. *F*-ratios measured with ^{15}N ammonium or nitrate showed that, except on 1 occasion in the upper estuary, >95 % of the N uptake was from ammonium. Despite the high nutrient concentrations in the estuary, algal productivity was strongly limited by light availability. This, combined with the freshwater flushing time for the Colne Estuary (14 d), probably meant that depletion of nutrients and imposition of nutrient limitation on algal growth would only occur outside the estuary in the coastal zone.

KEY WORDS: Phytoplankton · Eutrophication · Nutrient limitation · Nutrient depletion

INTRODUCTION

Hypernutrification and eutrophication of coastal ecosystems are consequences of increased anthropogenic inputs of nutrients such as dissolved nitrate and phosphate (Fisher et al. 1995, Lohrenz et al. 1997, Kinney & Roman 1998). Because of the problems associated with algal blooms, the factors regulating phytoplankton primary production in estuaries are attracting considerable attention (Harding 1994, Harding & Perry 1997). The debate about nutrient-limited phytoplankton production and eutrophication in estuarine and marine ecosystems (e.g. Hecky & Kilham 1988) has often been confused by imprecise definitions of what aspect of algal activity is being limited and the scales on which nutrient limitation takes place (Malone et al. 1996, Underwood & Kromkamp 1999). Regulation of estuarine phytoplankton primary production is complicated owing to the heterogenous conditions within estuaries, which exhibit strong chemical concentration gradients between their freshwater and marine ends. Phosphorus availability usually limits primary production in freshwaters while the marine environment is usually regarded as being N limited (Ryther & Dunstan 1971). Along an estuarine gradient, therefore, there may be a change from P regulation at the freshwater end to N regulation at the seaward end (Doering et al.

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1995). Seasonal variation may also regulate estuarine primary production in estuaries (D'Elia et al. 1986, Fisher et al. 1992, 1995, Pennock & Sharp 1994). Furthermore, the assumption that a single variable may regulate a biological process, including primary production, may not be valid. For example, primary production may be regulated simultaneously by light and nitrogen (MacIsaac & Dugdale 1969, 1972) or light and phosphate (Reshkin & Knauer 1979), while more than 1 nutrient may interact synergistically to simultaneously regulate primary production by coastal phytoplankton (Davies & Sleep 1986, Davies 1988).

As with other estuarine systems, the Colne Estuary has been subject to overenrichment due to nutrient inputs, especially nitrate and ammonium from the river and sewage treatment works, and exhibits a strong downstream decrease in nutrient concentrations. The nutrient inputs and nitrogen budget of the estuary are relatively well defined (Robinson 1996, Ogilvie et al. 1997, Dong et al. 2000). The dissolved inorganic nutrient concentrations, seasonal and spatial variation and light regulation of the rate of surface primary production and of biomass (as chl *a* concentration) have also been studied in the estuary (Kocum et al. 2002, this issue). The present work was undertaken to investigate the factors regulating primary production and their temporal and spatial variation along the Colne Estuary. A variety of approaches were taken to investigate the relative importance of environmental variables, including availability of major nutrients relative to phytoplankton requirements, multiple regression models of primary production, and nutrient addition, nutrient depletion and nitrogen preference experiments (^{15}N-uptake experiments).

MATERIALS AND METHODS

Sampling sites. The work was carried out in the estuary of the River Colne, Essex, UK. The general characteristics of the estuary and the 4 sampling sites studied, from Brightlingsea (Site 1) at the seaward end through Aldboro Point (Site 2) and Wivenhoe (Site 3) to the Hythe at Colchester (Site 4) at the river end of the estuary, have been described in Kocum et al. (2002). Water samples were always collected at high tide. The water column is well mixed, with no stratification, and only surface water samples were taken.

Determination of potential stoichiometric nutrient limitation along the Colne Estuary. Water samples (quadruplicates from each site) were taken at high tide at monthly intervals from all 4 sites along the estuary from June 1994 to November 1995 (see Kocum et al. [2002] for details of the chemical analyses). Indication of whether the nutrient ratios (N:Si:P) in the water were likely to be potentially limiting to algal growth was determined by comparison with the Redfield ratio (16:16:1, N:Si:P). A molar N:P ratio of <10 was used to indicate potential nitrogen limitation and a molar ratio of >20 was used to indicate potential phosphorus limitation of algal biomass formation (Justic et al. 1995).

Multiple regression analyses of primary production rates along the estuary. A number of stepwise multiple regression (SMR) models were constructed to examine factors apparently correlating with primary production, and hence possibly regulating it. The models had either primary production rate or algal biomass (chl *a* concentration) as the dependent variable, and temperature, salinity, critical mixing ratio (Z_{mix}:Z_{eu}), average light intensity in the water column (I_{av}), and dissolved inorganic nutrient concentrations (total oxidised nitrogen, ammonium, phosphate, silicate) as possible independent variables which might correlate with the dependent variable. The statistical analysis focused on determining the relative effects of major physical and chemical variables on primary production data. In order to achieve this, the stepwise multiple regression function of the Statistical Package for Social Sciences (SPSS) computer programme was used. This enabled construction of models to determine which combinations of variables could best explain the most variation in phytoplankton production or biomass in the estuary. Strictly, regression analysis depends upon the dependent variable being directly influenced by the independent variables, a condition that may be difficult to establish in ecological data sets. Where a direct causal relationship cannot be established, caution should be used in interpreting results, and a direct causal link should not be assumed. However, this does not prevent the use of the technique to assess the relative impact of different variables without assuming a direct causal link (e.g. Masson et al. 2000).

The SPSS regression takes various steps to avoid colinearity (a common problem of regression analysis which interferes with the predictive ability of the dependent) by calculating tolerance (the proportion of variability not explained by the other variables, 1-R^2) of a variable with other independent variables already in the equation before taking it into the equation. Thus, the tolerances of all variables in the equation are recomputed at each step. The minimum probability of F (p_{IN}) for a variable to enter the equation was set at 0.05 and probability of F to remove a variable (p_{OUT}) from the equation was set at 0.1. As multiple regression analysis assumes normal distributions of the variables, data were log-transformed before being used in the regression analyses.

Based on these criteria, stepwise multiple regression (SMR) was used to develop the models, using the given set of initial variables, and to determine which vari-

ables were significantly correlated with the rate of primary production either in the estuary as a whole (the complete data set), in different parts of the estuary (data from each sampling site) or for the data seasonally divided into winter (<11°C, November to March inclusive) and summer (April to October inclusive) periods. The multiple regression coefficient of each variable included in the model was used in an equation to predict the magnitude of primary production by multiplying the actual magnitude of the variable with the corresponding coefficient that the model yielded. The fit of each model to actual data was determined by the magnitude of R^2 of the model.

In order to determine the apparent relative impact of each variable tested the magnitude of the standardised multiple regression coefficients (also called β coefficients) of each variable were calculated. β coefficients are the regression coefficients when all variables are expressed in standardised (*z*-score) form. Transforming the independent variables to a standardised form makes the coefficients directly comparable to each other in magnitude, since all are in the same unit.

Nutrient addition experiments. From February to November 1996, the effects of nutrient additions on the rate of primary production (as ^{14}C uptake), on biomass (as changes in chl *a* concentration) and on chl *a*-normalised primary production (P^B) were examined. Experiments were carried out with water collected from the Hythe and Brightlingsea. Water samples were analysed first for *in situ* concentrations of ammonium, nitrate, nitrite, phosphate and silicate, and then the samples were supplied with nutrients from stock solutions of $NaNO_3$, NH_4Cl, KH_2PO_4 and $NaSiO_3$ solutions and a mixture of nitrogen, phosphate and silicate in order to examine the algal response to nutrient supplementation of N, P or Si. Those samples with N:P ratio <10 (potentially N limited) had the N concentration increased until N:P was 16. Nutrient-enriched and control (unenriched) samples (all in triplicate) were held in 10 l glass flasks (previously acid-washed and rinsed with ultra-high-purity water) in a water bath at *in situ* temperature. The light intensities applied were determined to be saturating for the algae in the water samples from *P* versus *E* curves determined at the same time as the nutrient enrichment experiment (see Kocum et al. 2002).

Response to experimental nutrient enrichment was determined after 2 d. Water samples from control and nutrient-supplemented mesocosms were removed and chl *a* concentrations were measured as described in Kocum et al. (2002). At the same time, triplicate subsamples of water from each treatment were taken to determine whether nutrient supplementation had any effect on primary production rates. Primary production was measured by addition of 100 µl of 5 µCi (185 kBq ml^{-1}) $NaH^{14}CO_3$ solution (see Kocum et al. 2002 for further details) to each bottle. The water samples were incubated for 2 h after addition of the radioisotope, under the same light intensity as used for the mesocosms. The samples were then filtered through membrane filters (Whatman GF/F papers, pore size 0.7 µm) and the radioactivity incorporated into the cells was measured as described by Kocum et al. (2002).

The mean final chl *a* concentration, ^{14}C uptake rate and mean P^B in the control and enriched samples were compared by 2-way ANOVA (Systat) followed by post-hoc Tukey tests. Any significance increase in chlorophyll *a* concentration, primary production and P^B of an enriched sample over those of control samples was regarded as a positive response to the added nutrient, and the added nutrient therefore was considered as being limiting.

At the end of nutrient addition experiments, the phytoplankton present in all water samples were identified with light microscopy to determine any changes in phytoplankton species composition due to nutrient additions compared to control samples.

Nutrient depletion experiments. On 4 occasions between May and September 1996, water samples (20 l) were taken at high tide from both ends of the estuary (Hythe and Brightlingsea). The samples from each site were kept in glass containers in a water bath at *in situ* temperature, under a 12:12 h dark:light cycle. A saturating light intensity was chosen for each occasion from a *P* versus *E* curve experiment (see Kocum et al. 2002) when each depletion experiment was carried out. The mesocosms were purged continually with humidified and filtered air. Samples of water were taken from each mesocosm at 24 h intervals for up to 14 d to determine the changes in nutrient (ammonium, nitrate, nitrite, phosphate and silicate) and chlorophyll *a* concentrations.

Measurements of *F* ratio by uptake of ^{15}N nitrate and ammonium. In order to determine the relative importance of ammonium or nitrate as a nitrogen source for phytoplankton, water was taken on 3 occasions (June and September 1996 and May 1997) at high tide from the Hythe and Brightlingsea. Triplicate light and dark samples (in 250 ml glass bottles) were supplemented with either $Na^{15}NO_3$ (99.3% ^{15}N; Europa Scientific, Crewe, UK) or $^{15}NH_4Cl$ (99.3% ^{15}N, Europa Scientific) to 10% of the *in situ* concentration of nitrate or ammonium, respectively, and triplicates were incubated both in the light and in the dark to detect any ^{15}N incorporation which might not be due to algae. In addition, light and dark controls unsupplemented with ^{15}N were also included. The bottles were incubated as for the nutrient addition experiments, under saturating light and at *in situ* temperature. After 2 h incubation, water samples were filtered through

aluminium oxide filters (Anodisc, pore size 0.2 µm, Whatman, UK), dried, and stored at –20°C. The at.% excess of ^{15}N in the samples was measured at the NERC Mass Spectrometry Unit at the Institute for Terrestrial Ecology, Merlewood, Cumbria, UK. The preference of the algae for ammonium or nitrate was determined from calculating the *F*-ratio according to Harrison (1983). Values of $F > 0.5$ indicate preferential uptake of nitrate, while those < 0.5 indicate preferential uptake of ammonium.

RESULTS

Stoichiometric nutrient limitation

Molar N:P values along the Colne Estuary are shown in Fig. 1. Total inorganic nitrogen (TIN) was predominantly nitrate, and the TIN:PO_4^- ratios were generally greatest at Brightlingsea, with more values >20, indicating an increased tendency towards potential P limitation down the estuary. Most TIN:PO_4^- ratios at Hythe and Wivenhoe were in the 10 to 20 range, providing no clear indication of potential limitation of either N or P. During winter and spring N:P ratios increased along the estuary, reaching peak values of 279 in May 1995 at Brightlingsea, while at the same time in the upper estuary ratios were >20. When ratios of individual nitrogen sources (as nitrate or ammonium) to phosphate were calculated NO_3^-:PO_4^- ratios were higher than the NH_4^+:PO_4^-. The NH_4^+:PO_4^- ratios ranged between <0.1 in October 1994 at Brightlingsea and >20 in October 1995 at the Hythe. At the Hythe, the ratios were <10 except for May and October 1995, and NH_4^+:PO_4^- ratios were <10 at the 3 lower sampling sites except for October 1995.

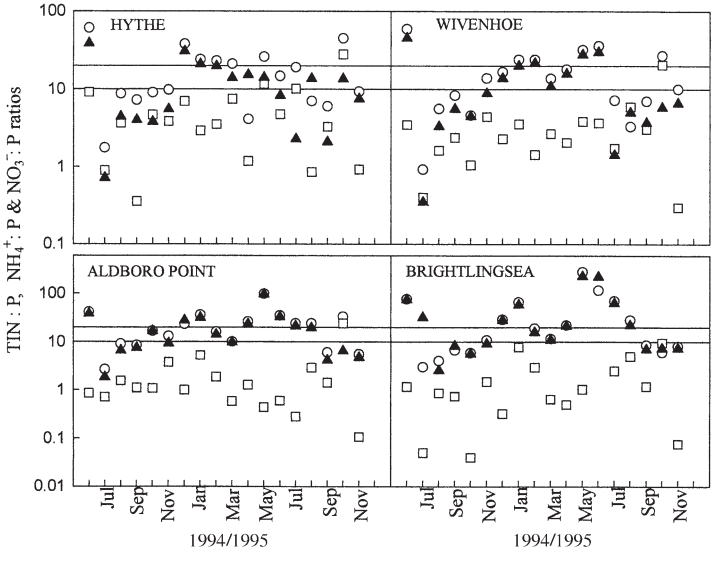

Fig. 1. Molar ratios of total inorganic nitrogen, TIN (= NH_4^+ + NO_3^-+NO_2^-) to phosphate (○), NH_4^+:PO_4^{2-} (□) and NO_3^-:PO_4^{2-} (▲) at 4 sites in the Colne estuary between June 1994 and November 1995. Horizontal lines indicate where N:P is 10 and 20, i.e. >20 indivates potential P limitation, and <10 indicates potential N linitation

Stepwise multiple regression analyses

The results of the multiple regression analyses of primary production rates and algal biomass (chlorophyll *a*) are shown in Tables 1 & 2.

Primary production

With the complete data set, SMR accounted for 38% of the total annual variance at all sites, increasing to 67% of the variance at all sites during summer. Segregation of the data either into sites or seasons increased the percentage of the variance explained. For the annual data (with the exception of the Hythe), temperature was the most significant variable with the largest β values of the significant variables. Phosphate and silicate concentrations were significant at the 2 upper sites. Apart from the significance of temperature in the annual data, there was no consistent pattern of any particular variables being significantly associated with production rates at all sites.

Chlorophyll *a*

The complete data set accounted for 59% of the variance in the total data set (Table 2), but only 40% of the summer data, when chlorophyll concentrations were highest. Temperature again dominated as the most influential variable, with highest β values, but there was generally a strong inverse relationship between chlorophyll and silicate, particularly in the summer data set.

Nutrient addition experiments

Table 3 summarises the results of the nutrient addition experiments on the rate of primary production, chlorophyll *a* concentration and P^B measured under saturating irradiances. Primary production rates were never stimulated by addition of P alone, but were generally stimulated by additions of N or mixtures of nutrients. When only mixtures of nutrients stimulated production, colimitation by more

Table 1. Standardised multiple regression analysis of primary production data, showing statistically significant variables. β is standardised multiple regression coefficient. TON: total oxidised nitrogen (nitrate + nitrite); I_{av}: monthly average light intensity; $Z_{mix:eu}$: critical mixing ratio

Site	Annual			Winter			Summer		
	Variable	β	R^2	Variable	β	R^2	Variable	β	R^2
Entire estuary	Temperature	0.71	0.38	Ammonium	−0.47	0.39	Temperature	0.82	0.67
	TON	0.44		Phosphate	0.53		TON	0.30	
	Phosphate	−0.25		$Z_{mix:eu}$	−0.25				
	I_{av}	−0.13		TON	−0.40				
Brightlingsea	Temperature	0.64	0.37	$Z_{mix:eu}$	−0.77	0.78	Ammonium	0.97	0.48
	Ammonium	0.45		TON	−0.69		Silicate	−0.63	
Aldboro Point	Temperature	0.58	0.37	Ammonium	−0.60	0.75	Temperature	0.67	0.65
	$Z_{mix:eu}$	0.30		Phosphate	0.91		$Z_{mix:eu}$	0.28	
				I_{av}	−0.72				
Wivenhoe	Temperature	0.56	0.65	$Z_{mix:eu}$	−0.84	0.74	$Z_{mix:eu}$	0.84	0.78
	$Z_{mix:eu}$	0.37		Silicate	0.28		Ammonium	0.41	
	Phosphate	0.34					Phosphate	0.25	
	Silicate	−0.24							
Hythe	Phosphate	−0.57	0.54	Phosphate	0.7	0.74	Temperature	0.86	0.89
	Silicate	−0.45		Ammonium	−0.65		Ammonium	−0.32	

Table 2. Standardised multiple regression analysis of chl *a* data, showing statistically significant variables. β is standardised multiple regression coefficient. TON: total oxidised nitrogen (nitrate + nitrite); I_{av}: monthly average light intensity; $Z_{mix:eu}$: critical mixing ratio

Site	Annual			Winter			Summer		
	Variable	β	R^2	Variable	β	R^2	Variable	β	R^2
Entire estuary	Temperature	0.58	0.59	Ammonium	0.53	0.63	Temperature	0.51	0.40
	Salinity	−0.23		I_{av}	−0.45		Phosphate	0.46	
	Silicate	−0.40		$Z_{mix:eu}$	0.39				
	Ammonium	0.390		Phosphate	0.40				
Brightlingsea	Temperature	0.58	0.47	$Z_{mix:eu}$	1.16	0.65	Temperature	0.56	0.42
	I_{av}	0.22		I_{av}	0.64		Silicate	−0.27	
Aldboro Point	Temperature	0.57	0.64	Ammonium	0.51	0.68	Temperature	0.67	0.68
	Silicate	−0.39		Silicate	−0.51		Silicate	−0.38	
Wivenhoe	Temperature	0.38	0.64	$Z_{mix:eu}$	0.97	0.56	Temperature	0.79	0.78
	Silicate	−0.36		TON	−0.68		$Z_{mix:eu}$	−0.75	
	I_{av}	0.23					Silicate	−0.70	
	TON	−0.17							
Hythe	Silicate	−0.85	0.75	$Z_{mix:eu}$	0.80	0.65	Silicate	−0.71	0.50
	TON	−0.48							

than 1 nutrient was indicated. Whereas Brightlingsea always showed a response to nutrient addition, on 2 occasions at the Hythe there were no responses to nutrient additions. Additions of nitrate and ammonium stimulated P^B at Brightlingsea and Hythe during June and July (Table 3). During October and November, phosphate addition stimulated P^B at both Brightlingsea and Hythe. Microscope examination indicated some changes in phytoplankton composition in enriched samples compared to controls. Silicate addition increased the number of diatom cells (which were mostly *Cylindrotheca closterium*) in all nutrient addition experiments. Nitrogen additions increased the number of flagellated euglenoids.

Nutrient depletion experiments

The residual nutrient concentrations during the depletion experiments were plotted as percentages of their initial concentrations to identify those nutrients that were depleted most rapidly. As an example, Fig. 2 illustrates a time course for water from the Hythe and Brightlingsea in August, when there was active algal growth and removal of silicate. Ammonium was removed before nitrate started to be used, especially at the Hythe (Table 4). In the nutrient depletion experiments, silicate and ammonium became completely depleted at the Hythe during July and August, while in Brightlingsea samples ammonium was completely

depleted (under detection levels) in 14 d during the May and November depletion experiments.

^{15}N measurements of *F*-ratios

Table 5 shows the results of the *F*-ratios measured at the Hythe and Brightlingsea in June and September 1996 and in May 1997, and corresponding *in situ* concentrations of nitrate and ammonium. At the Hythe both nitrate and ammonium concentrations were always high (>100 µM), being much lower at Brightlingsea. At the Hythe, both summer samples (June 1996 and May 1997) had *F*-ratios which were very low in both light and dark, confirming that the phytoplankton were assimilating >95% ammonium. In contrast, during September at the Hythe, 99% of N assimilation was from nitrate. We can provide no explanation for this result. At Brightlingsea the *F*-ratios were consistently higher than at the Hythe, but nevertheless during June and September 1996, >75% of N was assimilated as ammonium despite *in situ* ammonium concentrations being much smaller than those of nitrate (Table 5). In May 1997 at Brightlingsea, about half of the N was assimilated from each source.

Table 3. Significant increases in primary production, normalised primary production (P^B) and chl *a* concentrations in response to nutrient additions (Mix: addition of N, P and Si) at the freshwater (Hythe) and seawater (Brightlingsea: B'sea) ends of the Colne estuary. Significant responses at the $p \leq 0.05$ level are shown. –: no significant increase; nm: no measurements made

Date	Site	Production (µgC l^{-1} h^{-1})	P^B (µgC µg chl a^{-1} h^{-1})	Chl *a* (µg l^{-1})
6.6.96	Hythe	NO_3^-, Si	NO_3^-, Si	nm
17.7.96	B'sea	NH_4^+, NO_3^- Mix	NH_4^+	NH_4^+, NO_3^-, Mix, P, Si
22.7.96	Hythe	Mix	NH_4^+	Mix
1.10.96	Hythe	–	–	–
14.10.96	B'sea	NH_4^+, NO_3^-, Mix	P, Si	Mix
28.10.96	Hythe	–	–	–
1.11.96	B'sea	Mix	–	–
20.11.96	Hythe	NH_4^+	P, NO_3^-	NH_4^+

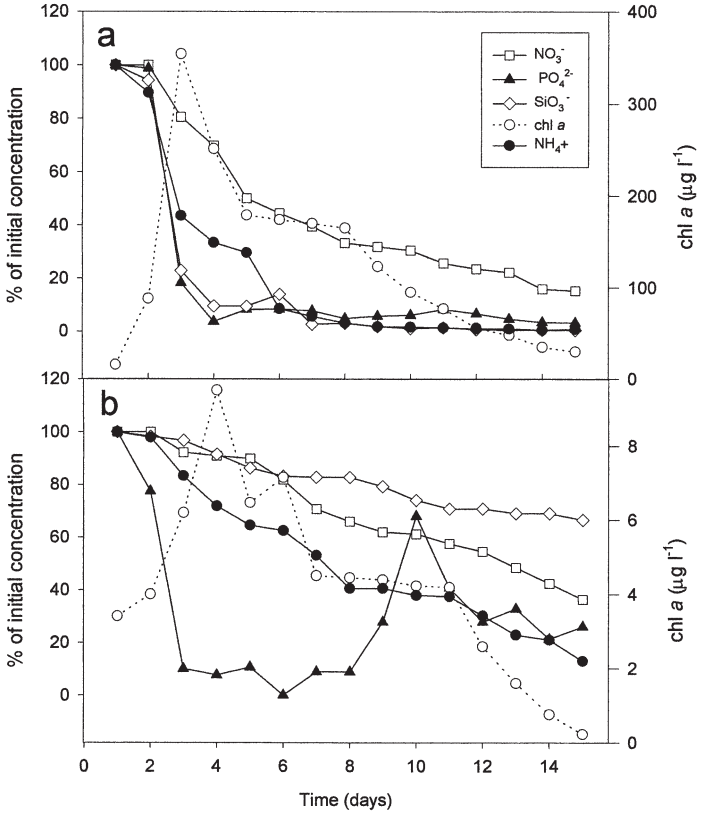

Fig. 2. Changes in nutrient concentrations (NH_4^+, NO_3^-, PO_4^{2-}, SiO_3^-), expressed as percentages of their intial concentrations, and water-column chl *a* concentrations during nutrient run-down experiments from Hythe (a) and Brightlingsea (b) during August 1996

DISCUSSION

Much attention is being paid to the environmental factors which control primary production in estuaries, particularly with respect to the influence of increased nutrient availability from riverine inputs (largely fertiliser run-off) and from inputs from sewage treatment works (STW) effluents (Nedwell et al. 1999). Increased nutrient loads to coastal waters have apparently increased nutrient concentrations, although the effect of this on estuarine and coastal primary production rates is by no means certain (e.g. Reid et al. 1990, Underwood & Kromkamp 1999). The Colne provides a good example of a hypernutrified estuary, with very high nutrient concentrations at the riverine end near the Hythe where there are inputs from both the Colchester STW and from the River Colne (e.g. see King & Nedwell 1987 and Ogilvie et al. 1997).

When considering the effect of nutrients on primary production, it is necessary to define clearly what is meant by 'limiting nutrient', as different measurements give different information. Any consideration of potential nutrient limitation by imbalance between the *in situ* ratios of nutrients such as N, P or Si and the ratio required for balanced growth (usually defined by the Redfield ratio) examines the potential for

Table 4. Nutrient depletion experiments at 2 sites along the River Colne Estuary. Nutrient that was depleted most rapidly, proportionate to its initial concentration, is indicated. –: no experiment performed on that date

Date (1996)	Site 1 (Brightlingsea)	Site 4 (Hythe)
8–20 May 1996	NO_3^-	NH_4^+
5–7 Jul 1996	–	NH_4^+
20 Aug–8 Sep 1996	NH_4^+ NO_3^-	NH_4^+
26 Nov–11 Dec 1996	NH_4^+	NO_3^-

limitation of ultimate biomass production (Liebig-type limitation) not the effect of the nutrient on *in situ* growth rate. Thus, N:P ratios <10 during summer (Fig. 1) were an indicator of potential N limitation of algal biomass formation. Because the N:P ratio in different algae may vary significantly from the Redfield ratio (from 10:1 to as much as 30:1; Boynton et al. 1982, Atkinson & Smith 1983), and may also alter if nutrients are supplied in pulses (Fong et al. 1993), such data should only be considered as indicative. Furthermore, these ratios do not necessarily imply that algal growth rates and primary production rates in the Colne Estuary were N-limited during summer, as the *in situ* nitrogen concentrations (particularly in the upper estuary) could still have been saturating in terms of the concentrations required to support maximum algal growth rates, despite the low N:P ratios. It is only after growth rates start to be limited by lowered nutrient concentrations (Michaelis-Menten or Blackman-type limitation) that Liebig-type limitation of biomass production begins.

The nutrient ratios suggested that Si was always low relative to N and P throughout the estuary, and silicate concentrations were always depleted after the initial spring bloom period, as reported in other estuaries and coastal waters (e.g. Franzs 1986, Justic et al. 1995, del Amo et al. 1997a,b). Such depletion would tend to select against diatoms, which require silicate. Microscopic examination confirmed that the majority of the phytoplankton in the Colne Estuary was euglenophytes and chlorophytes, planktonic diatoms being uncommon in the estuary (Kocum et al. 2002). The stimulation of diatom numbers by silicate additions during the summer nutrient-addition experiments again suggested that this was due to silicate deficiency. The SMRA (Tables 1 & 2) indicated that, at the 3 upper estuarine sites, silica concentrations always seemed to be a major variable inversely associated with high chlorophyll *a* and productivity, particularly during the summer. This again suggested that during the period of greatest production during the summer the algae were silica limited, and that the majority of the production in the summer was not due to diatoms.

Many hypernutrified estuaries become N-limited because of the large input of treated sewage effluent, which tends to have a low N:P ratio (Howarth 1988). The low N:P ratios in the Colne Estuary, particularly during the summer, suggested that algal biomass production would ultimately become limited by N availability, although as the freshwater flushing time of the Colne Estuary is of the order of 14 d the result of such limitation might only become apparent outside the estuary. Despite nitrogen being the nutrient usually assumed to limit primary production in seawater (Ryther & Dunstan 1971, Howarth 1988, Fisher et al. 1992, Doering et al. 1995, Oviatt et al. 1995), the euhaline part of the estuary at Brightlingsea often had higher N:P ratios than the oligohaline, upper estuary at the Hythe. This is opposite to what might be expected in a typical estuary (Pennock & Sharp 1994), but in the Colne Estuary appears to be the result of high inputs of P skewing the ambient nutrient ratios in the upper estuary. The changes in ratios down the estuary indicated that P decreased proportionately more rapidly than N, so that at Brightlingsea P limitation was sometimes evident (Fig. 1). The relatively rapid removal of P through the estuary may be the result of the adsorption of P to particulates in this very turbid estuary with high suspended particulate loads (Prastka et al. 1998, Nedwell et al. 1999) The indication of greater limitation by N at the top of the estuary was corroborated by the results of the nutrient addition and nutrient run-down experiments. The NH_4^+:PO_4^- ratios showed that stoichiometrically ammonium was potentially much more limiting to algal biomass and to production than was nitrate.

The magnitude of the β values of significant variables in the SMRA showed that seasonal changes in temperature had the greatest association with algal primary production, but

Table 5. Summary of the results of ^{15}N experiments with samples collected from Brightlingsea and Hythe. The *F*-ratios and *in situ* concentrations of ammonium and nitrate for each experiment are shown to compare availability of 2 types of nitrogen sources. *F*-ratio >0.5 shows preference for nitrate

Date	*F*-ratio				*In situ* nutrient concentrations (µM)			
	Brightlingsea		Hythe		Brightlingsea		Hythe	
	Light	Dark	Light	Dark	NH_4^+	NO_3^-	NH_4^+	NO_3^-
Jun 1996	0.22	0.12	0.01	0.03	1.4	3	197	190
Sep 1996	0.23	0.32	0.99	0.97	4.2	13	310	147
May 1997	0.5	0.44	0.04	0.11	0.98	21.3	235	150

that seasonal changes in light levels as I_{av} had little association. Our previous work (Kocum et al. 2002) identified light as a major regulator of primary production rates in the Colne Estuary and showed that, on average, phytoplankton photosynthesis was nearly always light-limited. It is therefore surprising that irradiance did not appear as a significant variable influencing either algal biomass or primary production in the SMRA. However, while the peak of irradiance was in June, temperature and algal biomass peaked in July, and primary production peaked in August. This lag of 1 to 2 mo in the response of both the water temperature and the phytoplankton population to the seasonal change of irradiance resulted in an uncoupling of the direct effect of irradiance on phytoplankton, and in an apparently significant effect of temperature. This significant effect is probably dependent on both temperature and algal activity lagging behind seasonal light availability, since when algae are light-limited, rates of photosynthesis are not significantly influenced by temperature (Underwood & Kromkamp 1999). Temperature affects rates of processes, and may therefore influence P^B, but will not directly influence algal biomass which is a function of a variety of variables. Standing crop (as chl *a*) will be influenced by a variety of factors such as flushing time, grazing, as well as algal growth rates. Usually the effect of temperature change on photosynthetic rate or efficiency is small compared to that of change in light intensity, especially when light is limiting (Geel et al. 1997, Underwood & Kromkamp 1999). For example, a Q_{10} response of approximately 2 would suggest that seasonal temperature changes (~10 to 15°C) would affect photosynthesis at most by a factor of ×2 to 4. P^B_{max} tends to be optimum at a temperature of 20 to 25°C, but such changes are not sufficient to explain the patterns observed in the estuary. In comparison, the seasonal differences in algal variables were about 2 to 4 orders of magnitude for chlorophyll *a* concentration, primary production rate and P^B. This provides an example of the difficulty in determining cause and effect in complex ecological systems, and in determining what the true independent and dependent variables are, and therefore underlines the requirement for careful interpretation of the results of SMRA.

The results of the SMRA suggested that algal primary production in the upper estuary was unlikely to be limited by N uptake, and indeed the nutrient concentrations at Sites 3 and 4 were greatly in excess of typical half-saturation values for uptake of these nutrients (K_m 0.1 to 0.5 µM for Si, 0.1 to 0.2 for PO_4^{2-} and 1 to 2 µM for N: Fisher et al. 1992, 1995, van Spaendonk et al. 1993). The nutrient addition experiments also suggested that there was no consistent stimulation of algal activity in response to supplementation by nutrients at the Hythe, where nutrient concentrations were high, although at Brightlingsea, where ambient nutrient concentrations were low, addition of nitrogen or mixed nutrients often caused significant stimulation. In the Cape Fear River estuary, Mallin et al. (1999) also reported that in an oligohaline, nutrient-rich station responses to nutrient additions were rare and primary production was light-limited. They also showed that at various times during summer at a mesohaline station limitation of primary production changed between N limitation, P limitation or N+P colimitation.

Had the nutrient-depletion experiments been continued until nutrients started to be exhausted, they might have indicated which nutrient would ultimately become limiting to biomass production, permitting comparison with predictions derived from nutrient ratios. However, algal growth rates were such that during the course of the experiments (usually 14 d) nutrients were not usually removed completely, thus preventing such comparisons. A prolonged experimental incubation would probably have been of decreasing relevance to the *in situ* situation, as the algal community in the experimental mesocosms would increasingly deviate from a community in the natural environment (Underwood & Kromkamp 1999). The time required to see significant nutrient depletion in these experiments also emphasized that any detectable response by the algae within the estuary to increased nutrients will be strongly influenced by the freshwater flushing time of the estuary, as when flushing is rapid the nutrients may be exported before inducing a detectable biological response. The average freshwater flushing time of the Colne Estuary is 14 d, which suggests that nutrients were unlikely to be significantly depleted by planktonic primary production before they were flushed from the estuary. Monbet (1992) has shown previously that the phytoplankton biomass within an estuary can be related to its flushing time.

Other biological processes, such as benthic primary production or denitrification (King & Nedwell 1987, Ogilvie et al. 1997, Dong et al. 2000), have a more significant impact on depleting at least some nutrients within the estuary than does water column primary production. The depletion experiments indicated (Table 4) that at both ends of the estuary it was the nitrogenous nutrients that were depleted proportionately most rapidly during the experiments, and that, when present, ammonium was removed preferentially to nitrate. This, as with the results of the nutrient addition experiments, suggested that were any nutrient likely to become limiting to growth rates (as opposed to stoichiometric potential for biomass limitation), then this would be nitrogen. A number of other estuaries also demonstrated that primary production in the summer became N-limited (Malone et al. 1996, Gallegos & Jordan 1997).

The importance of ammonium to estuarine phytoplankton production was confirmed by the ^{15}N experiments in which, during the summer throughout the estuary, the *F*-ratios were extremely low. This indicated a large preference for ammonium uptake by the algae, even at the seaward end at Brightlingsea, where the ammonium concentration was always very much lower than that of nitrate. At the Hythe, where ammonium concentrations during summer were often higher than nitrate, >95% of nitrogen was assimilated in the form of ammonium. We can offer no explanation why during September 1996 nitrate was used almost exclusively at the Hythe but not at Brightlingsea, although continued work may shed some light in the future. However, during spring and early summer, when primary production was most rapid (Kocum et al. 2002), ammonium was the most important source of nitrogen for estuarine phytoplankton primary production, corroborating the similar observation in the nutrient depletion experiments.

In conclusion, our previous work (Kocum et al. 2002) indicated the overall controlling importance of light in this hypernutrified and turbid estuary. The generally low N:P ratios, particularly during summer, when primary production was greatest, implied that biomass production would probably become N-limited eventually; and the data from the nutrient depletion experiments and the *F*-ratios suggested that ammonium concentrations were, after light availability, the most important factor regulating algal growth in this hypernutrified estuary. The nutrient depletion experiments, however, indicated that any eventual imposition of this limitation was likely to occur only outside the estuary in coastal seawater, as the time required for algae to deplete nutrients to limiting concentrations was approximately the same as the freshwater flushing time of the Colne Estuary. There was limitation of diatom growth and biomass production by low silicate in the Colne Estuary during summer, microflagellates being the dominant algae after the initial spring bloom.

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