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Evaluation of Kinetics of Nitrogen Uptake by Five Macroalgal Species in Eutrophic Coastal Waters Using ^{15}N

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ABSTRACT*. Kinetics of uptake of nitrate, ammonium, nitrite and urea in light and dark in five intertidal macroalgal species namely Stoechospermum marginatum, Sargassum tenerrimum, Ulva lactuca, Dictyota dichotoma and Padina tetrastomatica from the eutrophic waters of Goa, India were studied using* ^{15}*N as a tracer. Uptake of all the four nutrients by the five algal species followed substrate-saturable kinetics both in light and dark. Ammonium uptake in U. lactuca followed a biphasic pattern, with saturable kinetics up to a substrate concentration of 20 μM and linearity beyond. In most of the species, ammonium was taken up at greater rates than others in light and dark. Uptake of urea ranked generally the lowest. The* V_{max} *values for the four nutrients in all the algae were generally similar in light and dark. The orders of preference (affinity) and efficiencies to take up N in light and dark varied among species. The high uptake rates and safety factors observed indicate that the algae could play an important role in moderating the effects of excess N loading in coastal waters.*

INTRODUCTION

Leaching by rain and mobilization into cultivated crops are the two major mechanisms of removal of nitrogenous nutrients from topsoil in farm lands. Modern agricultural practices tend to compensate this loss by addition of Nitrogen (N) fertilizers (ammonium and nitrate salts, ammonium nitrate and urea), often in excess of immediate requirements. The rapid increase in intensive and extensive application of N fertilizers during last few decades and their subsequent leaching has been responsible for increase in dissolved N concentrations in rivers and lakes, causing eutrophication. However, evaluating the problem of N pollution is more complex, especially in estuaries and coastal waters since there are several sinks for this N (assimilation by phytoplankton, bacterial remineralization, removal by dilution, denitrification and assimilation by coastal vegetation, notably the macroalgae). Among them, the macroalgae, because of their high biomass, could have an important role in mitigating the effects of excess N loading.

This paper examines, using ^{15}N as the tracer, the ability of some common species of marine macroalgae to take up various forms of N at a range of concentrations in light and dark. Some earlier studies that have used a similar approach are those of Williams and Fisher (1985), O'Brien and Wheeler (1987), Dohler *et al.* (1995) and MacGlathery *et al.* (1997). However, the present study was the first study done in tropical waters considering the uptake of all the four assimilable forms of N; nitrate, nitrite, ammonium and urea.

The classical technique in such studies is evaluation of the responses of uptake rates to increasing additions of nutrients. These responses are often best described by Michaelis-Menten kinetics, with the derivation of two constants, V_{max} and K_s. The V_{max} is a measure of the potential rate of removal with which the algae can cope up with greater addition of a nutrient. The half-saturation constant K_s is useful as an indicator of whether or not an increase in available nutrients will increase nutrient acquisition and possibly the growth. The ratio of V_{max} to K_s (designated as α) quantifies the efficiency of nutrient uptake at low substrate concentrations and thus is a measure of the affinity of the algae for a particular nutrient. It is also a useful and reliable parameter compared to V_{max} or K_s alone, to evaluate the competition among macroalgal species for nutrients.

MATERIAL AND METHODS

Five species of intertidal macro-algae namely, *Stoechospermum marginatum* (C. Agardh) Kutzing, *Sargassum tenerrimum* J. Agardh, *Dictyota dichotoma* (Hudson) Lamouroux, *Padina tetrastomatica* Hauck and *Ulva lactuca* Linnaeus were used in this study. The first four are phaeophytes and the last one is a chlorophyte. The algae were collected from the intertidal regions of Goa (15° 35′ N; 73° 44′ E) with their holdfasts intact and brought to the laboratory where they were cleaned with filtered seawater to remove the epiphytes and sediments attached to the thalli. They were then held in filtered seawater with aeration for 1-2 h before experimentation.

Uptake measurements were carried out with approximately 0.5 g of algal thallus placed in beakers with 250 ml of seawater to which ammonium, nitrate, nitrite and urea were added at concentrations of 2, 4, 6, 8, 10, 20, 30, 40, 50 and 60 µM N. The spike in each case was prepared as a 9:1 mixture of unlabelled and ^{15}N-labelled (95-99 atom % excess) N salt. Uptake measurements were made in duplicate at each spike level. During incubation, the beakers were shaken periodically to prevent localized depletion of N in the medium. The incubations were carried out for 1 h at a light intensity of 800 µE/m/s and ambient temperature of 27-28°C. For measurements of uptake in dark, the beakers were covered with aluminum foils.

Algal material removed from the incubation medium was briefly rinsed with deionized water to remove the traces of N adsorbed on the thallus and dried to a constant weight at 70°C. The dried samples were homogenized to fine powder and used for estimation of particulate organic nitrogen (PON) content and ^{15}N enrichment. PON content was determined by Kjeldahl digestion, steam distillation and titration with a Metrohm 665 titrator. At the end of titration, the sample was re-acidified and an aliquot of this was retrieved in a capillary of known volume, dried and taken for measurements of ^{14}N:^{15}N isotopic ratios in a Jasco N-151 Heavy Nitrogen analyzer.

Concentrations of nutrients were measured by the methods described by Bendschneider and Robinson (1952) (nitrite), Wood *et al.* (1967) (nitrate), Koroleff (1970) (ammonium) and Aminot and Keroulé (1982) (urea).

The kinetic constants (V_{max} and K_s) were calculated from linear fits of S/V *vs* S where S is the substrate concentration (µM) and V is the uptake rate (µM/(g dry wt)/h). In all the cases, the fits were significant ($p<0.05$). The significance of the differences in uptake rates of a given nutrient by the five algal species or in uptake rates of different N nutrients by one algal species were tested by analysis of covariance. The safety factor for a given nutrient, which provides a simple estimate of the amount of surplus capacity for its uptake, was calculated as the ratio of its V_{max} to the rate at which it was taken up at the maximum ambient concentration of nutrient.

RESULTS

Uptake kinetics

Uptake of all the four nutrients by the five algal species followed substrate-saturable kinetics both in light and dark (Figs 1-5). Only in *U. lactuca* did ammonium uptake above a substrate concentration of 20 μM deviate from saturation kinetics to linearity in both light and dark.

Table 1. Values of uptake kinetic constants V_{max} (μM N/(g dry wt)/h) and K_s (μM N) and α for different species of alga in light and dark.

N form/Algal species	Light			Dark		
	V_{max}	K_s	α	V_{max}	K_s	α
Nitrate - N						
Stoechospermum marginatum	31.9	9.2	3.4	-	-	-
Sargassum tenerrimum	52.1	0.7	66.8	31.3	1.2	27.0
Ulva lactuca	84.2	8.3	10.2	85.0	11.6	7.3
Dictyota dichotoma	105.4	7.7	13.6	102.6	24.0	4.3
Padina tetrastomatica	32.6	6.7	4.8	34.7	4.4	7.9
Nitrite - N						
S. marginatum	47.4	10.6	4.4	-	-	-
S. tenerrimum	59.3	10.7	5.5	50.3	3.0	16.6
U. lactuca	127.2	6.7	19.0	92.2	4.8	19.0
D. dichotoma	54.2	1.6	33.4	56.9	1.7	32.7
P. tetrastomatica	32.7	2.7	12.0	27.7	1.3	21.4
Ammonium - N						
S. marginatum	96.5	28.8	3.3	-	-	-
S. tenerrimum	84.8	10.2	8.3	51.7	0.9	53.1
U. lactuca	38.6	0.6	65.1	105.8	5.6	18.7
D. dichotoma	161.9	29.5	5.5	54.1	10.7	5.0
P. tetrastomatica	64.3	17.8	3.6	56.3	2.1	26.2
Urea - N						
S. marginatum	46.9	21.7	2.1	-	-	-
S. tenerrimum	21.3	1.1	19.1	16.1	4.8	3.3
U. lactuca	37.2	3.9	9.5	45.6	4.9	9.2
D. dichotoma	20.8	1.9	10.9	22.4	5.9	3.7
P. tetrastomatica	44.7	0.9	49.6	28.7	10.8	2.6

The highest V_{max} values calculated for different species of N were almost similar in light and dark (Table 1). The highest V_{max} values for NO_3^- in light and dark were observed in *D. dichotoma*. With NO_2^-, NH_4^+ and urea, the highest V_{max} values in light were observed respectively in *U. lactuca*, *D. dichotoma* and *S. marginatum* whereas the greatest uptake rates for all three compounds in dark were seen in *U. lactuca*.

In most of the species, ammonium was taken up at greater rates than others in light and dark. Uptake of urea ranked generally the lowest. Differences in the uptake rates between the four nutrients or in the uptake rates of any given nutrient among the five algal species were more often statistically significant (Tables 2-6).

No definite pattern in the affinity to take up a particular N species was observed (Table 1). The highest affinity in light and dark for NO_3^- uptake was observed in *S. tenerrimum* and for nitrite, it was in *D. dichotoma*. In the case of ammonium, *U. lactuca* exhibited the highest affinity in light and *S. tenerrimum,* in dark. *P. tetrastomatica* and *U. lactuca* exhibited maximum affinity for urea uptake in light and dark respectively. The orders of preference (affinity) to take up N species by the 5 species of algae are given in Table 7.

The safety factor was in the range from 2 to 6 for nitrate, 1 to 6 for ammonium, 3 to 22 for nitrite and 1 to 9 for urea (Table 8).

Table 2. Variance ratios of uptake rates between two nutrients in light.

N form	SM		ST		UL		DD		PT	
	F	df	F	df	F	df	F	df	F	df
NO_3^- - NH_4^+	54.8^{**}	1,20	35.6^{**}	1,16	1.4	1,18	11.4^{**}	1,16	21.8^{**}	1,20
NO_3^- - NO_2^-	4.7^{*}	1,20	31.3^{**}	1,16	2.0	1,20	22.9^{**}	1,16	3.8	1,20
NO_3^- - Urea	10.0^{**}	1,20	2.6	1,16	17.6^{**}	1,20	32.4^{**}	1,16	12.7^{**}	1,20
NH_4^+ - NO_2^-	26.2^{**}	1,20	2.8	1,16	2.1	1,18	98.5^{**}	1,16	39.0^{**}	1,20
NH_4^+ - Urea	25.6^{**}	1,20	41.6^{**}	1,16	1.5	1,18	-	1,16	54.9^{**}	1,20
NO_2^- - Urea	0.3	1,20	40.0^{**}	1,16	20.9^{**}	1,20	5.4^{*}	1,16	2.9	1,20

Note: *S. marginatum* - SM; S. *tenerrimum* - ST; *U. lactuca* - UL; *D. dichotoma* - DD; *P. tetrastomatica* - PT; * $p < 0.05$, ** $p < 0.01$; df - degree of freedom.

Table 3. Variance ratios of uptake rates between two nutrients in dark.

N form	ST		UL		DD		PT	
	F	df	F	df	F	df	F	df
NO_3^- - NH_4^+	1.1	1,16	35.3^{**}	1,18	17.9^{**}	1,16	0.5	1,20
NO_3^- - NO_2^-	10.1^{**}	1,16	1.4	1,20	56.6^{**}	1,16	10.8^{**}	1,20
NO_3^- - Urea	1.0	1,16	16.4^{**}	1,20	69.4^{**}	1,16	2.3	1,20
NH_4^+ - NO_2^-	5.9^{**}	1,16	39.8^{**}	1,18	12.3^{**}	1,16	10.9^{**}	1,20
NH_4^+ - Urea	0.0	1,16	83.9^{**}	1,18	18.8^{**}	1,16	0.3	1,20
NO_2^- - Urea	7.1^{**}	1,16	6.2^{**}	1,20	0.4	1,16	25.7^{**}	1,20

Note: S. *tenerrimum* - ST; *U. lactuca* - UL; *D. dichotoma* - DD; *P. tetrastomatica* - PT;. * $p < 0.05$; ** $p < 0.01$; df - degree of freedom..

Table 4. **Variance ratios of N uptake rates between light and dark.**

N form	ST		UL		DD		PT	
	F	df	F	df	F	df	F	df
NO_3^-	0.2	1,16	0.3	1,20	0.4	1,16	0.4	1,20
NH_4^+	33.1^{**}	1,16	47.5^{**}	1,16	49.5^{**}	1,16	17.1^{**}	1,20
NO_2^-	9.0^{**}	1,16	3.6	1,20	0.1	1,16	4.9^{*}	1,20
Urea	5.6^{*}	1,16	1.6	1,20	6.9^{**}	1,16	21.3^{**}	1,20

Note: S. *tenerrimum* - ST; *U. lactuca* - UL; *D. dichotoma* - DD; *P. tetrastomatica* - PT;. * p< 0.05, ** p< 0.01; df - degree of freedom.

Table 5. **Variance ratios of uptake rates of a given N compound between 2 species of algae in light.**

Algal species	NO_3^-		NH_4^+		Urea		NO_2^-	
	F	df	F	df	F	df	F	df
SM- ST	14.4^{**}	1,16	1.1	1,16	71.6^{**}	1,16	1.2	1,16
SM- UL	13.8^{**}	1,16	14.8^{**}	1,16	12.9^{**}	1,16	10.3^{**}	1,16
SM- DD	17.2^{**}	1,16	16.5^{**}	1,16	58.8^{**}	1,16	11.4^{**}	1,16
SM- PT	0.1	1,16	7.3^{**}	1,16	50.4^{**}	1,16	15.4^{**}	1,16
ST- UL	29.8^{**}	1,16	4.6^{*}	1,16	14.5^{**}	1,16	6.3^{**}	1,16
ST- DD	30.7^{**}	1,16	20.0^{**}	1,16	1.4	1,16	17.1^{**}	1,16
ST- PT	11.1^{**}	1,16	1.1	1,16	2.5	1,16	21.1^{**}	1,16
UL- DD	0.8	1,16	23.7^{**}	1,16	9.8^{**}	1,16	22.6^{**}	1,16
UL- PT	14.7^{**}	1,16	5.1^{*}	1,16	7.3^{**}	1,16	24.5^{**}	1,16
DD- PT	18.1^{**}	1,16	37.5^{**}	1,16	0.3	1,16	0.2	1,16

Note: S. *marginatum - SM; S. tenerrimum - ST; U. lactuca - UL; D. dichotoma - DD; P. tetrastomatica – PT*; * p< 0.05, ** p< 0.01; df - degree of freedom.

Stoechospermum marginatum

Uptake of all four N forms for this species followed substrate-saturable kinetics (Fig. 1), with the highest V_{max} for ammonium, followed by those for nitrite, urea and nitrate (Table 1). The rates of uptake of ammonium were significantly higher than those of other 3 nutrients (Table 2) whereas the differences between uptake rates of nitrate, nitrite and urea were not significant. The highest safety factors for nitrite (22.27) and urea (9.68) in light (Table 8) were obtained with these species.

Table 6. Variance ratios of uptake rates of a given N compound between two species of algae in dark.

Algal species	NO_3^-		NH_4^+		Urea		NO_2^-	
	F	df	F	df	F	df	F	df
ST- UL	42.2^{**}	1,16	96.4^{**}	1,16	11.3^{**}	1,16	8.3^{**}	1,16
ST- DD	89.4^{**}	1,16	25.8^{**}	1,16	2.7	1,16	0.7	1,16
ST- PT	9.6^{**}	1,16	6.2^{**}	1,16	17.3^{**}	1,16	10.5^{**}	1,16
UL- DD	1.9	1,16	55.1^{**}	1,16	5.1^{*}	1,16	11.8^{**}	1,16
UL- PT	23.9^{**}	1,16	71.9^{**}	1,16	0.0	1,16	22.3^{**}	1,16
DD-PT	54.8^{**}	1,16	5.7^{*}	1,16	7.1^{**}	1,16	6.5^{**}	1,16

Note: S. *tenerrimum* - ST, *U. lactuca* - UL, *D. dichotoma* - DD, *P. tetrastomatica* – PT; * $p< 0.05$, ** $p< 0.01$; df - degree of freedom.

Sargassum tenerrimum

Substrate saturable kinetics of uptake was observed for all the four N species in light and dark (Fig. 2). The highest V_{max} was observed for ammonium in light followed by almost similar V_{max} for nitrate and nitrite (Table 1). The V_{max} for urea was four times less than that for ammonium in light and dark. Even though the V_{max} for ammonium (84 µM N/(g dry wt)/h) in light was much higher than that for nitrite, it was interesting to note that the V_{max} in dark incubations for both nutrients were almost similar (50 µM N/(g dry wt)/h). The highest safety factor (22.47) was obtained for nitrite in light (Table 8).

Ulva lactuca

Substrate saturable kinetics was observed for all the N species in light and dark except for ammonium (Fig. 3). Ammonium uptake saturated at 20 µM and later became linear. It was interesting to note that the V_{max} for ammonium in dark (105 µM N/(g dry wt)/h) was about thrice times higher than in light (38 µM N/(g dry wt)/h) but the affinity for ammonium in light was comparatively higher than in dark. Almost similar values of V_{max} (84 and 85 µM N/(g dry wt)/h) and α (8 and 11) were obtained in light and dark for nitrate (Tables 1 and 7). While the affinity values for nitrite was twice higher than that for urea, there were no differences in them between light and dark. Differences in uptake between light and dark incubations were pronounced only in the case of ammonium (Table 4). The highest safety factors were obtained for nitrite in both light (14.4) and dark (10.7) (Table 8).

Table 7. Order of preference for N nutrients by the five algal species in light and dark.

Algal species	Order of preference for N	
	Light	Dark
Stoechospermum marginatum	$NO_2^- > NO_3^- > NH_4^+ >$ urea	-
Sargassum tenerrimum	$NO_3^- >$ urea $> NH_4^+ > NO_2$	$NH_4^+ > NO_3^- > NO_2^- >$ urea
Ulva lactuca	$NH_4^+ > NO_2^- > NO_3^- >$ urea	$NO_2^- > NH_4^+ >$ urea $> NO_3^-$
Dictyota dichotoma	$NO_2^- > NO_3^- >$ urea $> NH_4^+$	$NO_2^- > NH_4^+ > NO_3^- >$ urea
Padina tetrastomatica	urea $> NO_2^- > NO_3^- > NH_4^+$	$NH_4^+ > NO_2^- > NO_3^- >$ urea

Table 8. Safety factors for NO_3^-, NH_4^+, NO_2^- and urea at maximum ambient concentrations of 17, 6, 0.5 and 2.45 µM N.

Algal species	NO_3^-		NH_4		NO_2^-		urea	
	Sf	N	Sf	N	Sf	N	Sf	N
Stoechospermum marginatum (L)	1.8	9	5.8	12	22.3	11	9.7	13
Sargassum tenerrimum (L)	3.0	12	2.7	12	22.5	16	1.4	8
S. tenerrimum (D)	1.8	9	1.1	12	7.0	12	2.9	8
Ulva lactuca (L)	4.9	14	1.1	8	14.4	9	2.5	14
U. lactuca (D)	5.0	12	1.9	8	10.7	9	2.9	14
Dictyota dichotoma (L)	6.2	12	5.9	10	4.2	14	1.8	10
D. dichotoma (D)	6.0	12	2.7	9	4.5	13	3.4	12
Padina tetrastomatica (L)	1.9	16	3.9	8	6.4	16	1.3	15
P. tetrastomatica (D)	2.0	17	1.3	6	3.6	12	5.3	11

Note: L - light; D - dark.

Dictyota dichotoma

Uptake of all 4 N nutrients followed substrate-saturable kinetics (Fig. 4). The V_{max} values in light and dark were almost similar for NO_3^-, NO_2^- and urea where as in the case of ammonium, V_{max} in light was thrice higher than in dark (Table 1). Affinity for nitrite in light and dark was of the same order (α=32) and was the highest among all. Uptake rates of different N forms were in general significantly different except in the case of ammonium and urea in light and nitrite and urea in dark (Tables 2 and 3). Among the four nutrients only ammonium and urea showed significant differences in uptake rates between light and dark (Table 4). Safety factors for NO_3^- and NO_2^- were similar in light and dark whereas in the case of NH_4 and urea the fifty factors were twice higher in light than in dark (Table 8).

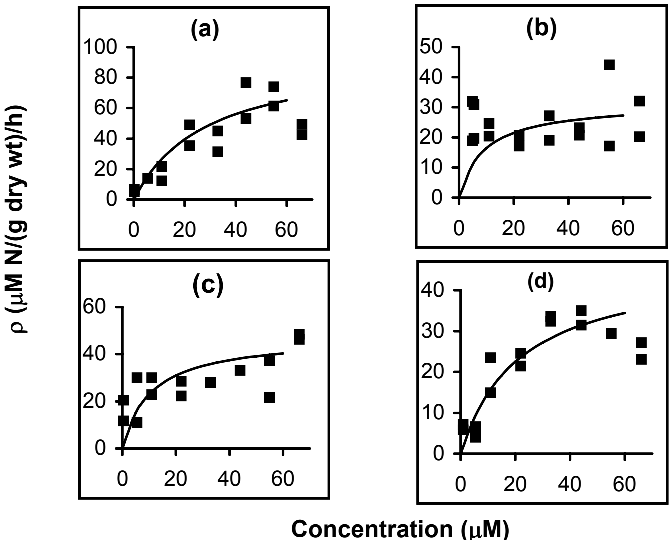

Fig. 1. Rates of uptake of NH_4^+ (a), NO_3^- (b), NO_2^- (c) and urea (d) as a function of substrate concentrations in *Stoechospermum marginatum* in light.

Padina tetrastomatica

Uptake of all four N forms followed substrate-saturable kinetics (Fig. 5). The V_{max} for nitrate and nitrite in light and dark were similar but the affinity for them in light was comparatively less than that in dark (Tables 1 and 7). Differences in uptake between light and dark were significant in the case of ammonium, nitrite and urea (Table 4). Safety factors for ammonium and nitrite were higher in light than in dark whereas in the case of urea, they were higher in dark than in light (Table 8).

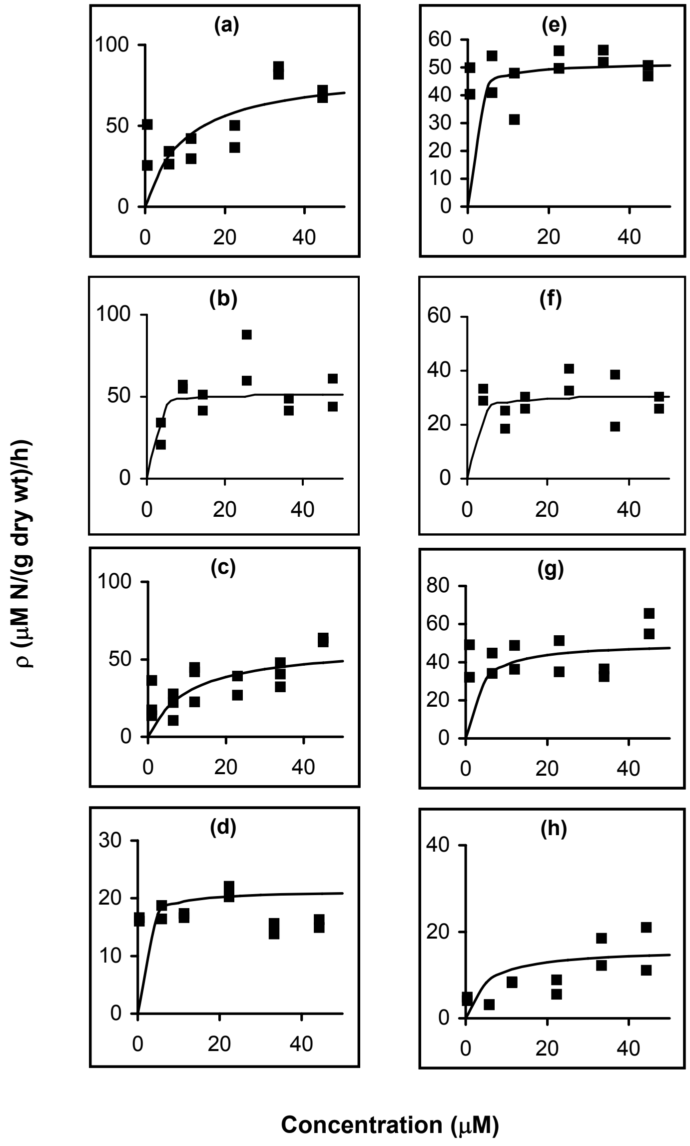

Fig. 2. Rates of uptake of NH_4^+ (a, e), NO_3^- (b, f), NO_2^- (c, g) and urea (d, h) as a function of substrate concentrations in *Sargassum tenerrimum* in light and dark, respectively.

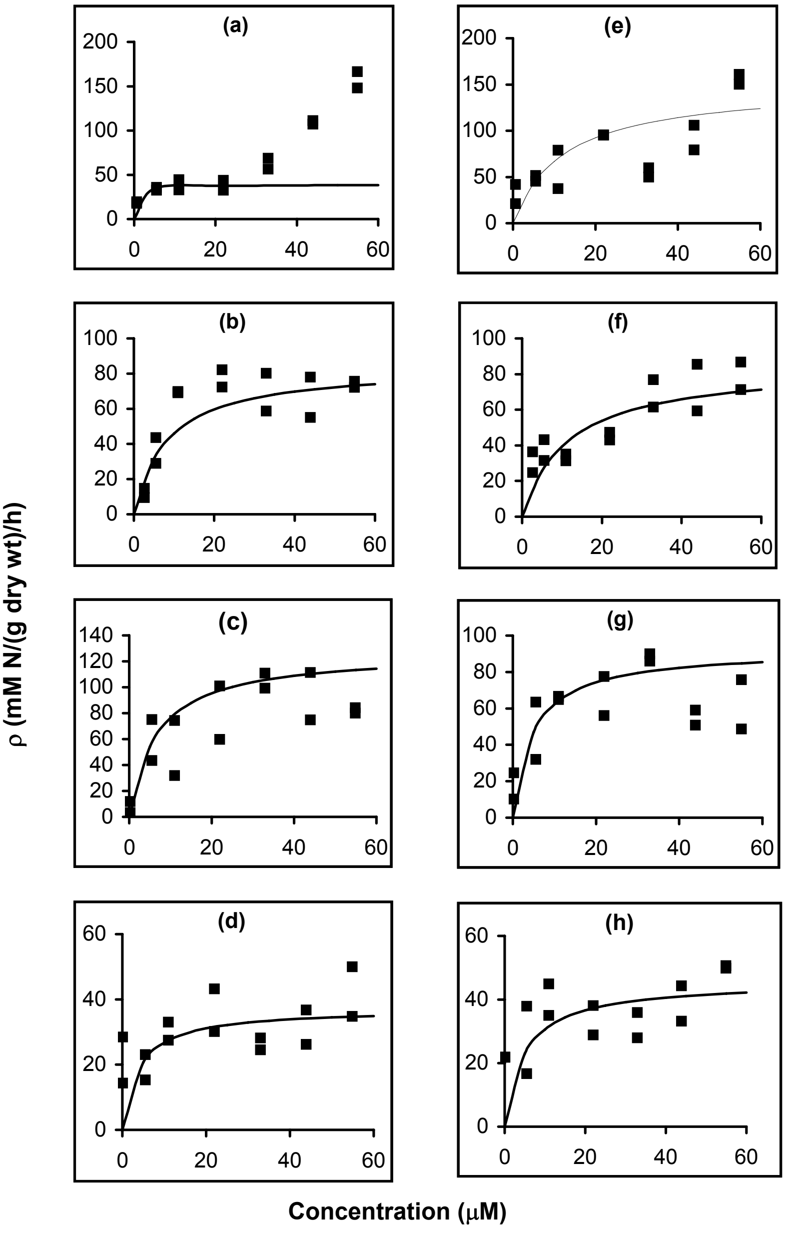

Fig. 3. Rates of uptake of NH_4^+ (a, e), NO_3^- (b, f), NO_2^- (c, g) and urea (d, h) as a function of substrate concentrations in *Ulva lactuca* in light and dark, respectively.

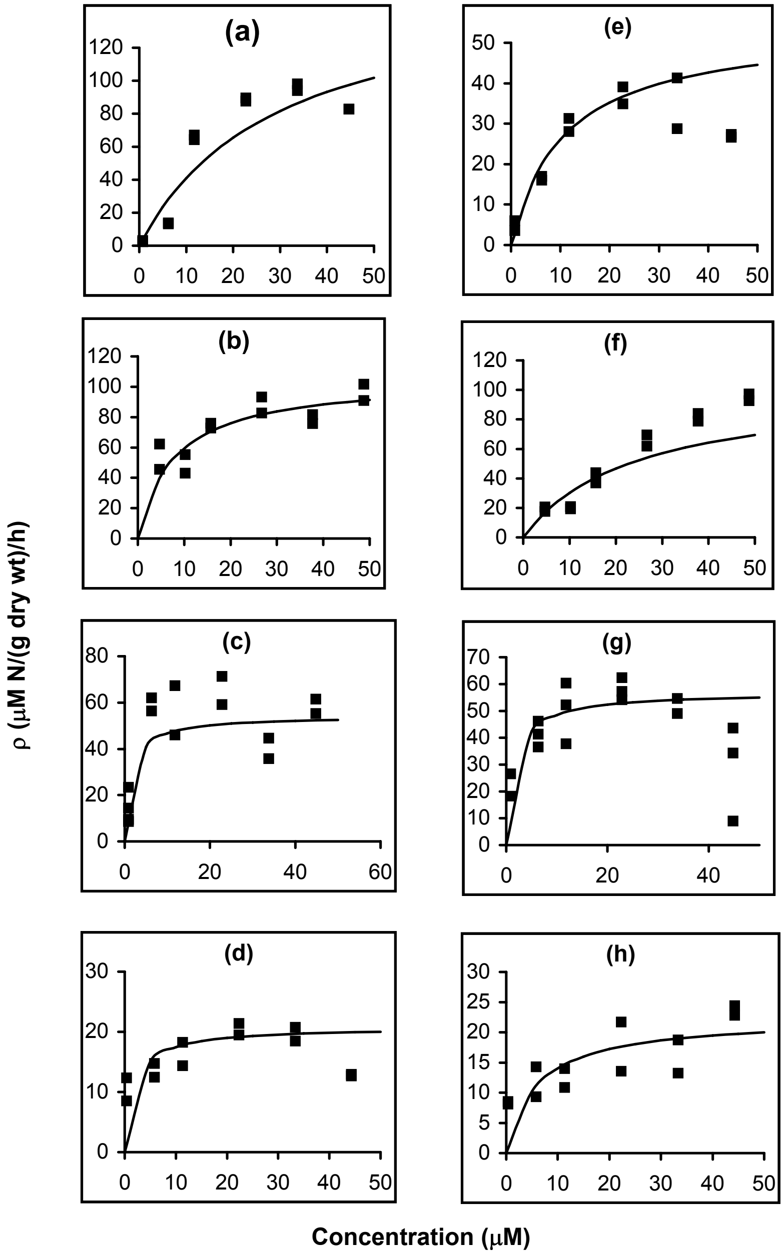

Fig. 4. **Rates of uptake of NH_4^+ (a, e), NO_3^- (b, f), NO_2^- (c, g) and urea (d, h) as a function of substrate concentrations in *Dictyota dichotoma* in light and dark, respectively.**

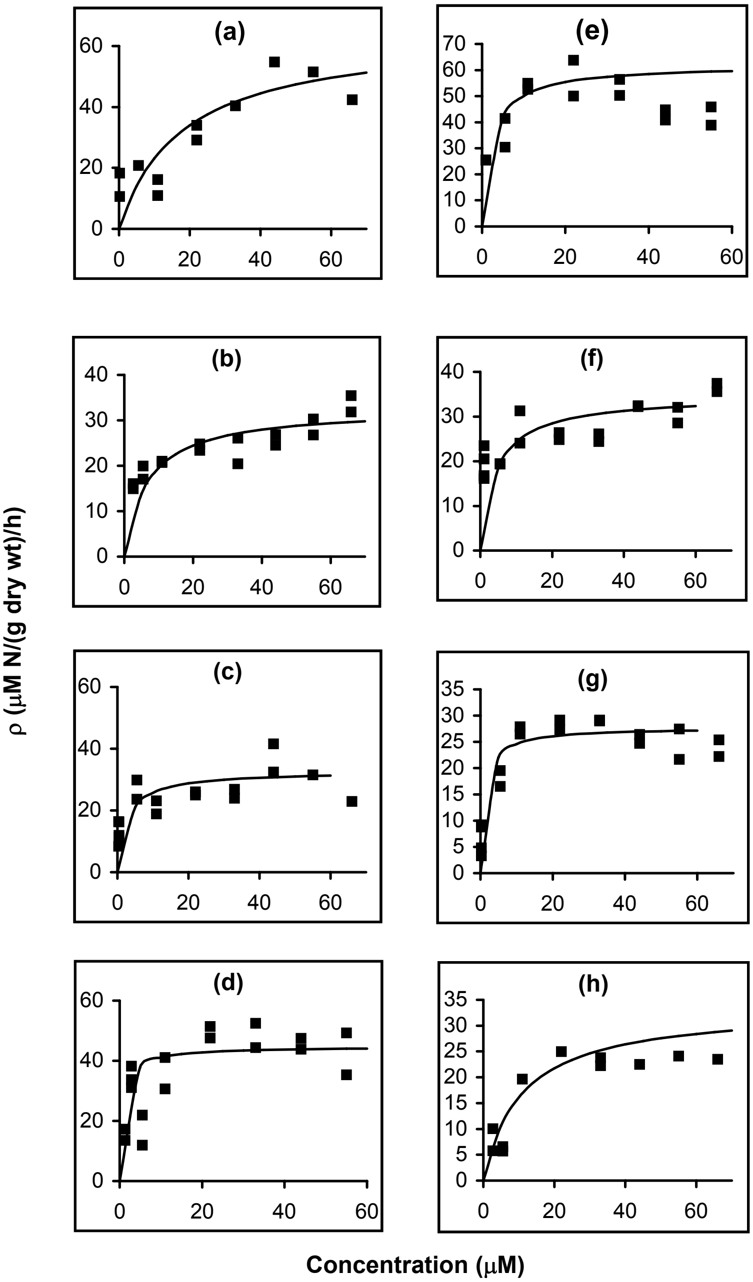

Fig. 5. Rates of uptake of NH_4^+ (a, e), NO_3^- (b, f), NO_2^- (c, g) and urea (d, h) as a function of substrate concentrations in *Padina tetrastomatica* in light and dark, respectively.

DISCUSSION

The five species of macroalgae growing in an eutrophic habitat have different capacities to take up N nutrients and this could be seen clearly from the results (Figs 1 to 5). The relationships between substrate concentrations and the uptake rates of all N nutrients were hyperbolic and could be described by Michaelis-Menten uptake kinetics. Only in the case of ammonium uptake in *U. lactuca*, could the uptake be described as biphasic - active removal up to a substrate concentration of 20 µM followed by a diffusive flux.

Nitrite uptake

The present study is one of the very few studies (Hanisak and Harlin 1978; Topinka 1978) where nitrite uptake in macroalgae has been measured. The nitrite uptake of the present study showed substrate saturable kinetics indicating active transport in both light and dark (Figs. 1 to 5), suggesting clearly that, when provided in excess, all the five algae species were capable of harvesting nitrite. Maximum nitrite uptake rates in light and dark were different between species, but within the species, the uptake rates in light were not much different from those in dark, suggesting that substrate availability rather than light was more important as a regulating factor for nitrite uptake. This is different from the observations of Topinka (1978) where V_{max} for nitrite were lower in dark than in light indicating that light stimulate nitrite uptake rates. The high V_{max} values in the present study also suggested that all the algae could respond readily to high nitrite pulses.

The α (affinity) values that were used as a measure of uptake capacities at low concentrations (Healey, 1979) also support the present inference. For example, the highest affinity for nitrite (33) was seen in *D. dichotoma* in both light and dark (Table 1) and this was 6 times higher than that for *U. lactuca*, which showed maximum uptake rates. Such high α values for NO_2^- uptake point to the ability of the algae to use nitrite at lower concentrations. The wide range of α values among the different species, however, suggests that the affinity for nitrite varies among species.

Nitrate uptake

Uptake of nitrate by macroalgae has been shown, depending upon the environment where they grow and their past nutritional history to follow saturable, linear and/or biphasic patterns. Generally, nitrate uptake exhibits saturation kinetics (Harlin and Craigie 1978; Topinka, 1978; Haines and Wheeler, 1978) but linear uptake with the increase in nitrate concentration for *Gracilaria pacifica* (Thomas *et al*., 1987) has also been reported. In the present study, nitrate uptake in all the species could be described as substrate-saturable. Relatively more measurements of nitrate uptake are available only in the case of *U. lactuca*. The V_{max} values in reported here are similar to those reported by Tarutani *et al*. (2004) for *U. pertusa* (59-85 µM/(g dry wt)/h). The half saturation constants for *U. lactuca* on the other hand, are comparatively less than those reported (18-34 µM) by them. Pedersen and Borum (1997) reported V_{max} and K_s values of 20 µM/(g dry wt)/h and 5 µM for *U. lactuca* from Denmark. These are substantially lower than the value reported here, which may be due to differences in environmental conditions, particularly the temperature. Similarly, the V_{max} (13 µM/(g dry wt)/h) and α (2.71) values reported for *Sargassum baccularia* from the Great Barrier Reef waters (Schaffelke and Klumpp, 1998) are also lower than the values calculated for *S. tenerrimum* in this study. These results imply that the uptake rates of nitrate vary within the genera and could also be species-specific as was also observed in the case of nitrite.

Ammonium uptake

Uptake of ammonium has been of greater interest because this is one of the two N forms (nitrate and ammonium) that are responsible for eutrophication of coastal waters. As this N is in the reduced form, it is the most preferable nutrient for the macroalgae, thus enabling the latter to serve as a sink for ammonium. The uptake rates of ammonium generally exceed those of nitrate (Topinka, 1978; Haines and Wheeler, 1978; Wallentinus, 1984; Pedersen and Borum, 1997; Naldi and Wheeler, 2002; Phillips and Hurd, 2004).

Ammonium uptake has been shown to follow generally saturable kinetics. Fujita (1985), Pedersen (1994) and Pedersen and Borum (1997) reported such a kinetics for *Ulva lactuca* and *Catenella nipae*. Hanisak and Harlin (1978) showed a similar active transport in *Codium fragile*. Some non-saturating kinetics with linear increase in the uptake rates due to diffusion of ions has also been reported. Patterns of linear uptake have been reported in *Macrocystis pyrifera* (Haines and Wheeler, 1978), *Gracilaria pacifica* (Thomas *et al.*, 1987), *G. tikvahiae* (Friedlander and Dawes, 1985) and *Enteromorpha* sp. (Fujita, 1985).

Uptake of ammonium was generally greater than those of other three nutrients, with the notable difference in four out of five species, which could be described by saturable kinetics. Only in *U. lactuca*, a bi-phasic transport mechanism could be observed. This is more in accordance with the generally held notion of this species being more opportunistic and ephemeral in nutrient harvesting than others. The maximum uptake rate and the half saturation constant for ammonium in *U. lactuca* were within the ranges reported for *U. pertusa* (19-240 μM/(g dry wt)/h and 5-27 μM respectively) by Tarutani *et al.* (2004).

Urea uptake

Urea could become available to intertidal seaweeds through excretion by a variety of benthic heterotrophs, agricultural runoff, sewage and bacterial degradation of purines and pyrimidines. Urea thus could become a non-negligible source of N for seaweeds. Though an active uptake of urea has been shown in microalgae (Rees and Syrett, 1979), measurements of urea uptake in macroalgae are known from only two studies. Probyn and Chapman (1982) who were the first to report on urea uptake by macroalgae, showed that *Chordaria flagelliformis* exhibit saturable kinetics of urea uptake. In another study, Phillips and Hurd (2004) reported saturable uptake for urea in *Stictosiphonia arbuscula*, *Apophlaea lyallii*, *Scytothamnus australis*, *Xiphophora gladiata* during winter and summer and linear uptake in *Stictosiphonia arbuscula* during winter. In the present study, urea uptake in all the five species followed saturable kinetics indicating active transport.

Comparison between different N sources

While the macroalage showed capability to take up all the four N nutrients, differences in their uptake rates in light and dark were not always similar among species or even between any two nutrients (Tables 2 and 3). However, some patterns could still be made out. The first is that the differences in uptake of ammonium and those of other three nutrients were often significant in all the species. In *U. lactuca*, such differences were pronounced in dark (Table 3) but not in light (Table 2). This could be interpreted as the opportunistic nature of *U. lactuca* to take up any form of N in light and a possible greater uptake of ammonium in dark that is by nature light-independent, especially in shorter time intervals. Among the other three N nutrients, the differences in uptake of nitrate and urea or nitrite and urea were often significant than those of nitrate and nitrite, which could be explained from the differences in the nature (oxidized or reduced) of, and hence preference for, these compounds.

Effect of light

In all the species, the differences in light and dark uptake rates were significant in the case of ammonium, but not in nitrate (Table 4). Urea and nitrite showed significant differences in one and two species, respectively. Though nitrate uptake was expected to be light-dependent, absence of differences in light and dark uptake was surprising. It is possible that the macroalgae, unlike the phytoplankton, may have a capacity for dark uptake of nitrate or that there could be a certain amount of diffusive flux of nitrate that may occur independent of light.

Safety factor

While uptake kinetics could indicate potential maximum uptake rates, how much of this could actually happen in normal circumstances could only be evaluated with a knowledge of safety factor (Diamond, 1998; 2002) that is indicative of the surplus capacity for uptake and storage relative to the maximum concentrations of the nutrient that an alga is likely to encounter in nature. The safety factors calculated for the different species of algae showed relatively high values for nitrite (Table 8), compared to those for other N nutrients. This observation suggests that the concentration of nitrite in the vicinity of seaweed is unpredictable. The unpredictable increase in nitrite concentration may occur due to sporadic events like phytoplankton blooms. The macroalgae with high safety factors have a greater potential to take advantage of the increased nutrient concentrations than macroalgae with low safety factors.

CONCLUSIONS

All the five algal species studied showed the capacity to take up ammonium, nitrate, nitrite and urea in light and dark. However, rates of uptake varied among species. The high uptake rates and safety factors observed indicate that the algae could play an important role in moderating the effects of excess N loading in coastal waters.

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