ELSEVIER

Journal of Experimental Marine Biology and Ecology
269 (2002) 65–83

Journal of EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

www.elsevier.com/locate/jembe

Nitrate uptake and storage in the seaweed *Ulva rigida* C. Agardh in relation to nitrate availability and thallus nitrate content in a eutrophic coastal lagoon (Sacca di Goro, Po River Delta, Italy)

Mariachiara Naldi *, Pierluigi Viaroli

Dipartimento di Scienze Ambientali, Università di Parma, Parco Area delle Scienze 11/A, 43100 Parma, Italy

Received 26 March 2001; received in revised form 14 June 2001; accepted 26 November 2001

Abstract

The seasonal cycle of biomass and tissue composition of *Ulva rigida* C. Agardh, in relation to nitrogen availability in the water column, was studied in 1991–1992 in the Sacca di Goro, a highly eutrophic lagoon in the Po River Delta (Italy). Nitrate uptake rates and storage capacity were also determined in laboratory experiments. The seasonal growth of *U. rigida* was related to the seasonal trend of nitrogen concentration in the water column. *U. rigida* biomass increased exponentially during spring and attained peaks of about 300–400 g dry mass (DM) m^{-2} in June. As biomass increased, *U. rigida* depleted nitrate in the water column. Thallus nitrate reserves also declined from 100 μmol N (g DM)$^{-1}$ to almost undetectable levels, and total thallus nitrogen declined from 4% to 2.5% DM and 1.25% DM in 1991 and 1992, respectively. During summer, *U. rigida* decomposition increased, and organic nitrogen concentrations in the water column increased. The uptake experiments demonstrated an inverse relationship between thallus nitrate content and nitrate uptake rates. A modified Michaelis–Menten equation that accounts for thallus nitrate fit the uptake data well. *U. rigida* can accumulate up to about 400–500 μmol nitrate (g DM)$^{-1}$ in cellular reserves. *U. rigida* in the Sacca di Goro has higher K_m and lower V_{max}/K_m ratios for nitrate uptake than other chlorophycean species, indicating a low efficiency of uptake at low nitrate concentrations. This low uptake efficiency, and the ability to exploit N availability by storing cellular nitrate pools in excess of immediate growth needs, may represent a physiological response to an eutrophic environment where nitrate is in large supply for most of the year.

Keywords: Coastal lagoon; Eutrophication; Nitrate; *Ulva rigida*; Uptake

* Corresponding author. Tel.: +39-0521-905976; fax: +39-0521-905402.
E-mail address: chiara@dsa.unipr.it (M. Naldi).

0022-0981/02/$ - see front matter
PII: S0022-0981(01)00387-2

1. Introduction

In many temperate coastal systems, the anthropogenic increase in nutrients, especially nitrogen (N), has led to periodic and massive blooms of ephemeral seaweeds such as *Ulva*, *Cladophora*, *Enteromorpha* and *Chaetomorpha*. These seaweeds often replace the original rooted phanerogam and perennial seaweed communities (Duarte, 1995; Borum and Sand-Jensen, 1996; Valiela et al., 1997). In Northern Europe, the development of dense green macroalgal mats has been recorded in bays, estuaries and lagoons along the Atlantic coasts of France and the coasts of the North Sea (e.g. Ménesguen and Piriou, 1995; Fletcher, 1996; Nienhuis, 1996; Wallentinus, 1996). In coastal systems of the Mediterranean Sea, many of these "green tides" are due to blooms of *Ulva rigida* C. Agardh. Mediterranean coastal lagoons are generally eutrophic, due to the high nutrient loading coming from the surrounding land, their morphological and hydrodynamic characteristics (shallow and semi-enclosed systems, with low water exchange) and to climatic conditions (Bellan, 1987). In recent years, *U. rigida* blooms have become even more frequent. In the French Etang du Prévost (Viaroli et al., 1996a), and in the Italian Venice lagoon (Sfriso et al., 1992) and Po River Delta lagoons (Viaroli et al., 1993) dense mats of this macroalga develop in spring. The decomposition of the large *U. rigida* standing stocks (500–2000 g dry mass m^{-2}) in summer causes oxygen depletion in the water column, and often dystrophic crises, characterized by anaerobic processes and strongly reducing conditions that last several days (Sfriso et al., 1992; Viaroli et al., 1993).

Species of the genus *Ulva* have been described as opportunistic due to their thallus morphology (thin, undifferentiated thalli), fast growth rates and rapid uptake of inorganic nutrients (Littler and Littler, 1980). Efficient nitrogen uptake even at low substrate concentrations has been reported for these species (Wallentinus, 1984; Smith and Horne, 1988; Fujita et al., 1989; Hein et al., 1995; Pedersen and Borum, 1997). In addition, the capacity to store N in cellular reserves is used to exploit periodic episodes of high N availability ("pulses"), which occur in many coastal environments (Rosenberg et al., 1984; Fong et al., 1994). Growth of *Ulva* and other chlorophycean species can be sustained by N accumulated over such periods of excess availability (Hanisak, 1979; Rosenberg and Ramus, 1982; Asare and Harlin, 1983; Fujita, 1985; Pedersen and Borum, 1996). However, most of these studies of chlorophycean species concern ammonium uptake. Uptake kinetics and storage of nitrate, which is often the dominant inorganic N source in coastal waters, have been well studied in Phaeophyceae and Rhodophyceae (e.g. Chapman and Craigie, 1977; Bird et al., 1982; Thomas and Harrison, 1985; Thomas et al., 1987), but more sparsely studied in Chlorophyceae (Hanisak and Harlin, 1978; Wallentinus, 1984; O'Brien and Wheeler, 1987; Lavery and McComb, 1991; McGlathery et al., 1996; Pedersen and Borum, 1997).

The present work aimed to investigate some aspects of the nitrate metabolism of *U. rigida* in a highly eutrophic lagoon of the Po River Delta by conducting both field and laboratory studies. The seasonal trends in standing stocks of *U. rigida* were studied in relation to nutrient availability in the lagoon. Laboratory experiments were designed to test *U. rigida* efficiency at nitrate uptake and its capacity to store nitrate in cellular reserves, in relation to substrate concentration and thallus nitrate content.

2. Materials and methods

2.1. Study area

The Po River (Northern Italy) drains a wide, intensively cultivated and populated plain, and discharges considerable amounts of nutrients in its deltaic lagoons and the Northern Adriatic Sea. The present research was carried out in one of the brackish shallow-water embayments of the the Po River Delta, locally called a "sacca". The Sacca di Goro (Fig. 1) has an area of 26 km^2, an average depth of about 1.5 m, and is connected to the sea by a 1.5-km wide mouth. The bottom is flat and the sediment is composed of alluvial muds, with a high-sand content near to the southern shoreline. Nutrient-rich freshwater flows into the lagoon from the Po di Goro, the Po's southernmost deltaic branch, and the Po di Volano, a highly polluted canal. In 1992, the Po di Volano discharged about 1200 tons of total nitrogen, which correspond to an areal loading of 3 mol N m^{-2} $year^{-1}$ (Alvisi et al., 1993). The eastern part of the Sacca di Goro, the Valle di Gorino, accounts for about half of the total surface area and is isolated from the sea by a long sand bank. The Valle di Gorino is shallow (average depth of about 0.8 m) and sheltered and has a slow water renewal (about 20 days in summer months). In this area, extensive spring blooms of *U. rigida* have been observed since 1987. During summer, the algal mats often undergo fast

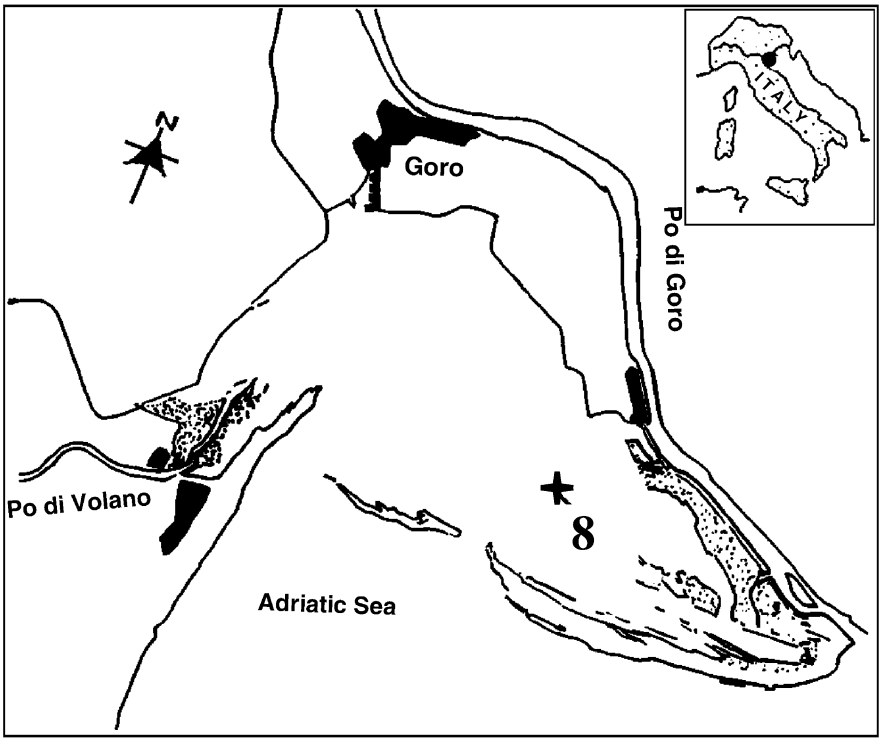


Fig. 1. Map of the Sacca di Goro (Po River Delta, Italy), showing the location of the sampling station (station 8).

decomposition, causing oxygen depletion over vast areas of the Valle di Gorino and even prolonged anoxic crises (Viaroli et al., 1993).

2.2. Water sampling and analysis

From February 1991 to December 1992, one water sample was collected approximately once per month from a fixed station in the Valle di Gorino (station 8) at a depth of about 0.5 m with a Ruttner bottle. After filtration (Whatman GF/C), the sample was analyzed in duplicates for nitrogen (N) and phosphorus (P). Nitrite was determined spectrophotometrically after diazotation; nitrate was measured as nitrite following cadmium reduction (A.P.H.A. et al., 1975); ammonium was determined by the indophenol-blue method (Koroleff, 1970). We consider dissolved inorganic nitrogen (DIN) as the sum of nitrate, nitrite and ammonium. Total dissolved nitrogen (TDN) was determined by persulphate oxidation (Valderrama, 1981) and dissolved organic nitrogen (DON) was obtained by subtraction of DIN from TDN. Particulate organic nitrogen (PON) was determined on filters by persulphate oxidation. Soluble reactive phosphorus (SRP) was determined by the ascorbic acid method (Valderrama, 1977).

2.3. U. rigida sampling and tissue analyses

Along with water sampling, *U. rigida* samples were collected around station 8 within a 0.5-km^2 area. *U. rigida* biomass was harvested with a rake-like device over an area ranging from 2 to 20 m^2 depending on biomass density. Two or three samples were collected on each date. Thalli were cleaned by hand to remove visible epiphytes, rinsed briefly with tap water to remove salt, and oven-dried at 70 °C to determine the dry mass (DM). *U. rigida* biomass was then expressed as g DM m^{-2}. Coefficient of variation for biomass estimates ranged from 34% to 57%.

Total nitrogen (TN) and phosphorus (TP) and extractable nitrate content were determined on triplicate subsamples of dried, powdered thalli. TN was determined by CHN analysis (LECO 600). For TP 1 g subsamples were ignited overnight at 550 °C in a muffle furnace and TP content was determined spectrophotometrically after acid extraction of the ash (Isaac, 1990). Extractable nitrate content was determined according to Corzo and Niell (1992), by shaking 100 mg subsamples in 50 ml of distilled water for 2 h. Nitrate and nitrite were then determined on the filtered extract as previously described. Coefficients of variation for TN, TP and nitrate analysis were $<10\%$.

2.4. Nitrate uptake experiments

Nitrate uptake kinetics were studied over a wide range of tissue nitrate contents. In May 1992, we conducted three uptake experiments with *U. rigida* collected at station 8. Prior to the experiments, *U. rigida* discs ($d=17$ mm) were maintained for 7–10 days in the laboratory, at ambient light and temperature conditions, under three different regimes: filtered lagoon water with very low nitrogen (DIN <2 μM) and phosphorus (SRP $=0.1$ μM) and no nitrogen and phosphorus additions ($-N-P$), with only P (as KH_2PO_4; 6 μM P day^{-1}) ($-N+P$), and with both N (as KNO_3; 71 μM N day^{-1}) and P ($+N+P$). Uptake

rates were then determined for each set of preconditioned thalli, with a combination of the multiple flask and the perturbation methods. For each experiment, we incubated 50–100 *U. rigida* discs, corresponding to 0.4–0.8 g of dry algal mass, in glass jars with 1 liter of filtered lagoon water enriched to four KNO_3 concentrations over the range 9–60 μM, each in triplicate; KH_2PO_4 was added at the non-limiting concentration of 16 μM. The cultures were illuminated by cool white fluorescent tubes (Philips TLD 16W/840) at 160 μE m^{-2} s^{-1} and mixed by bubbling air through an air-stone. At the beginning of the incubation (t_0), thallus nitrate content was determined on a subsample as described previously. Water nitrate concentrations were determined at t_0 and after 30 (t_{30}), 60 (t_{60}), 120 (t_{120}) min; in the +N+P cultures nitrate concentrations were determined also after 180 min (t_{180}).

With the same experimental set up, in 1995 we conducted three uptake experiments without preconditioning. Freshly collected thalli very rich in nitrate were maintained in the laboratory for less than 48 h, at ambient light and temperature conditions, in filtered lagoon water (100 μM N) changed daily. We tested uptake at five nitrate concentrations over the range 7–83 μM. Water nitrate was determined at t_0, t_{30} and t_{60}.

Uptake rates were calculated from changes in substrate concentration during each sampling interval, normalized for time and biomass; uptake rates are thus reported in μmol N (g DM)$^{-1}$ h^{-1}. In a preliminary experiment conducted without *U. rigida* no significant decrease of water nitrate concentration was observed during the incubation. Observed uptake rates (V) for each time interval and the corresponding initial substrate concentrations (S) were fit to a modified Michaelis–Menten function with the Marquardt method for non-linear regression, using the NLIN procedure of SAS statistical package for computations (SAS Institute, 1985). An additional parameter, the threshold substrate concentration S_T, was introduced in the Michaelis–Menten function

$$V = \frac{V_{max}(S - S_T)}{K_m + (S - S_T)} \quad (1)$$

where V_{max} is the theoretical maximum uptake rate, K_m is the half-saturation constant for uptake, and S_T is the value of S below which no uptake is observed, and removes forcing of the fitted curve through the origin. The Student's t-test was used to compare the parameters estimated in the various experiments (Sokal and Rohlf, 1981).

2.5. Nitrate storage experiments

The storage capacity of *U. rigida* for nitrate was tested in the laboratory with experimental designs similar to those described for uptake. A 12-h experiment was conducted in 1991 with freshly collected thalli cut into discs. Two 24-h experiments (16:8 light/dark cycle) were performed in 1992 with thallus discs maintained in the laboratory at ambient light and temperature, under two conditions: with nitrate (as KNO_3; 100 μM N day^{-1}) for 7 days or without nitrate addition for 14 days. These two different preconditioning periods were necessary to obtain thalli with, respectively, very high and undetectable cellular nitrate pools. For each experiment, 0.1–0.5 g DM of *U. rigida* discs were incubated in glass jars with 1 l of filtered lagoon water enriched to four concentrations of KNO_3 in the range 33–580 μM, each in triplicate. KH_2PO_4 was added in various concentrations to ensure that nitrogen was always the potential limiting nutrient. Water

nitrate concentrations were determined at t_0 and at the end (t_f) of the incubation. Thallus nitrate content was determined, as described above, on a subsample taken at t_0 and at t_f.

3. Results

3.1. Field data

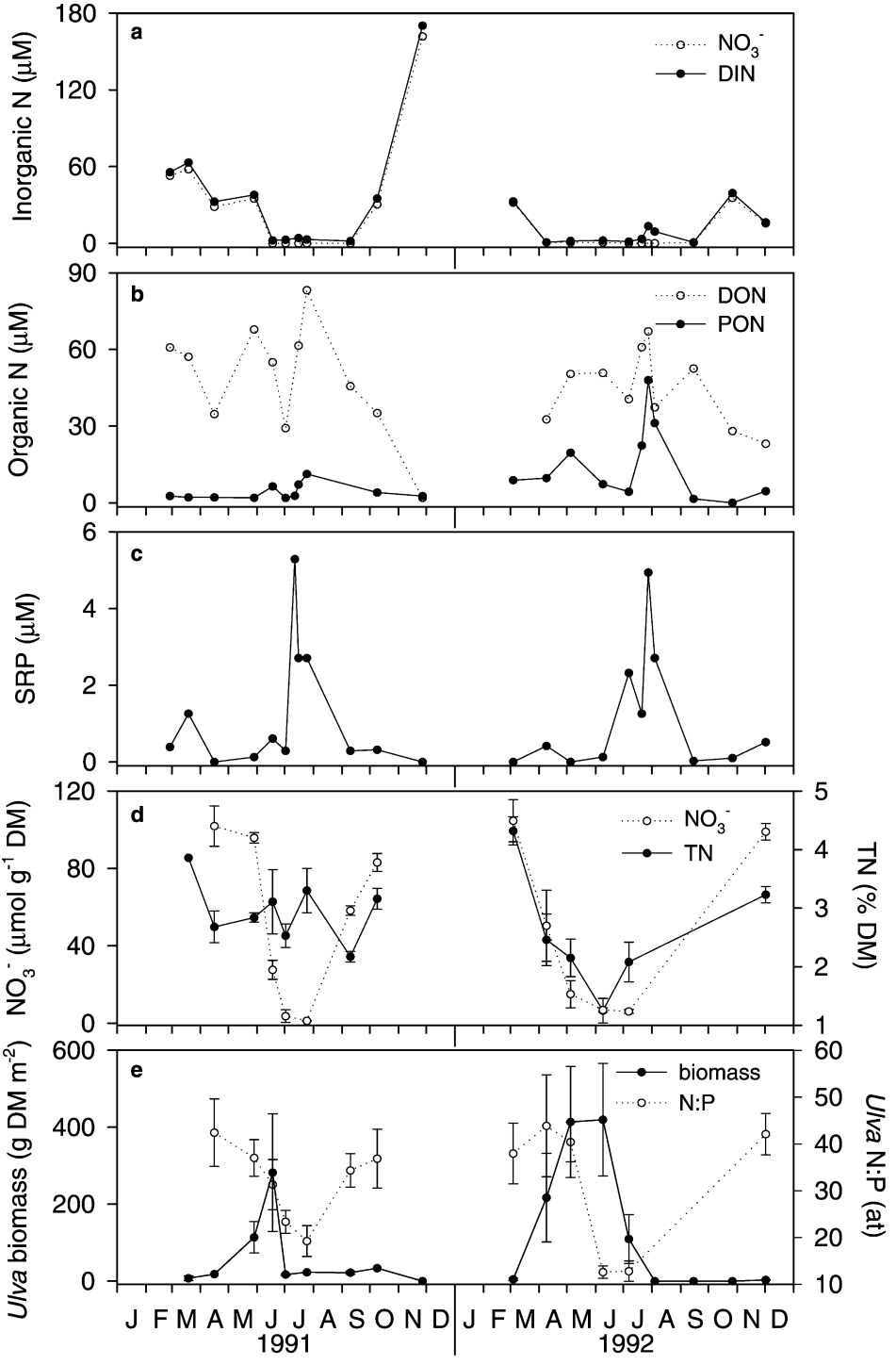
The seasonal trends of nitrogen and phosphorus concentrations in the water column in Sacca di Goro (station 8) are shown in Fig. 2. Nitrate generally contributed more than 90% of DIN (Fig. 2a), and we measured the highest nitrate concentrations in late autumn (162 and 38 μM in 1991 and 1992, respectively). The nitrate concentrations decreased rapidly in spring, and DIN was almost depleted from May–June through September. DON and PON concentrations were usually higher than 30 and 2 μM, respectively (Fig. 2b), and increased during dystrophic events, which occurred in mid-July 1991 and from the end of July through early August 1992. During dystrophic crises SRP also increased sharply, with peaks of 5.3 μM in 1991 and 4.9 μM in 1992 (Fig. 2c).

A strong seasonal pattern was shown in the nitrate and TN content of *U. rigida* thalli (Fig. 2d) and in *U. rigida* biomass, that increased exponentially during spring and attained peaks of about 300 and 400 g DM m^{-2} in June 1991 and 1992, respectively (Fig. 2e). In both years, the difference in *U. rigida* biomass from March to April was significant ($P < 0.05$). In the following months, the average biomass incrased, but, due to the higher variability of the estimates, the differences were not statistically significant. After June, *U. rigida* biomass crashed ($P < 0.05$). The increase in *U. rigida* biomass was correlated with a decrease in tissue TN content, which was more pronounced in 1992 than in 1991 (Fig. 2d). TN values were around 4% of DM in winter and fell to 2.5% of DM in June 1991 and to 1.25% of DM in June 1992 ($P < 0.05$ in both years). The nitrate content of thalli (Fig. 2d) decreased from about 100 μmol N (g DM)$^{-1}$ in March to almost undetectable levels in June/July, concomitant with the biomass peaks. All differences in nitrate content were significant ($P < 0.05$) or highly significant ($P < 0.01$), except from May to June 1992 and from June to July 1992. The atomic ratio between TN and TP content (N:P) of thalli also varied seasonally (Fig. 2e), with winter–spring values greater than 40:1, decreasing significantly ($P < 0.05$) to 19:1 and 13:1 in July 1991 and 1992, respectively.

3.2. Nitrate uptake experiments

U. rigida thalli maintained in the cultures without nitrogen addition ($-N-P$; $-N+P$) contained less than 2 μmol N (g DM)$^{-1}$, while thalli preconditioned with nitrate ($+N+P$) maintained the nitrate content they had immediately after collection (14 μmol N (g DM)$^{-1}$). The difference in nitrate content influenced the uptake kinetics of nitrate. Thus,

Fig. 2. Seasonal trends of water column nutrient concentrations, *U. rigida* biomass and elemental composition in Sacca di Goro (station 8) in 1991 and 1992: (a) concentrations of nitrate and dissolved inorganic nitrogen (DIN); (b) concentrations of dissolved organic nitrogen (DON) and particulate organic nitrogen (PON); (c) concentrations of soluble reactive phosphorus; (d) nitrate and total nitrogen (TN) contents of *U. rigida* thalli (mean values ± SD, $n = 3$); (e) *U. rigida* biomass and N:P atomic ratio of *U. rigida* thalli (mean values ± SD, $n = 3$).


a
b
c
d
e
Inorganic N (µM)
Organic N (µM)
SRP (µM)
NO3- (µmol g-1 DM)
Ulva biomass (g DM m-2)
TN (% DM)
Ulva N:P (at)
NO3-
DIN
DON
PON
TN
biomass
N:P
J F M A M J J A S O N D J F M A M J J A S O N D
1991
1992

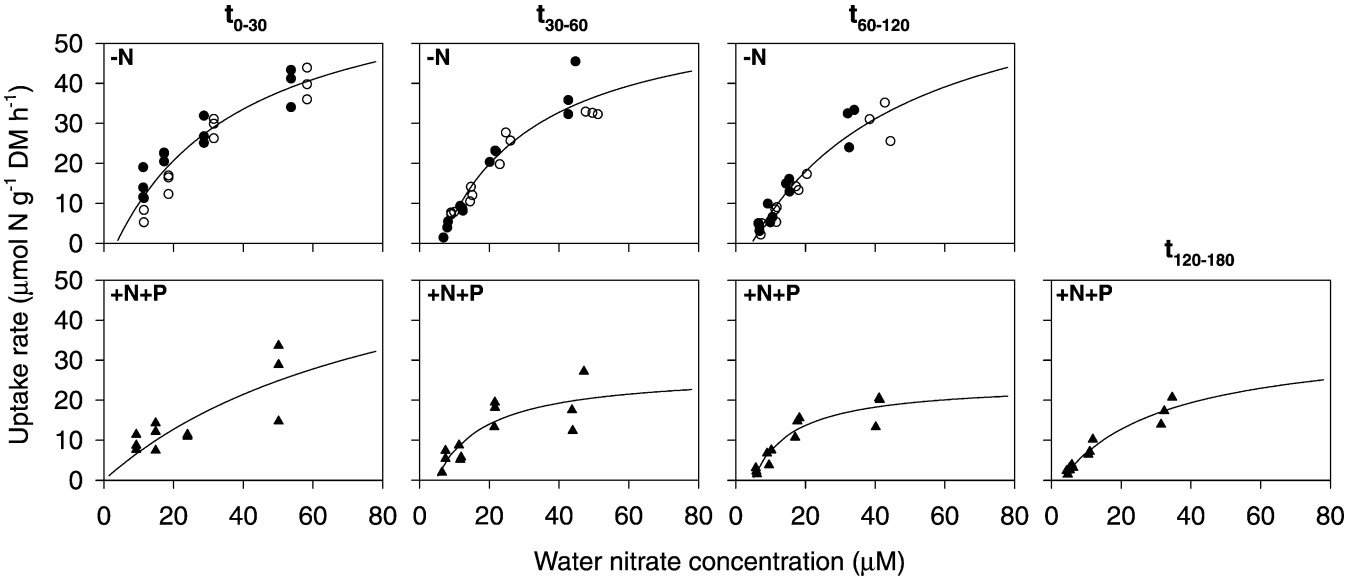


Fig. 3. Nitrate uptake experiments conducted with *U. rigida* preconditioned with nitrate (black triangles: + N + P) and without nitrate addition (open circles: − N − P; black circles: − N + P). Nitrate uptake rates vs. substrate concentrations during the different incubation periods are shown.

the uptake rates determined on thalli preconditioned without nitrate addition could be fit to the Michaelis–Menten function in all time intervals, although the uptake capacity of *U. rigida* was not saturated (Fig. 3). Preconditioning with P had no effect on V_{max} and K_m. The threshold value S_T was 6.92 μM N in the −N−P cultures and 0 in the −N+P cultures, but this difference was not significant. Since the results for the −N−P and −N+P experiments were not significantly different, we fit the pooled data for the corresponding time intervals to the Michaelis–Menten equation (Table 1). During t_{0-30} V_{max} was 69 μmol N (g DM)$^{-1}$ h^{-1}, K_m was 38 μM N and S_T was 3.56 μM N. Nitrate depletion was linear with time (data not shown), and therefore the parameters did not differ significantly between time intervals.

U. rigida maintained with nitrate (+N+P) showed highly variable uptake rates during t_{0-30} (Fig. 3). Data from the three subsequent time intervals were better fit by the Michaelis–Menten function (Table 1). V_{max} values were 50% lower than the −N treatments (Table 1), and the differences were significant ($P<0.05$). In contrast, K_m and S_T did not differ significantly between treatments, and, overall, ranged from 12 to 46 and from 2.6 to 6.1 μM N, respectively (Table 1).

Nitrate content of *U. rigida* used for the 1995 experiments was very high (83–181 μmol N (g DM)$^{-1}$) compared to those determined in 1992, and the nitrate uptake kinetics were very different. Nitrate-rich thalli showed either low net uptake or even a net release of nitrate and showed no clear relationship with substrate concentration (Fig. 4). The data shown in Fig. 4 indicate that uptake decreases dramatically as thallus nitrate increases. We described this relationship by introducing an exponential term, that accounts for thallus nitrate content, in Eq. (1), which is rewritten in the form

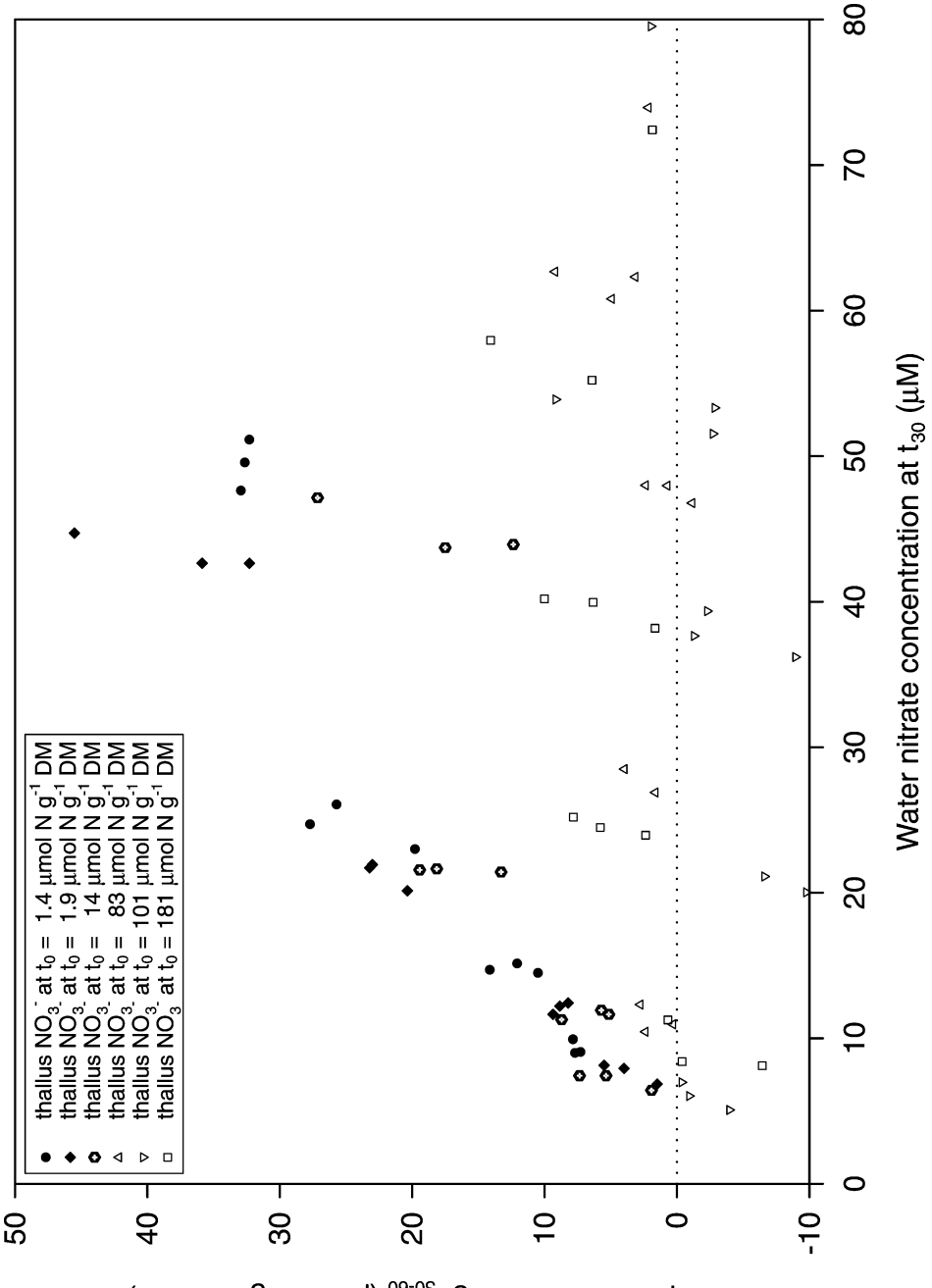
$$V = \frac{V_{max}(S - S_T)}{K_m + (S - S_T)} e^{(-cN_{th})} \quad (2)$$

where N_{th} is the nitrate content of thallus (in μmol N (g DM)$^{-1}$) and c is a parameter to be estimated by the above mentioned non-linear fitting procedure.

Table 1
Parameters of the Michaelis–Menten function estimated by non-linear fitting of data from the three uptake experiments conducted with *U. rigida* previously maintained in the laboratory with and without nitrate addition

Exp.	Time interval	V_{max} (μmol N g^{-1} h^{-1})	K_m (μM N)	S_T (μM N)	R^2
−N	0–30	68.61 (16.72)	38.04 (22.04)	3.56 (3.20)	0.89
	30–60	59.89 (9.45)	28.11 (9.81)	6.06 (1.00)	0.93
	60–120	71.50 (23.26)	45.90 (25.76)	4.60 (1.24)	0.92
+N+P	0–30	68.16 (125.72)	87.03 (266.97)	0.00 (13.39)	0.61
	30–60	27.06 (9.43)	14.34 (15.15)	4.65 (2.96)	0.71
	60–120	24.41 (4.50)	11.82 (6.43)	4.89 (1.05)	0.89
	120–180	35.33 (12.95)	30.80 (22.24)	2.61 (1.31)	0.95

−N: pooled data from the −N−P (without either nitrogen or phosphorus) and −N+P (with only phosphorus) treatments (these two treatments were pooled because the results were not significantly different); +N+P: thalli maintained with both nutrients. For each incubation time interval (in min), the estimated V_{max}, K_m, S_T (threshold substrate concentration, see text) and their standard error (in parenthesis), as well as coefficient of determination (R^2) of fitting, are reported ($n=24$ for −N fittings, $n=12$ for +N+P fittings). Underlined values differ significantly between treatments in the corresponding time intervals.


Uptake rate during t_{30-60} (µmol N g^{-1} DM h^{-1})
Water nitrate concentration at t_{30} (µM)
thallus NO_3^- at t_0 = 1.4 µmol N g^{-1} DM
thallus NO_3^- at t_0 = 1.9 µmol N g^{-1} DM
thallus NO_3^- at t_0 = 14 µmol N g^{-1} DM
thallus NO_3^- at t_0 = 83 µmol N g^{-1} DM
thallus NO_3^- at t_0 = 101 µmol N g^{-1} DM
thallus NO_3^- at t_0 = 181 µmol N g^{-1} DM
50
40
30
20
10
0
-10
0
10
20
30
40
50
60
70
80

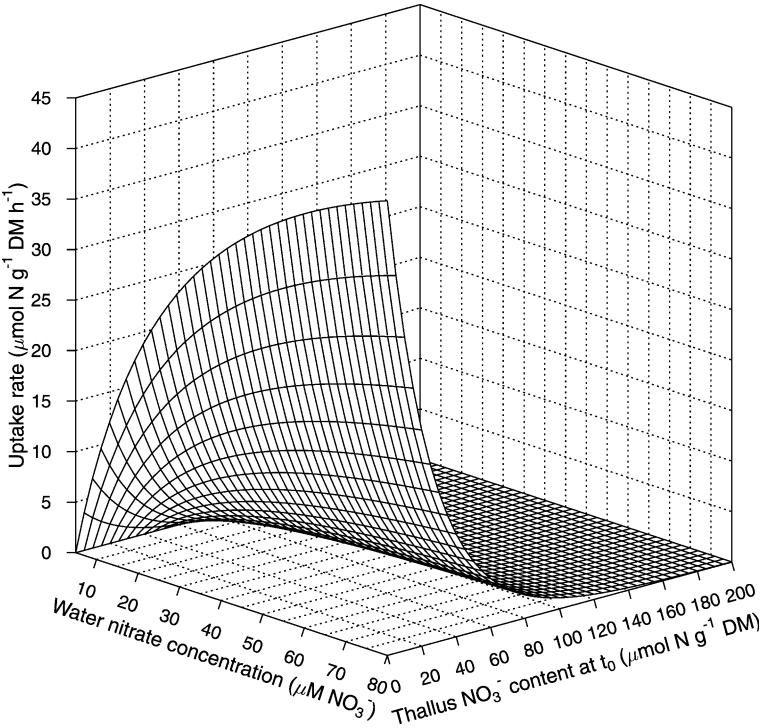


Fig. 5. Shape of Eq. (2) with parameters calculated from fitting of Fig. 4 data (substrate concentration at t_{30}, nitrate uptake rates during t_{30-60} and thallus nitrate content at t_0). Parameter values are given in the text.

Pooled uptake rates from the six experiments, determined in the t_{30-60} time interval, versus t_{30} substrate concentrations and thallus nitrate content are well described by Eq. (2) ($R^2 = 0.83$; $P < 0.01$; n = 72); parameters are: $V_{max} = 62$ μmol N (g DM)$^{-1}$ h^{-1} (SE = ± 13); $K_m = 27$ μM N (SE = ± 13); $S_T = 6$ μM N (SE = ± 1); $c = 0.0372$ h^{-1} (SE = ± 0.0063). These values are similar to those determined during t_{30-60} in the −N experiments with *U. rigida* of low thallus nitrate content (Table 1). The shape of the function with these parameter values is represented in Fig. 5.

3.3. Nitrate storage experiments

The three long-term incubations of *U. rigida* thalli at increasing nitrate concentrations demonstrate that the macroalga can accumulate large cellular nitrate pools. Highest nitrate accumulation was observed for thalli with no detectable initial thallus nitrate. After 24-h

Fig. 4. Nitrate uptake rates of *U. rigida* with different initial (t_0) thallus nitrate content. Uptake rates during the 30–60 min incubation period (t_{30-60}) are plotted vs. substrate concentration at the beginning of the same incubation period. Black symbols are data from the 1992 experiments with *U. rigida* preconditioned with different nutrient regimes (see Fig. 3), open symbols are data from the 1995 experiments without preconditioning treatments.

incubation at water nitrate concentrations higher than 800 μmol N (g DM)$^{-1}$, thallus nitrate increased on average to 391 μmol N (g DM)$^{-1}$ (SE = ± 25, n = 12) (Fig. 6). Fitting of the data to a hyperbolic function, with the non-linear fitting procedure described previously, results in a asymptotic nitrate content of 448 μmol N (g DM)$^{-1}$ (SE = ± 64), which may represent the maximum pool size possible.

Thallus nitrate content was 83 μmol N (g DM)$^{-1}$ at the beginning of the 12-h experiment, and it increased to 130–180 μmol N (g DM)$^{-1}$ in thalli cultured over a wide range of substrate concentrations (Fig. 6). For the second 24-h experiment, we used *U. rigida* thalli with an initial nitrate content of 167 μmol N (g DM)$^{-1}$. At the end of the incubation, cellular nitrate increased to 209–270 μmol N (g DM)$^{-1}$ in thalli exposed to water nitrate concentrations higher than 280 μmol N (g DM)$^{-1}$, while all others depleted the available substrate (Fig. 6).

Based on the initial nitrate content of thalli and the depletion of dissolved nitrate in the media (uptake), we could estimate an "expected" final thallus nitrate content. In most cases, the expected content was greater than the observed values, suggesting that nitrate was assimilated into organic N over the 12 or 24 h. Release of organic N could also contribute to the differences, however, assuming that they are due to assimilation only, thalli with no initial nitrate reserves assimilated 636 μmol N (g DM)$^{-1}$ (SE = ± 223,

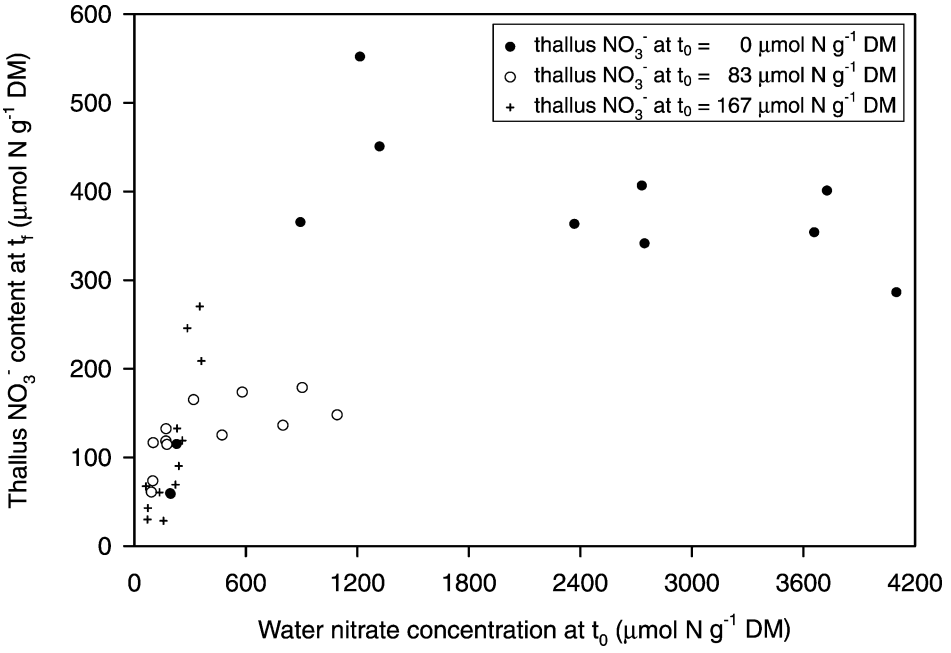


Fig. 6. Nitrate storage experiments with *U. rigida* of different initial thallus nitrate content. Nitrate concentration in the culture medium at the beginning of the experiment (t_0) is represented on the x-axis as nitrate "available" to the macroalgal biomass of each culture (i.e. nitrate concentration in the water has been converted from μM to μmol N (g DM)$^{-1}$ of each bottle). Thallus nitrate contents at the end (t_f) of the 24-h (black symbols) and 12-h (open symbols) incubations are represented on the y-axis.

$n = 10$) in 24 h. Assimilation was 151 μmol N (g DM)$^{-1}$ (SE = ± 25, $n = 11$) in the 12 h experiment, and 207 μmol N (g DM)$^{-1}$ (SE = ± 22, $n = 9$) in the second 24 h experiment.

4. Discussion

4.1. U. rigida growth and N cycling in the Sacca di Goro

In eutrophic systems the amounts of nutrients stored as seaweed biomass are important at the level of the ecosystem (Valiela et al., 1997). In the Sacca di Goro the seasonal growth of *U. rigida* is related to the seasonal trend of nitrate concentration in the water column. *U. rigida* thalli have maximum TN and nitrate levels in winter and early spring, when biomass is low and nitrate availability in the water is high. Winter DIN concentrations in 1993–1997 ranged from 50 to 150 μM (Viaroli et al., 1996b and unpublished data). As biomass increases *U. rigida* depletes DIN in the water as well as its own cellular nitrate reserves, and in summer thallus TN levels decline to about one third to one half of the winter values. However, we show in Fig. 7 that, due to the high standing stocks, large amounts of N are immobilized in the macroalgal biomass at the beginning of summer. During the spring growth phases, as *U. rigida* standing stock increases, the amount of TN stored in the macroalga biomass, on an areal basis, increses 10-fold, and largely exceeds the amount of TN in the water column (Fig. 7). After June *U. rigida* biomass crashes resulting in intense decomposition and anoxic conditions in the water mass for several days (Viaroli et al., 1993). When *U. rigida* decomposition takes over, organic N concentrations increase in the water column, particularly PON in 1992, possibly due to

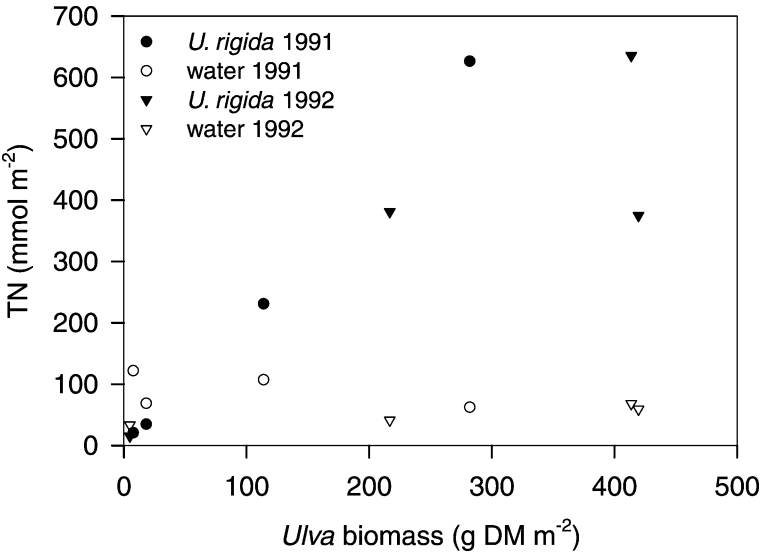


Fig. 7. Comparison between amounts of TN (DIN + DON + PON) in the water column and TN immobilized in *U. rigida* biomass at different algal densities during the spring growth phase (April–June 1991, March–June 1992).

the fragmentation of thalli. The observed organic nitrogen increases account for about 10% of the potential release from *U. rigida* biomasses. Since we measured nitrogen concentrations on a single water sample on each date, we cannot estimate the statistical significance of the differences. For example, in 1991 the DON concentrations following *U. rigida* decomposition are similar to the winter concentrations. However, winter concentrations are more likely due to high freshwater input. On the contrary, we believe that the summer increase of DON and PON concentrations is more likely of macroalgal origin, because freshwater inputs are low in the summer months, and because inorganic nitrogen increases only slightly (Fig. 2). In fact, at this time of the year inorganic nitrogen reserves in *U. rigida* thalli are depleted, and the very low oxygen concentrations in the water column do not allow a fast remineralization of DON.

Similar seasonal relationships between seaweed biomass, DIN, and tissue TN content, have been described in several European coastal systems (Sfriso et al., 1992; Ménesguen and Piriou, 1995; Pedersen and Borum, 1996; Hernández et al., 1997; Malta and Verschuure, 1997), while seasonal trends of cellular nitrate reserves have been described in *Ulva* sp. for other environments by Rosenberg and Ramus (1982), and for other macroalgal species by Asare and Harlin (1983). The seasonal development in tissue N suggests that *U. rigida* may become N-limited during summer, when thallus TN falls near to, or below, 2.4% DM. Fujita et al. (1989) reported that the critical N content (*sensu* Hanisak, 1979) for *U. rigida* grown on nitrate is $\leq 2.4\%$ DM. Summer N:P ratios lower than 20:1 also suggest N limitation (Atkinson and Smith, 1983; Björnsäter and Wheeler, 1990).

4.2. Nitrate uptake and storage

The observed V_{max} values for *U. rigida* are in the range reported for *Ulva* and other chlorophycean species (Table 2). In our experiments, we used only four to five initial nitrate concentrations, mostly below the asymptotic region of uptake. This could affect the calculation of a precise V_{max} (Conway et al., 1976; Harrison et al., 1989; Pedersen, 1994), however the estimated values seem adequate to support growth in the Sacca di Goro. In spring, at nitrate concentrations of about 30 μM, thalli with a TN of 2.5% DM could sustain a 10–20% daily growth rate with 6–12 h of uptake. In contrast, the estimated K_m is much higher than those earlier reported for Chlorophyceae, resulting in low V_{max}/K_m

Table 2
Kinetic parameters V_{max} (μmol N (g DM)$^{-1}$ h^{-1}), K_m (μM N) and affinity for uptake at low concentrations (V_{max}/K_m, Healey, 1980) for nitrate uptake in *U. rigida* and other chlorophycean species

Species	V_{max}	K_m	V_{max}/K_m	Reference
Ulva rigida	68.61	38.04	1.80	this study
U. rigida	58.57–85.21	17.86–33.50	2.54–3.28	Lavery and McComb, 1991
U. lactuca	20	5	4.5	Pedersen and Borum, 1997
Cladophora glomerata	69.50–161.93	1.10–12.93	11.24–63.18	Wallentinus, 1984
Codium fragile	2.80–10.93	1.93–7.64	1.25–1.59	Hanisak and Harlin, 1978
Enteromorpha spp.	129.43	16.57	7.81	Harlin, 1978
E. ahlneriana	27.79	1.73	16.07	Wallentinus, 1984
E. prolifera	75–169	2.31–13.30	12.7–32.6	O'Brien and Wheeler, 1987

values (Table 2). The ratio V_{max}/K_m is the slope of the Michaelis–Menten equation at lowest substrate concentrations, and quantifies the uptake efficiency at low substrate concentrations (Healey, 1980). High ratios can indicate a competitive advantage at nutrient procurement under low nutrient availability (Hein et al., 1995). A potential overestimation of K_m can arise from the type of experimental approach that we employed. In fact, several authors (e.g. Probyn and Chapman, 1982; Rosenberg et al., 1984) have found that uptake under continuous N addition is more efficient than in batch-type experiments. Our estimate of V_{max}/K_m is, however, similar to that determined by Lavery and McComb (1991) in *U. rigida* from the eutrophic Peel estuary in Australia. Low uptake efficiency may represent a local adaptation to N-rich environments, as several authors have shown that uptake kinetics for a given algal species are plastic, and can vary under different environmental conditions (Wallentinus, 1984; Thomas et al., 1987; Lavery and McComb, 1991; Fong et al., 1994).

We introduced the threshold term S_T in the Michaelis–Menten equation because the graphic representations of uptake rates vs. nitrate concentrations generally had a positive intercept with the *x*-axis. This phenomenon has also been reported by O'Brien and Wheeler (1987), Stapel et al. (1996), and O'Neill et al. (1989), who suggested substitution of the substrate term S with $(S - S_{min})$, where S_{min} corresponds to our S_T. Fitting of our data to the equation without S_T did not affect the derived V_{max}, but K_m increased by three to four times, or in some cases the fitting procedure failed to converge.

Preconditioning of thalli with nitrate greatly reduced nitrate uptake, which appears thus to be regulated, in the short term, by cellular nitrate pools, as also found by McGlathery et al. (1996) for *Chaetomorpha linum*. We quantified this effect of thallus nitrate content on uptake kinetics using Eq. (2), with an exponential term that accounts for nitrate content at t_0. Whatever mechanism is responsible for reducing uptake, it is unlikely that it affects the enzymatic affinity for nitrate. On the other hand, thallus nitrate content does affect uptake rates and V_{max}. In Eq. (2), V_{max} has a different meaning than in Eq. (1), since it represents the potential maximum uptake rate, at saturating nitrate concentrations, when cellular nitrate approaches zero. Eq. (2) can be applied only in short incubations, however it can explain uptake curves that depart from typical Michelis–Menten kinetics, and allows calculation of a more realistic V_{max}. We suggest, therefore, that tissue nitrate content should be determined prior to nitrate uptake experiments, because it will likely affect uptake rates.

Low uptake by nitrate-rich thalli may be the result of a feed-back inhibition, either by nitrate pool itself, or by ammonium or amino acid pools. Some authors have suggested, and modeled, a regulatory control of uptake by the TN content of macroalgae and macrophytes (Rosenberg et al., 1984; Fujita, 1985; O'Brien and Wheeler, 1987; Zimmerman et al., 1987; Duke et al., 1989). Pedersen (1994), however, showed that the transient nature of ammonium uptake rates by *Ulva lactuca* appeared to be related to rapid changes in small cellular pools of inorganic N or amino acids, rather than to changes in TN content. An inverse correlation between both ammonium and nitrate uptake and cellular inorganic N pools has also been reported in *Porphyra perforata* (Thomas and Harrison, 1985) and *C. linum* (McGlathery et al., 1996). It is probable that large nitrate pools can only persist in N-sufficient algae, in which TN content exceeds growth needs. In this condition, reactions that reduce nitrate to ammonium and assimilate it into organic molecules could proceed at

a slower rate, thus delaying the depletion of the inorganic pool, and causing slower uptake. For example, a lag phase in N uptake by N-rich thalli, due to slow nitrate reductase activity, has been described in *P. perforata* (Thomas and Harrison, 1985). Our results strongly support the hypothesis that nitrate pool size, rather than TN, directly affects nitrate uptake, since a small increase in nitrate content, from 2 to 14 μmol N (g DM)$^{-1}$, caused a pronounced decrease of uptake rates.

The nitrate storage experiments showed that the inhibitory effect of thallus nitrate content on nitrate uptake was less significant than in the short term uptake experiments. In fact, *U. rigida* thalli with different nitrate contents at t_0 accumulated comparable nitrate pools over 12–24 h periods. *U. rigida* can accumulate nitrate up to 400–500 μmol N (g DM)$^{-1}$ (15% of TN), and it can probably assimilate another 200–600 μmol N (g DM)$^{-1}$ d^{-1} in organic N pools. Cellular nitrate pools of similar size have been reported in *Ulva* species (Lopez-Figueroa and Rüdiger, 1991; Duke et al., 1986; Naldi and Wheeler, 1999) and in *C. linum* (McGlathery et al., 1996). Chapman and Craigie (1977) showed an important reserve role for nitrate in *Laminaria longicruris* (30% of TN), while Rosenberg and Ramus (1982) and Bird et al. (1982) did not consider nitrate a reserve for *Gracilaria* species, since it accounted for less than 10% of TN. *U. rigida* is a fast growing species, and a nitrate pool of 100–150 μmol N (g DM)$^{-1}$, as observed in the field samples, can sustain 10–15% day^{-1} growth rates for only 1 day, given a critical TN content of 2.4% of DM. Nitrate, therefore, represents a short term reserve. Amino acids and proteins are likely larger N storage pools (Rosenberg and Ramus, 1982; McGlathery et al., 1996) and could sustain growth for longer periods. Cellular nitrate pools, however, influence nitrate uptake kinetics, and as *U. rigida* nitrate reserves decrease in spring, uptake rates will be faster, thus accelerating nitrate depletion from the water column.

In summary, the seasonal cycle of biomass and tissue composition of *U. rigida* was correlated with the N cycle in the Sacca di Goro. The macroalga attained high standing stocks in late spring, due to elevated nitrate availability in the previous months. The growth phase with its high demand for N, in turn, influenced the seasonal dynamics of N cycle, causing nitrate depletion from the water column. Laboratory experiments demonstrated that *U. rigida* has low V_{max}/K_m ratios for nitrate uptake, and that thallus nitrate content strongly influences nitrate uptake rates. The low uptake efficiency, and the ability to exploit N availability by storing excess nitrate in cellular pools, may represent a physiological response to an eutrophic environment where nitrate is in large supply for most of the year. When *U. rigida* biomass declines in summer, large amounts of DON are released to the water column, thus contributing to N recycling, and sustaining the highly eutrophic nature of this ecosystem.

Acknowledgements

This work is a contribution to the European Union ELOISE thematic network (ELOISE No. 262/6) in the framework of the NICE (NItrogen Cycling in Estuaries) project carried out under contract MAS3-CT96-0048. This research was also supported by Grants No. EV4V-0131-I of the C.E.C.-4th Environmental R and D Programme and MIUR-COFIN 99 (Prof. I. Ferrari). We are indebt to Tony Zappata (Battello Hydra, Amministrazione

Provinciale di Ferrara, Assessorato Ambiente) for the support during sampling in the Sacca di Goro. We thank Ivan Fumagalli (ENEL-CRTN, Milano) for CHN analysis. We are grateful to S. Leonardi, Patricia A. Wheeler, Robert R. Christian and David T. Welsh for helpful discussions and critical reading of the manuscript. *[SS]*

References

Alvisi, A., Marzocchi, D., Edelwais, S., 1993. Indagine quali-quantitativa delle acque superficiali dei bacini Burana-Volano e Canal Bianco. Technical Report, Dimensione Ambiente, Provincia di Ferrara, Servizio Ambiente.

A.P.H.A., A.W.W.A., W.P.C.F., 1975. Standard Methods for the Examination of Water and Wastewater, 14th edn. AOHA, Washington.

Asare, S.O., Harlin, M.M., 1983. Seasonal fluctuations in tissue nitrogen for five species of perennial macroalgae in Rhode Island Sound. J. Phycol. 19, 254–257.

Atkinson, M.J., Smith, S.V., 1983. C:N:P ratios of benthic marine plants. Limnol. Oceanogr. 28, 568–574.

Bellan, G., 1987. Ecologie littorale méditerranéenne. Bull. Ecol. 18, 99–254.

Bird, K.T., Habig, C., DeBusk, T., 1982. Nitrogen allocation and storage patterns in *Gracilaria tikvahiae*. J. Phycol. 18, 344–348.

Björnsäter, B.R., Wheeler, P.A., 1990. Effect of nitrogen and phosphorus supply on growth and tissue composition of *Ulva fenestrata* and *Enteromorpha intestinalis* (Ulvales, Chlorophyta). J. Phycol. 26, 603–611.

Borum, J., Sand-Jensen, K., 1996. Is total primary production in shallow coastal marine waters stimulated by nitrogen loading? Oikos 76, 406–410.

Chapman, A.R.O., Craigie, J.S., 1977. Seasonal growth in *Laminaria longicruris*: relations with dissolved inorganic nutrients and internal reserves of nitrogen. Mar. Biol. 40, 197–205.

Conway, H.L., Harrison, P.J., Davis, C.O., 1976. Marine diatoms grown in chemostats under silicate or ammonium limitation. II. Transient response of *Skeletonema costatum* to a single addition of the limiting nutrient. Mar. Biol. 35, 187–199.

Corzo, A., Niell, F.X., 1992. Inorganic nitrogen metabolism in *Ulva rigida* illuminated with blue light. Mar. Biol. 112, 223–228.

Duarte, C.M., 1995. Submerged aquatic vegetation in relation to different nutrient regimes. Ophelia 41, 87–112.

Duke, C.S., Lapointe, B.E., Ramus, J., 1986. Effects of light on growth, RuBPCase activity and chemical composition of *Ulva* species (Chlorophyta). J. Phycol. 22, 362–370.

Duke, C.S., Litaker, W., Ramus, J., 1989. Effects of temperature on nitrogen-limited growth rate and chemical composition of *Ulva curvata* (Ulvales: Chlorophyta). Mar. Biol. 100, 143–150.

Fletcher, R.L., 1996. The British isles. In: Schramm, W., Nienhuis, P.H. (Eds.), Marine Benthic Vegetation. Recent Changes and the Effects of Eutrophication. Springer Verlag, Berlin, pp. 223–250.

Fong, P., Foin, T.C., Zedler, J.B., 1994. A simulation model of lagoon algae based on nitrogen competition and internal storage. Ecol. Monogr. 64, 225–247.

Fujita, R.M., 1985. The role of nitrogen status in regulating transient ammonium uptake and nitrogen storage by macroalgae. J. Exp. Mar. Biol. Ecol. 92, 283–301.

Fujita, R.M., Wheeler, P.A., Edwards, R.L., 1989. Assessment of macroalgal nitrogen limitation in a seasonal upwelling region. Mar. Ecol. Prog. Ser. 53, 293–303.

Hanisak, M.D., 1979. Nitrogen limitation of *Codium fragile* subsp. *tomentosoides* as determined by tissue analysis. Mar. Biol. 50, 333–337.

Hanisak, M.D., Harlin, M.M., 1978. Uptake of inorganic nitrogen by *Codium fragile* subsp. *tomentosoides* (Chlorophyta). J. Phycol. 14, 450–454.

Harlin, M.M., 1978. Nitrate uptake by *Enteromorpha* spp. (Chlorophyceae): applications to aquaculture systems. Aquaculture 15, 373–376.

Harrison, P.J., Parslow, J.S., Conway, H.L., 1989. Determination of nutrient uptake kinetic parameters: a comparison of methods. Mar. Ecol. Prog. Ser. 52, 301–312.

Healey, F.P., 1980. Slope of the Monod equation as an indicator of advantage in nutrient competition. Microb. Ecol. 5, 281–286.

Hein, M., Pedersen, M.F., Sand-Jensen, K., 1995. Size-dependent nitrogen uptake in micro- and macroalgae. Mar. Ecol. Prog. Ser. 118, 247–253.

Hernández, I., Peralta, G., Pérez-Lloréns, J.L., Vergara, J.J., Niell, F.X., 1997. Biomass and dynamics of growth of *Ulva* species in Palmones River Estuary. J. Phycol. 33, 764–772.

Isaac, R.A., 1990. Plants. In: Helrich, K. (Ed.), 15th edn. Official Methods of Analysis of the Association of Official Analytical Chemists, vol. 1. A.O.A.C, Arlington, pp. 40–68.

Koroleff, F., 1970. Direct determination of ammonia in natural waters as indophenol blue. I.C.E.S. Interlaboratory rep. No. 3: Information on techniques and methods for seawater analysis.

Lavery, P.S., McComb, A.J., 1991. The nutritional eco-physiology of *Chaetomorpha linum* and *Ulva rigida* in Peel Inlet, Western Australia. Bot. Mar. 34, 251–260.

Littler, M.M., Littler, D.S., 1980. The evolution of thallus form and survival strategies in benthic marine macroalgae: field and laboratory tests of a functional form model. Am. Nat. 116, 25–44.

Lopez-Figueroa, F., Rüdiger, W., 1991. Stimulation of nitrate net uptake and reduction by red and blue light and reversion by far-red light in the green alga *Ulva rigida*. J. Phycol. 27, 389–394.

Malta, E., Verschuure, J.M., 1997. Effects of environmental variables on between-year variation of *Ulva* growth and biomass in a eutrophic brackish lake. J. Sea Res. 38, 71–84.

McGlathery, K.J., Pedersen, M.F., Borum, J., 1996. Changes in intracellular nitrogen pools and feedback controls on nitrogen uptake in *Cheatomorpha linum* (Chlorophyta). J. Phycol. 32, 393–401.

Ménesguen, A., Piriou, J.-Y., 1995. Nitrogen loadings and macroalgal (*Ulva* sp.) mass accumulation in Brittany (France). Ophelia 42, 227–237.

Naldi, M., Wheeler, P.A., 1999. Changes in nitrogen pools in *Ulva fenestrata* (Chlorophyta) and *Gracilaria pacifica* (Rhodophyta) under nitrate and ammonium enrichment. J. Phycol. 35, 70–77.

Nienhuis, P.H., 1996. The North Sea coasts of Denmark, Germany and The Netherlands. In: Schramm, W., Nienhuis, P.H. (Eds.), Marine benthic vegetation. Recent changes and the effects of eutrophication. Springer Verlag, Berlin, pp. 187–221.

O'Brien, M.C., Wheeler, P.A., 1987. Short term uptake of nutrients by *Enteromorpha prolifera* (Chlorophyceae). J. Phycol. 23, 547–556.

O'Neill, R.V., DeAngelis, D.L., Pastor, J.J., Jackson, B.J., Post, W.M., 1989. Multiple nutrient limitations in ecological models. Ecol. Model. 46, 147–163.

Pedersen, M.F., 1994. Transient ammonium uptake in the macroalga *Ulva lactuca* (Chlorophyta): nature, regulation, and the consequences for choice of measuring technique. J. Phycol. 30, 980–986.

Pedersen, M.F., Borum, J., 1996. Nutrient control of algal growth in estuarine waters. Nutrient limitation and the importance of nitrogen requirements and nitrogen storage among phytoplankton and species of macroalgae. Mar. Ecol. Prog. Ser. 142, 261–272.

Pedersen, M.F., Borum, J., 1997. Nutrient control of estuarine macroalgae: growth strategy and the balance between nitrogen requirements and uptake. Mar. Ecol. Prog. Ser. 161, 155–163.

Probyn, T.A., Chapman, A.R.O., 1982. Nitrogen uptake characteristics of *Chordaria flagelliformis* (Phaeophyta) in batch mode and continuous mode experiments. Mar. Biol. 71, 129–133.

Rosenberg, G., Ramus, J., 1982. Ecological growth stratgies in the seaweeds *Gracilaria foliifera* (Rhodophyceae) and *Ulva* sp. (Chlorophyceae): soluble nitrogen and reserve carbohydrates. Mar. Biol. 66, 251–259.

Rosenberg, G., Probyn, T.A., Mann, K.H., 1984. Nutrient uptake and growth kinetics in brown seaweeds: response to continuous and single additions of ammonium. J. Exp. Mar. Biol. Ecol. 80, 125–146.

SAS Institute, 1985. SAS User's Guide: Statistical Version, 5th edn. SAS Institute, Cary.

Sfriso, A., Pavoni, B., Marcomini, A., Orio, A.A., 1992. Macroalgae, nutrient cycles, and pollutants in the Lagoon of Venice. Estuaries 15, 517–528.

Smith, D.W., Horne, A.J., 1988. Experimental measurement of resource competition between planktonic microalgae and macroalgae (seaweeds) in mesocosms simulating the San Francisco Bay-Estuary, California. Hydrobiologia 159, 259–268.

Sokal, R.R., Rohlf, F.J., 1981. Biometry, 2nd edn. WH Freeman, New York.

Stapel, J., Aarts, T.L., van Duynhoven, B.H.M., de Groot, J.D., van den Hoogen, P.H.W., Hemminga, M.A., 1996. Nutrient uptake by leaves and roots of the seagrass *Thalassia hemprichii*. Mar. Ecol. Prog. Ser. 134, 195–206.

Thomas, T.E., Harrison, P.J., 1985. Effect of nitrogen supply on nitrogen uptake, accumulation and assimilation in *Porphyra perforata*. Mar. Biol. 85, 269–278.

Thomas, T.E., Harrison, P.J., Turpin, D.H., 1987. Adaptations of *Gracilaria pacifica* (Rhodophyta) to nitrogen procurement at different intertidal locations. Mar. Biol. 93, 569–580.

Valderrama, J.C., 1977. Methods used by the Hydrographic Department of National Board of Fisheries, Sweden. In: Grasshoff, K. (Ed.), Report of the Baltic Intercalibration Workshop. Annex Interim Commission for the Protection of the Environment of the Baltic Sea, pp. 14–34.

Valderrama, J.C., 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Mar. Chem. 10, 109–122.

Valiela, I., McClelland, J., Hauxwell, J., Behr, P.J., Hersh, D., Foreman, K., 1997. Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. Limnol. Oceanogr. 42, 1105–1118.

Viaroli, P., Naldi, M., Christian, R.R., Fumagalli, I., 1993. The role of macroalgae and detritus in the nutrient cycles in a shallow-water dystrophic lagoon. Verh. Int. Verein. Limnol. 25, 1048–1051.

Viaroli, P., Bartoli, M., Bondavalli, C., Christian, R.R., Giordani, G., Naldi, M., 1996a. Macrophyte communities and their impact on benthic fluxes of oxygen, sulphide and nutrients in shallow eutrophic environments. Hydrobiologia 329, 105–119.

Viaroli, P., Naldi, M., Bondavalli, C., Bencivelli, S., 1996b. Growth of the seaweed *Ulva rigida* C. Agardh in relation to biomass densities, internal nutrient pools and external nutrient supply in the Sacca di Goro lagoon (Northern Italy). Hydrobiologia 329, 93–103.

Wallentinus, I., 1984. Comparison of nutrient uptake rates for Baltic macroalgae with different thallus morphologies. Mar. Biol. 80, 215–225.

Wallentinus, I., 1996. The Northern Atlantic coasts (the Swedish west coast, Norway and Iceland). In: Schramm, W., Nienhuis, P.H. (Eds.), Marine benthic vegetation. Recent changes and the effects of eutrophication. Springer Verlag, Berlin, pp. 165–184.

Zimmerman, R.C., Smith, R.D., Alberte, R.S., 1987. Is growth of eelgrass nitrogen limited? A numerical simulation of the effects of light and nitrogen on the growth dynamics of *Zostera marina*. Mar. Ecol. Prog. Ser. 41, 167–176.