# Nutrient and iron limitation to *Ulva* blooms in a eutrophic coastal lagoon (Sacca di Goro, Italy)

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#### Abstract

Growth patterns and bloom formation of the green seaweed Ulva rigida were analysed in the eutrophic Sacca di Goro lagoon (Po River Delta, Italy). Variations of standing biomasses and elemental composition of Ulva were analysed through an annual cycle with respect to nitrogen, phosphorus and iron. Growth rates, nutrient and iron uptake and nitrate storage by macroalgal thalli were also assessed with field experiments during the formation of a spring bloom. The control of Ulva growth and the bloom formation depended on multiple factors, especially on nitrogen availability and iron deficiency. In the nitrate rich waters of the Sacca di Goro lagoon, nitrate accumulation in Ulva thalli was inversely related with Fe uptake, indicating an influence of Fe limitation on N acquisition. Since length and magnitude of nitrate luxury uptake are inversely related to the size of the intracellular nitrate pools, in nitrate rich waters the fast growing Ulva may face risk of N-limitation not only when exposed to low N concentrations or at high biomass levels, but also when exposed to pulsed dissolved nitrate concentrations at low iron availability. The potential Fe limitation could be affected by processes controlled by geochemical reactions and by macroalgal growth and decomposition. Both Fe oxidation during the active macroalgal growth and the formation of insoluble FeS and FeS<sub>2</sub> during bloom collapse can result in a drastic decrease of soluble iron. Thus, a potential limitation of Fe to macroalgae can occur, determining positive feedbacks and potentially controlling the extent of bloom development and persistence.

#### Introduction

Watershed exploitation is thought to influence coastal lagoons and nearshore coastal waters throughout spatial dependent and time-lagged processes, which control the delivery of nutrients and pollutants (Valiela et al., 1997a). The excess of organic matter and nutrient inputs from the watershed can contribute significantly to eutrophication processes (Nixon, 1995; Cloern, 2001). Altered nutrient cycles have been recognised as one of the main causes of mass blooms of nuisance seaweeds, which seem to be stimulated especially by nitrogen availability (Borum, 1996; Valiela et al., 1997b; Raven & Taylor, 2003). In turn, the development of macroalgae plays a key role in oxygen production and consumption, and regulation of the entire benthic metabolism (Viaroli & Christian, 2003 and references therein). Rapid macroalgal growth followed by the decomposition of the accumulated biomass is considered to be one of the most important factors in the occurrence of dystrophic crises (Viaroli et al., 1996a; de Wit et al., 2001). Dystrophic crises are characterised by a strong perturbation of sulphur and inorganic carbon cycles, mostly due to the increased sulphide release by the mineralisation of macroalgal biomass and by bacterial sulphate reduction activity (Castel et al., 1996). The sulphide cycling not only depends on decomposition processes but is also influenced by sedimentary reactions of the iron cycle, which in turn regulate iron and phosphorus recycling and their availability to macroalgae (de Wit et al., 2001; Rozan et al., 2002 and references therein).

In temperate ecosystems, macroalgal blooms the so called "green tides" – are characterised by the dominance of a relatively small number of chlorophyceans, mostly belonging to the genera Ulva, Enteromorpha, Cladophora and Chaetomorpha (Flechter, 1996; Morand & Briand, 1996; Valiela et al., 1997b). The causes of green tides have attracted several speculations and a wealth of information exists on the ecophysiological traits of the bloom-forming species (Raven & Taylor, 2003) as well as on biogeochemical processes in macroalgal dominated ecosystems (Bartoli et al., 2001; Evre & Ferguson, 2002). Nevertheless, little is known on mode of benthic interaction that can trigger the onset and self-sustain the proliferation and persistence of green tides (Pihl et al., 1999; Sundbäck et al., 2003). Macroalgal growth is likely due to multiple factors that include bottomup controls and internal biochemical and physiological regulators as well as physical constraints (Lapointe, 1997; Valiela et al., 1997b; Cohen & Fong, 2004). A general precept is that the relative abundance of the macro-nutrients, especially nitrogen, controls the macroalgal productivity and the onset of biomass collapse (Pedersen & Borum, 1997 and references therein). In carbonatic systems, where geochemical processes control phosphate availability, phosphorus rather than nitrogen is the main limiting factor (Lapointe et al., 1992; McGlatery et al., 1994; Lapointe, 1997; Chambers et al., 2001). Generally, fast growing macroalgae exhibit nitrogen uptake and storage greatly in excess of their immediate physiological needs. This could result in the competitive ability to exploit and capitalise transient nutrient supply. In eutrophic nitrate rich waters, Ulva species exhibit a high capacity of storing mineral nitrate (Naldi & Wheeler, 1999; Naldi & Viaroli, 2002). Nitrate delivery to coastal areas

often depends on terrestrial run-off and river discharge. Consequently, nitrate availability to primary producers undergoes seasonal patterns and pulsed fluctuations that can favour this kind of macroalgae. Although macroalgal growth and bloom formation seem to be stimulated by exogenous nitrate availability, intracellular nitrate strongly influences nitrate uptake rates (Naldi & Viaroli, 2002).

More recently, the debate on oceanic primary productivity suggested a possible role for iron as a limiting factor (Martin et al., 1994). Urbanisation and changes in coastal areas may have also implications for primary productivity in coastal waters, leading to a further reduction of bioavailable iron (Lewitus et al., 2004). The importance of Fe as a limiting factor has been demonstrated to some extent for seagrasses growing above carbonate sediments (Duarte et al., 1995; Chambers et al., 2001). The Fe transported by terrestrial runoff may also have a significant impact on the production of macroalgae in coastal waters (Wheeler & Naldi, pers. comm.), especially in nitrate rich waters. Nitrate utilisation depends on Fe-containing enzymes and needs high cellular Fe quotas. Therefore, in nitrate rich and iron deficient waters nitrate utilisation by macrolagae could be controlled by iron availability rather than nitrate supply as previously demonstrated for phytoplankton (Geider & La Roche, 1994; Maldonado & Price, 1996).

This paper aims to analyse growth patterns and the bloom formation of the green seaweed *Ulva rigida* in the Sacca di Goro lagoon (Po River Delta, Italy). Variations of standing biomasses and elemental composition of *Ulva* are analysed through an annual cycle with respect to nitrogen, phosphorus and iron. Growth rates, nutrient uptake and retention by *Ulva* are also assessed with field experiments during the formation of a spring bloom.

#### Study area

The Sacca di Goro lagoon is a shallow-water embayment (44° 47′–44° 50′ N and 12° 15′–12° 20′ E), located in the southern part of the Po River Delta (Fig. 1). The surface area is  $26 \text{ km}^2$ , the total water volume is approximately  $26 \times 10^6 \text{ m}^3$  and the



*Figure 1.* Map of the Sacca di Goro showing the sampling stations (4, 11 and 17) and the area covered by *Ulva* on 12 March (continuos line) and 10 June (dotted line) 1997. PV, Po di Volano canal; GI, Giralda canal; CB, Canal Bianco canal.

average depth is approximately 1.5 m. The main freshwater inputs are the Po di Volano (PV), Canal Bianco (CB) and Giralda (GI) canals and the Po di Goro river. A detailed description of the lagoon characteristics and interactions with the watershed and the adjacent sea is reported by Viaroli et al. (2005). From 1987 to 1998, the lagoon was affected by frequent *Ulva* blooms, followed by dystrophic crises (Viaroli et al., 2001; 2005 and references therein). Since 1999, due to canal dredging and the improvement of hydrodynamics, macroalgal growth has decreased and blooms have become rare.

#### Materials and methods

The annual survey started on 23 January 1997 and was performed as shown in Table 1. Samplings in

the lagoon were carried out at station 17 that is located in the zone characterised by the maximum growth of *Ulva*, and at station 11, where a moderate macroalgal development was attained (Fig. 1). Additional samplings were also performed at station 4, which was at the edge of the area influenced by macroalgal blooms.

#### Canals – Water chemistry and nutrient loadings

The PV, CB and GI canals were sampled monthly (Table 1). Sampling stations were located at a certain distance from the mouth of the canals in order to minimise effects of marine water intrusion (Fig. 1). Samplings were performed with a Ruttner bottle from near-surface and near-bottom in the CB and GI canals. In the PV water samples were collected at 0.5, 2, 4 m depths. Soluble reactive (SRP), particulate (PP) and total phosphorus (TP); nitrite (NO<sub>2</sub>-N), nitrate (NO<sub>3</sub>-N), ammonium (NH<sub>4</sub>-N), dissolved (DON) and particulate organic nitrogen (PON) and reactive iron (RFe) were determined. The corresponding loadings were estimated from depth-weighted means of concentrations and the freshwater discharge values. Freshwater discharge data were kindly provided by the water authority of the Sacca di Goro watershed (Consorzio di Bonifica Primo Circondario Ferrarese).

#### Lagoon – Water chemistry

Measurements of water temperature, salinity, oxygen and pH were determined approximately every 10 days by the research vessel "Hydra" (Sacca di Goro Unit, Environmental Service of the Province of Ferrara) with an automatic

Table 1. Sampling frequencies from January to December 1997 at station 17 in the Sacca di Goro lagoon

Date	23/1	8/2	12/3	24/3	3/4	8/4	17/4	30/4	13/5	27/5	10/6	24/6	8/7	5/8	18/9	16/10	25/11	16/12
WL	х	х	х	х	х	х	x	х	х	х	x	х	х	х	x	х	x	х
WC	х	х	х		х		х		х		х		х	х	х	х	х	х
Ulva	х	х	х		х			х		х	х		х		х	х	х	
Cha		х	х	х			х	х		х	х	х				х		х
$P_{\rm max}$ -R	х	х	х	х		х		х	х	х	х	х						
Cage	х		х			х		х		х								

WL, water in lagoon; WC, water in canals; Ulva, biomass and elemental composition of Ulva; Cha, chlorophyll content of Ulva thalli;  $P_{max}$ -R, production and respiration of Ulva; Cage, incubation of Ulva inside cages.

multiparametric probe (IDRONAUT OCEAN SEVEN, MILAN, ITALY). Water samples were collected with a Ruttner bottle from near-surface and near-bottom (Table 1). SRP, NO<sub>2</sub>-N, NO<sub>3</sub>-N and NH<sub>4</sub>-N were determined on water subsamples obtained by mixing samples collected from both surface and near-bottom layers.

#### Ulva biomass

Three to five *Ulva* samples were collected quantitatively using a benthic grab from an area of  $2-10 \text{ m}^2$  of sediment surface. In the laboratory, *Ulva* thalli were cleaned to remove epiphytes, rinsed with tap water to remove salt, and ovendried at 70 °C to determine the dry weight (DW). We computed the areal biomass from the dry weight (DW) and surface area sampled.

Dry matter was processed for Ash Free Dry Weight (AFDW), total carbon (TC), total nitrogen (TN), extractable nitrate, total phosphorus (TP) and total iron (TP). Wet samples were processed for chlorophyll-*a*.

## Oxygen production and respiration and photosynthetic parameters of Ulva

Oxygen production and respiration by Ulva were measured in the laboratory 10 times, from 23 January until 24 June (Table 1). Thalli were sorted randomly and were cut into small disks (diameter = 10 mm). Disks were maintained overnight at the in situ conditions in order to minimise effects of cell overgrowth. Four beakers were then filled with 100 ml of filtered lagoon water and five discs added to each. Beakers were sealed with floating lids, incubated under slow stirring at increasing irradiances (I = 25, 50, 100, 200, 400 and800  $\mu \text{Em}^{-2} \text{ s}^{-1}$ ) for 30 min each and in the dark for 3 h. The incubations were performed sequentially, changing both macroalgal disks and medium. Dissolved oxygen was determined at the beginning and at the end of each incubation interval. Net oxygen production and consumption were measured as mg  $O_2$  gDW<sup>-1</sup> h<sup>-1</sup>. Light was supplied by a halogen-cold white lamp regulated by a rheostat (LAMPITALIA R7S 500 W, ITALY). The irradiance was recorded using a quantum radiometric detector HD 9021 RAD/ PAR with cosine correction (DELTA OHM,

PADOVA, ITALY). The main photosynthesis parameters were obtained with the light-saturation model proposed by Zimmerman et al. (1994). Here we consider the light-saturated oxygen production  $(P_{\text{max}})$  and the dark respiration (R).

#### Macroalgal growth rates and nutrient uptake

Net growth rates (NGR) of *Ulva* were estimated *in situ* by means of thalli incubations inside five cylindrical enclosures positioned 20 cm above the sediment surface to minimise cage burial. Incubations were performed at a fixed site located between station 17 and 11, where the *in situ* macroalgal biomass never exceeded 300 gDW m<sup>-2</sup>. Since the density effect was minimised, the measured NGR represents the potential growth rate. Moreover, the net mesh was large enough to allow grazing and losses by thallus fragmentation. Therefore, we consider the measured NGR as the potential net biomass increase resulting from the difference between potential production and losses.

Enclosures (diameter = 20 cm, volume = 10.5 l) were built using a plastic fencing net (mesh = 8 mm) to allow free water exchange. Incubations were carried out from 23 January to 6 February, from 12 to 24 March, from 8 to 17 April, from 30 April to 13 May, and from 27 May to 10 June (Table 1). At the beginning of each experiment, each cage was filled with 20 g of wet Ulva discs (diameter = 50 mm). At the end of each incubation, Ulva wet and dry weights were determined. Net growth rates were estimated considering an exponential growth, according to  $B_t = B_i * e^{t * NGR},$ equation: the where  $B_i$  = initial biomass (g DW),  $B_t$  = biomass (g DW) after t days of incubation.

Subsamples of the initial  $(B_i)$  and final biomasses  $(B_i)$  were analysed for chlorophyll-*a*, AFDW, TC, extractable NO<sub>3</sub>-N, TN, TP and TFe. The measured TN, TP and TFe concentrations in the macroalgal tissues at the end of the incubation are the net result of both dilution of the initial concentration due to growth and uptake from the external medium. Hypothetically, if no uptake or losses occur, an expected final concentration can be calculated assuming the product of algal biomass by tissue concentration as a constant during the incubation time. On the contrary, if uptake occurs the final quantity will be higher than the initial one, and a daily uptake can be calculated as the difference between the final and initial quantity. Nitrate can be reduced and assimilated in the organic cellular pools, therefore its intracellular quota do not represent the nitrate uptake but the net nitrate storage.

#### Analytical techniques

#### Water

Nutrient analyses were performed with conventional techniques: SRP (Valderrama, 1977), PP and TP (Valderrama, 1981), NO<sub>2</sub>-N and NO<sub>3</sub>-N (A.P.H.A. et al., 1975), NH<sub>4</sub>-N (Koroleff, 1970), DON and PON (Valderrama, 1981). For RFe, one litre of water was dried at 70 °C and the residue was dissolved in 30 ml of 0.5 M HCl. The reactive iron was determined after reduction with hydroxylamine HCl with the ferrozine method (Lovley & Phillips, 1987).

#### Ulva biomass

Triplicate wet subsamples (approx. 100 mg) were extracted overnight with 90% acetone and chlorophyll-a was determined according to Jensen (1978).

Dry subsamples ranging from 20 to 50 mg were analysed for TC and TN by elemental analysis (PERKIN ELMER 4200 CHNS). The extractable nitrate content of the thalli was determined according to Corzo & Niell (1992). The AFDW was measured following the ignition of 1 g dry matter overnight at 550 °C in a muffle furnace. TP was determined by spectrophotometry after acid extraction of the ash (Aspila et al., 1976). TFe was extracted on 0.5 g dry matter in 6 ml HNO<sub>3</sub> 60% + 1 ml H<sub>2</sub>O<sub>2</sub> 30% in a Milestone MCS-1200 MEGA microwave furnace. Iron was then determined on the filtered extract with inductively coupled plasma emission spectrometry (ICP AES Philips Analyticals 7450).

#### Statistical analysis

Normality of data was assumed, and homoscedasticity was confirmed using the Cochran test. Data were analysed with *a posteriori* one-way ANOVA (Sokal & Rohlf, 1995).

#### Results

#### Riverine loadings

In 1997, the annual freshwater discharge from the main canals was  $405 \times 10^6$  m<sup>3</sup>, of which 83.0% from PV, 9.5% from GI and 7.5% from CB. Freshwater flows were partially independent of rain events, since the overall hydrographic system was man regulated. TP and RFe loadings attained summer maxima, which were due to the delivery of drainage water from irrigated landfarms. Maximum TN loadings were recorded in January and May. The resulting annual loadings were 21 t RFe, 36 t TP and 1220 t TN. On average, TN was composed by 38.7% DON, 15.5% PON, 21.8% NH<sub>4</sub>-N, 24.0% NO<sub>3</sub>-N and, to a much lesser extent, NO<sub>2</sub>-N.

#### Main characteristics of the lagoon water column

The main physico-chemical variables of the water column at station 17 are reported in Figure 2. Salinity was in the typical range for temperate brackish systems, with the lowest values coinciding with the highest freshwater inputs due either to the natural riverine discharge or man-regulated inflows (Fig. 2a). The temperature peaked in June, and temperatures up to 25-30 °C persisted throughout the summer (Fig. 2b). The temporal variations of dissolved oxygen were partially related to Ulva bloom development (Figs. 2c and 3a). In spring, oxygen supersaturation occurred throughout the water column. From June onwards, hypoxia and anoxia frequently occurred mostly in the bottom water layers. Strict anoxic conditions persisted from 14 to 18 June. On 18 June, we also measured 85  $\mu$ M total sulphide in the water column at st. 17, coinciding with a pH of 7.8 (data not shown). Dissolved inorganic nitrogen concentrations in the water mass were related to macroalgal biomass and riverine discharges, as shown by the low spring concentrations and the autumn peak which coincided with freshwater inputs and low macroalgal biomasses. SRP concentrations were below the detection limits (5  $\mu$ g P l<sup>-1</sup>) during macroalgal growth but attained a peak (305  $\mu$ g P l<sup>-1</sup>) during the dystrophy in June (Fig. 2f).



*Figure 2.* Annual variations of salinity (a), temperature (b) and dissolved oxygen saturation (c) in the surface and near bottom waters and dissolved inorganic nitrogen (DIN) and nitrate (e) and soluble reactive phosphorus (SRP) concentrations (f) at station 17 from January to December 1997. Continuous line: surface water; dotted line: bottom waters.

#### Bloom formation and biomass characteristics

An overwintering *Ulva* bed persisted in the eastern part of the lagoon, with biomasses up to 100 g DW m<sup>-2</sup>. Active macroalgal growth began in March when the water temperature was 10–12 °C and light conditions became favourable. Afterwards, the biomass increased exponentially and reached the maximum spreading at the end of May-early June (Figs. 1 and 3a). The biomass peak was attained at station 17 on 27 May (878  $\pm$  302 g DW m<sup>-2</sup>), whilst at st. 11 at the edge of the macroalgal beds, a maximum biomass of 296  $\pm$  92 g DW m<sup>-2</sup> was reached on 10 June. On the latter date, the zone of the lagoon east of st. 11 (approximately 11 km<sup>2</sup>) had an almost 100% *Ulva* coverage, whilst in the central area (approximately 5 km<sup>2</sup>) the macroalgal coverage ranged from 25 to 50%. The standing biomass peak was followed by a sudden collapse of the macroalgal mats, which started to decompose causing anoxia and sulphide release, mostly at station 17. In the sheltered area of the lagoon, *Ulva* growth recommenced in mid September and persisted through the following winter. Thallus TN, NO<sub>3</sub>-N, TP and TFe followed clear seasonal patterns (Fig. 3b–e).



*Figure 3*. Annual variations of *Ulva* standing biomass (dry weight), its elemental composition and chlorophyll-*a* content at station 17 from January to December 1997. (a) biomass, (b) total nitrogen, (c) nitrate nitrogen, (d) total phosphorus, (e) total iron, (f) chlorophyll-*a*, (g) C:N, (h) N:P, (i) N:Fe, (j) Fe:P molar ratios. Error bars represent 1 standard deviation of 3 replicates. CTC: critical concentration. C:N = 9.6 and N:P = 35 are considered as ratios for a balanced growth.

NO<sub>3</sub>-N peaked in early April, when it represented 12% of thallus TN content. Thallus TN attained maximum values from January to February and decreased during the spring growth phase of Ulva, when it was close to the critical concentration (CTC) threshold (Pedersen & Borum, 1997). Thallus TP was very high in winter, whilst from late March to the dystrophic period it remained at significantly lower values, close or below the CTC limit (Lyngby et al., 1999). Both TN and TP rose again in the autumn macroalgal growth phase. TFe underwent a similar pattern, but with an autumn peak. Chlorophyll-a in macroalgal tissues attained the highest values in March and early April, whilst the minimum coincided with the biomass peak (Fig. 3f). The relative ratios of C, N, P and Fe in *Ulva* thalli are shown in Figure 3g-j. During the bloom formation, the C:N ratio increased, indicating a potential N limitation. Moreover, during the same period C:N ratio was inversely correlated with chlorophyll-a (Fig. 4). The N to P ratio indicated an alternance of P and N limiting conditions, with a slight N-limitation during the biomass peak. In April-May, a Fe deficiency relative to both N and P was also observed.



### *Figure 4*. Relationship between chlorophyll-*a* and C to N ratios. Empty circles: T < 10 °C; dark circles: 12 < T < 25 °C.

#### Macroalgal growth potential in relation to nitrogen, phosphorus and iron

The macroalgal photosynthesis and respiration followed a clear seasonal pattern (Fig. 5). The optimal photosynthetic performance was attained at the end of March, with the highest  $P_{\text{max}}$  and minimum R. In the subsequent period, coinciding with the biomass bloom,  $P_{\text{max}}$  decreased progressively. The maximum R rates were attained in mid May, and coincided with the highest biomass value. In the latest period of the *Ulva* bloom, coinciding with temperatures up to 30 °C, the lower R rates were probably indicative of increasing physiological stress.

The net biomass increase measured as NGR attained maximum values  $(0.143 \pm 0.040 \text{ d}^{-1})$  in April, whereas minimum rates coincided with low temperatures in January and with high temperatures and the biomass peak at the end of May-early June (Table 2). The NGR rates were consistent with TN, NO<sub>3</sub>-N and TP uptake rates. TFe uptake was negligible during the maximum growth phase of *Ulva*. A comparison among dates is reported in Table 3.



*Figure 5.* Variations of maximum net photosynthetic and dark respiration rates determined for *Ulva* from January to June 1997. Error bars represent 1 standard deviation of 5 replicates.

Incubation period	NGR $(d^{-1})$	Net uptake						
		Total N $\mu$ gN g <sup>-1</sup> d <sup>-1</sup>	Nitrate $\mu$ gN g <sup>-1</sup> d <sup>-1</sup>	Total P $\mu$ gP g <sup>-1</sup> d <sup>-1</sup>	Total Fe $\mu$ gFe g <sup>-1</sup> d <sup>-1</sup>			
23/01-06/02	0.025 (0.004)	1467 (551)	25 (8)	144 (9)	52 (20)			
12/03-24/03	0.105 (0.010)	3169 (437)	431 (58)	233 (37)	3 (3)			
08/04-17/04	0.146 (0.049)	4903 (873)	808 (194)	295 (88)	5 (18)			
30/04-13/05	0.081 (0.027)	2252 (751)	294 (28)	146 (65)	23 (10)			
27/05-10/06	0.048 (0.025)	1466 (471)	211 (66)	174 (45)	40 (6.2)			

Mean values and standard deviations (in brackets) of five replicates are reported.

#### Discussion

In polluted coastal systems ephemeral macroalgae may predominate and undergo abnormal growth and rapid extinction, which are usually unpredictable. The causes of these blooms have been described considering the ecophysiological characteristics of the bloom-forming species as well as the boundary conditions (for an updated review see Valiela et al., 1997b; Martins et al., 2001; Raven & Taylor, 2003). Amongst these, many chlorophytes have been found to exploit short-term nutrient availability taking competitive advantages in fluctuating and extreme environments. When macroalgal densities exceed a certain critical threshold, the capacity of the macroalgae to control their biogeochemical environment ceases and the occurrence of biogeochemical and internal feedbacks may cause the collapse of the bloom (Viaroli et al., 1996b). These feedbacks are likely

*Table 3.* Differences between dates for NGR (a), total nitrogen uptake (b), net nitrate storage (c), total phosphorus uptake (d), iron uptake (e)

	23 Jan– 06 Feb	12–23 March	8–17 April	30 Apr- 13 May
23 Jan-06 Feb				
12-23 March	abde			
8–17 April	abcde	bd		
30 Apr-13 May	ade	ns	abcd	
27 May-10 June	d	abde	abce	ns

Differences were tested with one-way ANOVA. Letters indicate significant differences between dates at  $p \le 0.01$ ; ns, statistically not significant.

multifactorial and include grazing and bottom-up controls as well as internal biochemical and physiological regulators (Lapointe, 1997; Balducci et al., 2001; Raven & Taylor; 2003; Cohen & Fong 2004). A general precept is that the relative abundance of carbon, nitrogen and phosphorus controls the macroalgal productivity. Hence, the deficit of one of these nutrients may act as a switch determining the onset of the bloom collapse.

The concentration of chlorophyll-a provides a specific adaptative response to seasonally changing radiation and canopy stratification (Lobban & Harrison, 1994; Vergara et al., 1998). Nitrogen availability may be also responsible of pigment level variations. In this study, during bloom formation, chlorophyll-a correlated directly to the tissue nitrate concentrations and attained a minimum during N depletion. Moreover, at temperatures above 10 °C, the chlorophyll-a content inversely correlated with the C:N ratio. The strong seasonality of the nutritional status of Ulva and its dependence on the biogeochemical conditions was evident for the C to N and N to P atomic ratios. During the bloom formation, the C to N ratio increased up to 16.9  $\pm$  3.6, which coincided with the critical N content as defined by Fujita et al. (1989), Lavery & McComb (1991) and Pedersen & Borum (1997). The increased C to N ratio may result not only from the nitrogen depletion but also from the accumulation of carbonaceous compatible solutes (Welsh, 2000). These substances, which act either as energy reserve or protection of macroalgal cells against environmental stresses, are probably used or leached when the macroalgal biomasses attain critical levels causing a subsequent decrease of the C to N ratio. 66

The N to P ratio, which was minimum in winter and peaked in late March, decreased during the spring growth phase. The variability of the N to P ratio evidenced that the relative N to P limitation was not permanent and underwent a seasonal evolution, with a potential P-limitation in the intermediate growth phase, when the thallus P-content was close to the CTC reported by Lyngby et al. (1999). It is currently accepted that availability of N limits the productivity of macroalgae in temperate siliciclastic waters, whilst availability of P is the critical factor in limiting macroalgal productivity in carbonate-rich tropical waters (Lapointe et al., 1992; Lapointe 1997; Chambers et al., 2001). In the Sacca di Goro lagoon the sedimentary carbonate concentration ranged from approximately 10% DW up to 20% DW (Dal Cin & Pambianchi, 1991) and most of the inorganic phosphorus was in the calciumbound pool (Azzoni et al., 1999). As a consequence, during the bloom formation P might not be available to Ulva. A decrease of P retention by the sedimentary Ca-bound pool can also occur as the eutrophication degree increases (McGlatery et al., 1994). This causes a release of phosphorus to the water column in summer during dystrophy and, likely, in autumn, when the intracellular P pool increases. The highest thallus P-content found in autumn and winter can be also related to the riverine discharge at lower growth rates.

Overall, the combined analysis of the relative C:N and N:P trends evidenced that nitrogen seemed to be only temporarily the critical factor in the Ulva life-cycle. During the formation of the spring bloom, Fe depletion, rather than N and P deficiency, seemed to be critical for Ulva to grow. Based on our knowledge, this point was not previously considered when assessing limiting factor of bloom formation. Only recently, iron and trace element availability have attracted more attention (Wheeler and Naldi, pers. comm.). From January to May, the C:Fe, N:Fe and P:Fe ratios increased progressively. During the maximum growth phase of Ulva, the stoichiometric ratio was 4224C:395N: 11P:1Fe, which approximate the stoichiometry of C, N and P for balanced growth (Atkinson & Smith, 1983; Viaroli et al., 1996b; Lyngby et al., 1999), but corresponded to the lowest level of Fe. Based on these results, the CTC for Fe seemed to be close to 0.3 mg Fe g DW<sup>-1</sup>. Likewise, part of this

Fe was not immediately available, since the thallus Fe-content comprises intracellular and adsorbed FeOOH quotas. Therefore, both CTC and elemental ratios can underestimate the Fe limitation.

The relative N and P limitation was reconsidered through the cage incubations of *Ulva* thalli, which were performed under controlled conditions during the spring bloom formation. The total nitrogen uptake followed a seasonal pattern that was consistent with the net nitrate storage. In January at T < 10 °C and at low NGR, when the dissolved nitrate availability was relatively high the net nitrate storage was negligible. At the temperatures occurring from early March onwards (T > 12 °C), there was a significant linear relationship between NGR and TN uptake, with NGR = 0.008 + 0.029TN,  $R^2 = 0.85$ , p = 0.0001 (Fig. 6a). The NGR correlation with nitrate uptake indicated the dependence of *Ulva* growth on nitrate availability, nitrate



*Figure 6*. Relationships between NGR and total nitrogen uptake (a) and NGR and net nitrate storage (b) from January to June 1997. Empty circles: T < 10 °C; dark circles: 10 < T < 25 °C.

being the main nitrogen source in the lagoon (Fig. 6b). The maximum nitrogen uptake  $(4.90 \pm 0.87 \text{ mg g } \text{DW}^{-1} \text{ d}^{-1})$  corresponded to a maximum NGR of 0.143  $\pm$  0.040 d<sup>-1</sup>. The NGR peak also coincided with the capacity of Ulva store nitrates at very high to rates  $(0.81 \pm 0.19 \ \mu g \ N \ g \ DW^{-1} \ d^{-1})$ , leading to intracellular concentrations up to 0.6% DW. This capacity has been described for ephemeral seaweeds which control the transient nitrate availability by means of a luxury uptake and can saturate the nitrate and nitrite reductases (Pedersen & Borum, 1997; Raven & Taylor, 2003). Among these, Ulva rigida can accumulate cellular nitrate reserves up to 0.7% DW, which appears to be a saturation threshold (Naldi & Viaroli, 2002). At lower temperatures (T < 10 °C), the nitrate accumulation was almost negligible whilst the TN demand was



*Figure 7*. Relationships between NGR and the C to N molar ratio (a) and between cellular concentrations of total nitrogen and total phosphorus (b) in *Ulva* thalli grown in cages. In the upper figure (a), empty circles refer to T < 10 °C; dark circles to 12 < T < 25 °C.

relatively high. Based on these data, one can argue that at slow growth rates *Ulva* can exploit immedi-

and assimilation (Pedersen & Borum, 1997). At temperatures above 10 °C, a significant inverse correlation (r = -0.735, p = 0.0003) was found between NGR and the C to N ratio (Fig. 7a). A linear relationship between TN and TP uptake rates by Ulva was also found (Fig. 7b). The regression equation was TN = 30.4TP-6.4, with  $R^2 = 0.59$ , p < 0.01. The slope 30.4 (confidence interval 20–41 at p = 0.05) was close to the N to P ratio occurring during a balanced growth of Ulva. Moreover, the highest NGR values were attained at C to N ratios ranging from 9 to 10, which are close to the value reported for an optimal growth of Ulva (Atkinson & Smith, 1983, Viaroli et al., 1996b). We assessed the C:N:P:Fe stoichiometry for Ulva thalli which were incubated in cages in situ. In March, in the early phase of bloom formation, the elemental composition of *Ulva* thalli was  $(5794 \pm 877)C:(594 \pm 91)$ N:(18  $\pm$  2)P:1Fe. During the NGR peak in April, the stoichiometry was  $(13,882 \pm 2595)$ C:  $(1442 \pm 227)$ N: $(41 \pm 15)$ P:1Fe. In both conditions, the macro-nutrient ratios were close to the stoichiometric conditions of a balanced growth, whilst the relative availability of Fe decreased 2.4 folds. Thus, a potential Fe-limitation was assumed.

ate nitrogen resources through an efficient uptake

The maximum NGR found in this study was approximately two times lower than those measured for *U. lactuca* from Roskilde Fijord in laboratory experiments with N enrichment (0.34–0.35 d<sup>-1</sup>, Pedersen & Borum, 1996) and for *Ulva* sp. with cage incubation in the Veerse Meer at low macroalgal density and high nitrate availability (0.38–0.39 d<sup>-1</sup>, Malta & Verschuure, 1997). In the Veerse Meer, under natural summer conditions and low nitrogen availability, NGR was much lower and close to 0.10 d<sup>-1</sup>. Similar values were also recorded in several eutrophic Mediterranean coastal lagoons, in a temperature range (10–25 °C) that was considered as optimal for *Ulva* growth (De Casabianca et al., 2002).

In order to assess the extent of nitrogen limitation to *Ulva* in the Sacca di Goro, we tentatively estimated the maximum NGR of *Ulva* that can be achieved at the actual intracellular N reserves under non-limiting temperature conditions (10 < T < 25 °C). We used the maximum specific growth rate  $(0.34 \text{ d}^{-1})$ , the subsistence N quota (0.71% DW) and the Droop's equation as reported by Pedersen & Borum (1996, 1997). With an intracellular N reserve ranging from 2.52 to 3.62% DW, we estimated a theoretical NGR =  $0.265 \pm 0.014$ , which was constant from March to June. On average, the difference between the theoretical NGR and the measured NGR attained a minimum in April (47%) and a maximum in June (80%). Therefore, the actual NGR was much less than the growth potential suggesting that Ulva growth cannot be explained only by N-limitation. Other factors, namely the top down control by grazing (Balducci et al., 2001) and selfshading (Krause-Jensen et al., 1996; Vergara et al., 1998) can affect macroalgal growth. In the conditions we performed incubations, the selfshading effect was likely low due to the low biomass density. Moreover, the incubated thalli did not show apparent damages by grazers.

In addition to the above-mentioned factors, we assumed that in nitrate rich waters iron could regulate *Ulva* growth, through the control of nitrate and nitrite reductase (NIR) activities. The influence of Fe limitation on N acquisition has been demonstrated for several phytoplankton species, which at low Fe concentrations showed a lower N assimilation capacity in nitrate rich waters rather than in ammonium rich waters (Geider & La Roche, 1994). In the nitrate-rich waters of the Sacca di Goro lagoon, free-floating macroalgae exhibited a NO<sub>3</sub>-N peak coinciding with the Fe minimum. During cage incubations, nitrate accu-

mulation within Ulva thalli was inversely related with Fe uptake (Fig. 8a). Moreover, at the end of the incubation the cellular NO<sub>3</sub>-N content inversely correlated with Fe-thallus concentration (Fig. 8b). Based on these finding, one can assume that the fast growing Ulva may face risk of N-limitation not only when exposed to low N concentrations or at high biomass levels, but also when exposed to pulsed dissolved nitrate concentrations at low iron availability. Albeit surge uptake is a competitive advantage to ephemeral macroalgae living under fluctuating N availability (Pedersen & Borum, 1997), length and magnitude of luxury uptake are inversely related to the size of the intracellular nitrate pools (Naldi & Viaroli, 2002). Therefore, the potential gain of N achieved during the nitrate luxury uptake at high transient nitrate concentrations could be contrasted by the nitrate accumulation within cells, which in turn depends upon Fe depletion. When opposite fluctuations of nitrate and Fe occur, surge uptake may not be as ecologically important for N acquisition as formerly suggested. Moreover, Pedersen & Borum (1997) suggested that ephemeral macroalgae are subjected to N limitation at higher DIN concentrations than slow growing species. Therefore, in nitrate rich waters DIN thresholds could be further increased by Fe limitation, making these macroalgae more sensitive to N stress.

In shallow eutrophic ecosystems, the potential Fe limitation could be also affected by processes controlled by geochemical reactions (Chambers et al., 2001; Rozan et al., 2002). During bloom formation, under oxic conditions Fe availability is



*Figure 8*. Relationship between net nitrogen storage and iron uptake (a) and cellular concentrations of nitrate and iron in *Ulva* thalli at the end of the incubation in cages (b).

controlled not only by macroalgal uptake, but also by the formation of the insoluble Fe oxidised species. In the latest stage of the bloom, anoxic conditions due to the biomass decomposition can favour Fe release, but the concurrent sulphide production acts as a Fe trap, with the formation of insoluble FeS and FeS<sub>2</sub>. The formation of FeS and pyrite results in a drastic decrease of soluble iron, thus a potential limitation of Fe to macroalgae can occur. Fe limitation is potentially higher in carbonate-rich sediments, due to Fe trapping by carbonates. Here iron, sulphur and carbonate have also implications for P cycling (Rozan et al., 2002). Overall, iron-sulphur-phosphorus interactions may determine positive feedbacks for macpotentially controlling bloom roalgae, development and persistence.

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