Science of The Total Environment
1 October 2007, Volume 384, Issues 1-3, Pages 293-305
http://dx.doi.org/10.1016/j.scitotenv.2007.05.007

In situ growth potential of the subtidal part of green tide forming Ulva spp. stocks

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Abstract:

Ulva spp., the algae most responsible for green tides in Brittany (France), are found on the foreshore and in the most beachward wave area (MBWA) of many bays during green tide phenomena. These algae have recently been seen drifting at greater depths (reaching − 20 m). In view of the significant quantities of algae found at these depths, and the less favorable conditions for algal growth than in the intertidal zone, we attempted to determine if they could grow there. For that, during their maximum growth period (from May to July), algae were picked up at three stations located on the foreshore, in the MBWA and in the subtidal (deep) zones of the Bay of Douarnenez, and their nitrogen, carbon and chlorophyll a + b contents were determined, and their photosynthetic activity was compared in the laboratory. The intracellular concentrations did not differ much from one station to another, although in the subtidal zone, the irradiance and the nitrogen concentration in the ambient water were much lower than those measured on the foreshore and in the MBWA. Photosynthetic activity characterized by maximum amounts of oxygen produced at different irradiances and by saturating and compensating irradiance levels, was also quite similar at the three stations. The irradiance, temperature and salinity of the subtidal environment, together with the chemical and photosynthetic characteristics of the algae found in that area, are consistent with the hypothesis that they grow there, and that their nitrogen supply comes from nitrogen releases from sediments. Nevertheless, their growth rate is probably less than that of algae in the MBWA.

Keywords: Eutrophication; Algal blooms; *Ulva* sp.; Photosynthesis; Irradiance; Nutrients.

1. INTRODUCTION

Macroalgal blooms (green tides) and phytoplankton blooms are the main evidence of coastal eutrophication, and are widespread throughout the world. Morand and Briand (1996) listed 37 countries including several American states affected by the phenomenon. This number was found to have increased in a recent review (Morand and Merceron, 2004). Schramm and Nienhuis (1996) listed 114 sites in Europe and Asia Minor, where the phenomenon has been observed and described. In France, several regions are affected including the coasts of Brittany (Dion and Le Bozec, 1996), the Arcachon basin (Auby et al., 1994) and the Mediterranean lagoons (De Casabianca, 1996).

Many studies have confirmed the limiting role played by nitrogen in this phenomenon (Sfriso et al., 1988; Fong et al., 1993; Ménesguen and Piriou, 1995; Pedersen, 1995). With only rare exceptions, such as in Western Australia, where intensive farming practices are recent and phosphorus stocks have not yet been created in marine sediments, nitrogen is considered to be the main limiting factor for algal blooms (Morand and Briand, 1996).

Ulva spp. are the most prevalent of the algae found in green tides, which explains why many studies have focussed on their growth (Fortes and Lüning, 1980; Pihl et al., 1996). Their speed of growth has often been linked to the morphology of their thalli (the thin blade facilitates exchanges with the environment) (Littler and Littler, 1980; Rosenberg and Ramus, 1984), to their capacity to store nutrients during pulses (Ramus and Venable, 1987), and to their ability to adapt to varied environmental conditions (Vermaat and Sand-Jensen, 1987; Geertz-Hansen and Sand-Jensen, 1992; Riccardi and Solidoro, 1996).

In Brittany, green tides are mainly due to blooms of two *Ulva* species, *U. rotundata* Bliding and *U. armoricana* Dion, de Reviers *et* Coat (Dion et al., 1998), whose morphology and ecological requirements are quite similar. Accumulations on small surface areas and some drifting mats of these algae can be seen on aerial photos taken in the 1950s. Since the 1970s, the phenomenon has increased both in terms of the number of areas affected and the extent of the areas involved, with the biggest increases at the most favorable sites. Two inventories recorded about 50 affected areas (Merceron, 1998, 1999). Sites that favor green tides have several typical characteristics: a gently sloping sandy cove or bay with a wide foreshore exposed at low tide and weak residual currents; water containing little suspended matter; nutrient inputs provided by small local streams loaded with inorganic nitrogen. Classically, algae proliferate in the most beachward wave area (MBWA) during spring and summer when temperature, irradiance and nutrients are optimal for their development. Depending on the local combination of tide, wind and sea conditions, the seaweed can be stranded on the beach or resuspended by incoming tides. The total "intertidal" biomass, i.e. located in the MBWA and foreshore zone of the 15 most affected sites in Brittany has been assessed at 20 000 tons of fresh *Ulva* spp. at the time of the seasonal maximum (Merceron, 1999).

Until recently, this "intertidal" *Ulva* spp. stock was the only one studied and preventive measures against green tides in Brittany aimed at it only. Yet another stock at a deeper (or subtidal) location, has been documented at several sites in Brittany: in the Bays of Saint-Brieuc, Lannion, Douarnenez and Concarneau (CEVA, 2000; Chaquin, 2000; Merceron and Morand, 2004). Between these two stocks, or rather between the two fractions of the same stock, there is a zone where macroalgae are absent most of the time. It constitutes a strip at the seaward edge of the surf zone, where waves just begin to break. This strip, which is parallel to the shore, is subject to hydrodynamics that prevents drifting, and creates a barrier the algae cannot cross in either direction, except when the sea is completely calm. The deep fraction of *Ulva* spp. is located beyond the surf zone, usually at depths ranging from 2 to 20 metres. The algae found there are also drifting. They lie lightly on the bottom and are gently resuspended by turbulence. The tonnage of subtidal *Ulva* spp. was estimated by Merceron and Morand (2004). In the Bays of Douarnenez and Concarneau, the subtidal tonnages were comparable to or higher than those observed in the MBWA and on the foreshore. At Saint-Michel-en-Grève, they were lower than the intertidal tonnages, and at Locquirec they were similar. A survey we conducted in 2000 indicated that this subtidal fraction was often present offshore from the intertidal fractions but also occurred at sites without intertidal algae.

For green tides we reported in the Bay of Douarnenez, with a mix of *U. rotundata* and *U. armoricana* lying on the seafloor off the surf zone as well as on the foreshore, we showed that temperature, salinity, irradiance and nitrogenous nutrients were suitable for growth of *Ulva* spp. in a subtidal position (Merceron and Morand, 2004). In view of the significant size of the subtidal fractions, and the fact that they occur despite nevertheless less favorable conditions for algal growth than in the intertidal zone (reduced light due to increased water depth, fewer nutrients due to dilution of stream inputs, lower temperature in spring and summer), the question is whether algae can grow on site, or whether they originate from the intertidal fraction and could return to the more favorable conditions on the foreshore. In order to test if the algae can really grow in the subtidal zone, we measured the nitrogen, carbon and chlorophyll contents of *Ulva* spp. sampled at three different sites, and estimated their photosynthetic activity in environments reconstructed in the laboratory.

2. MATERIALS AND METHODS

Study area

The Bay of Douarnenez, where seaweed was sampled, is located at the tip of Brittany (Fig. 1). The 8.7 km wide bay opens toward the west. Its surface area is 252 km². The water becomes steadily deeper from east to west, reaching a maximum depth of 35 m at the lowest astronomical tide in the area where the bay opens to the sea.

The catchment area feeding the bay has a total surface area of 298 km² (Anonymous, 1995), and is mainly used for intensive farming. Fresh water inputs come from several streams located on the southern and eastern edges of the bay. The overall average flow rate is low (approximately 4 $m^3 s^{-1}$). Salinities observed in the bay generally range from 34 to 35 PSU. Water surface temperatures usually range from 9 to 18 °C. However, there is greater variation in the surf zone due to the shallowness of the water and the fact that it covers the beach sand daily. The bottoms are generally soft, made up of pale fine sand (medium grain size ranging between 100 and 200 µm), with an increasing proportion of fine particles (<63 µm) with depth, but only significant at a depth of 20 m and over (Bourgoin et al., 1991). The maximum instantaneous currents in the study area are less than 10 cm s^{-1} (Obaton, 1996). Mathematical simulation of currents in the Bay of Douarnenez by SEAMER-CEVA (2001) indicates low residual circulation in the bay, particularly in the eastern part, which promotes eutrophication. The residual current computed was very low, since the residence time of the water in a 500 m x 500 m cell was over five days in the eastern half of the bay (Obaton, 1996). Intense phytoplankton blooms, some of them toxic, occur frequently (Birrien 1987). The Bay of Douarnenez is the most frequently affected site amongst those monitored by France's toxic phytoplankton monitoring network since 1983.

The bay is also affected by large and recurrent green tides, especially at the beaches fringing the bay between the Aber headland to the north and the town of Douarnenez to the south. Occasional biomass counts were made between 1996 and 1998. The intertidal biomasses present were between 2,173 and 3,385 t of fresh spin-dried seaweed. The total biomass of the subtidal fraction was about the same, whereas, per surface area unit, it varied considerably from almost nil to 1.5 kg of fresh spin-dried weight per m² (Merceron and Morand, 2004). The seabed can be totally overlaid by seaweed, particularly where algae accumulate in shallow depressions.

Algae and water samples were taken and *in situ* measurements made at the three stations in the southeastern part of the bay, on Le Ry beach and in the immediate offshore area, the first on the foreshore (station A), the second in the MBWA (station B), and the third in a subtidal location (station C) (Fig. 1). The bottom was sandy at all three stations. Station A was located at the mid-tide level and its area was big enough to include variations in the locations of stranded seaweed. Station B covered the western part of Le Ry beach, where algae were present in the MBWA. Station C was located one km offshore from station B, in an area where subtidal *Ulva* spp. beds are frequently observed.

Sampling

Seaweeds

Samples were taken seven times at each station in the morning from mid-May to mid-July, which is the period when Ulva spp. growth is thought to be maximal. By sampling weekly, we aimed to study the growth period as precisely as possible.

Samples were taken at stations A and B the same day during low tide, whereas at station C samples were taken two days earlier or later. This interval made it possible to measure the photosynthetic activity on the day following collection of the seaweed after an identical storage period and under controlled conditions. The time-lag was inverted randomly to avoid any possible bias due to a constant order. At station B, water depth at the time of sampling ranged from 0.5 m to 0.7 m; at station C, it ranged from 8 m to 11 m.

At least five large thalli, in apparently good condition, were randomly sampled at each site. The frequent moving of algae by currents and waves was assumed to prevent any distribution bias. At stations A and B, algae were gathered by hand. At station C, a small, purpose-built trawl was used. It had a metal frame of 1.00 m x 0.36 m in contact with the seabed, fitted with floaters at the top to maintain it in a vertical position and with chains at the bottom. A net with a 6 mm x 6 mm mesh was attached to it. Ulva spp. thalli were collected by slowly towing this little trawl for a few minutes. Thalli from each site were brought back separately in coolers filled with water from the corresponding site, except those from station A for which water from station B was used.

Water

A water sample was taken at each sampling time and at each of the three stations: at station A by digging a very shallow depression in the sand filled by the surrounding surface water film, at station B by filling a clean bucket in the waves, and at station C by sampling at 0.3 – 0.5 m above the seabed with a horizontal Niskin bottle.

The water fraction intended for nitrate (+nitrite) and ammonium determination was filtered through an 85 µm porosity membrane and stored in polyethylene flasks, which were immediately placed in a refrigerated cooler. This large mesh size is needed by the N analysis procedure of Aminot (1995), which requires a shorter filtering time at sea. Water was immediately cooled and quickly frozen (within 8 h) and special care was taken to avoid the N water content being altered by plankton growth, excretion or decay or by other means before analysis (see section 4/).

The water fraction to be used to measure photosynthetic activity was brought back to the laboratory in a non-refrigerated cooler with the Ulva spp. from the corresponding station, except for station A for which station B water was used. After being filtered through a 0.45 µm porosity membrane, this water was used both to wash the algae before measuring their photosynthetic activity and as the natural milieu of the algae in the apparatus used.

In situ measurements

Temperature and salinity

At station A, the temperature was measured using a mercury thermometer placed under the layer of stranded algae, whose thickness varied from 1 to 3 cm. The salinity of the water bathing the seaweed was measured using an ATAGO S/Mill refractometer with a salinity measurement range of 0-100. At stations B and C these two parameters were measured using an electrical probe (Kent-EIL 5005).

Irradiance

Photosynthetically active radiation (PAR: 400-700 nm) was measured using two quantum sensors (LI-COR). At station A (measurement in the air), we used a horizontally positioned cosine collector (LI-190) that measures downwelling irradiance. At stations B and C (measurement in the water), we used a 4π collector (LI-193) that measures scalar irradiance. As a matter of fact, seaweeds stranded in station A were generally accumulated in several layers. Thus, only the downwelling light was available for algae, and completely only for the top layer. Conversely, in stations B and C, seaweeds were suspended in the water column or gently lying on the bottom, and using a 4 π collector was suitable because of light diffusion by suspended matter and reflection by the pale sandy bottom.

At station B, the measurement was taken at mid depth of the water column. At station C, the irradiance measurement was taken at one meter intervals in the vertical water column so as to compute the average attenuation coefficient. All measurements were taken in the morning, sometimes quite early, when solar radiation was still fairly weak but the sea is generally calmer in the morning before the midday sea breezes start.

Nitrogenous nutrients in water

Nitrate plus nitrite concentration in water was determined colorimetrically using a Technicon AutoAnalyser II following the method of Tréguer and Le Corre (1974). Ammonium was analysed by fluorimetry with the same apparatus following the method of Kérouel and Aminot (1997). Special care was taken (i.e. very rapid thawing, and analysis within the hour) to avoid NH_4^+ being released from plankton through bacterial activity (Aminot, 1995). The analysis precision was 0.1 and 0.01 $\mu mol\ l^{-1}$ for nitrate and ammonium respectively. The results given by this analysis method have been checked with comparitive tests and have been systematically confirmed.

The concentrations of dissolved inorganic nitrogen (DIN) included $N\text{-}NO_3^-$, $N\text{-}NO_2^-$ and $N\text{-}NH_4^+$, but nitrite-nitrogen was not determined and separated from nitrate-nitrogen since, in comparison with other forms of nitrogen, it is usually very low in the marine coastal environment.

Analyses of *Ulva* spp. thalli

The thalli were rinsed in the laboratory. Two pieces of about 10 cm^2 were cut out from each of the five thalli collected to measure C, N and chlorophyll a and b contents, and one piece of 4 cm^2 to measure photosynthetic activity. The two 10 cm^2 pieces were frozen for the analyses.

Carbon, nitrogen and chlorophylls a and b in algae

One frozen piece was freeze-dried and ground to a powder. Its carbon and nitrogen contents were determined using a CHN analyser (LECO, model 800). The other frozen piece was used to extract the chlorophylls using acetone after grinding in the dark, and chlorophyll absorption was measured at 750, 664 and 647 nm using a spectrophotometer (UV-240-1 PC from Shimadzu). Chlorophyll a and b contents were calculated according to the equations of Jeffrey and Humphrey (1975).

Photosynthetic activity

On returning to the laboratory, the 4 cm^2 piece was put in a pillbox containing water sampled on site. The pillbox was placed in a thermostatically controlled basin at 15 °C for approximately 18 h.

Photosynthetic activity was measured using the oxygen production of a known quantity of *Ulva* spp. thallus incubated in the light. A Plant Efficiency Analyser (Hansatech) specially dedicated was used. All the necessary components were bunded inside. The thallus fragment was put into a closed oxygen electrode chamber with an adjustable light source (DW3). Each *Ulva* spp. fragment was placed in the unit vertically, held between two fine white plastic grids to keep it perpendicular to the unidirectional light beam. The light was red monochromatic ($\lambda = 650 \pm 25$ nm), emitted from a diode (LH36 type LED) whose intensity was adjustable. The DW3 reaction chamber (holding 18.5 ml), made of black acetal plastic, was filled with water from the corresponding station, filtered to 0.45 µm and temperature controlled at 15°C. A magnetic stirrer and a polarographic oxygen probe were placed at its base. To calibrate the oxygen measurements, zero was obtained by bubbling nitrogen in the chamber before it was closed to take measurements.

Each measurement was begun by 10 min of adaptation to dark. Changes of the dissolved oxygen during the last 500 s of this period were recorded (i.e. for a little over 8 min), and this was used to determine the respiration rate of the sample. Then increasing irradiance levels were applied (10, 30, 60, 100, 150, 300, 500 and 1000 µmol of photons $m^{-2}\ s^{-1}$), in successive five-minute sequences. For each irradiance level, the dissolved oxygen concentration in the tank was recorded every second during the last 200 s of the sequence (i.e. for a little over 3 min). The variation in the concentration of dissolved oxygen in the measurement unit was recorded along with the irradiance applied. Each *Ulva* spp. thallus sample was then dried in an autoclave at 60 °C for 24 h before weighing.

The net oxygen production value (P) is the difference between the gross rate of photosynthesis and the dark respiration (R). It is a function of the irradiance applied (I). Different equations have been proposed to represent it. We used the TableCurve software to see which of those we tested best corresponded to the series of pairs of values obtained (irradiance applied and oxygen production or consumption). Finally, we calculated photosynthesis indexes P_{max} P, I_k and I_c using the equation of Webb et al. (1974):

$$P + R = P_{max} [1 – \exp(-I/I_k)]$$

where P_{max}, photosynthesis maximum production, is O_2-based net photosynthetic activity (µmol O_2 h^{-1} g^{-1}dry weight); I_k, saturating irradiance for P_{max} (µmol photons m^{-2} s^{-1}). $I_{c,}$ compensating irradiance, i.e. irradiance when oxygen production by photosynthesis equilibrates respiration (µmol photons m^{-2} s^{-1}), is given by the value of I at the point where the curve intersects the abscissa axis.

Statistical data processing

At each sampling site and date and for the six parameters of *Ulva* spp. tissue (nitrogen, carbon and chlorophyll (a + b) contents, P_{max}, I_k and I_c) mean and standard deviation were obtained from values of the five individuals sampled.

For each station, the correlation coefficients between the six parameters were calculated. The homogeneity of the standard deviations was tested with the *MINITAB* software. This condition being fulfilled for N, C and P_{max}, two-way analyses of variance (ANOVA) were then used.

In addition, the similarity between the mean of each of the above-mentioned seaweed parameters at the three stations A, B and C was tested by an ANOVA. For this analysis, the six groups of samples taken in June and July were pooled.

3. RESULTS

Physical and chemical characteristics of the study areas

Table 1 shows temperature, salinity, irradiance, and the concentrations of nitrate + nitrite, ammonium and DIN in the sampling areas.

Temperature varied much more at station A than at the other two stations. This was due to the low thermal inertia of the layer of stranded seaweed and its sensitivity to solar radiation, which raises the temperature, and evaporation, which lowers it. Salinity at station A was also much more variable than at stations B and C. The nearby stream flowing onto the beach reduced the salinity, which once even reached a nil value, while evaporation at the surface of the sand was the cause of salinity often exceeding 35 PSU.

Overall, irradiance decreased sharply from station A towards station C. At the three stations light variation can be explained by the fluctuations in solar radiation due to the date, the time of day and the cloud cover. In the surf and subtidal zones, possible other factors were the state of the sea surface, water turbidity and depth. At the three stations, the irradiance was lower at the last visit (measurement made early in the morning on July 12; very cloudy weather on July 10). At station C, it was lower on July 3 because of cloudy weather.

At station B, DIN tended to decrease progressively as the season progressed. The same was true at station A, except for June 21, when salinity was also nil, showing that DIN was measured directly in the stream coming from land. At station C, the trend was also to a general decrease over time but no significant conclusion could be drawn. The ammonium values seemed to vary less over the study period and their proportion in DIN rose to reach 90% by the end of the study.

There was a decreasing gradient of DIN from station A towards station C that reflected the dilution of the fresh water nitrogen inputs by sea water. Although concentrations at station C were much lower than at stations A and B, they remained appreciable (between 3.6 and 8.3 µmol l^{-1}), except on June 28 and July 3 (between 1.5 and 2.0 µmol l^{-1}).

Nitrogen and carbon contents in algae

The nitrogen and carbon contents in *Ulva* spp. tissues tended to increase at station B until early June, whereas they were mostly stable at stations A and C (Fig. 2). In early June, the mean nitrogen values at the three stations ranged between 1.8 and 3.3% of the dry weight (DW) and carbon values ranged between 14 and 28%. Nitrogen and carbon contents decreased at stations B and C from early June until the end of the measurements in mid-July, while at station A they remained almost at the same level, although they were lower than at the beginning of June. At each of the three stations, nitrogen and carbon contents of tissues were significantly correlated (Table 2). However, whereas ANOVA revealed no significant differences in nitrogen ($p = 0.057$) at the three stations, there were significant differences in carbon ($p < 0.001$).

Chlorophyll and photosynthetic activity

The chlorophyll a + b content of the thalli tended to drop from late May to late June at the three stations and then to become stable (Fig. 2). The chlorophyll content was generally higher at the subtidal station (2.79 mg g^{-1}DW ± 1.24 SD at station C) than at the other two stations (2.36 ± 1.39 at station B and 2.01 ± 0.80 at station A) (Fig. 2). The decreasing gradient of chlorophyll content from station C towards stations B and A was in the opposite direction from the irradiance gradient. In addition, at each of the three stations, this parameter followed the same pattern as the nitrogen and the carbon content of thalli (Table 2).

Changes in each of these parameters over time were similar (Fig. 3): P_{max} decreased over the whole period, whereas I_k and I_c increased, except for the final observation at stations A and B, and the last two observations at station C (Fig. 2). This reversal in trends for I_k and I_c was concommitant with lower irradiance (Table 1) which was observed earlier at station C than at stations A and B. Indeed, the correlation between the irradiance and I_k was statistically significant (Fig. 4).

The P_{max} of the thalli collected at stations A, B and C was statistically different (ANOVA : $p < 0.001$), with an average of 762.8 µmol O_2 h^{-1} g^{-1}DW ± 151 (SD) at station A, 643.8 ± 162 at station B and 593.1 ± 159 at station C. At all three stations, the P_{max} values tended to decrease over the study period (station average: 870.3 µmol O_2 h^{-1} g^{-1}DW ± 144 (SD) in May and 474.3 ± 98.0 in mid-July). This decrease was significantly linked to that of nitrogen content (Fig. 5).

Comparison of I_c and I_k with irradiance observed at stations B and C shows that irradiance was mostly above I_k at station B, and mostly between I_c and I_k at station C (Fig. 6).

4. DISCUSSION

The present study, performed with irradiation ranges including those observed *in situ*, shows that *Ulva* spp. thalli can grow in their subtidal habitat, as our previous *in situ* study led us to assume (Merceron and Morand, 2004).

Firstly, the bottom irradiance measured at station C ranged from 12 to 100 µmol m^{-2} s^{-1}. This maximum value, much higher than the others, corresponded to a combination of very sunny weather, a calm and fairly transparent sea and a depth of only 8 m on June 28. Several authors working with various *Ulva* species have observed thallus growth at irradiances within this range or even lower, the irradiance threshold allowing growth being about 2 µmol m^{-2} s^{-1} (Table 3). This threshold is approximately as high as I_c values we found for station C, while irradiance was higher. The chlorophyll a + chlorophyll b content of the thalli tended to drop from late May to mid-July at all three stations. *Ulva* spp.'s very great adaptability to low or very low irradiance is well known (Table 3). The *Ulva* spp. thalli in the Bay of Douarnenez thus followed a classic pattern by adapting their chlorophyll content to irradiance variations linked to the location and the time of year. Moreover, the P_{max} values recorded in the present study, even at the subtidal station C, are of the same order of magnitude as those obtained by other authors (Table 4).

At station C three elements, lower I_c than observed irradiance, the fact the algae presented a normal adaptation pattern to irradiance, and the usual P_{max} value, all reinforce the hypothesis of *in situ* growth of *Ulva* spp..

Secondly, during the study period, water DIN ranged between 4.7 and 657 µmol l^{-1} at stations A and B, i.e. 0.07 and 9.2 mg N l^{-1}, whereas other studies showed that a concentration of 0.1 mg l^{-1} was the lowest nitrogen limit for proliferation of green algae to be possible (Morand and Briand, 1996). This limit was only reached in July. The very high DIN value recorded on June 21 at station A (656 µmol l^{-1}) corresponded to the totally fresh water composition of the sample with a very high nitrate load. Otherwise, the decrease in nitrate (+ nitrite) over time was likely due, at least in stations A and B, to the drop in input from streams (reduction in flow rates and concentrations as reported in a previous study (Merceron et al. 1999)). At station C, five water samples out of seven displayed DIN values under 7 µmol l^{-1} (i.e. 0.1 mg l^{-1}), and, five times, the DIN concentration in the water was much lower than at stations A and B (an average of 4.9 µmol l^{-1} at station C compared to 27.4 at station B).

Despite the differences in the DIN concentrations in the water surrounding the algae, the nitrogen content of *Ulva* spp. tissues in the three stations was very similar, i.e. always above 1% DW (Fig. 2) and thus above the threshold value of nitrogen which sharply limits growth, as determined by Dion et al. (1996). It was frequently close to or above the critical value, defined as the concentration at which the maximum growth rate of the alga is reached. This critical value has been estimated to be 2% DW for *Ulva rigida* for instance (Lavery and McComb, 1991).

Several hypotheses can be put forward to explain why the nitrogen contents of *Ulva* spp. at station C were sufficient to ensure their growth, even when the DIN content of the surrounding water was less than 2 µmol l^{-1}:

i) Hypothesis 1: The low DIN concentration can support prolonged growth at reduced rates because the growth rate is limited by the PAR intensity, or low DIP concentration. Thus, the lower DIN concentration results in a reduced uptake rate, but this rate is matched by the reduced N-demand of the algae.

ii) Hypothesis 2: *Ulva* colonizes greater depths mainly due to substantial advective transport which results in net import of biomass.

iii) Hypothesis 3: At greater depths *Ulva* growth depends mainly on nutrients delivered from the sediment by hydrodynamic pumping due to the ripple marks, a phenomenon that could locally increase NH_4^+ release from sediments (Huettel et al., 1998).

Ulva spp. adaptability to lower irradiance levels (Table 3) seems to contradict hypothesis 1, as far as a possible limitation by PAR is concerned. For P, the hypothesis remains to be tested, although a limitation of N coupled with a limitation of P would be expected to drastically reduce growth.

Indeed, despite the mobility of the intertidal fraction under specific weather conditions we previously observed (Merceron and Morand, 2004), an advection model showed that the residual current alone would enable *Ulva* spp. to travel only 500 m in more than 5 days. As stations B and C are at least one km apart and the seven samplings at these two stations were systematically made at only at an interval of two days, no mechanism can be proposed to support hypothesis 2.

Finally, the thalli in fact most often lie gently on the bottom. There, there are nutrient exchanges between the sediment pore water and the water lying immediately above it. DIN being released from the sediment could be captured by *Ulva* spp. and thus be much less perceptible or even imperceptible at 0.3 m above the bottom. Thybo-Christensen et al. (1993) and Trimmer et al. (2000) showed that sediment is probably a significant source of regenerated nutrients which can support the growth of established algal blooms. In addition, Sundbäck et al. (2003) indicated that nitrogen fluxes of benthic origin could provide from 55 to 100% of the estimated demand for an algal bloom at its outset. McGlathery et al. (1997) and Krause-Jensen et al. (1999) noted the absence of increase in nitrogen compounds in the water column above a mat of *Chaetomorpha linum* (O.F. Müller) Kützing whose base was abundantly supplied with NH_4^+ by the sediment. In the middle of the Bay of Douarnenez, a clearly subtidal area, Birrien (1987) observed significant NH_4^+ releases from the bottom in June and September. In the Le Frêt cove (10 m deep), in the Bay of Brest, a site similar to the Bay of Douarnenez, Douchement (1987) calculated the monthly diffusive flux of NH_4^+ from the sediment into the overlying water. The annual mean flux was 33.0 ± 11.6 µmol m^{-2} h^{-1} with a minimum in spring and a maximum from July to October. Auby et al. (1994) also measured considerable Summer releases of ammonium from subtidal sediments in the Arcachon Basin, another macrophytic bloom site on the Atlantic coast of France. Therefore, hypothesis 3 is the most likely. Nevertheless, subtidal growth of *Ulva* spp. should be slower than that in intertidal areas, since their maximum oxygen production is from 1 to 1.6 times lower. This could be explained by temperature (Table 1) and irradiance (Fig. 6). At station C, algae growth was partly limited by ambient irradiance mostly lower than I_k, in contrast to station B. Lower availability of nitrogen may also be involved. In this respect, subtidal sediments could have become loaded with nitrogen over successive years of green tides, due to the degradation of thalli, whether from the intertidal or subtidal stocks, as well as the migration of foreshore sediments to subtidal depths.

The next useful step would be to check if one *Ulva* spp. stock - intertidal or subtidal - appears sooner than the other in any given year that could initiate the growing season in both zones. This could have significant consequences for corrective measures for green tides.

Acknowledgements

We thank Jacqueline Le Grand for providing help during field sampling, and Roger Kerouel and Philippe Cann for chemical analyses. We are grateful to the referees and to the editor for their very useful comments.

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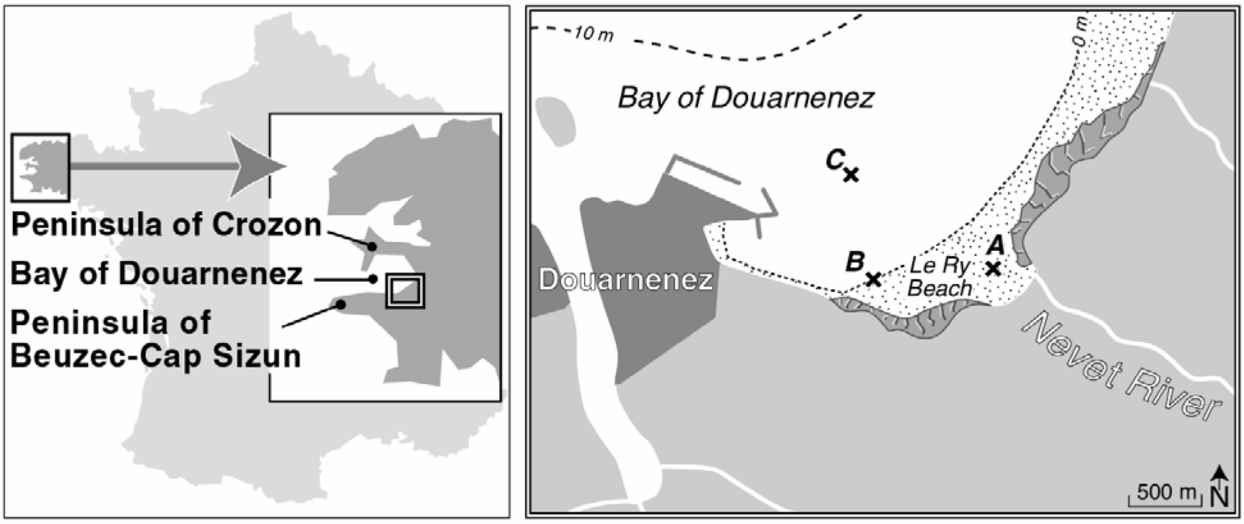


Fig. 1. Location of sampling sites in the Bay of Douarnenez. GPS position at station C: 04°18'739 W; 48°06'069 N.

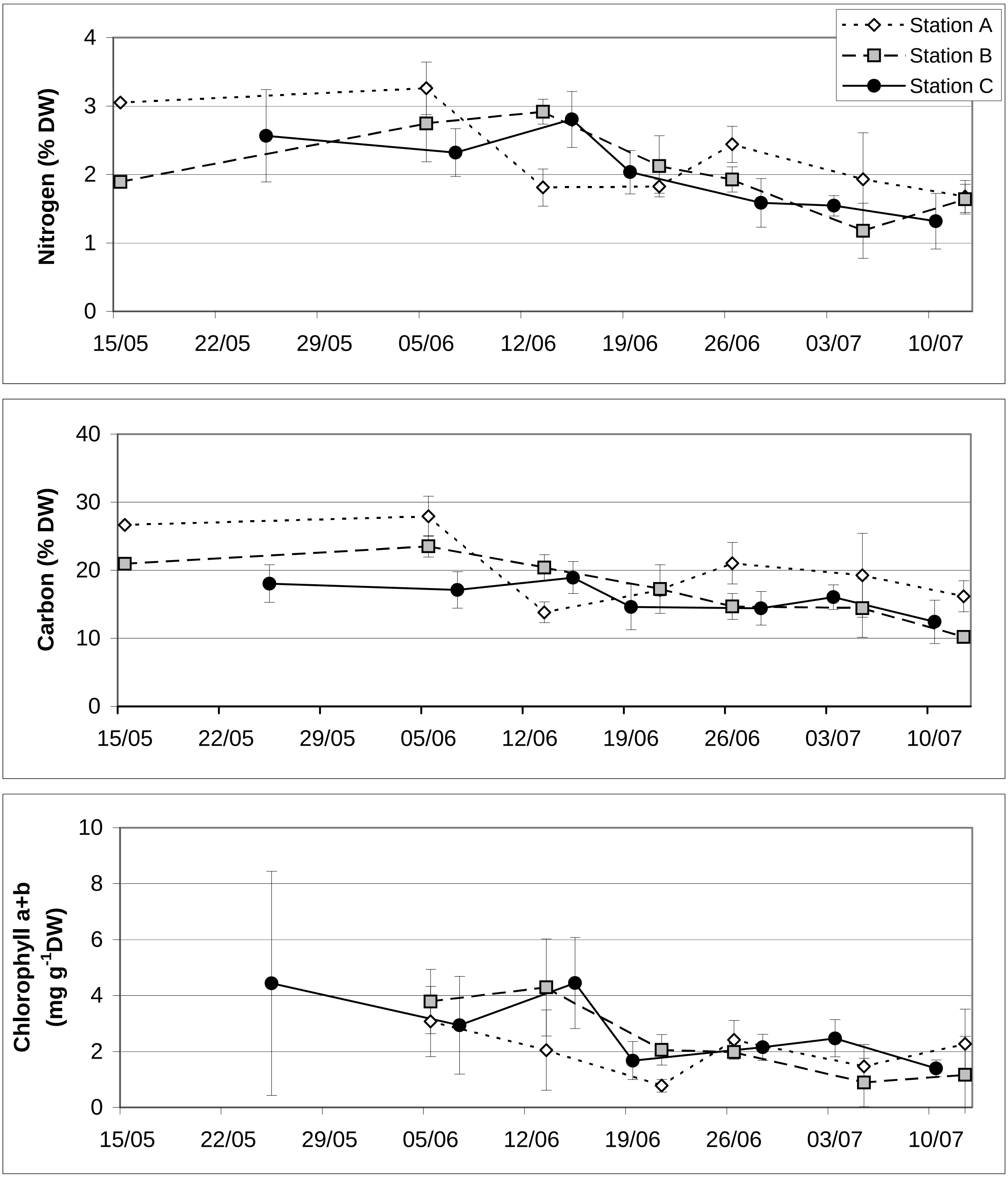


Fig. 2. Nitrogen, carbon and chlorophylls a + b content of *Ulva* during the period of maximum growth; means and standard deviations from five or six measurements except on May 15 (one measurement); station A: foreshore, station B: surf zone, station C: subtidal zone.

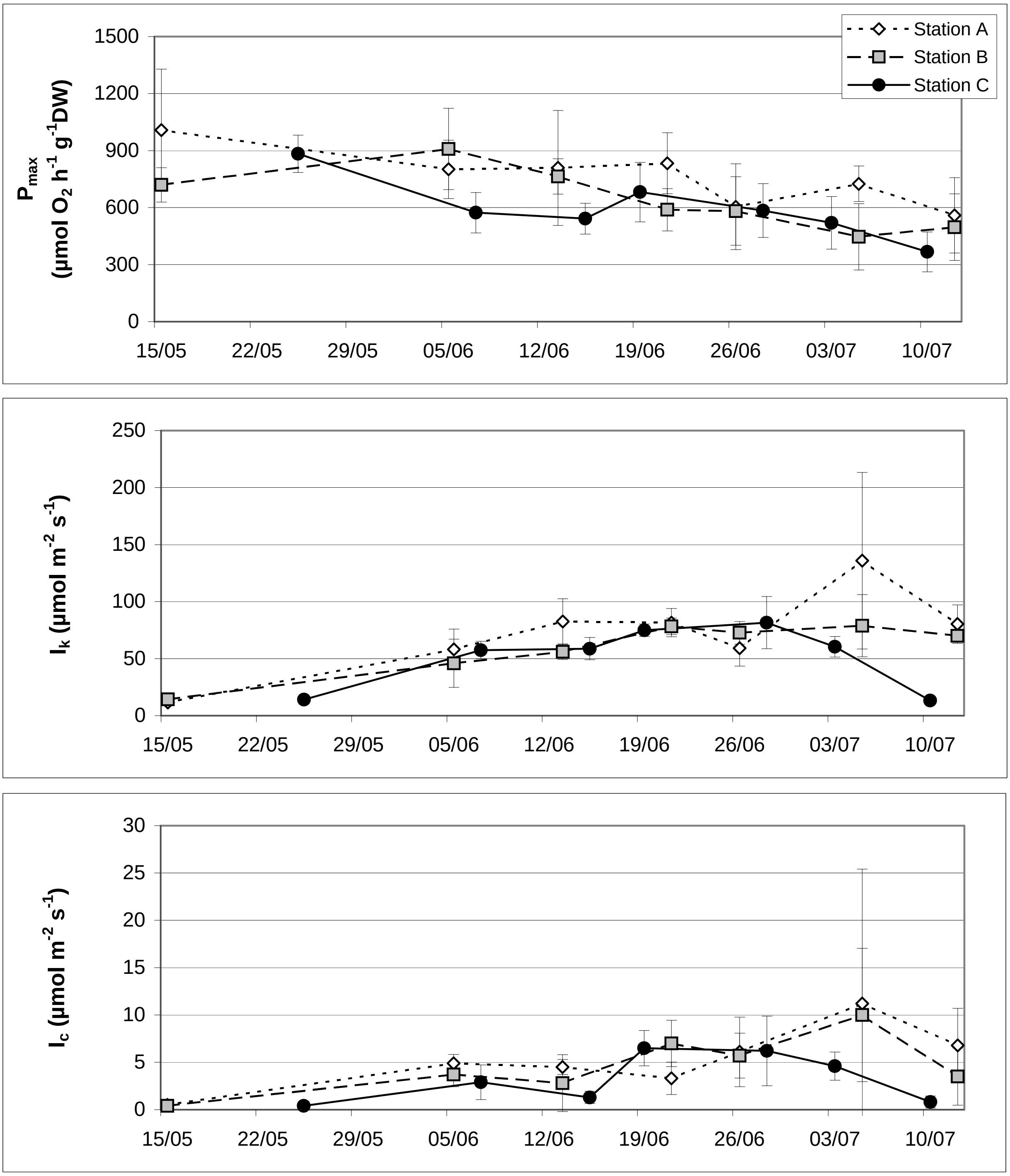


Fig. 3. Photosynthetic parameters of *Ulva* during the period of maximum growth: maximum photosynthesis production (P_{max}), saturating irradiance (I_k), compensating irradiance (I_c); means and standard deviations from five or six measurements; station A: foreshore, station B: surf zone, station C: subtidal zone.

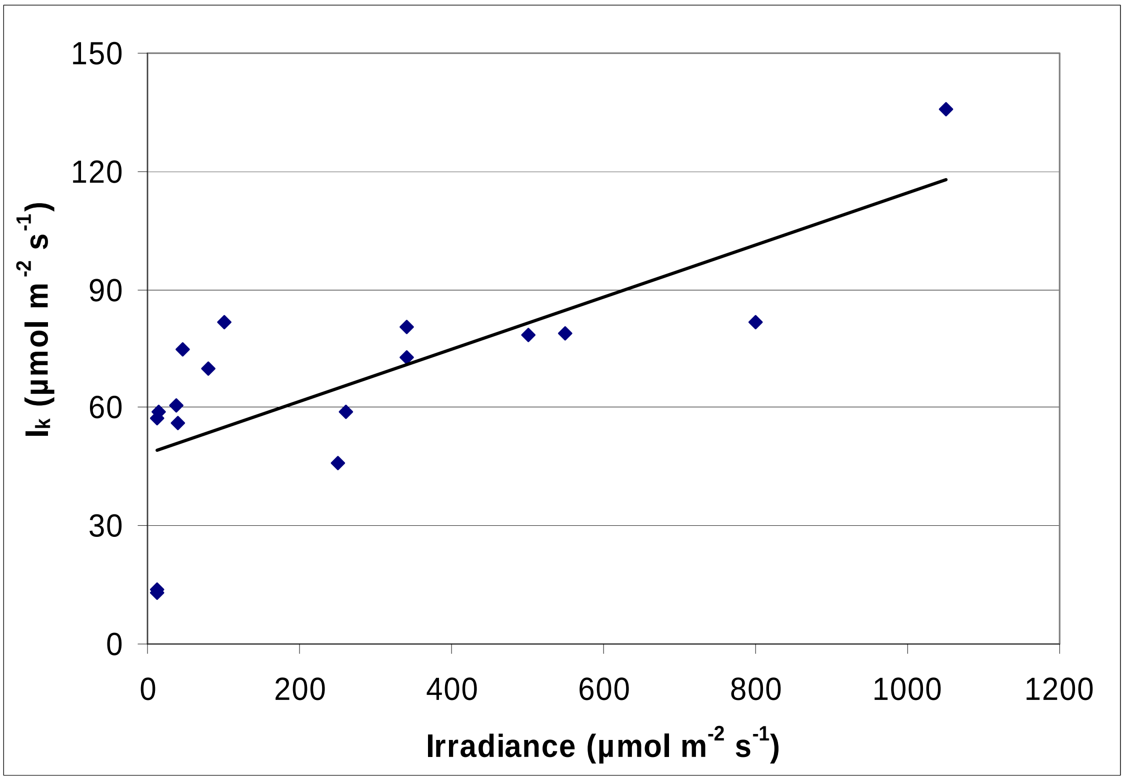


Fig. 4. Correlation between irradiance (I) and saturating irradiance (I_k) from data of stations A, B and C taken together. $I_k = 0.07\ I + 48.5$; n = 17; r = 0.738; p > 0.99.

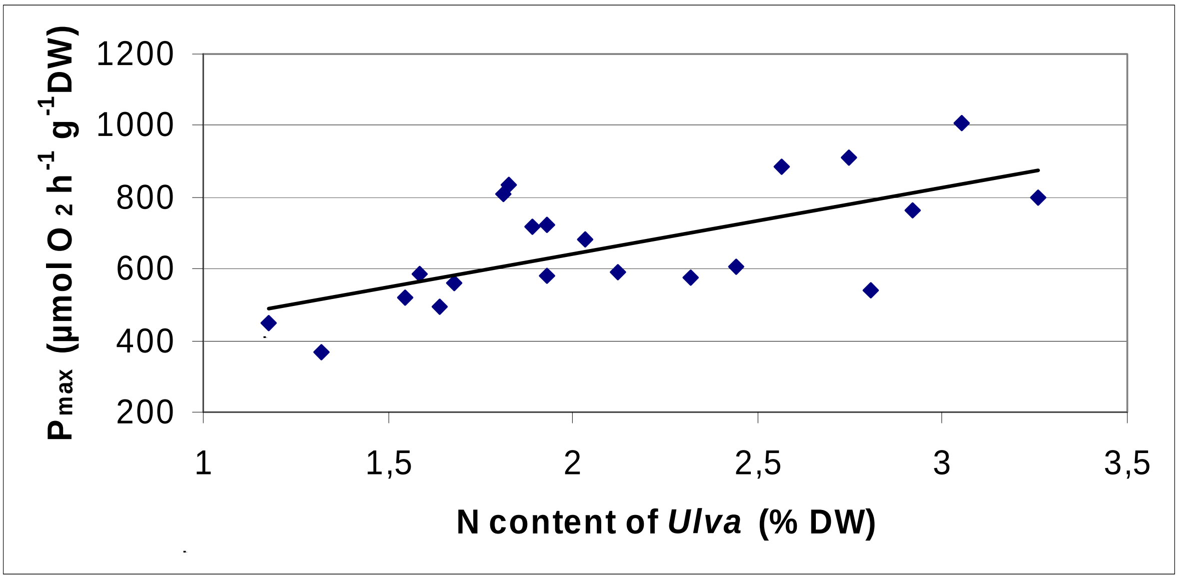
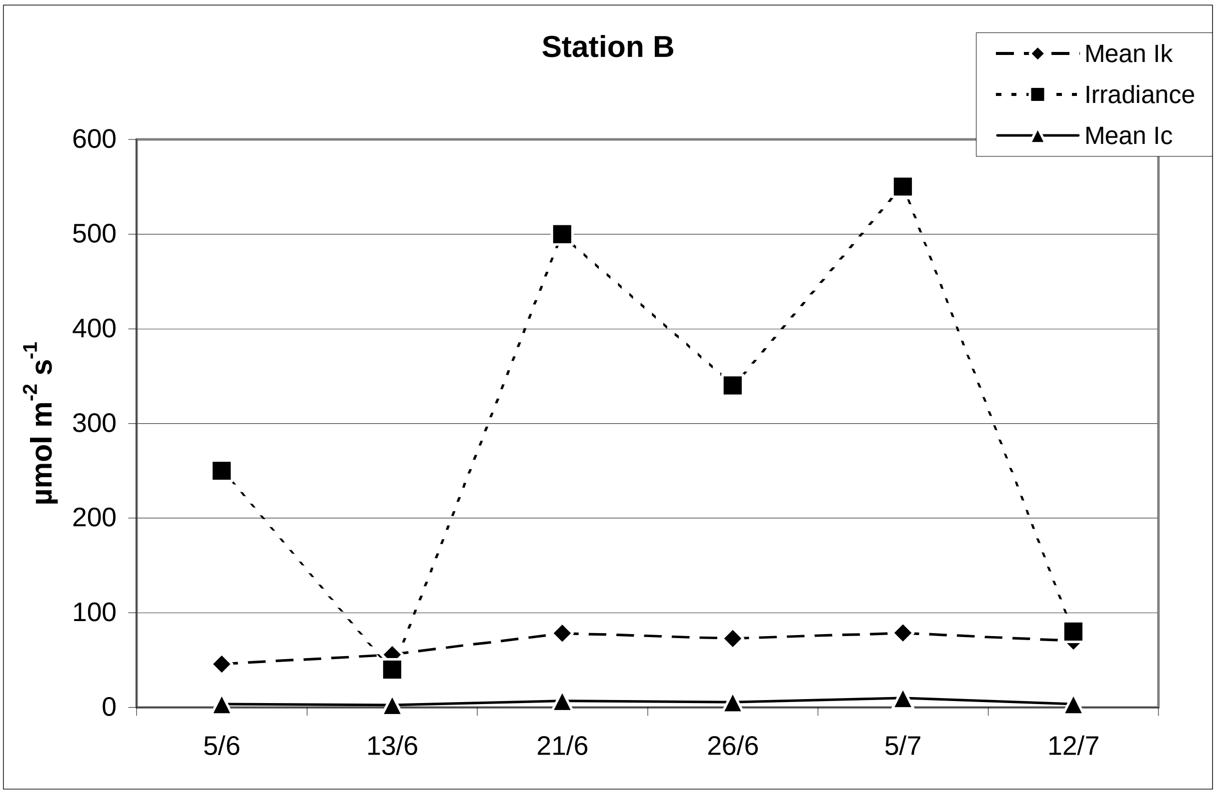


Fig. 5. Correlation between N content and P_{max} of *Ulva* spp. Data from stations A, B and C taken together. P_{max} = 185 N content + 273; n = 21; r = 0.654; p > 0.99.



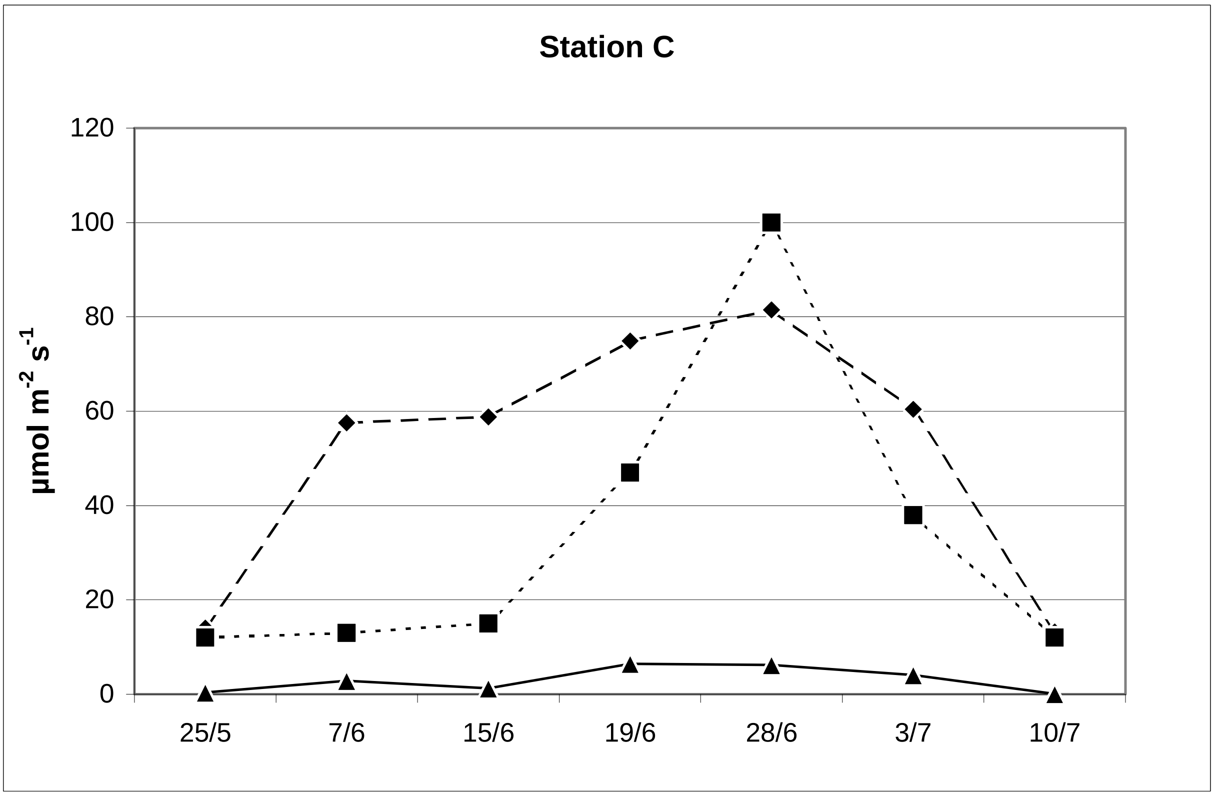


Fig. 6. Comparison between observed ambient irradiance and *Ulva* spp. I_k and I_c at stations B (surf zone) and C (subtidal zone).

Table 1

Physical and chemical characteristics of seawater in the nearby environment of sampled *Ulva*

Station[a]	Date	Depth (m)	Temperature (°C)	Salinity	Irradiance[b] (µmol m^{-2} s^{-1})	N-NO_3^-+N-NO_2^- (µmol l^{-1})	N-NH_4^+ (µmol l^{-1})	DIN[c] (µmol l^{-1})
A	15/5/00	-	24,6	37	n.r.[d]	177	4,24	181
"	5/6/00	-	17,0	28	n.r.	68,7	2,04	70,7
"	13/6/00	-	20,0	35	n.r.	40,5	3,09	43,6
"	21/6/00	-	24,5	0	800	654	2,80	657
"	26/6/00	-	12,6	37	261	3,25	6,60	9,85
"	5/7/00	-	24,1	40	1051	0,60	5,69	6,28
"	12/7/00	-	16,5	42	342	0,73	3,93	4,66
B	15/5/00	n.r.	16,5	32	n.r.	47,0	5,42	52,4
"	5/6/00	0,7	15,0	32	250	85,6	0,33	85,9
"	13/6/00	0,5	16,0	32	40	12,5	2,17	14,7
"	21/6/00	0,7	17,1	34	500	4,15	2,87	7,01
"	26/6/00	0,6	17,8	33	340	19,2	0,99	20,1
"	5/7/00	0,7	18,8	34	550	0,65	5,54	6,19
"	12/7/00	0,6	16,2	34	80	2,83	2,71	5,54
C	25/5/00	10	14,0	35	12	5,93	1,75	7,68
"	7/6/00	11	14,2	34	13	4,90	1,75	6,65
"	15/6/00	8	14,6	34	15	2,66	0,91	3,56
"	19/6/00	9	13,0	34	47	5,19	3,08	8,27
"	28/6/00	8	14,9	35	100	0,52	1,12	1,65
"	3/7/00	9	14,7	35	38	0,20	1,64	1,85
"	10/7/00	9	16,5	34	12	1,18	3,28	4,46

[a] Station A: foreshore, B: surf zone, C: subtidal zone

[b] for station A: downwelling irradiance; for stations B and C: scalar irradiance

[c] DIN: N-NO_3^- + N-NO_2^- + N-NH_4^+

[d] n.r.: not recorded.

Table 2

Correlation probability between the three *Ulva* parameters nitrogen, carbon and photosynthesis maximun productio (P_{max}) tested by ANOVA by station

Probability of correlation between parameters[a, b]		Nitrogen	Carbon	P_{max}
at station A[c]	Nitrogen		>0.99 (30)	n.s.[d] (29)
	Carbon			n.s. (29)
	P_{max}			
at station B[c]	Nitrogen		>0.99 (30)	>0.99 (28)
	Carbon			>0.99 (28)
	P_{max}			
at station C[c]	Nitrogen		>0.99 (34)	>0.99 (33)
	Carbon			n.s. (33)
	P_{max}			

[a] Pair numbers between parentheses.

[b] Correlation tested thallus by thallus, generally 5 thalli at each station and date; aberrant values discarded from tests.

[c] Stations A: foreshore, B: surf zone, C: subtidal zone.

[d] n.s.: not significant.

Table 3

Growth of different *Ulva* species under low irradiances

Species	Irradiance (μmol m^{-2} s^{-1})	Photoperiod (light:dark)	Growth (% j^{-1})	References
U. lactuca L.	30	16:08	6	Fortes and Lüning (1980)
	0,6	24:00	threshold	Vermaat and Sand-Jensen (1987)
	2,5	24:00	threshold	Sand-Jensen (1988)
U. curvata (Kütsing) *U. rotundata* Kützing	60	12:12	7	Pérez-Llorens et al. (1996)
U. rigida C. Agardh	1,9	(unknown)	threshold	Riccardi and Solidoro (1996)
U. curvata (Kütsing)	10	16:08	9,5	Taylor et al. (2001)

Table 4

Comparison of maximum photosynthesis production (P_{max}) of different *Ulva* species from several authors

Ulva species	P_{max} (μmol O_2 h^{-1} g^{-1}DW)[a]	References
U. rotundata	997	Henley et al. (1991)
	< = 2120[b]	Henley et al. (1992)
	623	Pérez-Llorens et al. (1996)
	1084	Mercado et al. (2003)
	523	Vergara et al. (1998)
U. rigida	997	" " "
U. pertusa	872	Pérez-Llorens et al. (1996)
	195-1344[c]	Han et al. (2003)
U. lactuca	450-1147[d]	Rivers and Peckol (1995)
	500-770	Brush and Nixon (2003)
U. sp.	403	Menéndez et al. (2001)
U. sp.	758-2342[e]	Levavasseur and Giraud (1982)
U. sp.	844-1219[f]	Levavasseur (1987)
U. spp.	505-457[g]	Plus et al. (2005)
U. spp.	763-644-593[h]	Present study

[a] original data expressed in relation to m² by some authors were converted using the ratio of 1 m² of thallus corresponding to 14.444 gDW.

[b] thalli stranded on foreshore subjected to a natural irradiance of 2 300 μmol photons m^{-2} s^{-1}.

[c] P_{max} measured respectively at base and apex of thallus.

[d] P_{max} respectively measured in summer and spring.

[e] P_{max} corresponding to measurements performed respectively in April under high irradiance and in July under low irradiance.

[f] P_{max} measured respectively in deep (subtidal) and intertidal *Ulva.*

[g] P_{max} measured respectively in May and July.

[h] the three values correspond respectively to the stations A (foreshore), B (surf zone) and C (subtidal zone) in the present study.