

BIOMASS AND DYNAMICS OF GROWTH OF *ULVA* SPECIES IN PALMONES RIVER ESTUARY¹

Ignacio Hernández,² Gloria Peralta, J. Lucas Pérez-Lloréns, Juan J. Vergara

Departamento de Ecología, Facultad de Ciencias del Mar, Universidad de Cádiz, 11510 Puerto Real, Cádiz, Spain

and

F. Xavier Niell

Departamento de Ecología, Facultad de Ciencias, Universidad de Málaga, 29071 Málaga, Spain

ABSTRACT

During the last decade, the Palmones River estuary has undergone severe eutrophication followed by a green tide episode; two species of *Ulva*, *rotundata* Blid. and *Ulva curvata* (Kütz.) De Toni, were the main macroalgae responsible for this bloom. From November 1993 to December 1994, we followed the biomass, the growth dynamics, and tissue elemental composition (C:N:P) of *Ulva* species, as well as some physicochemical variables in the estuary. Maximum biomass (up to 375 g dry wt·m⁻² in some spots, corresponding to a thallus area index of nearly 17 m² *Ulva*·m⁻² sediment) were observed in June and December. However, the biomass varied among the sampling stations. Water nitrate, ammonia, and phosphate showed high concentrations throughout the year, with extremely high transient pulses, sustaining the high growth rates observed. Growth rates were estimated directly in the field. The rates were generally higher in *Ulva* discs maintained in net cages than those estimated by changes in biomass standing stock between two consecutive samplings. The difference between both estimates was used to quantify the importance of the processes causing loss of biomass, which were attributable to grazing, exported biomass, and thallus decomposition under anaerobic conditions resulting from extreme self-shading. Maximum chlorophyll content was found in winter, whereas the minimum was in spring. Atomic N:P ratios were generally higher in the algae than in the water. However, the absolute concentrations of tissue N and P were always higher than the critical levels for maximum growth, which suggests that growth was not limited by inorganic N or P availability. The results suggested that the increase in nutrient loading in the river may have triggered the massive development of green algae and that light limitation and temperature stress in summer seem to be the main factors controlling the abundance of *Ulva* in the estuary. In addition to light availability and thermal stress, the different loss processes may have a decisive role in the dynamics of *Ulva* biomass.

Key index words: eutrophication; growth; primary production; *Ulva*

Local proliferations of masses of green algae are often found in estuaries and coastal waters of areas undergoing eutrophication (Geertz-Hansen et al. 1993, Kamermans and Nienhuis 1995, Anderson et

al. 1996). Usually, these accumulations are comprised of species of the order Ulvales (mainly *Ulva* and *Enteromorpha*). These algae are characterized by a double-celled, simple, sheet-like thallus (*Ulva*) or a single-celled, hollow thallus (*Enteromorpha*), with a high surface:volume ratio (Rosenberg and Ramus 1984). Because of this morphology, these species respond rapidly to nutrient inputs with high rates of nutrient uptake and growth (Duke et al. 1987, Markager and Sand-Jensen 1994).

Green tides produce changes in ecosystem structure and biodiversity (Rivers and Peckol 1995, Short et al. 1995). The thick mats of algae affect estuarine communities dominated by seagrasses, mainly by shading (Den Hartog 1994, Short et al. 1995). Furthermore, decomposition of the algal biomass produces underlying anoxic conditions, which may reduce the invertebrate fauna (Ferrari et al. 1993). Green tides may also influence local fisheries and contribute to aesthetically displeasing, noxious-smelling deposits of drift weed on shorelines. The ecological and economic problems derived from these large crops of macroalgae make further research necessary on the relationships between development of opportunistic algae and environmental factors.

Recent laboratory studies have established the effect of light, temperature, and nutrient concentrations for optimum growth in *Ulva* (e.g. Levvasseur et al. 1991, Henley et al. 1992, Markager 1993, Pérez-Lloréns et al. 1996). However, the occurrence of algal biomass depletion in nature is triggered by abrupt changes of physicochemical variables (Nilsen and Nordby 1975), which are smoothed under conditions of cultivation (Israel et al. 1995). Field experiments are essential to relate the impact of single or multiple stresses on the growth dynamics of macroalgal populations (Davison and Pearson 1996). Thus, both field and laboratory studies are necessary to gain insight into the dynamics of ulvacean populations in eutrophic areas (Pérez-Lloréns et al. 1996).

The estuary of the Palmones River in southern Spain is an example of a coastal system dramatically affected by an eutrophication process. The construction of a dam in the headwaters of the river in 1986, the sewage discharge in the estuary, and the prolonged drought between 1991 and 1995 caused an

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² Author for reprint requests; e-mail ignacio.hernandez@uca.es.

enhanced concentration of nutrients, in the water and sediment (Niell et al. 1996). The intertidal ecosystem was formerly covered by late successional species, such as the rhodophyte *Gracilaria bursa-pastoris* (S. G. Gmelin) Silva and, particularly, the aquatic angiosperm *Zostera noltii* Hornem. (Pérez-Lloréns and Niell 1993). The seagrass beds have been replaced by a community dominated by *Ulva* species, mainly *U. curvata* (Kütz.) De Toni and *U. rotundata* Bliding (Pérez-Lloréns et al. 1996). Furthermore, the benthic infauna, essentially the polychaete *Nereis diversicolor* O. F. Muller, practically disappeared (Carreira et al. 1995).

The aim of this study was to monitor the seasonal trends of biomass, growth rate, and elemental nutrient composition of *Ulva* spp. in the Palmones estuary and to assess the role of environmental conditions in the dynamics of the population. Light within the *Ulva* canopies and seasonal changes in photosynthesis of the two *Ulva* species existing at the estuary have also been thoroughly studied (Vergara et al. 1997), whereas the effect of *Ulva* biomass on the disappearance of *Zostera noltii* beds is at present under study.

MATERIALS AND METHODS

The study was carried out from November 1993 to December 1994 in the Palmones River estuary (36°11' N, 5°27' W). This shallow estuary can be regarded as eutrophic, with great energy input and high productivity (Pérez-Lloréns and Niell 1989). The maximum tidal amplitude of 1 m exposes extensive areas of mud at low tide twice daily. These mudflats are covered by green algae (primarily *Ulva*) and formerly, by meadows of the seagrass *Zostera noltii* (Pérez-Lloréns and Niell 1993).

Three locations were selected for sampling (Fig. 1), representing an inner (station 1), a central (station 2), and an outer (station 3) part of the estuary. Two main species of *Ulva* were found. *Ulva rotundata* grows as compact sheets on the sediment at the inner and central parts of the estuary. *Ulva curvata* accompanies the former species, especially at station 3. Thalli were found anchored to shells from March to July, in shallow pools and in the main flume.

Water samples were collected fortnightly at spring low tides and stored in an ice chest. Water samples were filtered (1.2 µm glass fiber filter, Whatmann GF/C) and analyzed for nutrients. Inorganic nutrients (nitrate, nitrite, ammonium, and orthophosphate) were analyzed in a continuous flow autoanalyzer (Bran+Luebbe Traacs-800). The nutrients were measured as shown in Grashoff et al. (1983). Salinity was measured with a hand refractometer (Atago). Air temperatures from the sampling site and global daily surface irradiance data were kindly provided by the Meteorological Institute of Andalucía.

Independent biomass samples were collected from each station. A 20 cm × 20 cm metallic frame (minimum representative area) was randomly thrown three times in each sampling station. All the algal biomass was transported to the laboratory in polyethylene bags. Samples were carefully rinsed with tap water to remove mud and invertebrates. The *Ulva* biomass was separated from other green algae that grew sporadically at the sampling stations (see Results), and was oven dried at 60° C to express biomass as g dry wt·m⁻² sediment. Percentage cover was estimated by a modification of the linear transect method (Hernández et al. 1993). Thallus area index (TAI), the total *Ulva* thalli surface area per unit of sediment area (a concept analogous to the leaf area index for vascular plant sporophytes), was estimated in the laboratory using an *Ulva* arrangement resembling that in the field. The slope of the regression line biomass (g dry wt) vs. num-

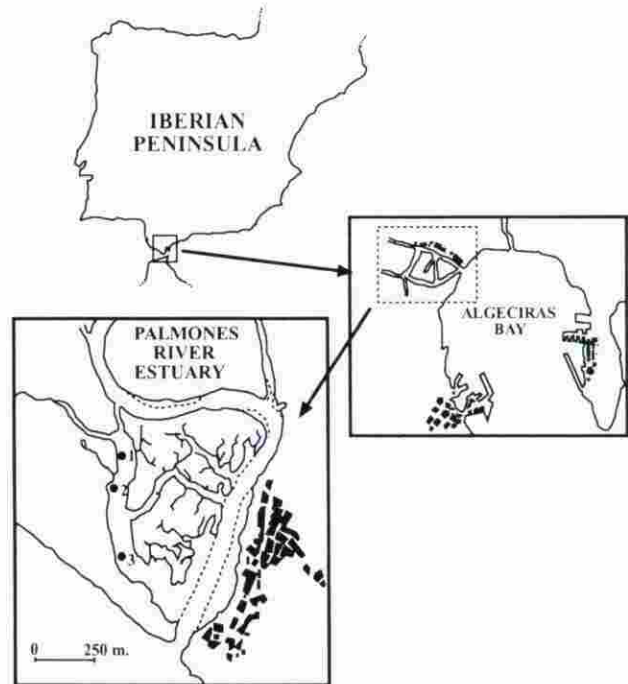


FIG. 1. Sampling stations (stations 1–3) in the Palmones River estuary, southern Spain.

ber of *Ulva* layers covering a surface of 0.04 m² yielded a biomass per *Ulva* layer of 20 g dry wt·m⁻² sediment ($r^2 = 0.98$). The TAI can be computed as $B/20$, where B is the biomass estimation at each sampling date. The index was considered a rough estimation, as in some cases, *Ulva* thalli were not evenly distributed on the sediment, especially at low biomass densities (less than 40 g dry wt·m⁻²).

Growth rates were estimated in the field as the area increase of *Ulva* discs, punched from free specimens with a sharpened steel tube, maintained in cages (two replicates) and as changes of *Ulva* biomass between two sampling dates. In the first case, 10 discs of 12 mm diameter were left in mesh cylindrical cages (7 cm diameter × 15 cm height) for 2 weeks (two replicates). The cages attenuated less than 10% of the incident light. The cages were then placed over the sediment and linked by a cord (1 m long) to large plastic rods that were sunk into the mud. This placement prevented the cages from being covered by large free-floating *Ulva* during the experiment because of the partial free movement of the cages during the ebb and flow of the tide. Preliminary studies showed that cages that were attached directly to a rod offered more resistance to the tidal currents, causing the cages to be covered by drifting *Ulva*. To avoid possible fouling, the cages were replaced every sampling day.

Growth rates (μ_s) were calculated assuming exponential growth:

$$\mu_s = (\ln A_t - \ln A_0)/t$$

where A_0 is the initial and A_t the final surface areas of the discs after t days of incubation. The discs were recovered, and those that were heavily damaged were discarded. In any case, the percentage of recovery was usually higher than 80%. A_t was computed according to the final diameter of the discs, assuming isodiametric growth, because the final shape of the discs stayed almost circular. Grazing was considered insignificant, because the mesh size (1 mm) prevented the main *Ulva* consumers (isopods and amphipods) from entering the cages (occasionally, few individuals were encountered).

Ulva growth rates were also estimated by the biomass standing stock variation between successive sampling days.

$$\mu_B = (\ln B_{t+1} - \ln B_t)/t$$

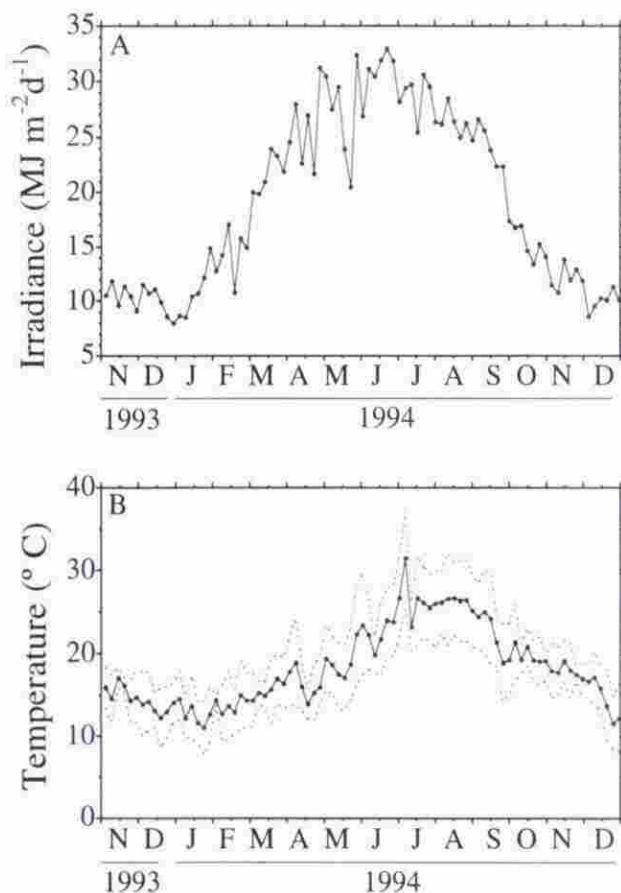


FIG. 2. Seasonal variation of A) daily integral irradiance in the estuary and B) daily mean temperature in air (solid line) and daily maximal and minimal temperatures (dashed lines). Data are the means of five consecutive days.

Where B_{t+1} and B_t are the *Ulva* biomass in the sampling stations at time $t + 1$ and t , and t is the interval between two consecutive samplings.

Total chlorophyll was quantified from field material. Three discs of 12 mm diameter were punched from specimens in the field with a sharpened steel tube. Chlorophyll was extracted in acetone (24 h, 4°C in darkness), and concentration was estimated according to Jeffrey and Humphrey (1975). Tissue C and N were determined in triplicate on samples of dry ground tissue using a Perkin-Elmer 240 CNH elemental analyzer. Total P was analyzed by acid digestion in triplicate samples of dry ground tissue (Sommer and Nelson 1972).

Net production throughout the study period was estimated as the product of the growth rate estimated in cages and the biomass abundance ($\mu_s \times B$). These values were expressed on the basis of tissue C according to the tissue nutrient composition on each sampling day.

Linear correlation between variables was tested by the Pearson correlation coefficient (Zar 1984). The null hypothesis was rejected at the 5% significance level.

RESULTS

Physicochemical variables. The daily irradiance (Fig. 2A) displayed an obvious bell-shaped seasonal pattern. The maximum daily irradiance ($33 \text{ MJ} \cdot \text{m}^{-2} \cdot \text{day}^{-1}$) was found at the end of June, and the minimum was found in December ($8 \text{ MJ} \cdot \text{m}^{-2} \cdot \text{day}^{-1}$). Figure 2B shows the air temperature at the sampling site. Maxi-

mal temperatures were observed at the beginning of July (37°C , mean of maximum temperatures during 5 days). Mean temperatures during winter 1994 were always greater than 11°C . At low tide, salinity was usually about 33‰ (data not shown). However, after heavy sporadic rains in winter and spring, values lower than 10‰ were recorded. Only on one occasion (July, station 1) was salinity greater than 36‰ (43‰, when the maximum temperature was recorded).

Nutrient analyses revealed high concentrations at spring low tides throughout the study (Fig. 3). Overall, dissolved inorganic nitrogen showed decreasing concentrations toward the mouth of the estuary. There were sporadic pulses of nitrate in spring and autumn (up to $18 \mu\text{M}$ in June, station 1) and lower values during the summer. Nitrite (Fig. 3B) was high in winter and spring, whereas the concentrations were close to the detection limit in summer ($0.2 \mu\text{M}$). A considerable concentration of ammonium was found throughout the year (about $15 \mu\text{M}$ in the inner station, $10 \mu\text{M}$ in the other stations) (Fig. 3C), with extremely high concentrations in winter and at the beginning of the spring (pulses greater than 0.18 mM at station 3). Orthophosphate concentration was also high during most of the year (Fig. 3D), with the greatest levels recorded in July and pulses greater than $5 \mu\text{M}$ recorded in winter. Water N:P ranged from 50 to 1 (Fig. 4). Maxima were recorded in February, when the highest ammonium concentrations occurred, and in September, as a consequence of a low orthophosphate level. During the rest of the year N:P averaged 9.4 (SD = 7.8).

Biomass and growth rates. Biomass showed a clear seasonal trend during the study period (Fig. 5). A bimodal pattern was evident at stations 1 and 3. Maximal densities were recorded in June and December (ca. $200 \text{ g dry wt} \cdot \text{m}^{-2}$), which corresponded to an approximate TAI of 9–10. Algal biomass was negligible in February and at the end of the summer; however, at station 2, the highest biomass was reached in January ($250 \text{ g dry wt} \cdot \text{m}^{-2}$) and in October ($375 \text{ g dry wt} \cdot \text{m}^{-2}$), yielding an approximate TAI of 17. At this station, biomass was always greater than $50 \text{ g dry wt} \cdot \text{m}^{-2}$. The percentage cover did not influence significantly the shape of the biomass curve (data not shown). Other ephemeral green algae (*Enteromorpha* sp., *Rhizoclonium* sp.) were important in spring and summer, reaching densities up to $100 \text{ g dry wt} \cdot \text{m}^{-2}$, an abundance similar or greater than that of *Ulva*.

Growth rates estimated in cages were greater than those computed by biomass changes (Fig. 6). A significant correlation between the two estimates was found only at station 1 ($P < 0.01$). The growth rates computed in cages reached the greatest values in September–October (0.25 day^{-1}). Station 2 showed the highest annual average ($0.128 \text{ day}^{-1} \pm 0.069$; mean \pm SD), whereas the lowest rate was found at the outer station ($0.08 \text{ day}^{-1} \pm 0.063$). Growth rates (in cages) at stations 2 and 3 were correlated signifi-

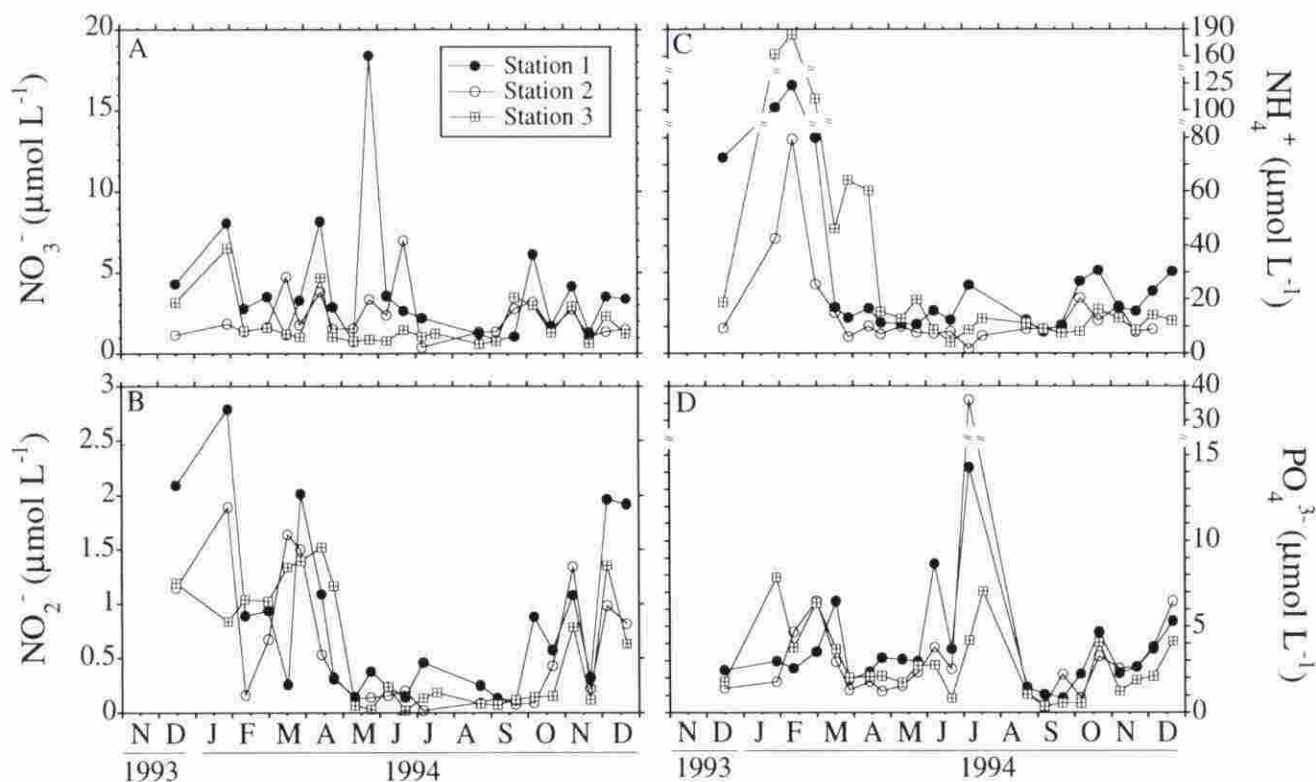


FIG. 3. Seasonal variation of A) nitrate, B) nitrite, C) ammonium, and D) phosphate in water samples collected at spring low tide from the inner (station 1), central (station 2), and outer (station 3) sampling sites.

icantly ($P < 0.01$). Either no discs or severely damaged discs were found at some sampling dates (e.g. August), which was attributable to sporulation. When the growth rates were estimated from biomass changes, a similar trend was noticed in the three stations. Positive rates were observed in spring and autumn, with a maximum of 0.1 day^{-1} , and negative growth rates (i.e. a net loss of biomass) usually occurred in winter and summer.

Total chlorophyll showed a similar seasonal pattern in the three stations (Fig. 7). Minimal values occurred during July and maximal pigment content was observed in the winter, with values about 120

$\text{mg} \cdot (\text{m thallus})^{-2}$. A second maximum was evident during August. At the three stations, an inverse correlation between total chlorophyll and global irradiance was observed ($P < 0.005$, $n = 20$). Pigment content was also positively correlated with tissue N ($P < 0.02$, $n = 19$).

Tissue nutrient composition. The tissue composition is shown in Figure 8. No clear pattern was observed for internal carbon content. Mean C values were about 30% dry wt. Overall, the C concentration was minimal in April (27%) and September (23%, station 2), reaching higher levels at the onset of the summer (36%, station 1). Tissue nitrogen, by contrast, showed a clear seasonal pattern (Fig. 8B). The maximum tissue N was found during the winter (up to 5% dry wt of N) and the minimum at the end of the spring (1.9%, stations 2 and 3). Tissue phosphorus was highest in January and decreased toward the spring (Fig. 8C). The lowest values were found in June, but no clear profiles were observed at any of the three stations.

Tissue C:N showed a clear increase during the spring (Fig. 9A), with values near 20 at stations 2 and 3. However, a less obvious pattern was recorded in the inner station, where tissue N content was fairly stable in summer. The rest of the year the ratio remained stable (ca. 9). The C:P ratio was greatest at the end of the spring (up to 800, Fig. 9B). The N:P did not show a clear pattern, although it in-

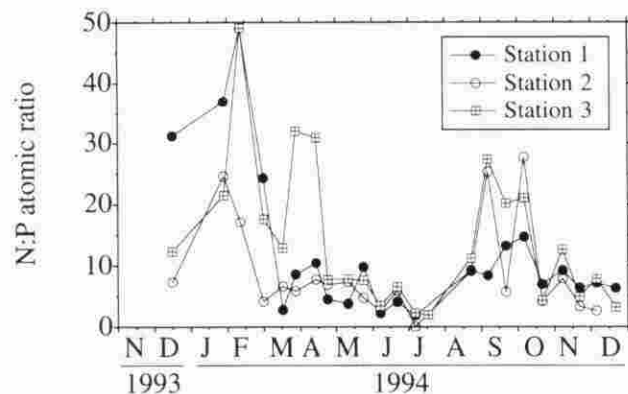


FIG. 4. Seasonal variation of N:P in water samples collected from the three sampling stations at spring low tide.

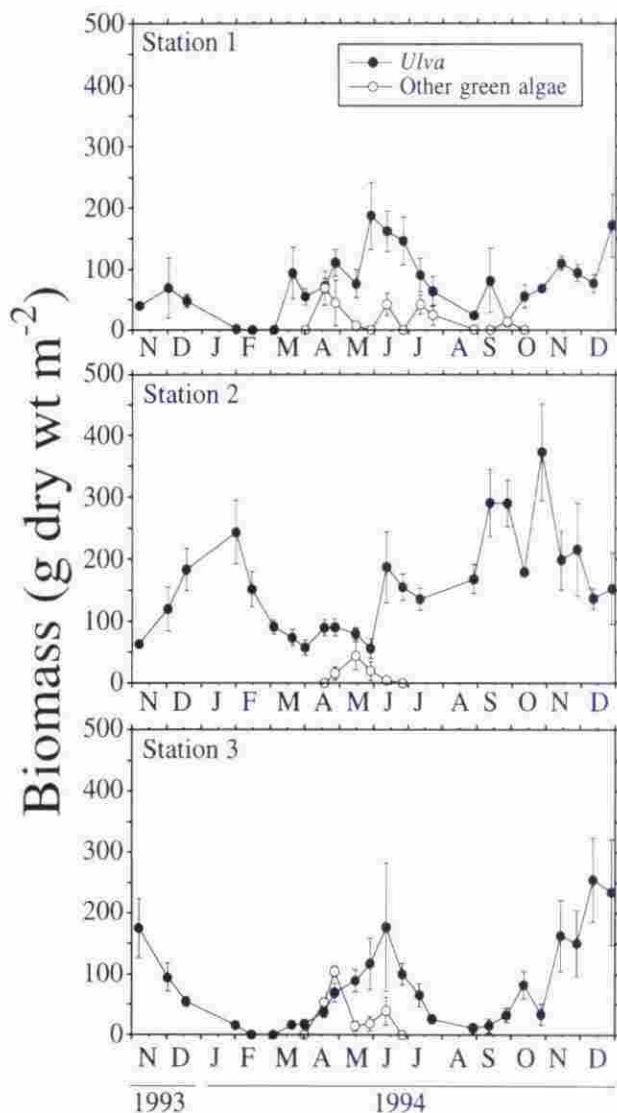


FIG. 5. Seasonal variation of *Ulva* and other green algae biomass at the three sampling stations. Data are means of three replicates and bars represent SE.

creased during the autumn (Fig. 9C). The ratio varied throughout the year between 25 (June, station 3) and 75 (November, station 1), averaging 45. Tissue N:P was usually higher than water N:P.

Net production. Table 1 shows the mean values of net production for each season calculated from biomass, %C, and disc growth rates. The highest mean value was achieved at station 2 during the summer and autumn, when high biomass and high growth rates were observed. At station 1, the mean net production was highest during the spring, whereas station 3 showed the highest production in autumn. Values were usually higher at the inner station than at the outer station.

DISCUSSION

Biomass and growth rate. Recent efforts in several European countries have been devoted to studying

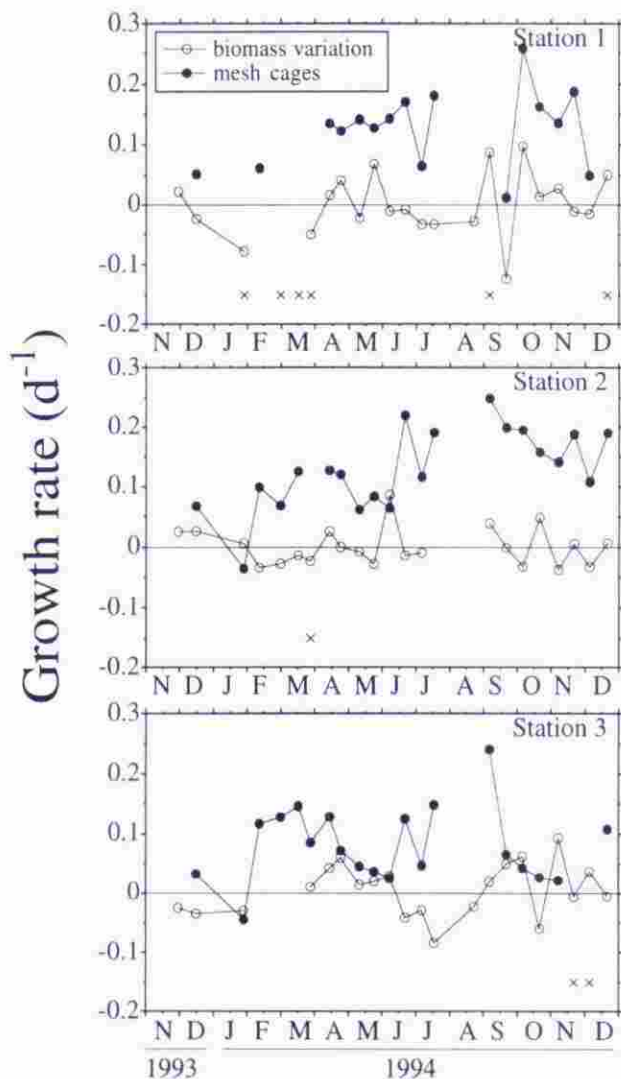


FIG. 6. Seasonal variation of *Ulva* growth rates estimated from the increase of area of at least 10 *Ulva* discs maintained in mesh cages and from biomass variation during two consecutive samplings. Crosses represent days on which no discs were found. Data are means of 10–20 replicates. SE (6% of mean) are omitted for clarity.

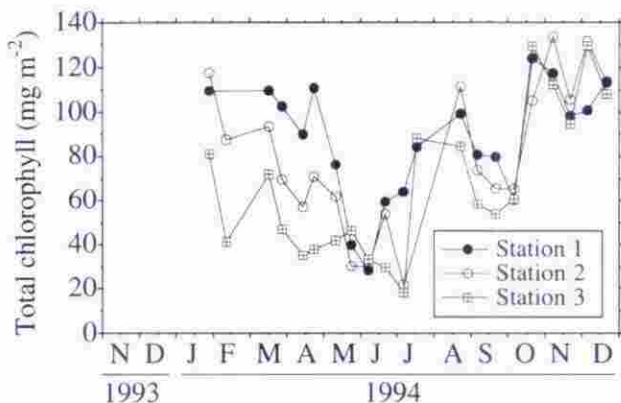


FIG. 7. Seasonal variation of total chlorophyll concentration in *Ulva* from the three sampling stations. Data are means of three replicates. SE (15% of mean) are omitted for clarity.

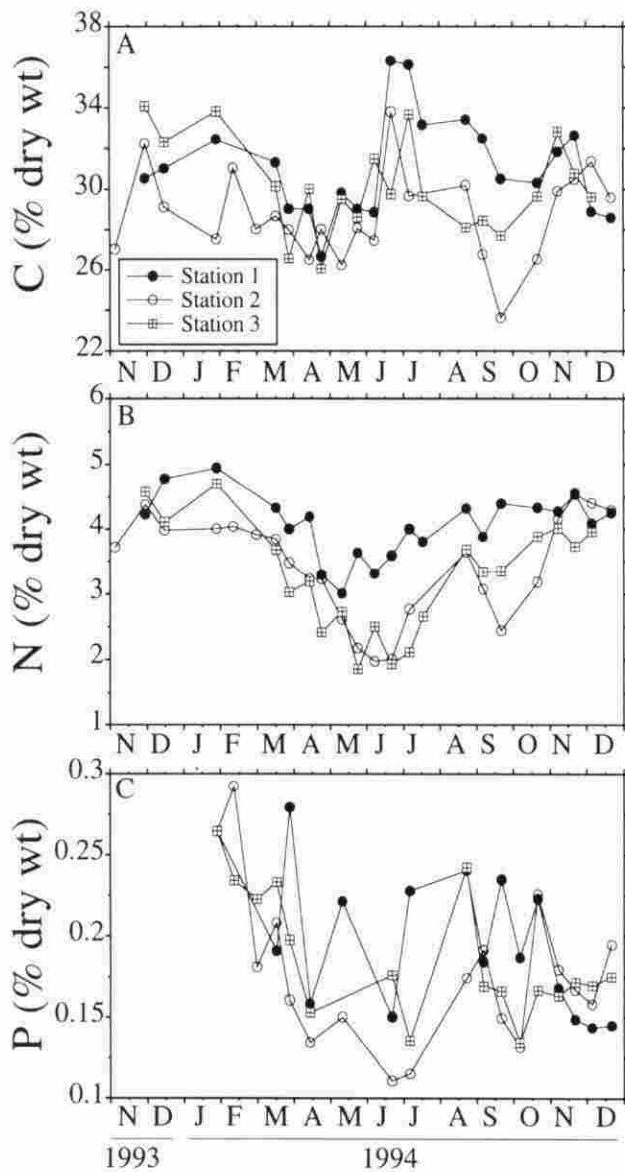


FIG. 8. Seasonal variation of A) carbon, B) nitrogen, and C) phosphorus content of *Ulva* species from the three sampling stations. Data are means of three replicates. SE. (4.5% of mean) are omitted for clarity.

the mass development of fast-growing green algae (Kamermans and Nienhuis 1995, Schramm and Nienhuis 1996), but specific studies to understand the complex mechanisms that trigger the appearance of the so-called green tides are scarce (Kamermans 1995). In the Palmones River estuary, *Ulva* biomass reached densities lower than those estimated in the Venice lagoon (higher than $2 \text{ kg dry wt} \cdot \text{m}^{-2}$, Sfriso and Pavoni 1994), but higher than, or similar to, the standing crops of other coastal zones where green tide episodes have been reported (e.g. Viaroli et al. 1993, Malta et al. 1995). The abundance of *Ulva* declined in winter and summer in most sites of the estuary (Fig. 5) and this pattern has been observed again during 1996 (our pers. observ.).

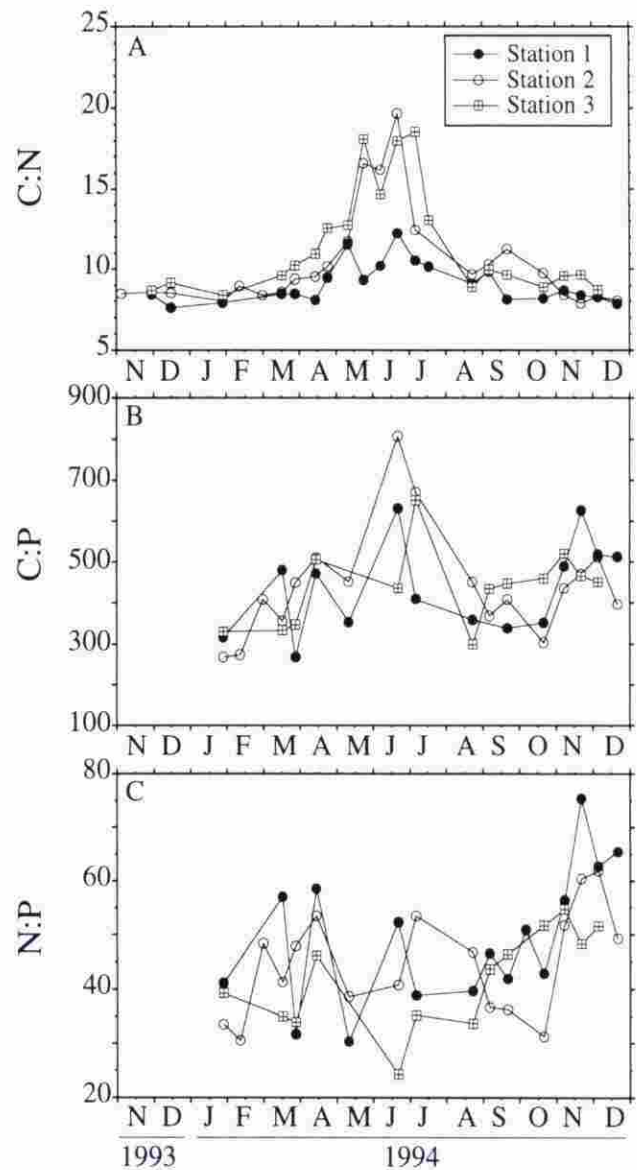


FIG. 9. Seasonal variation of A) C:N, B) C:P, and C) N:P ratios in *Ulva* from the three sampling stations.

However, the trend of station 2 was different. There are several possible explanations. The first is that the flat topography and low hydrodynamic energy input of station 2 favors biomass accumulation in this zone. Second, given the high biomass observed at

TABLE 1. Mean seasonal net production of *Ulva* (*U. curvata* and *U. rotundata*) in Palmones River estuary. Values were calculated from biomass, %C, and *Ulva* disc growth rates (Fig. 5, 6, 8A) and are means \pm SD; number of estimations (n) in parentheses.

Season	Net production ($\text{g C} \cdot \text{m}^{-2} \cdot \text{day}^{-1}$)		
	Station 1	Station 2	Station 3
Winter	=	4.4 ± 2.0 (3)	0.5 ± 0.1 (3)
Spring	4.6 ± 2.6 (6)	3.4 ± 3.9 (6)	1.9 ± 2.5 (6)
Summer	2.7 ± 2.6 (4)	11.1 ± 4.0 (5)	1.2 ± 1.2 (5)
Autumn	3.6 ± 2.6 (3)	10.1 ± 3.1 (4)	4.2 ± 5.6 (2)

this station, an upper *Ulva* layer can prevent the lower ones from desiccation by maintenance of humidity during emersion (Beer and Eshel 1983) and thus alleviate the summer dieback. At station 2, a pale, thin-textured *Ulva* layer covered the underlying biomass during the summer. Finally, the influence of other factors such as grazing pressure, thallus disintegration by release of zoospores and gametes, or competition between *Ulva* species may also be important (Ramus and Venable 1987, Rivers and Peckol 1995). Sporulation can be induced by abrupt physicochemical changes (Nilsen and Nordby 1975). In fact, a considerable amount of biomass is allocated monthly to reproduction in *Ulva* (from 20% to 60%, Niesembaum 1988).

A summer decline in *Ulva* biomass has been reported often (Sfriso and Pavoni 1994, Kamermans and Nienhuis 1995, Rivers and Peckol 1995), but in contrast to other studies (Rivers and Peckol 1995), the tissue nutrient content of *Ulva* in the Palmones estuary did not reach critical levels during this season (Fig. 8), and therefore, other factors seem to control biomass abundance. Temperature and light have been suggested as the main physical stresses leading to dieback of *Ulva* in summer (Kamermans and Nienhuis 1995). Although *Ulva* is able to withstand high temperatures for prolonged time periods (Einav et al. 1995), the extremely high temperatures reached during the first week in July (Fig. 2B) were correlated with a biomass die-off at the three sampling stations (Fig. 5) and a decrease in growth rate (Fig. 6). Besides thermal effects, photosynthetic performance of the two *Ulva* species reached a minimum in spring and summer (Vergara et al. 1997). Henley et al. (1992) demonstrated that high temperatures and photon irradiances have a synergetic effect, decreasing photosynthetic activity.

The growth rates showed marked differences when estimated per change in area or per biomass variations. Such differences, previously noted by Sfriso and Pavoni (1994), can be attributed to the processes causing loss that act on *Ulva* under these two different biomass estimations. In our study, three main loss processes are suggested: grazing pressure, the export of biomass out of the estuary, and thallus decomposition in the lower layers due to anoxic conditions. These three processes do not affect the algal discs growing within the cages. We assumed that other loss processes, such as thallus decomposition due to release of zoospores affect both growth rate estimations similarly. Geertz-Hansen et al. (1993) showed the importance of grazing by invertebrates on the control of an *Ulva* population. In addition, different bird species (*Anas platyrhynchos* L., *A. clypeata* L.) are abundant in the estuary and can also be active *Ulva* consumers. On the other hand, significant movements of free-floating thalli toward the mouth of the estuary were observed at high tide. The effects of floods after a storm event

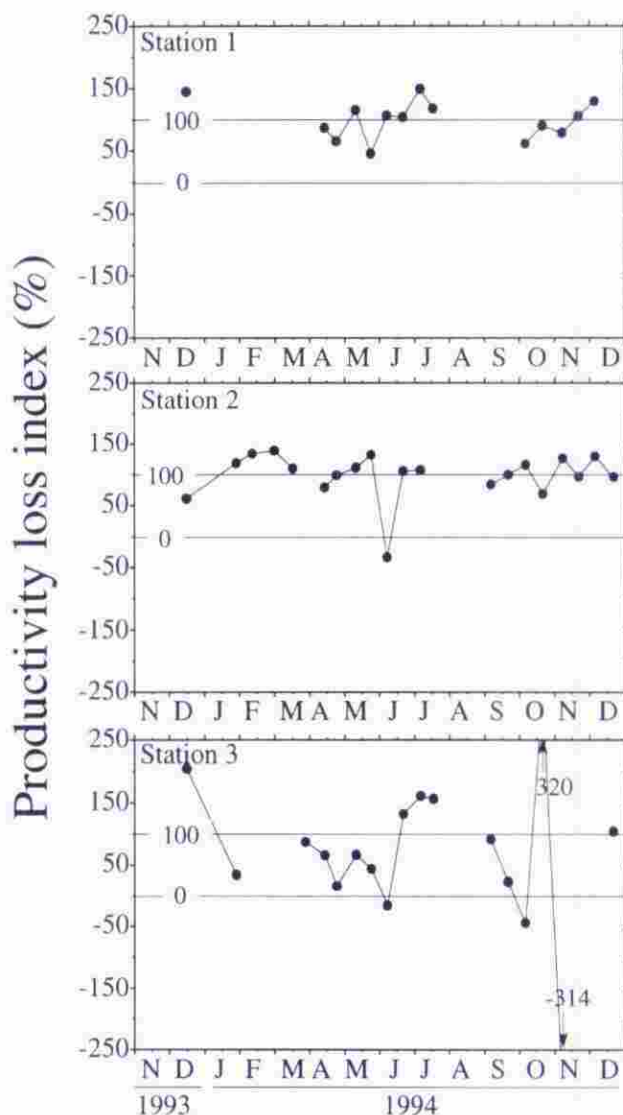


FIG. 10. Seasonal variation of the production loss index in *Ulva* from the three sampling stations. The two lines delimit the production losses extended between 0% and 100%. Gaps in the figure are due to a lack of either biomass and/or *in situ* growth rate estimations.

or the prevailing winds accentuate the loss process and also redistribute biomass within the estuary.

Based on these assumptions, a loss index is proposed as:

$$I_{\text{loss}} = [1 - (\mu_B/\mu_S)]100$$

where I_{loss} represents the percentage of net production lost, μ_S is the growth rate estimated from the increase of area of discs maintained in cages, and μ_B is the growth rate estimated from the changes in biomass.

Losses of the net *Ulva* production were important throughout the year (Fig. 10), occasionally reaching values greater than 100% meaning that not only all the biomass produced, but some remaining biomass, was either consumed, degraded, or exported.

The loss processes are less important at station 3 (mean $I_{\text{loss}} = 67 \pm 132$). By contrast, some net accumulations of *Ulva* from other parts of the estuary were noticeable ($I_{\text{loss}} < 0$), either in spring (station 2) or autumn (station 3). Further research in progress will try to elucidate the significance of these loss processes and the growth rate of the two *Ulva* species at different parts of the estuary.

Net production. Different methods have been proposed to estimate algae primary production, making comparison among values difficult. In this study, *Ulva* net production was estimated from growth rates (computed from the increase in area within cages), avoiding most of the biomass loss processes (see above). We assumed that all the biomass was growing at the same rate, but this assumption may be certain only for the algae growing in the first layers of the canopy (Vergara et al. 1997). Mean net production was highest at station 2 (Table 1), far from the maximum values reported by Sfriso et al. (1988) and Sfriso and Pavoni (1994). These authors estimated *Ulva* net production at up to 33 g C·m⁻²·day⁻¹ in the central Venice lagoon based on changes in biomass. However, the net production found in the Palmones River estuary is similar to values reported in other studies. Starting with an approximate stocking density of 200 g·m⁻² of *U. lactuca*, Israel et al. (1995) reported yields of biomass production at ~14 g C·m⁻²·day⁻¹ in outdoor tanks (assuming 20% dry wt per fresh wt and 30% dry wt of C), whereas Sand-Jensen (1988), also working with *U. lactuca*, reported a maximum photosynthesis of 600 nmol O₂·cm⁻²·h⁻¹. This value yielded approximately 2.9 g C·m⁻²·day⁻¹ (assuming a 12 h LD cycle, a photosynthetic quotient of 1.2 (O₂/CO₂) and a mean TAI of 4). Finally, in a recent laboratory study (Pérez-Lloréns et al. 1996), *U. curvata* and *U. rotundata* showed a significant correlation between observed growth rates and those estimated from photosynthesis, reaching rates of 0.2 day⁻¹. These rates are in accordance with the estimated production values observed in the field.

Tissue nutrient content. In contrast to other studies (Wheeler and Björnsäter 1992, Rivers and Peckol 1995), *Ulva* did not exhibit broad changes in N or P concentrations during the study period. The mean N and P contents were usually higher than the mean values given by Duarte (1992) for macroalgae as well as those found in other studies of *Ulva* spp. (Fagnelli et al. 1986, Le Bozec, unpubl.). Only values at the end of the spring might suggest a temporal N limitation, based on the critical 2% N level suggested by Hanisak (1983). However, nutrient availability was always very high (Fig. 4).

Water N:P and tissue N and P decreased during the spring (Fig. 8B). However, an increase of biomass (Fig. 5) was still observed. Therefore, nutrient limitation is unlikely. The decrease in tissue N and P content observed in the spring seems to be a dilution effect caused by a high growth rate during

this period (Figs. 6, 8). This decrease could also have originated from the decomposition of biomass due to high temperatures and anaerobic conditions under the lower layers (Viaroli et al. 1992), which may explain the peak of orthophosphate at the beginning of July (Fig. 3). Previous work with *U. rotundata* and *U. curvata* (Pérez-Lloréns et al. 1996) suggested that incipient N limitation may occur under high light conditions. However, the limitation may not be due to an external N depletion. Rather, the rate-limiting step appears to be the conversion of internal NH₄⁺ into soluble N compounds and proteins at high growth rates.

Ultimately, light and thermal stress seem to be the main factors controlling *Ulva* growth in intertidal mudflats at the estuary. *Ulva curvata* and *U. rotundata* have a compensation point for growth of 2 μmol·m⁻²·s⁻¹ (Pérez-Llorens et al. 1996). However, from the chlorophyll data (Fig. 7) and TAI values, it can be deduced that a few *Ulva* layers yield total chlorophyll greater than 300 mg·m⁻². These concentrations are enough to absorb practically all the incident light (Margalef 1986), thus leaving the lower layers in severe light limitation. Light quality also was affected, as the light crossing 2–3 *Ulva* layers became enriched in the green region of the spectrum (Vergara et al. 1997). Thermal stress, especially due to high summer temperatures, may be considered as a forcing function, leading to massive die-off and contributing to the observed changes in biomass.

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