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Seasonal variation in the concentrations of nutrients in two green macroalgae and nutrient levels in sediments in the Rías Baixas (NW Spain)

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Abstract

Seasonal monitoring of the levels of carbon, nitrogen and phosphorus in two green macroalgae (*Ulva* and *Enteromorpha*) was carried out at 22 sampling sites in four embayments (rías) on the northwest coast of Spain. Sediments were also analysed to determine the concentrations of these elements as well as organic matter, organic carbon, iron and texture. In addition, accumulations of *Ulva* were monitored. Nitrogen levels in algae were similar to those found in comparable studies, whereas carbon concentrations were generally higher and those of phosphorus were lower. As a result the macroalgae were relatively enriched by carbon and nitrogen compared with phosphorus. Seasonal variations in algal tissue nitrogen and phosphorus followed the usual pattern for temperate zones, with minimum levels in summer and maximum levels in winter. Variations in carbon concentrations were much less accentuated. The low levels of P in the algae appear to indicate limitation by this element, however, comparison of the monthly changes in nitrogen and phosphorus in *Ulva* with the critical concentrations of these elements suggests dual nutrient limitation in this alga in summer. By contrast, the sediments were more enriched by phosphorus; high retention of this element by the sediment may explain the low levels in algae. The highest accumulations of *Ulva* occurred in spring and summer; hydrodynamic factors appeared to be important in determining the extent of these accumulations.

Keywords: *Ulva*; *Enteromorpha*; sediments; nutrients; eutrophication; Spain

1. Introduction

Eutrophication has traditionally been considered to affect continental waters, but marine coastal eutrophication is now an increasing problem (Smetacek et al., 1991; Nixon, 1990, 1995; Goldberg, 1995; Wu, 1999). A small number of macroalgae are universally cited as occurring in eutrophic waters: *Chaetomorpha*, *Cladophora*, *Enteromorpha* and *Ulva* (Fletcher, 1996). Excessive proliferations of these algae are known as green tides; many examples of the occurrence of this phenomenon can be found throughout the world (see reviews by Morand and Briand, 1996; Fletcher, 1996). The proliferation of green macroalgae often takes place at the expense of perennial algal species (Duarte, 1995; Pedersen, 1995; Middelboe and Sand-Jensen, 2000; Díaz et al., 2002) and seagrasses (Duarte, 1995; Harlin, 1995; Short and Burdick, 1996; Short and Wyllie-Echevarria, 1996), which in turn negatively affects the numerous species associated with them (Fletcher, 1996). Changes in macrozoobenthic communities have commonly been found in ecosystems suffering green tides. In the study area, these phenomena are known to have negative effects on bivalve production (Rodríguez et al., 1987; Niell et al., 1996). The effects of eutrophication on fish communities have also been reported (Rosenberg, 1985; Hansson and Rudstam, 1990; Pihl et al., 1995).

The embayments under study, known collectively as the Rías Baixas, are naturally enriched by nutrients due to positive estuarine circulation, which concentrates

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nutrients (Fraga and Margalef, 1979), and to periodic upwelling events, which occur in spring and summer (Álvarez-Salgado et al., 1993). These zones are therefore susceptible to eutrophication because of this natural enrichment and also nutrient inputs from the dense population living in the surrounding areas. Although the proliferation of green macroalgae is still far from being considered serious, it appears to be an increasing problem, which could have important consequences in the future, given the huge economical and ecological importance of these natural systems to the region.

The objectives of this study, which was part of a wider investigation of green tides on this coast, were to monitor the levels of different nutrients in two genera of green macroalgae (*Ulva* and *Enteromorpha*) in different parts of the area, throughout one year, and to try to evaluate possible nutrient limitation in the algae. Tissue levels of nutrients in macroalgae result from the integration of the nutrient regime of the water during a certain period prior to sampling, therefore they provide a reliable indication of nutrient status (Wheeler and Björnsäter, 1992; Lyngby and Mortensen, 1994; Horrocks et al., 1995), and thus C : N : P ratios have often been used to determine limiting nutrients (Atkinson and Smith, 1983; Wheeler and Björnsäter, 1992; Hernández et al., 1993; Flores-Moya et al., 1995). Changes in the accumulations of *Ulva* were studied throughout the same period. Nutrient levels in sediments were also investigated to aid in the interpretation of the nutrient levels in macroalgae. Sediments play an essential role in the nutrient cycle in coastal areas (Jørgensen, 1996; Cloern, 2001; Tyler et al., 2001). They can receive material of terrestrial or oceanic origin, and material generated in situ; organic compounds are degraded more or less rapidly depending on environmental factors such as pH, temperature, available oxygen, salinity, etc. Regeneration of nutrients by sediment is a factor to be considered in the process of eutrophication (Raaphorst et al., 1988; Lavery and McComb, 1991; Jeffrey et al., 1995; Trimmer et al., 2000).

2. Materials and methods

2.1. Sampling

Intertidal macroalgal samples were collected monthly from 22 sampling sites (Fig. 1) at which a relatively high abundance of *Ulva* and *Enteromorpha* had previously been found. More intensive sampling (fortnightly) was carried out at five selected sites (A2, A8, P2, P5 and V4). Samples of *Ulva* were collected between March 1994 and February 1995, whereas sampling of *Enteromorpha* was suspended in September 1994 because it had stopped growing at many sites. The fortnightly sampling took place between April and November 1994. At each sampling site we collected at least 75 fronds of *Ulva* and at least samples from 30 mats of *Enteromorpha* along a transect of 100 m. The sediment was sampled from the 22 sites mentioned above, on two occasions—in April 1994 (representative of the rainy season) and in August 1994 (representative of the dry season). Sediment was collected using a plastic spatula and only the surface layer (0–1 cm) was removed. The algae were stored in mesh bags and the sediment in plastic bags; all samples were transported at 4 °C to the laboratory.

The macroalgae were then washed carefully with distilled water, dried at 50 ± 1 °C until constant weight, crushed using a mortar and pestle and stored in plastic bags until analysed. The sediment was also dried at 50 ± 1 °C, one part was kept for granulometric analysis, the rest was sieved (<0.2 mm) and stored in plastic bags.

Most of the fronds were tentatively identified as *Ulva rigida* and *Enteromorpha intestinalis*. Identification was not however made to species level because of the taxonomic complications of these genera, in which polymorphism is common (Provasoli and Pintner, 1980). Malta et al. (1999) demonstrated that specimens of *Ulva* are often assigned to different species when they are in fact different morphotypes of a single species. In the case of *Ulva*, the fronds were usually free living and often lacked a stipe, which further complicated their identification.

2.2. Chemical analysis

2.2.1. Macroalgae

Carbon and nitrogen were measured with a LECO CHN 1000 elemental analyser, using EDTA as a standard. For phosphorus analysis, the samples were combusted in a muffle furnace at 550 ± 5 °C for 3 h, and then digested in 2.5 ml of 2 N HCl on a hot plate at 200 ± 2 °C for 15 min. The extracts were then filtered and brought to 50 ml with distilled water (Chapman and Pratt, 1981). Phosphate in the extracts was determined using the vanadomolybdophosphoric acid colorimetric method, which is the most suitable for the range of concentrations involved here (APHA, AWWA, WEF, 1995). Certified reference plant material, BCR (Community Bureau of Reference) no. 129 (hay powder), was analysed along with the samples. Analysis of the fortnightly collected samples was carried out in triplicate.

2.2.2. Sediment

The sediment texture was determined using the methods described by Guitián and Carballas (1976). Carbon, nitrogen and phosphorus were measured using the same methods as for the algae. Estuarine sediment (BCR-277) was used as reference material in the analysis. Organic carbon was determined following

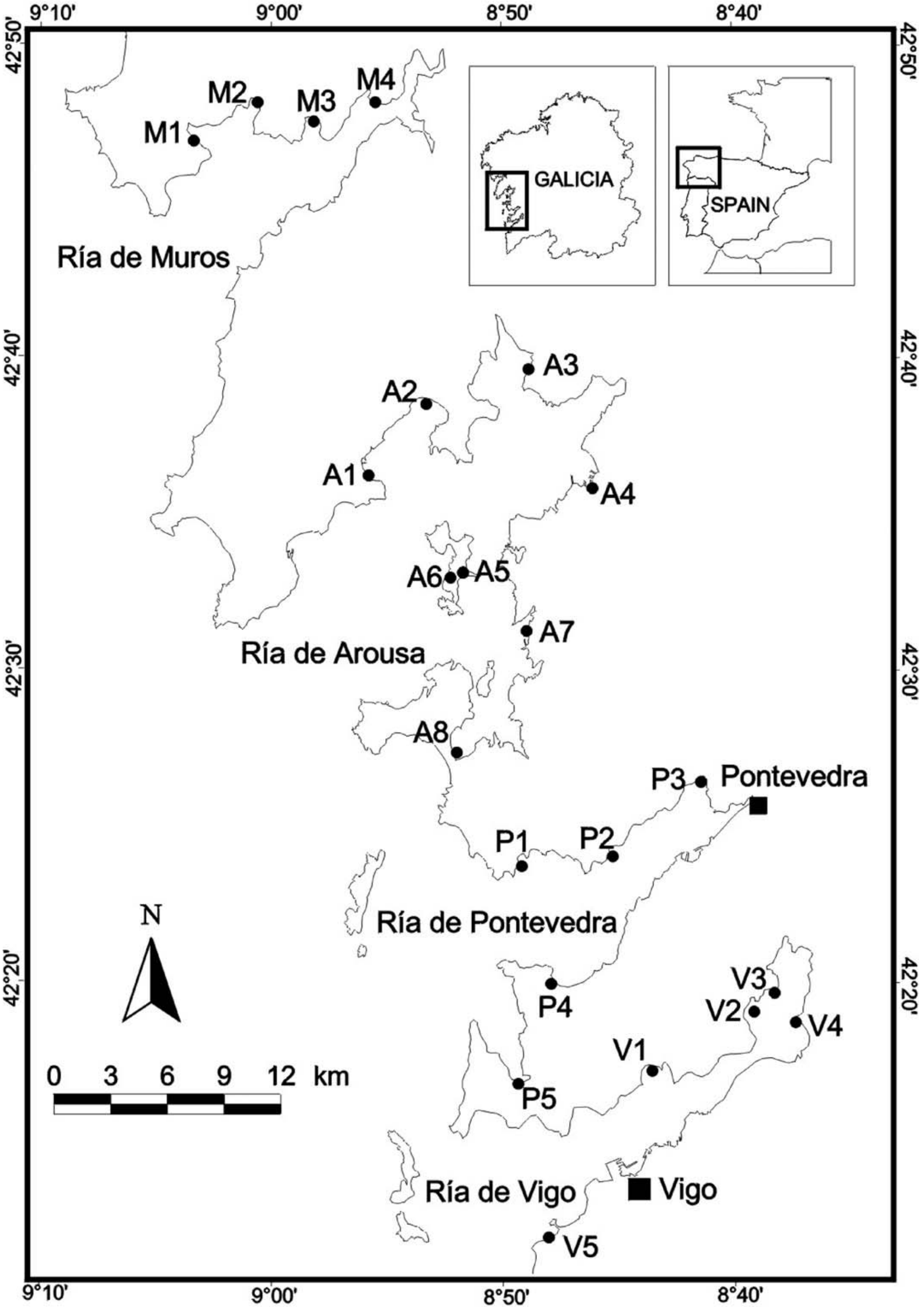

Fig. 1. Location of sampling sites.

treatment of the sample with 2 N HCl, the acid was then evaporated off at 120 °C, thereby avoiding loss through leaching; losses in the form of volatile compounds are very low using this method (Nieuwenhuize et al., 1994); the sample was then processed in a LECO CHN 1000 elemental analyser. Organic matter was determined as loss on ignition after combustion of samples at 400 °C for 6 h. Iron was extracted in Teflon® bombs in a microwave oven with aqua regia and HF. Concentrations were determined by flame atomic absorption spectrophotometry (Perkin–Elmer 2100). Certified reference material (MESS-2, marine sediment, National Research Council of Canada) was processed at the same time as the samples.

2.3. *Estimation of the abundance of* Ulva

A gross estimation of the abundance of *Ulva* at the different sampling sites was obtained by visual observation at low tide and by scoring the relative abundance on a scale between 0 (hardly any fronds present at the sampling site) and 6 (almost 100% cover of *Ulva* fronds at the sampling site). It was not intended to provide an exhaustive description of the temporal variations in the algal biomass, as this would have required a larger sampling effort. Although the estimates thus made are subjective, they do give an approximate idea of the temporal changes in the abundance of *Ulva* at the different sites.

3. Results and discussion

3.1. *Levels of nutrients in* Ulva *and* Enteromorpha

The levels of C found in the macroalgae in the present study (Table 1) were generally higher than those found by other authors in different species of *Ulva* and *Enteromorpha* (Table 2), whereas levels of N were similar to those reported in other studies and those of P were clearly lower. The coefficients of variation of the nutrient concentrations in both algae indicated large variation in the levels of P, followed by N, whereas those of C were more stable; Duarte (1992) obtained similar results in an extensive study of aquatic plants, as did Delgado et al. (1994) with *Cystoseira mediterranea*.

The median concentrations of N and P were significantly higher ($P < 0.001$) in samples of *Enteromorpha* than in samples of *Ulva* collected during the same period (March–September), whereas the levels of C were higher in *Ulva*, although at a level of significance of $P = 0.05$ (Mann–Whitney *U*-test). The presence of fine particles adhering to the algal shoots is usually taken into account in studies of metal contamination (Bryan et al., 1985; Villares et al., 2001) as it may interfere in the use of algae as biological indicators, particularly species such as *Enteromorpha*, because their tubular structure makes them difficult to clean. It is unlikely that the relatively high levels of nutrients in *Enteromorpha* were caused by this, given that the concentrations of these elements were generally much lower in the sediments than in the algae (Tables 1 and 4). A more feasible explanation for the relatively high concentrations of N and P in *Enteromorpha* is that the samples of this species comprised matted material fixed to the substrate, whereas the *Ulva* samples mainly comprised free-floating fronds. The *Enteromorpha* specimens were therefore in a better situation to take up nutrients regenerated from the sediment.

The ratios between the maximum and minimum values of the nutrients studied are shown in Table 1. The highest values corresponded to phosphorus in both algae and ratios were always higher in *Enteromorpha* than in *Ulva*. This may indicate that both species of algae, but especially *Enteromorpha*, have a higher capacity for storing P than for storing the other elements, or that they are more tolerant to a lack of this nutrient (Björnsäter and Wheeler, 1990). Furthermore, if the sampling period of *Enteromorpha* had been as long as for *Ulva*, the ratios obtained may have been even higher.

The possible relationships among tissue C, N and P were analysed using a Spearman's rank correlation test. The similar seasonal variation in the levels of nutrients found in this study may complicate interpretation of the results, thus the correlation analysis was carried out using the mean values of the concentrations of C, N and

Table 1
Descriptive statistics of the levels of carbon, nitrogen and phosphorus (concentrations are expressed in mg g^{-1} dry wt) and the C:N, C:P and N:P atomic ratios in the entire set of samples of *Ulva* and *Enteromorpha* in the present study

Ulva	C	N	P	*Enteromorpha*	C	N	P
n	282	282	282	*n*	166	166	166
Mean	397	28.8	0.965	Mean	385	30.4	0.958
Median	396	28.3	0.907	Median	386	29.4	0.881
S.D.	14.1	11.5	0.419	S.D.	22.5	11.7	0.444
Minimum	355	6.90	0.252	Minimum	252	7.10	0.248
Maximum	426	51.3	2.286	Maximum	448	59.3	2.602
Maximum/minimum	1.20	7.44	9.07	Maximum/minimum	1.78	8.37	10.49
C.V.	3.55	39.9	43.4	C.V.	5.83	38.3	46.4

	C:N	C:P	N:P		C:N	C:P	N:P
Mean	19.47	1309	69.9	Mean	17.6	1239	76.2
Median	16.38	1121	65.3	Median	15.2	1136	67.9
S.D.	9.57	666	23.6	S.D.	8.58	519	31.0
Minimum	9.61	463	27.4	Minimum	8.82	377	32.4
Maximum	65.1	3904	178	Maximum	58.7	3594	209
C.V.	49.2	50.8	33.7	C.V.	48.8	41.9	40.7

Table 2
Previously reported levels of carbon, nitrogen and phosphorus found in different species of *Ulva* and *Enteromorpha* growing under natural conditions

Species	C	N	P	Season	Locality	Reference
U. lactuca	235	8.80	1.40	Spring–summer	NE USA	Lapointe et al. (1992)
U. rigida	208.5 (180.1–250.0)	21.7 (10.2–29.3)	1.91 (0.38–2.91)	Average of 28 months	Venice	Sfriso et al. (1993)
U. rigida		27.7 (22.9–31.8)	2.50 (1.10–3.90)	Spring–summer	Venice	Sfriso et al. (1987)
U. fenestrata		(24.4–54.8)	(3.20–6.00)	Annual cycle	NW USA	Wheeler and Björnsäter (1992)
U. lactuca		(22.2–52.7)	(0.86–3.10)	Two annual cycles	Hong Kong	Ho (1987)
U. rigida		(21.0–42.0)		Period of upwelling	NW USA	Fujita et al. (1989)
U. lactuca		37.25 ± 11.37	2.17 ± 0.76	February, April	Hong Kong	Ho (1981)
U. fasciata	268 ± 5	21 ± 1		February	Hong Kong	Kaehler and Kennish (1996)
U. rigida		20–30	1.10–2.40	March–July	North Adriatic	Viaroli et al. (1996)
U. rigida	211–327	9.90–35.0	0.27–2.70	Annual cycle	SE France (lagoon)	De Casabianca and Posada (1998)
Ulva spp.		15–50	3.0–5.0	Annual cycle	Brittany	Ménesguen and Piriou (1995)
Ulva sp.	302.4	36.4	2.17	Autumn	SW USA	Smith et al. (1987)
E. intestinalis	228	18.8	1.60	Spring–summer	NE USA	Lapointe et al. (1992)
E. intestinalis		(20.2–51.1)	(3.72–7.33)	Annual cycle	NW USA	Wheeler and Björnsäter (1992)
E. clathrata	246 ± 1	14.0 ± 1		February	Hong Kong	Kaehler and Kennish (1996)

Concentrations are expressed in mg g^{-1} (dry wt). The range of values is given in brackets. The measure of dispersion, where included, is the standard deviation.

P for each sampling station ($n = 22$). The coefficients of correlation were significant in all cases, at $P < 0.001$ for C–N in *Ulva* and *Enteromorpha* and N–P in *Enteromorpha*; for the remaining comparisons the level of significance was slightly lower ($P < 0.01$). The strong relationships are not surprising because the metabolism of these elements is closely linked. The relationship between the metabolism of N and of C in algae has been pointed out by Turpin (1991); limitation by N may affect C fixation by means of the proteins necessary for the carboxylation of CO_2 (McGlathery, 1992). Pedersen and Borum (1996) showed that assimilation of dissolved inorganic phosphorus may be limited in N-depleted algae because of low enzyme activity and protein content, even though P availability is high. By contrast, the severe P limitation in *Gracilaria tikvahiae* found by Lapointe (1987) may affect the metabolism of N because of the importance of P in molecules of ATP, ADP and phospholipids.

3.2. C : N : P ratios

Atkinson and Smith (1983) showed that the Redfield ratio, $C:N:P = 106:16:1$ (Redfield et al., 1963), does not apply to marine macroalgae. In an extensive study of numerous species of seaweeds and also some cyanobacteria and seagrasses, they obtained a mean value for the C:N:P atomic ratio of 700:35:1 and a median of 550:30:1. More recently, Duarte (1992) reported an average value of 800:49:1, with a larger amount of macroalgae data.

In the present study, a mean value of 1309:70:1 and a median value of 1121:65:1 were obtained for *Ulva*; in *Enteromorpha* the mean was 1239:76:1 and the median was 1136:68:1 (Table 1). The C:N ratios are very similar to those reported in the previously mentioned studies. By contrast, the ratios involving P are quite different, although it should be taken into account that those studies were carried out by using average values from macroalgae from all over the world, with many different species and at different times of the year. Despite this, the C:N:P ratios are still high compared with those obtained by other authors for temperate zones (Table 3), and are more similar to those cited for tropical zones, where there is often acute P limitation (Lapointe, 1987; Lapointe et al., 1992). High ratios of this type have also been reported for the Mediterranean, and are attributed to a scarcity of phosphorus (Vidondo and Duarte, 1995; Flores-Moya et al., 1995). These results appear to indicate the existence of P limitation in the algae in the present study.

3.3. Temporal variations in nutrients

The levels of C in *Ulva* in the four rías (Fig. 2a) generally increased from the beginning of the study, in spring, to winter. There were, however, differences in the changes in each ría, with many increases and decreases in the levels in the Ría de Muros and more gradual increases in those in the Ría de Pontevedra. The changes in carbon levels in *Enteromorpha* and *Ulva* differed, especially in the Ría de Vigo. The most erratic changes in *Enteromorpha* were in the Ría de Muros. The levels of N (Fig. 2b) in both algae were similar in all four rías, with minimum values in summer. Trends in tissue concentrations of P (Fig. 3a) were similar in *Ulva* and *Enteromorpha*. In general, minimum values occurred in spring and summer, then increased in autumn and winter. The pattern in the Ría de Muros was different, with many increases and decreases in the levels of this nutrient.

Table 3
Previously reported atomic ratios of C : N, C : P, N : P, calculated for different species of macroalgae growing under natural conditions

Species	C : N	C : P	N : P	Season	Locality	Reference
Various spp.	20[a]	700	35	Variable	Worldwide	Atkinson and Smith (1983)
	18.3[b]	550	30			
Various spp.	16.3	800	49	Variable	Worldwide	Duarte (1992)
Various spp.	23.1	976	43.4	Spring–summer	Caribbean	Lapointe et al. (1992)
Various spp.	29.2	430	14.9	Spring–summer	NE USA	Lapointe et al. (1992)
Ulva rigida			30.9–49.4	March–July	North Adriatic	Viaroli et al. (1996)
Ulva lactuca	31.0	433	14.1	Spring–summer	NE USA	Lapointe et al. (1992)
Ulva sp.	9.73	360	37	Autumn	SW USA	Smith et al. (1987)
Enteromorpha intestinalis	14.1	366	26.1	Spring–summer	NE USA	Lapointe et al. (1992)
Gracilaria tikvahiae		1818	124	Summer and winter	SE USA	Lapointe (1987)
Codium bursa		1712	31.8	Summer	NW Mediterranean	Vidondo and Duarte (1995)
Caulerpa taxifolia	11–27	382–610	22–46	Annual cycle	NW Mediterranean	Delgado et al. (1996)
Penicillus capitatus			75	Summer	Bermuda	McGlathery et al. (1992)

[a] Mean.
[b] Median.

In the study of C : N : P ratios, the input of each component to the final result must be taken into account; the coefficient of variation of C was very low for both *Ulva* and *Enteromorpha* samples (Table 1), therefore the variations in the C : N and C : P ratios depended almost exclusively on the second term. Other authors have pointed this out for different macroalgae (Hardwick-Witman and Mathieson, 1986; Viaroli et al., 1992; Duarte, 1992; Delgado et al., 1994). We therefore have only shown the variations in the tissue N : P ratio. A spring peak in this ratio occurred in May (Fig. 3b) in all rías and in both algae, except for *Enteromorpha* in Pontevedra. The values then fell in summer, except in *Ulva* in the Ría de Vigo, for which there was no clearly defined pattern. There was then an increase in the ratio in *Ulva* in autumn and a decrease in winter. The changes in the monthly means for all the sampling sites (Fig. 4) were clearly very similar for *Ulva* and *Enteromorpha*, except for C, for which there was a contrasting pattern in the summer months. Apart from C in *Ulva*, the general trend was a decrease in the concentrations from spring to summer, with a later rise in autumn and winter.

Seasonal variations in C in macroalgae have been poorly studied in comparison with those of N and P. De Casabianca and Posada (1998) described a trend of maximum levels of C in winter and minimum values in summer for *Ulva rigida*, although the general rule is for nonseasonality of this element. Brown et al. (1990) found that in *Porphyra columbina* the concentration remained relatively constant over time and Hardwick-Witman and Mathieson (1986) did not find seasonal variations in this element in *Ascophyllum nodosum*. A similar situation was described by Hernández et al. (1997) in *Ulva* spp.

In contrast to the findings for C, many studies have shown that concentrations of N and P in macroalgae follow a seasonal pattern. In temperate zones the highest values are typically found in winter and the lowest in summer; this can be attributed firstly to a sharp increase in primary production, which is followed by a rapid decline in nutrients in solution and secondly to growth, which causes dilution of nutrients in the algal shoots when the metabolic demand exceeds assimilation of nutrients from the water (Viaroli et al., 1996; Pedersen and Borum, 1996; Hernández et al., 1997; De Casabianca and Posada, 1998). This trend has been described by many authors for N in different species of macroalgae (Delgado et al., 1994; Chopin et al., 1996; Pedersen and Borum, 1996; Hernández et al., 1997; De Casabianca and Posada, 1998; Lyngby et al., 1999). The situation concerning P appears to be different and there are many studies in which clear seasonal variation in this element was not found (Piriou and Ménesguen, 1992; Wheeler and Björnsäter, 1992; Hernández et al., 1997). Seasonal patterns have also been described in different macroalgae (Delgado et al., 1994; Chopin et al., 1996; Pedersen and Borum, 1996; De Casabianca and Posada, 1998; Lyngby et al., 1999). In short, of these three elements, N usually shows the most evident seasonal variation. This may be because on most occasions it is the most limiting for algal growth. In the present study, there was significant temporal variation (Kruskal–Wallis test) in the three elements studied, however the variations in the levels of C were relatively insignificant compared with the variations in levels of N and P, perhaps because of the structural importance of C.

The critical concentration (minimum tissue concentration of a nutrient in a plant needed to support maximum growth rates) was previously calculated for N and P in *Ulva* in the study area. The critical concentration of N in *Ulva* was calculated as 24.5 mg g^{-1} (dry wt); the average levels in all of the rías were always well below this level in summer and always above it in autumn–winter. The critical concentration established for P was 0.810 mg g^{-1} (dry wt); as with N, this value

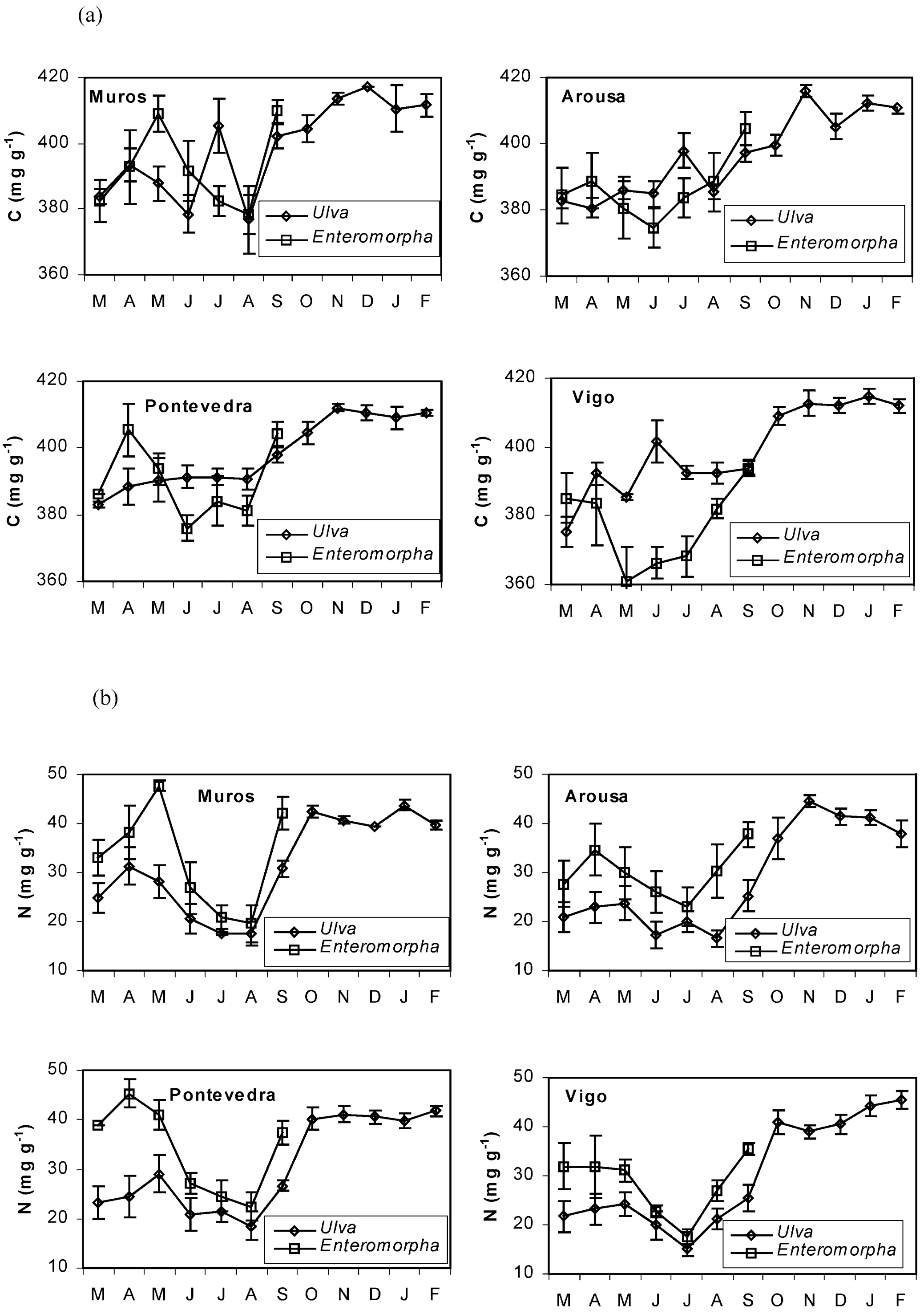

Fig. 2. Average monthly variations in the concentrations of carbon (a) and nitrogen (b) (mg g^{-1} dry wt) in *Ulva* and *Enteromorpha* in each ría.

was below the average levels for each ría in autumn and winter, except for Muros in October. The average levels for each ría in spring and summer followed a less clear pattern than for N, with increases and decreases from the critical concentration, similar results were obtained by Lyngby et al. (1999). Analysis of the variations in levels of nutrients compared with the critical concentrations allowed us to show that, in general, *Ulva* was limited by N and P in summer, whereas in spring it was close to being or was limited by these nutrients. In autumn and winter there was clear nutrient sufficiency. These results do not support the possibility of limitation exclusively by P, inferred from the C:N:P ratio; furthermore, the existence of simultaneous limitation

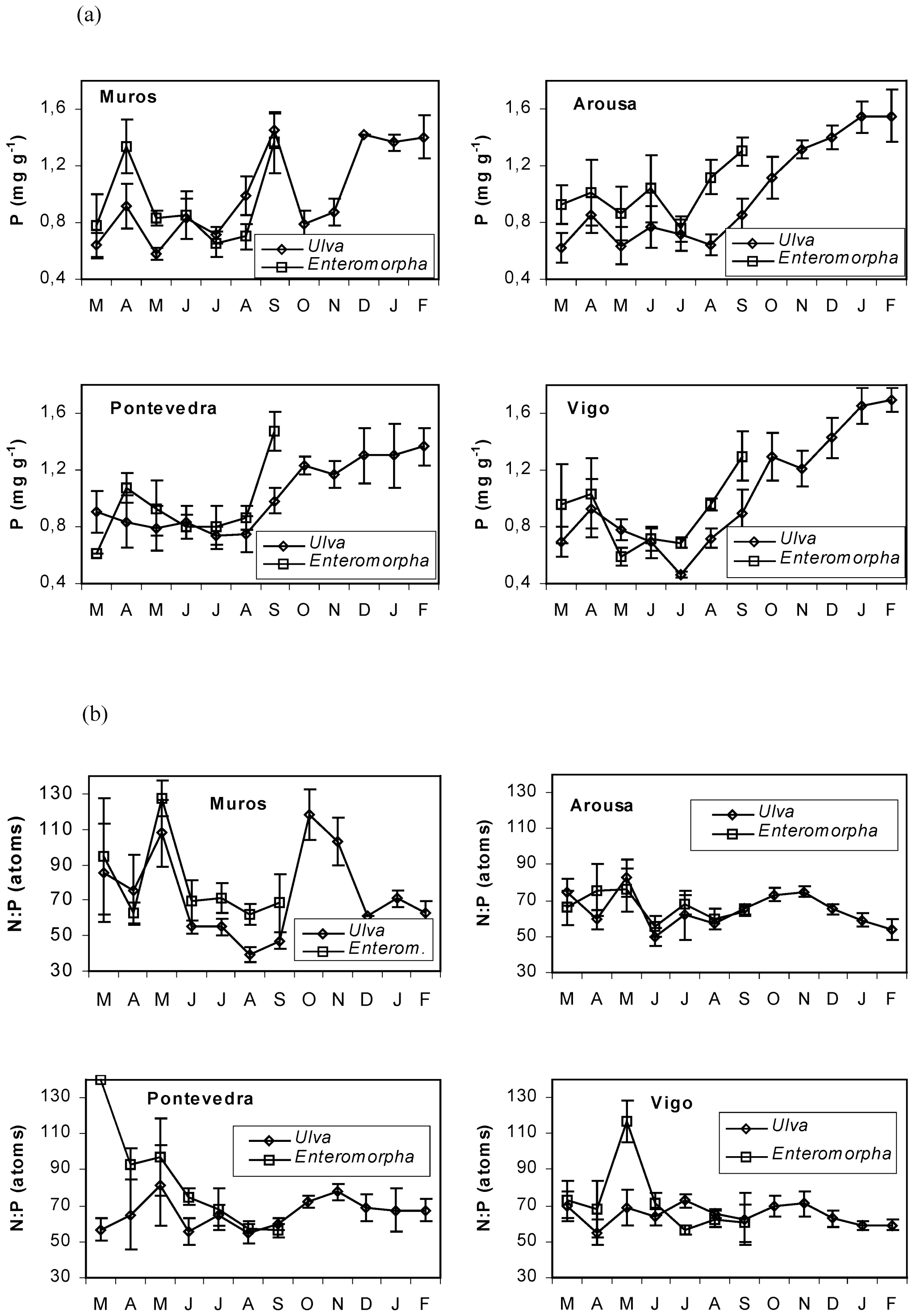

Fig. 3. Average monthly variations in the concentrations of phosphorus (a) (mg g^{-1} dry wt) and the N : P atomic ratio (b) in *Ulva* and *Enteromorpha* in each ría.

by two nutrients would not be consistent with Leibig's Law of the Minimum, although the idea of dual nutrient limitation in macroalgae has previously been suggested (Lapointe, 1987). Dual limitation may be caused by the already mentioned strong links in the metabolism of the two elements (Lapointe, 1987; Pedersen and Borum, 1996).

3.4. Sediments

Sediments may play an important role in maintaining green tides, as they supply nutrients originating from the mineralization of organic matter present in them. This may be especially relevant in the seasons of highest primary production, when the concentrations of

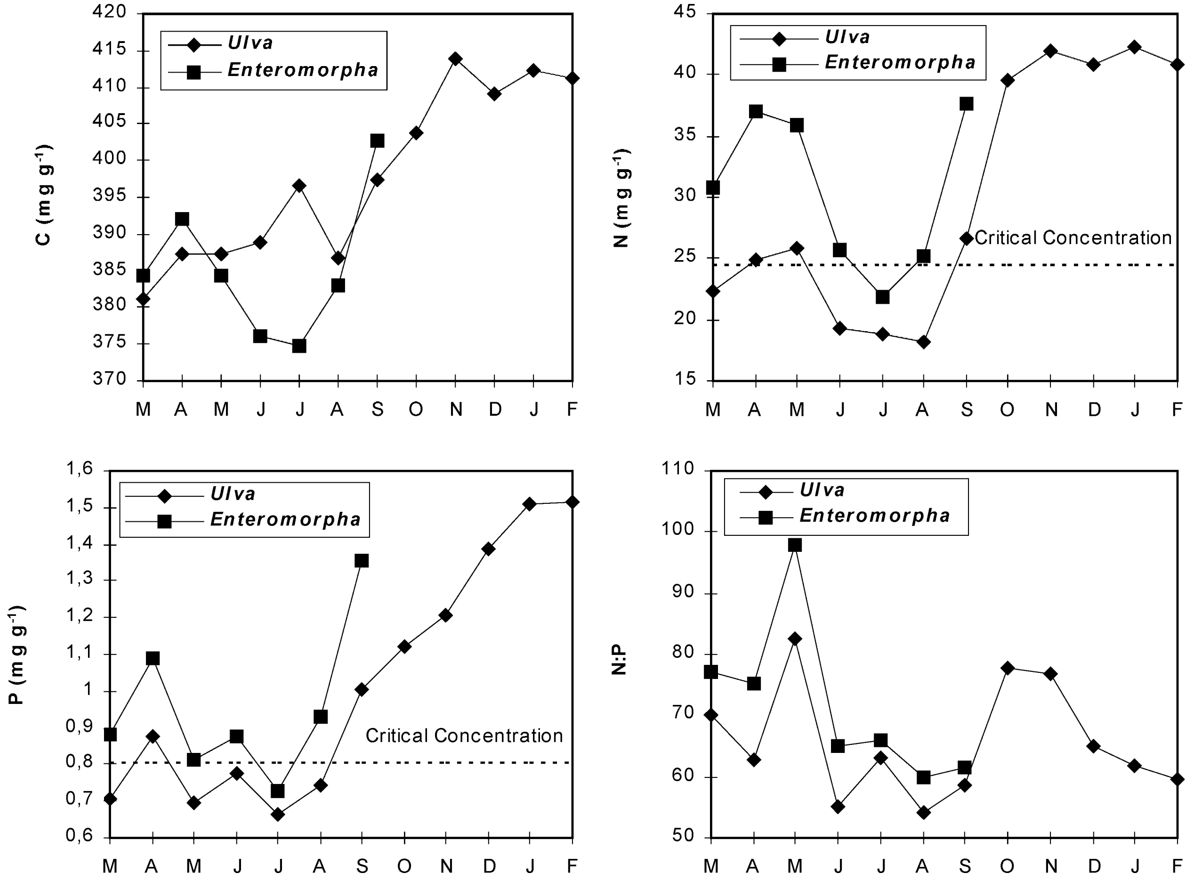

Fig. 4. Monthly variations (average of all the sampling sites) of the concentrations of carbon, nitrogen, phosphorus (mg g^{-1} dry wt) and the N : P atomic ratio in *Ulva* and *Enteromorpha*. The critical concentrations of nitrogen and phosphorus for *Ulva* are also shown.

nutrients in the water may decline (Lavery and McComb, 1991; Trimmer et al., 2000). In the present study, this compartment probably also has an essential role in the nutrient cycle, given the high ratio between the surface area of sediment and the volume of water at the sampling stations, as they are located in shallow and intertidal zones.

No significant differences were found in the concentrations of nutrients in the sediment samples collected in April and those collected in August (Mann–Whitney *U*-test), therefore the mean values of the two samplings were used. There was a large difference in the median and mean values obtained for C and therefore for the C : N and C : P ratios (Table 4), which was due to the presence of high levels of carbonates in some samples, in these cases the median value gave a better indication of the overall situation. The ratio of C_{org} : N confirmed the influence of inorganic C in the previously mentioned mean, and the median and mean values for this ratio were very similar.

The high relative abundance of *Ulva* and *Enteromorpha* at the sampling stations appears to suggest that the sediment nutrient content may be related to the nutrient content in the macroalgae; Sfriso et al. (1988) reported similar values of the N : P ratios in sediments and in macroalgae due to their decomposition. The average values of the C : N, C : P and N : P ratios that were obtained for the algae were higher than those corresponding to the sediment, indicating greater enrichment of C than of N and P, and of N than of P in algae. This finding is consistent with the nonsignificant Spearman's coefficients of rank correlation found

Table 4
Descriptive statistics of sediment characteristics (top 1 cm, <0.2 mm fraction), averages of two samples (*n* = 22)

	C	N	P	Fe	C_{org}	O.M.	C : N	C : P	N : P	C_{org} : N	C_{org} : P
Mean	11.04	0.641	0.281	8315	4.14	18.3	38.6	969	7.11	7.66	76.6
Median	6.59	0.405	0.259	7198	2.46	14.0	9.11	62.8	5.05	7.16	35.5
S.D.	12.73	0.556	0.154	4834	3.71	12.2	81.2	3812	9.65	2.20	143

Concentrations are expressed in mg g^{-1} (dry wt) and the proportions in atoms.

between nutrients in sediment and in algae. Taking the Redfield ratio as a reference value, it was found that the ratio of $C_{org} : N = 7.16$ was very similar to this. The median values of ratios involving P ($C : P = 63$, $N : P = 5.05$), however appear to indicate an enrichment of this element with respect to C and N.

The levels of P in the sediments therefore appear to be relatively high; Sfriso et al. (1988) cite higher values for the N:P ratio in Venice Lagoon sediments than those found in most of the samples in this study. Under anoxic conditions the sediment may release significant amounts of P to the overlying water (Rozan et al., 2002), favouring proliferation of algae. In the present study, because the sampling stations were situated in intertidal areas, the most superficial layer of the sediment was oxidised and therefore may have favoured retention of P, which migrates from the deepest, anoxic layers to the surface where it precipitates (Jørgensen, 1996). High retention of P relative to C may be a general occurrence in marine coastal sediments, largely associated with oxidised iron species (Jensen et al., 1995). In this study there was a highly significant ($P < 0.001$) correlation between the concentrations of Fe and P in the sediments. It is therefore possible that a large amount of P may have remained immobilized in the sediment and was not available to the algae. This fact may partly explain the relatively high concentrations of P compared with those of C and N in the sediment and the relatively low concentrations found in *Ulva* and *Enteromorpha*. Another possible explanation for the low N:P ratios in the sediments may be the existence of a significant loss of N by denitrification, however the $C_{org} : N$ ratios were lower than those found by other authors for coastal sediments (Hopkinson, 1987; Zimmerman and Benner, 1994; Middelburg et al., 1996), which may indicate that the losses of this element are not very significant. Further studies are required to evaluate the actual importance of nutrient flows between sediment and the water column in the study area.

3.5. Temporal variations in the abundance of *Ulva*

The factors controlling the abundance of *Ulva* are very diverse. One of the most obvious factors is the availability of N and P, which favours massive proliferation of the typical macroalgae of eutrophic zones. Other elements apart from N and P may be important, e.g. inorganic C (Frost-Christensen and Sand-Jensen, 1990; Levavasseur et al., 1991; Rivers and Peckol, 1995; Menéndez et al., 2001) and trace metals (Zhang, 2000) may also be limiting for algal growth. Additional factors that may affect growth are physical in nature, such as temperature (Sfriso et al., 1992; Hernández et al., 1997; De Casabianca and Posada, 1998) and light (Piriou and Ménesguen, 1992). Hydrodynamic conditions may favour the accumulation of macroalgae (Piriou and Ménesguen, 1992; Salomonsen et al., 1999; Martins et al., 2001) and the presence of a suitable substrate for attachment is also a relevant factor (Schories and Reise, 1993; Nedwell et al., 2002). Biotic factors such as interspecific competition or grazing may be important in green macroalgae, which are usually highly palatable (Einav et al., 1995; Valiela et al., 1997; Lotze et al., 1999; Ruiz, 1999; Lotze and Schramm, 2000; Kamermans et al., 2002).

The complex interaction of these factors means that different patterns of temporal abundance of green macroalgae have been described in different studies. In general, highest biomasses have been reported to occur in spring or spring–summer (Sfriso et al., 1992; Jeffrey et al., 1995; Flindt et al., 1997; De Casabianca and Posada, 1998). In the present study, separate analysis of each sampling site did not reveal a common pattern in the abundance of *Ulva*, and varying trends were found at the different sites. At some sites the highest abundances occurred in summer or summer–autumn, as at M4 or A4, whereas at sites, such as A6 and A8, the opposite was true, and at sites such as P5 there were high biomass levels throughout the whole year, with little monthly variation. Maximum mean values were found in spring and summer and minimum mean values in winter (Fig. 5). The spring peak may have been due to the increase in temperature and incident radiation at a time when the levels of nutrients in the water were probably still high. The accumulation decreased slightly during the summer, possibly because of a reduction in the bioavailability of nutrients in the water and the depletion of tissue reserves. The second peak at the end of the summer may have been maintained by regeneration of nutrients following the summer decline at a time when the temperature and light intensity were both still high. Piriou and Ménesguen (1992) found a similar trend in seasonal changes in *Ulva*, as estimated by a numerical model. The phytoplankton biomass in the Ría de Vigo follows a similar pattern, with a peak in May and another in September (Nogueira et al. 1997),

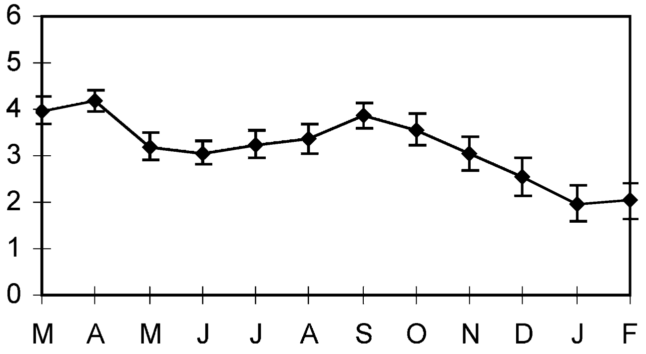

Fig. 5. Average monthly variations in the abundance of *Ulva* (estimated visually). Arbitrary relative scale. Error bars represent the standard error.

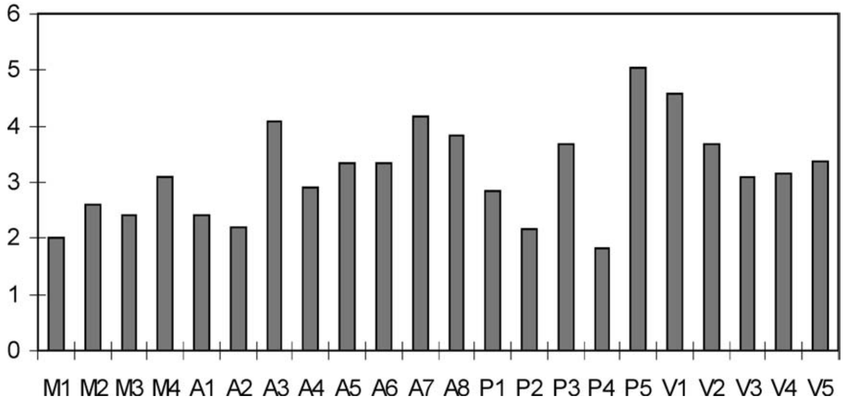

Fig. 6. Average annual abundances of *Ulva* at each sampling site. Arbitrary relative scale.

which appears to indicate that the optimum conditions for growth of phytoplankton and of *Ulva* in the study area are similar.

The average abundances of *Ulva* at each site are shown in Fig. 6; the sites with the highest abundances were P5, V1, A7 and A3, whereas the lowest abundances were at sites P4, M1, P2 and A2. To find if the nutrient contents in *Ulva* could explain this distribution, correlation analysis of the annual average nutrient contents in this algae and the average abundances was carried out. The results of the sediment analysis, granulometric data, were used as an indicator of the degree of hydrodynamic exposure. The correlations obtained (Table 5) show that there was a total lack of linear relationship between the abundance of *Ulva* and tissue nutrients. Within the granulometric data the coefficients of correlation for the coarse silt and the fine silt were significant and those corresponding to the clay fraction were almost significant. The coefficients of correlation corresponding to the <63 μm fraction (coarse silt, fine silt and clays) and to the <20 μm fraction (fine silt and clays) were also significant, whereas those corresponding to the coarse fractions (from fine sand to gravel) were very low.

These results show that the accumulations of *Ulva* appear to depend greatly on hydrodynamic factors, so that they are more abundant in areas with restricted circulation where the deposition of fine sediments is favoured. Obviously the macroalgae must also have sufficient nutrients to be able to proliferate, however, dilution of these elements in shoots during periods of rapid growth and the lack of storage of nutrients at these times mean that significant correlations between nutrient levels and algal abundance are not found. Jeffrey et al. (1995) and De Casabianca and Posada (1998) found minimum levels of N and P in macroalgae during the months when the biomass present in the study areas was maximal.

4. Conclusions

The levels of C found in the tissues of *Ulva* and *Enteromorpha* were generally higher than those reported by other authors for macroalgae of the same genera, whereas the levels of P were relatively low, and N was the only one of the nutrients found at levels consistent with those reported in the literature for temperate regions; the C:N:P ratios were therefore very high. These results indicate possible limitation by P. Temporal variations in the concentrations of N and P appeared to follow the typical trend found in macroalgae in temperate zones, with minimum levels in the summer and maximum levels in winter; this seasonal pattern was most evident for N. The changes in the tissue concentrations of these nutrients in *Ulva* and *Enteromorpha* were similar, whereas tissue levels of C followed a different pattern. Comparison of the levels of N and P in tissues with critical concentrations showed *Ulva* to be limited by N and P in summer; these nutrients did not

Table 5
Spearman's coefficients of rank correlation for abundance of *Ulva*, nutrients in *Ulva* and sediment granulometry (average of two samples)

	N *Ulva*	P *Ulva*	Gravel	Coarse sand	Medium sand	Fine sand
Abundance of *Ulva*	0.0656 n.s.	0.3324 n.s.	0.1436 n.s.	0.1063 n.s.	−0.0520 n.s.	0.1249 n.s.
	Coarse silt	Fine silt	Clay	% <63 μm	% <20 μm	
Abundance of *Ulva*	0.4251*	0.5221*	0.3944 n.s.	0.5427**	0.5351*	

Gravel (>2 mm), coarse sand (2–0.5 mm), medium sand (0.5–0.2 mm), fine sand (0.2–0.063 mm), coarse silt (0.063–0.020 mm), fine silt (0.020–0.002 mm), clays (<0.002 mm); $n = 22$. *Significant at $P < 0.05$, **significant at $P < 0.01$, n.s. = not significant.

appear to be limiting in autumn and winter, in spring the situation was intermediate. This is not consistent with the conclusions that may be drawn from the low concentrations of tissue P; it is possible that the close metabolic relationships between the different nutrients may have caused dual nutrient limitation.

Analysis of the C:N:P ratios in the sediments showed that these were relatively more enriched with P than with C and N, in contrast with the findings for *Ulva* and *Enteromorpha*. It may be that retention of P in the upper, oxidised layer produced low concentrations of phosphate in solution in the intertidal zone, which in turn produced low levels of P in algae.

The highest accumulations of *Ulva* were found in spring and summer. Hydrodynamic factors appeared to have an important influence on the accumulations of this alga, as this seaweed was abundant in areas with restricted water circulation, where deposition of fine sediments was favoured.

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