

Seasonal variations in fatty acid composition of *Caulerpa taxifolia* (M. Vahl.) C. Ag. in the northern Adriatic Sea (Malinska, Croatia)

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Abstract

Fatty acid composition was monitored in *Caulerpa taxifolia* collected from January to December 1998 in the northern Adriatic Sea. Saturated (45.2–73.7%), monounsaturated (10.3–25.9%) and polyunsaturated (16–34.3%) fatty acid proportions varied considerably during the period investigated. The *Caulerpa* growth cycle proceeded through a) minor changes during the latent and growth recruitment phase in spring, b) a gradual increase in unsaturated fatty acids during maximum growth in the summer/early autumn period and c) an abrupt increase, particularly of polyunsaturated fatty acids, during biomass maintenance and survival as lowering temperatures approached the lethal level. These variations were similar to those found in native algal species of temperate regions. Stimulation of growth and spreading can be partly explained by successful adaptation of *C. taxifolia* to the seasonality of environmental parameters (primarily temperature).

Keywords: *Caulerpa taxifolia*; fatty acids; northern Adriatic Sea.

Introduction

Fatty acid composition has been used for the chemotaxonomic classification of *Caulerpa* species (Shameel 1990, Shameel and Khan 1991, Aknin et al. 1992, Khotimchenko 1995, Aliya and Shameel 1998). However, very few studies have examined seasonal changes in fatty acids in algae (Kim et al. 1996, Nelson et al. 2002) and none that we are aware of for *Caulerpa taxifolia* (M. Vahl.) C. Ag. The significance of algal lipids lies, *inter alia*, in their participation in biological membranes. Their metabolic behavior is largely determined by the structure of the component fatty acids (Cohen et al. 1988, Kim et al. 1996). The changes in the degree of unsaturation of membrane fatty acids affect the maintenance of membrane functions and its resistance to cold stress (Terrados and Lopezjimenez 1996).

The strain of *Caulerpa taxifolia* that has colonized the Mediterranean Sea has some unusual morphological and physiological characteristics in comparison with tropical

populations. Among these are longer fronds, a higher population density, adaptation to a large spectrum of temperatures and light, and higher concentrations of toxic metabolites (Boudouresque et al. 1995, Gayol et al. 1995, Meinesz et al. 1995, Komatsu et al. 1997, Chisholm et al. 2000). It can form meadows of exceptional densities, whereas in its native tropical seas the alga is usually sparse, grows in small patches and does not cause problems (Meinesz and Hesse 1991). *Caulerpa* is difficult to control and, in the Mediterranean Sea, including the Adriatic Sea, its spreading is causing a "major ecological event" (Boudouresque et al. 1995, Žuljević et al. 1998). It invades beds of the dominant seagrass *Posidonia oceanica* (L.) Delile and those of various native seaweeds, repressing and more or less totally replacing them (Villèle and Verlaque 1995, Dumay et al. 2002). In the Adriatic Sea, *Caulerpa* meadows grow seasonally, with population expansion into new areas of habitat (Iveša 2001, Zavodnik et al. 2001, Žuljević 2001).

In this study, the seasonal changes in fatty acid composition in *Caulerpa taxifolia* thalli were examined and related to different stages of the vegetative cycle. These modifications are important because of their relationship to the maintenance of algal cell function and to the ecology of this invasive species in temperate regions.

Materials and methods

Sampling of the green alga *Caulerpa taxifolia* was performed monthly from January to December 1998 in the northern Adriatic Sea at Malinska, on the coast of the island Krk, Croatia (45°7'30"N, 14°31'56"E), the most northerly and coldest boundary position reported thus far (Figure 1). *C. taxifolia* was collected manually within quadrat frames at a depth of 8 m by SCUBA divers. At each sampling, three plots of 25×25 cm were harvested. In the laboratory, algal wet weight, frond length and frond number were measured. Two subsamples of *C. taxifolia* thalli were extracted in Soxhlet apparatus (Witeg, Wertheim, Germany) in CHCl₃-MeOH (1:1). The chloroform phases were purified by adding a salt solution and then evaporated to dryness using rotary evaporation at 30°C (De Rosa et al. 1988). After weighing, the total extracts were saponified following the addition of 1.2 M NaOH in 50% aqueous methanol solution. The tubes were placed in a boiling water bath for 30 min. After cooling, the saponificate was acidified with 6 M HCl (pH < 2), and 12% BF₃ in methanol was added and heated for 10 min in a boiling water bath. After cooling, the fatty acid methyl esters (FAME) were extracted in CH₂Cl₂.

FAME were analyzed by gas-liquid chromatography (GLC) on a 6890N Network GC System (Wilmington, DE,

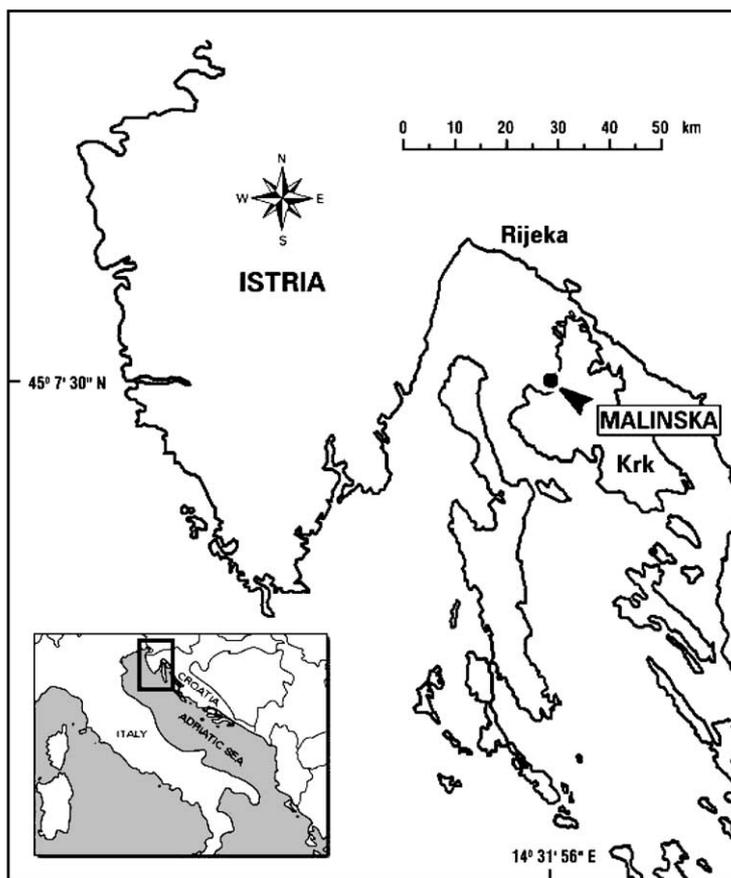


Figure 1 *Caulerpa taxifolia*: sampling location in the northern Adriatic Sea.

USA) equipped with a 5973 Network Mass Selective Detector (Wilmington, DE, USA) with a capillary column (25 m×0.3 mm×0.25 μm; cross linked 5% phenylmethylsiloxane) and ultra high purity helium as the carrier gas. The GLC settings were as follows: programmed column temperature 145°C by 4°C/min up to 270°C and constant column pressure 2.17 kPa. Retention times, peak areas and mass spectra were recorded on ChemStation (Palo Alto, CA, USA) software. FAME in seaweed samples were identified by mass spectral data and family plot of equivalent chain length data (ECL) for GC standards for the GC column used. Fatty acid methyl esters mix C18-C20 and polyunsaturated fatty acids standards (PUFA1 and PUFA3; Supelco, Bellefonte, PA, USA), cod liver oil and various individual pure standards of fatty acid methyl esters (Sigma, Steinheim, Germany) were used.

Cluster analysis was applied to the data. The analysis is based on the correlation coefficients matrix of each variable (fatty acid proportions) with every other. The samples are successively fused into groups, starting with samples with the highest mutual correlation coefficients and F-ratios, then gradually lowering the similarity level at which groups are fused and ending in a single cluster. The process is represented by a tree diagram or dendrogram (joining method). The most similar groups to those found by the joining procedure were identified by K-means method and represented by their ranges, means and standard deviations. The statistical data analysis was performed with PC Systat™ 10 (Richmond, CA, USA).

Results

The percent distributions of dominant components among the fatty acids isolated and identified from *Caulerpa taxifolia* were seasonally stable. Saturates represented the major fraction of the total (45.2–73.6%), and were always dominated by hexadecanoic acid (32.8–54.9%) and, to a lesser extent, by tetradecanoic (5.0–11.5%), tetracosanoic (2.1–5.6%); octadecanoic (0.9–2.8%) and docosanoic (0.5–1.1%) acids. Monoenes were mostly represented by hexadecenoic 16:1(n-7) acid. Octadecenoic, 18:1(n-9), docosenoic 22:1 and tetracosenoic 24:1 acids were present at a lower level but with a similar pattern of variation. Dienes and trienes were dominated by 18:2(n-6), 16:2(n-6), 18:3(n-3) and 16:3(n-3) acids. In all samples, two unusual diunsaturated fatty acids 17:2 and 19:2 (ECL values of 16.55 and 18.6, respectively) were detected. Polyunsaturated acids with more than 3 double bonds were mostly represented by 20:5(n-3), 16:4(n-3), and 18:4(n-3).

Seasonal changes were most evident in the relative distribution of saturates (SFA), mono- (MUFA) and polyunsaturated fatty acids (PUFA). Saturates gradually increased from February, attaining maximum values in July, then decreased to December, while monounsaturated acids varied seasonally in a reverse pattern. Seasonal changes in polyunsaturated fatty acids tracked those of monounsaturates (Figure 2). Over the year, a consistent dominance of 16 MUFA and 18 PUFA was found. Total lipids and the unsaturated/saturated (UNS/

SAT) ratio showed the converse behavior from January to May when the maximum in total lipids was measured. Later, the behavior of UNS/SAT ratio was similar to total lipids (Figure 2). Generally, the UNS/SAT ratio varied inversely with temperature, with the maximum values during late autumn/winter months.

To better understand the seasonal cycle in fatty acid characteristics, cluster analysis-complete linkage (Figure 3) and K-means (Table 1) methods analysis were performed. Three groups of samples with similar characteristics were identified. Group 1 included summer and early autumn (July–October 1998) samples, with the highest mean values and ranges for SFA (61.0–73.7; 66.3) and the lowest for MUFA (10.3–15.3; 12.9), PUFA (16.0–24.4; 20.8) and consequently the lowest UNS/SAT ratios (0.4–0.6; 0.5). The highest PUFA proportions (26.7–34.3; 30.5) and UNS/SAT ratios (1–1.2; 1.1) characterized Group 3, i.e., the samples collected in the late autumn and winter period (November–February). In addition, the proportions of 16 (8–8.8; 8.5) and 18 (10.5–16.3; 13.7) PUFA were higher than those obtained for the other two groups (samples collected during the March–October period). The proportions of 16 and 18 PUFA for Groups 1 and 2 were essentially the same, while proportions of MUFA clearly differed among three sample groups (Table 1).

Annual changes in total biomass (g wet wt/m²), density (number of fronds/m²) and frond length (mm) of *Caulerpa taxifolia*, are presented in Figure 4. All of these parameters display a similar pattern of variation, with minima in May, corresponding to the latent (covering) phase. The growth and vegetative reproduction phase began when the water temperature started to increase (May–June). In June *C. taxifolia* attained a constant number of fronds/m². In the period of rapid water cooling, increases in algal biomass were due to the lengthening of the fronds. In the March–May period, fronds disappeared rapidly.

Discussion

The fatty acid composition of *Caulerpa taxifolia* collected in the northern Adriatic sea shows a pattern of dominating components C16:0, C16:1(n-7) and C18:3(n-3) among saturated, monounsaturated and polyunsaturated fatty acids, respectively. Furthermore, some specific features taxonomically important for *Caulerpa* species, e.g., α -linolenic acid, 18:3(n-3), as the dominant C18 PUFA and 16:3(n-3) as the dominant C16 PUFA associated with low 18:4(n-3) and 16:4(n-3) contents, were revealed. These results are in agreement with previous reports on *C. taxifolia* (Aknin et al. 1992) and similar to fatty acid compositions reported for other *Caulerpa* species (Khotimchenko 1995). Additionally, we determined the presence of longer chain saturated fatty acids C22:0 and C24:0 together with their monounsaturated homologs, 22:1 and 24:1, including rare diunsaturated fatty acids C17:2 and C19:2. C17:2 reported for *C. veravalensis* Thivy et Chauhan (Shameel and Khan 1991) and *Halymenia porphyroides* Børg. (Shameel 1990) from the Karachi coast.

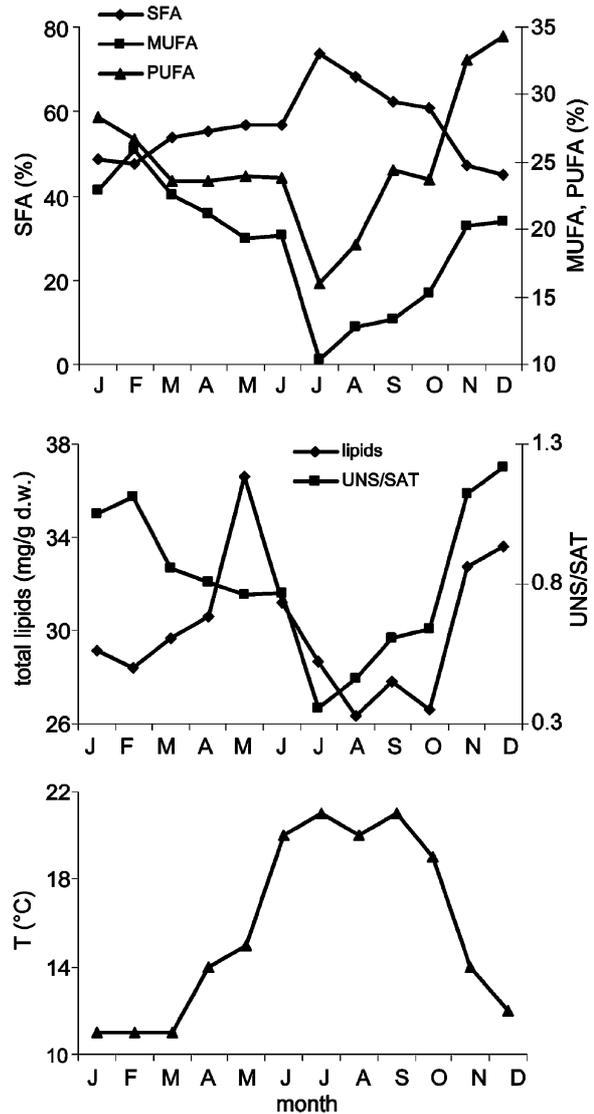


Figure 2 *Caulerpa taxifolia*: annual variations in the saturated (SFA), unsaturated (MUFA, PUFA) fatty acids, expressed as % total fatty acids, total lipids and unsaturated versus saturated fatty acids ratio (UNS/SAT) and seawater temperature.

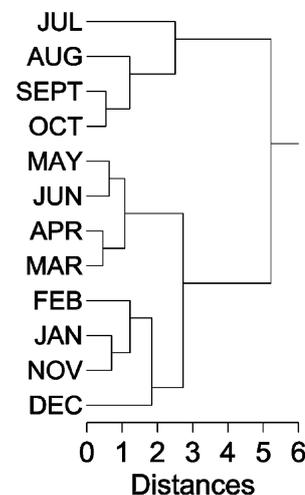


Figure 3 *Caulerpa taxifolia*: cluster analysis dendrogram of fatty acid composition during 1998.

Table 1 *Caulerpa taxifolia*: fatty acid composition in three groups of samples identified using K-means.

Fatty acid	Group 1				Group 2				Group 3			
	min	mean	max	SD	min	mean	max	SD	min	mean	max	SD
C14:0	9.7	10.7	11.5	0.8	10.3	10.7	11.2	0.4	5.0	7.9	9.6	2.0
C16:0	44.0	48.1	54.9	4.9	37.7	39.7	41.7	1.8	32.8	33.7	34.8	0.9
C18:0	1.4	1.9	2.8	0.6	1.7	2.0	2.3	0.3	0.9	1.4	1.9	0.4
C22:0	0.8	0.9	1.1	0.1	0.5	0.9	1.1	0.3	0.6	0.9	1.2	0.2
C24:0	4.0	4.7	5.6	0.8	2.2	2.3	2.4	0.1	2.1	3.1	4.9	1.3
C16:1(n-7)	6.3	7.2	8.2	0.8	10.1	11.4	12.7	1.1	10.3	12.5	15.3	2.1
C18:1(n-9)	1.9	2.1	2.3	0.2	3.3	3.5	3.9	0.3	2.2	3.0	3.5	0.6
C20:1	0.0	0.0	0.0	0.0	0.4	0.5	0.6	0.1	0.0	0.4	0.6	0.3
C22:1	0.6	1.3	1.9	0.6	1.5	2.3	3.0	0.7	2.5	2.7	2.9	0.2
C24:1	1.5	2.4	2.9	0.6	2.7	3.0	3.2	0.2	2.4	3.5	5.3	1.3
C16:2	0.6	1.0	1.5	0.5	0.6	0.8	1.0	0.2	1.3	1.8	2.2	0.4
C16:3	1.8	3.2	4.4	1.2	1.6	2.8	3.4	0.8	5.3	5.7	6.2	0.4
C16:4	0.0	0.0	0.0	0.0	1.2	1.3	1.4	0.1	0.5	1.0	1.4	0.4
C17:2	1.3	2.4	3.3	0.8	2.7	3.4	4.0	1.0	1.5	2.1	2.7	0.4
C18:3(n-6)	0.0	0.2	0.5	0.3	0.0	0.1	0.3	0.1	0.4	0.5	0.7	0.2
C18:4(n-3)	0.0	0.4	0.7	0.4	0.7	0.7	0.8	0.0	1.1	1.5	1.7	0.3
C18:2(n-6)	2.2	2.6	2.9	0.3	2.4	2.8	3.3	0.4	2.4	3.4	3.8	0.7
C18:3(n-3)	4.4	5.9	7.0	1.1	5.2	5.8	6.4	0.5	6.6	8.3	10.2	1.6
C19:2	1.0	1.3	1.6	0.3	1.0	1.2	1.3	0.1	1.3	1.9	2.1	0.4
C20:5(n-3)	0.0	0.9	1.8	1.0	0.4	1.0	1.9	0.8	1.5	2.3	3.0	0.8
C20:2	0.0	0.2	0.8	0.4	0.3	0.5	0.7	0.2	0.2	0.5	0.9	0.3
SFA	61.0	66.3	73.7	5.9	53.8	55.7	56.8	1.4	45.2	47.1	48.8	1.5
MUFA	10.3	12.9	15.3	2.1	19.4	20.7	22.6	1.5	20.3	22.4	25.9	2.6
PUFA	16.0	20.8	24.4	4.0	23.5	23.7	23.9	0.2	26.7	30.5	34.3	3.6
UNS/SAT	0.4	0.5	0.6	0.1	0.8	0.8	0.9	0.0	1.1	1.1	1.2	0.1

Group 1: July–October; Group 2: March–June; Group 3: November–February assigned with dendrogram (Figure 3). SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, UNS/SAT: unsaturated/saturated fatty acids ratio.

Seasonal changes in fatty acid composition are usually related to the combined influence of environmental factors such as temperature, light or nutrient availability, and to physiological state. Sorting out fatty acid profiles representative of each of these influences is made difficult by many co-variations involved. Cluster analysis helps to resolve some of this complexity and leads to the definition of groups of samples with similar behavior from various periods. Hierarchical clustering clearly separated the periods of summer/autumn (Group 1) from winter/spring (Group 2 and 3), corresponding to variations observed in the seasonal growth of *Caulerpa taxifolia* in our study. These included maximum growth values in summer/autumn and minimum values during the winter/spring period. Such a growing cycle appears to be common for this species within the Mediterranean Sea (Belsher et al. 1994, Dumay et al. 2002). In the period from July to October (Group 1), when the optimal temperature conditions for growth (19°C–21°C) were attained, *C. taxifolia* grew rapidly producing new stolons, and the length of the fronds increased gradually. The density attained in June did not change notably until October. The healthy growth of fronds and stolons was associated with decreasing total lipids, and increases in poly- and monounsaturated fatty acids with consequent decreases in saturated fatty acids. A quite similar seasonal pattern (summer → autumn) has been observed in some algae belonging to the Phaeophyta, Rhodophyta and Chlorophyta in the northeastern Pacific Ocean (Nelson et al. 2002). However, the simultaneous increase in density of newly developed stolons and fronds and their lengths, between September and October, correlated with a decrease in total lipids and with a slight increase in unsaturation.

The fatty acid composition during the period of minimum growth can be further separated into the late autumn/winter (Group 3) and the spring periods (Group 2). Both periods are characterized by biomass maintenance (November, December) or biomass loss caused by frond degeneration (January–May). As previously described (Komatsu et al. 1997), during winter algal growth was retarded when the water temperature decreased below 15°C. In the late autumn from October to November, data indicated a progressive trend toward highest total lipids as well as the proportions of both mono- and polyunsaturated fatty acids. Rapid desaturation of increasing lipids could be attributed primarily to a sharp and continuous decrease in water temperature during the winter months. In general, increases in the level of polyunsaturated as well as monounsaturated fatty acids are considered to provide a mechanism for the thermo-adaptive regulation of membrane lipid fluidity (Pohl and Zurheide 1979). This conclusion is also in agreement with results for *C. prolifera* in Mar Menor (Terrados and Lopezjimenez 1996) and it suggests that, in the winter months, increased levels, particularly of polyunsaturated fatty acids, are needed for survival and acclimatization of *C. taxifolia* at low temperatures that approach the lower lethal level.

Although during the spring period (March–June) *Caulerpa taxifolia* thalli faced significant changes in environmental parameters, the response in fatty acid composition of this group is the most consistent and invariant. During the greatest biomass loss, caused by frond degeneration, *C. taxifolia* entered the covering latent phase with a maximum for total lipids in May. Conversely, after the unsaturated fatty acids decreased and

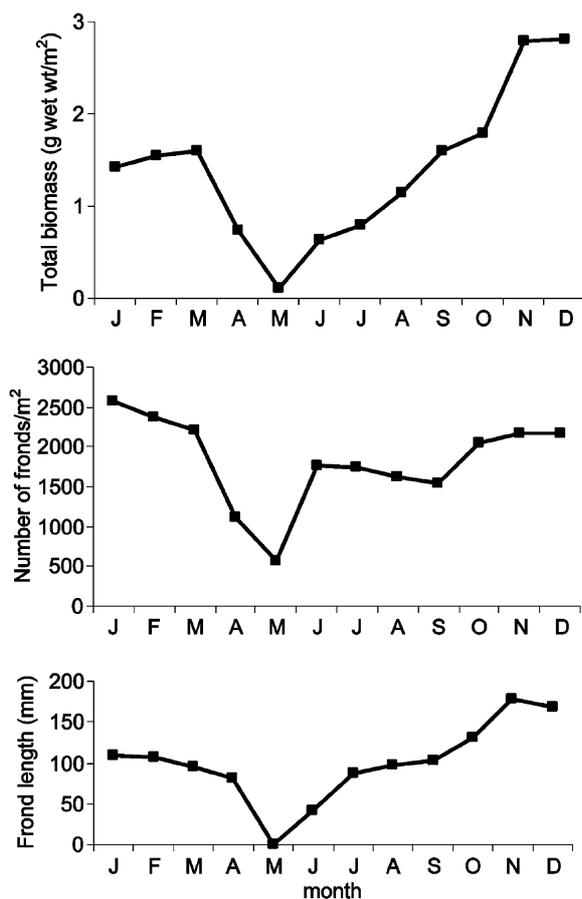


Figure 4 *Caulerpa taxifolia*: annual variations in total biomass (g wet wt/m²), density (fronds/m²) and frond length (mm).

saturated acids increased from February to March, the relative proportions of the three main components remain more or less constant until June. Finally, from May to June, total biomass increased with the appearance of new fronds and stolons (correlated with favorable temperature increase from 15°C to 20°C), while total lipids decreased. Such a growth pattern is observed in some other rapidly growing plants that usually contain high levels of total lipids in their young, recruiting phase, gradually losing them during biomass increase (Nelson et al. 2002). Therefore, the constant profiles in saturated and polyunsaturated fatty acids might have resulted from the overlapping effects of temperature increases and changes in growth of *C. taxifolia*. The effect of temperature on the fatty acid composition alone should cause the saturation. Conversely, the growth of *C. taxifolia* should cause desaturation, as was observed in this study during rapid growth from July to October. However, the combined influence of water temperature increases from May to June and the most rapid appearance of new fronds and stolons resulted in minor changes in fatty acid composition.

The results demonstrated that the physiological condition of *Caulerpa taxifolia* during the annual growth cycle is reflected in the variation of the fatty acid composition. The annual changes observed proceeded through a) minor changes during the latent and growth recruitment phase in the spring, b) a gradual increase in unsaturated fatty acids during maximum growth in the summer/early autumn period and c) an abrupt increase, particularly of

polyunsaturated fatty acids during maintenance and survival in the coldest months.

The seasonal variations in the fatty acid composition of *Caulerpa taxifolia* in the northern Adriatic Sea were similar to those found in native algal species of temperate regions (Kim et al. 1996, Nelson et al. 2002). Due to these adaptive mechanisms, seasonal variations of environmental parameters (primarily temperature) do not present a limiting factor. On the contrary, these changes, particularly in the recruitment phase, have a significant impact on growth stimulating the spreading of *C. taxifolia*.

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