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Seasonal rubisco enzyme activities and caulerpenyne levels in invasive *Caulerpa racemosa* var. *cylindracea* and native *Caulerpa prolifera*

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Abstract

Caulerpa racemosa var.*cylindracea* (*C.racemosa*) is an invasive marine seaweed in the Mediterranean Sea. Since no valid eradication method has been existed in the scientific literature on this species, it has currently been continuing its invasion along the coastlines of 13 Mediterranean countries. One of the important factors responsible for its invasion is thought to be its toxic secondary metabolite, i. e. caulerpenyne (CYN). The present paper investigates seasonal changes in the secondary metabolite CYN and rubisco enzyme (EC 4.1.1.39) activities of invasive *C. racemosa* and native *C. prolifera*. Inasmuch as no correlation between CYN level and rubisco enzymic activity was observed in these species, it is considered that the regulation of CYN synthesis and rubisco enzymic activity might be controlled independently. In conclusion, the further analysis on the rubisco enzymic activity determinations with methyl-erythritol-4-phosphate and mevalonate pathways considered to be responsible for CYN bio-synthesis in invasive and native *Caulerpa* species in the Mediterranean Sea and these subjects should be studied in great detail to get the overall picture.

Keywords: *Caulerpa racemosa* var. *cylindracea*, *Caulerpa prolifera*, caulerpenyne, invasive species, Rubisco enzyme.

Introduction

Caulerpa racemosa var. *cylindracea* (Sonder) Verlaque, Huisman, et Boudouresque (hereafter *C. racemosa*) is one of the well-known, well-spread and well-studied invasive species in the Mediterranean Sea (Klein&Verlaque, 2008). So far, the invasive *C. racemosa* has been observed in 13 Mediterranean countries including Albania, Croatia, Cyprus, France, Greece, Italy, Libya, Malta, Spain, Tunisia and Turkey, Monaco (M. Verlaque, pers. commun.) and Algeria, and also large islands (Balearic Islands, Corsica, Crete, Cyprus, Sicily), the Atlantic (Canary Islands) (Verlaque *et al.,* 2003; Verlaque *et al.*, 2004; Ould-Ahmed& Meinesz, 2007; Klein&Verlaque, 2008). Since no valid eradication method has been published on this species, *C. racemosa* has been continuing its invasion in the Mediterranean Sea. Its effects on the sublittoral ecosystem of the Mediterranean Sea have been mentioned in many reports (Terlizzi *et al.,* 2011; Katsanevakis et al, 2011; Box et al, 2010; Deudero *et al,* 2010; Klein&Verlaque, 2008). *Caulerpa prolifera* (Forsskål) J.V. Lamouroux (hereafter *C. prolifera*) is a native species of *Caulerpa* genus in the Mediterranean Sea and it is very common along Turkish coastlines. Members of the genus *Caulerpa* have special secondary metabolites for defensive purposes. One of the important secondary metabolites in *Caulerpales* is a sesquiterpenoid caulerpenyne (CYN) (Cavas & Pohnert, 2010). The structure of the CYN isolated from the *C. prolifera* was firstly identified by Amico *et al.* (1978). The importance of the CYN in biochemical and pharmacological research areas has been evaluated in several published papers (Mayer *et al.*, 1993; Fischel *et al.*, 1995; Bitou *et al.*, 1999; Nicoletti *et al.*, 1999; Barbier *et al.*, 2001; Cavas *et al.*, 2006; Cavas & Pohnert, 2010). There are a few numbers of published papers in the literature where caulerpenyne levels were compared among *Caulerpa* spp (Paul & Fenical, 1986; Meyer&Paul, 1992; Gavagnin *et al.*, 1994; Amade&Lemée, 1998; Jung *et al.*, 2002; Box *et al.*, 2010a,b). The common point of the literatures mentioned above is that CYN is the main or one of the main metabolites of *Caulerpa* species (Jung *et al.*, 2002). Meyer and Paul (1992) found that 0.2-0.5 % of dry weight of *C. racemosa* collected from Guam consisted of CYN. The CYN content was found as 1.9 % and 0.4 % of the algal dry weight of *C. taxifolia* which was collected in January and March, respectively (Amade& Lemée, 1998). Jung *et al.* (2002) modified Amade and

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Lemée's method. They froze the algae with liquid nitrogen before extraction and reported that the yield of modified method was two-fold higher than that of Amade and Lemée's method. According to a very recent paper, the CYN levels in native *C. prolifera* were higher than those of invasive *Caulerpa* members, the *C. taxifolia* and the *C. racemosa* (Box *et al.* , 2010a). All green members of the Kingdom Plantae have an important enzyme called ribulose biphosphate carboxylase/oxygenase (rubisco) for dark reactions of photosynthesis. It is the half of the proteins in the plant chloroplasts. Carboxylase activity of rubisco catalyses the reaction of adding carbondioxide to ribulose-1,5-bisphosphate and then cleavages the product (β-keto acid intermediate) into two 3-phosphoglicerate (3PG) (Pratt & Cornelly, 2011). So far, there has only been one published report on the bio-synthesis of the CYN in the Caulerpales in the scientific literature (Pohnert & Jung, 2003). In the exclusive report of Pohnert & Jung (2003), they reported that backbone of sesquiterpene (CYN) is synthesised in chloroplast via methyl-erythritol-4-phosphate (MEP) pathway and then it is acetylated with cytoplasm-derived acetyl residues based on a labelling study. Since rubisco plays vital roles in the fixation of atmospheric carbondioxide, and also has responsibility for many carbon based biomolecules in the plant physiology, we did want to investigate if there might be a relationship between the seasonal CYN levels and rubisco enzymic activities in the invasive *C. racemosa* and native *C. prolifera*. To the best of our knowledge, this is the first report on the relationship between CYN contents and the rubisco enzymic activities of the *C. racemosa* and the *C. prolifera* from the Turkish coastlines.

Materials and Methods

Collection of algae species

C. racemosa and *C. prolifera* were collected from Çesme coastline (İzmir-Turkey) in October 2009, January 2010, April 2010 and June 2010. The sampling depth was -1 meter for both species. The geographical coordinates of the sampling site for *C. racemosa* and *C. prolifera* were 38^0 19' 36.89''N-26^0 17' 55.78''E and 38^0 20' 40.20''N-26^0 23' 17.48''E, respectively. The algae were transported to the laboratory immediately and they were washed with tap water and then with distilled water to remove salts and epiphytes. The samples for enzyme activity and protein determinations were stored at -20°C until their use. Since the CYN is degraded very fast because of esterase enzymic activity (Jung *et al.*, 2002), the CYN determinations were done immediately after washing protocol on the same day of sampling. Liquid nitrogen was used to prevent esterase-based decomposition. The data on average air and seawater temperature and sunshine duration were provided from local state meteorological station in Çeşme.

Isolation of CYN from C. prolifera

In order to obtain the standard CYN, the purification protocol described by Jung *et al.* (2002) was conducted. The enzymatic degradation of CYN into its derivatives was prevented by extracting the algae with ethyl acetate under liquid nitrogen. The mixture was centrifuged at 4000 rpm for 1-2 minutes at 25 °C and the supernatant was evaporated in vacuum. The crude product was purified from two silica gel column using the mixture of petroleum ether/ethyl acetate (7/3, v/v) and petroleum ether/diethyl ether (6/4, v/v) as eluents, respectively. Reverse phase HPLC and ^{1}H NMR spectroscopic analyses were conducted in order to check the purity of the CYN (Cengiz *et al.*, 2009). The NMR data were identical with those reported in the literature (Amico *et al.* , 1978).

CYN determination in Caulerpa species

The CYN concentration of *Caulerpa* species were identified according to procedure described by Box *et al.* (2008). For this analysis, 1 g fresh algae was extracted with 5 mL of methanol in cold conditions. The homogenate was centrifuged at 10000 rpm for 5 minutes at 4°C. The crude extracts were filtered on SepPack columns (Waters, WAT020515), then the bounded CYN on the columns was eluted with a mixture of methanol/ethyl acetate (50/50, v/v). The estimation of CYN concentration in fresh materials was done through HPLC method depended on a calibration curve which was plotted with the known concentration of CYN standards was used. Teknokroma C18 150 x 4 mm HPLC column was used in the analyses. Mobile phase was set as water/methanol (20/80, v/v) mixture with 0.4 $mL.min^{-1}$ flow rate.

Preparation of algal samples for rubisco enzyme activities

1 g of algal material was ground in a pre-cooled mortar. 0.1 g of the ground algae was placed into each eppendorf tube. 1 mL of extraction buffer containing 0.1 M Tris-HCl, 2 mM EDTA, 10 mM $MgCl_2$, % 20 glycerol, % 10 PVPP, % 1 Tritone X-100, 50 mM DTT, 10 mM sodium ascorbate, 10 mM $NaHCO_3$, at pH 7.6 (Gerard&Driscoll, 1996) were added into each tube. The homogenates were centrifuged at 10000 rpm for 5 minutes at 4 °C and the supernatant was stored at 4 °C until used.

Protein determination

Protein concentration in the supernatant was measured according to the method of Bradford (1976). Bovine serum albumin (BSA, Sigma) was used to obtain the standard curve of protein contents.

Determination of rubisco enzyme activity

Rubisco enzyme activity was determined according to the procedure described by Gerard & Driscoll (1996).

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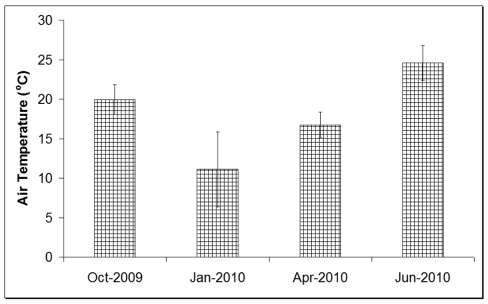


Fig. 1: Seasonal variations of the air temperatures in the sampling site. The values are the mean of the daily temperatures for each month. Error bars show the standard deviation.

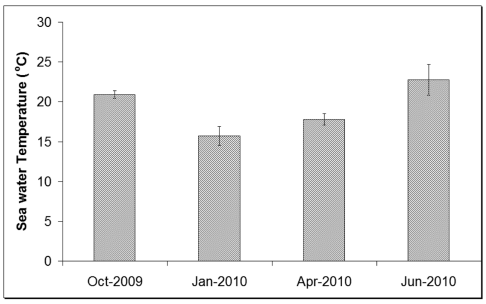


Fig. 2: Seasonal variations of the seawater temperatures in the sampling site. The values are the mean of the temperatures of the days of the months. Error bars show the standard deviation.

Reaction mixture consisted of 50 mM HEPES, 10 mM $NaHCO_3$, 20 mM $MgCl_2$, 0.2 mM NADH, 5 mM ATP, 5 mM phosphocreatine, 5 units of creatine phosphokinase, 5 units of gliceraldehyde-3-phosphate dehydrogenase and 1 unit of phosphoglycerate kinase at pH 7.8. The rate of the reaction was observed by the decrease in absorbance at 340 nm against air (Gerard & Driscoll, 1996; Bischof *et al.*, 2002).

Statistical Analysis

Minitab 16.0 statistical package was used to evaluate the experimental data statistically. One-way ANOVA test followed by Tukey's test was selected for multiple comparisons. The statistical significance was set at $p<0.05$. The pearson correlation test was also applied to the data for possible relations among values.

Results

The average air and seawater temperatures and sunshine duration in sampling months were depicted in Figures 1-3. The highest air-seawater temperatures and sunshine duration were found as 24.62±2.20 °C, 22.76±1.95 °C and 10.85± 2.74 hours in June 2010, respectively. The lowest values were observed in January 2010. Statistical comparison of these data was given in Table 1a-c.

The seasonal variations of the CYN contents in *C. racemosa* and *C. prolifera* were presented in Figure 4. The CYN contents in *C. racemosa* were calculated as 0.200±0.003 mg g^{-1} fresh algae, 0.202±0.007 mg g^{-1} fresh algae, 0.028±0.005 mg g^{-1} fresh algae and 0.307±0.171 mg g^{-1} fresh algae for October 2009, January 2010, April 2010 and June 2010, respectively. The seasonal CYN variations in *C. prolifera* were determined as 0.608±0.405 mg g^{-1} fresh algae, 0.372±0.071 mg g^{-1} fresh algae, 1.523±0.552 mg g^{-1} fresh algae and 0.825±0.535 mg g^{-1} fresh algae for October 2009, January 2010, April 2010 and June 2010, respectively. The CYN content in *C. racemosa* was raised from October 2009 to June 2010 except the one in April 2010. The CYN content in *C. racemosa* collected in April was statistically ($p<0.05$) different from that of October 2009. The highest value was observed from the samples of June 2010. In contrast to *C. racemosa*, the highest CYN content of *C. prolifera* was determined from the samples collected in April 2010. The statistical comparison for all CYN data was given in Table 2a.

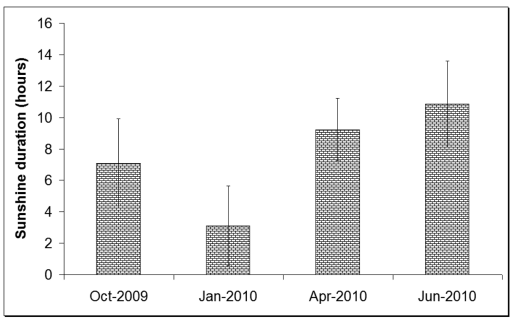


Fig. 3: Seasonal variations of the sunshine durations in the sampling site. The values are the mean of the sunshine hours of the days of the months. Error bars show the standard deviation.

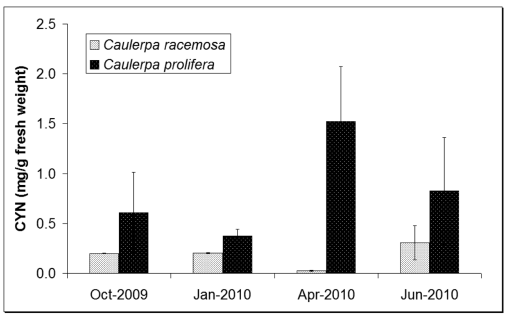


Fig. 4: Seasonal caulerpenyne concentrations in *Caulerpa racemosa* and *in Caulerpa prolifera.* Error bars show the standard deviation.

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Table 1. Statistical comparison of the air temparatures (a), seawater (b) and sunshine duration (b) values. The data were analysed by one-way ANOVA (Tukey's test was also used for multiple comparison).

a) Air temperatures

Months	P value
October 2009 - January 2010	P<0.05
October 2009 - April 2010	P<0.05
October 2009 - June 2010	P<0.05
January 2010 - April 2010	P<0.05
January 2010 – June 2010	P<0.05
April 2010 - June 2010	P<0.05

b) Seawater

Months	P value
October 2009 - January 2010	P<0.05
October 2009 - April 2010	P<0.05
October 2009 - June 2010	P<0.05
January 2010 - April 2010	P<0.05
January 2010 – June 2010	P<0.05
April 2010 - June 2010	P<0.05

c) Sunshine

Months	P value
October 2009 - January 2010	P<0.05
October 2009 - April 2010	P<0.05
October 2009 - June 2010	P<0.05
January 2010 - April 2010	P<0.05
January 2010 – June 2010	P<0.05

The seasonal protein concentrations in these species were shown in Figure 5. The values of *C. racemosa* were detected as 0.178±0.001 mg protein mL supernatant^{-1}, 0.550±0.010 mg protein mL supernatant^{-1}, 0.456±0.006 mg protein mL supernatant^{-1} and 0.574±0.013 mg protein mL supernatant^{-1} for October 2009, January 2010, April 2010 and June 2010, respectively. Although the protein contents of January, April and June samples were statistically different from that of October 2009 samples ($p<0.05$), there was no statistical difference among them ($p>0.05$). The results of *C. prolifera* samples could be summarized as 0.587±0.001 mg protein mL supernatant^{-1}, 0.553±0.002 mg protein mL supernatant^{-1}, 0.527±0.013 mg protein mL supernatant^{-1} and 0.645±0.007 mg protein mL supernatant^{-1} for October 2009, January 2010, April 2010 and June 2010, respectively. According to the results of *C.prolifera*, a decreasing trend was observed from October 2009 to April 2010 and an increase was seen for the samples collected in June. The statistical comparisons of all protein concentrations were depicted in Table 2b.

In order to determine the specific carboxylase activity values of rubisco enzyme in these species, the volumetric

Table 2. Statistical comparison of the caulerpenyne (a), protein (b) and rubisco (c) values. The data were analysed by one-way ANOVA (Tukey's test was also used for multiple comparison). Abreviations: CR, *Caulerpa racemosa*; CP, *Caulerpa prolifera*; OCT, October; JAN, January; APR, April; JUN, June.

a) Caulerpenyne

Data	P value
CR2009OCT – CP2010APR	P<0.05
CR2010JAN – CP2010APR	P<0.05
CR2010APR – CP2010APR	P<0.05
CR2010APR – CP2010JUN	P<0.05
CR2010JUN – CP2010APR	P<0.05
CP2009OCT – CP2010APR	P<0.05
CP2010JAN – CP2010APR	P<0.05
CP2010APR – CP2010JUN	P<0.05

b) Protein

Data	P value
CR2009OCT – CR2010JAN	P<0.05
CR2009OCT – CR2010APR	P<0.05
CR2009OCT – CR2010JUN	P<0.05
CR2009OCT – CP2009OCT	P<0.05
CR2009OCT – CP2010JAN	P<0.05
CR2009OCT – CP2010APR	P<0.05
CR2009OCT – CP2010JUN	P<0.05
CR2010JAN – CR2010APR	P<0.05
CR2010JAN – CR2010JUN	P<0.05
CR2010JAN – CP2009OCT	P<0.05
CR2010JAN – CP2010APR	P<0.05
CR2010JAN – CP2010JUN	P<0.05
CR2010APR – CR2010JUN	P<0.05
CR2010APR – CP2009OCT	P<0.05
CR2010APR – CP2010JAN	P<0.05
CR2010APR – CP2010APR	P<0.05
CR2010APR – CP2010JUN	P<0.05
CR2010JUN – CP2010APR	P<0.05
CR2010JUN – CP2010JUN	P<0.05
CP2009OCT – CP2010JAN	P<0.05
CP2009OCT – CP2010APR	P<0.05
CP2009OCT – CP2010JUN	P<0.05
CP2010JAN – CP2010APR	P<0.05
CP2010JAN – CP2010JUN	P<0.05
CP2010APR – CP2010JUN	P<0.05

c) Rubisco

Data	P value
CR2009OCT – CR2010JAN	P<0.05
CR2009OCT – CP2009OCT	P<0.05
CR2010JAN – CR2010APR	P<0.05
CR2010JAN – CR2010JUN	P<0.05
CR2010JAN – CP2010JAN	P<0.05
CR2010JAN – CP2010APR	P<0.05
CR2010JAN – CP2010JUN	P<0.05
CR2010APR – CP2009OCT	P<0.05
CR2010APR – CP2010JUN	P<0.05
CP2009OCT – CP2010APR	P<0.05

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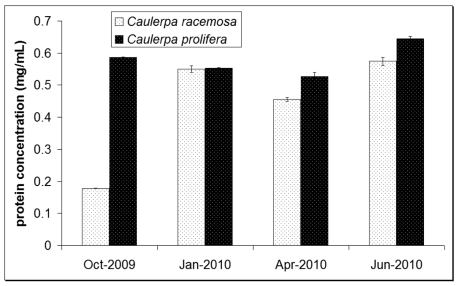


Fig. 5: Seasonal protein concentrations in *Caulerpa racemosa* and *Caulerpa prolifera.* Error bars show the standard deviation.

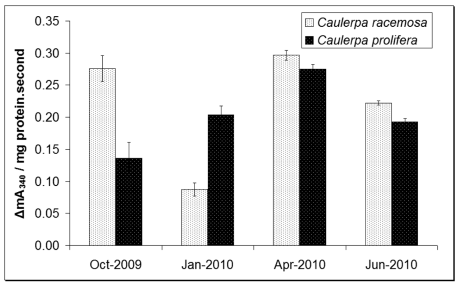


Fig. 6: Seasonal rubisco enzymic activities in *Caulerpa racemosa* and *Caulerpa prolifera.* Error bars show the standard deviation.

activity values were corrected by the protein content of related species. The results for *C. racemosa* and *C. prolifera* were presented in Figure 6. The rubisco activities of *C. racemosa* were determined as 0.276±0.020 ΔmA_{340} mg protein^{-1}second^{-1}, 0.088±0.010 ΔmA_{340} mg protein^{-1}second^{-1}, 0.297±0.008 ΔmA_{340} mg protein^{-1}second^{-1} and 0.222±0.003 ΔmA_{340} mg protein^{-1}second^{-1} for October 2009, January 2010, April 2010 and June 2010, respectively. The result observed for January 2010 was statistically ($p<0.05$) lower than that of the value of October 2009. As can be seen from the Figure 6, the lowest specific activity value of *C. racemosa* was observed in January 2010. The rubisco activities of *C. prolifera* were observed as 0.136±0.025 ΔmA_{340} mg protein^{-1}second^{-1}, 0.203±0.014 ΔmA_{340} mg protein^{-1}second^{-1}, 0.275±0.007 ΔmA_{340} mg protein^{-1}second^{-1} and 0.193±0.005 ΔmA_{340} mg protein^{-1}second^{-1} for October 2009, January 2010, April 2010 and June 2010, respectively. The statistical comparisons for all rubisco activities were given in Table 2c.

The Pearson correlation test was used in order to evaluate the existences of correlations among seasonal air-seawater temperatures, sunshine duration, CYN concentrations, protein contents and rubisco enzyme activities (Table 3). No statistical correlation was observed among the observed parameters except significant positive correlation between seawater and air temperatures, (Table 3).

Discussion

The major secondary metabolite of *Caulerpa* spp. is called CYN. The amount of this metabolite varies among

Table 3. The correlation results for the parameters studied. The numbers in the cell are pearson correlation and p-value, respectively. Abbreviations: AT, air temperature; SWT, seawater temperature; SSD, sunshine duration; CYN-CR and CYN-CP, caulerpenyne levels in *C.racemosa* and *C.prolifera,* respectively; Rub-CR and Rub-CP, rubisco enzymic activity in *C.racemosa* and *C.prolifera,* respectively; Pro-CR and Pro-CP, protein concentrations in *C.racemosa* and *C.prolifera,* respectively.

	AT	SWT	SSD	CYN-CR	CYN-CP	Rub-CR	Rub-CP	Pro-CR
SWT	0.983 0.017							
SSD	0.867 0.133	0.761 0.239						
CYN-CR	0.465 0.535	0.569 0.431	0.047 0.953					
CYN-CP	0.214 0.786	0.046 0.954	0.658 0.342	-0.702 0.298				
Rub-CR	0.586 0.414	0.514 0.486	0.739 0.261	-0.406 0.594	0.724 0.276			
Rub-CP	-0.299 0.701	-0.470 0.530	0.204 0.796	-0.685 0.315	0.761 0.239	0.107 0.893		
Pro-CR	-0.129 0.871	-0.220 0.780	0.056 0.944	0.188 0.812	0.066 0.934	-0.540 0.460	0.558 0.442	
Pro-CP	0.804 0.196	0.858 0.142	0.473 0.527	0.899 0.101	-0.346 0.654	0.003 0.997	-0.569 0.431	0.110 0.890

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the members of *Caulerpa* genus. In addition, it was reported that the CYN level may change depending on some factors such as season, temperature, depth, herbivorism, etc. (Amade & Lemée, 1998; Jung *et al.* , 2002; Cavas & Yurdakoc, 2005; Sureda *et al.* , 2009). In the present study, the CYN levels, protein concentrations and the rubisco enzyme activities in the invasive *C. racemosa* and native *C. prolifera* were seasonally determined and the obtained results were analysed comparatively. So far, no study has been reported for the relationship between the rubisco enzyme activities and the CYN levels on *Caulerpa* spp. in the scientific literature. According to the results of this investigation, the highest CYN content in *C. racemosa* was observed in June 2010. An increase in sea water temperature might cause the rise in epiphytic activity and in metabolic rate. This result also confirmed the results of Box et al's study where *C. taxifolia* epiphytised by red algae (by *Lophocladia lallemandii*) secreted more CYN than that of the samples without *L. lallemandii* (Box *et al.* , 2008). It was reported that the *C. racemosa* epiphytised by red algae showed low catalase enzyme activity which could be a result of hydrogen peroxide based defence (Cavas & Yurdakoc, 2005). The sudden decrease of the CYN content in *C. racemosa* observed in April may be due to an intense rain in March 2010 and April 2010. Since the collection depth was -1m, the sea water concentration might have temporarily been changed by heavy rains. However, heavy rains not only affect the CYN concentrations, but also frond morphologies of *C.racemosa*. This observation, which is related to fronds of *C.racemosa,* should be investigated in great detail inasmuch as we observed different frond morphologies after the rains. It is very important to note that similar frond anomalilies after the rain were not observed for *C.prolifera.* As can be seen in Figure 4, the CYN content of *C. racemosa* raised from April to June and the results observed in this season were statistically different from each other. For *C. prolifera*, the maximum and the minimum CYN contents were determined in April 2010 and January 2010, respectively. As a result of this investigation, it can be said that *C. prolifera* may have not been affected by the heavy rain as it was observed in the case of *C. racemosa*. Similar results were reported by Amade & Lemée (1998) where the CYN content in *C. taxifolia* was at maximum in summer and autumn. They claimed that the increase in CYN content also affected the feeding behaviours of *Paracentrotus lividus* (sea urchin) that use this alga as a food source only in winter and spring due to the lower CYN content. The rapid increase in CYN content of *C. prolifera* observed in April may be caused by the rise in epiphytic activity that occurred in that season. Indeed, Verlaque & Frytaire (1994) emphasized that the maximum amount of fouling organisms were observed in spring where the CYN content was also at its maximum. Another reason of the increased CYN content in summer would be the herbivore pressures and, also, space competition with other species in the same habitat (Amade & Lemée, 1998). *Lobiger serradifalci* and *Oxynoe olivacea,* two common seaslugs in the Mediterranean Sea, also consumed these species as food sources (Cavas *et al.* , 2005). Since *L. serradifalci* fragments *C. racemosa* and fragmented parts are regenerated, this seaslug can contribute the invasion of this species in the Mediterranean Sea. The total protein levels of these algae species were also determined in this investigation. Seasonal protein concentrations in invasive *C.racemosa* and native *C.prolifera* showed different trends. The protein concentrations in *C.prolifera* were higher than those of *C.racemosa.* However, no significant difference ($p>0.05$) was observed between protein concentrations of two species in January 2010. Since different trends were observed for the protein concentrations in the species studied, it can be said that there might have been different factors in the synthesis of proteins in these species. Rubisco enzyme is very important for photosynthetic plants and the major role of this enzyme for algae is the fixation of CO_2 solved in sea water. In the first step of the reaction, the fixation of CO_2 into ribulose-1,5-bisphosphate is occurred. The product of this step is a highly unstable product, which is known as 3-keto-2-carboxyarabinitol-1,5-bisphosphate, and then it is decomposed into two molecules of 3-phosphoglycerate (Boyer, 2006; Andersson & Backlund, 2008). Although two different functions of this enzyme are known, its carboxylase activity was determined in the present study. There have been very limited scientific studies on the rubisco enzymic activities and secondary metabolites of seaweeds in the literature. Therefore, it is very hard to compare our experimental results with the published papers. However, the papers focusing on the rubisco were compared with our results. The effect of solar radiation on the activity of rubisco enzyme activity and the photosynthetic pigments of *Ulva lactuca* (L.) was investigated by Bischof *et al.* (2002). It was reported that the blocking of UV radiation caused an increase in concentration and total activity of rubisco enzyme. Therefore, it can be said that UV radiation is of great importance for rubisco enzyme activity (Bischof *et al.* , 2002). In another investigation of this group, the effect of UV radiation on the rubisco enzyme activities of 5 different macroalgae species (*Monostroma arcticum, Laminaria solidungula, Alaria esculenta, Palmaria palmata, Phycodrys rubens*) was evaluated. According to the results of this study, the enzyme activity of rubisco was associated with the glyceraldehyde-3-phosphate dehydrogenase. Moreover, it was determined that the proportion of maximal electron transport (ETRmax) was also associated with the rubisco enzyme activity (Bischof *et al.* , 2000). The variation of photosynthetic capacity of *Gracilaria tenuistipitata* along with monochromatic blue light was determined by

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Mercado *et al.* (2002). No statistically-significant difference was observed for the photosynthetic capacity of *Gracilaria tenuistipitata* under blue light and white light conditions. Delrio *et al.* (1995) investigated the photosynthetic rate and carbonic anhydrase activities of *Ulva rigida* C. Agardh (Chlorophyta) grown in seawater and NH_4Cl enriched seawater. The results of the study showed that the growth and O_2 formation rate and the activity of carbonic anhydrase enzyme were higher in *U. rigida* grown in NH_4Cl enriched seawater than that of *U. rigida* grown in seawater. It was concluded that the main environmental factor affecting the assimilation of CO_2 and the activities of the rubisco and carbonic anhydrase enzymes of *U. rigida* was low nitrogen concentration (Delrio *et al.* , 1995). According to our results, we did not find any relationship between seasonal rubisco enzyme activies and the seasonal parameters such as air-sea water temperature and sunshine durations. Moreover, we did not find any relationship between solar radiation level (Aksoy, 1997) which is directly associated with light reactions of photosynthesis and the parameters studied (data not shown). No significant correlation was observed between seasonal rubisco enzyme activies and CYN levels. Since CYN contents of the *C.racemosa* and *C.prolifera* showed different trends seasonally, it might be concluded that the CYN synthesis might be regulated differently in invasive and native *Caulerpa* species, but this regulation should be studied in great detail in future studies. Similar cases were also observed for rubisco activities in the species studied; therefore, different regulatory mechanisms might have had some roles in the regulation of rubisco activities. In the MEP pathway, one of the starting molecules is glyceraldehyde-3-phosphate (G3P). During this pathway, G3P is enzymatically converted into isopentenyl-pyrophosphate (IPP) (Rohmer, 1999). IPP is the precursor of the CYN (Pohnert and Jung, 2003). In the beginning of this study, we aimed to find a relationship between rubisco enzyme activities and CYN levels since rubisco activity produces two molecules of 3-phosphoglicerate (3PG). Then, 3PG can be metabolically transformed into G3P (Plaxton & Podestá, 2006) so that it supports the synthesis of CYN via MEP pathway. However, we did not observe this relationship between the CYN and rubisco in our study. This can be explained by other enzymes and control mechanisms might be involved in the synthesis of the CYN and regulation of rubisco.

In conclusion, *C. racemosa* has still been invading new localities in the Mediterranean Sea. Studying biochemical mechanisms of *C.racemosa* can help us understand the invasive property of this species. Therefore, the further analysis on the rubisco enzymic activity determinations with MEP and mevalonate pathways considered to be responsible for CYN bio-synthesis in invasive and native *Caulerpa* species in the Mediterranean Sea should be studied in great detail to get the overall picture

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