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SESQUITERPENOID CAULERPENYNE LEVELS OF NEWLY IDENTIFIED *CAULERPA TAXIFOLIA* VAR. *DISTICHOPHYLLA* FROM THE ISKENDERUN BAY, TURKEY

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ABSTRACT

The seasonal variations of caulerpenyne (CYN) levels, secondary metabolite of *Caulerpa* genus, were investigated in the fronds and stolons of *Caulerpa taxifolia* var. *distichophylla* recently present at three stations in the Gulf of İskenderun, the Eastern Mediterranean Sea, including its relationships with various physicochemical parameters of the sea water. The CYN levels were highest in the spring, and decreased in autumn. Also, the CYN contents in the fronds were statistically higher than the stolons ($p<0.05$). The highest seasonal mean CYN content was determined in the fronds as 0.82±0.3 ‰ wet weight during summer season. However, there were no statistically significant differences in the CYN levels among sampling stations ($p<0.05$). It was also determined that the CYN levels had positive correlations with temperature and oPO4-P concentrations.

KEYWORDS:
Alien species, Mediterranean Sea, *Caulerpa taxifolia* var. *distichophylla*, caulerpenyne

INTRODUCTION

Caulerpa taxifolia (M. Vahl) C. Agardh is invasive green marine seaweed in the Mediterranean Sea. It was accidentally introduced to the Mediterranean Sea from Oceanographic Museum of Monaco in 1984 [1]. After its first observation in there, it has successfully invaded the sublittoral ecosystem of the Mediterranean Sea. Because of its effective invasive trait, this species is called as "Killer Alga" [2]. Up to date, this species has been observed in the seven Mediterranean countries: Croatia, France, Italy, Monaco, Spain, Tunisia and Turkey. The first observation of *C. taxifolia* at Turkish coastlines (Gulf of İskenderun, Eastern Mediterranean) was reported in 2007 [3]. Although its first observation was reported from the Gulf of İskenderun, currently its existence in Antalya (Western coastlines of Turkey) and Sicily has been reported by Jongma et al. (2013) [4]. Most recently, it was reported in Cyprus [5].

Cevik et al. (2007) [3] reported that the morphology of *C. taxifolia* observed in the Gulf of İskenderun was different from the invasive aquarium strain of *C. taxifolia*. According to Cevik et al. (2007) [3], stolons of the strain observed in the Gulf of İskenderun were more slender and its rhizoidal pillars were shorter than the aquarium strain of *C. taxifolia*. Moreover, first molecular analyses (ITS1-5.8S-ITS2 rDNA sequences) on *C. taxifolia* from the region revealed that this species has a different genetic structure compared to the well-known *Caulerpa taxifolia* in the Western Mediterranean region [3]. In a more recent study, the identity and origins of *C. taxifolia* observed in Sicily (Italy) were discussed in great detail by Jongma et al. (2013) [4]. *C. taxifolia* observed in Sicily was genetically compared with *C. distichophylla* (from Australia), *C. taxifolia* (the invasive aquarium strain) and *C. taxifolia* (observed in Gulf of İskenderun) by Jongma et al. (2013) [4] through the use of the tufA gene, rDNA ITS and cp 16S rDNA intron-2 sequences in molecular analyses. Jongma et al. (2013) [4] have found that both *C. taxifolia* var. *distichophylla* strains observed in Sicily and Gulf of İskenderun were genetically identical to each other. They were in the same clade with *C. distichophylla* from Australia, and according to the analyses of tufA cpDNA gene and of the cp 16S rDNA intron-2 sequences; they were genetically different from the aquarium strain of *C. taxifolia* only with a single nucleotide polymorphism. Therefore, due to the presence of morphological and genetic differences between C. *distichophylla* and *C. taxifolia*, Jongma et al. (2013) [4] have proposed the use of *Caulerpa taxifolia* (Vahl) C. Agardh var. *distichophylla* (Sonder)

Verlaque, Huisman and Procaccini, stat. nov. instead of *C. distichophylla*.

Members of the Caulerpales have chemical defences against herbivores and epiphytes like many macroalgae. One of the best known among them is a secondary metabolite produced by species of the genus *Caulerpa*, caulerpenyne (CYN) [6-9]. Besides its production with defensive purposes, CYN also has important bioactivities like cytotoxic effects, growth inhibition of marine fungi, alteration of sexual reproduction in sea urchins, prevention of herbivory grazing by gastropods and fishes, and toxicity against fishes [12]. CYN can also be transformed into more toxic forms like oxytoxins and reactive aldehydes by wound-activated enzymatic reactions [10, 11]. The aim of the study was to investigates the seasonal CYN levels of *C. taxifolia* var. *distichophylla*, colonising in the Gulf of Iskenderun, through examining the possible effects of various hydrographical conditions (temperature, light levels, depth etc.). Absence of the investigation related to CYN levels of this newly-introduced *C. taxifolia* var. *distichophylla* strain from the Gulf of Iskenderun motivated us to study the secondary metabolite chemistry.

MATERIALS AND METHODS

The names and the geographical coordinates of the stations (Figure 1) are Çevlik (L1) (36°08′05″ N; 35°53′48″ E), Arsuz (L2) (36°25′49″ N; 35°53′29″ E), İsdemir (36°41′48″ N; 36°11′17″ E), Toros (36°53′58″ N; 36°57′38″ E), Yumurtalık (L3) (36°47′43″ N; 35°51′04″ E) and Kokar (36°38′32N; 35°40′32″ E). However, among the stations mentioned in Figure1, sufficient amount of samples was not found in Isdemir, Toros and Kokar stations at all seasons. Therefore, these stations were not included in the analyses. The Figure 1 was obtained by using Basemap extension of Matplotlib package [12] within Enthought Canopy Academic software (Austin, TX, USA). The samples from three stations were collected at 10m, 15m, 20m, 25m and 30m depth contours as a transect. The samples were collected from defined quadrates (2x10 m). The sampling dates were as follows: spring 2010 (from May 27 to June 5), summer 2010 (from July 31 to Aug. 8), autumn 2010 (from Oct. 24 to Nov. 3), and winter 2011 (from Jan. 13 to Feb. 1), respectively. The method of Sureda et al. (2009) [13] was used to estimate the CYN levels in the samples. Shortly, the seaweed materials were washed with tap water to remove salts and also epiphytes and then frozen with liquid nitrogen until used. The methanolic extracts were prepared by using 50 mL methanol for 5 g fresh seaweed and then extracts were applied to sep-pak cartridges (WATERS, WAT020515). The CYN transformation by esterases was blocked by liquid nitrogen. The samples were eluted with 5 mL of a mixture of methanol: ethyl acetate (50:50). The CYN concentrations in the elutions were determined by using a HPLC system. The mobile phase and column were methanol:water (80:20) and hyperpack ODS 3 µm, 15x0.4 cm (Teknokroma), respectively. CYN levels were measured at 254 nm and quantified by using standard curve. The isolation of CYN as a standard was carried out according to the method of Cengiz et al. (2009) [14].

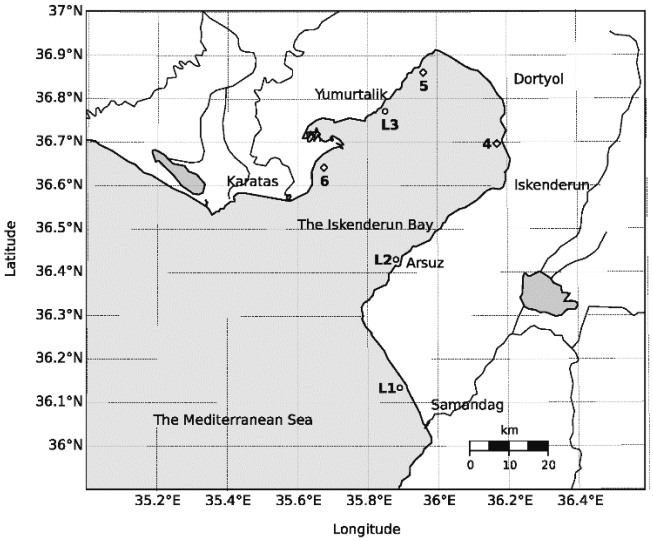

FIGURE 1
Sampling stations. (Location 4 (İsdemir), Location 5 (Toros) and Location 6 (Kokar) were excluded from analyses due to the insufficient sample size)

Abiotic parameters indicating seawater quality such as temperature, dissolved oxygen, salinity, pH, secchi disk and nutrients (TNO_x (NO_3+NO_2), NH_4, oPO_4-P) were measured at each station. Temperature, pH, salinity, dissolved oxygen and depth measurements were performed with a multiparameter CTD profiler (Model 6600, YSI Inc., USA). Seawater samples for laboratory analyses were collected 0.5 m above the sediment from -5, -10, -15, -20, -25 and -30 m depth contours at each station by using a non-transparent universal water sampler (Hydrobios GmbH, Germany). The seawater samples were kept in a freezer and stored frozen at -20 °C until the analyses. Inorganic phosphate (o-PO_4-P) was determined by the reduction of phosphomolybdate complex in the presence of ascorbic acid [15]. Nitrite (NO_2) and nitrate (NO_3), initially converted to nitrite by the cadmium reduction, were determined by the sulphanilamide method [15]. Ammonia (NH_4) was analyzed by using the indophenol blue method [15]. The suspended particulate matter analysis was included as an additional abiotic parameter after the second sampling. The light penetration measurements conducted with a luminometer both at the air and at the various depths of seawater were evaluated as percent penetration (%). The sediment samples were collected by using an Ekman type grab sampler and the sediments were stored in polyethylene bags which were kept in ice during the transport to the laboratory. Moisture content of the sediment samples were determined from the weight difference of wet and dried (at 105 °C) samples (APHA, 1998). The sediment weight loss on ignition was used as an indicator of organic matter in sediments. For loss on ignition analysis, sediments placed into porcelain crucibles were first dried at 105 °C to remove moisture and then transferred to a furnace and kept at 550 °C for 5 h [16]. For sediment texture analysis, the density and temperature measurements were performed sequentially with a hygrometer following the pre-treatment and dilution steps on the wet sediment samples which were initially taken to include a minimum of 50 g of dry matter. Then, the clay, silt and sand percentages were determined with a set of sieves [17]. Following the ignition, total phosphorus in the homogenized sediments was determined spectrophotometrically [18]. Total Kjeldahl nitrogen in homogenized sediments was determined titrimetrically following the ignition and distillation [18].

The data obtained during the study were given in the form of mean ± standard deviation. Before the ANOVA tests, the data were checked using the Shapiro-Wilk normality test for fitting the normal distribution and using Levene's homogeneity test for homogeneity of the variances. In cases where the data did not fit the normal distribution, mathematical transformation (Ln) was used to normalize the data [19].

One-way ANOVA test was used to analyze whether the sampling depths (10m, 15m, 20m, 25m, 30m) demonstrate any effects on the CYN and temperature. The differences in the CYN between stations or seasons were tested by discarding a group (station or season) using one-way ANOVA test. Two-way ANOVA test was also applied to check any interactions between the stations and seasons. An another two-way ANOVA test was used to determine whether there are any differences between the CYN levels in the fronds and stolons seasonally. In addition, presence of any interactions was checked. Tukey multiple comparison test was used to determine the factors showing differences as a result of variance analyses. The significance of main effects in two-way ANOVA was analyzed using the Bonferroni test. Pearson multiple correlation test was used to determine the correlation between the temperature and the CYN. Multiple correlation test was also used to determine significant correlations between the CYN and environmental factors. Principal component analysis (PCA) was performed among 8 factors (CYN, temperature, dissolved oxygen, salinity, pH, TNO_x, oPO_4-P and NH_4). K-means clustering of the factors used in the PCA was also performed. Significance level was determined as 0.05 for all analyses. IBM SPSS 20 [20] and R package software [21] were used for statistical analyses.

RESULTS

The relationships of CYN and seawater temperature with sampling depths were compared statistically by One-Way ANOVA and there were no significant differences among the groups ($p<0.05$, Table 1). The seasonal variation of environmental parameters together with CYN levels within stations were given in Table 2. According to the Two-Way ANOVA test between seasons, stations and CYN levels, a significant decrease in CYN levels were found. According to results, CYN levels were decreased in the seasonal order of spring, summer, autumn and winter (Table 2). Spring and summer can be grouped separately than autumn and winter ($p<0.05$, Table 2). However, there were no statistically significant differences between stations and CYN levels ($p<0.05$). Also, the interaction between seasons and stations was not significant ($p<0.05$).

The sediment measurements were given in Table 3. It can be seen in Table 3, there were no significant difference between location and season.

TABLE 1
The CYN levels and seawater temperatures at the sampling depths (p<0.05)

Depth	CYN	Temperature
10 m	0.4±0.2[a]	22.24±4.5 [a]
15 m	0.3±0.2[a]	22.03±4.4 [a]
20 m	0.3±0.1 [a]	22.09±4.3 [a]
25 m	0.4±0.2[a]	22.15±4.1 [a]
30 m	0.4±0.2[a]	22.06±4.1 [a]
Sig.	0.864	1
Between Group Mean Square	0.204	0.242

TABLE 2
Means of the CYN levels and other environmental factors grouped according to the seasons and stations

Season	Location	CYN (‰ ww)	Temp (°C)	DO (mg/L)	TNO_x (µM)	oPO_4-P (µM)	NH_4 (µM)
Spring	L1	0.6±0.1[1A**]	21±0.3	5.9±0.1	0.1±0.04	0.5±0.6	1.6±0.6
	L2	0.6±0.1[1A]	22±0.5	6.8±0.2	0.2±0.06	0.7±0.5	0.8±0.2
	L3	0.6±0.03[1A]	22±0.5	6.6±0.3	0.2±0.07	0.2±0.1	0.7±0.2
$\bar{x}_{sp} \pm S.D.$		0.6±01[a]	22.1±0.6	6.6±0.4	0.2±0.07	0.4±0.49	0.9±0.4
Summer	L1	0.4±0.1[1A]	26.9±0.3	5.7±2.1	1.25±0.86	0.8±0.2	1.6±0.3
	L2	0.7±0.1[1A]	27±0.6	6.5±0.3	0.51±0.23	0.9±0.2	1.3±0.2
	L3	0.5±0.4[1A]	27.1±0.9	5.7±0.4	0.39±0.19	1.05±1.2	1.5±0.4
$\bar{x}_{su} \pm S.D.$		0.6±0.25[a]	27.05±0.76	6.02±0.9	0.57±0.48	0.96±0.85	1.48±0.4
Autumn*	L1	0.3±0.1[1A]	25.5±0.3	7.1±0.4	0.26±0.12	0.05±0.01	0.7±0.6
	L2	0.3±0.1[1A]	25.7±0.4	6.4±0.4	0.46±0.26	0.05±0.03	0.8±0.6
	L3	0.05	24.9±0.3	6.0±0.6	0.87±1.04	0.03±0.02	0.7±0.6
$\bar{x}_{au} \pm S.D.$		0.3±0.13[b]	25.32±0.53	6.49±0.6	0.63±0.79	0.04±0.02	0.78±0.6
Winter*	L1	0.2±0.1[1A]	17.2±1,07	7.3±0.8	1±0.54	0.03±0.01	1.2±1.3
	L2	0.2±0.1[1A]	17.1±0.7	7.3±0.9	0.9±0.73	0.03±0.02	0.9±0.8
	L3	0.27	16.2±0.7	7.7±0.4	0.8±0.51	0.03±0.02	0.9±0.8
$\bar{x}_{wi} \pm S.D.$		0.250.13[b]	16.6±0.94	7.5±0.7	0.9±0.59	0.03±0.02	0.9±0.9

*Since there were single measurements at least in one station for winter or autumn seasons, two sample t-test was used instead of variance analysis (p<0.05).
**Superscripted numbers indicate statistically significant differences between CYN levels and stations within seasons (p<0.05).
Superscripted capital letters indicate statistically significant differences only between CYN levels and stations (p>0.05).
Superscripted small letters indicate statistically significant differences only between CYN levels and seasons (p<0.05).

The CYN levels in the fronds were statistically higher than stolons in all seasons (Table 4). When seasonal means of CYN levels in fronds were compared, both spring and summer seasons were significantly higher than those of the values observed in the autumn and winter seasons (p<0.05). However, the seasonal mean CYN levels in the stolons were only statistically lower in winter compared to other seasons. According to the Two-Way ANOVA tests between season, stations and CYN levels in the fronds or stolons, significant differences were only found at Yumurtalık (L3) station both in the fronds and the stolons. The variations of CYN levels in the fronds and the stolons with respect to stations were depicted in Figure 2.

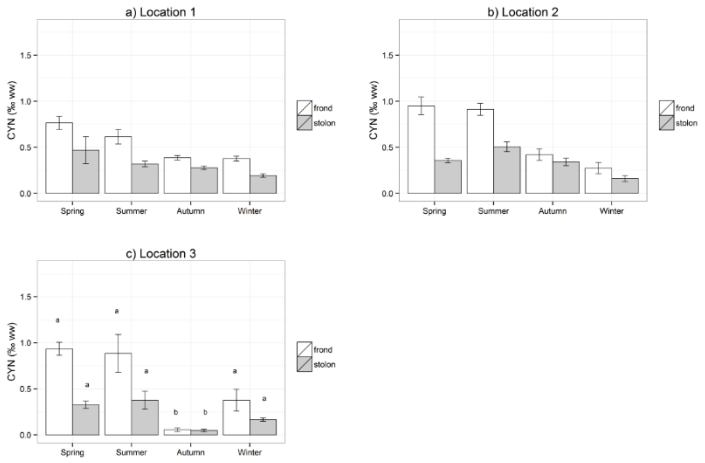

FIGURE 2
Seasonal variation of CYN levels in frond and stolons at different locations. Lowercase indicates differences from Anova tables

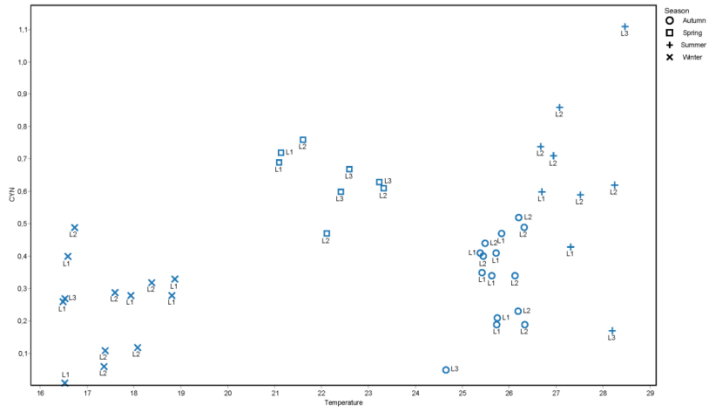

FIGURE 3
Seasonal variation of CYN levels vs temperature at locations

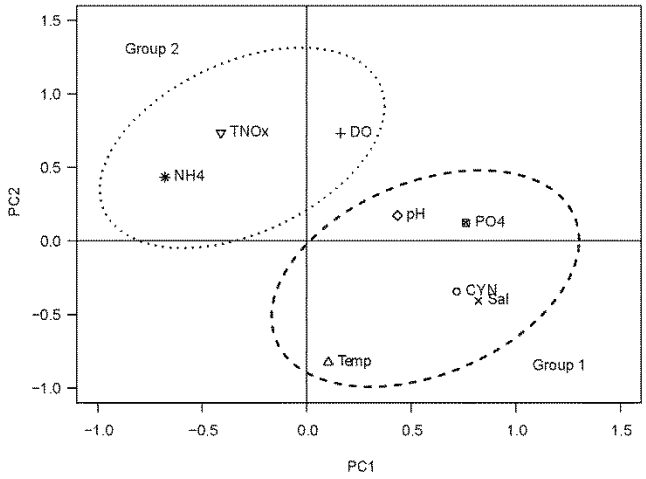

FIGURE 4
Bivariate score plots of the PCA between CYN, Temperature, pH,) PO_4, Dissolved Oxygen (DO), Salinity (Sal), NH_4 and TNO_x

TABLE 3
Means of the sediment measurements factors grouped according to the seasons and stations

Season*	Loc*	S_{clay} (%)	S_{silt} (%)	S_{sand} (%)	S_{moist} (%)	S_{ncs} (%)	SKN (ppm)	STP (ppm)
Spring	L1	7.7±1.7	25.6±10.5	66.6±12.1	29.5±4.7	4.3±1.3	3793±1843	417±130.5
	L2	10.6±6.7	17.1±16.4	72.1±22.9	29.9±7.1	5.8±2.1	1397±817.9	326±156.2
	L3	20.6±12.1	39.5±25.2	39.7±37.1	37.7±9.5	7.5±3.5	1992±809.3	486±84.3
$\bar{x}_{sp} \pm S.D.$		15.6±11.1	31.2±22.8	53.1±33.4	34.3±9.1	6.5±3.1	2201±1316	436±126.4
Summer	L1	7.7±2.6	22.3±10.2	69.9±12.8	30.8±2.19	2.79±0.9	1379±571.5	384±64.4
	L2	9.7±5.1	22.2±24.2	67.9±28.1	31.82±8.2	4.03±1.7	1758±542.2	365±209.2
	L3	13.1±6.4	49.2±30.7	37.6±34.5	36.67±10.4	5.72±2.7	3315±2151	477±92.4
$\bar{x}_{su} \pm S.D.$		11.35±5.9	37.8±29.1	50.8±33.1	34.44±9.1	4.7±2.5	2584±1864	433±130.1
Autumn	L1	3.2±2.4	23.7±11.9	73.1±13.9	31.49±9.2	3.59±1.3	2065±1239	371±67.2
	L2	6.9±8.3	19.5±19.1	73.4±26.7	28.62±6.9	4.68±2.1	2700±1715	295±172.8
	L3	14.2±10.3	47±23.9	38.7±31.3	36.18±9.3	5.65±3.1	3740±2758	464±70.7
$\bar{x}_{au} \pm S.D.$		10.30±9.8	35.7±24.2	53.9±32.4	33.43±9.2	5.00±2.7	3155±2371	405±124.4
Winter	L1	2.8±1.7	23.4±10.9	73.7±12.2	26.8±4.3	4.01±1.5	3226±2624	351±60.2
	L2	5.6±8.8	12.7±15.2	81.6±22.8	27.2±6.6	5±2.1	2786±2080	263±161.6
	L3	15.4±10.9	41.2±21.7	43.2±30.3	36.4±9.3	6.4±3.1	3397±2399	430±143.3
$\bar{x}_{wi} \pm S.D.$		10.4±10.7	30.3±22.2	59.1±31.1	32.2±9.1	5.60±2.7	3215±2338	374±151.9

*Superscripts indicate column-wise statistically not significant differences among groups (p>0.05).

TABLE 4
The seasonal and locational variations of CYN levels in the fronds and stolons

Season	Station	CYN		Seasonal Mean±S.D	
		Frond	Stolon	Frond	Stolon
Spring	L1	0.76^{a*}	0.47^{a}	$0.85±0.2^{a}$	$0.41±0.3^{a}$
	L2	0.95^{a}	0.35^{a}		
	L3	0.93^{a}	0.32^{a}		
Sig		0.21	0.942		
Summer	L1	0.61^{a}	0.31^{a}	$0.81±0.3^{a}$	$0.41±0.2^{a}$
	L2	0.91^{a}	0.50^{a}		
	L3	0.88^{a}	0.37^{a}		
Sig		0.128	0.062		
Autumn	L1	0.38^{a}	0.28^{a}	$0.38±0.2^{b}$	$0.28±0.1^{a}$
	L2	0.42^{a}	0.34^{a}		
	L3	0.057^{b}	0.05^{b}		
Sig		0.000	0.000		
Winter	L1	0.37^{a}	0.19^{a}	$0.34±0.2^{b}$	$0.18±0.1^{b}$
	L2	0.27^{a}	0.16^{a}		
	L3	0.37^{a}	0.16^{a}		
Sig		0.246	0.64		
Mean±S.D		$0.56±0.3^{a}$	$0.31±0.2^{b}$		
Sig.		0.00		0.00	0.00

*Superscripted small lettters indicate column-wise statistically significant differences among groups (p<0.05).

TABLE 5
Multiple correlation analysis results between the CYN levels and envrionmental parameters (*p<0.05; n.c: not calculated)

Variable	CYN	Temp	DO	TNO_x	PO_4	NH_4	SPM	Chl-a	%LP
Temp	**.399***								
DO	-.227	**-.710***							
TNO_x	-.096	**-.213***	.135						
PO_4	**.491***	**.361***	-.166	-.037					
NH_4	-.044	.147	**-.390***	.065	.129				
SPM	**-.342***	.145	-.035	-.039	-.021	.038			
Chl-a	.031	.024	-.050	-.076	n.c	-.006	.091		
%LP	.515	.143	-.055	-.081	n.c	-.224	-.123	-.212	
SD	-.140	.011	-.075	-.083	-.084	.124	**.195***	-.149	.168

A positive and statistically significant correlation between seawater temperatures and CYN levels were found as 0.399 ($p<0.05$). Correlation between the temperature and the CYN levels with regards to seasons and stations was given in Figure 3. According to the Figure 3, the CYN levels were consistently changed with the increase or decrease of the seawater temperature between seasons. The correlations between other environmental parameters and CYN were given in Table 5. A positive and significant correlation was found between oPO_4-P and CYN levels ($p<0.05$). Another significant correlation was found between suspended particulate matter (SPM) and CYN levels as -0.342 ($p<0.05$). Also, high positive, but not significant, correlations were also found between CYN levels and dissolved oxygen/light penetration parameters ($p<0.05$). According to the PCA, estimated 2 principal components were statistically sufficient to model the factors ($p<0.05$). The factors used in the PCA were also classified by K-means clustering method and revealed the presence of two distinct groups ($p<0.05$). Following the clustering analysis, CYN, temperature, salinity, pH and oPO_4-P were classified as Group 1, whereas Group 2 was formed by TNO_x, NH_4 and DO. The results of the PCA and K-means clustering analysis were given in Figure 4.

DISCUSSION

This study examines, for the first time, the seasonal variations of the CYN levels in the fronds and stolons of *Caulerpa taxifolia* var. *distichophylla* present in the Levantine coast of Turkey, the Gulf of Iskenderun, including its relationships with various physicochemical parameters of the sea water such as temperature and light.

The sampling stations were comprised of 6 different sites (five of them were in the Gulf of Iskenderun and one station was at the outside of the Gulf) at the beginning of the study. However, it was only possible to collect sufficient amounts of *Caulerpa taxifolia* var. *distichophylla* samples at five different depths from L1, L2 and L3 stations. First, the possible effects of sampling depths on the CYN levels and the temperature were investigated statistically, but there were no significant differences among the studied groups ($p<0.05$, Table 1). Following this finding, the sampling depth was eliminated as a factor for statistical analyses and different sampling depths were taken into consideration as a single station. It was found that the seasonal variation of mean CYN levels both in the fronds and in the stolons were statistically higher in spring and summer compared to autumn and winter ($p<0.05$, Table 2). Also, the CYN levels in the fronds were statistically higher than stolons in all seasons ($p<0.05$, Table 4).

In the literature, number of the studies that support seasonal variations of the secondary metabolite CYN from Caulerpales with (or without) environmental factors are limited [22-25]. On the other hand, there are studies that associate the CYN levels with the seasonal changes of epiphytic organisms present on the marine algae [13, 26, 27]. In the studies performed in the Western Mediterranean, the highest CYN levels from *C. taxifolia* were reported in the seasonal order of summer or autumn, winter and spring [22-25].

Amade and Lemée (1998) [22] have studied the CYN contents in fronds and stolons of *C. taxifolia* from Cap Martin, Monaco at depths of 5, 10, 15, 20 and 30 m. The highest CYN levels were determined in the summer and autumn seasons. It was also reported that the CYN levels were decreased with increasing depth and the highest CYN contents were recorded at 5 m depth above 19 °C. Therefore, the seawater temperature was stated as another factor supporting the CYN production in *C. taxifolia* [22]. The effect of the seawater temperature on CYN production was also discussed

by Millar (2002) [28] for *C. taxifolia* strains found at New South Wales, Australia. It was reported that CYN levels below 10 meters of depth was low in winter and herbivores are seen on *Caulerpa* genus most frequently in winter [28]. In the present study, CYN levels were found higher in spring/summer than autumn/winter seasons. Therefore, our results from Iskenderun strain confirm the increase of CYN production in temperate and warm seasons, especially when the herbivore and epiphyte pressure increases, and its decrease in cold seasons when interspecies competition increases [13, 25, 29].

The results of our study also demonstrated that the mean CYN levels in fronds were higher than the mean CYN levels in stolons. Similar studies carried on this subject were mostly showed similar results [22, 24]. It is known that *Caulerpa* fronds are exposed to higher amounts of herbivore and fouling organism pressure than stolons and thus, the upper sections contain more CYN [30]. However, Dumay et al. (2002) [25] have reported that over a one year period, there were no significant differences between CYN levels in *C. taxifolia* and *C. racemosa* frond and stolons, and that both parts of the tallus employed similar protection against herbivores. On the contrary, it was found by Box et al. (2010) [24] that the CYN levels in the stolons of the native *Caulerpa* species (*C. prolifera*) from the Mediterranean were higher compared to the fronds. Therefore, it cannot be stated that the CYN levels found in fronds and stolons are always the same. In studies performed by Box et al. (2010) [24], highest CYN levels in fronds were found in May, and the highest levels in stolons were found in summer and autumn.

Cavas et al. (2012) [6] investigated seasonal rubisco enzyme activities and caulerpenyne levels in invasive *C. cylindracea* and native *C. prolifera*. They found that no correlation was existed between production of CYN and rubisco enzyme activities in *C. cylindracea* and *C. prolifera.*

Cevik et al. (2012) [29] have reported the presence of many epiphytic organisms and herbivores such as *Bittium reticulatum* on *C. taxifolia* colonies in highest numbers during the winter and it was the dominant species in winter, spring and summer seasons. As a result, variations in CYN levels can be depended not only on the temperature but also on other environmental factors. In the present study, a high positive correlation was found between CYN and oPO_4-P and might be related to the phosphorus limited seawater characteristics of the Eastern Mediterranean. Also, temperature, salinity, pH and oPO_4-P were classified as a closely related group following the clustering and principal component analyses. Therefore, it is obvious that more detailed studies are necessary to determine whether the CYN production is due to fishes, epiphytes, invertebrate herbivores or the hydrographical structure of the area.

The CYN levels found in our study were determined to be much lower than the levels determined for invasive *C. taxifolia* in the Western Mediterranean. Among the Caulerpales members in the Mediterranean, *C. taxifolia* from the Western Mediterranean shows the highest CYN levels. Amade et al. (1994) [31]. reported that the wet weight of CYN can be 0.2% of the total weight in spring and 13% of the total weight in summer in fronds of the Western Mediterranean strain. Our findings showed that even though the CYN contents were lower than the levels in the Mediterranean strain, it was closer to the CYN levels determined at the original range of *C. taxifolia* var. *distichophylla*.

According to the literature, the CYN levels in *C. taxifolia* from the Western Mediterranean is higher than the CYN levels in the natural range of *C. taxifolia* which is a result of the biotic and abiotic conditions of the Mediterranean not considered at all. Comprehensive studies on this subject has demonstrated that the *C. taxifolia* found in the Mediterranean is genetically different and thus demonstrates different physiological (CYN levels etc.) and morphological characteristics compared to its natural range [1, 2, 32-37]. The number of *Caulerpa taxifolia* taxons in the Mediterranean Sea has been increasing because of the heavy ship traffic in the region. The most important prevention method, the control of balast waters, must be taken into consideration by the countries in the region

According to our data, *C. taxifolia* var. *distichophylla* has not revealed remarkable invasive property in our stations yet. However, it's newly existence in Cyprus and Italy might be an indication of danger. Consequently, this new taxon should be monitored regularly.

ACKNOWLEDGEMENTS

The authors thank to Turkish Scientific and Technological Research Council of Turkey (TÜBİTAK) for financial support of the project 109Y284.

REFERENCES

[1] Meinesz, A., Hesse, B. (1991). Introduction of the tropical alga *Caulerpa taxifolia* and its invasion of the Northwestern Mediterranean. Oceanol acta 14(4): 415-426.

[2] Meinesz, A. (1997). Origine et expansion de la forme méditerranéenne de *Caulerpa taxifolia*. Dynamique d'especes marines invasives: application a I'expansion de *Caulerpa*

taxifolia en Méditerranée Paris, France: Lavoisier, 197-207.

[3] Cevik, C., Yokes, M.B., Cavas, L., Erkol, L.I., Derici, O.B, Verlaque, M. (2007). First report of *Caulerpa taxifolia* (Bryopsidales, Chlorophyta) on the Levantine coast (Turkey, eastern Mediterranean). Est Coast Shelf Sci 74(3): 549-556.

[4] Jongma, D.N., Campo, D., Dattolo, E., D'Esposito, D., Duchi, A., Grewe, P., Huisman, J., Verlaque, M., Yokes., M.B., Procaccini, G. (2013). Identity and origin of a slender *Caulerpa taxifolia* strain introduced into the Mediterranean Sea. Bot mar 56(1): 27-39.

[5] Çiçek, B.A., Kurt, O., Taşkın, E., Öztürk, M. (2013). First report of *Caulerpa taxifolia* var. *distichophylla* (Sonder) Verlaque, Huisman and Procaccini (Caulerpaceae, Chlorophyta) from Northern Cyprus. In 40th CIESM Congress–Marseille, France, 28 October-1 November 2013.

[6] Cavas, L., Cengiz, S., Karabay, Z.A. (2012). Seasonal rubisco enzyme activities and caulerpenyne levels in invasive Caulerpa racemosa var. *cylindracea* and native *Caulerpa prolifera*. Medit Mar Sci 13(1): 126-133.

[7] Cavas, L., Pohnert, G. (2010). The potential of Caulerpa spp. for biotechnological and pharmacological applications. In Seaweeds and their role in globally changing environments. pp. 385-397.

[8] Erickson, A.A., Paul, V.J., Van Alstyne, K.L., Kwiatkowski. L.M. (2006). Palatability of macroalgae that use different types of chemical defenses. J Chem Ecol 32(9): 1883-1895.

[9] Pohnert, G., Jung, V (2003). Intracellular compartmentation in the biosynthesis of caulerpenyne: study on intact macroalgae using stable-isotope-labeled precursors. Org lett 5(26): 5091-5093.

[10] Gavagnin, M, Marin, A., Castelluccio, F., Villani, G., Cimino, G. (1994). Defensive relationships between *Caulerpa prolifera* and its shelled sacoglossan predators. J Exp Mar Biol Ecol 175(2): 197-210.

[11] Jung, V., Pohnert, G. (2001). Rapid wound-activated transformation of the green algal defensive metabolite caulerpenyne. Tetrahedron 57(33): 7169-7172.

[12] Hunter, J.D., (2007). Matplotlib: A 2D graphics environment. Computing in science and engineering,. 9(3): p. 90-95.

[13] Sureda, A., Box, A., Deudero, S., Pons, A. (2009). Reciprocal effects of caulerpenyne and intense herbivorism on the antioxidant response of Bittium reticulatum and *Caulerpa taxifolia*. Ecotox environ safe 72(3): 795-801.

[14] Cengiz, S., Cavas, L., Yurdakoc, K., Pohnert, G. (2009). Chromatographic isolation of bioactive agent caulerpenyne from *Caulerpa prolifera*. Turkish Journal of Biochemistry 34: 103-104.

[15] Grasshoff, K., Kremling, K., Ehrhardt, M. (1999). Methods of seawater analysis. Third, completely revised and extended edition. Wiley-VCH, Weinheim 77(89): 160.

[16] Dean Jr., W.E. (1974). Determination of carbonate and organic matter in calcareous sediments and sedimentary rocks by loss on ignition: comparison with other methods. J Sediment Res 44(1).

[17] Bouyoucos, G.J. (1951). A recalibration of the hydrometer method for making mechanical analysis of soils. Agronomy journal 43(9): 434-438.

[18] Kacar, B. (1995). 'Bitki ve topragin kimyasal analizleri: III.' (AÜ Ziraat Fak. Egitim, Arastirma ve Gelistirme Vakfi Yayinlari: Ankara)

[19] Gündoğdu, S. (2014). Su ürünlerinde çoğunlukla uygulanan çoklu karşılaştırma (Post-Hoc) testleri. Journal of FisheriesSciences. com 8(4): 310-316.

[20] SPSS, I. (2011). IBM SPSS statistics for Windows, version 20.0. New York: IBM Corp.

[21] Team, R.C. (2014). A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. (Austria)

[22] Amade, P., Lemee. R, (1998). Chemical defence of the Mediterranean alga *Caulerpa taxifolia*: variations in caulerpenyne production. Aquat Toxicol 43(4): 287-300.

[23] Amade, P., Lemée, R., Pesando, D., Valls, R., Meinesz, A. (1996). Variations de la production de caulerpényne dans *Caulerpa taxifolia* de Méditerranée. In 'Second International Workshop on *Caulerpa taxifolia* Universitat de Barcelona Publ Spain pp. 223-231

[24] Box, A., Sureda, A., Tauler, P., Terrados, J., Marba, N., Pons, A., Deudero, S. (2010). Seasonality of caulerpenyne content in native *Caulerpa prolifera* and invasive *C. taxifolia* and *C. racemosa* var. *cylindracea* in the western Mediterranean Sea. Bot mar 53(4): 367-375.

[25] Dumay, O., Pergent, G., Pergent-Martini, C., Amade, P. (2002). Variations in caulerpenyne contents in *Caulerpa taxifolia* and Caulerpa racemosa. J Chem Ecol 28(2): 343-352.

[26] Baumgartner, F.A., Motti, C.A., de Nys, R., Paul, N.A. (2009). Feeding preferences and host associations of specialist marine herbivores align with quantitative variation in

seaweed secondary metabolites. Mar Ecol Prog Ser 396: 1-12.
[27] Box, A., Sureda, A., Terrados, J., Pons, A., Deudero, S. (2008). Antioxidant response and caulerpenyne production of the alien *Caulerpa taxifolia* (Vahl) epiphytized by the invasive algae Lophocladia lallemandii (Montagne). J Exp Mar Biol Ecol 364(1): 24-28.
[28] Millar, A., Talbot, B. (2002). The introduction of *Caulerpa taxifolia* in New South Wales, Australia. In 'Abstract, international *Caulerpa taxifolia* conference.
[29] Cevik, C., Cavas, L., Mavruk, S., Derici, O.B., Cevik, F. (2012). Macrobenthic assemblages of newly introduced *Caulerpa taxifolia* from the Eastern Mediterranean coast of Turkey. Biol Invasions 14(3): 499-501.
[30] Meyer, K.D. (1992). Intraplant variation in secondary metabolite concentration in three species of Caulerpa (Chlorophyta: Caulerpales) and its effects on herbivorous fishes. Mar Ecol Prog Ser 82: 249-257.
[31] Amade, P., Valls, R., Bouaicha, N, Lemée, R., Artaud, J. (1994). Méthodes de dosage de la caulerpényne produite par *Caulerpa taxifolia*. In 'First International Workshop on *Caulerpa taxifolia*. GIS Posidonie publ., Fr', pp. 163-167
[32] Boudouresque, C.F. (1997). Dynamique des populations de *Caulerpa taxifolia* en Méditerranée, y compris les mécanismes de la compétition interspécifique. Dynamique d'espèces marine invasives: application à l'expansion de *Caulerpa taxifolia* en Méditerranée. Académie des Sciences, Paris. Tec & Doc, Paris, 145-162.
[33] Caye, G., Chambet, P., Delahaye, L., Meinesz, A., Pietkiewicz, D., Ryder, K. (1996). Différences entre *Caulerpa taxifolia* de Méditerranée et *C. taxifolia* des mers tropicales. Second International Worshop on *Caulerpa taxifolia*. Universitat de Barcelona, Barcelona, Spain, 171-176.
[34] Jousson, O., Pawlowski, J., Zaninetti, L., Meinesz, A., Boudouresque, C.F. (1998). Molecular evidence for the aquarium origin of the green alga *Caulerpa taxifolia* introduced to the Mediterranean Sea. Mar Ecol Prog Ser 172(0): 275-280.
[35] Komatsu, T. (1997). Temperature and light responses of the alga *Caulerpa taxifolia* introduced into the Mediterranean Sea. Mar Ecol Prog Ser 146 : 145-153.
[36] Meinesz, A., Boudouresque, C.F. (1996). Sur l'origine de *Caulerpa taxifolia* en Méditerranée. CR Acad Sci Paris Ser III Sci Vie 319: 603–613
[37] Meinesz, A., Pietkiewicz, D., Komatsu, T., Caye, G., Blachier, J., Lemee, R., Renoux-Meunier, A. (1994). Notes taxinomiques preliminaires sur *Caulerpa taxifolia* et Caulerpa mexicana. In: Boudouresque, C-F, Meinesz, A. and Gravez, V. (eds) First International Workshop on *Caulerpa taxifolia*, Nice, 17–18 January 1994, pp 105–114. GIS Posidonie, Marseille

Received: **24.08.2015**
Accepted: **18.12.2015**

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