Seasonality of caulerpenyne content in native *Caulerpa prolifera* and invasive *C. taxifolia* and *C. racemosa* var. *cylindracea* in the western Mediterranean Sea

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Abstract

Fronds had higher mean caulerpenyne concentration than stolons in *Caulerpa prolifera, C. taxifolia* and *C. racemosa*. *C. prolifera* had higher mean caulerpenyne content [$7.29 \pm 0.64\%$ caulerpenyne/frond dry weight (DW)] than *C. taxifolia* ($5.47 \pm 0.32\%$ caulerpenyne/frond DW) and *C. racemosa* ($0.43 \pm 0.07\%$ caulerpenyne/frond DW). *C. prolifera* had reduced content from May to October in fronds, with no clear seasonal pattern in stolons. *C. taxifolia* reached a maximum caulerpenyne value in fronds in May, with a slight decrease until the onset of winter; there was no clear seasonal trend in stolons. Caulerpenyne content remained relatively constant through the year in fronds of *C. racemosa*; stolons showed a clear seasonal pattern of increasing caulerpenyne content in spring, reaching maximum values at the end of summer, and decreasing during winter. *C. prolifera* caulerpenyne contents were positively correlated with densities of the *Caulerpa*-feeding gastropod *Ascobulla fragilis* ($\rho = 0.84$, $p < 0.05$). The main finding of the present work is that *C. prolifera* has higher caulerpenyne concentrations than invasive *Caulerpa* species under similar climate conditions.

Keywords: algal toxins; *Caulerpa*; caulerpenyne; exotic species; toxicity.

Introduction

The presence of introduced species in marine ecosystems is an increasing problem around the world, and is related to the development of international shipping, aquaculture, and the aquarium trade (Verlaque and Fritayre 1994, Boudouresque et al. 1995, Boudouresque 1998, Boudouresque and Verlaque 2002). An introduced species is considered as invasive when self-sustaining populations outside its native area spread and are able to modify the structure of the invaded ecosystems causing ecological and/or economic impact (Sakai et al. 2001, Boudouresque and Verlaque 2002). Escape from biotic constraints (competitors, predators, grazers, parasites) may be one of the mechanisms by which introduced species become successful invaders (Mack et al. 2000, Sakai et al. 2001).

Several macroalgae can deter herbivores by using chemical defenses (Hay and Fenical 1988, Erickson et al. 2006). Species of the order Caulerpales (Chlorophyta) produce sesquiterpenoid and diterpenoid compounds that are toxic and actively deter generalist herbivores (Paul and Fenical 1986, Hay and Fenical 1988, Paul and Van Alstyne 1992). Caulerpenyne is a secondary metabolite synthesized by species of the genus *Caulerpa*; it plays a major role in their chemical defence (Paul and Fenical 1986, Pohnert and Jung 2003) against epiphytes and herbivores (Erickson et al. 2006). Caulerpenyne has also cytotoxic effects on mammalian cells (Parent-Massin et al. 1996, Barbier et al. 2001), inhibits the growth of marine fungi (Paul and Fenical 1986), interferes with the sexual reproduction of sea urchins at different stages (Paul and Fenical 1986, Lemee et al. 1996, Pesando et al. 1996), deters herbivory by gastropods and fishes, and is toxic to fishes (Paul and Fenical 1986). There are examples, however, in which caulerpenyne does not deter fish grazing (Meyer and Paul 1992), indicating that the toxicity is not intrinsic to the compound, but is a result of metabolite-consumer interactions (Paul 1992). Recent studies demonstrated that caulerpenyne is transformed into more toxic and deterrent oxytoxins when the algae are wounded (Gavagnin et al. 1994, Jung and Pohnert 2001). For instance, caulerpenyne has been shown to act as inducer of antioxidant response in the Mediterranean labrid *Coris julis* (Linnaeus) (Sureda et al. 2006).

Three species of *Caulerpa* are present in the western Mediterranean, *C. prolifera* (Forsskål) Lamouroux, *C. taxifolia* (Vahl) C. Agardh, and *C. racemosa* var. *cylindracea* (Sonder) Verlaque, Huisman *et* Boudouresque. *C. prolifera* is native and widely distributed throughout the Mediterranean Sea. An aquarium-maintained strain of *C. taxifolia* was introduced in the Mediterranean Sea in 1984 (Meinesz and Hesse 1991) and rapidly spread through the western basin (Meinesz et al. 2001). This species seems to interfere with vegetative development of the seagrass *Posidonia oceanica* L. Delile (Devillele and Verlaque 1995, Dumay et al. 2002) and reduces algal

diversity of invaded communities (Balata et al. 2004, Verlaque et al. 2004); it has thereby greatly altered the Mediterranean shallow benthic communities in certain locations (Meinesz et al. 1993, Meinesz 2004). *C. racemosa* has been present in the eastern Mediterranean Sea since the early 20th century, likely to have been introduced from the Red Sea through the Suez Canal. However, it is the invasive *C. racemosa* var *cylindracea* variety, originally from southwestern Australia (Verlaque et al. 2003) that has rapidly spread throughout the western Mediterranean during the last 20 years (Verlaque et al. 2000), and has even reached the Canary Islands in the Atlantic Ocean (Verlaque et al. 2004). *C. racemosa* var *cylindracea* affects the development of seagrasses (Ceccherelli and Campo 2002, Dumay et al. 2002), reduces species richness and diversity of macroalgae in the invaded communities (Piazzi et al. 2001, Balata et al. 2004), and changes benthic invertebrate communities (Argyrou et al. 1999, Buia et al. 2001).

The aim of this study was to compare the caulerpenyne content of the native *Caulerpa prolifera* with those of the two invasive species of *Caulerpa* present in the Mediterranean Sea (*C. taxifolia* and *C. racemosa* var. *cylindracea*). We estimated the content of caulerpenyne at monthly intervals over one year taking into account previous studies showing that the content of caulerpenyne changes seasonally in *Caulerpa* species (Amade and Lemee 1998, Dumay et al. 2002), and evaluated its relationship to seasonal conditions reflected by seawater temperature, the biomass of *Caulerpa*, and the abundance of *Caulerpa*-specialized herbivores. The study was performed in three mixed *Caulerpa-Posidonia oceanica* meadows in Mallorca (Balearic Islands, western Mediterranean), all experiencing similar seasonal conditions.

Materials and methods

Sampling locations

Samples of *Caulerpa* species were collected monthly from November 2004 to October 2005 in Mallorca (Balearic Islands, western Mediterranean). Because the three *Caulerpa* species do not co-occur at any location off the island we chose two sampling locations: *C. taxifolia* and *C. prolifera* were collected in Cala D'Or Bay, off southeastern Mallorca (39°22.164′ N/3°13.887′ E and 39°22.028′ N/3°13.739′ E, respectively), and *C. racemosa* var. *cylindracea* (hereafter *C. racemosa*) was collected in Portals Vells Bay, southwestern Mallorca (39°28.321′ N/2°31.320′ E). Both bays face east, and all the *Caulerpa* samples were collected at similar depth (5–6 m) over the rhizome mat of the seagrass *Posidonia oceanica*. This sampling design avoids the effect of depth on the caulerpenyne content of the three species. Seawater temperature was recorded with StowAway® Tidbit® (Onset Computer Corporation, Pocasset, MA, USA) temperature loggers installed permanently at the sampling depths in both bays.

Extraction and quantification of caulerpenyne

Caulerpa species were visually identified and carefully hand-collected by SCUBA divers to prevent the transformation of caulerpenyne into reactive aldehydes, a process driven by the fragmentation of the thallus (Gavagnin et al. 1994, Jung and Pohnert 2001). The algal material was maintained in fresh seawater at ambient temperature until the extraction of caulerpenyne (undertaken within 1 h of collection). In the laboratory, *Caulerpa* fragments were quickly washed in freshwater to remove external salt, dried with absorbent paper, and the fronds and stolons were separated. Each sample of fresh algal material [5 g wet weight (WW)] was placed in 50 ml of methanol for 24 h at 4°C to obtain the crude methanolic extract; for each sampling, five extracts (replicates) of each *Caulerpa* species and algal part (frond, stolon) were prepared. In addition, five replicate samples of 5 g (WW) of each *Caulerpa* species were dried at 70°C for 24 h, then re-weighed to estimate the ratio of WW/dry weight (DW) and in order to compare the concentrations of caulerpenyne that were measured with values reported in the literature.

The methanolic extracts were filtered through silica columns and eluted with 5 ml of a mixture of methanol/ethyl acetate (50:50). The concentration of caulerpenyne was determined using HPLC. The mobile phase consisted of 80:20 methanol/H_2O. The HPLC was a Shimadzu (Shimadzu Europe, Duisburg, Germany) with a diode array detector, and the column was a Nova Pak C_{18}, 3.9×150 mm (Waters HPLC columns, Dublin, Ireland). Caulerpenyne was determined at 254 nm and quantified by comparison with a standard curve of known concentrations. Pure caulerpenyne used to establish a standard curve was provided by Dr. P. Amade (Equipe Antitumoraux Naturels, Université Nice, Sophia Antipolis).

Biomass of *Caulerpa* species and abundance of *Caulerpa*-specialized herbivores

Biomass samples were collected from six quadrats of 20×20 cm in 100% cover patches of each *Caulerpa* species on several dates during the study period (November, December 2004 and February, May, June, July, August, September and October 2005). Gaps in the sampling data (when compared with caulerpenyne assays) were due to the fact that these samples were initially collected for different purposes with a different sampling periodicity, and were only subsequently coupled to the caulerpernyne sampling when some correlations were observed. These samples were collected by hand by SCUBA divers. The algal material was sorted into fronds and stolons, dried for 48 h at 70°C, then weighed to determine biomass as DW. *Caulerpa*-specialized herbivores, such as the sea slug *Ascobulla fragilis* (Jeffreys, 1856) were sorted from samples using a dissecting microscope.

Statistical analysis

Statistical analyses were carried out using the statistical package SPSS 14.0 for Windows. The statistical signifi-

cances of the differences between the concentration of cau-lerpenyne in the fronds and stolons of the three *Caulerpa* species were evaluated using the ANOVA for two groups test, while two-way ANOVA was used to test for significant differences in the content of caulerpenyne between *Caulerpa* species and sampling dates for each thallus part (frond, stolon).

The relationships among the biomass and the concentration of caulerpenyne in the thallus part of each *Caulerpa* species and seawater temperature was evaluated by performing a linear correlation (Pearson) analysis. The relationship between the abundance of *Ascobulla fragilis* in *C. prolifera* and the biomass and caulerpenyne content of this species were also evaluated by linear correlation. Pearson correlations of caulerpenyne concentration between *Caulerpa* species were also used to evaluate the temporal coherence of the concentration of caulerpenyne among species.

Results

The average [±1 standard error (SE)] biomasses (sum of frond and stolons) in the *Caulerpa* meadows were

83.9 ± 10.7 g algae DW m^{-2} for *C. prolifera*, 43.0 ± 6.3 g algae DW m^{-2} for *C. taxifolia* and 16.2 ± 4.2 g algae DW m^{-2} for *C. racemosa* var *cylindracea*. Both stolon and frond biomasses of all *Caulerpa* species changed temporally during our study. *C. prolifera* biomass remained low during the summer months (from July to September 2005) and had maximum values in spring (February 2005 and May 2005 for fronds and February 2005, May 2005 and June 2005 for stolons) (Figure 1A,B). The biomass of *C. taxifolia* fronds showed a clear seasonal pattern, with a maximum at the end of summer (September 2005) and a minimum value at the end of winter (February 2005). The biomass of stolons was higher in summer (May 2005, June 2005 and August 2005) than in the rest of the year, with a maximum stolon biomass peak in May (Figure 1C,D). *C. racemosa* biomass showed a clear seasonal pattern with maximum values for both fronds and stolons in August 2005, and a minimum in February 2005 for fronds and October 2005 for stolons (Figure 1E,F).

All *Caulerpa* species had a higher content of caulerpenyne in fronds than in stolons (Table 1). These differences were significant for *C. prolifera* and *C. taxifolia* but not for *C. racemosa* (Table 1). The content of caulerpenyne in fronds and stolons was highest in *C. prolifera*, while *C. racemosa*
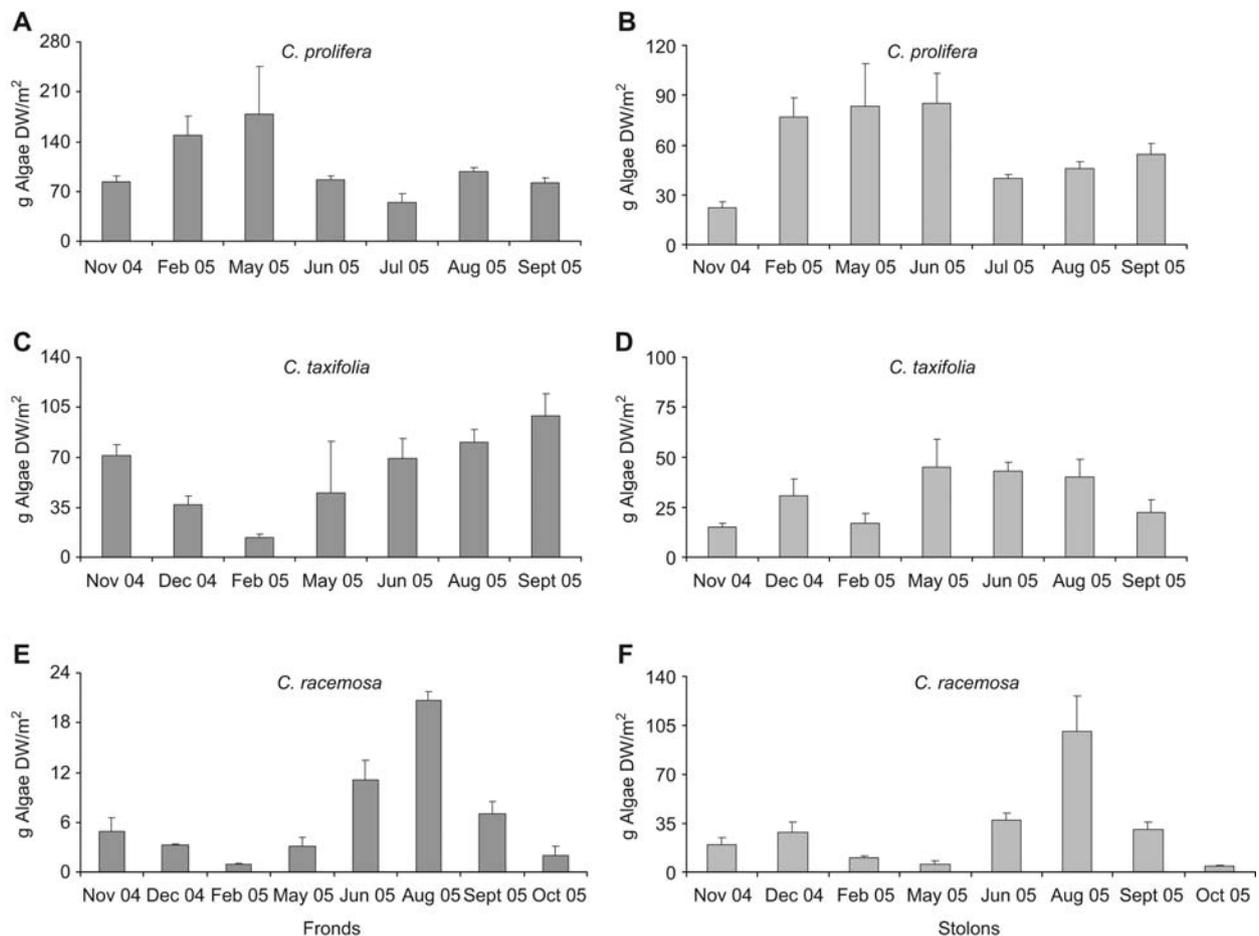

Figure 1 Biomass of fronds (left panels) and stolons (right panels) of three *Caulerpa* species in Mallorca (western Mediterranean) from November 2004 to October 2005.
Values are means+SE.

Table 1 Caulerpenyne mean concentration in *Caulerpa* species (mg caulerpenyne g^{-1} algal dry weight±SE).

Caulerpa species	Thallus part		
	Frond	Stolon	
C. prolifera	72.92±6.44[#]	46.35±2.51[#]	***
C. taxifolia	54.70±3.21[*]	12.73±0.37[*]	***
C. racemosa	4.31±0.79[+]	2.97±1.30[*]	NS

Post hoc DMS results compare caulerpenyne concentrations in each tissue among meadows. Different symbols (#, *, +) denote significantly different groups in columns (ANOVA); *** denotes significant differences between thallus parts within species (in rows) (ANOVA p<0.01); NS indicates no significant differences between thallus parts.

Table 2 Two-way ANOVA (factors: *Caulerpa* species and time) for fronds and stolons.

Source of variation	Thallus part			
	Frond		Stolon	
	Df	MS	Df	MS
Caulerpa species	2	30,840.53***	2	49,256.71***
Time	8	159.40	9	2009.51***
Caulerpa species×time	16	777.92***	18	2896.16***
Residual	81	139.02	90	182.15

*** Denotes significant differences (ANOVA p<0.01).

had the lowest levels (Table 1). The content of caulerpenyne was different between *Caulerpa* species and sampling dates; there was also a significant interaction between species and date (Table 2).

The content of caulerpenyne in fronds of *Caulerpa prolifera* was lower from May 2005 to October 2005 than during the rest of the year, while the content of caulerpenyne in the stolons showed no clear seasonal pattern (Figure 2A,B). None of the correlations among biomass and caulerpenyne content in the fronds and stolons of this species and temperature of the water were significant (Table 3).

The content of caulerpenyne in fronds of *Caulerpa taxifolia* was lowest at the end of winter (February 2005) and then reached a maximum value in May 2005 before decreasing slowly until the last sampling date in October 2005. Except for the relatively high values in April 2005, September 2005, and October 2005, the content of caulerpenyne in the stolons of this species did not change during the year (Figure 2C,D). The content of caulerpenyne in fronds of *C. taxifolia* was positively correlated with water temperature but not with frond biomass (Table 3). The content of caulerpenyne in stolons was not correlated with temperature or stolon biomass.

The content of caulerpenyne in fronds of *Caulerpa racemosa* remained low during the year except for a high value recorded in October 2005. However, the caulerpenyne content in stolons showed clear seasonal pattern, with minimum values at the end of winter (February 2005) and in early spring (March 2005 and April 2005), increasing to reach maximum values at the end of summer (August 2005–October 2005) and (Figure 2E,F). The content of caulerpenyne in the stolons of this species was positively correlated with water temperature (Table 3).

Ascobulla fragilis was the only *Caulerpa*-specialized opisthobranch found amongst the three *Caulerpa* species *A. fragilis* was more frequent and abundant on *C. prolifera* than in the two non-native *Caulerpa* species studied, on which it was found on only one sampling date for *C. taxifolia* and two for *C. racemosa* (Table 4). The abundance of *A. fragilis* in *C. prolifera* mats was higher from November to January than during the rest of the year (Table 4) and it was positively correlated (ρ=0.82, significance p<0.05) with the content of caulerpenyne in the fronds of *C. prolifera*.

The concentration of caulerpenyne in the stolons of *Caulerpa prolifera* was negatively correlated with those of *C. taxifolia* (ρ=-0.36, significance p<0.05) and *C. racemosa* (ρ=-0.30, significance p<0.05), and it was correlated positively between the last two species (ρ=0.49, significance p<0.01). The concentration of caulerpenyne in the fronds was not correlated between *Caulerpa* species.

Discussion

Our results indicate that caulerpenyne concentration differs significantly among *Caulerpa* species, decreasing from *C. prolifera* to *C. taxifolia* and again to *C. racemosa.* The concentration of caulerpenyne was also different between fronds and stolons in *C. prolifera* and *C. taxifolia* but not in *C. racemosa*. The fronds of *C. prolifera* and *C. taxifolia* had higher concentration of caulerpenyne than their respective stolons. Differences in the concentration of caulerpenyne among *Caulerpa* species has been reported previously by other authors (Paul and Hay 1986, Amade and Lemee 1998, Dumay et al. 2002, Jung et al. 2002, Sureda et al. 2006). The concentrations of caulerpenyne quantified in this study fall within the upper range of the concentrations of this compound reported for these *Caulerpa* species elsewhere (Table 5). In addition, and similar to previous studies (Amade and Lemee 1998, Dumay et al. 2002), we found that the concentration of caulerpenyne changes during the year.

Temperature and biomass seasonality were not the main factors determining caulerpenyne concentrations in *C. prolifera* thallus parts. Neither was correlated with caulerpenyne concentration seasonal variations. High densities of *Ascobulla fragilis* were found in *C. prolifera* mats but not in the other studies of *Caulerpa* species *A. fragilis* is a specialized *Caulerpa prolifera* herbivore that uses caulerpenyne derivates as chemical defence (Gavagnin et al. 1994, Marin and Ros 2004). Caulerpenyne concentration in *C. prolifera* fronds was positively correlated with the abundance of *A. fragilis.* Previous studies concluded that algal toxicity could be increased as result of herbivore pressure, which could drive an increase in concentration in the defensive metabolites in the algae (Van Alstyne 1988, Paul and Van Alstyne 1992, Erickson et al. 2006, Sureda et al. 2009).
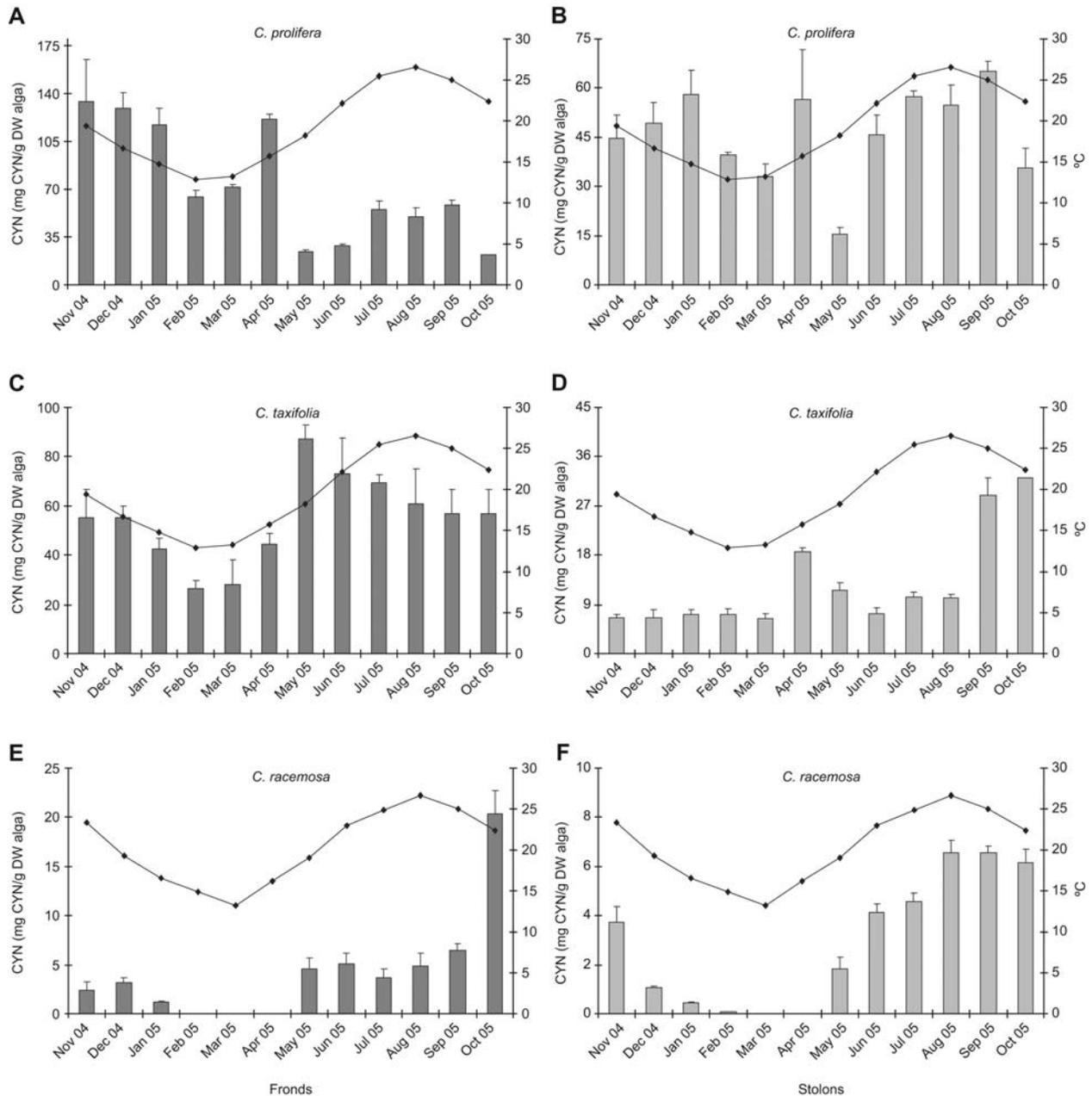
Figure 2 Concentration of caulerpenyne (CYN) expressed as mg CYN g^{-1} dry weight of alga in the fronds (left panels) and stolons (right panels) of three *Caulerpa* species in Mallorca (western Mediterranean) from November 2004 to October 2005.
The solid line with triangles represents temperature of seawater. Caulerpenyne values are mean+SE.

Caulerpenyne concentration in *Caulerpa taxifolia* was 2–10 times higher in fronds than stolons, depending on the period of the year. In *C. taxifolia,* the highest caulerpenyne concentration was found in May 2005 in fronds and in summer-autumn (September 2005/October 2005) in stolons. The increase in caulerpenyne concentration during spring was observed earlier in stolons (April) than in fronds (May). These values corresponded to a low biomass period of fronds and stolons of *C. taxifolia* and appeared just before the increase in *C. taxifolia* biomass. *C. taxifolia* colonization around the Balearic Islands has been patchy and, where present, it does not form dense meadows. Moreover, only a few

C. taxifolia fronds and stolons remain in seagrass meadows over dead *Posidonia oceanica* rhizomes in winter. An increase in water temperature provides a better environment for the growth of *C. taxifolia*, which increases its biomass and protects the new growth with secondary metabolites. Caulerpenyne concentration in *C. taxifolia* stolons also exhibited another seasonal pattern, with an important increase at the end of summer. These results are similar to those obtained in previous studies (Amade and Lemee 1998, Dumay et al. 2002), which reported higher caulerpenyne concentration in summer and autumn, but these studies were performed in monospecific *Caulerpa* meadows that had high

Table 3   Pearson bivariate correlations between caulerpenyne concentration in thallus parts and biomass and temperature.

	Fronds		Stolons	
	Biomass	Temperature	Biomass	Temperature
C. prolifera	NS	NS	NS	NS
C. taxifolia	NS	0.644[*]	NS	NS
C. racemosa	NS	NS	NS	0.922[**]

NS denotes no significant correlation; * denotes significance $p<0.05$; ** denotes significance $p<0.01$.

Table 4   *Ascobulla fragilis* abundance (individuals m[-2]) in *Caulerpa* mats.

Dates	*Ascobulla fragilis*		
	C. prolifera	*C. taxifolia*	*C. racemosa*
November 2004	408±144.01	16.67±5.89	33.33±15.90
December 2004	300±89.02		33.33±23.57
January 2005	442±123.01		
February 2005	58±15.53		
May 2005	175±58.00		
June 2005	67±43.12		
July 2005	59±41.14		
August 2005	8±4.12		

Values are means±SE.

biomass values throughout the year. Caulerpenyne concentration in *C. taxifolia* fronds was positively correlated with temperature; when the temperature was over 18°C (May 2005), a significant increase in caulerpenyne concentration occurred. Amade and Lemee (1998) found this important increase in caulerpenyne concentration in June at temperatures over 20°C in samples obtained over a rocky substratum in Cap Martin (with high *C. taxifolia* winter biomass).

Caulerpa racemosa is the most recent *Caulerpa* species in Balearic Islands waters (Ballesteros et al. 1999). It has colonized larger areas around these islands than *C. taxifolia* (personal observations). In agreement with previous works (Dumay et al. 2002, Jung et al. 2002), *C. racemosa* had lower caulerpenyne concentration than the other two *Caulerpa* species studied (Table 5). The influence of temperature on caulerpenyne concentration in *C. racemosa* was observed in stolons (positive correlation). The seasonality observed in caulerpenyne concentration was similar to that described by Dumay et al. (2002) over a 1 year cycle. Caulerpenyne concentration did not differ significantly between fronds and stolons, which suggests that both thallus parts are similarly protected against herbivores.

This study showed different seasonal variations in caulerpenyne content among three *Caulerpa* species inhabiting the

Table 5   Concentration of caulerpenyne in *Caulerpa* species.

Reference	*Caulerpa* species	Caulerpenyne concentration in fronds and stolons (‰ WW)	Caulerpenyne concentration in fronds (% DW)	Caulerpenyne concentration in stolons (% DW)	Caulerpenyne concentration in fronds and stolons (% DW)	Location
Amico et al. 1978	*C. prolifera*				0.35	Mediterranean
Gavagnin et al. 1994	*C. prolifera*		0.16–0.5	0.22–0.66		Italy, Naples Bay
Jung et al. 2002	*C. prolifera*	6			3.22[a]	South France
Sureda et al. 2006	*C. prolifera*				4.5	Balearic Islands, Spain
This study	*C. prolifera*		7.29±0.64	4.64±0.25		Balearic Islands, Spain
Guerriero et al. 1992	*C. taxifolia*	1.95			0.12[b]	France, Cap Martin
Amade et al. 1994	*C. taxifolia*				0.16	France, Cap Martin
Amade and Lemee 1998	*C. taxifolia*	1.5–12.5			1.16–9.69[b]	France, Cap Martin, 10 m depth
Dumay et al. 2002	*C. taxifolia*	3.70 (max. value)			2.87[b]	Alpes Maritime, France
Jung et al. 2002	*C. taxifolia*	6			4.65[b]	South France
Sureda et al. 2006	*C. taxifolia*				3.0	Balearic Islands, Spain
This study	*C. taxifolia*		5.47±0.32	1.27±0.03		Balearic Islands, Spain
Meyer and Paul 1992	*C. racemosa*		0.5	0.3		Guam
Meyer and Paul 1992	*C. racemosa*		0.75	0.25		Bahamas
Dumay et al. 2002	*C. racemosa*	0.07 (max. value)			0.08[c]	Alpes Maritimes, France
Jung et al. 2002	*C. racemosa*	3			0.33[c]	South France
This study	*C. racemosa*		0.43±0.07	0.30±0.13		Balearic Islands, Spain

[a] Calculated using a WW to DW conversion factor of 0.19. [b] Calculated using a WW to DW conversion factor of 0.13. [c] Calculated using a WW to DW conversion factor of 0.09.

Balearic Islands under similar seasonal and climate conditions. Caulerpenyne concentrations in stolons of *C. prolifera* were negatively correlated with the caulerpenyne content in stolons of both invasive *Caulerpa* species as the Pearson results show. Caulerpenyne concentrations in stolons of both invasive *Caulerpa* species were positively correlated between species, confirming similar trends in the caulerpenyne concentrations for invasive species. The highest caulerpenyne concentrations in stolons were recorded in the native Mediterranean *Caulerpa* species (*C. prolifera).* This may be explained as a response to grazing pressure, with *C. prolifera* being more chemically protected against direct herbivory than invasive *Caulerpa* species. Similarly, Sureda et al. (2009) found an increase in caulerpenyne concentration in *C. taxifolia* fronds grazed by *Bittium reticulatum* (da Costa, 1778). This increase in caulerpenyne concentration in fronds was reached in less than a week after the introduction of the *Caulerpa* consumer under controlled conditions (Sureda et al. 2009). Therefore, a time-lag between the presence of *A. fragilis* and caulerpenyne concentration in *C. prolifera* is not expected. To explain caulerpenyne production, factors other than herbivores, such as epiphytes, must be considered. Epiphytism of *Caulerpa* species by other macroalgae might also activate caulerpenyne production as a method of reducing the growth of epiphytes (Box et al. 2008). Further research is needed to determine the possible real effects of epiphytes on calulerpenyne production.

In invasive *Caulerpa* species, temperature was correlated with the concentration of caulerpenyne in fronds of *C. taxifolia* and stolons of *C. racemosa*. Previous studies have also shown the highest concentrations of caulerpenyne to be coincident with higher temperatures (Amade and Lemee 1998, Dumay et al. 2002). *C. taxifolia* fronds have higher caulerpenyne concentrations than stolons, while in *C. racemosa*, similar concentrations of caulerpenyne were found in both fronds and stolons. Each *Caulerpa* species showed the highest concentration of caulerpenyne in the thallus part where the highest proportion of biomass is recorded (*C. prolifera* and *C. taxifolia* highest biomass and caulerpenyne concentration in fronds and *C. racemosa* had similar caulerpenyne concentration in both tissues). Field results for *C. prolifera* did not reflect the seasonal cycle in fronds and stolons with the temperature in our region of study, and the presence of small *Caulerpa* herbivores may influence caulerpenyne production.

All three *Caulerpa* species contain higher caulerpenyne concentrations in fronds than stolons, probably due to the photosynthetic activity and higher exposure of fronds to consumers than stolons. This is the only coincidence between the three species because, under similar climate conditions and seasonality, *C. prolifera* is the species with the highest caulerpenyne concentration in Balearic Islands followed by *C. taxifolia* and *C. racemosa*. Despite the fact that the expansion of invasive *Caulerpa* species does not seem to depend on the caulerpenyne concentration, the massive *Caulerpa* biomass degradation observed in invasive species, could lead to a caulerpenyne accumulation (or its derivates) in the substratum that may affect possible competitors, as previously reported for *Cymodocea nodosa* (Ucria) Ascherson (Raniello et al. 2007). Temperature is an important factor influencing the caulerpenyne concentration in invasive *Caulerpa* species, but not in *C. prolifera*. However, the concentration of caulerpenyne in *C. prolifera* fronds was correlated with the abundance of the specialised herbivore *Ascobulla fragilis*, which might suggest that the feeding activity of this herbivore influences the concentration of caulerpenyne. Therefore, further studies are needed to evaluate different aspects of the physiology and seasonality on the toxicity of *Caulerpa*, on the biotic interactions with other plant species, on the effects of temperature and on herbivore pressure to discern the main factors involved in caulerpenyne production.

Acknowledgements

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