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REPORT

Reproductive cycle and trophic ecology in deep versus shallow populations of the Mediterranean gorgonian *Eunicella singularis* (Cap de Creus, northwestern Mediterranean Sea)

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Abstract The annual gonad development of a shallow (20 m depth) population of the Mediterranean gorgonian *Eunicella singularis* was found to be closely synchronized with that of a deep (60 m depth) population, but differences were observed in the gonadal output, with the shallow population producing more and larger sexual products. Lipid content in the shallow population showed a marked seasonality, peaking during summer. In contrast, lipid content remained persistently lower in the deep population. Fatty acids as well as C/N composition were also seasonal in the shallow population and more constant in the deep one. The isotopic composition (δ^{15}N and δ^{13}C) of the shallow colonies was similar to values observed for passive suspension feeders with symbiotic algae, whereas the deep colonies exhibited values similar to those of aposymbiotic passive suspension feeders that primarily feed on micro-zooplankton and particulate organic matter. These results highlight the importance of considering the depth-related variability among populations in order to achieve a better understanding of the ecology of sessile benthic suspension feeders.

Keywords Gorgonian · Reproduction · Energy storage · Fatty acids · δ^{15}N–δ^{13}C · Mediterranean Sea

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Introduction

The study of spatial variability in the ecology of sessile benthic suspension feeders is vital for understanding the potential adaptability of species to local conditions and to achieve a better understanding of their ecology and the role they play in marine benthic ecosystems. Both the ecology and physiology of these organisms are strongly dependent on environmental features such as water temperature, current speed, light, and the quantity and quality of available food (Gili and Coma 1998; Gardner 2000). In coastal areas, these factors may change considerably with depth, even among close locations (Rossi et al. 2003). Strong hydrodynamic forces influence shallow bottoms; by contrast, deeper sublittoral bottoms are mostly sheltered from the direct physical damage caused by strong storm-induced waves (Hiscock 1983). Light intensity decreases exponentially with depth (Drew 1983), and in temperate seas, high irradiance during the summer induces strong water column stratification that can result in severe depletion of suspended material in shallower waters (Coma et al. 2000).

Marine hard-bottom communities located at intermediate depths, particularly coastal rocky bottoms at 40–150 m, have received relatively little attention because they are below scuba depth (Menza et al. 2008; Rooney et al. 2010), and most submersible-based research has been traditionally conducted at depths below 150 m (Sink et al. 2006; Virgilio et al. 2006; Hinderstein et al. 2010). Research in

tropical coastal areas has primarily focused on coral reefs within the depth range of traditional scuba diving (approximately 40 m depth), and much of our understanding of coral reef ecology is based on these relatively shallow depths (Rooney et al. 2010). Far less is known about the mesophotic zone (Menza et al. 2008), which is defined as the deeper region of the photic zone in which light-dependent coral communities develop (Ginsburg 2007). This zone ranges between 30 and 40 m depth to the deeper part of the photic zone, which varies by location and extends to over 150 m in some regions (Hinderstein et al. 2010). This depth range accounts for two-thirds of the total depth range of symbiotic coral environments but has remained largely unexplored (Bongaerts et al. 2010). Enabled by advanced technologies, mesophotic coral ecosystem studies have revealed extensive, productive and rich communities, which differ significantly from their shallow-water counterparts (Bongaerts et al. 2010; Kahng et al. 2010). Additionally, mesophotic zone processes may have a global relevance in the biogeochemical cycles, as well as the maintenance of biodiversity, which has yet to be understood (Buesseler et al. 2007; Hinderstein et al. 2010).

Recently, several studies using remotely operated vehicles (ROVs) have provided quantitative information on Mediterranean sublittoral hard-bottom communities located 40–150 m deep. These studies have reported dense populations of corals and gorgonians dwelling on sublittoral bottoms as deep as 100 m (Rossi et al. 2008; Bo et al. 2009, 2011; Gori et al. 2011a). Gorgonians (Octocorallia, Alcyonacea) are among the main structural species (as defined by Jones et al. 1994) of the Mediterranean coralligenous and precoralligenous communities (True 1970; Gili and Ros 1985; Harmelin and Garrabou 2005), whose diversity and richness in animal species is comparable with those of tropical coral reefs (Ros et al. 1985; Ballesteros 2006). Among Mediterranean gorgonians, the white gorgonian *Eunicella singularis* (Esper, 1794) is one of the most abundant species in both the shallow and deep sublittoral bottoms of the Western Mediterranean (Carpine and Grasshoff 1975). This species is long-lived and gonochoric, and its shallow populations reproduce annually in late May and June (Ribes et al. 2007; Gori et al. 2007). It is an internal brooder, and the planula larvae settle within a few days near the parental colony (Théodor 1967; Weinberg and Weinberg 1979). Small, non-reproductive colonies have been shown to dominate the shallow populations of this species (Linares et al. 2008), which contrasts with the dominating medium-sized colonies and larger gorgonian patches at 60 m (Gori et al. 2011b). Depth-related morphological variation has been described wherein two distinct morphotypes exist above and below the summer thermocline; the presence of symbiotic algae (*Symbiodinium* spp.) is the main characteristic that distinguishes the shallow morphotype (Théodor 1969; Gori et al. 2012). The presence of symbiotic algae, as well as depth-related differences in the key environmental features, vitally shapes the trophic ecology of octocoral species (Fitt and Pardy 1981; Tsounis et al. 2006a) and strongly influences processes such as growth and reproduction (Sebens 1987; Grigg 1977; Benayahu and Loya 1983) even within the depth range between 40 m and the surface. However, few studies have analyzed how the biological processes of a gorgonian species vary over more extensive depth ranges, taking into account deep populations that are not stressed by the summer constraints occurring above the thermocline (Coma et al. 2000).

In this work, several approaches and methodologies have been used to address a comparison of the reproduction and trophic ecology of the gorgonian *E. singularis* in shallow (20 m) versus deep (60 m) sublittoral populations and to relate them with the main environmental features experienced by the species at the two different depths. The main goal was begin to explore the depth-related variability in the ecology of a benthic suspension feeder among populations settled in the same area but affected by different environmental conditions. We analyzed annual gonad development, carbohydrate, protein and lipid content, and the fatty acids and the stable isotope composition over an annual cycle to address the following questions: (1) Are there differences in the reproductive timing and gonadal output between shallow and deep populations? (2) Are there differences in the energy storage between shallow and deep populations? (3) Are there differences in the primary food sources between shallow and deep populations?

Methods

Study area and sampling procedure

Two populations of *E. singularis* were studied on the Cap de Creus (42°18′44″N; 003°19′05″E) in the northwestern Mediterranean Sea (Fig. 1). The first population was located at a depth of 18–20 m and extended over an area of approximately 4,000 m^2, whereas the second population was located at a depth of 55–60 m and extended over an area of approximately 2,500 m^2. Gorgonian colonies were haphazardly sampled by scuba diving monthly from June 2009 to July 2010; due to bad weather conditions, samples from October and December 2009, February and March 2010 are absent. *E. singularis* colonies 20–30 cm (±0.5 cm) in maximum height (the distance from the base to the highest point) were sampled (sexually mature colonies, Ribes et al. 2007). During each sampling, a fragment of a primary branch from each of 15 different colonies

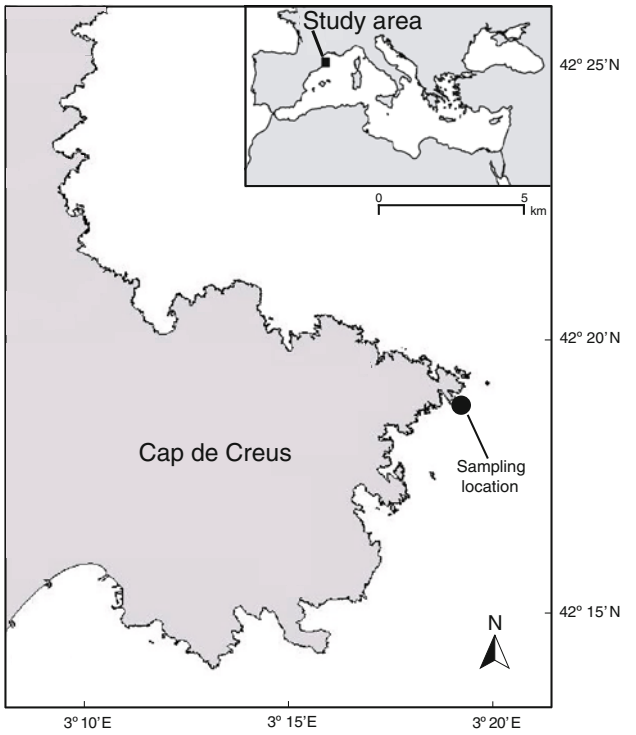


Fig. 1 Map of the study area. The position of the sampling location along the Cap de Creus coast is indicated

haphazardly selected from each population was collected and divided into two portions. To study the annual gonad development, one portion was fixed in 10 % formalin, whereas the other portion was frozen at −20 °C for biochemical analyses. The portions stored for biochemical testing were lyophilized for 16 h at −110 °C and at 100 mbar pressure with a Telstar Lyo Alfa 6 lyophilizer, due to a problem during the lyophilization, samples from the deep population from July 2009 are absent. A total of 150 and 135 branches, sampled from colonies haphazardly selected at each sampling interval, were collected and examined for the shallow and the deep populations, respectively.

Annual gonad development

Colonies of the two populations were examined monthly for the presence of mature gonads inside the polyps to determine their reproductive state. The sex of each sample was determined by the gonadal color and appearance (Ribes et al. 2007). Colonies lacking gonads inside the polyps were considered indeterminate. For gonads with appearance that yielded a doubtful sex determination, histological analysis was used to confirm colony sex. Samples were rinsed in distilled water and dehydrated in 70, 96, and 100 % ethanol solutions. Next, the samples were submerged in ethanol:resin (1:1) for 2 h, embedded in the biphasic resin (Technovit 7100) and stored in the dark at 4 °C for 48 h. After dehydration, the samples were encased in the resin and left to harden at room temperature. The branches were cut into 3 µm longitudinal sections, stained with hematoxylin and eosin, and observed under a microscope (Meiji, 100x) to determine their sex. Between 3 and 10 sections, with approximately 5–7 polyps each, were examined from each sample.

The timing of the annual gonad development was assessed with a stereo microscope (Wild, 50x) by measuring the diameter of all oocytes and spermaries in 5 polyps of each mature sampled colony while avoiding apical-ends (3 cm from the branch tip), where gonadal output may be affected by annual growth. Gonad diameter was measured with an eyepiece micrometer (± 10 µm). When the gonads looked like ellipses, both minor (a) and major (b) diameters were measured, and the diameter of a sphere with an equivalent volume was calculated ($d = 2\ ((a/2)^2 \times b/2)^{1/3}$). To estimate the gonadal volume per polyp, diameters (d) were transformed into volume ($V = 4/3\pi(d/2)^3$). A total of 750 polyps from each population were examined, and more than 1,500 and 900 gonads were measured from the shallow and the deep populations, respectively.

Biochemical analyses

Gorgonian organic matter in the coenenchyme was assessed using monthly samples of 7 colonies from each population. Approximately 12 mg (±0.01 mg) of coenenchyme dry weight from each sample was reduced to ash for 4 h at 500 °C in a muffle (Relp 2H-M9), and the weight of organic matter (OM hereafter) was calculated as the difference between the coenenchyme dry weight and ash weight (Slattery and McClintock 1995).

Five colonies were sampled monthly from each depth in order to determine carbohydrate, protein, and lipid content. Approximately 6 mg (±0.01 mg) of coenenchyme dry weight from each sample was homogenized in 3 ml of double distilled water, and carbohydrates were quantified colorimetrically (Dubois et al. 1956) with glucose as a standard. Approximately 6 mg (±0.01 mg) of coenenchyme dry weight from each sample was homogenized in 2 ml 1 N NaOH, and proteins were quantified colorimetrically (Lowry et al. 1951) with albumin as a standard. Finally, approximately 10 mg (±0.01 mg) of coenenchyme dry weight from each sample was homogenized in 3 ml of chloroform:methanol (2:1), and total lipids were quantified colorimetrically (Barnes and Blackstock 1973) with cholesterol as a standard. The results are presented in µg carbohydrate–protein–lipid mg^{-1} of OM.

Trophic markers

Three colonies from each population were sampled monthly to determine fatty acid composition. Approximately 12 mg (±0.01 mg) of coenenchyme dry weight from each sample was dissolved in dichloromethane/methanol (3:1) and fatty acids were quantified with gas chromatography technique (Electronic Supplementary Material, ESM 1) (Soler-Membrives et al. 2011). The carbon/nitrogen (C/N) ratio and stable isotope (δ^{15}N and δ^{13}C) composition of the gorgonian tissue were assessed from monthly samples of 3 colonies from each population. Approximately 6 mg (±0.01 mg) of coenenchyme dry weight from each sample was fumed with concentrated HCl for 48 h to eliminate the inorganic fraction, and the C/N ratio and stable isotope composition were determined with a Thermo FlashEA 1112 analyzer and a Thermo Delta V Advantage spectrometer (Jacob et al. 2006; Carlier et al. 2007).

Environmental conditions

Environmental conditions at the sampling location were monitored monthly from September 2009 to August 2010. Water temperature, salinity, fluorescence, turbidity, and photosynthetically active radiation (PAR, 400–700 nm) were measured at 1 m depth intervals from 5 to 60 m with Seabird 25 and Seabird 19 conductivity temperature and depth sensors (CTDs) equipped with a Seapoint Chlorophyll Fluorometer, a Seapoint Turbidity Meter, a Biospherical Instruments Inc QSP-2300 and a Li-Cor underwater spherical quantum sensor LI-193. Recorded turbidity values expressed in formazin turbidity units (FTU) were subsequently converted into suspended sediment concentrations (SSC, mg/L) (SSC = 1.21 FTU + 0.43, Guillén et al. 2000). Sea water temperatures at 20 and 60 m were recorded every hour from August 2009 to August 2010 with Hobo Pro V2 temperature data loggers placed in the middle of each studied gorgonian population. Unfortunately, data loggers were repeatedly stolen during the study period, causing a partial loss of data recorded in May, June and August 2010 at 20 m as well as of data recorded in July 2010 at 60 m.

Statistical analyses

Monthly differences between populations in gonadal volume per polyp, organic matter in the coenenchyme, content of carbohydrates, proteins, lipids, and the C/N ratio were tested using the non-parametric Wilcoxon–Mann–Whitney test because the data were not normality distributed. The test was performed with the R-language function Wilcox.test of the R software platform (R Development Core Team 2007). Seasonal differences in organic matter, carbohydrates, proteins, lipids, and C/N within each population were tested using the distance-based permutational multivariate analysis of variance (PERMANOVA) (Anderson 2001) performed using the PERMANOVA.exe software (Anderson 2005). Each term of the analysis was tested using 9,999 random permutations of appropriate units (Anderson and ter Braak 2003), and significant terms were investigated using a posteriori pairwise comparisons with the PERMANOVA t statistic and 9,999 permutations.

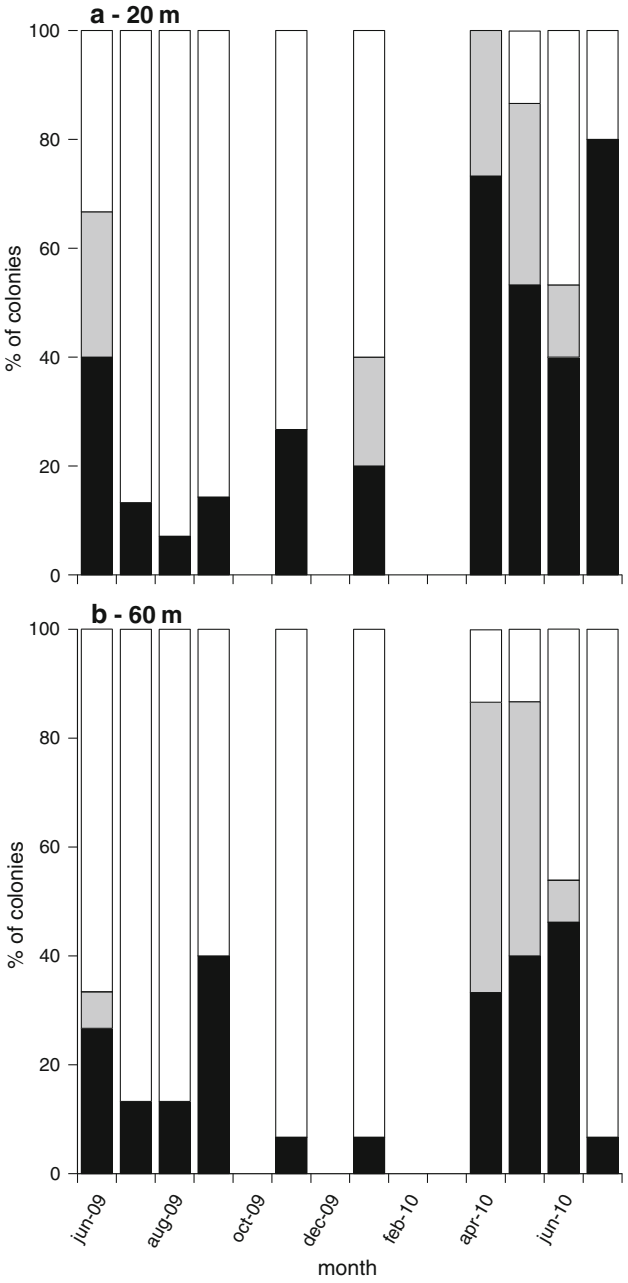


Fig. 2 Reproductive state of *E. singularis* colonies from the *shallow* (**a**) and *deep* (**b**) populations; female colonies are indicated in *black*, male colonies are indicated in *gray*, and indeterminate colonies are indicated in *white*

An ordination of the analyzed colonies ($n = 53$) based on their fatty acid composition was obtained with a principal component analysis (PCA) performed on transformed data ($p' = \arcsin(p^{1/2})$) with the R-language function Princomp, which is available in the Vegan library (Oksanen et al. 2005) of the R software platform.

Results

Annual gonad development

Female colonies were found in both populations throughout the entire study period; conversely, male colonies were present in both populations from April to June and in the shallow population also in January (Fig. 2). In both the shallow and deep populations, *E. singularis* female colonies exhibited two overlapping oocyte size cohorts. The first consisted of oocytes with diameters between 50 and 300 μm, whereas the second cohort consisted of oocytes with diameters between 300 and 900 μm. Eggs matured during the summer and planulae were released between May and July, and between May and June in the shallow and deep populations, respectively (Fig. 3, Table 1). In the shallow population, an average of 0.03 ± 0.18 (total number of larvae observed, $n = 1$) and 0.30 ± 0.48 (total number of larvae observed, $n = 3$) larvae per female polyp was observed in June and July 2009, respectively, whereas an average of 0.93 ± 1.31 (total number of larvae observed, $n = 28$) and 0.45 ± 0.77 (total number of larvae observed, $n = 27$) larvae per female polyp was observed in June and July 2010, respectively. By contrast, in the deep population, an average of 0.10 ± 0.31 (total number of larvae observed, $n = 2$) larvae per female polyp was observed in June 2009, and an average of 0.30 ± 0.47 (total number of larvae observed, $n = 9$) larvae per female polyp was observed in June 2010. Male gonadal development cycle began earlier in the shallow than in the deep population (Fig. 4), with male gametes spawned in both populations between May and June (the larger amount) and between June and July (the remaining ones) (Fig. 4). The shallow population exhibited predominantly higher gonadal volumes. Significant differences (Wilcoxon–Mann–Whitney test, $p < 0.05$) were observed in the gonadal volume in the female colonies, in June 2009, January and June 2010 (Fig. 5a) in the female colonies, whereas significant differences between the male colonies were observed in June 2009 and April 2010 (Fig. 5b).

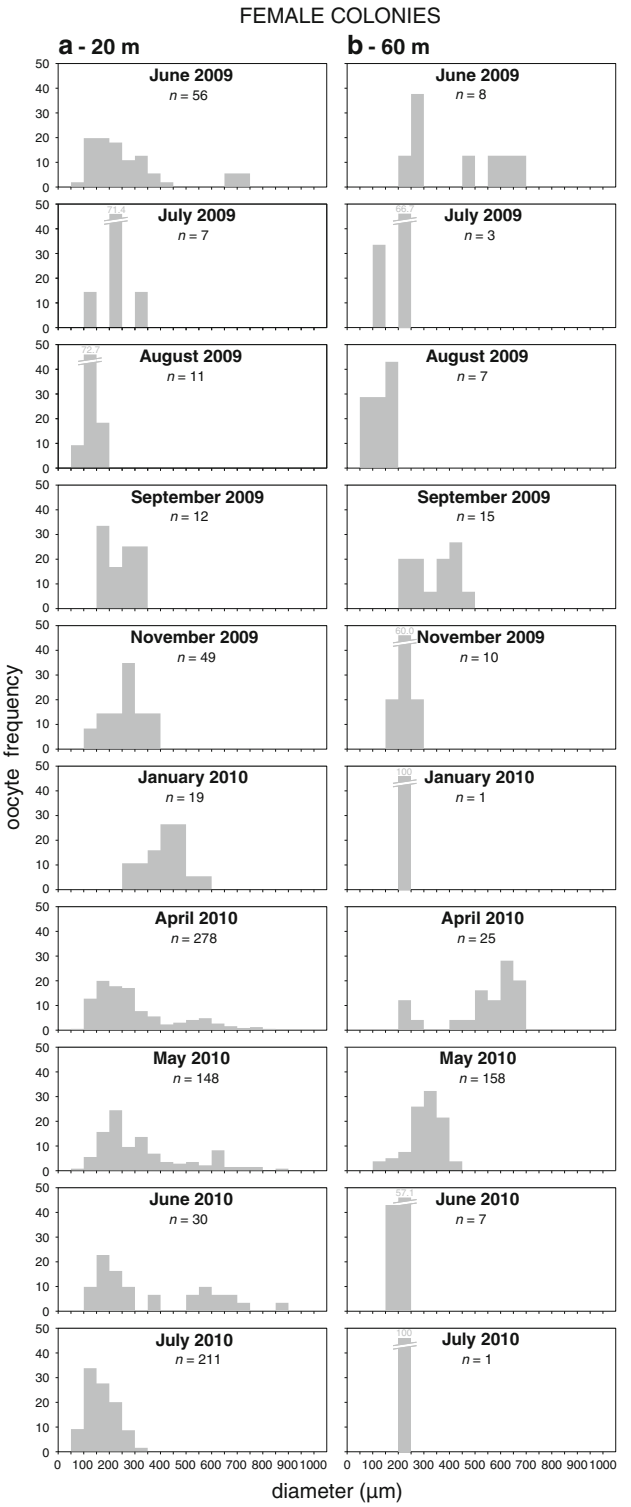


Fig. 3 Frequency of gonadal diameter (μm) in female *E. singularis* colonies from the *shallow* (**a**) and *deep* (**b**) populations (n = gonad number)

Biochemical analyses

Annually, organic matter averaged 26.2 ± 4.7 % of the coenenchyma dry weight in the shallow population and 21.9 ± 4.5 % in the deep population. The percentage of organic matter in the coenenchyme was variable throughout the studied period in the shallow population (Tables 2

Table 1 Monthly changes in diameter and number of *Eunicella singularis* gonads in the studied populations (mean ± SE)

Month	Gonad diameter (μm)		Gonad number/polyp	
	20 m	60 m	20 m	60 m
Female colonies				
June 2009	266 ± 23	423 ± 65	1.9 ± 0.3	0.4 ± 0.2
July 2009	215 ± 21	177 ± 23	0.7 ± 0.3	0.3 ± 0.2
August 2009	131 ± 8	129 ± 14	2.2 ± 0.4	0.6 ± 0.4
September 2009	247 ± 18	339 ± 23	1.2 ± 0.5	0.5 ± 0.1
November 2009	155 ± 11	221 ± 9	2.5 ± 0.4	2.5 ± 0.6
January 2010	414 ± 19	200 ± 0	1.3 ± 0.3	0.2 ± 0.2
April 2010	285 ± 10	535 ± 30	5.1 ± 0.5	1.0 ± 0.2
May 2010	320 ± 14	300 ± 5	3.7 ± 0.7	5.3 ± 0.9
June 2010	351 ± 40	187 ± 8	0.9 ± 0.2	0.3 ± 0.3
July 2010	163 ± 4	200 ± 0	3.5 ± 0.7	0.2 ± 0.2
Male colonies				
June 2009	340 ± 11	250 ± 0	3.3 ± 0.7	0.2 ± 0.2
July 2009				
August 2009				
September 2009				
November 2009				
January 2010	217 ± 11		3.3 ± 0.6	
April 2010	234 ± 5	244 ± 4	13.7 ± 1.5	8.8 ± 1.0
May 2010	270 ± 5	304 ± 4	10.7 ± 1.7	6.9 ± 1.1
June 2010	142 ± 8	158 ± 7	4.3 ± 2.0	4.2 ± 1.0
July 2010				

and 3) where it was significantly higher then in the deep population (Wilcoxon–Mann–Whitney test, $p < 0.05$) in January and from May to July 2010 (Fig. 6). Carbohydrate content was variable between seasons in both populations (Tables 2 and 3), and significantly higher (Wilcoxon–Mann–Whitney test, $p < 0.05$) in the shallow population throughout the entire study period (Fig. 7a). Protein content exhibited lower values (Wilcoxon–Mann–Whitney test, $p < 0.05$) in the shallow than in the deep population in August 2009 and July 2010, but overall there were no significant differences between populations as well as no seasonality (Table 2 and 3) (Fig. 7b). Conversely, lipid content exhibited seasonal changes in the shallow population (Tables 2 and 3), with significantly (Wilcoxon–Mann–Whitney test, $p < 0.05$) higher values in August 2009, January and from May to July 2010 than those of the deep population, which showed lower and more constant values (Fig. 7c).

Trophic markers

A total of 36 fatty acids were identified in colonies from both populations (ESM 2). The first two principal components explained 72.4 % of the data variance in the fatty acid composition of the colonies; the first axis explained 41.8 %. The PCA revealed two groups formed by the shallow and deep colonies sampled during the spring and summer. Conversely, colonies from both populations sampled during the winter were mixed in a third, weaker group (Fig. 8). A covariance was noted between C20:4(n-6) and C20:5(n-3), and among C18:1(n-7), C18:0 and C24:1(n-9). A weaker covariance was observed among C18:2(n-6), C16:1(n-7), C22:6(n-3), and C16:0 (Fig. 8).

The C/N ratio exhibited strong seasonality in the shallow population, whereas seasonality was much weaker in the deep one (Tables 2 and 3). In the shallow population, the summer values were significantly higher (Wilcoxon–Mann–Whitney test, $p < 0.05$) than in the deep population (Fig. 9). Finally, the samples from the deep and shallow populations exhibited a clear separation within their stable isotope composition with the deep population colonies universally exhibited higher $\delta^{15}N$ values than the shallow colonies. The $\delta^{13}C$ composition was more variable from month to month in the shallow than in the deep population (Fig. 10).

Environmental conditions

Water column stratification began to develop in April, wherein a pycnocline formed around 30 m but partially broke at the end of June due to strong winds. The water column was

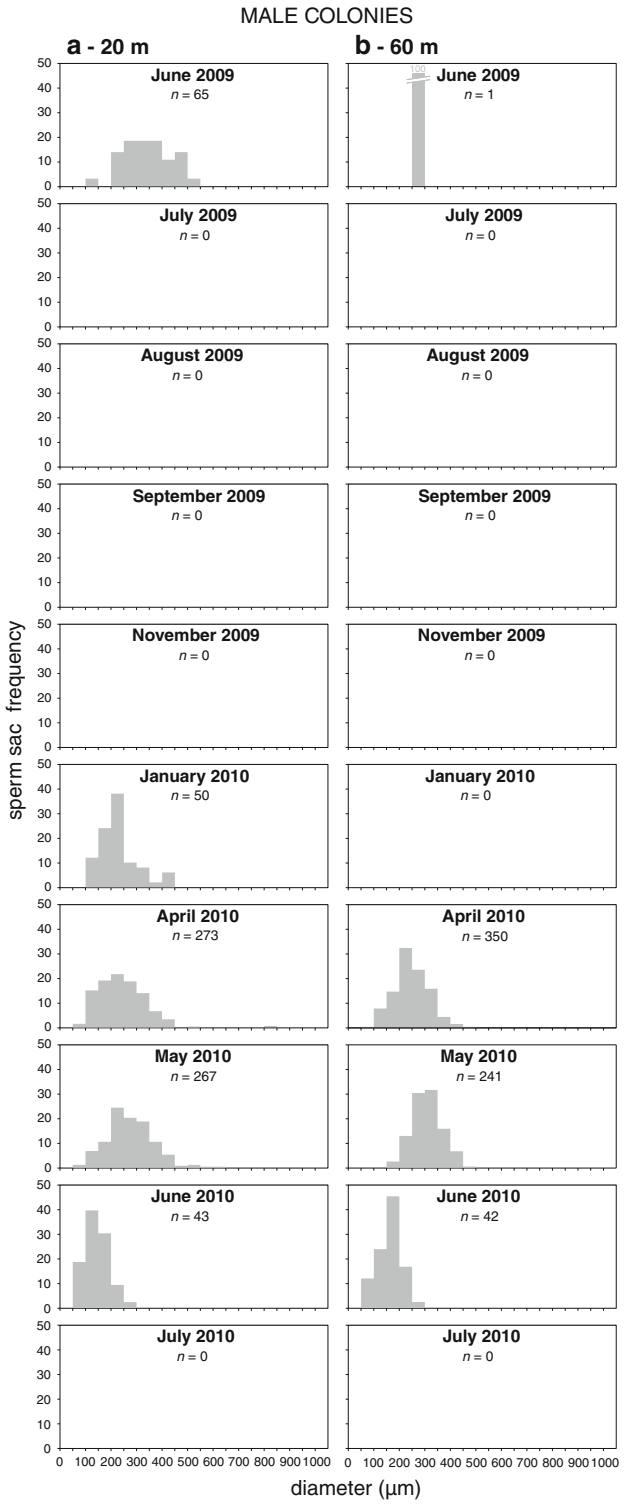


Fig. 4 Frequency of gonadal diameter (μm) in male *E. singularis* colonies from the *shallow* (**a**) and *deep* (**b**) populations (n = gonad number)

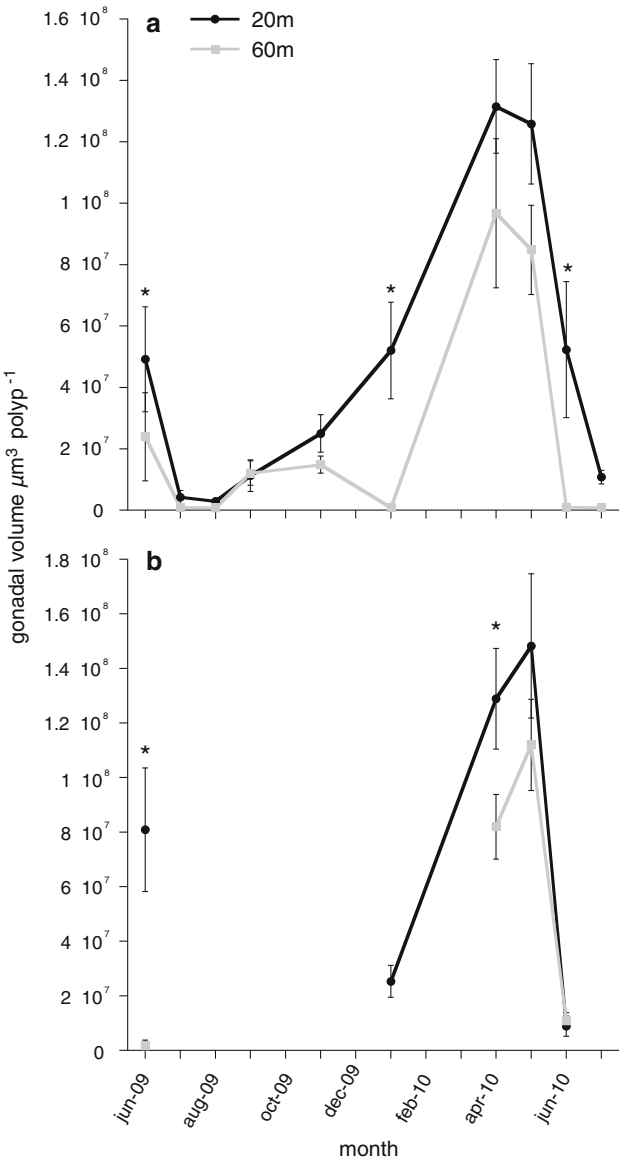


Fig. 5 Monthly changes in mean polyp volume of female (**a**) and male (**b**) gonads (μm^3 per polyp) in *E. singularis* colonies from *shallow* and *deep* populations (mean ± SE); *asterisk* indicates significant differences between the two populations (Wilcoxon–Mann–Whitney test, $p < 0.05$)

fully stratified in July and August when a pycnocline formed at 35 m. Finally, stratification was stretched out in September (Fig. 11). PAR decreased exponentially with depth (Fig. 11). Annually, if compared with 20 m, PAR averaged 1.4 ± 1.0 % at 60 m depth. Fluorescence was higher below the summer pycnocline during the water column stratification period from March to September. The suspended sediment concentration (SSC) was much more variable, being high in shallow waters between April and June, and in deeper waters in May, June, August, and September (Fig. 11).

Discussion

Depth-related differences in the reproductive cycle of the gorgonian *E. singularis* were observed in the gonadal

Table 2 Two-way PERMANOVA comparing the organic matter, content of carbohydrates, proteins, lipids, and carbon/nitrogen (C/N) ratio, among populations and seasons

	df	SS	MS	Pseudo-F	*p* value
Organic matter					
Population	1	739.2	739.2	39.8	<0.001***
Season	4	374.1	93.5	5.0	<0.001***
Population*Season	4	277.2	69.3	3.7	0.006**
Residual	116	2,154.4	18.6		
Carbohydrate content					
Population	1	5,866.0	5,866	89.1	<0.001***
Season	4	693.4	173.3	2.6	0.042*
Population*Season	4	390.6	97.7	1.5	0.212
Residual	80	5,269.3	65.9		
Protein content					
Population	1	77,570	77,570	3.2	0.077
Season	4	2.4 E05	60,028	2.4	0.051
Population*Season	4	70,571	17,643	0.7	0.579
Residual	80	1.0 E05	24,534		
Lipid content					
Population	1	3.7 E05	3.7 E05	54.3	<0.001***
Season	4	89,695	22,424	3.2	0.018**
Population*Season	4	53,171	13,293	1.9	0.112
Residual	80	5.5 E05	6,906		
C:N					
Population	1	9.2	9.2	60.9	<0.001***
Season	4	2.5	0.6	4.2	0.007**
Population*Season	4	2.1	0.5	3.6	0.013*
Residual	44	6.6	0.1		

Significant *p* values are indicated with * (*p* value <0.05), ** (*p* value <0.01), or *** (*p* value <0.001)

output, but not in the reproductive timing. Deep and shallow populations were found to release their gametes almost simultaneously, although the release of larvae from the female colonies was more prolonged in the shallow than in the deep population. Synchronous broadcast spawning between deep colonies located at a depth of 33–42 m and shallower colonies located at 16 m was previously observed in tropical corals (Vize 2006). However, the synchronization observed in *E. singularis* was surprising because previous studies reported a delay in the release of sexual products with increasing depth in this species (Weinberg and Weinberg 1979; Gori et al. 2007), in other gorgonian species from the Mediterranean Sea (Tsounis et al. 2006b; Rossi and Gili 2009), as well as in gorgonians and soft corals species from other areas (Grigg 1977; Benayahu and Loya 1983, 1986; West et al. 1993). Temperature is a major factor determining gonadal development and gamete cycles in many corals and gorgonians (Harrison 2011), and time lags in spawning at greater depths have been attributed to differences in the timing of peak water temperatures along the depth gradient (Grigg 1977; Benayahu and Loya 1983, 1986). The synchronization in the reproduction observed in *E. singularis* suggests that the spring increase in water temperature that occurred until mid-April (rising simultaneously at the two depths, Fig. 12), was one of the main cues that regulates reproductive timing. The subsequent differences between depths in terms of water temperature did not affect the timing of reproduction. However, temperature alone may not be the only factor affecting the reproductive timing of gorgonians (Rossi and Gili 2009) because reproduction may be sensitive to seasonal fluctuations in food availability (Ben-David-Zaslow and Benayahu 1999) as well as to lunar and solar light cues (Alino and Coll 1989; Jokiel et al. 1995; Vize 2006). Moreover, annual variability in reproductive cycles (Brazeau and Lasker 1989; Dahan and Benayahu 1997) have been previously shown to also affect the synchronization between deep and shallow populations, which can spawn simultaneously or not depending on the environmental conditions of each year (Tsounis et al. 2006b).

In contrast to reproductive timing, gonadal volume was different between the two populations. It was higher in the

Table 3 Pairwise test for the seasonal comparison of the organic matter, content of carbohydrates, proteins, lipids, and carbon/nitrogen (C/N) ratio, within the two populations

	Organic matter		Carbohydrate content		Protein content		Lipid content		C:N	
	t	*p* value	*t*	*p* value	*t*	*p* value	*t*	*p* value	*t*	*p* value
20 m										
Summer 09/Autumn 09	0.41	0.685	0.55	0.578	0.26	0.790	2.83	0.013*	1.98	0.067
Summer 09/Winter 10	1.91	0.068	0.71	0.500	0.33	0.750	1.98	0.072	3.68	<0.001***
Summer 09/Spring 10	4.89	<0.001***	3.05	0.005**	0.92	0.375	0.06	0.949	0.46	0.657
Summer 09/Summer 10	1.56	0.138	0.80	0.448	0.81	0.420	0.90	0.379	2.03	0.068
Autumn 09/Winter 10	2.09	0.053	0.25	0.807	0.19	0.858	0.45	0.667	1.23	0.293
Autumn 09/Spring 10	5.01	<0.001***	1.74	0.098	0.84	0.426	2.46	0.027*	1.71	0.117
Autumn 09/Summer 10	1.86	0.074	1.09	0.287	1.36	0.188	3.49	0.003**	3.81	0.002**
Winter 10/Spring 10	1.96	0.062	0.96	0.376	0.48	0.665	1.64	0.125	2.05	0.098
Winter 10/Summer 10	0.53	0.602	1.00	0.337	1.22	0.246	2.45	0.026*	5.87	<0.001***
Spring 10/Summer 10	3.03	0.005**	2.83	0.007**	1.90	0.075	0.74	0.466	0.71	0.473
60 m										
Summer 09/Autumn 09	0.14	0.882	2.82	0.009**	0.50	0.625	0.59	0.572	2.48	0.017**
Summer 09/Winter 10	1.77	0.093	1.29	0.216	2.46	0.023	1.21	0.246	2.80	0.013**
Summer 09/Spring 10	0.71	0.477	1.17	0.250	0.23	0.819	0.62	0.532	1.34	0.215
Summer 09/Summer 10	1.48	0.148	2.25	0.026*	2.23	0.039	0.41	0.679	0.42	0.742
Autumn 09/Winter 10	1.33	0.203	0.82	0.427	1.54	0.151	0.56	0.653	2.95	0.012**
Autumn 09/Spring 10	0.44	0.662	1.29	0.216	0.60	0.547	0.06	0.943	0.40	0.705
Autumn 09/Summer 10	1.17	0.259	0.18	0.869	1.44	0.166	0.26	0.804	1.27	0.241
Winter 10/Spring 10	2.06	0.053	0.24	0.814	1.76	0.105	0.81	0.506	1.81	0.099
Winter 10/Summer 10	1.17	0.262	0.71	0.513	0.38	0.704	1.04	0.314	1.25	0.249
Spring 10/Summer 10	2.08	0.050	1.17	0.263	1.88	0.077	0.23	0.820	0.72	0.474

Significant *p* values are indicated with * (*p* value <0.05), ** (*p* value <0.01), or *** (*p* value <0.001)

shallow then in the deep population, as were the maximum gonadal diameters. Similar depth-related differences were previously observed in tropical gorgonian and coral species, whose colonies from shallow waters produced more and larger oocytes (West et al. 1993) and larvae than those from deep waters (Kojis and Quinn 1984; Rinkevich and Loya 1987). The Cap de Creus shallow population displayed lower gonadal volumes as compared to other southern shallow *E. singularis* populations (Ribes et al. 2007; Gori et al. 2007). Such spatial variability in gonadal output has been previously reported for coral species being attributed to differences in the allocation of resources to gamete production in response to unstable environmental conditions experienced at higher latitudes or in shallow waters (Sier and Olive 1994; Fan and Dai 1995; Kruger et al. 1998). Because reproduction involves a major energy investment, differences in gonadal output are likely related to differences in the nutritional state of gorgonians as a consequence of food quality and availability (Stimson 1987; Harland et al. 1992; Ben-David-Zaslow and Benayahu 1999). Energy storage observed in the studied populations of *E. singularis* seems to support this interpretation, since the higher lipid content in the shallow population,

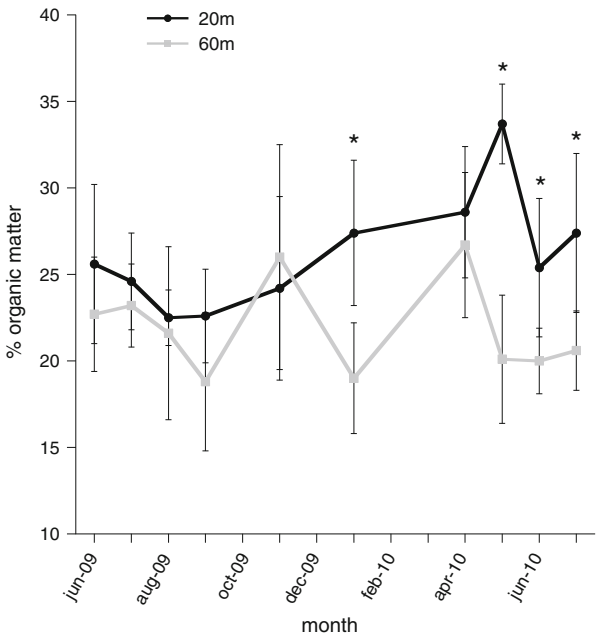
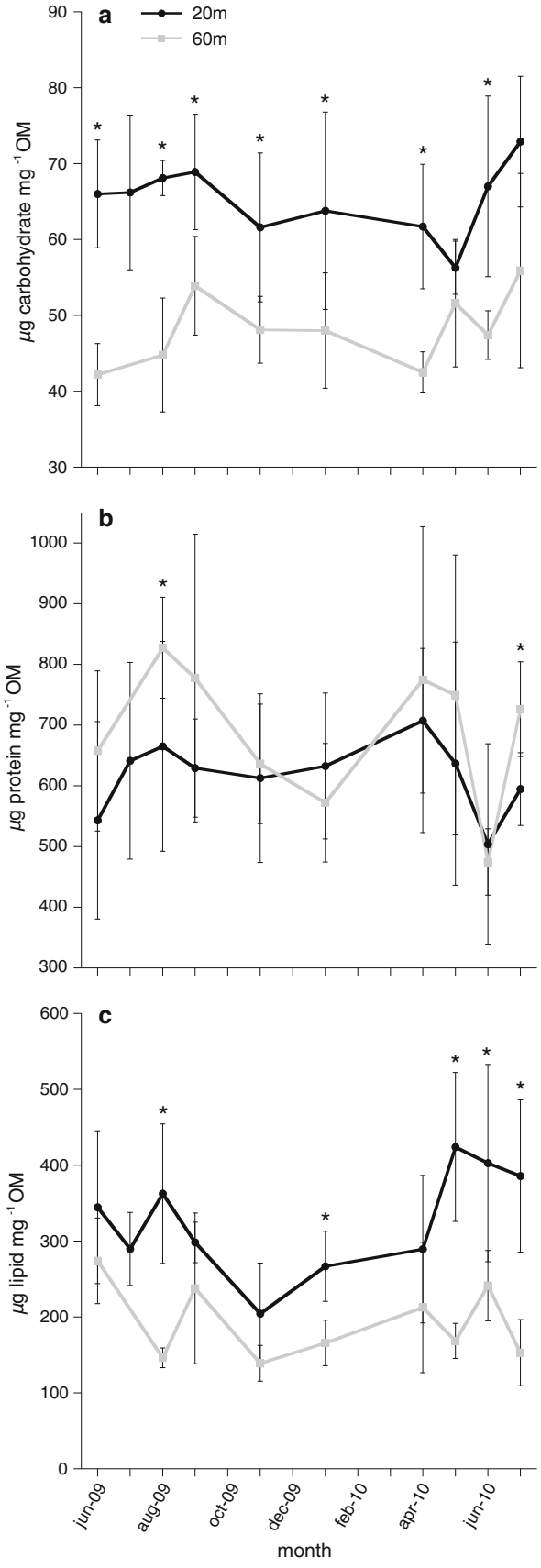


Fig. 6 Annual cycle (June 2009–July 2010) of organic matter in the coenenchyme of *E. singularis* colonies ($n = 7$) from *shallow* and *deep* populations (mean ± SD); *asterisk* indicates significant differences between the two populations (Wilcoxon–Mann–Whitney test, $p < 0.05$)



◀**Fig. 7** Annual cycle (June 2009–July 2010) of carbohydrate (**a**), protein (**b**), and lipid (**c**) content (μg · mg OM) in tissue of *E. singularis* colonies ($n = 5$) from the *shallow* and *deep* population (mean ± SD); *asterisk* indicates significant differences between the two populations (Wilcoxon–Mann–Whitney test, $p < 0.05$)

peaking during summer, contrasts with the lower and more constant content observed in the deep population. Several studies have highlighted the importance of symbiotic algae in the translocation of lipids from algal cells to cnidarian tissue (Patton et al. 1983; Tolosa et al. 2011), and depth-related differences in lipid storage by symbiotic corals were previously observed, indicative of a light constraint with increasing depth (Harland et al. 1992). Conversely, the lower but more constant lipid content observed in the deep population, which lack symbiotic algae (Théodor 1969; Gori et al. 2012), may result from a more constant food availability when presence of phytoplankton and high concentrations of suspended material were observed below the pycnocline (Fig. 11). This is in line with previous results from the Mediterranean aposymbiotic gorgonian *Corallium rubrum*, whose colonies located above the summer pycnocline displayed lower and more variable annual prey capture rates than deeper colonies (Tsounis et al. 2006a), resulting in lower lipid content in shallower populations than in deeper ones (Rossi and Tsounis 2007), which are not stressed by the summer constraints occurring above the pycnocline (Coma et al. 2000; Rossi et al. 2006).

Fatty acid profiles also revealed depth-related differences in trophic structure. The dominant fatty acid marker in the shallow population was 18:2(n-3), which proceeds from macroalgal origins (detritus) in the Mediterranean Sea (Soler-Membrives et al. 2011), whereas the 20:4 (n-3) marker is indicative of ciliate and flagellate (heterotrophic) origins (Zhukova and Kharlamenko 1999; Broglio et al. 2003) and was clearly dominant in the deep population. As observed for the lipid content, a greater seasonality was observed in the fatty acid composition of the shallow population with respect to the deep one. Trophic markers of microalgal origin (16:1(n-7) and 22:6(n-3), Dalsgaard et al. 2003) were more abundant and followed a seasonal trend in the shallow population, whereas the deep colonies presented a significantly lower proportion without following any clear seasonality (Online Resource 2). In a similar way, the C/N ratio was almost constant in the deep population, while it followed a greater seasonality in the shallow one, possibly reflecting the contribution of the primary production from symbiotic algae during the summer, and the transfer of lipids to the host (Treignier et al. 2008). The two populations were clearly differentiated with respect to their δ^{15}N isotopic signature: the deep population exhibited high δ^{15}N values close to those of other aposymbiotic passive suspension feeder species, which primarily feed on

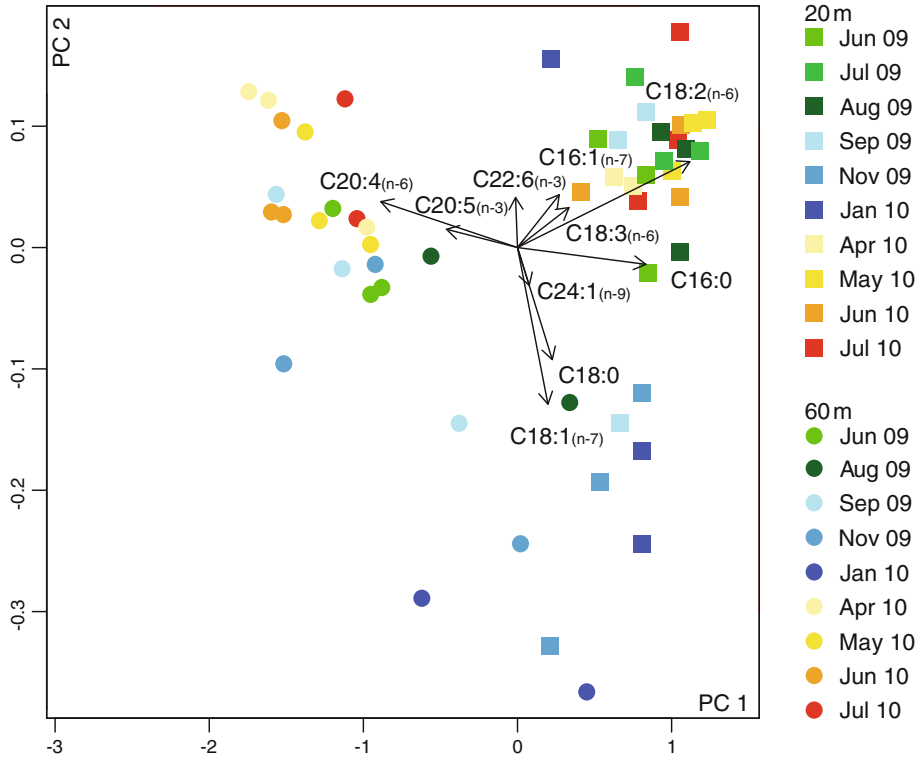


Fig. 8 Principal component analysis (PCA) biplot illustrating the ordination of the studied colonies with regard to their fatty acid composition, and the roles of the first 10 fatty acids sorted according to the explained variance

microzooplankton and particulate organic matter (Carlier et al. 2007), whereas the lower δ^{15}N values observed in the shallow population throughout the year are similar to those from symbiotic passive suspension feeders (Carlier et al. 2007). This result suggest that shallow colonies were relying more on dissolved sources of nitrogen absorbed by the symbiotic algae than on the organic nitrogen derived from feeding, as was previously observed in the Mediterranean symbiotic coral *Cladocora caespitosa* (Ferrier-Pagès et al. 2011). Conversely, the deeper colonies that lack symbiotic algae seem to rely on heterotrophic food

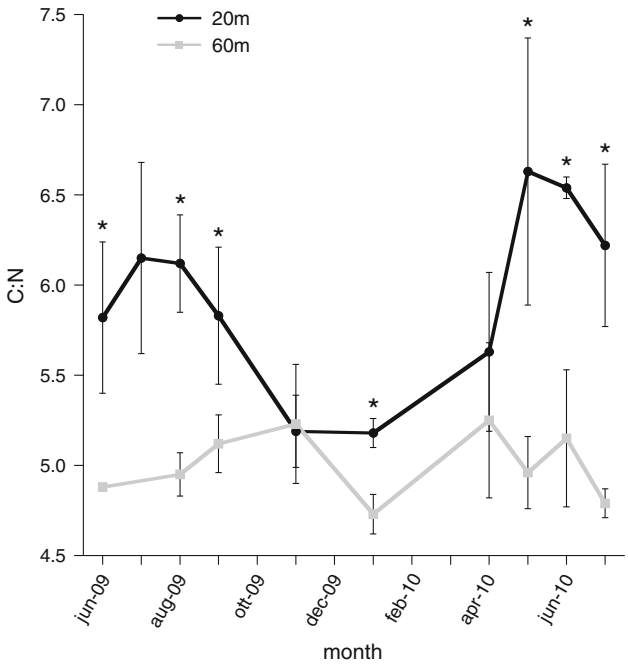


Fig. 9 Annual cycle (June 2009–July 2010) of C/N ratio in the tissue of *E. singularis* colonies ($n = 3$) from *shallow* and *deep* populations (mean ± SD); *asterisk* indicates significant differences between the two populations (Wilcoxon–Mann–Whitney test, $p < 0.05$)

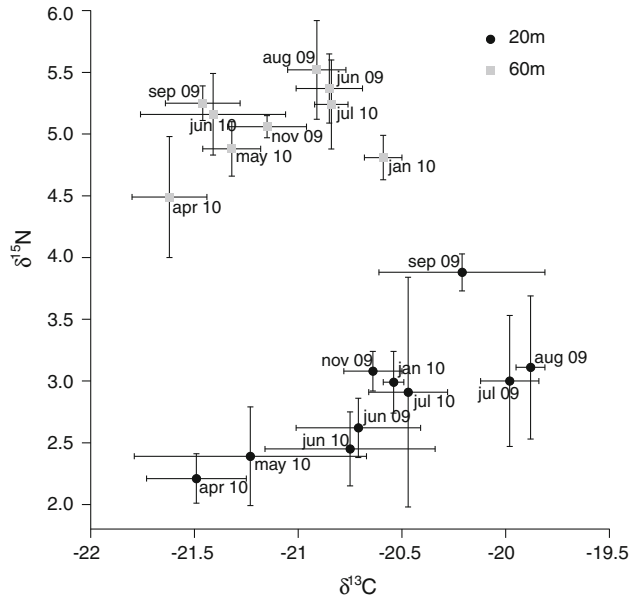


Fig. 10 Stable isotope (δ^{15}N and δ^{13}C) composition of *E. singularis* colonies ($n = 3$) from *shallow* and *deep* populations (mean ± SD)

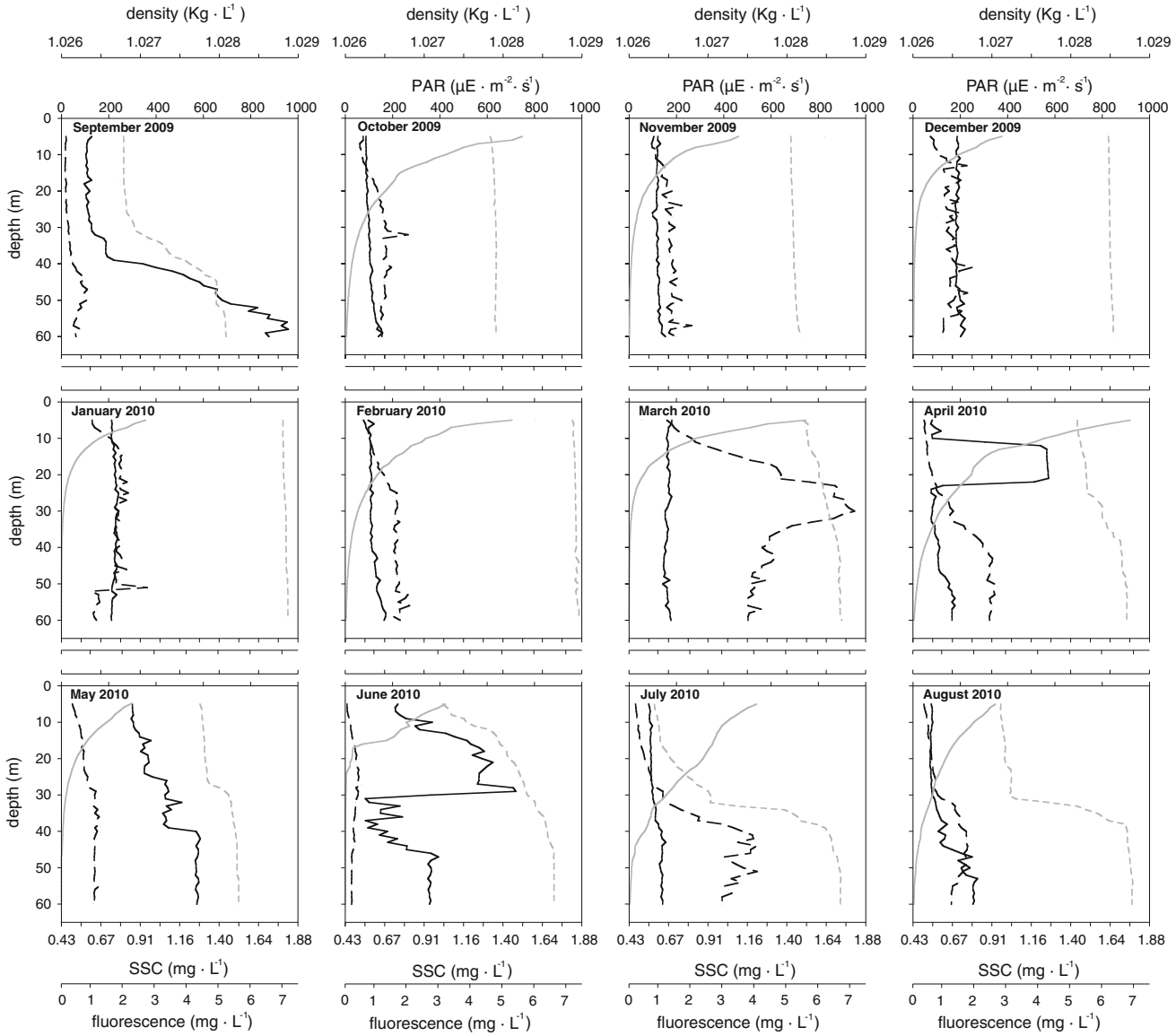


Fig. 11 Water column characterization in the sampling location during the study period; *gray line* represents photosynthetically active radiation (PAR), *gray dashed line* represents water density, *black line* represents suspended sediment concentration (SSC), and *black dashed line* represents fluorescence

sources, microzooplankton being the most predominant prey group as already reported for other aposymbiotic Mediterranean gorgonian species (Ribes et al. 1999, 2003). The δ^{13}C isotopic signature was similar between the two populations, despite it was more variable at 20 m than at 60 m suggesting a more stable source for carbon in the deep population during the entire year. However, the heavier δ^{13}C values observed in the shallow population were much lighter than those observed in *C. caespitosa* (Ferrier-Pagès et al. 2011), suggesting that also during summer a part of the carbon uptake proceeds from feeding, possibly mainly detritus of macroalgal origins as suggested by the fatty acid markers analysis.

In conclusion, differences in the reproductive cycle were observed between shallow and deep populations of *E. singularis*. The energy storage, as well as the studied trophic markers, was also different between populations and showed a greater seasonality in the shallow than in the deep population. The results of this study, together with those obtained in previous ones (Ribes et al. 2007; Gori et al. 2007, 2011b), showed that large differences may occur among shallow and deep sublittoral populations of the same benthic suspension feeder species and highlight the importance of considering depth-related variability among populations in order to achieve a better understanding of their ecology.

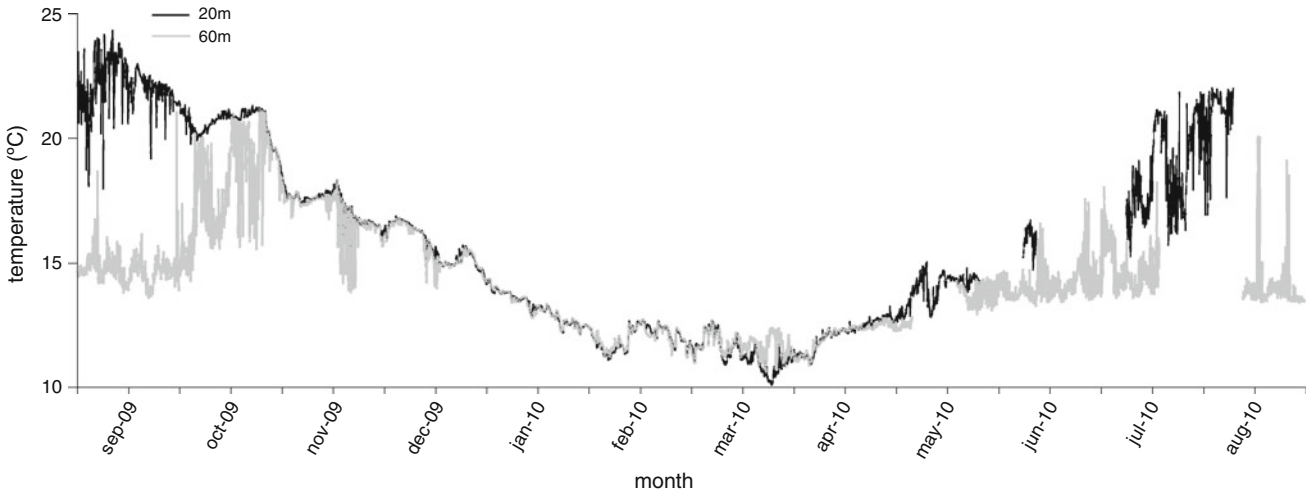


Fig. 12 Water temperature in the sampling location at depths of 20 and 60 m

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