Size, spatial, and bathymetrical distribution of the ascidian *Halocynthia papillosa* in Mediterranean coastal bottoms: benthic–pelagic coupling implications

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Abstract Benthic suspension feeders are abundant in Mediterranean coastal environments, though most of them are threatened due to overexploitation, climate change impacts, and unregulated diving or fishing practices. Little is known about most of the coastal communities in terms of large-scale distribution and realistic benthic–pelagic coupling implications, which are keys to understand and manage those threatened ecosystems. The active suspension feeder *Halocynthia papillosa* (one of the most common ascidian species of the Mediterranean Sea) was selected as a model organism to help to understand the ecological role in benthic–pelagic coupling processes and its importance as a carbon sink (an essential ecosystem service). The spatial and bathymetrical distribution of this organism has been studied using remotely operated vehicle video transects. The species was distributed throughout the study area, with a maximum density of 4 specimens m^{-2}. The highest abundances and the biggest sizes were observed on the range of 20–50 m depth. The role as carbon and nitrogen sink of this suspension feeder has been quantified coupling distribution data with existing in situ studies of feeding and respiration. Along the 1.24 ha of the study area, *H. papillosa* yearly ingested 519.4 g C and 31.4 g N and retained 20.2 g C. As long as the physiological data are known, this new methodology could be very useful in assessing bentho–pelagic links and the capacity of being C and N sinks of a wide range of species. This new approach may be essential for the future management of benthic communities.

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Introduction

Benthic suspension feeders are the main constituents of hard-bottom benthic communities in dark or low-light zones (Witman and Dayton 2000) in cold (Gili et al. 2001), temperate (Gili and Coma 1998; Orejas et al. 2009), and tropical environments (Lesser 2006; Wild et al. 2011). These sessile organisms play a key role as ecosystems engineers (Jones et al. 1994), enhancing the structural heterogeneity of the community and thus promoting a diversification of the associated fauna (Krieger and Wing 2002; Henry and Roberts 2007; Rossi 2013). The three-dimensional complexity originated by their populations may influence the water flow at a local scale and increase the residence time of suspended particles (Leicher and Witman 1997; Gili and Coma 1998). These complex and well-structured communities (mainly composed by sponges, gorgonians, corals, and bryozoans) are called "animal forests," and probably accumulate one of the highest biomasses within world oceans (Rossi et al. 2012; Rossi 2013).

Depending on the feeding strategy, these animals can be classified as passive or active suspension feeders (Shimeta and Jumars 1991). Passive suspension feeders (e.g., gorgonians and corals) solely rely on the movement of water

masses with respect to their capturing structures and on the particle concentration in the water for food capture (Best 1988). Conversely, active suspension feeders (e.g., sponges, bivalves, and ascidians) pump water through a filtering structure that separates food particles from the water; the particle capture mechanisms can be different depending on the organism, ranging from cirri trapping to mucus net filter feeding (see Riisgård and Larsen 2000 for further details). By means of their trophic activity, both passive and active benthic suspension feeders generate an important flux of energy and matter from the pelagic to the benthic ecosystems (Gili and Coma 1998). Their populations may act as carbon and/or nitrogen sinks by removing organic matter from the highly dynamic and yet poorly structured pelagic system (where both carbon (C) and nitrogen (N) are quickly transmitted and recycled through the trophic net), and incorporating this organic matter into the long-lived and highly structured benthic system (Smith 1981; Graf 1992; Gili and Coma 1998; Coma et al. 2001; Rossi et al. 2012).

Ascidians show an heterogeneous diet mainly composed of particulate organic matter (POM) (Petersen and Riisgård 1992; Ribes et al. 1998; Petersen 2007). Active feeding behavior allows many ascidian species to reach high filtration rates, especially if occurring in dense populations (Riisgård et al. 1995). In many hard-bottom communities, ascidians act as key organisms in specific seasons contributing to control the density of phytoplankton (Petersen and Riisgård 1992), as well as reducing eutrophication or contaminant concentrations (Officer et al. 1982; Loo and Rosemberg 1989; Hily 1991; Naranjo et al. 1996). Ascidians may also be significant carbon sinks in areas where they occur in high densities (Petersen and Riisgård 1992).

In the Mediterranean Sea, *Halocynthia papillosa* (Linnaeus, 1767) (Order: Stolidobranchia, Family: Pyuridae) is one of the most common ascidian species inhabiting coralligenous and sciaphilic benthic communities (Turon 1985, 1990), especially in crevices and overhangs in non-perturbed rocky bottoms (Naranjo et al. 1996). The information about the distribution and abundance pattern of this species is currently restricted to the very shallow depths, lacking information about its populations below 30 m depth. The recent development of remotely operated vehicles (ROVs) and their decreased operational cost and increased accessibility makes it possible to quantitatively sampling rocky bottoms at depths that cannot be sampled with traditional SCUBA dive (Mortensen and Buhl-Mortensen 2004; Rossi et al. 2008; Gori et al. 2011a). One of the main advantages of this methodology is that ROV sampling coupled with quantitative video analysis allows for the study of large extensions of sea bottom with no impact on the benthic communities (Bianchi et al. 2004, 2012; Mortensen and Buhl-Mortensen 2004). On the other hand, ROV-based studies have lower accuracy compared to SCUBA dive sampling especially with species that tends to occupy cryptic locations (Gili and Ballesteros 1991).

Recent studies using ROV have shown that some of the most common coastal benthic suspension feeder species display greater abundances below 30–40 m depth (Rossi et al. 2008; Bo et al. 2011; Gori et al. 2011a; Ambroso et al. 2013), highlighting the importance of studies beyond the SCUBA limit depth. The large-extent approach proposed in this work allowed to obtain quantitative information of the distribution pattern of benthic sessile species along the entire bathymetrical range. This knowledge is essential for the management of large areas [including marine protected areas (MPA)] and to obtain a clear picture of the overall system.

The availability of in situ experimental data on the clearance and respiration rates of *H. papillosa* (Ribes et al. 1999; Coma et al. 2002) allowed to explore the possible influence of trophic constrains on the species distribution, as well as to infer the role played by this active suspension feeder in coastal marine biogeochemical cycles over a large geographical and bathymetrical extension. The novelty of the methodology proposed here lays in combining these data with quantitative data on the distribution pattern and population structure. To reach the study goal, three different steps have been performed: (1) characterize the spatial distribution pattern of *H. papillosa* over a large geographical and bathymetrical extent, following previous ROV and quantitative video analysis methods (Gori et al. 2011a; Ambroso et al. 2013); (2) describe the size structure of the studied species; and (3) estimate the trophic impact (in terms of C and N uptake) of this species over a large representative area of Mediterranean coastal bottoms. This is the first application of such large-scale approach to one of the species of the coralligenous community as previous studies focused on coral reefs (Kinsey and Hopley 1991), sea grass meadows (Duarte et al. 2010), or mangrove forests (Eong 1993). The results of this study will increase our understanding about the distribution pattern of one important species of the coralligenous community, and will provide some insight into the benthic–pelagic coupling in coastal areas. Both information sets have to be taken into account for the establishment of management and conservation measures, as well as to make accurate calculation of ecosystem services played by the animal forest as carbon sinks.

Materials and methods

Distribution and size structure of *Halocynthia papillosa*

Study area

The study area extends along the Catalan Coast (northwestern Mediterranean Sea), from the boundary with France,
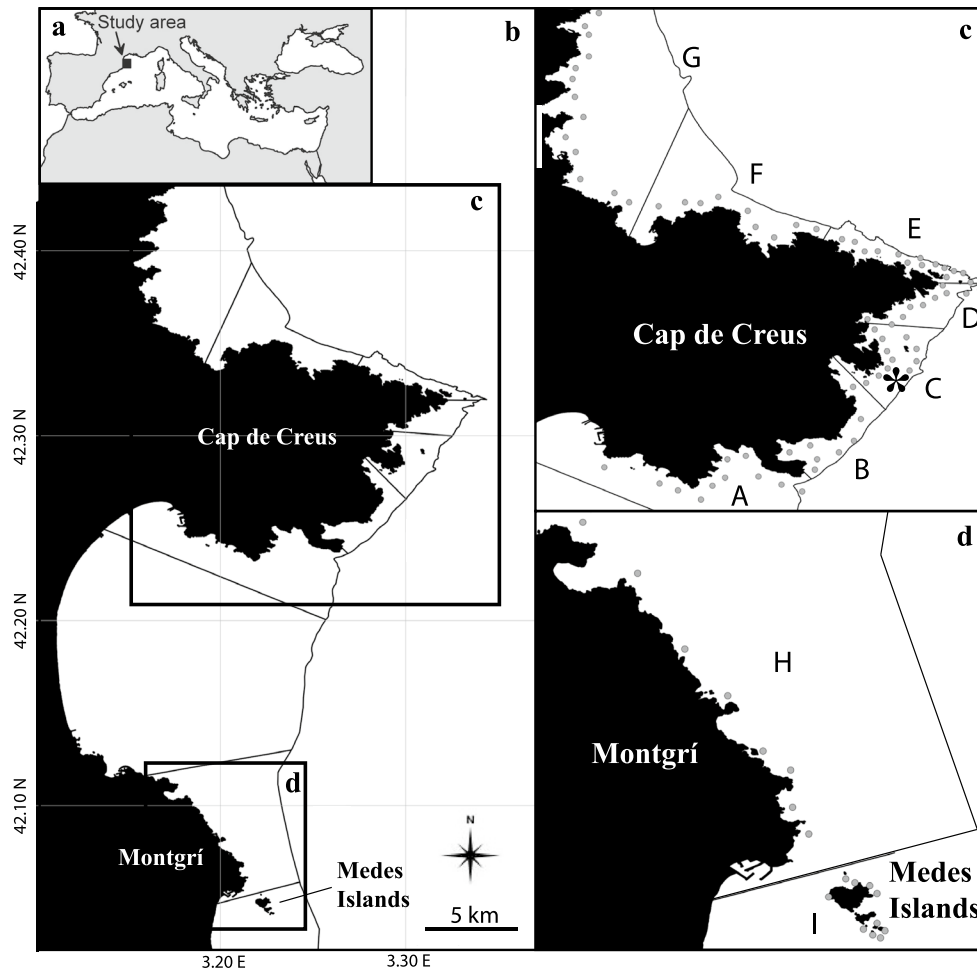
Fig. 1 Map of the study area: location of the study area (**a**), study area in details (**b**), Cap de Creus area showing the seven subareas and the transect positions; the *black asterisk* indicates the position of the sampling site Punta s'Oliguera (**c**), Montgrí and Medes Islands area showing the others two subareas and transect positions (**d**)

going southward across the Cap de Creus (42°19′12″N; 003°19′34″E) and the coast belonging to the natural park of Montgrí (42°02′38″N; 003°07′43″E) and the marine protected area of Medes Islands (40°02′55″N; 003°13′30″E). According to the main hydrodynamic patterns in the zone, and the specific features of the studied coast (Fig. 1), the study area was subdivided into nine subareas (A–I). Subareas from A to G are located in the Cap de Creus area (see also Gori et al. 2011a), subarea H corresponds to the Montgrí, and subarea I corresponds to the Medes Islands. The general circulation pattern is characterized by the dominance of the Liguro-Provençal-Catalan current (or Northern current), which flows south-westward creating an east-to-west circulation (DeGeest et al. 2008). The study area receives sediment inputs from the northern Gulf of Lions (Durrieu de Madron et al. 2000), especially by the Rhone that supplies for about 90 % of the total freshwater in the Gulf of Lions (Palanques et al. 2006). Besides, Medes Island are influenced by the Ter river, which show

seasonal differences in runoff with heavy discharges between November and February and low discharges in spring–summer (Rossi et al. 2003). The most important winds influencing the Cap de Creus area are the northerly Tramuntana and the northwesterly Mistral that occur for 41 and 28 % of the time, respectively. Also in Montgrí and Medes Islands, Tramuntana and Mistral are the most important winds, although the northeasterly Gregal and the easterly Levante are the ones that bring the biggest storm waves, being as high as 5 m (Pascual and Flos 1984). South-easterly and easterly marine winds are rare (<6 % of the time) and brief (<3 days), in contrast to the northerly ones that can last up than 1 month (Ulses et al. 2008). Consequently, subarea A is the most sheltered area of the surveyed coast; subareas B, C, and D are affected only by easterly winds, and they are not directly influenced by the main near-bottom currents (Ulses et al. 2008; DeGeest et al. 2008). Subareas E and F are directly exposed to the main winds and wave actions in the study area (Ulses et al.

2008), as well as to the main near-bottom currents that accelerate around the cape (DeGeest et al. 2008). Subarea G is characterized by sediment deposition processes due to the reduced influence of the main near-bottom currents. The bionomic characteristics vary between different subareas of the Cap de Creus: the northern part is characterized by higher slopes and mostly rocky-bottom communities, while the southern part is much flatter and mostly contained soft-bottom habitats (Sardá et al. 2012). Likewise, the Medes Islands topography is markedly asymmetrical, with prominent slopes on the northern side and gentle slopes on the southern side (Gili and Ros 1985). Coralligenous and precoralligenous communities predominate on the east side of the Medes Island, whereas sand and shards bottoms predominate on the west side (Gili and Ros 1985). The Montgrí area is characterized by the presence of rocky bottoms close to the coast, whereas coralligenous and sandy bottoms gain presence alternating each other at increasing distance from the coast (Hereu et al. 2010).

Sampling procedure

Fieldwork was conducted in October–November 2004 and in November 2009 for the Cap de Creus and Montgrí-Medes areas, respectively. In Cap de Creus, transects were video-recorded with the ROV Phanthom XTL equipped with a SONY FCB S3000P 3CCD camera (resolution of 700 horizontal lines), a depth sensor, a compass, and two laser beams that provide a scale to define a fixed width of the transects (0.5 m) for subsequent video analysis. In Montgrí-Medes, transects were video-recorded with the ROV Triggerfish equipped with a DOE 18:1 color zoom camera unit (resolution of 470 horizontal lines), a depth sensor, a compass, and two laser beams. Videos were recorded on tapes in DV format. In each sampled location,
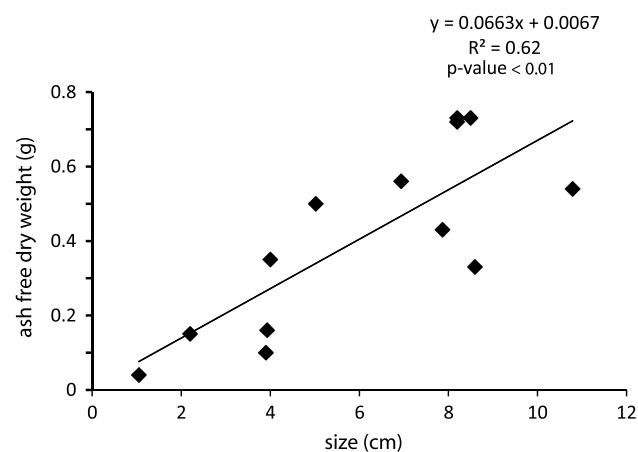

Fig. 2 Relationship between *H. papillosa* size (cm) and AFDW (g)

seabed video recording started at the deepest position and proceeded toward the shallows, until the ROV surfaced close to shore. The ROV speed was kept constant, approximately 0.4 knots for Cap de Creus and 0.5 knots for Montgrí-Medes. Transects started at a depth between 12 and 71 m in Cap de Creus, and between 10 and 52 m in Montgrí-Medes, depending on the geographical characteristics of each location. Transect length varied between 92.6 and 907.1 m in Cap de Creus and between 13.9 and 249.2 m in Montgrí-Medes. On the whole, 76 video transects were recorded in the 2004 field campaign (Fig. 1c) and 18 in the 2009 (Fig. 1d) covering a total distance of 28.3 km in Cape de Creus and 2.1 km in Montgrí-Medes.

Video analysis

Quantitative video analysis was performed according to the methodology described in Gori et al. (2011a) and Ambroso et al. (2013). Videos were transferred from tapes to hard disk and then analyzed using Final Cut Pro 6.06 software (Apple). As speed was constant, all the pauses in the movement of the ROV were removed to correctly estimate the total length of each transect. Sequences with poor image quality, due to bad visibility or too much distance from the bottom, were discarded from the analysis. Each transect has been divided into sampling units of 2.5 m² (0.5 m width and 5 m long); all the sampling units, except the ones that correspond completely or partially to no analyzable sequences, have been examined. The sampling unit area was chosen from Weinberg (1978), who estimated that a sample size of 2 m² was a representative sampling area for studying invertebrates in the Mediterranean rocky substrata. A total of 4,559 useful sampling units were obtained from the 76 transects of the Cap de Creus that correspond to 0.011 km² and 381 were obtained from the 19 transects of Montgrí-Medes equal to 0.0095 km². For each sampling unit, the number of *H. papillosa* was determined, together with its depth and the percentage of the abundance of each seabed substrate type. Substrates were classified into five different categories: soft bottoms (mud, sand, detritic, and *Posidonia oceanica* cover), maërl (species of coralline algae growing loosely in beds of fragmented nodules), pebbles, rock, and coralline rock. The distance occupied by each substrate type has been recorded and then converted into % of coverage. The cumulative percentage was converted into number of sampling units considering 100 % of a determinate substrate type as one sampling unit. To study the size structure of its populations, the maximum height of each observed *H. papillosa* was measured using the Carnoy software package (Schols and Smets 2001) on still images extracted from the videos. Only the organisms lain perpendicular to the video and on the same plane of the laser beams have been measured (Gori et al. 2011b), considering
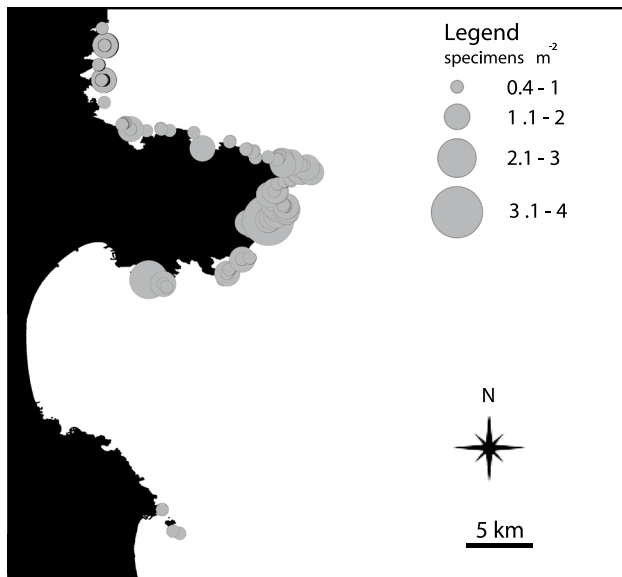
Fig. 3 Spatial distribution of *H. papillosa* in the study area. *Bubbles* indicate the density of *H. papillosa* (specimens m^{-2})

the distance between the laser beams as calibration for the extracted images. This methodological constraint entails that not all the specimens encountered could be measured; hence, only a subsample of the *H. papillosa* observed has been included in the study of the size structure.

Data treatment

The presence of *H. papillosa* was quantified both by occupancy (frequency of occurrence in the set of sampling units) and by abundance (number of specimens per sampling unit). The spatial distribution was studied by mapping the densities observed in each sampling unit with respect to its position on a geographically referenced map using software Quantum Gis 1.7.2 (Quantum GIS Development Team 2009). The position of the sampling units was estimated from the recorded geographical coordinates of the initial and final point of each transect. The bathymetrical distribution of the species was studied in each subarea, taking into account the average depth of each sampling unit and estimating the median density at depth intervals of 5 m. The R-language function lm of the R software platform (R Development Core Team 2012) was used to perform multiple linear regressions between *H. papillosa* abundance, and depth and the presence of hard substrate (rock and coralline rock). Afterward, the R-language function summary was used to test the adequacy of the obtained equation by means of an analysis of variance (ANOVA), calculate the multiple regression coefficient of determination (R^2), and assess the significance of each variable in the explanation of the species abundance.

The size structure of *H. papillosa* populations was analyzed with descriptive statistics using distribution parameters such as skewness and kurtosis. Skewness is a measure of the symmetry of a distribution using its mean; if skewness is significant, the distribution is asymmetric. The prevalence of small size class in a population is indicated by positive skewness, whereas negative skewness indicates the dominance of large size classes. Kurtosis indicates the peakedness of a distribution near its central mode. A significant value of kurtosis indicates that the variable has longer tails than a normal distribution and therefore the prevalence of a particular size class in a population. Skewness and kurtosis were calculated by the R-language functions agostino.test (Komsta and Novomestky 2012) and anscombe.test (Anscombe and Glynn 1983), which are available in the moments library of the R software platform.

Trophic impact of *Halocynthia papillosa*

Size versus dry weight

The relationship between *H. papillosa* size and dry weight (essential to calculate the biomass) was determined by sampling by SCUBA dive 13 ascidians ranging from small- to medium- and large-size class (Punta s'Oliguera, Cap de Creus, 42°17′1.62″N, 003° 17′ 57.18″E, Fig. 1c). Ascidians were photographed in situ with a ruler on their side, to infer the biomass from the image analysis performed with Carnoy software package (Schols and Smets 2001). Samples were collected at depths ranging from 10 to 30 m, in April 2012. Once in the laboratory, ascidians were freeze-dried, weighed, and then combusted for 5 h at 500 °C and weighed again to determine their ash-free dry weight (AFDW). The relationship between size and AFDW highlighted a lineal dependence between height and weight (Fig. 2). This relation was used to convert the size of the *H. papillosa* observed along the video transects, to their equivalent AFDW.

Carbon and nitrogen estimation

Total amount and changes with depth in the seasonal ingested C and N, as well as respired C, were then estimated for the entire study area based on the distribution of *H. papillosa* by means of the data from previous in situ experiments (Ribes et al. 1998; Coma et al. 2002). By combining the above-mentioned relationship between size and dry weight with the data about size and density of the species in the study area, the ingested C and N, and the respired C were estimated in 5-m-depth intervals. Ingestion and respiration rates are related to ascidian size following an allometric relation: $F = aW^b$ where a is the filtration or respiration rate of an organism of 1 g, W is the weight of

Table 1 Presence and spatial distribution of *H. papillosa* in the study area

Subarea	Sampling units			Max. densities (Ascidians m^{-2})	Mean densities ± SD (Ascidians m^{-2})	Mean height ± SD (cm)
	Numbers	With ascidians	%			
A	803	10	1.3	2.8	0.8 ± 0.7	4.7 ± 1.6
B	456	28	6.1	2	0.7 ± 0.5	4.7 ± 1.1
C	630	87	13.8	4	0.7 ± 0.5	5.4 ± 1.3
D	652	54	8.3	2	0.6 ± 0.4	5.5 ± 1.4
E	787	39	5.0	1.6	0.6 ± 0.3	6.2 ± 1.6
F	450	17	3.8	1.2	0.5 ± 0.2	6.2 ± 1.1
G	777	58	7.5	2	0.6 ± 0.4	6.9 ± 1.9
H	176	1	0.6	0.4	–	–
I	205	4	2.0	0.4	0.4	8.2 ± 1.1

Occupancy (frequency of occurrence in the set of sampling units), maximum, and mean densities, and mean size are given for each subarea. For subarea H, the mean density and the mean size are not reported because only one specimen was encountered. For subarea I, the SD of the mean density is not reported because the four sampling units with ascidians had the same density

the individual, and *b* is the rate of change in metabolic rates with size. Since the value of *b* is not known for *H. papillosa*, we used the value 0.67 and 0.70, for filtration and respiration, respectively, based on the previous studies on ascidians (Fisher 1976; Robbins 1983; Klumpp 1984; Jiang et al. 2008). Respiration also depends on seawater temperature (Riisgård and Larsen 2000); therefore, respiration rates from Coma et al. (2002) were chosen based on the following seasonal temperatures: shallow ascidians (from 0 to 35 m depth) 16 °C for spring, 20 °C for summer, 16 °C for autumn, and 12 °C for winter time; deep ascidians (deeper than 35 m) 14 °C for spring, 15 °C for summer, 16 °C for autumn, and 12 °C for winter (Pascual and Flos 1984; Gori et al. 2012; Fiorillo et al. 2013). The seasonal oxygen consumption was converted into seasonal carbon dioxide production using the respiratory quotient (RQ = $CO_{2\ evolved}$/$O_{2\ consumed}$). Considering that most aquatic organisms, in particular ascidian species, release ammonia as excretory product (Markus and Lambert 1983), the RQ = 0.949 estimated for ammonotelic organisms (Lampert 1984) was chosen for our conversion.

Environmental conditions

Water temperature, salinity, and turbidity were measured monthly at 1-m-depth intervals from 5 to 60 m with Seabird 25 and Seabird 19 conductivity temperature and depth sensors (CTDs) (see Gori et al. 2012 for further details). These data were used to characterize annual variation in water temperature, density, and turbidity along the studied depth range using the Surfer 8 software (Surfer 8 Manual 2006). Moreover, organic C/N composition of the POM was quantified seasonally. Water samples were collected at 20 and 60 m depth by means of a 5-l Niskin bottle. Three 800 ml replicates of seawater were filtered through GF/F pre-combusted

Table 2 Size structure distribution parameters of studied populations of *H. papillosa*: skewness and kurtosis

Subarea	Skewness	*p* value	Kurtosis	*p* value
A	0.58	0.43	2.61	0.88
B	1.00	0.14	3.63	0.22
C	0.74	0.10	3.86	0.11
D	0.37	0.52	2.40	0.59
E	1.32	0.15	3.79	0.08
F	0.20	0.82	1.55	0.16
G	1.01	0.23	3.30	0.28
I	–	–	–	–

Skewness and kurtosis are considered significant if the *p* value is equal or <0.05. Skewness and kurtosis for subarea I could not be calculated due to the few number of specimens measured

(450 °C for 5 h) glass fiber filters. Filters were immediately frozen in liquid nitrogen and stored at −80 °C until particulate organic carbon (POC) and particulate organic nitrogen (PON) analysis were performed. Filters were acidified with HCl, dried at 60 °C for 24 h, and therefore analyzed using a C/N autoanalyzer (Perkin-Elmer 2040).

Results

Distribution and size structure of *Halocynthia papillosa*

Halocynthia papillosa was recorded in 6.45 % of the total number of 4,559 sampling units in Cap de Creus and in 1.31 % of the total number of 381 sampling units in Montgrí-Medes area. A total of 483 specimens were counted and 169 of them measured in Cap de Creus, with the highest maximum density of 4 individuals m^{-2}. In Montgrí-Medes area,

five specimens were counted and three measured. *H. papillosa* was observed all along the coast of the Cap de Creus (Fig. 3), with a higher density on the east side of the cape (subareas C and D), and in some locations along the south side of the cape (subarea A). The studied species was very rare in the Montgrí-Medes area (Fig. 3). Bathymetrical distribution of *H. papillosa* ranged from 17 to 58 m depth, with the highest frequencies and abundances observed between 20 and 50 m depth (Fig. 4). Below this depth, sandy bottoms were dominant in almost all the areas. Subarea E was

the unique subarea where the species was present at all depths. Multiple linear regressions adequately represented the relationship between *H. papillosa* abundance, depth and the presence of hard substrate ($y = -0.00378 + 0.00019$ depth $+ 0.00033$ rock $+ 0.00229$ coralline rock; ANOVA, p value <0.001), even if the regression coefficient of determination was low ($R^2 = 0.163$). Species abundance was not significantly related to depth (t test, p value $= 0.339$), whereas it was significantly related to rock (t test, p value <0.001) and coralline rock (t test, p value <0.001) coverage.

Fig. 4 Bathymetrical distribution of the density of *H. papillosa* in each subarea **a–i**: the *black square* indicates the median value of the density; the *box* indicates the first and the third quartiles; and the *line* indicates the range between minimum and maximum and median value. *Gray-scale histograms* represent the total number of sampling units for each substrate type (see legend) over the studied bathymetrical range. *Numbers* at the *right side* indicate the percentage of sampling units with the presence of the species. Total number of specimens (n) and sampling units (m) are indicated for each subarea
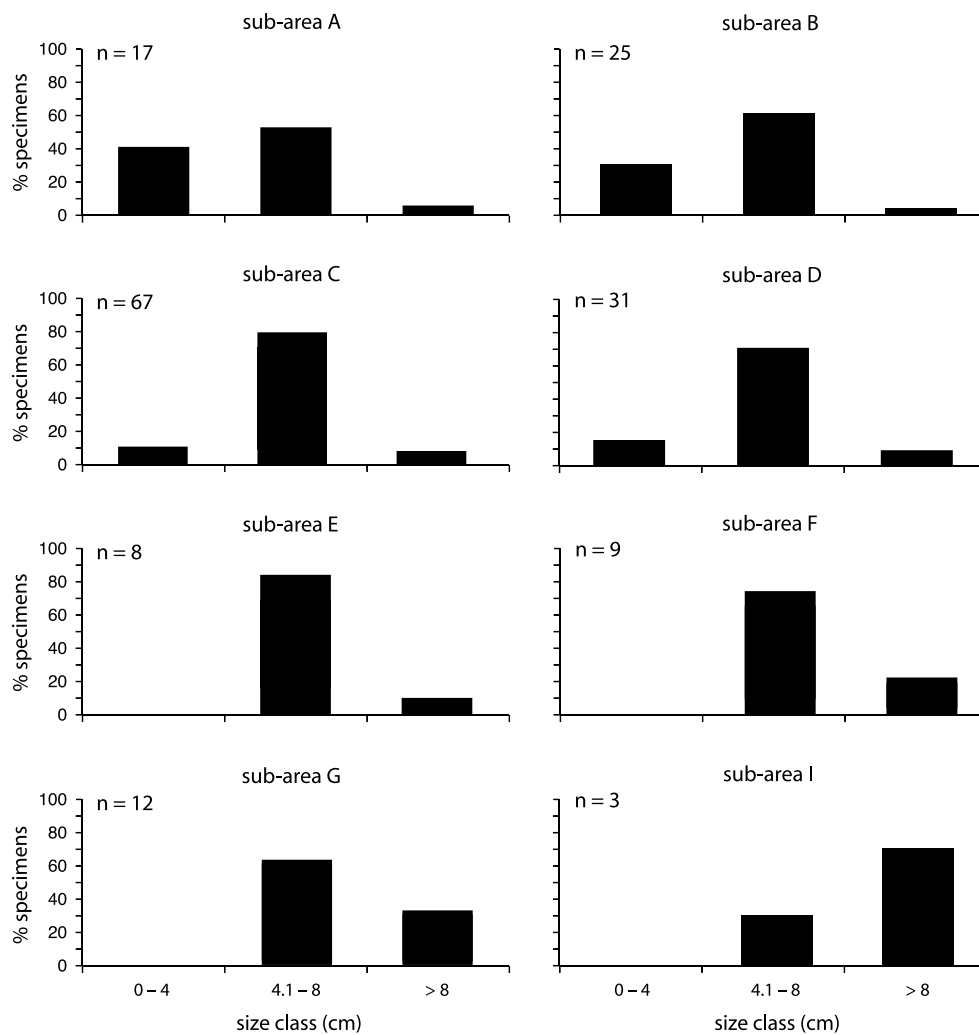
Fig. 5 Size–frequency distribution of *H. papillosa* in all the subareas (*n* = number of specimens)

Along all the study area, the smallest, the medium, and the largest ascidians encountered had a dimension of 2.35, 5.31, and 11.26 cm, respectively. Medium-sized specimens were dominant in all the subareas (but the subarea I where only three specimens were observed), and small specimens of *H. papillosa* were only observed in the south and east coast of the Cap de Creus. Average heights of *H. papillosa* for each subarea are shown in Table 1. Skewness and kurtosis analysis were not significant in any of the subareas (Table 2), highlighting that the size structure of the populations of this species showed a normal distribution in all the subareas with the dominance of the medium-size class (Fig. 5). Data for the subarea H were not shown because none of the encountered specimens could be measured. Skewness and kurtosis for subarea I could not be calculated because of the low number of specimens encountered and measured (Fig. 5, Table 2).

Trophic impact of *Halocynthia papillosa*

The 172 *H. papillosa* observed and measured in the analyzed sampling units (total area: 1.24 ha), ingested each year 519.4 g of C: 380.8 g C in spring, 64.3 g C in summer, 40.0 g C in autumn, and 34.3 g C in winter. A total of 499.2 g of C were estimated to be respired each year: 121.3 g C in spring, 198.5 g C in summer, 136.3 g C in autumn, and 43.1 g C in winter (Table 3). Regarding the N ingestion, *H. papillosa* ingested 22.9 g of N in spring, 4.9 g of N in summer, 1.4 g of N in autumn, and 2.3 g of N in winter, accumulating a total of 31.4 g of N each year (Table 4). From these data, the balance between ingested and respired C results in a net surplus of 259.6 g of C during spring, whereas a deficit of 134.2 g results in summer, 96.2 g in autumn, and 8.9 g in winter. Overall, the balance between ingested and respired C for *H. papillosa*

Table 3 Seasonal ingested (I) and respired (R) carbon (g) along the considered depth range in the study area, and their total (TOT)

Depth (m)	Spring		Summer		Autumn		Winter	
	I	R	I	R	I	R	I	R
0–5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5–10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10–15	2.6	0.9	0.4	1.6	0.3	0.9	0.2	0.3
15–20	5.2	1.9	0.9	3.3	0.5	1.9	0.5	0.6
20–25	50.6	18.3	8.5	31.6	5.3	18.1	4.5	5.7
25–30	104.0	37.5	17.6	64.9	10.9	37.1	9.4	11.7
30–35	92.0	33.3	15.5	57.6	9.7	33.0	8.3	10.4
35–40	63.0	14.6	10.6	19.7	6.6	22.5	5.7	7.1
40–45	25.3	5.8	4.3	7.9	2.7	9.0	2.3	2.9
45–50	25.9	6.0	4.4	8.1	2.7	9.3	2.3	2.9
50–55	7.8	1.8	1.3	2.4	0.8	2.8	0.7	0.9
55–60	4.5	1.0	0.8	1.4	0.5	1.6	0.4	0.5
60–65	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
65–70	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TOT (g C)	380.8	121.3	64.3	198.5	40.0	136.3	34.3	43.1

Table 4 Seasonal ingested nitrogen (g) along the considered depth range in the study area, and its total (TOT)

Depth (m)	Spring	Summer	Autumn	Winter
0–5	0.0	0.0	0.0	0.0
5–10	0.0	0.0	0.0	0.0
10–15	0.2	0.0	0.0	0.0
15–20	0.3	0.1	0.0	0.0
20–25	3.0	0.7	0.2	0.3
25–30	6.3	1.3	0.4	0.6
30–35	5.5	1.2	0.3	0.5
35–40	3.8	0.8	0.2	0.4
40–45	1.5	0.3	0.1	0.2
45–50	1.6	0.3	0.1	0.2
50–55	0.5	0.1	0.0	0.0
55–60	0.3	0.1	0.0	0.0
60–65	0.0	0.0	0.0	0.0
65–70	0.0	0.0	0.0	0.0
TOT (g N)	22.9	4.9	1.4	2.3

in the study area results in a net surplus of 20.2 g of C per year.

The impact of this species per m² was also estimated: *H. papillosa* was able to ingest 1.28 ± 0.6 g C m^{-2} in spring, 0.22 ± 0.10 g C m^{-2} in summer, 0.13 ± 0.06 g C m^{-2} in autumn, and 0.12 ± 0.05 g C m^{-2} in winter. Conversely, this species respired 0.41 ± 0.21 g C m^{-2} in spring, 0.67 ± 0.37 g C m^{-2} in summer, 0.46 ± 0.22 g C m^{-2} in autumn, and 0.15 ± 0.07 g C m^{-2} in winter. Regarding N ingestion, 0.08 ± 0.04 g N m^{-2} was ingested in spring, 0.017 ± 0.008 g N m^{-2} in summer, 0.005 ± 0.002 g N m^{-2}

in autumn, and 0.01 ± 0.003 g N m^{-2} in winter. The highest ingestion and respiration of C, as well as ingestion of N, occur between 20 and 35 m depth in spring (Figs. 6, 7, 8), due to the elevated number of specimens observed in this depth range (Fig. 4).

Environmental conditions

Stratification of the water column began to develop in April with a thermo- and pycnocline developing at 30 m depth. The water column was fully stratified in July and August with a thermo- and pycnocline at 35 m depth, whereas stratification was stretched out in September (Fig. 9a, b). Turbidity was highly variable with the highest value in November and in April–June in shallow waters, and in September and May in deeper waters (Fig. 9c). Organic C/N composition of the POM changed during season but without significant difference between depths (Table 5).

Discussion

This study assessed the distribution and population size structure of the active suspension feeder *H. papillosa* over a large Mediterranean coastal area, and inferred the trophic impact and the potential role of this species as C and N sinks.

Large-extent distribution of *Halocynthia papillosa*

Halocynthia papillosa has a more homogeneous distribution along the Cap de Creus coastline (Fig. 3) than

Fig. 6 Seasonal ingested carbon (mg C m^{-2}, mean ± SE) estimated every 5-m-depth interval
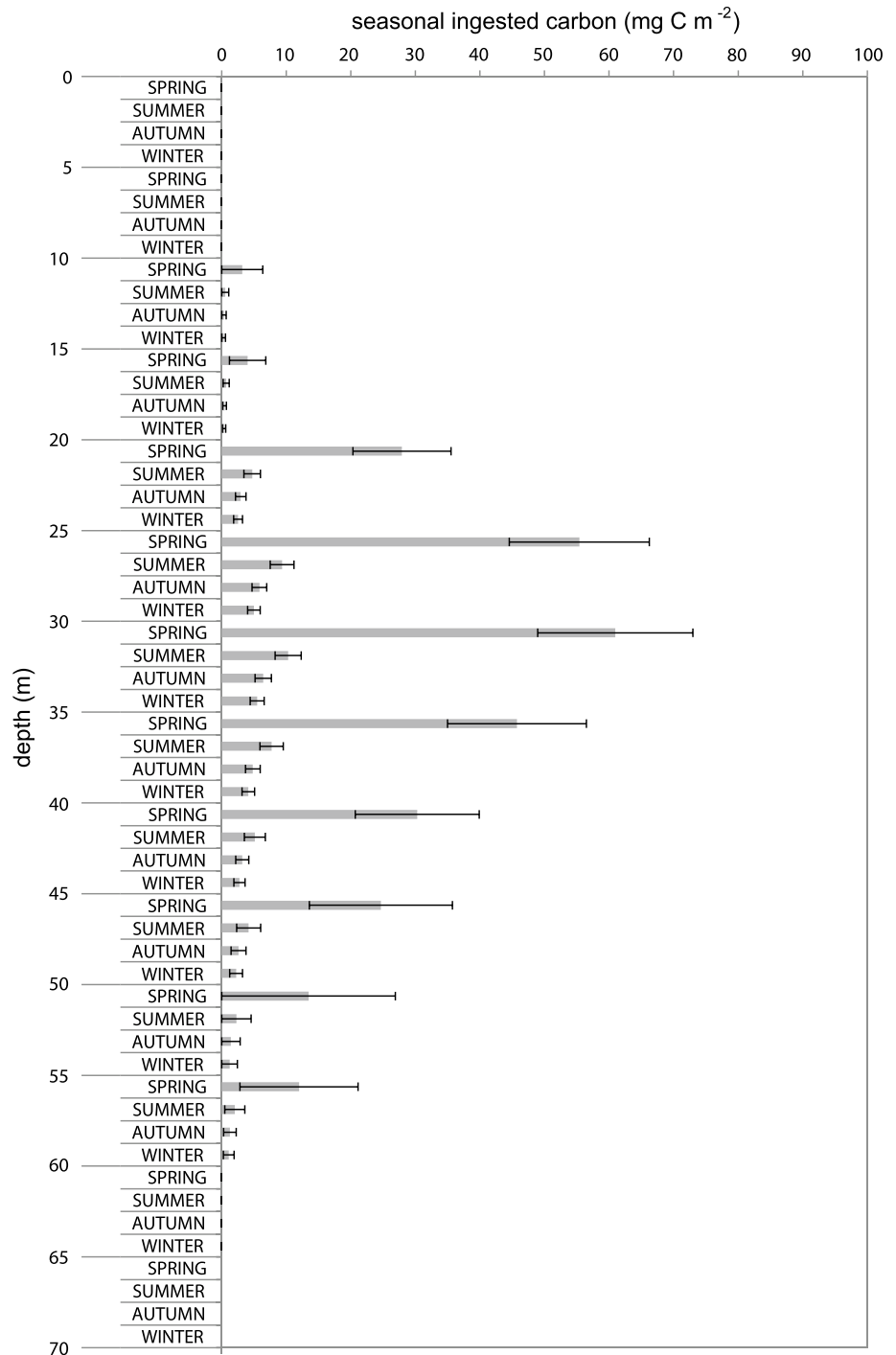
previously studied passive suspension feeder species (*Corallium rubrum*, *Eunicella singularis*, *Paramuricea clavata*, *Leptogorgia sarmentosa*, *Alcyonium acaule*; Rossi et al. 2008; Gori et al. 2011a; Ambroso et al. 2013). These other species have their higher abundance and density concentrated on the north side of the cape, in the area directly exposed to the main currents (DeGeest et al. 2008; Ulses et al. 2008). The active feeding of *H. papillosa* may explain its more homogeneous distribution along the coast,

independently from the main hydrodynamic conditions of the area (Ribes et al. 1999; Armsworthy et al. 2001; Petersen 2007). The maximum density of *H. papillosa* observed in this study (4 individuals m^{-2}) is in line with previous data from Banyuls-sur Mer (10–15 km north from Cap de Creus), where densities of ~3.5 specimens m^{-2} were found on walls and crevices, and 1 specimens m^{-2} on boulders (Weinberg 1978). These data confirm that even if the ROV-based approach has its limitations in accuracy,

Fig. 7 Seasonal respired carbon (mg C m^{-2}, mean ± SE) estimated every 5-m-depth interval
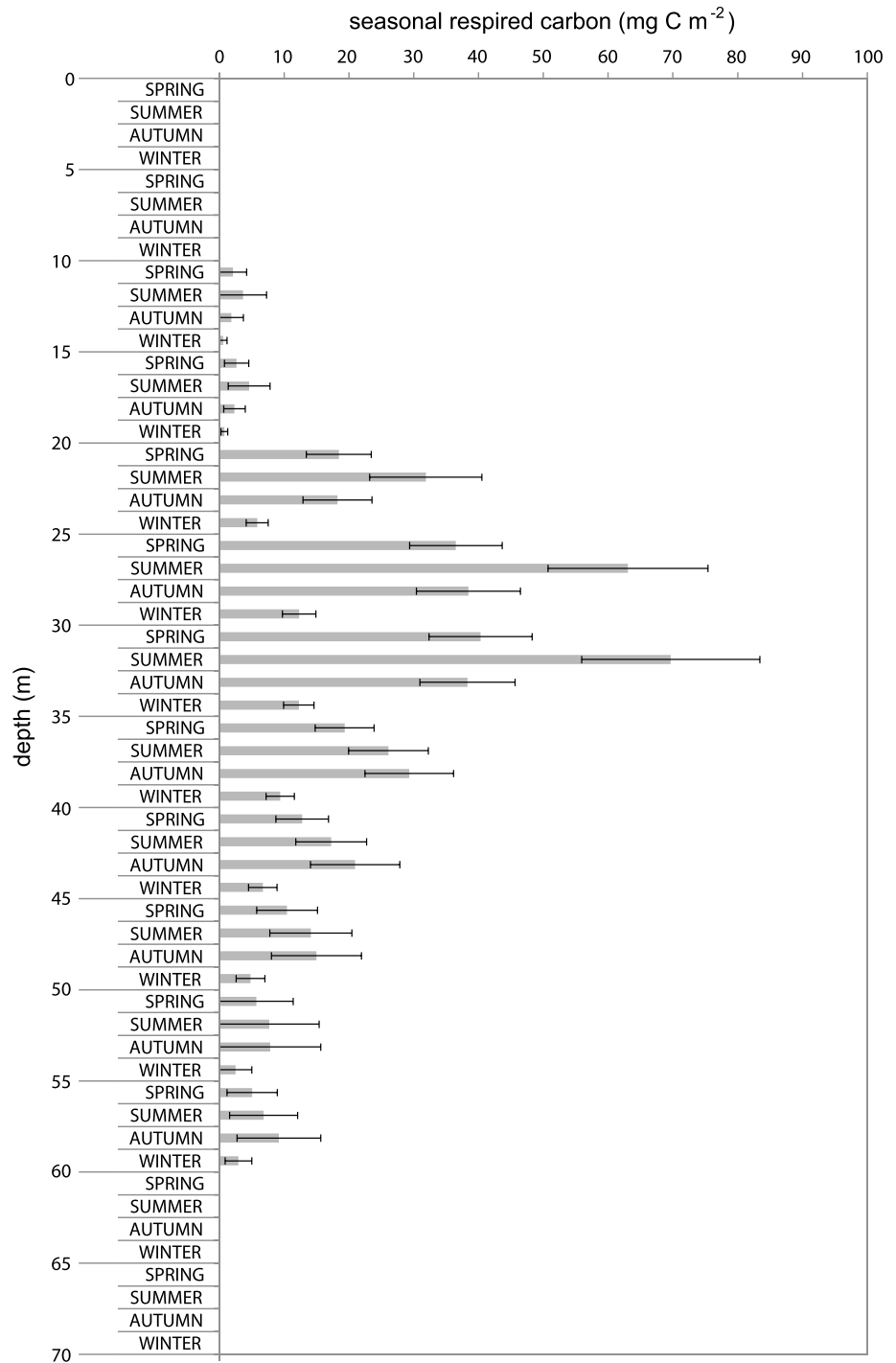

the numbers gathered with this methodology are realistic. In fact, similar densities of ascidian species have been recorded in other shallow water tropical, cold temperate, or polar environments (McClintock et al. 1991; Riisgård et al. 1995; Shenkar and Loya 2008). High-density patches of *H. papillosa* were not observed in the present or in the previous studies. This lack of patchiness could be related to the 12–24 h free-swimming period of its larvae (Lübbering

et al. 1993), which may allow large-extent dispersion of the species (Fletcher et al. 2013).

The presence of the highest densities of *H. papillosa* between 20 and 50 m depth is in line with previous data about gorgonians, soft corals, and hydrozoans (Rossi et al. 2008; Gori et al. 2011a; Ambroso et al. 2013; Cúrdia et al. 2013; Di Camillo et al. 2013). This highlights that maximum biomass of many coastal suspension feeder species

Fig. 8 Seasonal ingested nitrogen (mg N m^{-2}, mean $\pm$ SE) estimated every 5-m-depth interval
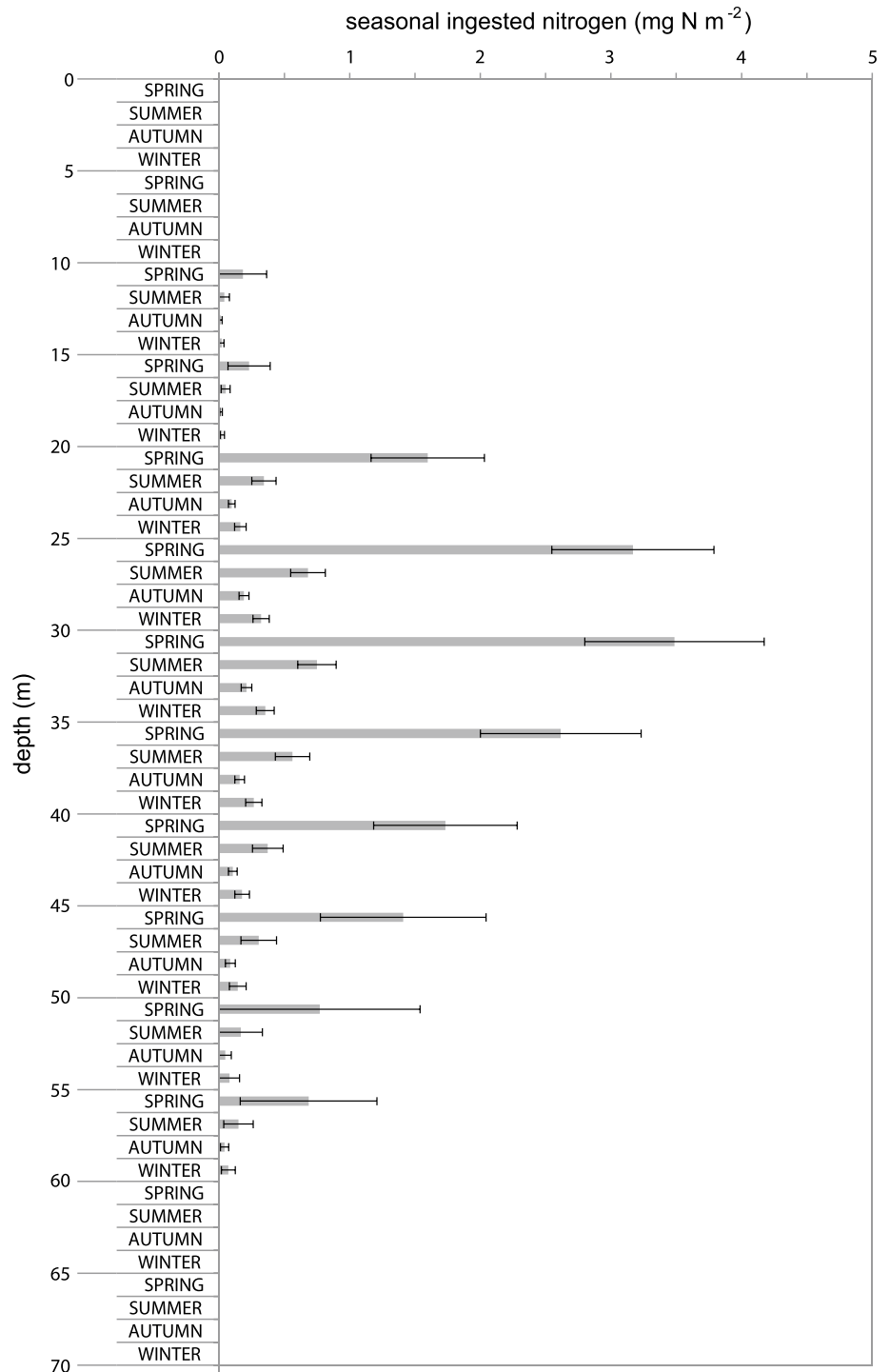
may be concentrated in deep sublittoral bottoms, a largely disregarded area by previous studies, as well as by conservation and management decisions (Bongaerts et al. 2010; Bridge and Guinotte 2013). As the highest abundance of rock and especially coralligenous substrate suitable for the studied species is concentrated between 20 and 50 m in the study area (Sardá et al. 2012), substrate availability could explain the higher densities observed in this depth

range (Fig. 4). The very low presence of *H. papillosa* in the Montgrí and Medes area contrasts with the occurrence and abundance of this species in Cap de Creus, and could be related to several factors. The elevated number of SCUBA divers visiting the Medes Islands each year (up to 10,000, Sala et al. 1996; Linares et al. 2012) might have impacted the studied species in the area; indeed, SCUBA divers have a significant impact on the abundance of *H.*

Fig. 9 Main environmental characteristics at the study area along an annual cycle: Water temperature in °C (**a**), density g cm^{-3} (**b**), and water turbidity expressed in suspended sediment concentration (SSC) mg l^{-1} (**c**)
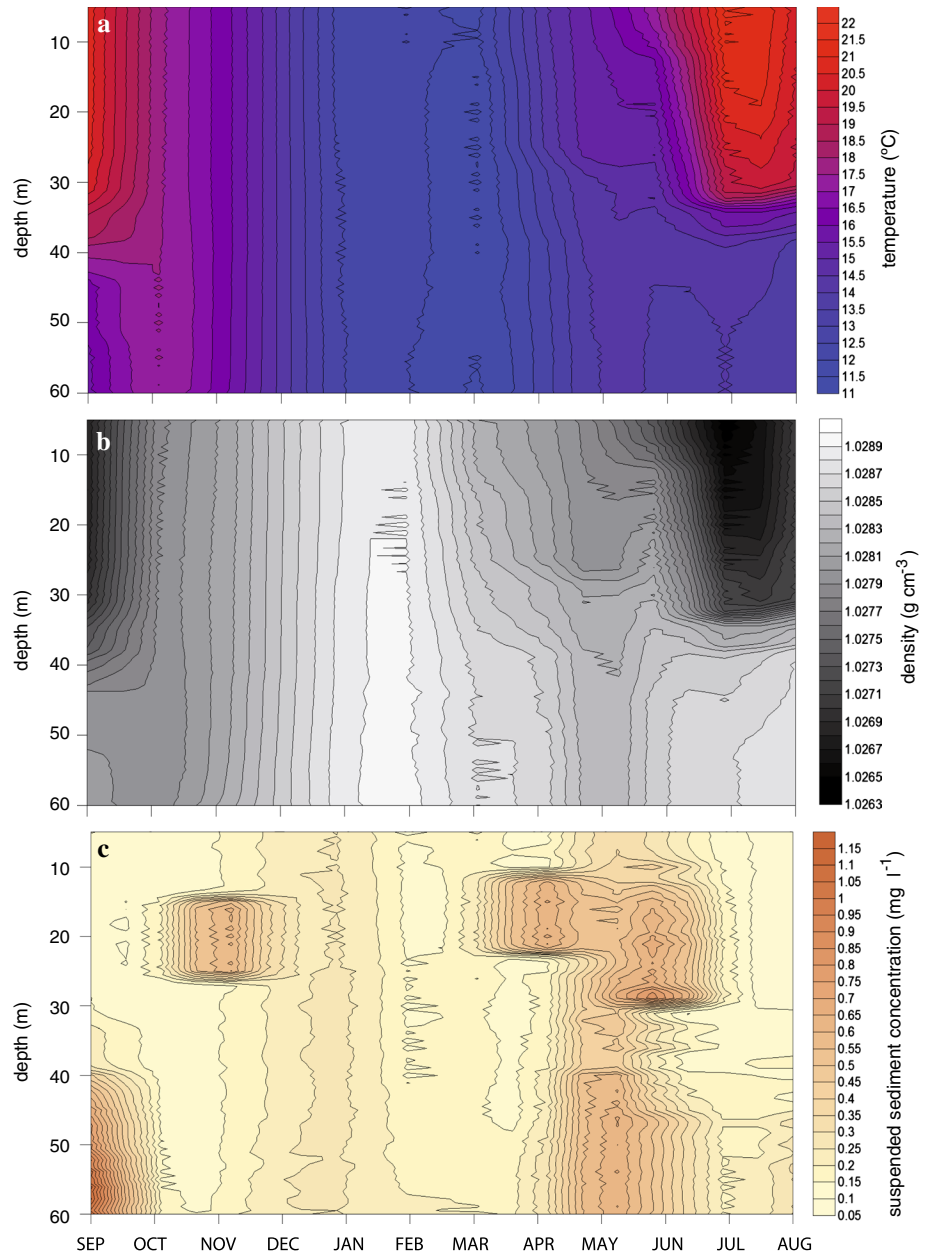
Table 5 Seasonal organic C/N composition (mean ± SD) of the particulate organic matter at 20 and 60 m depth

Depth (m)	Season	C/N	
		Mean	SD
20	Spring	11.40	4.12
	Summer	8.97	0.61
	Autumn	5.79	0.38
	Winter	8.18	2.26
60	Spring	10.19	1.08
	Summer	9.71	1.59
	Autumn	7.01	2.92
	Winter	8.57	3.43

papillosa (Luna-Pérez et al. 2010). However, no *H. papillosa* was observed along the Montgrí coast (Fig. 3), where diver pressure is one order of magnitudes lower than in the Medes Islands (Coma et al. 2004; Linares et al. 2012). The survey in Montgrí-Medes area was performed after the heavy storm that took place in December 26th 2008, which caused up to 80 % of mortality rates of several coastal benthic organisms (Garcia-Rubies et al. 2009; Navarro et al. 2011; Teixidó et al. 2013). Although the storm might have affected the shallow organisms, deep *H. papillosa* would not have been affected by this punctual perturbation. The disappearance from the same area of the ascidian *Microcosmus sabatieri* long before this heavy storm (Martinez Ricart 2011) also reinforce the idea that other reasons may

explain the sparse distribution of *H. papillosa* in Montgrí and Medes. It has been demonstrated that the abundances of many species has severely declined in Montgrí-Medes area, during the last two decades (Sala et al. 1996; Coma et al. 2004; Tsounis et al. 2006; Teixidó et al. 2013). The institution of the MPA in Medes Islands in 1983, with consequent fishing prohibition, resulted in a sudden increase in fish density, which consequently increase the feeding pressure on benthic organisms (García-Rubies and Zabala 1990; Sala et al. 1998), and might have reduced the recruitment of suspension feeder species (Rius and Zabala 2008; Santangelo et al. 2012). Since the highest abundances of *H. papillosa* are concentrated on rocky and coralligenous bottoms at 20–50 m depth (Fig. 4a–g), the lower presence of *H. papillosa* in Montgrí-Medes may also be related to the scarcity of these substrate in the 20–50 m depth range in this area (Fig. 4h, i; Gili and Ros 1985; Martinez Ricart 2011; Sardá et al. 2012).

Large-scale trophic impact of an active suspension feeder

Our results show that *H. papillosa* is not relevant in terms of global C coastal budget. This fact might be related with the low density and completely lack of patchiness of the studied species. *H. papillosa* yearly ingested 519.4 g C and retained 20.2 g C along the 1.24 ha of the explored coastal area. The C retained represents the C sink (difference between ingested and respired C); this C could return to the system partially in the form of reproductive output (Becerro and Turon 1992). We found out that *H. papillosa* had a trophic impact as high as 5.194×10^{-4} t C ha^{-1} year^{-1} and 3.14×10^{-5} t N ha^{-1} year^{-1}, and that the retention is equal to 2.02×10^{-5} t C ha^{-1} year^{-1}. Therefore, the trophic impact of *H. papillosa* is five orders of magnitude lower compared with previous studies performed on sea grass meadows (6.7 t C ha^{-1} year^{-1} of C stored in 1 year; Duarte et al. 2010) and mangrove forests (1.5 t C ha^{-1} year^{-1} of C stored in 1 year, Eong 1993).

Due to the higher densities and bigger specimens of *H. papillosa* concentrated between 20 and 50 m depth, this depth range shows the highest quantity of C retained and N ingested, compared to shallower or deeper bottoms where very low impact is recorded. The low presence of the species at depths shallower than 15–20 m may be due to high hydrodynamics and temperature constraints (shallow waters temperature rise up to 20–22 °C in the study area, Rossi and Gili 2005; Gori et al. 2012; Fig. 9), as well as to the presence of faster growing seaweeds competing for space (Young and Chia 1984; Garrabou et al. 2002). Although the quality of food is similar in shallow and deep waters (Table 5), temperature rising in spring, summer, and early autumn in shallow waters (Fig. 9) determines a negative balance between ingested and respired C at depths

shallower than 15–20 m. In fact, respiration of *H. papillosa* increases in the warmer seasons (Coma et al. 2002), while clearance rates fall to suboptimal levels (Petersen 2007). This could also explain the low abundance of *H. papillosa* in shallow waters. Lesser (2006) provided evidence of a similar bottom-up control in sponge in coral reefs, where the availability of picoplankton is one of the key factors to understand their bathymetrical distribution.

Spring is the only period in which there is a clear positive balance between ingested and respired C, possibly due to the high quality and quantity of the near-bottom seston available for the species (Grèmare et al. 1997; Estrada 1996; Ribes et al. 1999; Rossi et al. 2003). Indeed, the studied species had to stand for the other periods of the year with the energy stored in this favorable season, allowing secondary production, growth, and reproduction taking place in September until early November (Becerro and Turon 1992). In high seasonal environments, benthic suspension feeders can accumulate large quantities of lipids in favorable seasons to face the less favorable part of the year, where food input may be drastically lower (Cavaletto and Gardner 1999; Rossi et al. 2006; Elias-Piera et al. 2013).

Conclusion

The present study confirms that in Mediterranean Sea coastal areas, many benthic suspension feeder species concentrate their presence and abundance below 30 m depth (Rossi et al. 2008; Bo et al. 2011; Gori et al. 2011a; Ambroso et al. 2013). This should be carefully taken into account when conservation and management measures of coastal areas are established. Although our results shows that the studied species is not important in terms of C sink, it is the perfect case study to test the applicability of the novel approach proposed in this study for the quantification of the functional role of benthic species. The application of this methodology to the main species that compose the animal forests will provide a quantitative evidence of the ecosystem services performed by these assemblages in the overall budget of C as they may retain organic matter during decades or, in some cases, even centuries.

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