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The role of Mediterranean sponges in benthic–pelagic coupling processes: *Aplysina aerophoba* and *Axinella polypoides* case studies

Martina Coppari [a,*], Andrea Gori [b], Núria Viladrich [a], Luca Saponari [c], Antonio Canepa [c], Jordi Grinyó [c], Alejandro Olariaga [c], Sergio Rossi [a]

[a] Institut de Ciència i Tecnologia Ambientals, Universitat Autònoma de Barcelona, Edifici Z Campus UAB, 08193 Cerdanyola del Vallès, Spain
[b] Universitat de Barcelona, Departament d'Ecologia, Av. Diagonal 643, 08028 Barcelona, Spain
[c] Institut de Ciències del Mar, Consejo Superior de Investigaciones Científicas, Passeig Marítim de la Barceloneta 37–49, 08003 Barcelona, Spain

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ABSTRACT

Sponges are important components of marine benthic communities with a worldwide distribution ranging from polar to tropical regions. They play a key role in benthic–pelagic coupling processes through their active suspension feeding, providing a trophic link between the benthos and the overlying water column. Little is known about their broad-scale distribution and feeding ecology. The general tendency is to quantify their trophic impact through small patch estimations. In this work, two of the most abundant sponges in Mediterranean coastal bottoms (*Aplysina aerophoba* and *Axinella polypoides*) were studied combining remotely operated vehicle (ROV) survey with in situ feeding experiments. Spatial, bathymetrical distribution and population size structure of these species were analysed, together with their trophic ecology, in spring and autumn. We found that *A. aerophoba* is distributed between 5 and 20 m depth, with maximum densities of 1.6 sponges m^{-2}. This species ingested 0.12–0.39 mg of carbon (C) g $AFDW^{-1}$ (ash free dry weight) day^{-1} in spring and 0.09–0.13 mg C g $AFDW^{-1}$ day^{-1} in autumn. Conversely, *A. polypoides* was found between 10 and 70 m depth, with maximum densities of 7.6 sponges m^{-2}. This species ingested 0.07–0.17 mg C g $AFDW^{-1}$ day^{-1} 1 in spring, and 0.18–0.60 mg C g $AFDW^{-1}$ day^{-1} in autumn. The highest uptake of C concentrated between 5 and 15 m depth for *A. aerophoba* and between 65 and 70 m depth for *A. polypoides*. In the 1.14 ha of studied coastal bottom, *A. aerophoba* ingested 1.87 g C during spring and 0.19 g C during autumn, whereas *A. polypoides* 13.60 g C and 29.36 g C during spring and autumn, respectively. The present approach allowed a spatially explicit quantification of benthic–pelagic coupling processes produced by two of the most common sponges in a Mediterranean coastal area. This methodology, applied to benthic communities, mirrors similar approaches used in terrestrial forestry studies for C flux estimation.

1. Introduction

Sponges (Porifera) have been important components of benthic fauna since the Early Cambrian (Zhang and Pratt, 1994). There are more than 8000 known species (World Porifera Database; www.marinespecies.org/porifera; Van soest et al., 2014) distributed worldwide in marine and freshwater systems (Hooper and Van Soest, 2002). These metazoans became dominant during climate change shifts, forming transitional reefs that substituted calcium carbonate bioconstructions (Copper, 1994).

Sponges play different functional and structural roles (Bell, 2008), either bio-eroding or consolidating substrata, providing protection from predation and enhancing survival of associated species, thus increasing biodiversity (Marliave et al., 2009). They represent important carbon (C) sinks, accumulating biomass in three-dimensional or encrusting long-lived structures (Maldonado et al., 2012), and play an important role in the biogeochemical cycles of C, nitrogen (N) or silicon (Si) (Nixon et al., 1976; Richter et al., 2001; Maldonado et al., 2005; de Goeij et al., 2008). Although sponges can use food sources ranging from dissolved organic matter (DOM) (de Goeij et al., 2008) to small crustaceans (<1 mm) (Vacelet and Boury-Esnault, 1995), they primarily feed on picoplankton (<2 μm) with efficiencies up to 99% (Pile et al., 1996; Ribes et al., 2005).

The C transfer from pelagic to benthic systems has been estimated in shallow (Ribes et al., 1999a; Ribes et al., 2005) and deep environments (Pile and Young, 2006; Yahel et al., 2007; Kahn et al., 2015). However, most studies have quantified the C captured per m^{-2} only on small patches (Rossi et al., 2004) and substantial work is still needed to quantify the influence of sponges in benthic–pelagic coupling and biogeochemical cycles on a broad spatial scale. If these estimates are coupled with species distribution, density and population structure data over large areas, the impact of the sponge feeding can be quantified at the ecosystem-level. This approach is commonly used in landscape ecology (Bekkby et al., 2002) to infer the

* Corresponding author.
E-mail addresses: martysve85@gmail.com, Martina.Coppari@uab.cat (M. Coppari).

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role of forests, crops or grasslands as C sinks. This approach might help to bridge the gap of knowledge in between landscape and seascape ecology (Pittman et al., 2011).

Here we study the role in benthic–pelagic coupling processes of two of the most common Mediterranean coastal sponges, *Aplysina aerophoba* (Nardo, 1833) (Orden: Verongida, Family: Aplysinidae) and *Axinella polypoides* Schmidt, 1862 (Order: Halichondrida, Family: Axinellidae) (Fig. 1). The two species inhabit different communities and show a different morphology, feeding and physiological strategy; the first is characteristic of Mediterranean photofilic algae and precoralligenous communities, whereas the second is among the main constituents of the coralligenous community (Ballesteros, 2006; Gili et al., 2014). *A. aerophoba* is a massive species, organized in chimney-like structures with high symbiotic microbial abundance (HMA), which is partially constituted by photosynthetic cyanobacteria (Vacelet, 1970). *A. polypoides* is an erect, tree-like sponge with low microbial abundance (LMA). The microbial density in the tissues of HMA sponges is 2 to 4 orders higher than that of the surrounding water (Vacelet and Donaday, 1977; Friedrich et al., 2001). Conversely, for LMA species, it is the same as in the surrounding water (Hentschel et al., 2003). Different densities of bacteria in the sponge tissue contribute to determine the distribution pattern of the species as well as its clearance rates and feeding strategy (Vacelet and Donaday, 1977; Friedrich et al., 2001), depending on the phototrophic contribution of endosymbiotic bacteria or algae (Wilkinson, 1983).

Based on Coppari et al. (2014), quantitative analysis of video transects performed by a remotely operated vehicle (ROV) were coupled to data from in situ feeding experiments to estimate the trophic impact and the C flux mediated by these coastal Mediterranean sponges over a large extent. ROV surveys allow to determine the abundance and distribution pattern of megabenthic species over large areas and depths that cannot be sampled by SCUBA diving (Mortensen and Buhl-Mortensen, 2004; Gori et al., 2011a), and where several benthic suspension feeders are often the most abundant organisms (Rossi et al., 2008; Bo et al., 2011; Gori et al., 2011a; Ambroso et al., 2013; Coppari et al., 2014). In situ experiments enable the determination of the feeding habits of benthic suspension feeders under natural conditions (Ribes et al., 1999b; Tsounis et al., 2006) taking into account seasonal changes in food availability and retention rates (Ribes et al., 1998).

This study was consequently organized as follows: (1) characterize the spatial distribution pattern of *A. aerophoba* and *A. polypoides* over a broad geographical and bathymetrical extent; (2) describe their population size structure; (3) perform in situ experiments to quantify the feeding habits and C uptake of the two species in spring and autumn and to test the effect of the seasonality; and (4) estimate the total amount of C ingested by the studied species and how it changes with depth over the entire study area.

This study will increase our understanding of the distribution pattern of two important sponge species of the Mediterranean coastal bottoms, and will provide quantitative data about their role in benthic–pelagic coupling processes.

2. Methods

2.1. Study area

Fieldwork was performed in Cap de Creus (42° 19′ 12″ N; 003° 19′ 34″ E) on the northern extreme of the Catalan Coast (northwestern Mediterranean Sea), bordering France. The study area was sub-divided into 7 subareas, from A to G (see also Gori et al., 2011a) according to the main hydrodynamic patterns in the zone, and the specific features of the studied coast (Fig. 2). 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 River that supplies ~90% of the total freshwater in the gulf (Palanques et al., 2006). The most important winds influencing the study area are the northerly Tramuntana and the northwesterly Mistral that occur for 41% and 28% of the time, respectively. Strong south-easterly and easterly marine winds are rare (<6% of the time) and brief (less than 3 days), in contrast to the northerly ones that can last up to one month (Ulses et al., 2008). Consequently, subarea A is the most sheltered area of the surveyed coast; subareas B, C, D are affected mainly by easterly winds and 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 which accelerate around the cape (DeGeest et al., 2008). Due to the reduced influence of the main near-bottom currents, subarea G is characterized by sediment deposition processes.

2.2. Sponge distribution and size structure

2.2.1. Sampling procedure

Fieldwork was conducted in October–November 2004. Video transects were performed with the ROV Phanthom XTL equipped with a SONY FCB S3000P 3CCD camera (with a resolution of 700 horizontal lines), a depth sensor, a compass, and two parallel laser beams that provide the scale to define a fixed width of the transects (0.5 m) for

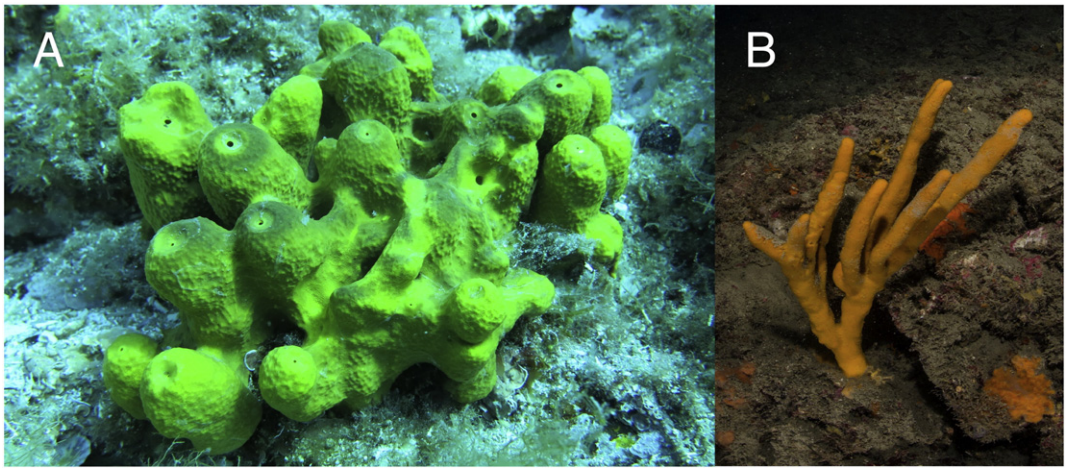

Fig. 1. Sponge species investigated in this study: *Aplysina aerophoba* (A), and *Axinella polypoides* (B). Photos by Núria Viladrich and Federico Betti.

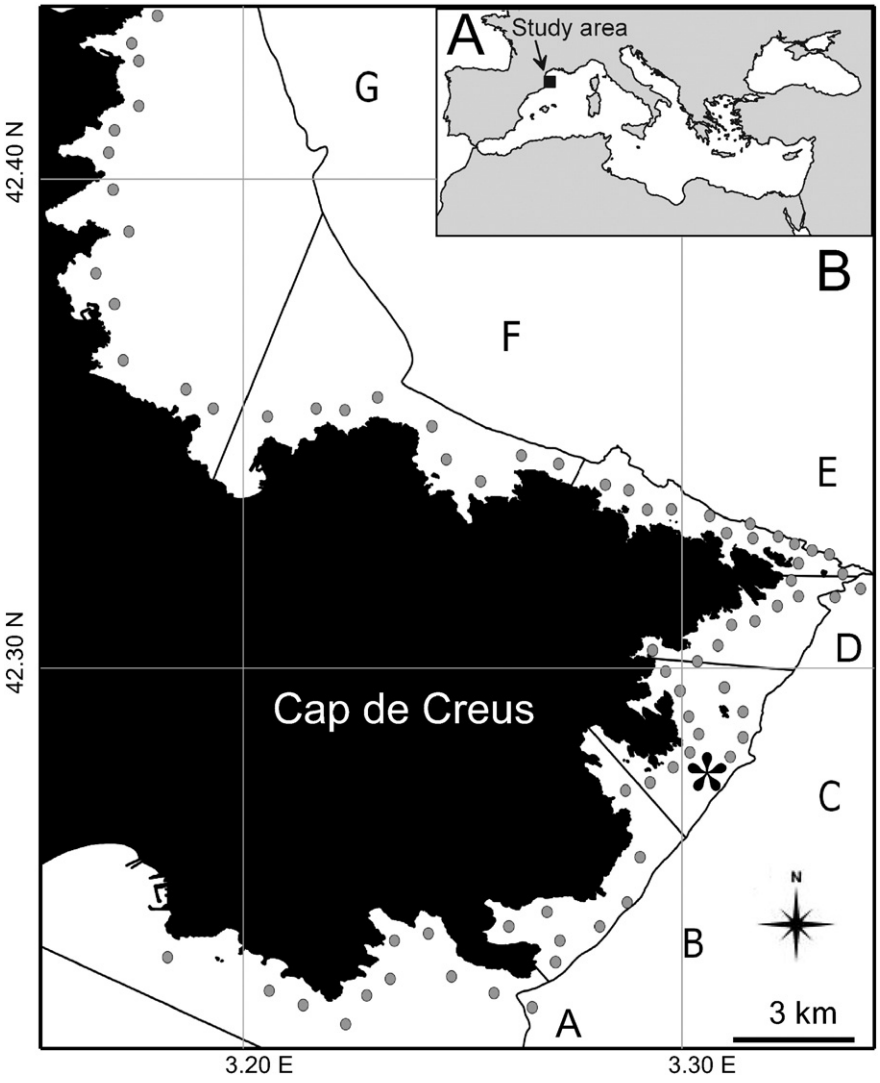

Fig. 2. Map of the study area: location of the study area (A), Cap de Creus area showing the seven subareas and the transect positions; the black star indicates the position of the sampling site Punta s'Oliguera (B).

subsequent video analysis. The ROV speed was kept constant, approximately 0.4 knots. In each sampled location, seabed video recording started at the deepest position and proceeded toward the shallows, until the ROV surfaced close to shore. Depending on the geographical characteristics of each location, transects started at a depth between 12 and 71 m, and their length varied in between 92.6 m and 907.1 m. On the whole, 76 video transects were recorded (Fig. 2B) covering a total distance of 28.3 km.

2.2.2. Video analysis

Quantitative video analysis was performed according to the methodology described in Gori et al. (2011a). Videos were analysed using Final Cut Pro 6.0.6 software (Apple). As speed was constant, all the pauses in the movement of the ROV were removed to estimate the total length of each transect. Sequences with poor image quality, due to bad visibility or distance from the bottom, were discarded from the analysis. Each transect was divided in sampling units of 2.5 m^2 (0.5 m width and 5 m long). The sampling unit area was chosen from Weinberg (1978), who estimated a sample size of 2 m^2 as representative for studying invertebrates in the Mediterranean rocky substrata. A total of 4559 useful sampling units were obtained from the 76 transects. For each sampling unit, the number of *A. aerophoba* and *A. polypoides* was determined, together with their depth and the seabed substrate type classified in five categories: soft bottoms (mud, sand, detritic and *Posidonia oceanica* cover), maerl (species of coralline algae growing loosely in beds of fragmented nodules), pebbles, rock and coralline rock. The distance occupied by each substrate type was converted into percentage of coverage. The cumulative percentage was converted into number of sampling units considering 100% of a given substrate type as one sampling unit.

To study the size structure of their populations, the maximum height of each observed *A. aerophoba* and *A. polypoides* was measured on still images extracted from the videos using the Macnification 1.8 software (Schols and Lorson, 2008). Only the organisms situated perpendicular to the video and on the same plane of the laser beams were measured, considering the distance between the laser beams as calibration for the still images (Gori et al., 2011b). This methodological constraint entails that only a subsample of the observed sponges could be measured for the study of the size structure (see Results).

2.2.3. Data treatment

The presence of *A. aerophoba* and *A. polypoides* was quantified 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 mapping the densities observed in each sampling unit on a geographically referenced map using Quantum GIS 1.7.2

software (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 5 m depth intervals.

Skewness and kurtosis were used to analyse the size structure of the sponge populations. 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 (R Development Core Team, 2012).

2.3. Trophic impact

2.3.1. In situ feeding experiments

In situ feeding experiments were conducted at 5 m depth (Punta s'Oliguera, Cap de Creus, 42° 17′ 1.62″ N; 003° 17′ 57.18″ E, Fig. 2B) in May and November 2013 for *A. aerophoba*, and in November 2013 and May 2014 for *A. polypoides*. The timing was chosen to obtain feeding rate data for both species in spring and autumn, which are the most contrasting seasons in the Mediterranean Sea in terms of seston quantity and quality (Rossi et al., 2003; Rossi and Gili, 2005). Experiments were performed following Ribes et al. (2000) and Tsounis et al. (2006) using incubation chambers of approximately 4.5 l volume each (Science O'Matic, www.science-o-matic.com) (Fig. 3 and Supplementary data, see Appendix 1 in Supplementary data). The system is designed to switch from open circulation (with water continuously entering the

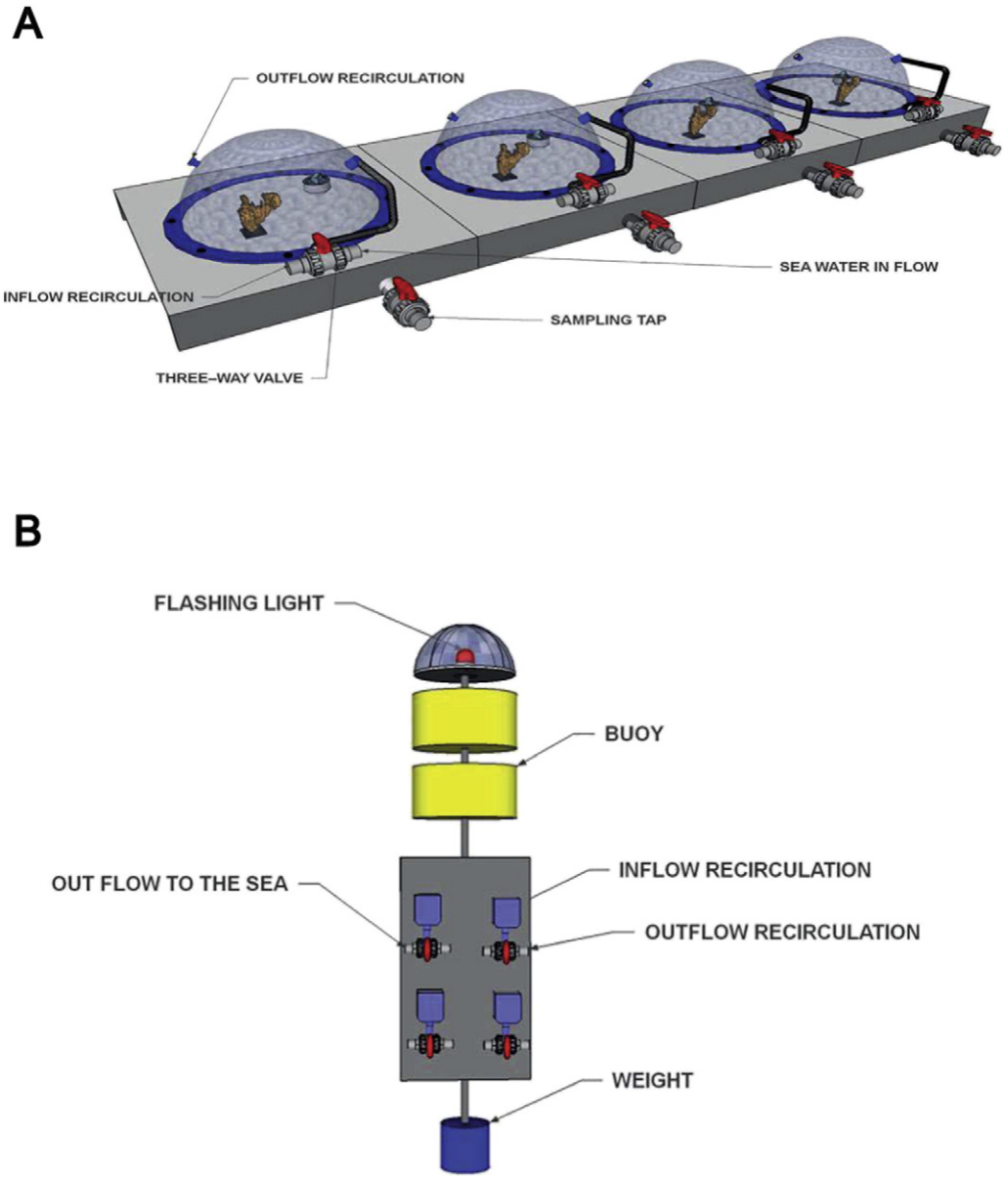

Fig. 3. In situ incubation chambers: the three way valve allows switching from open to closed recirculation, the sampling tap allows the plastic bag opening and consequently the final water sampling (A); floating system equipped with 4 pumps and 4 taps (one for each chamber); the "outflow to the sea" indicates where the initial and final waters were sampled (B).

chamber from outside) to closed recirculation of the water inside each chamber. For each species and season, three experiments with sponges (n = 3) and three controls without sponges (n = 3) were performed. Sponges were collected by SCUBA diving, one-day prior to the start of the experiment. In order to reduce the stress as much as possible, sponges were removed together with a piece of substrate, which was carefully cleaned of any epibionts. Sponges were then positioned at 5 m close to the flow chambers where they were left acclimate for 24 h. After this time, specimens were positioned into the chambers, and left to acclimate for 1 h with the system in open circulation mode before the experiment started. Three initial samples of 1.8 ml of water were collected from each chamber and fixed for further analysis (see below), and the system was then switched to closed recirculation mode. Two hours later, 3 final samples of 1.8 ml of water were collected from each chamber. All water samples were immediately fixed with 1% paraformaldehyde + 0.05% gluteraldehyde, frozen in liquid nitrogen and stored at −80 °C until analysis by means of a Becton Dickson FACSCalibur flow cytometer to quantify the abundance of heterotrophic and autotrophic bacteria, and autotrophic pico- and nanoplankton following Gasol and Moran (1999). Orange fluorescence (from phycoerythrin), red fluorescence (from chlorophyll), and green fluorescence (from DNA stained with SYBR Green) were collected through band-pass interference filters at 650, 585 and 530 nm, respectively. The five measured parameters (forward and right-angle light scatter (FALS and RALS), and orange, red and green fluorescence) were recorded on 3-decade logarithmic scales, sorted in list mode, and analysed using Flowing Software (Perttu, 2013, Turku Centre for Biotechnology, Finland, www.flowingsoftware.com). Differences between cell counts from initial and final water samples were tested with generalized linear models (GLM) assuming a negative binomial error family distribution with log-link (Zuur et al., 2009). Models were fitted using the function glm.nb available in MASS package of the R software platform (Venables and Ripley, 2002). Grazing rates were calculated following Ribes et al. (1998), and the number of cells removed converted to equivalent content of C using the following conversion factors: 10 fg C $cell^{-1}$ for heterotrophic bacteria (Gundersen et al., 2002); 46 and 470 fg C $cell^{-1}$, respectively for *Prochlorococcus* sp. and *Synechococcus* sp. (Campbell and Vaulot, 1993; Bertilsson et al., 2003). C conversions for pico- and nanoeukaryotes were based on their mean biovolume, 5.13 μm^3 and 20 μm^3, respectively (Montagnes et al., 1994; Caron et al., 1995). Difference in ingested C between *A. aerophoba* and *A. polypoides* in spring and autumn were tested using ANOVA. Data were log-transformed to meet the assumptions of normality and homogeneity of variance.

2.3.2. Sponge size versus dry weight

To determine the relationship between *A. aerophoba* and *A. polypoides* size and dry weight, 14 *A. aerophoba* (4–16 m depth) and 26 *A. polypoides* (10–30 m depth) were sampled in May 2014 (Punta s'Oliguera, Cap de Creus, Fig. 2B). Sponges were photographed underwater with a ruler on their side to infer their height from the image analysis performed with the Macnification software (Schols and Lorson, 2008). Once in the laboratory, sponges were freeze-dried, weighed (dry weight, DW), and then combusted for 5 h at 500 °C and weighed again to determine their ash free dry weight (AFDW). A power law and exponential relationships between size and AFDW were found for *A. aerophoba* and *A. polypoides*, respectively (Fig. 4). These relations were used to convert the size of the specimens observed along the video transects to their equivalent AFDW as in Coppari et al. (2014).

2.3.3. Carbon flux estimation

Based on either the distribution of *A. aerophoba* and *A. polypoides* and the results of the in situ feeding experiments, the total amount of seasonal ingested C was estimated for the entire study area. By combining the above-mentioned relationship between sponge size and AFDW,

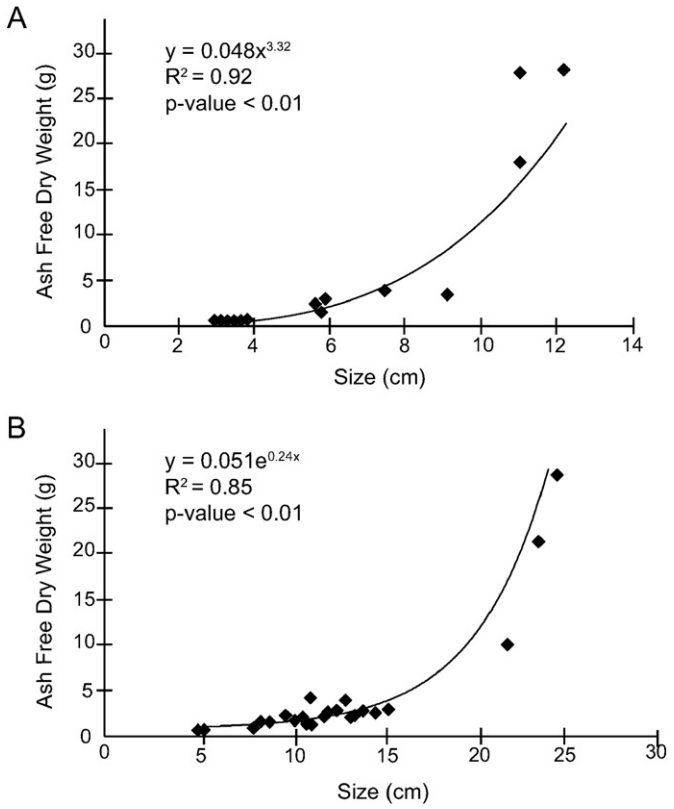

Fig. 4. Relationship between *Aplysina aerophoba* size (cm) and AFDW (g); 14 specimens were collected to obtain this relationship (A), relationship between *Axinella polypoides* size (cm) and AFDW (g); 26 specimens were collected to obtain this relationship (B).

with the data about sponge size and density in the study area, the ingested C was estimated in 5 m depth intervals. Ingestion rates (F) are related to sponge size following an allometric relation (Thomassen and Riisgård, 1995):

$$F = aW^b$$

where a is the filtration rate of an organism of 1 g (DW), W is the weight (DW) of the individual and b is the rate of change of metabolic rates with size. Since the value of b is not known for the two study species, we used the value 0.914 based on previous study from Thomassen and Riisgård (1995).

3. Results

3.1. Sponges distribution and size structure

A total of 20 *A. aerophoba* were measured out of the 56 observed, and a total of 703 *A. polypoides* out of 1050. The highest density recorded was 1.6 *A. aerophoba* m^{-2} and 7.6 *A. polypoides* m^{-2} (Table 1). *A. aerophoba* mainly occurred in the south and east side of the cape, whereas *A. polypoides* was distributed throughout all the study area, with maximum abundances in subareas C and D (Fig. 5). Bathymetrical distribution of *A. aerophoba* ranged from 5 to 20 m depth, whereas *A. polypoides* was encountered between 10 and 70 m depth. Below this depth, sandy bottoms, unsuitable for both species, were dominant in almost all study areas (Figs. 6 and 7).

Size of *A. aerophoba* specimens varies between 2.38 and 20.86 cm height, and between 1.07 and 46.20 cm for *A. polypoides*. In subarea B, medium sized sponges dominated, whereas in subarea D, small and big specimens were found in the same percentage; only small sponges were encountered in subarea E and, small and medium sized specimens were found in the same percentage in subarea G. Small sized specimens

Table 1
Presence and spatial distribution of *Aplysina aerophoba* (a) and *Axinella polypoides* (b) in the study area. Occupancy (frequency of occurrence in the set of sampling units), maximum and mean densities, and mean size are given for each subarea.

Subarea	Sampling units			Max densities	Mean densities ± SD	Mean height ± SD
	Number	With sponges	%	(sponges m^{-2})	(sponges m^{-2})	(cm)
a)						
A	803	–	–	–	–	–
B	456	10	2.19	1.6	0.96 ± 0.43	10.07 ± 5.61
C	630	–	–	–	–	–
D	652	6	0.92	1.6	0.73 ± 0.53	8.42 ± 6.34
E	787	3	0.38	1.2	0.67 ± 0.46	3.31 ± 1.21
F	450	–	–	–	–	–
G	777	8	1.03	0.8	0.6 ± 0.21	5.68 ± 2.45
b)						
A	803	11	1.37	5.6	2 ± 1.46	11.31 ± 6.42
B	456	18	3.95	1.6	0.84 ± 0.43	8.6 ± 4.09
C	630	151	23.97	4	0.96 ± 0.76	12.08 ± 6.99
D	652	43	6.66	2.8	0.87 ± 0.62	12.02 ± 7.09
E	787	76	9.66	5.2	0.96 ± 0.81	13.2 ± 7.57
F	450	47	10.44	7.6	1.17 ± 1.37	9.75 ± 7.71
G	777	4	0.51	2	0.8 ± 0.8	2.61 ± 1.08

of *A. polypoides* dominated all but subarea E, were the medium sized sponges were dominant (Table 2). Skewness and kurtosis were not calculated for *A. aerophoba* in any of the subareas, and for *A. polypoides* in subarea G due to the low number of specimens encountered and measured. Skewness and kurtosis for *A. polypoides* were both significant in subareas C, D and F highlighting the dominance of small size specimens. Conversely, both skewness and kurtosis were not significant in subareas A and B indicating a normal distribution of the sizes and dominance of medium size specimens. In subarea E only skewness was significant, probably due to the absence of large specimens (Table 3).

3.2. In situ feeding experiment

In spring, the presence of *A. aerophoba* caused a significant reduction in all prey items analysed ($p < 0.05$) except for heterotrophic bacteria ($p = 0.96$) (Table S1a), whereas the presence of *A. polypoides* caused a significant reduction in all prey items analysed ($p < 0.05$) (Table S2a). *Prochlorococcus* sp. was not detected in any of the spring samples. In autumn, both species caused a significant reduction in the concentration of *Synecococcus* sp. ($p < 0.05$), with *A. polypoides* significantly removing heterotrophic bacteria ($p < 0.05$) (Table S1b and S2b).

No significant difference in the amount of C acquired was observed between seasons ($p = 0.13$), whereas a significant difference in the C ingested was observed between species, with *A. polypoides* ingesting significantly more C than *A. aerophoba* ($p < 0.05$). The interaction between species and seasons was significant ($p < 0.05$) (Fig. 8 and Table S3), highlighting an opposite trend in the C consumption: *A. aerophoba* ingested more C in spring, whereas *A. polypoides* in autumn. Overall, *Synecococcus* sp. constituted the major food source of *A. aerophoba* in terms of particle abundance in both season, even though the main source of C originated from autotrophic nanoeukaryotes in spring and from *Synecococcus* sp. in autumn. Heterotrophic bacteria were the major food source in terms of particle abundance for *A. polypoides* (both in spring and autumn), but the main source of C originated from nanoeukaryotic cells. *A. aerophoba* ingested 0.12–0.39 mg C g AFDW^{-1} day^{-1} in spring and 0.09–-0.13 mg C g AFDW^{-1} day^{-1} in autumn, whereas *A. polypoides* ingested 0.07–0.17 mg C g AFDW^{-1} day^{-1} in spring and 0.18–-0.60 mg C g AFDW^{-1} day^{-1} in autumn. The impact of the two sponge species per m^2 was also estimated: *A. aerophoba* was able to ingest 0.81 ± 0.3 mg C m^{-2} day^{-1} (mean ± SE) in spring and 0.09 ± 0.03 mg C m^{-2} day^{-1} in autumn. *A. polypoides* ingested 0.19 ± 0.02 mg C m^{-2} day^{-1} in spring and 0.42 ± 0.04 mg C m^{-2} day^{-1} in autumn.

3.3. Carbon fluxes estimation

Over the entire study area (1.14 ha), the 20 measured *A. aerophoba* ingested 1.87 g of C in spring and 0.19 g in autumn (Table 4a); whereas the 703 measured *A. polypoides* ingested 13.60 and 29.36 g of C in spring and autumn, respectively (Table 4b). The highest estimated trophic impact was concentrated between 5 and 15 m depth for *A. aerophoba* (Fig. 9), and between 65 and 70 m depth for *A. polypoides* (Fig. 10).

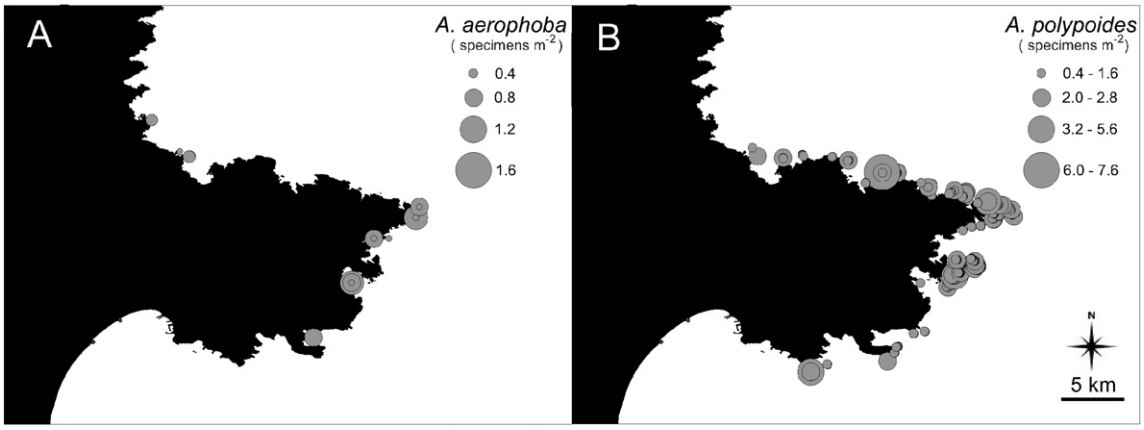

Fig. 5. Spatial distribution of *Aplysina aerophoba* (A) and *Axinella polypoides* (B) in the study area. *Bubbles* indicate the density of the two sponge species (specimens m^{-2}).

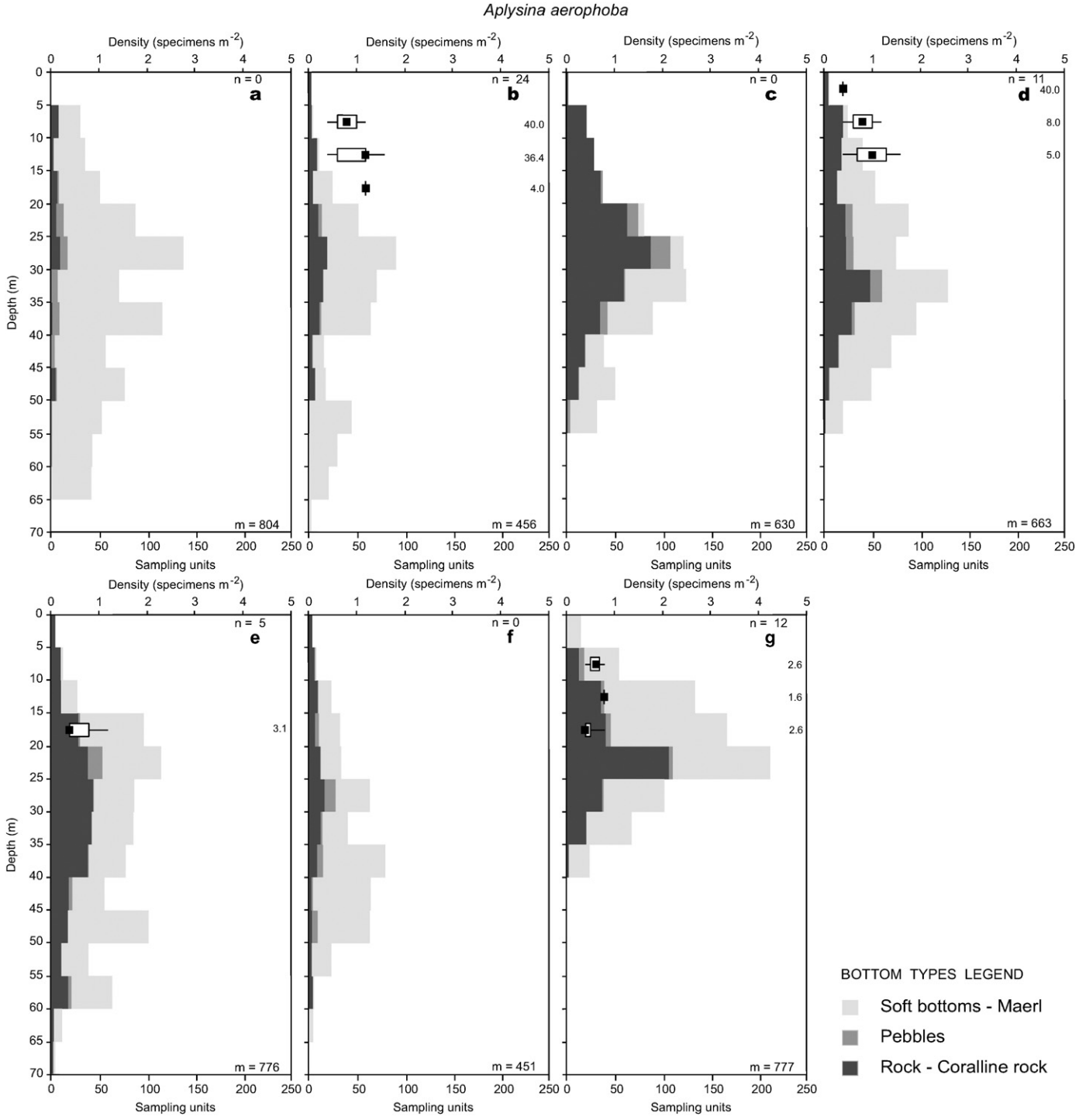

Fig. 6. Bathymetrical distribution of the density of *Aplysina aerophoba* in each subarea (a–g): the *black square* indicates the median value of the density; the *box* indicates the first and the third quartiles; and the *line* indicates the range between minimum, maximum and median values. *Grey-scale 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.

4. Discussion

The studied species showed contrasting spatial and bathymetrical distribution. *A. aerophoba* was mainly concentrated on the east side of the cape, where they are likely sheltered from strong hydrodynamics caused by wind waves (Ulses et al., 2008). HMA sponges that need light for their symbiotic autotrophic bacteria prefer sheltered, low hydrodynamic conditions to survive (Imhoff and Trüper, 1976) as well as a shallow environment to maintain photosynthetic activity (Becerro et al., 2003; Pfannkuchen et al., 2009; Perez Castro, 2014). Contrarily, *A. polypoides* presents a more homogenous distribution along the studied coast, similar to the ascidian *Halocynthia papillosa* (Coppari et al., 2014). The continuous distribution of sponges and ascidians differs from the abundances of passive suspension feeders mainly distributed in the northern face of the cape (Rossi et al., 2008; Gori et al., 2011a; Ambroso et al., 2013), in zones directly exposed to the main near-

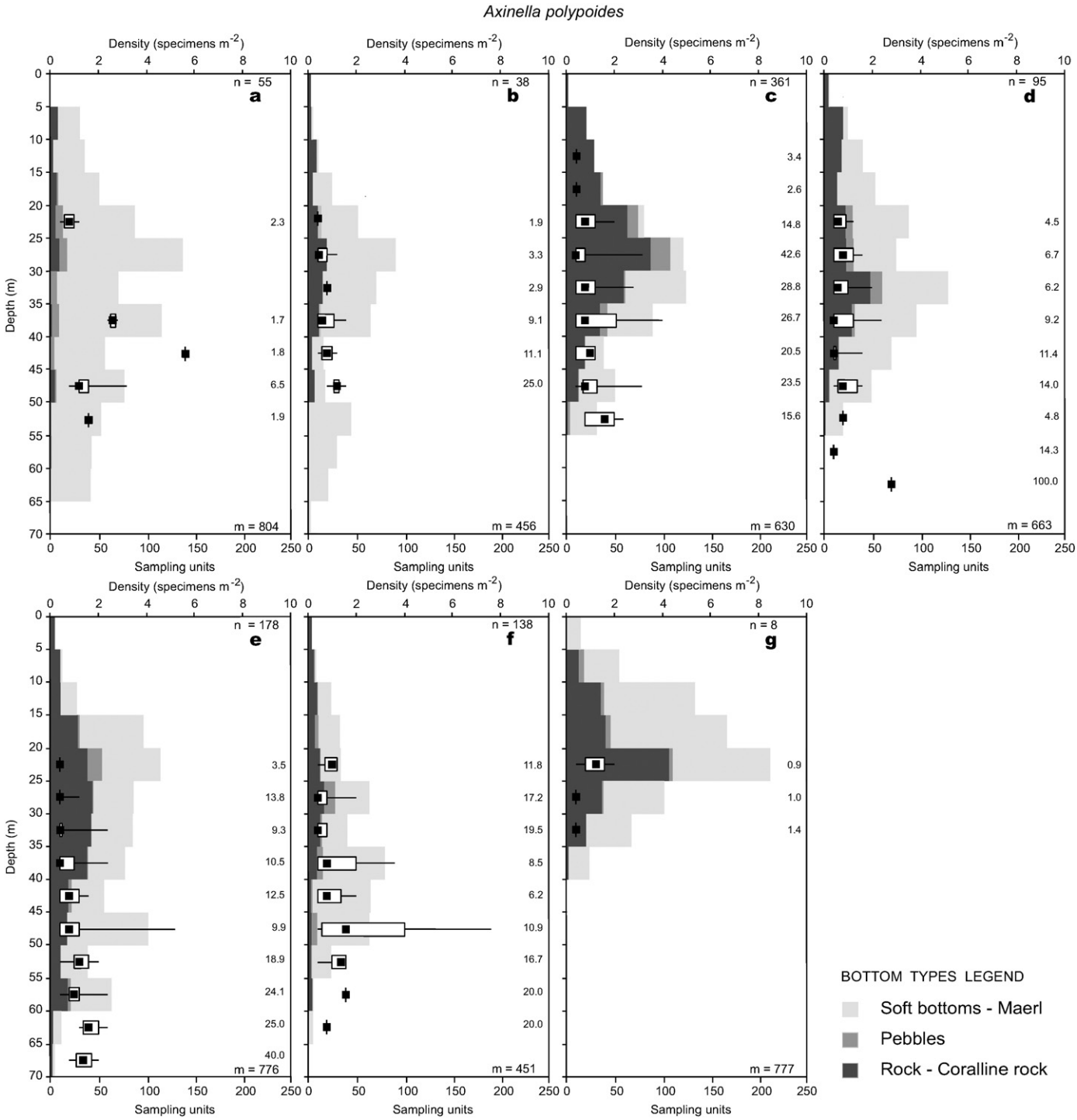

Fig. 7. Bathymetrical distribution of the density of *Axinella polypoides* in each subarea (a–g): the *black square* indicates the median value of the density; the *box* indicates the first and the third quartiles; and the *line* indicates the range between minimum, maximum and median values. *Grey-scale 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.

bottom currents (DeGeest et al., 2008). In fact, active suspension feeders may be more independent from near-bottom currents for food supply (Ribes et al., 1999a; Armsworthy et al., 2001).

The maximum density of *A. aerophoba* observed in this study (1.6 individuals m^{-2}) was much lower than previously reported (11 individuals along a 5 m transect, Becerro et al., 2003), possibly due to differences between the studied areas or to differences in the methodology applied. Conversely, the maximum density of *A. polypoides* (7.6 individuals m^{-2}) was higher than previously reported (0.5 individuals m^{-2}, at 23 m depth in Banyuls sur Mer, Weinberg, 1978) possibly because the use of the ROV allowed us to reach and quantitatively study the depth range where this species shows its highest densities (Fig. 7). *A. polypoides* is concentrated below 35 m depth; the arborescent-shape morphology of this species, which can easily break down in wave-exposed shallower depths (Bell and Barnes, 2000; Bell, 2007), might explain its distribution. Moreover, similar to other LMA species, *A. polypoides* mainly depends on near bottom live and detrital POM for its nutrition (Weisz et al., 2008;

Table 2
Size frequency distribution of *Aplysina aerophoba* in each subarea (a) with specimens classified as follows: small (0–5 cm), medium (5–10 cm), large (>10 cm); size frequency distribution of *Axinella polypoides* in each subarea (b) with specimens classified as follows: small (0–10 cm), medium (10–20 cm), large (>20 cm).

Subarea	n	%		
		Small	Medium	Large
a)				
A	–	–	–	–
B	11	18	45	36
C	–	–	–	–
D	2	50	–	50
E	3	100	–	–
F	–	–	–	–
G	4	50	50	–
b)				
A	38	51	41	8
B	34	68	32	–
C	240	45	41	14
D	80	48	42	10
E	186	38	42	20
F	118	60	29	11
G	8	100	–	–

Hadas et al., 2009). The summer oligotrophic condition of shallow Mediterranean waters (Rossi and Gili, 2005) might drive the distribution of this species in deeper waters (below 35 m). The observed bathymetrical distribution of *A. polypoides* supports the recent observation that the high biomass of coastal megabenthic suspension feeders is generally concentrated in deep sublittoral bottoms (30–90 m depth) (Gori et al., 2011a; Ambroso et al., 2013; Coppari et al., 2014), possibly due to the stability of the main environmental features under the summer thermocline (Gori et al., 2012; Coppari et al., 2014).

In agreement with Ribes et al. (1999a), both studied species showed a clear seasonal change in their feeding habits, mainly related to the natural variation in the composition of the near bottom seston in the Mediterranean Sea (Ribes et al., 1999b; Rossi and Gili, 2005). According to Ferrier-Pagès et al. (1998) and Ribes et al. (1999b), no *Prochlorococcus* sp. was detected in spring. Conversely, *Prochlorococcus* sp. was observed in autumn with abundances one order of magnitude less than in Ribes et al. (1999a). This discrepancy was also observed for picoeukaryotes, whose low concentration in the ambient water impeded detection of any possible decrease due to sponge feeding. In the Mediterranean Sea, autumn storms and river runoff result in high concentration of low quality POM mainly composed by inorganic sediment (Grémare et al., 2003; Rossi et al., 2003). Most of the C ingested by *A. aerophoba* was derived from *Synecococcus* sp., as previously observed by Pile et al. (1996, 1997), and nanoeukaryotes, whilst C mainly proceeded from nanoeukaryotes in *A. polypoides*, as described by Topcu et al. (2010) for *Spongia officinalis*. The detrital component of POM and DOM have also been considered important C input for sponges (de Goeij et al., 2008; Hadas et al., 2009; Gibson, 2011). In our study, no evidence of detrital POM feeding by either sponge was detected (data not shown); in accordance to Jiménez (2011) flow incubation chambers did not allow detection of differences neither in detrital POM nor in DOM

Table 3
Size structure distribution parameters of studied populations of *Axinella polypoides*; skewness and kurtosis are considered significant if the p-value is equal or less than 0.05.

Subarea	Skeweness	p-Value	Kurtosis	p-Value
A	1.027	0.086	3.830	0.152
B	0.260	0.641	2.068	0.135
C	1.297	<0.001	5.368	<0.001
D	1.594	0.002	6.419	0.001
E	0.723	0.012	2.911	0.981
F	1.327	0.001	4.859	0.004
G	–	–	–	–

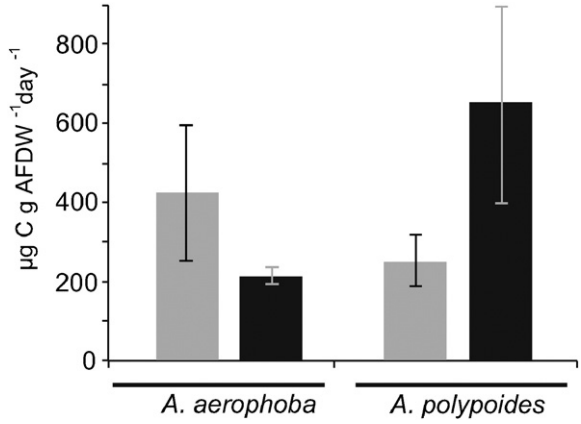

Fig. 8. Ingested C (µg C g AFDW^{-1} day^{-1}) (mean ± SE) in spring (grey histogram) and autumn (black histogram) by the two sponge species.

between initial and final water samples. Even if other methodologies (e.g. InEx; Yahel et al., 2005) can be more appropriate to completely identify all the food sources of sponge species, they can only be applied to species with osculum diameter big enough to allow for sampling of exhalant water (see Yahel et al., 2005 for further details), and this was not the case of *A. polypoides*.

The similarity in C ingested between seasons is consistent with previous study performed on *Dysidea avara* (Ribes et al., 1999a), whereas the higher C intake of *A. polypoides* with respect to *A. aerophoba* might be related to its deeper bathymetrical distribution concentrated at depths where food availability is constant throughout the year (Gori et al., 2012; Coppari et al., 2014). The elevated growth rate of *A. polypoides* demonstrated by Basile et al. (2009) might also explain the higher uptake of C compared to *A. aerophoba*. The high growth

Table 4
Carbon (g) ingested seasonally by *Aplysina aerophoba* along the considered depth range in the study area, and its total (TOT) (a), carbon (g) ingested seasonally by *Axinella polypoides* along the considered depth range in the study area, and its total (TOT) (b).

Depth (m)	Spring	Autumn
a)		
0–5	0.00	0.00
5–10	0.66	0.07
10–15	1.10	0.11
15–20	0.11	0.01
20–25	0.00	0.00
25–30	0.00	0.00
30–35	0.00	0.00
35–40	0.00	0.00
40–45	0.00	0.00
45–50	0.00	0.00
50–55	0.00	0.00
55–60	0.00	0.00
60–65	0.00	0.00
65–70	0.00	0.00
TOT (g C)	1.87	0.19
b)		
0–5	0.00	0.00
5–10	0.00	0.00
10–15	0.00	0.00
15–20	0.01	0.01
20–25	0.60	1.30
25–30	2.61	5.64
30–35	1.73	3.73
35–40	2.20	4.74
40–45	0.82	1.77
45–50	2.38	5.14
50–55	1.94	4.19
55–60	0.74	1.59
60–65	0.11	0.25
65–70	0.47	1.00
TOT (g C)	13.60	29.36

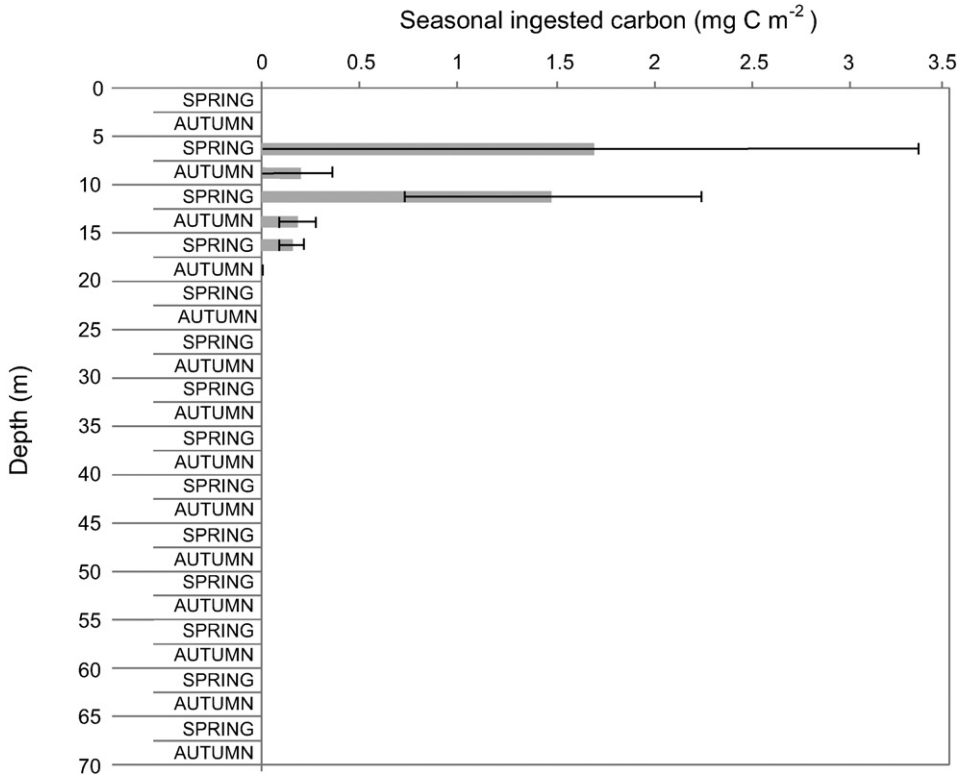

Fig. 9. Seasonal carbon (mg C m^{-2}, mean ± SE) ingested by *Aplysina aerophoba* estimated every 5 m depth interval.

rate of *A. polypoides* is possibly related to the lack of chemical defences against microbial fouling and feeding deterrence against predators. Indeed, chemically undefended species might invest more energy in growth and reproduction, and tolerating partial predation as a cost of being only marginally defended by means of physical defence (i.e. spicules) (Haber et al., 2011). Moreover, as demonstrated in *Haliclona*

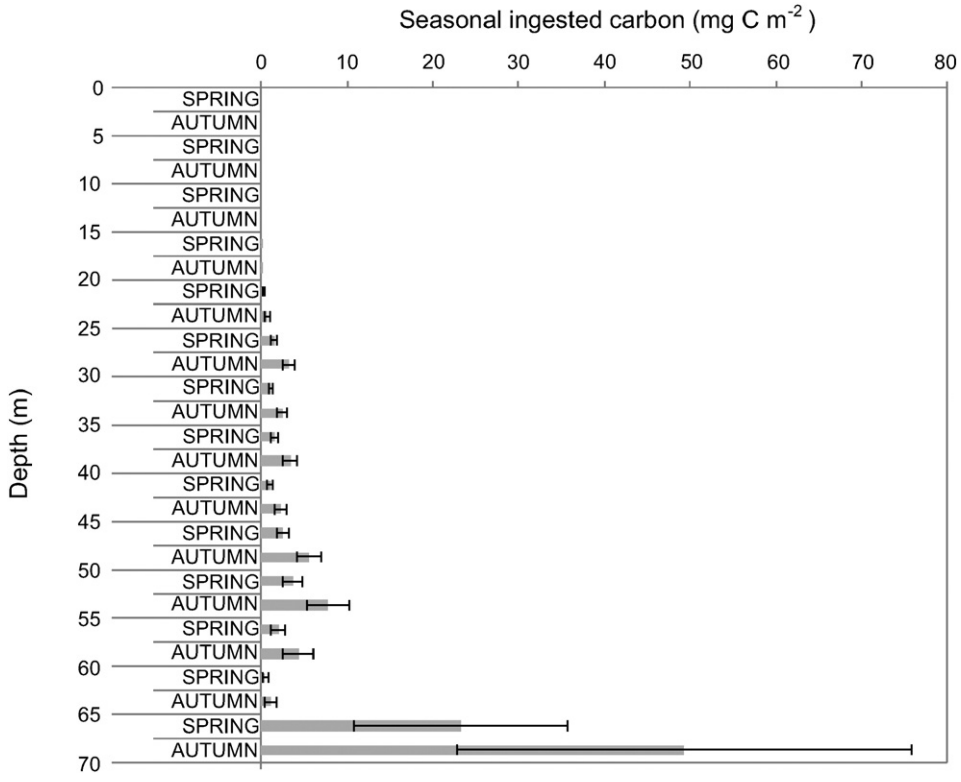

Fig. 10. Seasonal carbon (mg C m^{-2}, mean ± SE) ingested by *Axinella polypoides* estimated every 5 m depth interval.

oculata (i.e. LMA species), the quantity of POC in the water column is directly related to the growth of the species (Koopmans and Wijffels, 2008), thus, the constant food availability in deep waters of the study area might support the elevated growth rate of *A. polypoides*. Previous research demonstrated that DOC is not a food source for LMA species (Ribes et al., 1999a; Gibson, 2011; Ribes et al., 2012). However, other studies highlighted that LMA species might also consume DOC as a part of their diet (Mueller et al., 2014). Up to now, no study documenting the consumption of DOC by *A. polypoides* is available, but it could not be excluded, with the potential underestimation of the role played by this species in benthic–pelagic coupling processes. Contrarily, DOC is likely to be an important food source for *A. aerophoba*, as already demonstrated in other HMA species (de Goeij et al., 2008; Gibson, 2011). This additional food source, together with the possible nutritional contribution from symbiotic cyanobacteria, could explain the lower C ingested by this species compared to *A. polypoides*.

Considering the density of the studied species and their population size structure, *A. aerophoba* removed 0.81 ± 0.3 mg C m^{-2} day^{-1} in spring and 0.09 ± 0.03 mg C m^{-2} day^{-1} in autumn, whereas *A. polypoides* removed 0.19 ± 0.02 mg C m^{-2} day^{-1} in spring and 0.42 ± 0.04 mg C m^{-2} day^{-1} in autumn. These are low values compared to other sponges (*Mycale lingua*: 29 mg C m^{-2} day^{-1}, Pile et al. (1996); *Sericolophus hawaiicus*: 55 mg C m^{-2} day^{-1}, Pile and Young (2006); *Aphrocallistes vastus*: 3400 mg C m^{-2} day^{-1}, Kahn et al. (2015)), octocoral species (*Leptogorgia sarmentosa*: 2.3–16.8 mg C m^{-2} day^{-1}, Rossi et al. (2004); *Corallium rubrum*: 0.4–9.6 mg C m^{-2} day^{-1}, Tsounis et al. (2006)), or ascidians (*Halocynthia papillosa*: 13.9 mg C m^{-2} day^{-1} and 1.5 mg C m^{-2} day^{-1}, in spring and autumn respectively, Coppari et al. (2014)). This discrepancy could be related to the low densities of both species in the study area, or to the neglecting of some of the potential food sources (e.g. DOC). Overall, along the entire Cap de Creus coast, *A. aerophoba* removed 1.87 g C during spring and 0.19 g during autumn, whilst *A. polypoides* removed 13.60 g C during spring and 29.36 g C during autumn, as a consequence of the higher abundance and the larger specimens of *A. polypoides* compared to *A. aerophoba*.

During the last decade, an effort has been made to understand the role of terrestrial ecosystems in capturing part of the anthropogenic produced C (Bellassen and Luyssaert, 2014) by coupling forest ecology to broad-scale cartography and landscape studies based on remote sensing (Janssens et al., 2005). The resolution of spatial and temporal data in the marine environment is not as high as in the land environment (Bekkby et al., 2002), due to the difficulty of studying marine ecosystems compared to the terrestrial ones (Robbins and Bell, 1994). This study provides an application of landscape techniques to the study of seascape ecology coupling broad-scale quantification of species distribution pattern, to in situ assessment of their trophic impact (Coppari et al., 2014). Using this approach, the role played by one of the main components of the marine animal forests in benthic–pelagic coupling processes has been estimated, and can be used for further broad-scale C flux estimations (Rossi, 2013).

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