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Nutrient acquisition in four Mediterranean gorgonian species

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ABSTRACT: Carbon and nitrogen isotope abundance values ($\delta^{13}C$ and $\delta^{15}N$, respectively) were measured for the first time in the soft tissue, axial skeleton, and spicules of 4 Mediterranean gorgonians, 3 asymbiotic (*Leptogorgia sarmentosa, Paramuricea clavata,* and *Eunicella verrucosa*) and 1 symbiotic with autotrophic dinoflagellates (*Eunicella singularis*). The isotopic composition of their diet, i.e. zooplankton, particulate organic matter (POM), and sedimentary organic matter (SOM), was also measured to understand gorgonian feeding ecology. (1) Carbon and nitrogen signatures of the symbiotic *E. singularis* tissue in summer differed significantly from the signatures of the other species; (2) carbon and nitrogen signatures of the axial skeleton were similar to those of the tissue, because the skeleton is primarily made of gorgonin secreted by the tissue; and (3) spicules had a high $\delta^{13}C$ signature because they are made by a combination of 60 to 76% of respiratory CO_2 and of external CO_2, with a high $\delta^{13}C$ signature. Comparison of the isotopic signatures of the gorgonian tissues and the food sources indicated that *E. singularis* and *P. clavata* had the same diet in both winter and summer, either zooplankton for *E. singularis* or POM and SOM for *P. clavata*. Conversely, *L. sarmentosa* and *E. verrucosa* shifted from zooplankton in winter to SOM in summer. These results bring insights into the feeding ecology of temperate gorgonians and explain their distribution, abundance, and role in the flow of particulate matter between the water column and the benthos.

KEY WORDS: Temperate gorgonian · Feeding ecology · Symbiosis · Isotope · Spicule

INTRODUCTION

Gorgonians are among the most abundant and important suspension feeders of Mediterranean benthic communities (Weinberg 1976). Large gorgonians form dense aggregations that reduce water flow and affect sediment deposition, and species like *Paramuricea clavata* are responsible for a large share (~40%) of the biomass of the benthic community. They play a paramount role in shaping these communities by providing shelter for other animals and algae (Gili & Coma 1998). They significantly affect the carbon and nitrogen cycles of the water column either by capturing planktonic prey and filtering large amounts of particulate and dissolved organic matter, or by releasing mucus and other metabolic wastes into seawater (Gili & Coma 1998, Wild et al. 2004). Knowledge of their feeding ecology is there-

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fore essential at different levels. It is important to understand the biology and distribution of gorgonians, as well as their patterns of environmental adaptation, because acquisition of energy is a determinant factor for growth, fecundity, and thus the demographic distribution of the species. Distribution and abundance in turn determine the ecological role of gorgonians in littoral ecosystems. Finally, uptake and release of nutrients by gorgonian communities influence by energy flow between the pelagic and the benthic systems (Gili & Coma 1998, Coma et al. 2001). Despite much evidence suggesting that the feeding ecology of benthic suspension feeders may be important in understanding the functioning of littoral ecosystems, especially in areas with major environmental changes, natural diets and feeding rates of benthic suspension feeders are still poorly known.

It is difficult to study the trophic ecology of temperate gorgonians because they feed on a wide spectrum of food types, from pico- and nanoplankton to microplankton including particulate organic matter (POM) (Coma et al. 1994, Ribes et al. 1999, 2003). Like many anthozoans, they can also feed on organic matter contained in re-suspended sediment (Anthony & Fabricius 2000, Orejas et al. 2001, Mills & Sebens 2004). Food abundance and composition may change considerably with depth, season, or among locations (Garrabou et al. 2002, Coma & Ribes 2003, Rossi et al. 2004). Although pico- and nanoplankton show low seasonality (Coma & Ribes 2003), detrital POM and microplankton exhibit marked seasonal patterns, with high values in winter and spring and low values in summer. Therefore, food may occasionally become a constraining factor for gorgonians, which are continuously subject to random pulses of food availability (Gili & Ros 1985, Coma & Ribes 2003, Rossi et al. 2004, 2006).

Previously, feeding rates have mainly been studied in 2 species, *Paramuricea clavata* and *Leptogorgia sarmentosa*, by analyzing polyp contents or by enclosing specimens in chambers and measuring the depletion of natural particles (Coma et al. 1994, Ribes et al. 1999, 2003). Another approach to assess the physiology and nutritional ecology of gorgonians consists of measuring the stable carbon and nitrogen isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$, respectively) of their soft tissue. Indeed, stable isotopes have been increasingly used to provide time-integrated information on trophic relationships (Riera 2007), especially in coastal environments characterized by many local and imported food sources. Carbon isotopic signatures of the consumers are usually similar, or increased by 1‰ compared to their diet, whereas nitrogen signatures are enriched by 2.5 to 3.4‰ depending on tissue composition, nutritional status, and mode of nitrogen excretion (Minagawa & Wada 1984, Vander Zanden & Rasmussen 2001, Gollety et al. 2010). In anthozoans containing symbiotic dinoflagellates, nitrogen fractionation can be different compared to heterotrophic species, as the nitrogen isotopic signature of the host is either similar or increased by a few permille compared to its symbionts (Reynaud et al. 2009, Ferrier-Pagès et al. 2011). Although this isotopic approach has been applied to tropical gorgonians to monitor seawater pollution (Ward-Paige et al. 2005, Risk et al. 2009, Baker et al. 2010, Sherwood et al. 2010) and to deep-sea gorgonians to understand their trophic patterns (Carlier et al. 2009, Sherwood et al. 2011), the isotopic signature of Mediterranean gorgonians from shallow waters has been investigated only in *Eunicella singularis* (Gori et al. 2012).

We sampled 4 Mediterranean gorgonian species, *Paramuricea clavata*, *Leptogorgia sarmentosa*, *Eunicella verrucosa*, and *E. singularis*, in both winter and summer, from the Gulf of La Spezia, where the species are particularly abundant and live together (Cocito et al. 2002). The isotopic signatures of the gorgonian soft tissue, axial skeleton, and spicules were compared to the signature of their potential diet, i.e. zooplankton, POM in seawater, and sedimentary organic matter (SOM). We aimed at linking the isotopic signature of the gorgonian tissue to a specific food source and to highlight trophic differences among species or between seasons. *E. singularis*, in particular, hosts symbiotic dinoflagellates, which are expected to transfer a large part of their photosynthetic carbon to their host. We therefore hypothesized that the carbon signature of the host should have been largely affected by this autotrophic carbon and different from the signature of the other species, which are fully heterotrophic. The second aim was to assess the carbon signature of the spicules to understand the origin of the carbon deposited within them. In scleractinian tropical corals, 70% of the carbon deposited in the skeleton comes from internal respiration, while 30% has an external origin (Furla et al. 2000). In gorgonians, this point has yet to be clarified since Allemand & Grillo (1992) concluded that metabolic CO_2 may serve as a main source of carbon in the red coral *Corallium rubrum*, whereas Lucas & Knapp (1997) found the contrary. Finally, the gorgonians' trophic ecology is discussed to explain their particular abundance in the Gulf of La Spezia.

MATERIALS AND METHODS

Sampling and treatment of gorgonian species

Three asymbiotic gorgonian species were investigated: *Paramuricea clavata* (Risso, 1826), *Leptogorgia sarmentosa* (Esper, 1789), *Eunicella verrucosa* (Pallas, 1766), and the zooxanthellate *E. singularis* (Esper, 1791). These species are among the most representative gorgonians of the western Mediterranean sublittoral communities (Carpine & Grasshoff 1975). *P. clavata* and *E. verrucosa* are typically found between 10 and 110 m depth on shaded rocky walls and rocky bottoms, respectively; *L. sarmentosa* is an ubiquitous species living between 10 and 200 m depth on pebbles and boulders buried in sediment (Weinberg 1976); and *E. singularis* is abundant on horizontal and gently sloping rocky bottoms but at a more shallow depth (5 to 60 m) because its symbiotic dinoflagellates require light.

Sampling was performed at Tinetto Island (44° 01′ N, 09° 50′ E) in the western part of the Gulf of La Spezia (eastern Ligurian Sea). The area is characterized by high turbidity due to terrestrial runoff from the Magra River and sewage discharge from the city of La Spezia (Cocito et al. 2002). Sedimentation is high and constant over the year, with total suspended solids ranging between 1.61 and 2.65 mg l^{-1} and a rate (mean + SD) on the vertical cliff ranging between 188 ± 40 and 375 ± 61 g m^{-2} yr^{-1} (Cupido et al. 2009). The visual range is very low in autumn and winter (4.5 m) and increases in spring (8 m) and summer (10.5 m). All gorgonian species were sampled between 22 and 25 m at the western side of the island, where the walls descend steeply to a depth of 26 m on a flat, muddy bottom. *P. clavata* forms canopies on the vertical cliffs between 17 and 25 m, whereas *E. singularis, E. verrucosa*, and *Leptogorgia sarmentosa* live on boulders on the muddy bottom (Cocito et al. 2002). Gorgonians were collected by SCUBA diving in winter (March 2) and summer (August 18) 2009. From each of 3 colonies per gorgonian species, 15 apical fragments (15 cm long) were cut enclosed in plastic bags containing the surrounding seawater and brought to the laboratory.

For the analysis of carbon and nitrogen isotopic signatures of the soft tissue, 2 different protocols were compared, each allocating 5 fragments per colony × 3 colonies per species × 2 seasons (Fig. 1). The first protocol envisaged incubating the fragments in seawater enriched with magnesium chloride to relax the polyps. About 200 polyps per fragment were detached from the gorgonian axis using a dissecting needle and scalpel under a binocular microscope, frozen in liquid nitrogen and freeze-dried prior to subsequent analysis. The second protocol (applied on 5 fragments per colony × 3 colonies per species × 2 seasons) arranged for precisely separating the soft tissue from the axial skeleton of frozen fragments with a scalpel. The isolated soft tissue was then ground in a mortar under liquid nitrogen, homogenized with a Potter blender, and centrifuged at 500 × *g* and 4°C for 10 min. Centrifugation allowed separation of soft tissue from spicules (remaining at the bottom of the tube). Tissue samples were then frozen in liquid nitrogen and freeze-dried. All materials used for these extractions were previously heat-treated at 450°C for 5 h or rinsed in distilled water. All samples were analysed for their ^{15}N and ^{13}C isotopic composition, in triplicate, as described below. Stable isotope analyses of tissue samples prepared by either method yielded comparable results (ANOVA, no significant difference, $p = 0.18$) and were therefore pooled for subsequent data analysis ($n = 30$ per species and season).

For the determination of ^{15}N and ^{13}C isotopic signatures of the axial skeleton and spicules, 5 fragments per colony × 3 colonies per species × 2 seasons were considered. Each fragment was cut into a few pieces using clean scissors and macerated at 80°C for 10 min in a 50 ml Falcon tube containing a solution of sodium hydroxide (NaOH, 1 N), leading to a clean skeleton (Houlbrèque et al. 2011). Pieces of axial skeleton were then isolated with clean forceps,

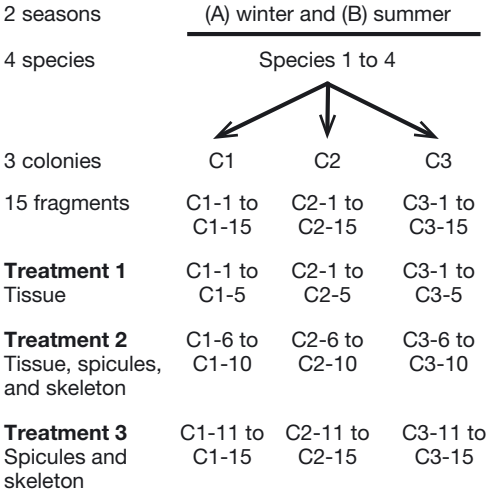

Fig. 1. Matrix of the sampling and treatment procedures. For each of the 4 gorgonian species, 3 colonies (C1 to C3) were collected in (A) winter and (B) summer. From each colony, 15 fragments were taken and distributed to the Treatments 1, 2, and 3. Each fragment was analyzed in triplicate for its $\delta^{13}C$ and $\delta^{15}N$ signature

rinsed with distilled water, ground under liquid nitrogen, and freeze-dried. The remaining solution of NaOH, containing the spicules and solubilized soft tissue, was centrifuged at 1000 × *g* and 4°C for 10 min. The supernatant was discarded, and the remaining spicules were rinsed several times with distilled water, frozen in liquid nitrogen, and freeze-dried prior to subsequent analysis. Results for skeleton and spicule samples obtained using this technique were compared to those obtained using the scalpel technique. All samples were analysed for their ^{15}N and ^{13}C isotopic composition, in triplicate, as described below. There was no significant difference between the 2 techniques for axial skeleton samples (ANOVA, $p = 0.12$), but data for spicules were different (ANOVA, $p < 0.001$), pointing towards a contamination of spicule samples with soft tissue material when prepared using the scalpel. For this reason, the latter data were not included in any subsequent data analysis.

Sampling and treatment of particulate organic matter

To characterize the isotopic signature of POM suspended in seawater, in each season, 3 separate volumes of 10 l of seawater were sampled near the gorgonians using a Niskin® bottle. Back at the laboratory, at least 5 l of seawater was filtered in triplicate through previously heat-treated (450°C, for 5 h) 25 mm Whatman GF/F filters under low vacuum. To remove carbonates, filters were quickly acidified (1 mol l^{-1} HCl) and rinsed with distilled water. They were then freeze-dried and kept frozen until isotopic analyses. Due to their low abundance in seawater, only a few zooplankters were collected on the filters. So, to obtain reliable isotope abundance data for zooplankton, concentrated samples were collected using a plankton net (type WP2; 5 min hauls). Plankton were then collected on filters and treated as described above.

Sampling and treatment of suspended organic matter

In each season, replicate sediment samples from the muddy bottom near the gorgonians were collected using plastic jars and by scraping the upper 1 cm of the surface (Riera et al. 1996). Samples were then sieved to a grain size of <50 µm to separate sand grains from sedimentary POM. Three sub-samples of ~5 g were freeze-dried and ground using a pestle and mortar. A 200 mg aliquot from each sub-sample was acidified with 1 N HCl to remove inorganic carbon. To prevent any loss of dissolved organics, these samples were not rinsed, but acidification was performed on a hot plate to quickly evaporate the acid. Samples were dried overnight at 50°C in a fume hood (Riera 1998). Once dried, the sediment was mixed with Milli-Q water, freeze-dried, ground again to a fine powder, and kept frozen (–80°C) until analysis.

Stable isotope analysis

Samples were analysed for their ^{15}N and ^{13}C isotopic composition using an elemental analyser (Flash EA-1112) coupled to a Delta V Advantage isotope ratio mass spectrometer (both ThermoFisher). International reference materials (USGS40 and IAEA-CH6, International Atomic Energy Agency) were analysed with each sample batch and used for scale calibration of results of ^{13}C isotope analyses to Vienna PeeDee Belemnite (VPDB). Similarly, results of ^{15}N isotope analyses were scale-calibrated to N_2 in air using USGS40 and an in-house standard (leucine, $\delta^{15}N = 10.77‰$; Fluka), whose $\delta^{15}N$ had been independently validated (Iso-Analytical). Two different analytical control samples were also analysed with each batch for quality control. Precision as determined by repeat analysis of the reference materials and quality controls was better than ±0.20 and ±0.15‰ for measured $\delta^{15}N$ and $\delta^{13}C$ values, respectively. Data are expressed in the standard δ-unit notation for the heavier isotope h of a given element X:

$$\delta^{h}X = [(R_{sample}/R_{reference}) - 1] \times 10^{3} \quad (1)$$

where $R = {}^{13}C/{}^{12}C$ for X = carbon and $^{15}N/^{14}N$ for X = nitrogen, with δ values reported relative to VPDB and air for ^{13}C and ^{15}N, respectively.

Statistics and models

The isotopic signatures of gorgonian soft tissue, axial skeleton, and spicules (4 species × 3 colonies per species × 5 to 10 samples per colony × 2 seasons) were compared by a 2-way ANOVA using the software package Statistica® with 2 independent factors, species (4 levels) and seasons (2 levels). Variance homogeneity was checked via Cochran's test. A Tukey test was used for post-hoc comparison of

levels. The isotopic signatures of POM, SOM, and zooplankton, sampled at the 2 seasons, were compared with a 1-way ANOVA using the software package Statistica®. To give an estimation of the isotopic contribution of each determined food source to the isotopic signal of the gorgonian tissue, a computer program (IsoSource) developed by Phillips & Gregg (2003) was used.

RESULTS

For all gorgonian species, spicules showed higher $\delta^{13}C$ values compared to the axial skeleton and tissue signatures, ranging from −6.81 to −7.97‰, while the tissue and axial skeleton signatures were comparable, with lower $\delta^{13}C$ values ranging from −18 to −24‰ (Fig. 2). Soft tissue and axial skeleton $\delta^{13}C$ signatures (Fig. 2) were not significantly different (Tukey test, $p = 0.99$ and 0.78, respectively) between *Paramuricea clavata*, *Eunicella verrucosa*, and *Leptogorgia sarmentosa*. However, soft tissues and the axial skeleton of *E. singularis* had distinct signatures of their own (Tukey test, $p < 0.0001$ and $p < 0.001$, respectively), with $\delta^{13}C$ values 1 to 2‰ more positive than the values of the other species. As discussed later, this difference could be due to the presence of symbiotic algae in the tissue. Finally, seasonal differences were small, with soft tissue $\delta^{13}C$ signatures of *L. sarmentosa* and *E. singularis* slightly more depleted in summer than in winter.

Concerning the carbon isotopic signature of the food (Fig. 3), POM and SOM were significantly more depleted than the zooplankton, in both winter and summer (1-way ANOVA, $p < 0.0001$), but there was no seasonal difference in the signature of each food source (ANOVA, $p = 0.04$).

As for $\delta^{13}C$, the $\delta^{15}N$ signature was also different between species and seasons (ANOVA, $p < 0.001$). The tissue of *Paramuricea clavata* (Fig. 4) had the lowest $\delta^{15}N$ values of the gorgonian species, in both summer and winter (Tukey test, $p < 0.01$, from 5.07 to 5.61‰). The isotopic signature of the spicules of *Eunicella verrucosa* was also higher in winter compared to the signatures of the other gorgonians (Tukey test, $p < 0.04$). Conversely, the $\delta^{15}N$ value of the axial skeleton was consistent between species and seasons and ranged between 7.48 and 8.20‰ (2-way ANOVA; Tukey test, $p > 0.05$). Concerning seasonal differences, $\delta^{15}N$ values of the tissues of *Leptogorgia sarmentosa* and *E. singularis* were significantly higher in winter than in summer (Tukey test, $p < 0.01$). Concerning the $\delta^{15}N$ signature of the food (Fig. 3), only POM showed a significant seasonal effect, with $\delta^{15}N$ values higher in summer than in winter ($p < 0.01$).

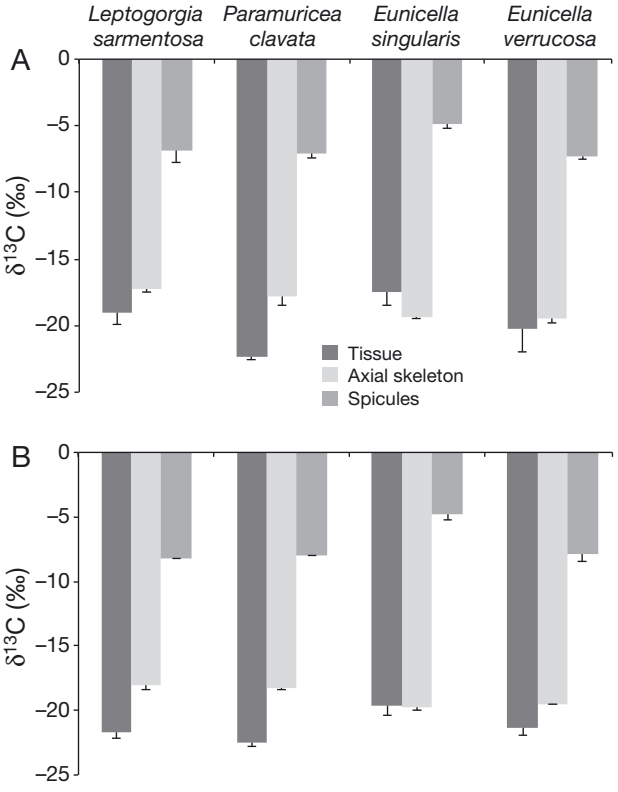

Fig. 2. $\delta^{13}C$ values of the tissue, axial skeleton, and spicules of the 4 gorgonian species, sampled in (A) winter and (B) summer. Mean + SD of 5 samples

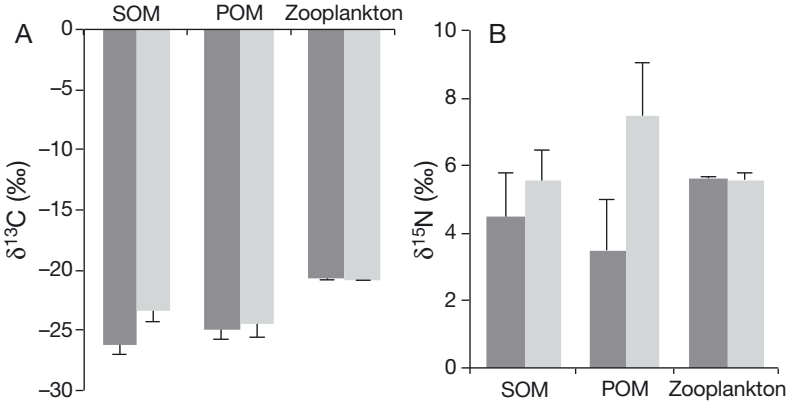

Fig. 3. (A) $\delta^{13}C$ and (B) $\delta^{15}N$ values of the sedimentary organic matter (SOM), particulate organic matter (POM) and zooplankton in winter (dark) and summer (pale). Means + SD of 5 samples

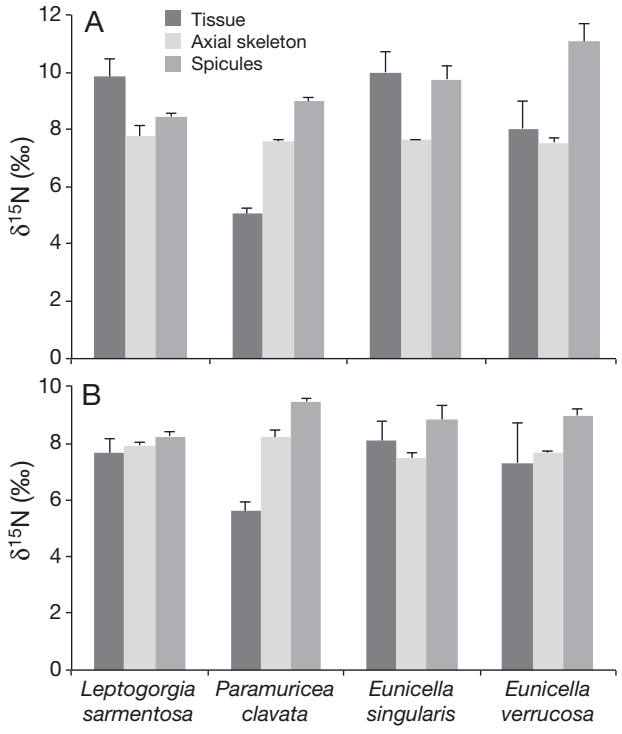

Fig. 4. $\delta^{15}N$ values of the tissue, axial skeleton, and spicules of the 4 gorgonian species, sampled in (A) winter and (B) summer. Mean + SD of 5 samples

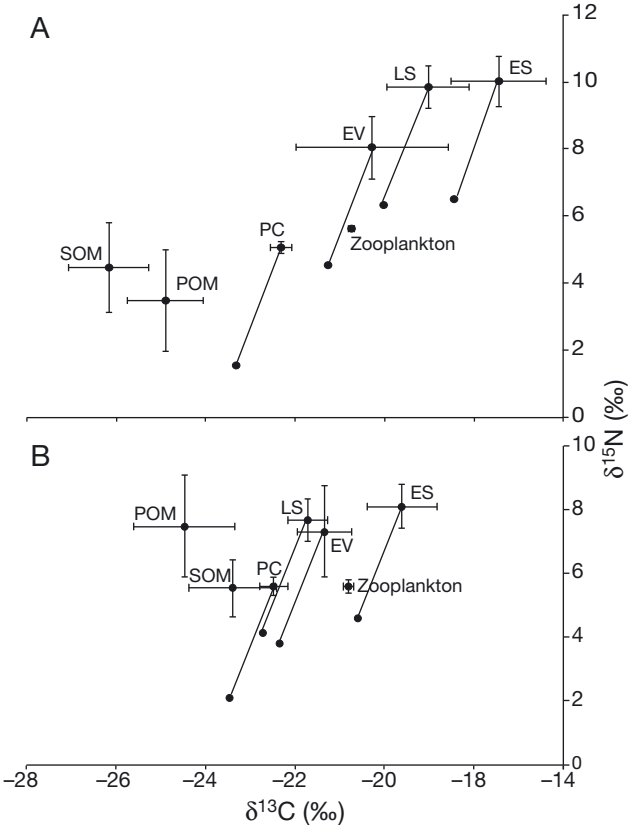

Fig. 5. $\delta^{13}C$ versus $\delta^{15}N$ (mean ± SD) for the gorgonians (ES = *Eunicella singularis*, EV = *E. verrucosa*, LS = *Leptogorgia sarmentosa*, PC = *Paramuricea clavata*), sedimentary organic matter (SOM), particulate organic matter (POM), and zooplankton for (A) winter and (B) summer. The lines, terminated by (●) correspond to the theoretical food source of the corals, taking into account the trophic enrichment of 1 and 3.5‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively

Carbon and nitrogen signatures of the different samples were simultaneously plotted in Fig. 5 to highlight the preferentially exploited food sources of gorgonians in each season. Such plots are usually used in complex food chains to determine the food source of a specific predator (Riera et al. 1999, 2009), assuming a mean trophic enrichment of 1‰ in $\delta^{13}C$ (Rau et al. 1990) and 2.5 to 3.4‰ in $\delta^{15}N$ (Minagawa & Wada 1984, Gollety et al. 2010) for the predator because of the assimilation of food. The exact value of the isotopic fractionation of carbon and nitrogen between the gorgonians and their prey is not known. We therefore applied the usual mean value of 3.4‰ (represented by ● in Fig. 5), considering that a value of 2.5‰ resulted in the same conclusions. Application of the above model to the gorgonians suggests different feeding behaviours, depending on the gorgonian species and the season. In winter, the isotopic signatures of the tissues of *Leptogorgia sarmentosa*, *Eunicella singularis*, and *E. verrucosa* were close to the zooplankton signature, while *Paramuricea clavata* tissue was closer to the POM and SOM signatures. In summer, the isotopic signatures of *L. sarmentosa*, *P. clavata*, and *E. verrucosa* were close to the SOM signature, while *E. singularis* tissue was closer to the zooplankton signature. According to the model of Phillips & Gregg (2003), SOM indeed contributed ca. 58% of the tissue signature of *L. sarmentosa*, *P. clavata*, and *E. verrucosa*, while zooplankton and POM contributed 34 and 8%, respectively. Conversely, there was a 94% contribution of the zooplankton to the tissue signature of *E. singularis*.

DISCUSSION

We demonstrated the acquisition of food and nutrients by 4 gorgonian species of the Mediterranean Sea at 2 different seasons. Results will contribute to the establishment of the role of this important component of the benthic Mediterranean community to biogeochemical fluxes in sublittoral ecosystems and, in general, to an understanding of trophodynamics in these communities. In addition to addressing primary research questions, the study may help in under-

standing the mechanisms which sustain suspension or affect nutrient dynamics. Overall, results have shown that each gorgonian species has its own isotopic signature, which depends on its diet and its relationship with the external environment as well as its symbiotic status. In summary, *Eunicella singularis* clearly had a higher $\delta^{13}C$ signature than the other species, independent of the season, due to its symbiosis with photosynthesizing dinoflagellates. All gorgonian species shifted from one diet to another depending on the season and food availability. Three out of the 4 gorgonian species (*Leptogorgia sarmentosa*, *E. verrucosa*, and *E. singularis*) consumed plankton in winter, when densities were relatively abundant, whereas they relied on detritic matter in summer.

Spicules of the 4 gorgonians had a very high carbon isotopic signature compared to the axial skeleton or the soft tissue due to a different carbon origin. In anthozoans, carbon used for calcium carbonate production can either come from the external dissolved inorganic carbon (DIC) in seawater (CO_2 or bicarbonate, HCO_3^-) or from respiratory (or metabolic) CO_2 (Erez 1978, Furla et al. 2000). Although it is clear now that metabolic CO_2 contributes to at least 70% of the skeletal carbon in scleractinian corals (Furla et al. 2000, Hughes et al. 2010), only 2 attempts have been made to address which source of inorganic carbon is predominantly used in spicule production. These studies led to opposite conclusions but also used different methods to constrain carbon partitioning, and each has its own relative strengths and weaknesses. While Allemand & Grillo (1992), using ^{45}Ca labelling of the skeleton, concluded that metabolic CO_2 may serve as a main source of carbon in the red coral *Corallium rubrum*, Lucas & Knapp (1997) used ^{14}C-labelling and found the contrary (70% of external DIC) in the gorgonian *Leptogorgia virgulata*. Our $\delta^{13}C$ analyses suggest that the carbon origin in spicules is species dependent. According to Swart (1983), there is a 7‰ enrichment between the internal pool of CO_2 and the pool of HCO_3^- and again ~1‰ enrichment between the HCO_3^- and the calcium carbonate of the skeleton or spicules. From a mean $\delta^{13}C$ value of the spicules equal to −8‰ in the asymbiotic gorgonians, the back-calculated value for the internal CO_2 pool at the origin of spicule formation is −16‰ [(−8‰) + (−1‰) + (−7‰)]. Considering that the respired CO_2 has the same isotopic signature as the tissue (ca. −21‰ in this study), the carbon in the spicules is a mix of 76% of respired CO_2 and 24% of external seawater CO_2 (76% of respired CO_2 at −21‰ + 24% of external CO_2 at 0.8‰; Reynaud et al. 2002). So, the major part of the carbon used for spicule formation seems to come from respired carbon. In *Eunicella singularis*, showing a mean $\delta^{13}C$ value of −4.8‰ for the spicules, the same calculations suggest a contribution of ~60 and 40% of respired and seawater CO_2, respectively. In this case, a larger fraction of the carbon used for spicule formation seems to come from external CO_2. This difference might be due to the presence of photosynthetic symbionts in *E. singularis*, which actively take up DIC from seawater for their own needs of photosynthesis, and a part of this CO_2 (with a higher $\delta^{13}C$ signature than that of respiration) might be diverted to spicule formation. Overall, since the above techniques are different, with all their uncertainties, more measurements are necessary to completely understand the origin of the carbon used for spicule formation.

The axial skeleton of the 4 gorgonian species was much more depleted in ^{13}C than were the spicules. This is explained by the fact that the skeleton is formed both by calcite and by a horn-like structural protein called gorgonin. Gorgonin is secreted by the soft tissues, and the ingested food serves as the source of amino acids and other molecules used in its synthesis (Roche et al. 1960, Heikoop et al. 2002, Sherwood 2006). Such skeleton is very enriched in carbon and nitrogen (35 and 12% of the total dry weight, respectively) and has therefore carbon and nitrogen signatures close to the signatures of the tissue influenced by the food source (Heikoop et al. 2002, Sherwood 2006) or by the external levels of dissolved inorganic nitrogen (Ward-Paige et al. 2005, Risk et al. 2009, Sherwood et al. 2010). The external influences are discussed below with the tissue signatures.

The soft tissue isotopic signatures have highlighted seasonal and species-specific differences. *Eunicella singularis* had slightly but significantly higher $\delta^{13}C$ and $\delta^{15}N$ signatures compared to the other non-symbiotic gorgonians, due to the presence of symbionts in its tissue. Indeed, symbiont photosynthetic activity uses dissolved HCO_3^- as a precursor pool for carbon, which in the case of seawater HCO_3^- has a naturally high ^{13}C signature of 0.8‰ (Reynaud et al. 2002). The resulting photosynthetic compounds therefore exhibit a higher ^{13}C signature of typically −12‰ (Reynaud et al. 2002) compared to $\delta^{13}C$ values of −22‰ typically observed in planktonic food or POM (Fry 2007). Also, in symbiotic organisms, nitrogen resulting from metabolic waste products is not excreted into seawater as in heterotrophic organisms. Instead, it is re-absorbed by the symbionts and therefore recycled within the symbiosis, a process which

increases the nitrogen isotopic signature. However, the carbon autotrophic input in *E. singularis* did not significantly increase from winter to summer, as expected if we consider that rates of photosynthesis are enhanced in summer by higher light and temperature levels (Ferrier-Pagès et al. 2011). This suggests that either *E. singularis* keep a constant rate of photosynthesis under the varying light intensities of the 2 seasons or that the depth at which they live, coupled with the turbidity of the water, induces a constant light environment. Conversely, the $\delta^{15}N$ signature of *E. singularis* decreased from 10 to 8‰, suggesting a more important input of dissolved inorganic nitrogen in summer, presumably taken up through the activity of the zooxanthellae.

The seasonal difference in the isotopic composition of the gorgonian tissue was linked to the seasonal changes in food availability in the surrounding seawater, as suggested by the $\delta^{13}C$-$\delta^{15}N$ plots generally used in complex food chains to determine the food source of a specific predator (Riera et al. 1999, 2009). In the Mediterranean Sea, large seasonal changes in the abundance and composition of phyto- and zooplankton are indeed observed, with high concentrations in winter due to the upwelling of nutrients to the surface and very low concentrations in summer after the establishment of the thermocline. Summer is therefore generally considered a very unfavourable season for all benthic suspension feeders (Coma et al. 1998), because most of these species are entirely dependent on food supply to maintain their metabolism (Coma et al. 2000). The expected mean winter isotopic ratios for the preferentially exploited food resources were close to the ratio of zooplankton for *Eunicella singularis*, *E. verrucosa*, and *Leptogorgia sarmentosa* and to the ratios of POM and SOM, i.e. organic matter from seawater and sediment, for *Paramuricea clavata*. These conclusions are corroborated by the results obtained using the model of Phillips & Gregg (2003), with zooplankton contributing >90% of the diet of the first 3 gorgonian species and POM and SOM contributing 75% of the diet of *P. clavata*. Summer results suggested 2 different feeding strategies depending on the gorgonian species. Indeed, *L. sarmentosa* and *E. verrucosa* showed seasonality in their feeding habits, as already observed with other benthic suspension feeders (Coma et al. 2000). Their isotopic signature shifted from zooplankton to SOM, suggesting that in summer, zooplankton concentration was no longer sufficient to sustain the metabolism of these species (Coma et al. 2000), which relied on the dominant food source available, i.e. detrital organic matter from re-suspended sediment. SOM can provide significant amounts of nutrients, such as nitrogen to anthozoans, and even sustain their metabolic needs in some conditions (Mills & Sebens 2004, Anthony & Fabricius 2000). This detrital material is abundant all year in the Gulf of La Spezia, because the Magra River continuously discharges a high quantity of sediment and particles on the vertical cliff where gorgonians thrive (Cupido et al. 2009). Conversely, the 2 other species showed little seasonality in their food sources. The *P. clavata* signature remained close to the SOM signature, as in winter, and zooplankton seemed to remain the preferential food source for *E. singularis*. In contrast to the other 2 gorgonian species relying on zooplankton in winter, the symbiotic status of *E. singularis* perhaps allowed it to survive with this sole heterotrophic food source in summer, despite its scarcity.

Only 2 out of the 4 gorgonians of this study had been previously investigated for their feeding habits, using punctual measurements of prey disappearance in incubation chambers (Ribes et al. 1999, 2003). These previous studies confirm the validity of our approach using stable isotopes. Ribes et al. (1999) observed that *Paramuricea clavata* from the Medes Islands mainly relied on detrital POC (86% of the total diet), especially during winter, when particles are re-suspended in the water column. Concerning *Leptogorgia sarmentosa*, Ribes et al. (2003) concluded that detritus and zooplankton were the 2 main food sources, which also confirms our findings that zooplankton account for the major $\delta^{13}C$ signal of the tissue of this species.

In conclusion, this is one of the first studies on the feeding habits of 4 predominant gorgonian species of the Mediterranean Sea under natural conditions. It has shown a high predation rate on zooplankton in winter by 3 out of the 4 species and a shift to detrital material in summer, when plankton is no longer sufficiently abundant to sustain gorgonian metabolism. The Gulf of La Spezia has a high detrital load all year, and this might explain the high gorgonian abundance in this zone, since they can rely on this food source during nutrient shortage. Because the Gulf is also under terrestrial influence, zooplankton is particularly abundant in winter and therefore constitutes a high energetic food source at this season. The symbiotic *Eunicella singularis* acquires additional carbon and nitrogen from its symbionts. Finally, the carbon used to build the spicules, is mainly produced from metabolic respiration, except again for *E. singularis,* which derives a larger fraction of carbon from the external medium via the activity of its symbiotic zooxanthellae.

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