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# Summer autotrophy and winter heterotrophy in the temperate symbiotic coral *Cladocora caespitosa*

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## Abstract

Carbon and nitrogen isotope ratios ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) were determined in the zooxanthellae, host tissue, and whole symbiotic association of the symbiotic temperate coral *Cladocora caespitosa*, as well as in the different components of the coral's potential food sources (plankton, particulate organic matter in seawater and in the sediment). Data were collected both in winter and summer at three different locations, to assess the extent of auto- and heterotrophy in this species and get a better understanding of the functioning of temperate symbioses. There was a marked seasonal difference in the signature of the zooxanthellae and host tissue, highlighting two clear feeding patterns. In summer,  $\delta^{13}\text{C}$  signatures of the coral host and the zooxanthellae were similar ( $-17\text{‰}$ ) and very different from the signature of the food sources (from  $-21\text{‰}$  to  $-25\text{‰}$ ), suggesting that corals were relying on autotrophy for the acquisition of carbon.  $\delta^{15}\text{N}$  values also suggested that nitrogen was not acquired through feeding. Conversely, in winter, the  $\delta^{13}\text{C}$  signature of the host decreased by ca.  $-23\text{‰}$  to  $-28\text{‰}$ , and was more comparable to the signature of the external food sources (between  $-24\text{‰}$  and  $-25\text{‰}$ ), suggesting a substantial reliance of *C. caespitosa* on external food sources during this season. There was also a  $3\text{‰}$  enrichment between the  $\delta^{15}\text{N}$  signatures of the food ( $4\text{--}5\text{‰}$ ) and the signature of the symbiotic association ( $7\text{--}8\text{‰}$ ), suggesting that nitrogen was also acquired through feeding. Overall, these results give evidence that *C. caespitosa* and temperate corals in general derive a large fraction of their energy from heterotrophic feeding in winter.

*Cladocora caespitosa* is a Mediterranean scleractinian coral (Faviidae family), comparable to its tropical congeners because it is colonial, lives in symbiosis with dinoflagellates commonly called zooxanthellae, and can form large banks or extensive bioherms (Kružić and Požar-Domac 2003; Kružić and Benković 2008; Peirano et al. 2004). This coral has, however, a wider habitat range, from well-lit shallow-water environments to relatively dark conditions (no more than  $20\text{ }\mu\text{mol photons m}^{-2}\text{ s}^{-1}$ ) such as those occurring at 40-m depth (Peirano 2007) or in the turbid waters of La Spezia (Italy, Eastern Ligurian Sea), which receives loads of sediment from the Magra plume (Peirano et al. 1999). Laboratory experiments have shown that its association with zooxanthellae can supply most of its carbon requirements, except under low light conditions, under which the coral has to rely more on the heterotrophic feeding (Schiller 1993; Hoogenboom et al. 2010). *C. caespitosa*, and corals in general, are indeed able to increase their feeding effort when prey are available and photosynthesis is suppressed or lowered (Anthony and Fabricius 2000; Ferrier-Pagès et al. 2010; Hoogenboom et al. 2010). They can prey on a wide range of particles, from bacteria and dissolved organic matter to macrozooplankton (reviewed in Houlbrèque and Ferrier-Pagès 2008). This heterotrophic mode of nutrient acquisition is particularly

important in temperate symbiotic anthozoans, because they often live in habitats with lower light levels, colder temperatures, and higher nutrient concentrations compared to tropical areas (Muller-Parker and Davy 2001). Studies performed on the temperate sea anemone *Anthopleura elegantissima* (Engebretson and Muller-Parker 1999; Bergschneider and Muller-Parker 2008) or on the temperate coral *Astrangia* (Szmant-Froelich and Pilson 1980, 1984; Dimond and Carrington 2007), both from the Pacific, indeed concluded that during unfavorable conditions, the host derives most of its nutrition via heterotrophy. The question is therefore to know if heterotrophy plays the same important role for the Mediterranean coral *C. caespitosa*. Although this issue has been assessed in two laboratory experiments with artificial food, supplied in high concentrations (Rodolfo-Metalpa et al. 2008b; Hoogenboom et al. 2010), the natural heterotrophic carbon and nitrogen acquisition of this species is still unknown.

The aim of this study was therefore to investigate the extent of auto- and heterotrophy in colonies of *C. caespitosa* living under natural conditions and to determine the potential food sources for this coral. This knowledge will bring a better understanding of the functioning of temperate symbioses, and of their response to stresses (Rodolfo-Metalpa et al. 2006, 2008c), which usually induce a decrease in symbiont density, as well as in photosynthetic capacities and autotrophic carbon acquisition (Rodolfo-Metalpa et al. 2006).

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In coastal environments, however, there are generally a large number of food sources for benthic consumers, and the diversity in the origin of the organic matter makes it difficult to determine which are the main food sources. Stable carbon and nitrogen isotope ratio ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) analysis has proven to be a useful tool in delineating carbon flows and trophic relationships in a large variety of marine ecosystems (Fry and Sherr 1984; Peterson and Fry 1987), and serves as a long-term index of carbon (and nitrogen) assimilation (Muscatine et al. 1989; Bergschneider and Muller-Parker 2008). While carbon isotope composition is, in many cases, characteristic of each primary source of organic matter (Farquhar et al. 1989) and the  $^{13}\text{C}:^{12}\text{C}$  ratio of consumers is similar to those of their diet (DeNiro and Epstein 1978), the enrichment factor in  $\delta^{15}\text{N}$  between two consecutive trophic levels is higher, from 2.3‰ to 3.4‰ (Minagawa and Wada 1984; McCutchan et al. 2003). Isotopic analyses of anthozoan tissue have already been used, either in tropical species (Muscatine et al. 1989; Muscatine and Kaplan 1994; Reynaud et al. 2009) or in a temperate sea anemone (Bergschneider and Muller-Parker 2008), to estimate the importance of heterotrophy in their diet. This technique has, however, never been applied to temperate corals, except in cold-water corals (Carlier et al. 2009), which are fundamentally different from shallow-water symbiotic ones.

To study the nutritional mode of *C. caespitosa*, and to assess the productivity of the zooxanthellae and their contribution to the animal host, several colonies were sampled, in both winter and summer, from three localities in the Ligurian Sea (northwest Mediterranean Sea) where this species is particularly abundant (Peirano et al. 1999). The isotopic signature of the coral tissue was compared to the signature of the zooxanthellae, its autotrophic source of nutrition, and to the signature of the particles in seawater (from bacteria to macrozooplankton) and sediment, which are its potential heterotrophic food sources.

## Methods

**Study site**—Corals were collected at ca. 8–10-m depth at three locations (Fig. 1) in the Ligurian Sea (northwest Mediterranean Sea). The first sampling site, named Lerici, was located in front of the town of Lerici (44°04.33'N, 09°54.41'E), and the second site, named Punta Bianca (44°02.16'N, 09°58.76'E), was in front of the Magra River, 8 km away from Lerici. These two sites have silt-sandy bottoms, because they are under the influence of the Magra plume, which carries high loads of particles and sediment, especially during wintertime. At these locations, colonies live in a very turbid environment and are often covered by a thin layer of sediment. Underwater photosynthetic active irradiance (PAR) at 9-m depth in both locations varied from a mean of 50  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  in winter (mean seawater temperature of 13°C) to 206–230  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in summer (mean temperature of 23°C). The third site, called Bonassola (44°10.88'N, 09°34.72'E), was at ca. 50 km from Lerici. Here, corals are at the same depth, and experience the same temperatures, but they are located in clear waters and on rocky substrate. Underwater PAR therefore varies

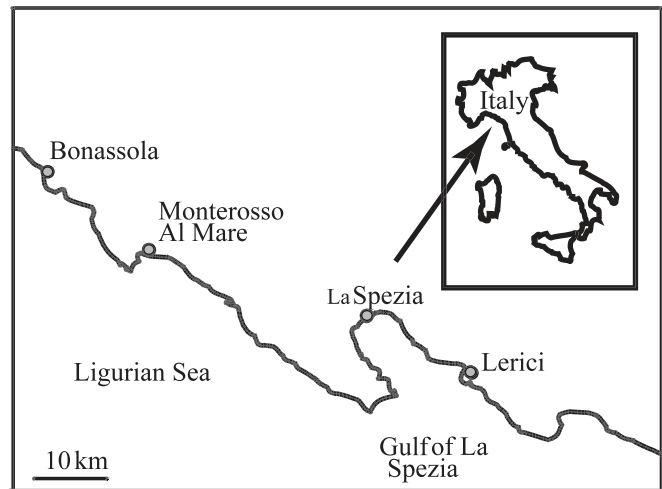


Fig. 1. Map of the three locations studied.

between a mean of 114–145  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in winter (Peirano et al. 1999) and a mean value of 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in summer. The other characteristics of these Ligurian waters are fully described in Peirano et al. (2005).

**Sampling and treatments**—Corals: Coral samples were collected during 2–3 d in winter (January) and summer (August) 2009. Five colonies (with ca. 30 polyps, or a total surface of 30–40 cm<sup>2</sup>) were randomly selected at each site and each season. They were enclosed underwater in a plastic bag containing the surrounding seawater and brought back to the laboratory, where they were cleaned of epiphytes and sediment, divided into two samples of ca. 20 and 10 polyps respectively, and frozen until subsequent analysis.

No more than a week after sampling, the 20 polyp samples from each mother colony were thawed and individually placed in 100-mL beakers (precombusted at 480°C for at least 4 h in a Thermolyne® 62700 oven) containing 20 mL of filtered seawater. For each sample, tissue was completely removed from the skeleton with an air pick and homogenized with a Potter tissue grinder (also precombusted at 480°C for at least 4 h). Half of the homogenate was directly flash frozen and freeze-dried. It will be referred as to the symbiotic association in the following sections and represents the mean signal of both the host tissue and zooxanthellae. The other half of the homogenate was divided into a host fraction and a zooxanthellae fraction. For the host fraction, the homogenate was centrifuged (Biofuge 17RS Heraeus) at 3000 g for 10 min at 4°C to pellet most of the zooxanthellae. The supernatant was recentrifuged twice to eliminate the remaining zooxanthellae (as checked under a microscope), then transferred in 50-mL Pyrex tubes, flash frozen with liquid nitrogen, and freeze-dried using a Heto (model CT 60) drier. For the zooxanthellae fraction, the zooxanthellae pellet was washed several times with filtered seawater (following Muscatine et al. 1989), until almost no contamination by host cells was visible under the binocular, and then also flash frozen and freeze-dried. Isotopic

analyses were performed on these three sets of freeze-dried samples, with each colony treated individually.

Samples from each colony containing ca. 10 polyps were used for protein (five polyps per colony) chlorophyll (Chl) and zooxanthellae (five polyps per colony) determination. For Chl and zooxanthellae measurements, coral tissue was detached from the skeleton using a Water Pick® in 12 mL of 0.45- $\mu\text{m}$ -filtered seawater. The slurry was homogenized using a Potter tissue grinder and a 2-mL subsample was taken for zooxanthellae density determination using the Histolab® 5.2.3 image analysis software (Rodolfo-Metalpa et al. 2006). The remaining 10-mL subsamples were centrifuged at 5000  $g$  for 10 min at 4°C and the pellet containing the zooxanthellae was resuspended in 10 mL of pure acetone. Pigments were extracted at 4°C during 24 h. The extract was centrifuged at 10,000  $g$  for 15 min, and Chl  $a$  and  $c_2$  were determined according to Jeffrey and Humphrey (1975). Data were normalized to the polyp surface area determined as in Rodolfo-Metalpa et al. (2006). For protein content measurement, tissue was extracted in 1 mol L<sup>-1</sup> NaOH at 90°C for 30 min and proteins were determined using the bicinchoninic acid assay kit (Interchim). The standard curve was established with bovine serum albumin and the absorbance was measured with a multiscan bichromatic spectrophotometer (Labsystem®). Data were normalized to the polyp surface area.

**Particulate organic matter:** To characterize the isotopic signature of the particulate organic matter (POM) suspended in seawater, five 10-liter bottles of seawater were sampled near the corals, at each site and each season, using a Niskin® bottle. Back to the laboratory, seawater was size fractionated using an inverse filtration device to avoid cell breakage. The following size fractions were achieved: particles above 105  $\mu\text{m}$  (macroplankton), between 105 and 50  $\mu\text{m}$  (microplankton), between 50 and 10  $\mu\text{m}$  (nanoplankton), and between 10 and 1  $\mu\text{m}$  (small nanoplankton and picoplankton). Each fraction was then filtered onto precombusted (450°C for 5 h) 25-mm Whatman GF/F filters under low vacuum. To remove carbonates, filters were quickly acidified (1 mol L<sup>-1</sup> HCl) and rinsed with distilled water. They were then freeze-dried and kept frozen until isotopic analyses. Only a few macrozooplankton cells were collected on the filters, because of their low abundance in seawater. Therefore, to get the isotopic signal of the zooplankton for the winter and summer seasons, concentrated samples were also collected in a 5-min haul using a plankton net (type WP2). A fraction of this zooplankton was counted, whereas another fraction was collected on filters and treated as described above.

**Sedimentary organic matter:** Muddy sediment samples were taken at each station and each season near the corals by scraping the upper 1-cm surface of sediment (Riera et al. 1996). For the measurements of stable isotopic ratios of the sedimentary organic matter (SOM), the sand was sieved to a grain size of < 50  $\mu\text{m}$  to separate sand grains from most of the sedimentary POM. A subsample of approximately 5 g was freeze-dried, and ground using a mortar and pestle. Then, 200 mg was acidified with 1 mol L<sup>-1</sup> HCl to remove inorganic carbon. In order to prevent any loss of dissolved

organics, these samples were not rinsed, but the acidification was performed on a hot plate to quickly evaporate the acid, and samples were dried overnight at 50°C under a fume extractor (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 analyzed for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  using an automated nitrogen-carbon analyzer (ANCA) coupled to a Geo-20:20 isotope ratio mass spectrometer (SerCon®). International reference materials (IAEA-600 and IAEA-CH6, International Atomic Energy Agency) were used for scale calibration of results to Vienna PeeDee Belemnite (VPDB) and air, and two different analytical quality control samples were also analyzed with each batch for quality control purposes. Precision as determined by repeat analysis of the reference materials and quality controls was better than  $\pm 0.20\text{‰}$  and  $\pm 0.15\text{‰}$  for measured  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values, respectively. Data are expressed in the standard  $\delta$  unit notation where

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

where  $R = {}^{13}\text{C} : {}^{12}\text{C}$  for carbon and  ${}^{15}\text{N} : {}^{14}\text{N}$  for nitrogen, and reported relative to VPDB for carbon and to air  $\text{N}_2$  for nitrogen.

**Statistics and models**—The isotopic signatures of the different samples (coral tissue, zooxanthellae, symbiotic association, POM, SOM, and plankton) taken at the three locations (Lerici, Punta Bianca, and Bonassola) and in two seasons (winter and summer) were compared using a three-way analysis of variance (ANOVA) and StatView® software. In order to give an estimation of the isotopic contribution of each determined food source to the isotopic signal of the host tissue, a computer program (IsoSource) developed by Phillips and Gregg (2003) and freely available at <http://www.epa.gov/wed/pages/models.htm> was used.

## Results

Colonies of *C. caespitosa* from the three locations contained equivalent concentrations of zooxanthellae, Chl, and protein per skeletal surface area during the winter season (Fig. 2; one-way ANOVA  $p = 0.6$ , 0.3, and 0.2 for the protein, zooxanthellae, and Chl, respectively). In summer, colonies from Bonassola showed significant lower zooxanthellae density and Chl concentration per skeletal surface area than in Lerici and Punta Bianca (one-way ANOVA,  $p = 0.016$  and 0.049 for the Chl and zooxanthellae, respectively). However, the amount of Chl per zooxanthella remained stable and equal to 3–4 pg cell<sup>-1</sup> (data not shown). Proteins were not significantly different (one-way ANOVA,  $p = 0.9$ ). Also, for Lerici and Punta Bianca, there was no significant difference between winter and summer for protein, zooxanthellae, and Chl concentrations (Fig. 2; ANOVA,  $p > 0.05$  for all parameters), suggesting a seasonal stability. Conversely, for Bonassola, Chl and zooxanthellae concentrations were significantly lower in summer (Fig. 2; ANOVA,  $p = 0.004$  and  $p = 0.02$ , respectively) than in winter.

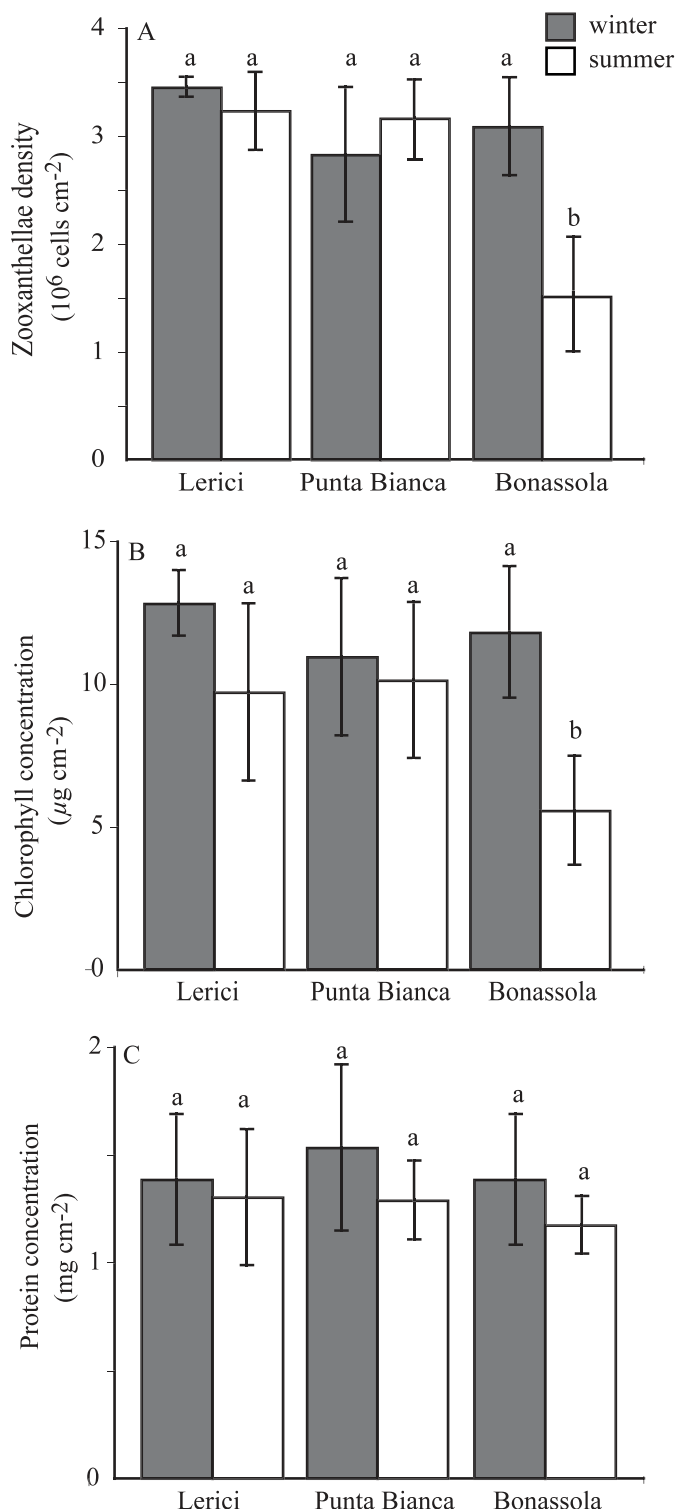


Fig. 2. (A) Zooxanthellae density (cells cm<sup>-2</sup>), (B) total Chl (μg cm<sup>-2</sup>), and (C) protein (mg cm<sup>-2</sup>) concentrations in corals sampled from Lerici, Punta Bianca, and Bonassola in winter and summer. Values are mean ± SD of five samples.

The carbon isotopic signature ( $\delta^{13}\text{C}$ ) of the different samples is represented in Fig. 3 and Table 1. Within each location and each season,  $\delta^{13}\text{C}$  of the different seawater POM fractions remained in a very close range, differing less

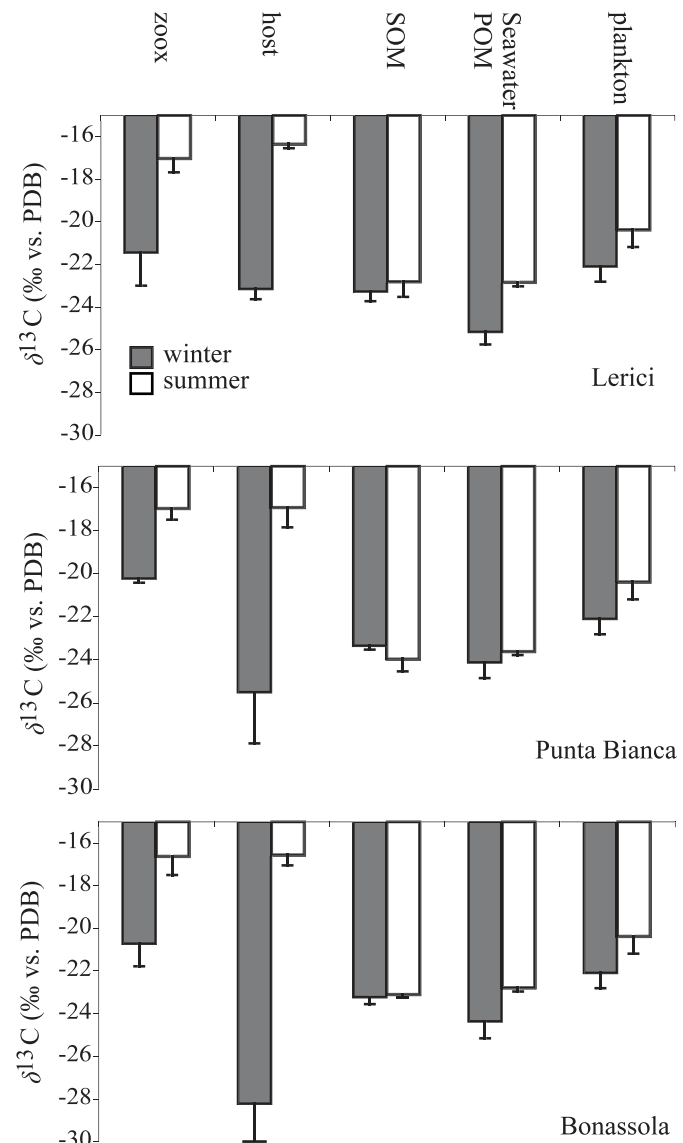


Fig. 3.  $\delta^{13}\text{C}$  values of zooxanthellae (zoox), host tissue (host), SOM, POM in seawater (seawater POM), and zooplankton (plankton), sampled in the three locations of Lerici, Punta Bianca, and Bonassola in winter and summer. Values are mean ± SD of five samples.

than 1‰ (Table 1). Therefore, for the following results and discussion, a mean value of seawater POM was calculated for each location and will be considered. Also, the  $\delta^{13}\text{C}$  signature of the entire symbiotic association (zooxanthellae + coral host) was equal to  $-22.57 \pm 1.60\text{‰}$ ,  $-21.24 \pm 0.32\text{‰}$ , and  $-22.22 \pm 0.80\text{‰}$  in winter and  $-15.8 \pm 0.42\text{‰}$ ,  $-16.31 \pm 0.21\text{‰}$ ,  $-15.71 \pm 1.36\text{‰}$  in summer for corals sampled in Punta Bianca, Lerici, and Bonassola, respectively.

$\delta^{13}\text{C}$  values were not different between locations (Table 2). They highlighted a marked seasonal difference in the signature of the symbiotic association of zooxanthellae and host tissue (Fig. 3; Table 2). Indeed, the  $\delta^{13}\text{C}$  of the symbiotic association and zooxanthellae decreased from ca.  $-16\text{‰}$  and  $-17\text{‰}$  in summer to ca.  $-21\text{‰}$  and



Table 1.  $\delta^{13}\text{C}$  mean values of the different fractions of POM and mean  $\delta^{13}\text{C}$  values of the total POM.  $1 < \text{POM} < 10$ , particles with a size between 1 and 10  $\mu\text{m}$ ;  $10 < \text{POM} < 50$ , particles with a size between 10 and 50  $\mu\text{m}$ ;  $50 < \text{POM} < 105$ , particles with a size between 50 and 105  $\mu\text{m}$ ;  $\text{POM} > 105$ , all particles whose size is more than 105  $\mu\text{m}$ . Values in parentheses are standard deviations.

	Lerici		Punta Bianca		Bonassola	
	Winter	Summer	Winter	Summer	Winter	Summer
$1 < \text{POM} < 10$	-24.8(0.5)	-23.1(0.4)	-24.6(0.1)	-22.7(0.6)	-24.3(0.5)	-23.1(0.6)
$10 < \text{POM} < 50$	-25.0(0.2)	-22.5(0.1)	-24.4(0.2)	-22.4(0.4)	-24.3(0.6)	-22.6(0.5)
$50 < \text{POM} < 105$	-25.6(0.7)	-22.9(0.6)	-24.1(0.6)	-22.7(0.2)	-23.7(0.7)	-22.8(0.7)
$\text{POM} > 105$	-25.7(0.7)	-22.6(0.2)	-24.6(0.4)	-22.5(0.3)	-24.2(0.3)	-23.1(0.5)
Mean	-25.3(0.6)	-22.8(0.4)	-24.1(0.7)	-22.6(0.2)	-24.3(0.8)	-22.8(0.4)

-22‰ in winter. Also, the  $\delta^{13}\text{C}$  of the host decreased from -17‰ in summer to -23‰, -25‰ and -28‰ in winter, depending on the location considered. Although the host tissue was significantly more depleted than the zooxanthellae in winter (from 2‰ to 7‰ depending on the location, Tukey test,  $p < 0.001$ ; Fig. 3),  $\delta^{13}\text{C}$  values of the two fractions were identical in summer (Tukey test,  $p = 0.351$ ). Also, the comparison of the  $\delta^{13}\text{C}$  of the symbiotic association with the  $\delta^{13}\text{C}$  of the coral potential food sources (POM, SOM, and plankton) showed two different trends, according to the season. In summer, the  $\delta^{13}\text{C}$  of the symbiotic association (ca. -15‰) was highly enriched compared to the food sources (-20 to 23‰; Fig. 3; Tukey test,  $p < 0.001$  between host tissue and all sources). Conversely, in winter, the  $\delta^{13}\text{C}$  signature of the symbiotic association was more depleted (-22‰), reaching the same low values as for POM, SOM, and plankton (ANOVA,  $p = 0.11$ , 0.06 and 0.25, respectively).

Concerning the  $\delta^{15}\text{N}$  signature, all POM size fractions were pooled into one fraction, as for carbon (Table 3). Overall, the nitrogen isotopic signature was different between samples, locations, and seasons (Table 3). As for carbon, results showed a marked seasonal difference in the  $\delta^{15}\text{N}$  of the host tissue and POM (Tukey test,  $p < 0.001$ ), which were significantly enriched by 3–5‰ in winter. Overall, all samples presented higher  $\delta^{15}\text{N}$  values in winter than in summer. In winter, coral tissue had higher  $\delta^{15}\text{N}$  values compared to zooxanthellae (by 0.3–2.3‰), water, and sediment (by 3–6.6‰; Tukey test,  $p < 0.01$ ). In summer, values were comparable (Tukey test,  $p > 0.05$ ), except for SOM (Tukey test,  $p < 0.001$ ).

Estimation of the isotopic contribution of each food source to the isotopic signal of the host tissue (Phillips and

Gregg 2003) has given the following information: in summer, the zooxanthellae isotopic contribution ranged from 81% to 100%. The external food sources (POM, SOM, plankton) accounted for only between 0% and 18% of the isotopic host signature in all sampling locations. Conversely, in winter, zooxanthellae contributed only 0–24% to the isotopic signal of the host, while the external food sources equally contributed between 10% and 90%. No external food source was predominant in terms of contribution, except in Punta Bianca, where POM had a main contribution of 55–75%.

## Discussion

In order to better understand the functioning of temperate symbioses in terms of acquisition of energy, this study has assessed, under natural winter and summer conditions, the contribution of algal symbionts and external food to the carbon and nitrogen budgets of the temperate coral *C. caespitosa*. Results obtained have shown that heterotrophy increased in importance in winter, and supplied, during this season, the major part of the carbon and nitrogen needs of this coral. Conversely, autotrophy seemed to play a major role in summer.

The biomass data indicated that population densities of zooxanthellae, as well as Chl contents of the colonies sampled in Lerici and Punta Bianca, were similar between summer and winter, in spite of large seasonal differences in temperature and irradiance. Such stability of the symbiosis has previously been observed in the same coral species (Rodolfo-Metalpa et al. 2008c) and in other temperate corals and sea anemones (Farrant et al. 1987; Muller-Parker and Davy 2001; Bergschneider and Muller-Parker

Table 2. Three-way ANOVA comparing the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures between samples (host tissue, zooxanthellae, symbiotic association, POM, SOM, plankton), locations (Lerici, Punta Bianca, Bonassola), and seasons (winter, summer).  $p$  values below 0.05 show significant differences.

Parameter	df	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
		$F$	$p$	$F$	$p$
Sample	5	120.9	<0.0001	1.3	<0.001
Location	2	2.1	0.1290	7.4	0.001
Season	1	317.9	<0.0001	9.3	0.003
Sample–location	10	4.7	0.0008	1.9	0.189
Sample–season	5	94.6	<0.0011	90.1	<0.001
Location–season	2	3.4	0.5224	11.3	<0.001
Sample–location–season	10	3.4	0.0069	5.2	0.042

Table 3.  $\delta^{15}\text{N}$  values for the different categories (host tissue, zooxanthellae, symbiotic association, POM, SOM, and plankton) in the three locations, for winter and summer.

Location and category	Winter		Summer	
	Mean	SD	Mean	SD
<b>Punta Bianca</b>				
Zooxanthellae	8.80	0.45	7.32	1.02
Host tissue	10.18	1.01	6.94	1.18
Symbiotic association	7.93	1.12	7.02	0.04
SOM	5.01	0.30	5.02	0.79
POM	4.60	1.15	9.30	1.32
Plankton	5.70	0.12	5.43	0.13
<b>Lerici</b>				
Zooxanthellae	7.51	0.99	7.73	0.70
Host tissue	8.58	1.51	8.20	0.65
Symbiotic association	7.42	0.10	7.68	0.07
SOM	3.15	0.19	2.48	1.09
POM	4.15	1.27	8.40	0.86
Plankton	5.72	0.17	5.40	0.10
<b>Bonassola</b>				
Zooxanthellae	6.86	1.10	5.45	0.80
Host tissue	7.46	1.68	5.93	0.70
Symbiotic association	7.17	0.25	5.06	0.14
SOM	4.51	1.09	2.27	0.20
POM	4.74	1.83	7.29	1.24
Plankton	5.75	0.09	5.45	0.11

2008), and might result from different algal expulsion rates between summer and winter, or a balanced growth of zooxanthellae and host (Verde and McCloskey 2007). The fact that corals sampled in Bonassola, only 50 km away from Lerici, presented significantly lower zooxanthellae density in summer compared to the other locations suggests that algae are rather expelled by the host when irradiance levels are high (Fitt et al. 2000; Rodolfo-Metalpa et al. 2008c). Bonassola is indeed the location that receives the highest light level in summer, compared to the other two more-shaded locations (Peirano et al. 2005). The lack of seasonal variation in the Chl content per zooxanthella was also observed in Mediterranean corals (Rodolfo-Metalpa et al. 2008c) and several other temperate anthozoans (Farrant et al. 1987; Verde and McCloskey 2002), and contrasts with tropical corals, which often exhibit a decrease in zooxanthellae density and pigment concentrations per zooxanthella (Fitt et al. 2000).

Isotopes of carbon and nitrogen have proven to be useful in assessing the importance of heterotrophy and autotrophy in corals. Concerning nitrogen isotopes, a pure heterotrophic predator is generally enriched by 2.5–4.5‰ compared to its prey, because of the excretion of light nitrogen ( $^{14}\text{N}$ ) as waste products (Minagawa and Wada 1984; Gollety et al. 2010). In symbiotic corals, nitrogen ingested by the host is not excreted, but rather recycled by the zooxanthellae, inducing a 1–3‰ enrichment of the host compared to the zooxanthellae, not to the food (Reynaud et al. 2009). Such enrichment was observed in this study in winter, confirming the previous observations of an important recycling of ingested nutrients within the symbiosis. When the whole symbiotic association (host

plus zooxanthellae) is however considered as “the predator,” there can indeed be observed, in winter, a 2.5–3.4‰ enrichment between the food (POM, SOM) and the symbiotic association, showing that corals derive a large fraction of their nitrogen needs from food during this season. Conversely, in summer, the isotopic signature of the host and the zooxanthellae was comparable, and it was much lower than in winter. The isotopic signature of the symbiotic association did not follow the classical enrichment values compared to the food, suggesting that corals were relying more on dissolved sources (ammonium, nitrate) absorbed by the zooxanthellae than on the organic nitrogen derived from particulate feeding.

$\delta^{13}\text{C}$  values confirmed our conclusions based on  $\delta^{15}\text{N}$  isotopes. In summer, the host and zooxanthellae presented the same  $\delta^{13}\text{C}$  signature, suggesting that most of the host’s carbon originated from its algae. Indeed, the zooxanthellae signature was significantly higher than those of the POM, SOM, and zooplankton, because of their intense photosynthetic activity of the zooxanthellae and partly because of the use of bicarbonate with a high  $\delta^{13}\text{C}$  signature (0.8‰; Reynaud et al. 2002). Inside the coral, bicarbonate undergoes several processes before reaching the photosynthetic site of the zooxanthellae, such as dehydration, which produces a large fractionation of –7‰ (Deuser and Degens 1967). It is also mixed with some respiratory carbon, leading to a mean isotopic signature of –10‰ to –16‰ in a wide range of zooxanthellae living in tropical coral species (Muscantine et al. 1989; Risk et al. 1994; Reynaud et al. 2002). Because the  $\delta^{13}\text{C}$  signal of *C. caespitosa* was very close to the signature of tropical corals, known to mostly be autotroph for carbon, it is likely that autotrophy is also

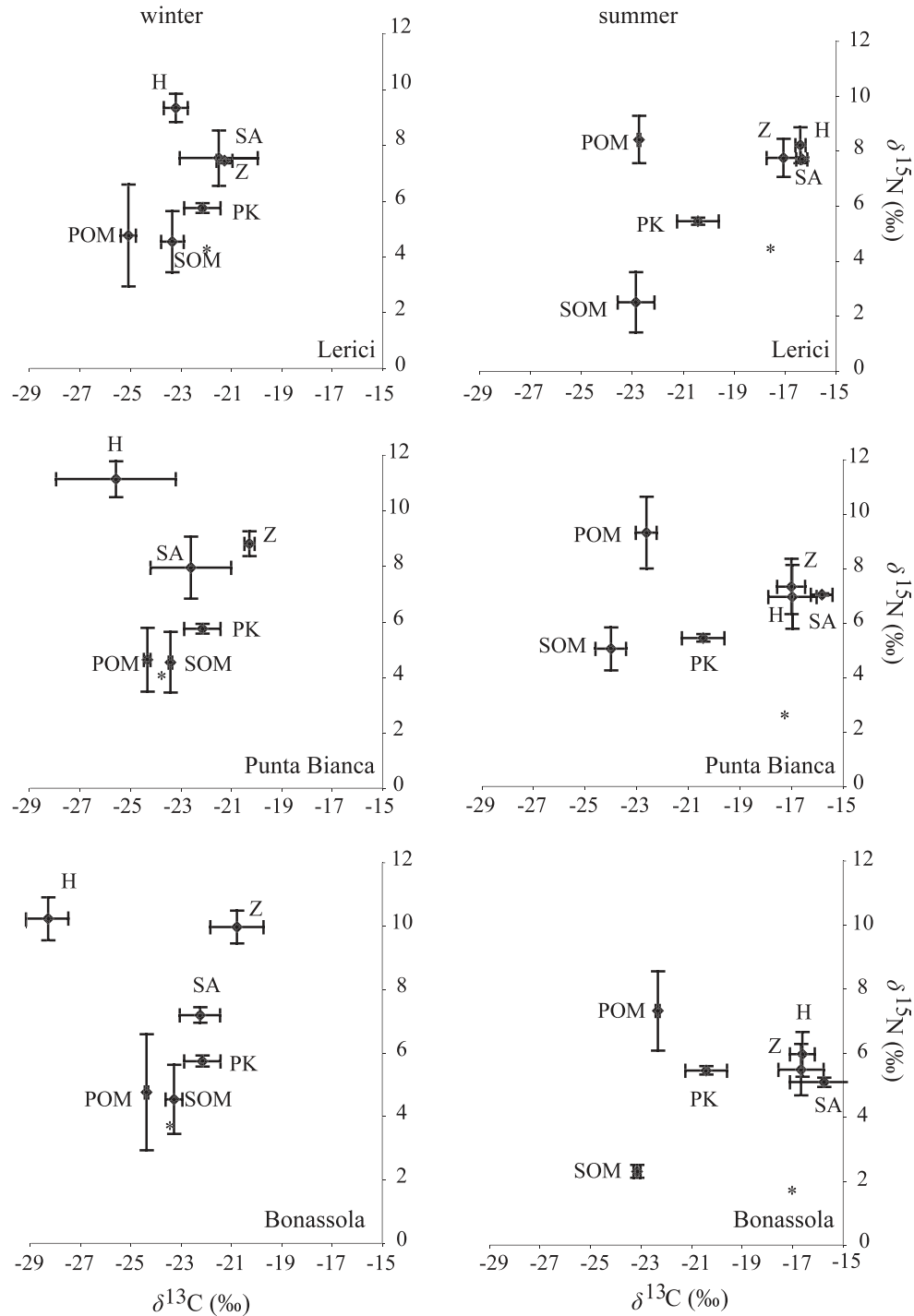


Fig. 4.  $\delta^{13}\text{C}$  vs.  $\delta^{15}\text{N}$  (mean  $\pm$  SD) for the corals of the three locations, stranded sediment, zooplankton, and seawater samples for winter and summer. Asterisks correspond to the theoretical food source of the corals, taking into account the trophic enrichment of 1‰ and 3.5‰ for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively. H, host; Z, zooxanthellae; SA, symbiotic association; PK, plankton.

important for the carbon budget of *C. caespitosa* in summer. This autotrophy might have been due to a lower availability of POM, SOM, and plankton during this season, or more probably to higher rates of zooxanthellae photosynthesis, and a higher contribution to the host's

energy demand. High zooxanthellae productivity and photosynthesis during summer or under high light conditions has indeed been recorded for the same coral species (Rodolfo-Metalpa et al. 2008a) and for other temperate anthozoans (Verde and McCloskey 2007; Bergschneider



and Muller-Parker 2008). In winter, the host  $\delta^{13}\text{C}$  signature was highly depleted compared to its summer time value or to the zooxanthellae  $\delta^{13}\text{C}$ , reaching the low values of POM, SOM, and plankton. These results give evidence that more carbon was obtained through external feeding. In a previous experimental study performed on the same temperate coral, heterotrophy was found to function as an alternative source of carbon when corals were maintained in the dark, and was sufficient to maintain calcification rates at a basal value (Hoogenboom et al. 2010). The same observation, that feeding sustains carbon requirements in winter, was also made for temperate anemones and zoanthids (Davy et al. 1996; Bergschneider and Muller-Parker 2008). Also, in winter conditions, zooxanthellae  $\delta^{13}\text{C}$  was more depleted than in summer, showing that they might have relied to a greater extent to host-derived carbon sources.

Carbon and nitrogen signature of the different samples were simultaneously plotted in Fig. 4 in order to highlight the preferentially exploited food sources of corals. Such kinds of plots are usually used in complex food chains to determine the food source of a specific predator (Riera et al. 1999, 2009), considering a mean trophic enrichment of 1‰ in  $\delta^{13}\text{C}$  (Rau et al. 1990) and of 2.5–3.4‰ in  $\delta^{15}\text{N}$  (Minagawa and Wada 1984; Gollety et al. 2010) for the predator as a result of the assimilation of food. It has to be noticed that the exact value of the isotopic fractionation of carbon and nitrogen between the coral host predator and its prey is not known, as it also is not known how zooxanthellae autotrophy is changing this signal. Nevertheless, application of the above model to *C. caespitosa* showed that the expected mean isotopic ratios for the preferentially exploited food resources (represented by an asterisk in Fig. 4) were close to the ratios of POM, SOM, and plankton in winter, and did not correspond to any of the food prey in summer. This model therefore suggests a major contribution to the host metabolism of the heterotrophic food sources in winter, and of the zooxanthellae in summer. These conclusions are corroborated by the results obtained using the model of Phillips and Gregg (2003), which calculated a contribution of the zooxanthellae to the host metabolism of 80–100% in summer and 0% to 20% in winter.

In conclusion, this is one of the first studies investigating, under natural conditions, the importance of auto- and heterotrophy in a temperate coral. In winter, the utilization of external food sources enables the symbiosis to persist when temperature and light conditions are unfavorable. Heterotrophy also helps in keeping a basic metabolism and some calcification when autotrophic input is not sufficient, as experimentally demonstrated in a previous study (Hoogenboom et al. 2010). Even if the photosynthetic contribution to the carbon and nitrogen budget of the coral is less important than heterotrophy during winter, there might be some aspects of the symbiosis, not measured in this study, that are beneficial to the host. Conversely, in summer, a larger autotrophic contribution and some heterotrophic feeding may help corals to increase their rates of calcification and build tissue reserves. Future work should focus on measuring the rates of photosynthesis during an annual

cycle, as well as the amount and nature of metabolites translocated from the zooxanthellae to the host.

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