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# Nutritional ecology of temperate octocorals in a warming ocean

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Global warming of the oceans poses a double threat to benthic organisms as they reach their thermal limits and starve to death due to declines in plankton abundance during heat waves. Under these circumstances, dissolved nutrients become an important alternative food source, especially in temperate regions where they can reach high concentrations. Although octocorals play a central role in temperate Marine Animal Forests and benthic-pelagic coupling, our knowledge of their feeding ecology is still limited. We used <sup>13</sup>C and <sup>15</sup>N isotope labeling to investigate feeding rates on dissolved inorganic (carbon, ammonium, nitrate) and organic (urea, amino acids) nutrients of the two most common Mediterranean gorgonians, the mixotrophic species *Eunicella singularis* and the heterotrophic species *Paramuricea clavata*. We also measured the uptake of natural dissolved organic matter (DOM) provided at different concentrations. Measurements were conducted at 17°C and 24°C to anticipate the effects of ocean warming. Our findings indicate that gorgonians exhibit notably low uptake rates of dissolved inorganic and organic nutrients, both at 17°C and 24°C. At 24°C, gorgonians experienced heat stress, as evidenced by elevated respiration rates, the loss of symbionts in *E. singularis*, and decreased lipid reserves in *P. clavata*. However, we did not observe a significant increase in the uptake rates of dissolved inorganic or organic nutrients, except for dissolved organic carbon in *E. singularis*. This study provides valuable insight into the nutritional requirements of temperate octocorals. The results demonstrate that dissolved nutrients, in particular nitrogen, do not play a substantial role in the diet of gorgonians, suggesting that these organisms primarily rely on the capture of plankton and detrital material for their nitrogen requirements. Therefore, temperate octocorals may be at high risk of severe starvation in the future.

## KEYWORDS

carbon, dissolved nutrients, gorgonians, heat stress, heterotrophy, marine animal forest, nitrogen, symbiosis

## 1 Introduction

Carbon (C) and nitrogen (N) elements are essential to any biological system, as they enter into the composition of important cellular components such as proteins, genetic material or enzymes. In various living organisms, their metabolisms are therefore closely linked, and an excess or shortage of one element strongly affects the metabolism of the other (Zhang et al., 2018). In the oceans, N is often a limiting nutrient for animal and plant growth (Howarth, 1988). Therefore, the ability of organisms to efficiently use the various available N sources also affects their distribution and abundance in a given environment (Klais et al., 2017). To cope with N limitation, many benthic marine organisms form holobionts (*sensu* Rohwer et al., 2002), in which the animal host lives in symbiosis with a variety of microorganisms, including protists, fungi, bacteria, archaea, and viruses (Knowlton and Rohwer, 2003). Each partner makes a specific contribution to the N requirements of the holobiont. The animal host feeds heterotrophically on particulate and dissolved organic nitrogen (PON and DON respectively), found in plankton, or in the form of dissolved free amino acids and urea (Grover et al., 2006; Rix et al., 2020). Microbial symbionts such as diazotrophs can provide N to the holobiont through N fixation (Bednarz et al., 2012). Other microbes, such as photosynthetic dinoflagellates from the Symbiodiniaceae family fix dissolved inorganic carbon (DIC) and nitrogen (DIN; ammonium, nitrate) to produce organic compounds that they share with the host to meet its nutrient needs (Rädecker et al., 2015; Freeman et al., 2016). However, the contribution of each partner to the N requirements of the holobiont is species-specific and varies greatly depending on dissolved N concentrations, seawater temperature or other environmental conditions (Baker et al., 2013; Pupier et al., 2021).

Corals are one of the most widespread holobionts in coastal benthic ecosystems. They can form large marine animal forests (MAFs, *sensu* Rossi et al., 2017), such as coral reefs and coralligenous assemblages, which are probably among the most widespread ecosystems on Earth, and provide services of great economic value to hundreds of millions of people worldwide (Rossi and Rizzo, 2020). Nitrogen availability is a key factor in coral functioning and health, as it determines their ability to grow, reproduce and survive (Rädecker et al., 2015). However, the acquisition and allocation of N in coral holobionts is not well understood, especially in octocorals (Baker et al., 2011; Pupier et al., 2019a; Pupier et al., 2021), despite the fact that octocorals play an important structural and functional role in MAFs, and are key players in the benthic-pelagic energy fluxes through the uptake and release of nutrients (Gili and Coma, 1998; Schubert et al., 2016; Rossi and Rizzo, 2021). Coral feeding ecology has indeed mainly focused on the Hexacorallia subclass, which consists of stony corals (76% of studies; Santos et al., 2020), while only 22% of studies were dedicated to Octocorallia. Moreover, half of the studies were related to feeding behaviour (Santos et al., 2020), so the uptake rates and assimilation of nutrients such as dissolved C and N by Octocorallia are still very poorly understood (Baker et al., 2015; Pupier et al., 2019b; Pupier et al., 2021).

In temperate coastal waters of the Mediterranean Sea, gorgonian corals can reach very high densities in both shallow and deep waters (Gori et al., 2011a; Gori et al., 2011b), and contribute greatly to the biological and economic value of the ecosystems they form. Gorgonians (with an average size of 20–40 cm height) can indeed reach densities from 20 to more than 60 colonies m<sup>-2</sup> in some locations (Sini et al., 2015; Grinyó et al., 2016). Our understanding of their ecology, distribution patterns or reproductive strategies has increased in recent decades (Schubert et al., 2016). However, as with all Octocorallia, much less is known about their feeding strategies for dissolved organic and inorganic C and N. This lack of knowledge becomes particularly concerning in the face of climate change, which poses a serious threat to the survival of these octocorals inhabiting shallow waters (Garrabou et al., 2022). Indeed, the Mediterranean Sea has been suffering from repeated heat waves for the last decades, leading to mass mortality of gorgonians that thrive in shallow waters (Garrabou et al., 2022). In the summer of 2022, which was one of the hottest ever observed in this region, seawater temperature rose above 25°C at depths of 20–30 m, where most gorgonians thrive, reaching threshold for these organisms (Pey et al., 2013; Tignat-Perrier et al., 2022).

To fill the knowledge gaps on N requirements of temperate octocorals both under normal growth conditions and heat stress, the uptake rates of DIN and DON were measured in the two most abundant gorgonian species of the western Mediterranean: the only symbiotic species *Eunicella singularis* (Esper, 1794), and the non-symbiotic species *Paramuricea clavata* (Risso, 1826) (Weinberg, 1976). In parallel, measurements of DIC and DOC uptake rates were also carried out, to link the uptake rates of C and N. Measurements were performed under normal growth temperature (17°C) and under heat stress (24°C) (Garrabou et al., 2022). To achieve our objectives, we used the techniques of stable isotopes tracers of N and C (<sup>15</sup>N and <sup>13</sup>C respectively), as well as an approach based on the measurement of nutrient depletion in seawater. Our study addresses several questions. First, to what extent Mediterranean gorgonians rely on dissolved nutrients? Second, how does the existence of a symbiosis with photosynthetic dinoflagellates influence the nutrition in temperate gorgonians? Third, how do their nutritional capacities change with heat stress? We hypothesized that (1) both species can assimilate dissolved C and N; (2) the symbiotic relationship in *E. singularis* allows for greater dissolved N uptake through the uptake of DIN. Finally, we hypothesized that (3) heat stress had a different effect on the symbiotic than on the non-symbiotic gorgonian. By assessing how gorgonians take up dissolved organic and inorganic nutrients in different seawater temperature conditions, this comparative study will provide a better understanding of the nutritional factors, which regulate the distribution of symbiotic *versus* non-symbiotic gorgonians in the Mediterranean Sea. In addition, understanding the nutritional requirements of these gorgonians can help inform management practices aimed at improving their health and resilience, particularly in the face of stressors such as climate change and ocean acidification.

## 2 Materials and methods

### 2.1 Gorgonian collection and experimental setup

Experiments were carried out with two Mediterranean gorgonians: the symbiotic species *Eunicella singularis* (Esper, 1794), which is mixotroph, and the asymbiotic species *Paramuricea clavata* (Risso, 1826), which is only heterotroph. Ten colonies of each species were collected in May 2022 in Marseille, France at 30 meters depth by SCUBA diving and transported directly to the aquarium facilities of the laboratory where experiments were performed. For each species, a total of 100 nubbins (8 cm) were generated (10 nubbins per colony) and evenly distributed among ten 20-L aquaria for acclimation (10 nubbins from different colonies per aquarium). The aquaria were continuously supplied with Mediterranean seawater, at a flow rate of 20 L h<sup>-1</sup>. Seawater temperature was kept constant at 17 ± 0.1°C using heaters (Visi-Therm VTX 300 W, Aquarium Systems, France) connected to temperature controllers (Ponsel, France). The gorgonians received a photosynthetically active radiation (PAR) of 120 μmol photons m<sup>-2</sup> s<sup>-1</sup> (on a 12:12 photoperiod) provided by HQI lamps (Tiger 230V/50Hz 250 W, Faerber, Italy). The chosen PAR corresponded to the summer average irradiance received by the gorgonians in their natural environment (Gori et al., 2012). During the acclimation phase (3 weeks), nubbins were fed a mixture of live *Artemia salina* nauplii and frozen plankton five times per week. After this acclimation phase, half of the aquaria were subjected to heat stress, with temperature increasing at a rate of 0.3°C per day to reach 24°C in 3 weeks, while the other aquaria remained at 17°C as controls. Therefore, each gorgonian colony had nubbins exposed to both temperatures. Feeding was then stopped in order to recreate natural summer conditions, during which the thermocline depletes the upper water column with nutrients and particulate food.

Measurements started two weeks after the temperature of 24°C was reached in the aquaria, when visible signs of heat stress (tissue loss) could be observed in few nubbins of *P. clavata* (n=2). These nubbins were discarded from measurements, which were therefore performed on the remaining ones. Six nubbins per species and temperature, coming from 6 different colonies and different aquaria, were directly frozen at -20°C for protein and lipid content measurements. The other nubbins were used to assess their feeding strategy on C and N as described in detail in the following chapters. For C, two sources were considered: i) the dissolved organic carbon (DOC) acquired heterotrophically through the uptake of natural dissolved organic matter (DOM), and ii) the dissolved inorganic carbon (DIC) acquired through photosynthesis. For N, several sources were considered: i) dissolved organic nitrogen (DON) acquired from a natural DOM mixture or specifically from urea and dissolved free amino acids (DFAAs), and ii) dissolved inorganic nitrogen (DIN) acquired specifically from ammonium and nitrate.

### 2.2 Acquisition of DIC assessed through oxygen fluxes

Measurements of net photosynthesis ( $P_n$ ) for *E. singularis* and respiration rates (R) for both species were performed in the dark and at 120 μmol photons m<sup>-2</sup> s<sup>-1</sup>, respectively, on 6 nubbins per species and temperature, from 6 different colonies and aquaria. Nubbins were individually placed in 50 mL transparent Plexiglas chambers, hermetically sealed and filled with 0.45-μm filtered seawater (FSW). Seawater mixing was ensured with magnetic stirrers. The chambers were equipped with a Unisense optode (oxygen-sensitive mini sensor) connected to the Oxy-4 software (Chanel fiber-optic oxygen-meter, Presens®, Regensburg, Germany). The optodes were calibrated before each measurement using nitrogen-saturated and air-saturated seawater for 0 and 100% oxygen, respectively.  $P_n$  and R were then estimated using a regression of the oxygen data against time, and gross photosynthesis ( $P_g$ ) was calculated as the sum of the absolute values of R and  $P_n$ . Finally, we assumed a mole-to-mole relationship from CO<sub>2</sub> consumed to O<sub>2</sub> produced during photosynthesis, and inversely from O<sub>2</sub> consumed to CO<sub>2</sub> produced during respiration. For *E. singularis*, the contribution of photosynthesis to the respiratory needs of the holobiont (P:R) was determined as  $P:R=(P_g \times 12)/(R \times 24)$ . We considered 12 h of C acquisition from photosynthesis and 24 h of C consumed through the respiration. Samples were then frozen at -20°C for the latter determination of their chlorophyll content and symbiont density (*E. singularis*), and ash-free dry weight (AFDW; both species) as described below.

### 2.3 Acquisition of DOC and DON assessed through a mixture of natural DOM

To evaluate the acquisition of DOC and DON, a new set of incubations was carried out with the two species and for each temperature conditions (17°C and 24°C), using 3 different concentrations of DOM: ambient seawater DOM concentration (80 μmol C L<sup>-1</sup> + 10 μmol N L<sup>-1</sup>, [DOM<sub>nat</sub>]); a medium DOM enrichment (3 x [DOM<sub>nat</sub>]); and a high DOM enrichment (6 x [DOM<sub>nat</sub>]). Each combination of temperature and DOM concentration was performed at 120 μmol photons<sup>-1</sup> m<sup>-2</sup> s<sup>-1</sup>. DOM used for the enrichment was obtained from frozen microplankton (Ocean nutrition<sup>TM</sup>, Switzerland) homogenized at 20,500 rpm, sonicated and centrifuged. The supernatant was then filtered through a 0.20-μm filter before being stored at -20°C.

At the beginning of the incubation, nubbins were individually transferred into 400 mL beakers placed beforehand in the aquaria. This prevented gorgonians to stress by being in contact with air. The beakers were then removed from the aquaria and put on a stirring plate with a magnetic stirrer at the correct temperature and light conditions. Nubbins were incubated for 7 h at the three DOM concentrations. In addition, each series of incubations included three control beakers (without nubbins), whose average DOM fluxes was subtracted from the fluxes calculated in the other beakers, to account for changes related to microbial activity and C and N exchanges at the air-water interface.

At the beginning and end of the incubation, 10 mL seawater samples were collected in duplicate, filtered on a 0.45- $\mu\text{m}$  sterile syringe filter and stored at  $-20^{\circ}\text{C}$  to determine the DIN concentrations in the beakers using a Xenius spectrophotometer (SAFAS<sup>®</sup>, Monaco) (Table S1; full description in the Supplementary material). In addition, 30 mL of seawater were collected in duplicate using a sterile syringe, then transferred to a pre-burnt (4 h,  $480^{\circ}\text{C}$ ) Pyrex tube, and stored at  $-20^{\circ}\text{C}$  for the determination of DOC and dissolved nitrogen (DN) concentrations using a TOC-L analyzer (Shimadzu, Japan) (full description in the Supplementary Material). At the end of the incubations, nubbins were frozen and stored at  $-20^{\circ}\text{C}$  for data normalization.

DON concentrations were calculated from the difference between DN and DIN concentrations. DOC and DON fluxes were then assessed through the difference in DOC and DON concentrations measured at the beginning and end of the incubation, and normalized to the AFDW of each nubbin. Negative fluxes indicated a net uptake of DOC or/and DON, whereas positive fluxes indicated a net release by the gorgonians. Finally, the contribution of DOC heterotrophy to the animal respiration ( $\text{CHAR}_{\text{DOC}}$ , %), was calculated according to:

$$\text{CHAR}_{\text{DOC}} = \frac{\text{DOC flux in the light} + \text{DOC flux in the dark}}{R \times 24} \times 100$$

where R is the respiration rate previously calculated (see 2.2).

## 2.4 Acquisition of DIC and DIN using the isotopic tracer techniques $^{13}\text{C}$ and $^{15}\text{N}$

$^{15}\text{N}$  stable isotope tracers were used to assess the uptake rates of the different DIN (ammonium and nitrate,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  respectively) and DON (urea, DFAAs) sources by gorgonians, according to Grover et al (2002; 2003). Therefore,  $^{15}\text{NH}_4\text{Cl}$ ,  $\text{Na}^{15}\text{NO}_3$ ,  $^{15}\text{N}$ -urea (all 98 atomic %  $^{15}\text{N}$ , Sigma-Aldrich, USA) or  $^{15}\text{N}$ -DFAAs (98 atomic %  $^{15}\text{N}$ , Algal Amino Acid mixture, CortecNet, France) were used at a final concentration of 2  $\mu\text{M}$  (note that since the urea molecule is composed of two N atoms, the enrichment concentration of N-urea is 4  $\mu\text{M}$ ). This concentration was chosen as it is in the same order of magnitude as the *in-situ* concentrations measured in the Mediterranean Sea (Doval et al., 1999; Ribes et al., 1999b; Reinthaler et al., 2008; Segura-Noguera et al., 2016). In addition,  $^{13}\text{C}$ -bicarbonate ( $\text{NaH}^{13}\text{CO}_3$ ) tracer was also used to estimate the rates at which DIC was fixed by *E. singularis* and *P. clavata* nubbins. For this purpose, 0.5 mM of  $\text{NaH}^{13}\text{CO}_3$  (98 atomic %  $^{13}\text{C}$ , Sigma-Aldrich, USA) was added to the beakers enriched with  $^{15}\text{NH}_4^+$  or  $^{15}\text{NO}_3^-$ .

For each N source, 6 nubbins per species and temperature, coming from 6 different colonies, were incubated under magnetic stirring for 7 h in individual beakers placed in a thermostatic bath and containing 220 mL of 0.45- $\mu\text{m}$  FSW enriched with the  $^{15}\text{N}$  and  $^{13}\text{C}$  tracers. Incubations were carried out at  $17^{\circ}\text{C}$  and  $24^{\circ}\text{C}$ , and at 120  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . At the end of the incubation, nubbins were rinsed in non-enriched FSW and frozen at  $-20^{\circ}\text{C}$  until further analysis. To determine the natural isotopic abundance of  $^{15}\text{N}$  and

$^{13}\text{C}$  of the gorgonians, three control nubbins of each species were incubated in 220 mL of 0.45- $\mu\text{m}$  FSW in the same conditions.

In order to distinguish uptake of  $^{15}\text{N}$  by the gorgonian host from uptake by the mucus-associated microbes, an additional experiment was performed at the control temperature. Ten nubbins of each species were incubated for 10 h in individual beakers containing 200 mL of 0.45- $\mu\text{m}$  FSW under magnetic stirring, to let them release mucus. Three control beakers filled with 0.45- $\mu\text{m}$  FSW without gorgonians were also added. After 10 h, nubbins were removed from the beakers leaving mucus behind. Four beakers per species were used to measure the natural isotopic abundance of the mucus. The remaining 6 beakers per species, as well as the 3 control beakers, were enriched with  $^{15}\text{NH}_4\text{Cl}$  to a final concentration of 2  $\mu\text{M}$  and incubated for 7 h. The mucus from each beaker, as well as the seawater in control beakers, were then filtered onto glass microfiber filters grade GF/F (Whatman<sup>®</sup>, United Kingdom) pre-burned for 4 h at  $480^{\circ}\text{C}$ . Filters were frozen at  $-20^{\circ}\text{C}$  before being freeze-dried. The control beakers were used to estimate if some  $^{15}\text{N}$  was taken up by the few particles which could have remained in the filtered seawater. The nubbins used were also frozen and stored at  $-20^{\circ}\text{C}$  for data normalization.

## 2.5 Sample processing

The surface area of all gorgonian samples was first determined using a caliper to measure the length and width of each gorgonian nubbin and considering that each nubbin was a cylinder. The samples were then freeze-dried, weighted to determine their dry weight (DW), and the tissue was separated from the axial skeleton with a scalpel. The tissue was then ground to a powder in a mortar, a fraction was taken for the AFDW determination according to Pupier et al. (2018), while the remaining powder was homogenized with 10 mL of Milli-Q water and centrifuged at 20 x g for 1 minute (adapted from Shirur et al., 2014). This gentle centrifugation allowed the separation of the soft tissue from the sclerites, which remained in the pellet. For *P. clavata* nubbins, the supernatant, considered as the host fraction, was immediately collected and freeze-dried for the corresponding analysis. For *E. singularis* nubbins, the supernatant was either directly freeze-dried (for protein and lipid content measurement) or was centrifuged at 1750 x g for 2 min to separate the soft tissue (supernatant; host fraction) from the symbionts (pellet; washed twice with Milli-Q water) for the other measurements. Both fractions were then freeze-dried, and different measurements were performed according to the samples considered.

### 2.5.1 Symbiont density and chlorophyll content of *E. singularis*

Symbiont density and total chlorophyll content of *E. singularis* were measured from nubbins used for the measurements of photosynthesis and respiration. For this purpose, from each nubbin, symbiont pellets were resuspended in 6 mL FSW. A subsample of 500  $\mu\text{L}$  was used for the determination of symbiont density by microscope counting via 8 replicate haemocytometer



counts (Neubauer haemocytometer, Marienfeld, Germany). Another subsample of 5 mL was taken for the determination of the total chlorophyll (a + c<sub>2</sub>) quantification. The subsample was centrifuged at 8,000 x g for 10 min at 4°C. The supernatant was removed, and the pellet was washed twice with Milli-Q water, resuspended into 5 mL of acetone (>99%, Sigma-Alrich, USA) and stored at 4°C for 24 h. After a centrifugation at 11,000 x g for 15 min at 4°C, absorbances at 616 nm and 663 nm were measured using a Xenius spectrophotometer (SAFAS<sup>®</sup>, Monaco) and the total chlorophyll content was assessed according to Jeffrey and Humphrey (1975).

### 2.5.2 Protein and lipid content

Measurements of protein and lipid content were carried out on 6 additional nubbins per species and temperature. After elimination of the spicules, 5 mg of freeze-dried tissue was resuspended in 200 µL of NaOH 1M and incubated in a dry bath for 30 min at 90°C. Protein content was then quantified using a BC assay kit (Interchim, France) (Smith et al., 1985) and protein standards (from 0 to 2000 µg mL<sup>-1</sup>) were prepared using bovine serum albumin (Interchim, France). The absorbance was measured at 562 nm by a Xenius spectrophotometer (SAFAS<sup>®</sup>, Monaco).

For total lipid content determination, 10 mg of freeze-dried tissue was added to 1.5 mL of chloroform/methanol (2v:1v) in a Pyrex tube and put on an orbital shaker (400 rpm) for 20 min. After a 5 min centrifugation at 1050 x g at 4°C, 150 µL of the supernatant were sampled and placed on a quartz microplate. Lipid standards (from 0 to 160 µg) were prepared using a cholesterol stock solution. Then lipid content was assessed according to Cheng et al. (2011). Briefly, the microplate was incubated for 10 min in a dry bath at 90°C for solvent evaporation, before adding 100 µL of sulfuric acid and incubating for 20 min at 90°C. The background absorbance at 540 nm was then measured using a Xenius spectrophotometer (SAFAS<sup>®</sup>, Monaco). Then, 50 µL of vanillin-phosphoric acid reagent was added for color developing and the absorbance at 540 nm was measured again. Finally, the background absorbance was subtracted from the second measured absorbance to avoid bias from the color developed by sulfuric acid.

### 2.5.3 Percentage of enrichment in <sup>13</sup>C and <sup>15</sup>N as well as the total carbon and nitrogen content of the symbionts and host compartments

These data were acquired from nubbins used in the <sup>13</sup>C and <sup>15</sup>N incubations. For this purpose, tin capsules containing approximately 600 µg of freeze-dried material, were prepared and analyzed using an isotopic ratio mass spectrometer (IRMS Delta Plus, ThermoFisher Scientific, Bremen, Germany) coupled to a C/N analyzer (Flash EA, ThermoFisher Scientific). Phosphorus (P) content in symbionts and host fractions was also determined using a Xenius spectrophotometer (SAFAS<sup>®</sup>, Monaco) (see detailed protocol in Supplementary Material). For the experiment regarding <sup>15</sup>N enrichment of the mucus, the whole filter was analyzed in a tin capsule using an Integra II mass spectrometer (Sercon<sup>®</sup>, United Kingdom). Uptake rates of each form of DIN (N-NH<sub>4</sub><sup>+</sup>, N-NO<sub>3</sub><sup>-</sup>) and DON (N-urea and N-DFAAs) were calculated according to Grover et al (2002; 2003). (see Table 1 for details):

$$\rho = \frac{N_{mes} - N_{nat}}{(N_{enr} - N_{mes}) \times T_{inc} \times M_{tot}} \times M_s \times M_N \times 10^3$$

$$\text{where } N_{enr} = \frac{(N_{exp} \times N_{comp}) + (N_{nat} \times N_{init})}{N_{exp} + N_{init}}$$

The same formulas were adapted and used for the uptake rates of <sup>13</sup>C - DIC (see Supplementary material 1.2.2, Table S6).

## 2.6 Statistical analysis

Analyses were performed using R software (R Foundation for Statistical Computing, version 4.0.2). All data were expressed as mean ± standard deviation. A preliminary identification of outliers was performed by Grubb's test, and values were excluded if the p-value was significant. The assumptions of normality and homoscedasticity of variances were verified by Shapiro's and Bartlett's test as well as graphical analysis of the residuals. When they were satisfied, a two-way analysis of variance (ANOVA) was performed to test the effect of species and temperature. When there were significant differences between treatments, Tukey's HSD test was performed. Otherwise, a Kruskal-Wallis test was used, looking separately at the effect of temperature and species. When the effect was significant, Dunn's test was performed. For the uptake rates of C, N using the stable isotope tracers technique, and the cellular CNP content, the effect of the fraction (host or symbiont) was also considered. Regarding the uptake rates of <sup>15</sup>N, the potential effect of the N source (NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, urea or DFAAs) was considered as well. Similarly, the separate effect of DOC and DON concentration on DOC and DON fluxes was also looked at. Finally, a Student T-test was performed to test the effect of temperature on *E. singularis* symbiosis-related parameters (P<sub>n</sub>, P<sub>g</sub>, symbiont density, total chlorophyll content and P:R). Differences were considered significant when p < 0.05. The data were normalized both by the nubbin's AFDW and surface (cm<sup>2</sup>) and the statistical

TABLE 1 Abbreviations and units of the parameters used for uptake rates calculations.

Symbol	Definition	Units
ρ	Uptake rates	nmol N g <sup>-1</sup> AFDW h <sup>-1</sup>
N <sub>mes</sub>	<sup>15</sup> N measured in the sample	%
N <sub>nat</sub>	Natural <sup>15</sup> N abundance in control nubbins	%
N <sub>enr</sub>	<sup>15</sup> N enrichment of the incubation medium	%
T <sub>inc</sub>	Incubation time (here 7 h)	h
M <sub>tot</sub>	Total AFDW of nubbin	mg
M <sub>s</sub>	Total mass of the sample	mg
M <sub>N</sub>	Particulate nitrogen concentration	µmol mg <sup>-1</sup>
N <sub>exp</sub>	Concentration added for the incubation (here 2 µM)	µM
N <sub>comp</sub>	<sup>15</sup> N of product added (here 98%)	%
N <sub>init</sub>	Initial concentration of either NH <sub>4</sub> <sup>+</sup> , NO <sub>3</sub> <sup>-</sup> , urea or DFAAs in beaker	µM

results obtained were essentially the same. Only the data normalized by AFDW will be used to discuss the results, and the data normalized by surface area are available as [Supplementary Information](#) (Figure S1; Tables S2-4).

### 3 Results

#### 3.1 Effect of heat stress on physiological and tissue parameters.

The results of the statistical tests are reported in the [Supplementary Material](#) in [Table S7](#) for symbiotic-related parameters of *E. singularis* and in [Table S8](#) for physiological and tissue parameters.

In *E. singularis*, heat stress induced a 40% decrease in symbiont density (Figure 1A;  $p < 0.05$ ), although there was no significant

decrease in chlorophyll content (Figure S2). As a result, net photosynthetic rates decreased by nearly 20% (Figure 1B;  $p < 0.05$ ), without any change in the gross photosynthesis ( $P_g$ ; Figure 1C), but decreased P:R ratios by 19% (Figure 1D,  $p < 0.05$ ). Therefore, photosynthesis could not sustain respiration at 24°C ( $P:R < 1$ ). Heat stress did not change the lipid or protein content (Figures 2A, B), but increased respiration rates by 47% (Figure 2C;  $p < 0.01$ ). It also induced a 14, 21 and 30% decrease in C, N and P content, in the symbiont fraction ( $p < 0.01$ ,  $p < 0.01$  and  $p < 0.01$ , respectively), as well as a 20% decrease in both N and P content in the host fraction (Figures 3A-C;  $p < 0.0001$  and  $p < 0.01$ , respectively). As a result, C:N and C:P ratios significantly increased in both fractions ( $p < 0.01$  and  $p < 0.0001$ , respectively), with also an increase of the N:P ratio in the host fraction (Figures 3D-F;  $p < 0.05$ ).

In *P. clavata*, heat stress increased respiration rates by 73% ( $p < 0.01$ ), along with a 30% decrease in lipid reserves (Figures 2A, B;

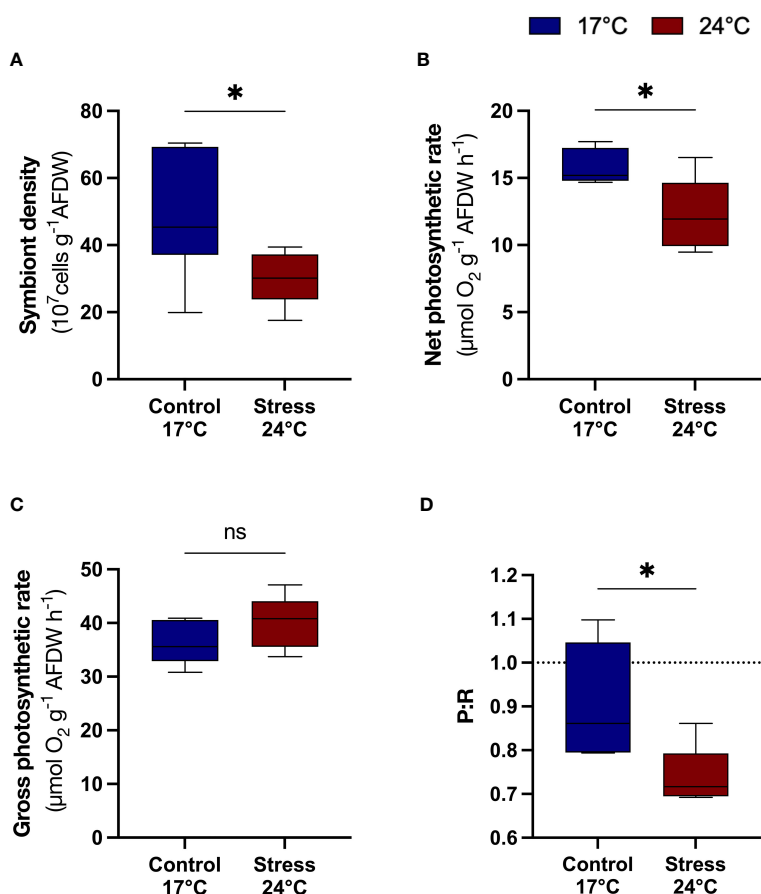


FIGURE 1

Symbiotic-related physiological parameters of *E. singularis* at 17°C (blue plots) and 24°C (red plots). The boxplot represent symbiont density ( $10^7$  cells  $g^{-1}$  AFDW) (A), net (B) and gross (C) photosynthesis rates ( $\mu\text{mol O}_2 g^{-1}$  AFDW  $h^{-1}$ ) and P:R (D) ( $n = 6$ ). AFDW, ash-free dry weight; P:R, contribution of photosynthesis to the holobiont respiratory requirements. Significant differences are displayed with asterisks ( $p$  value  $< 0.05$ ).

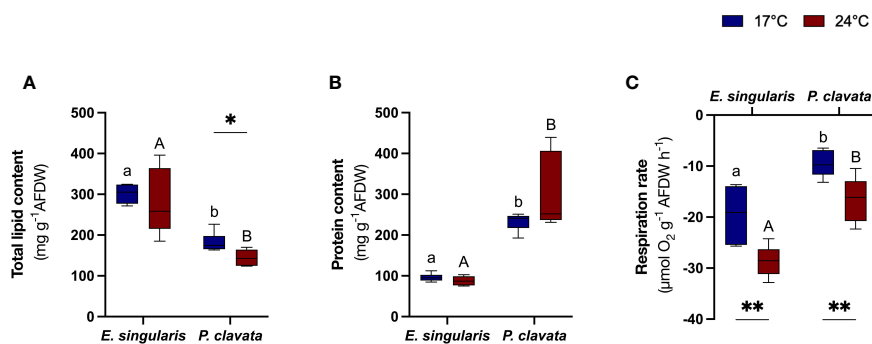


FIGURE 2

Gorgonian physiological parameters at 17°C (blue plots) and 24°C (red plots). The boxplots represent total lipid (A) and protein (B) content ( $\text{mg g}^{-1}$  AFDW), and respiration rates ( $\mu\text{mol O}_2 \text{g}^{-1}$  AFDW  $\text{h}^{-1}$ ) (C) ( $n = 6$ ). AFDW, ash-free dry weight. Significant differences between temperatures for each species are displayed with asterisks ( $p$  value < 0.05). Significant differences between species are distinguished with letters, for each temperature separately.

$p < 0.05$ ). These parameters were however significantly lower in *P. clavata* than in *E. singularis* ( $p < 0.01$ ). On the contrary, its protein content was two times higher than *E. singularis* ( $p < 0.001$ ). Regarding tissue content, heat stress only impacted the C content of *P. clavata* ( $p < 0.01$ ), which induced a decrease in the C:N ratio ( $p < 0.01$ ). On the contrary, the N:P ratio increased with heat stress ( $p < 0.001$ ).

## 3.2 Uptake of DOC and DIC

The results of the statistical tests are reported in the [Supplementary Material](#) in [Table S9](#) for DOC fluxes, in [Table S10](#) for  $\text{CHAR}_{\text{DOC}}$  and in [Table S11](#) for DIC uptake.

At 17°C, DOC fluxes were positive for ambient DOM conditions ( $[\text{DOM}_{\text{nat}}]$ ) for both species, suggesting a net release of DOC ([Figure 4A](#)). However, under DOM enrichment, there was a significant DOC uptake for both species ( $p < 0.05$ ). Similarly,  $\text{CHAR}_{\text{DOC}}$  significantly increased with DOM enrichment for both species ([Table 2](#);  $p < 0.05$  for *E. singularis* and  $p < 0.01$  for *P. clavata*), although DOM could not supply 100% of the respiratory needs of both species in any condition. Heat stress increased DOC uptake rates of *E. singularis*, at both  $3 \times [\text{DOM}_{\text{nat}}]$  and  $6 \times [\text{DOM}_{\text{nat}}]$  ( $p < 0.01$ ), leading to a significant increase of its  $\text{CHAR}_{\text{DOC}}$  above 100% of the respiratory needs ( $p < 0.05$ ). On the contrary, heat stress increased DOC release rates of *P. clavata* at  $[\text{DOM}_{\text{nat}}]$  ( $p < 0.01$ ) and had no significant impact at higher DOM concentrations. Finally, under heat stress and DOM enrichment, DOC uptake rates of *E. singularis* were significantly higher than those of *P. clavata* ( $p < 0.05$ ).

In contrast to *P. clavata*, *E. singularis* could also use autotrophic C through the fixation of DIC by the symbionts ([Figure 4B](#)). Measurements performed with  $^{13}\text{C}$ -bicarbonate showed that the C taken up by *E. singularis* after 7 h incubation was mainly found in the symbionts (80% assimilated from a total of  $13.2 \pm 3.4 \mu\text{mol C g}^{-1}$  AFDW  $\text{h}^{-1}$ ; [Figure S3A](#)). Under heat stress, DIC uptake rates of the holobiont remained constant ([Figure S3B](#)) but the contribution of each fraction was different. Indeed, heat stress increased the DIC uptake rates by the host fraction (36% of total assimilated C;  $p < 0.01$ ), while uptake by the symbiont fraction decreased to 64% ( $p < 0.05$ ).

## 3.3 Uptake of DON and DIN

The results of the statistical tests are reported in the [Supplementary Material](#) in [Table S9](#) for DON fluxes and in [Table S11](#) for N uptake using labelled  $^{15}\text{N}$ -compound.

Measurements of DON fluxes showed that, at 17°C, there was no DON net uptake by the gorgonians, even under high enrichment ([Figure 5A](#)), suggesting that excretion of DON by the gorgonians exceeded the DON uptake. Indeed, there was a constant net release of DON by *E. singularis*, independently of DOM enrichments, while DOM enrichment slightly reduced the release of DON by *P. clavata* ( $p < 0.01$ ). Heat stress increased DON release for both species at  $[\text{DOM}_{\text{nat}}]$  ( $p < 0.05$ ), as well as at  $3 \times [\text{DOM}_{\text{nat}}]$  for *P. clavata* ( $p < 0.01$ ).

Experiments with labelled  $^{15}\text{N}$ -compounds showed a significant uptake of all sources of dissolved N by both gorgonian species, with a higher uptake of  $\text{N-NH}_4^+$  and N-DFAAs compared to the other forms ([Figure 5B](#)). Note that, for the urea source, a given N uptake rate implies an uptake of urea molecules twice as low. In *E. singularis*, symbionts contributed more than 50% to  $\text{N-NH}_4^+$  uptake and 80% to N-DFAAs uptake ([Figure 5B](#); [S4A](#)). On the contrary, they assimilated less  $\text{N-NO}_3^-$  and N-urea than the host ( $p < 0.01$  and  $p < 0.05$ , respectively). At the holobiont level, there was no major difference in total dissolved N uptake between species. While *E. singularis* holobiont assimilated twice more  $\text{N-NH}_4^+$  than *P. clavata* (ca  $120 \text{ nmol N g}^{-1}$  AFDW  $\text{h}^{-1}$ ; [Figure S4B](#);  $p < 0.01$ ), it assimilated less DON ( $p < 0.01$ ). The additional investigation of N uptake by isolated mucus of gorgonians showed that only 7% to 8% of  $\text{N-NH}_4^+$  uptake by the holobiont (for *P. clavata* and *E. singularis*, respectively; [Table S5](#)) was carried out by the mucus-associated microbes.

Under heat stress,  $\text{N-NH}_4^+$  uptake rates decreased in the symbionts of *E. singularis*, while  $\text{N-NO}_3^-$  uptake rates increased ([Figure 5B](#);  $p < 0.05$  and  $p < 0.05$ , respectively). The host fraction, however, increased its N-DFAAs uptake, thus reducing the symbionts relative contribution to total N-DFAAs uptake ([Figure 5B](#) and [S4A](#);  $p < 0.05$  and  $p < 0.05$ , respectively). At the holobiont level, *E. singularis* assimilated more N-DFAAs and  $\text{N-NO}_3^-$  than under normal temperature ([Figure S4B](#);  $p < 0.05$  and  $p < 0.05$ ).



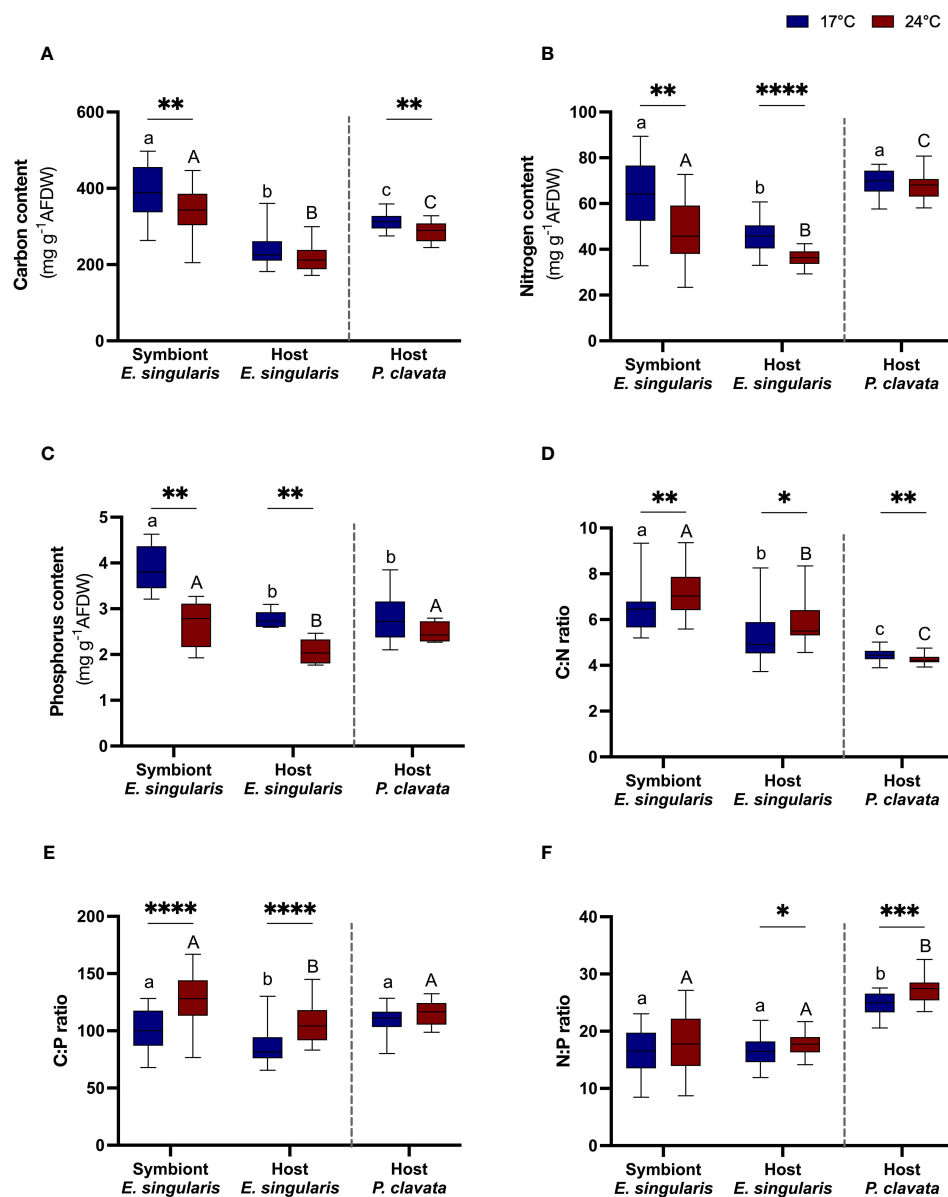


FIGURE 3

Gorgonian tissue parameters at 17°C (blue plots) and 24°C (red plots). The boxplot represent carbon (A), nitrogen (B), phosphorus (C) content in tissue (mg g<sup>-1</sup> AFDW), and C:N (D), C:P (E) N:P (F) ratio in tissue (n = 6). AFDW, ash-free dry weight. Significant differences between temperatures for each species are displayed with asterisks (p value < 0.05). Significant differences between species and/or fractions are distinguished with letters, for each temperature separately.

0.01, respectively), while *P. clavata* stopped assimilating N-urea and N-NO<sub>3</sub><sup>-</sup> (p < 0.01 and p < 0.05, respectively). However, *E. singularis* total N-DFAAs uptake was still lower than for *P. clavata*, along with a higher N-NH<sub>4</sub><sup>+</sup> uptake (p < 0.05 and p < 0.01, respectively).

## 4 Discussion

Understanding the nutritional requirements of Mediterranean gorgonians, which are essential in forming large Marine Animal Forests (Rossi et al., 2017), is critical for developing effective management practices that can improve gorgonian health and

resilience, especially in the face of climate change stressors. Despite high concentrations of dissolved inorganic and organic nitrogen (DIN, DON) in the mesotrophic Mediterranean Sea (Coste et al., 1988; Bethoux et al., 1992), the extent to which gorgonians rely on these nutrients is unknown. The main results of the study, summarized in Table 3 and discussed below, indicate that temperate gorgonians can assimilate all forms of DIN and DON, but to a much lesser extent than tropical octocorals and scleractinian corals, despite being exposed to higher nutrient concentrations. As a result, dissolved N does not contribute significantly to their diet. In addition, at natural concentrations, they do not rely on dissolved organic matter (DOM) to feed, but they can use it when concentrations of DOM are increased.

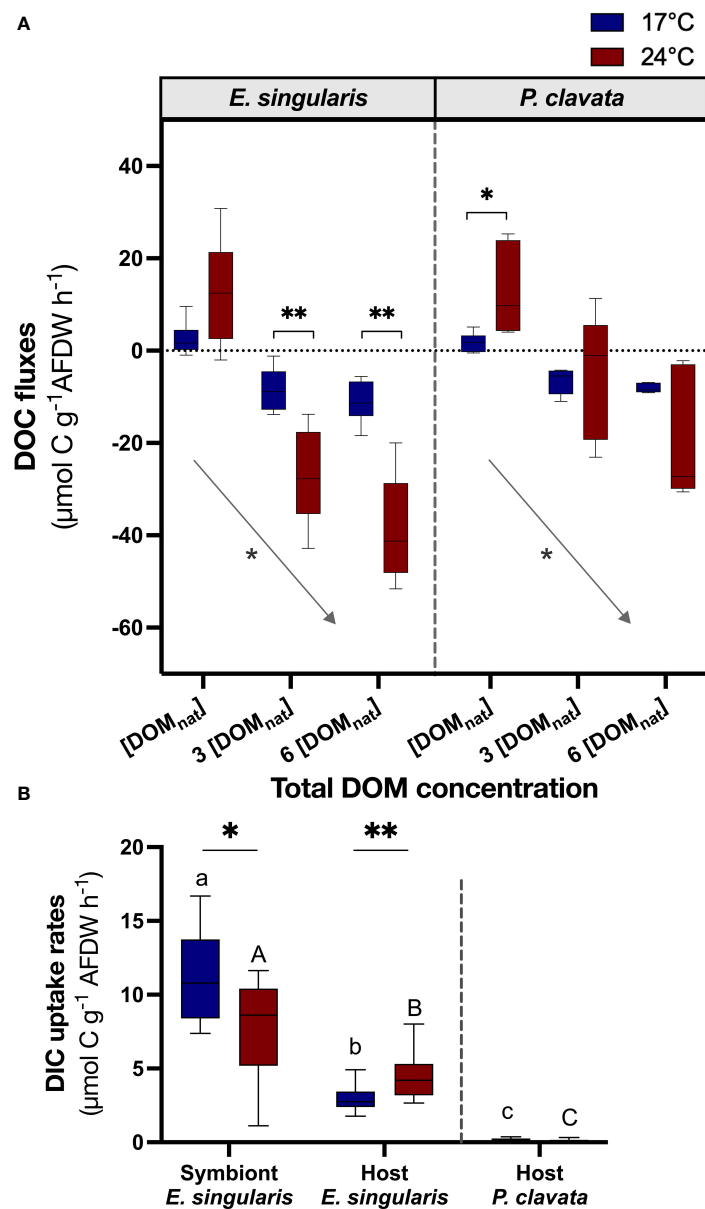


FIGURE 4

Assimilation of dissolved carbon by gorgonians at 17°C (blue bars) and 24°C (red bars). The boxplots represent the DOC fluxes ( $\mu\text{mol C g}^{-1} \text{AFDW h}^{-1}$ ) using a mixture of natural DOM at different concentrations (A) ( $n = 6$ ) and DIC uptake rates ( $\mu\text{mol C g}^{-1} \text{AFDW h}^{-1}$ ) of each fraction of the gorgonians, using labelled  $^{13}\text{C}$  (B) ( $n = 12$ ). AFDW, ash-free dry weight; DOC, dissolved organic carbon; DIC, dissolved inorganic carbon; DOM, dissolved organic matter. For the graph (A), a negative flux implies an uptake of DOC, whereas a positive flux implies a release of DOC. Significant differences in DOC fluxes between the lowest and highest DOC concentration (A: [DOM<sub>nat</sub>] and 6 x [DOM<sub>nat</sub>], respectively) are displayed with an arrow. For the graph (B), significant differences in DIC uptake rates between fractions are distinguished with letters, for each temperature separately. For both graphs, significant differences between temperatures are displayed with asterisks ( $p$  value < 0.05).

The asymbiotic gorgonian *Paramuricea clavata* was also able to take up relatively high amounts of ammonium ( $\text{NH}_4^+$ ), despite the absence of Symbiodiniaceae, which are usually the main drivers of  $\text{NH}_4^+$  uptake in many coral species (Muscatine et al., 1979; Grover et al., 2002; Grover et al., 2003; Pernice et al., 2012). Finally, an increase in temperature to 24°C for 2 weeks significantly affected the health of the gorgonians and their ability to take up nutrients.

The two gorgonian species studied, the asymbiotic *P. clavata* and the symbiotic *E. singularis*, thrive in mesotrophic waters with high

concentrations of DON (up to 14  $\mu\text{M}$ , Morganti et al., 2017) and DIN (1–3  $\mu\text{M}$ ); that vary depending on the season (Ribes et al., 1999b; Aminot and K erouel, 2004; Segura-Noguera et al., 2016). These values are significantly higher than those typically found in reef waters (DON: 3.8–5.6  $\mu\text{M}$  (Tanaka et al., 2011), DIN: 1–2  $\mu\text{M}$ ). Despite being able to take up DIN, mainly in the form of  $\text{NH}_4^+$  (at rates of 60–120  $\text{nmol N g}^{-1} \text{AFDW h}^{-1}$ ), the uptake rates of both gorgonian species were lower than those of tropical scleractinian corals (in the  $\mu\text{mol}$  range, Grover et al., 2002; Pupier et al., 2021) and

TABLE 2 Contribution of DOC heterotrophy relative to the holobiont respiration ( $\text{CHAR}_{\text{DOC}}$ , %) regarding species, temperature, and DOC concentration.

Species	Temperature (°C)	DOC concentration ( $\mu\text{mol C L}^{-1}$ )	$\text{CHAR}_{\text{DOC}}$ (%)
<i>E. singularis</i>	17°C	[DOM]	-13 ± 17
		3 x [DOM]	43 ± 33
		6 x [DOM]	56 ± 20
	24°C	[DOM]	-64 ± 52
		3 x [DOM]	138 ± 19
		6 x [DOM]	196 ± 60
<i>P. clavata</i>	17°C	[DOM]	-9 ± 9
		3 x [DOM]	33 ± 15
		6 x [DOM]	41 ± 6
	24°C	[DOM]	-65 ± 52
		3 x [DOM]	24 ± 72
		6 x [DOM]	101 ± 76

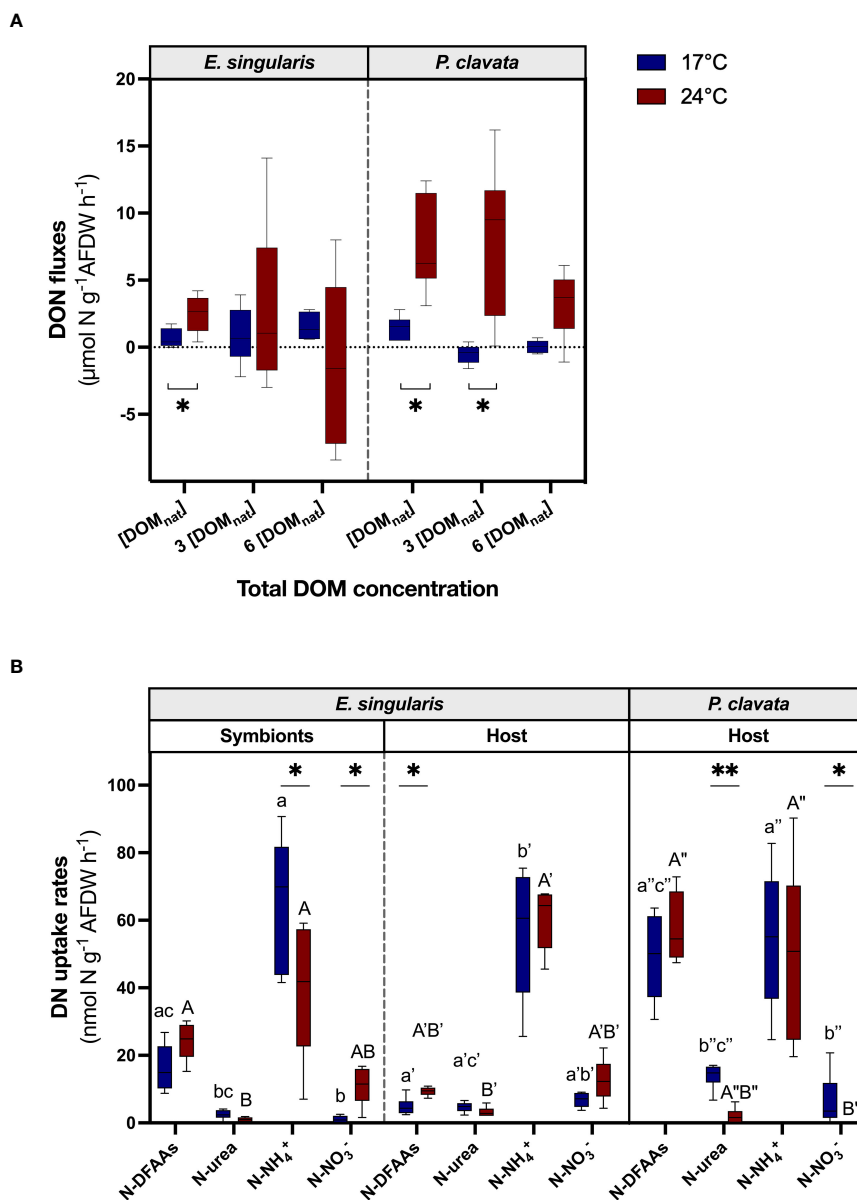
tropical octocorals of the suborder Alcyonacea (Pupier et al., 2021, ranging from 150 to 640  $\text{nmol N g}^{-1}$  AFDW  $\text{h}^{-1}$ , depending on the species). The most meaningful comparison can be made between *E. singularis*, which lives in symbiosis with Symbiodiniaceae dinoflagellates (LaJeunesse et al., 2018), and the tropical species mentioned above, all of which live symbiotically. On the contrary to tropical soft corals, gorgonian tissue is thin and cannot act as a barrier to N diffusion (Pupier et al., 2021). Therefore the lower uptake rates observed in *E. singularis* compared to tropical octocorals could be due to the lower irradiance it receives (50 – 150  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (Gori et al., 2012), compared to > 400  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  for tropical species). Indeed, a positive correlation has been demonstrated between irradiance, symbiont photosynthesis, and N uptake in corals, with increased photosynthesis and N uptake under high light conditions (Grover et al., 2002). In addition, the Mediterranean seawater temperature varies between 12°C and 24°C depending on the season, compared to a minimum of 25°C for tropical species. Therefore, *E. singularis* may have a lower metabolism than tropical species due to the lower temperatures at which it thrives (Previati et al., 2010).

An interesting observation is that the asymbiotic gorgonian *P. clavata* exhibited the same rates of  $\text{NH}_4^+$  uptake into host tissue as the symbiotic *E. singularis*. Normally, the first site of  $\text{NH}_4^+$  uptake in corals is the Symbiodiniaceae component, which assimilates 90% of the  $\text{NH}_4^+$  taken up via the glutamine synthetase/glutamine oxoglutarate aminotransferase pathway (GS/GOGAT) (Pernice et al., 2012; Roberty et al., 2020). Although the coral host can also assimilate  $\text{NH}_4^+$  via two pathways (GS/GOGAT and glutamate dehydrogenase pathway), and has ammonium transporters (Capasso et al., 2022), direct  $\text{NH}_4^+$  uptake is generally low (Pernice et al., 2012), except under certain stress conditions (Tang et al., 2020; Xiang et al., 2020). Unlike Symbiodiniaceae-bearing corals, non-symbiotic corals are generally unable to assimilate DIN

(Muscatine et al., 1979) and instead excrete  $\text{NH}_4^+$  and urea into the surrounding water (Rahav et al., 1989). Since microbes in the mucus layer accounted for only 7% of the total  $\text{NH}_4^+$  uptake of gorgonians, *P. clavata* must have evolved specific adaptations to assimilate  $\text{NH}_4^+$  and benefit from this additional N source. Alternatively,  $\text{NH}_4^+$  uptake may derive from the activity of microbes inside the gorgonian tissue, although the measured uptake rates are very high for such a presumed tiny microbial biomass. In addition to  $\text{NH}_4^+$  uptake, there was also very low uptake of nitrate ( $\text{NO}_3^-$ ) in both gorgonians (although 8 to 20 times lower than  $\text{NH}_4^+$ , which in turn is lower than in tropical corals). This uptake may be attributed to the microbes associated with the gorgonians, in particular from the bacterial symbiont Endozoicomonas. This bacteria is very abundant in Mediterranean gorgonians (Van De Water et al., 2017; Van De Water et al., 2018) and can take up  $\text{NO}_3^-$  and reduce it into  $\text{NH}_4^+$  (Neave et al., 2014; Neave et al., 2017).

Gorgonians were also able to assimilate labile DON, especially dissolved free amino acids (DFAAs). However, uptake rates were relatively low, in the nanomolar range (20 to 50  $\text{nmol N g}^{-1}$  AFDW  $\text{h}^{-1}$ ) and were at the lower end of rates measured for tropical soft corals (20 to 200  $\text{nmol N g}^{-1}$  AFDW  $\text{h}^{-1}$ ), themselves lower than for scleractinian corals (Grover et al., 2008; Pupier et al., 2021). DON uptake rates were however higher in the host tissue of the heterotrophic gorgonian *P. clavata* than in the symbiotic *E. singularis*, suggesting that *P. clavata* had to compensate for lower DIN uptake due to the absence of Symbiodiniaceae (total DN uptake of *P. clavata*: 120  $\text{nmol N g}^{-1}$  AFDW  $\text{h}^{-1}$  vs 160 for *E. singularis* holobiont). The uptake of nanomolar amounts of DFAAs suggests that the gorgonians either do not have a large requirement for DON or are unable to assimilate large amounts of DON. In addition, such nanomolar uptake of DFAAs cannot be measured through the incubations with natural DOM, which contains both labile and nonlabile DON at concentrations of 10  $\mu\text{mol N L}^{-1}$ . This differs from tropical octocorals, which are able to assimilate large amounts of DON when it is present in seawater (Lange et al., 2023). Considering the low uptake rates of DIN and DON, our results suggest that Mediterranean gorgonians have a low capacity to feed on dissolved N sources. However, their low C:N ratio of 5–6 indicates that gorgonians are not limited in N. In fact, the C:N ratio is in the same range as the Redfield ratio (C:N = 6.6) and much lower than the C:N ratio of N-depleted phytoplankton or of deep-sea corals (C:N > 10, Losh et al., 2012; Ferrier-Pagès et al., 2022). Mediterranean gorgonians should therefore meet their N requirements through heterotrophic feeding on particulate organic material (plankton). To our knowledge, no previous study has assessed the amount of N supplied to gorgonians through heterotrophy. However, if we assume a C:N ratio of 6 for plankton and a heterotrophic uptake of 450  $\mu\text{g C g}^{-1}$  AFDW  $\text{h}^{-1}$  measured for the Mediterranean gorgonian *Leptogorgia sarmentosa* (Ribes et al., 2003), the amount of N that can be obtained by heterotrophy may be ca. 75  $\mu\text{g N g}^{-1}$  AFDW  $\text{h}^{-1}$  or 5.4  $\mu\text{mol N g}^{-1}$  AFDW  $\text{h}^{-1}$ , which is in fact more than 30 times the amount of N obtained by the uptake of DIN and DON.

From the measurements of the dissolved organic carbon (DOC) fluxes, it appears that, as with N, the gorgonians release more DOC than they take up from the natural DOM at ambient concentration (80  $\mu\text{mol C L}^{-1}$ ), which is consistent with a previous study on



**FIGURE 5**  
 Assimilation of dissolved nitrogen by gorgonians at 17°C (blue bars) and 24°C (red bars). The boxplots represent the DON fluxes ( $\mu\text{mol N g}^{-1}\text{AFDW h}^{-1}$ ) using a mixture of natural DOM at different concentrations (A) ( $n = 6$ ) and DN uptake rates according to different nitrogen sources ( $\text{nmol N g}^{-1}\text{AFDW h}^{-1}$ ) for each fraction of the two gorgonian species, using labelled <sup>15</sup>N (B) ( $n = 6$ ). AFDW, ash-free dry weight; DFAAs, dissolved free amino acids; DON, dissolved organic nitrogen; DOM, dissolved organic matter. For the graph (A), a negative flux implies an uptake of DON, whereas a positive flux implies a release of DON. For the graph (B), for each fraction and temperature independently, significant differences in DN uptake rates between nitrogen sources are distinguished with letters. For both graphs, significant differences between temperatures are displayed with asterisks ( $p$  value < 0.05).

Mediterranean gorgonians (Ribes et al., 1999a; Ribes et al., 2003). The ratio between the release of DOC and the net photosynthetic rate of *E. singularis* (13%) is in the same range as previous measurements on tropical corals (Ferrier-Pagès et al., 1998; Tanaka et al., 2009). Nevertheless, our results show that gorgonians were able to take up significant amounts of DOC under enrichment conditions (3 and 6 x [DOM<sub>nat</sub>]), suggesting that they require a certain threshold for DOC utilization, as previously observed in sponges (Wooster et al., 2019; Ribes et al., 2023). Such high concentrations can be observed in

coastal areas receiving DOM from rivers (Cauwet et al., 1990; Ejarque et al., 2017; Sánchez-Pérez et al., 2020). *Eunicella singularis* can also acquire C as DIC thanks to the presence of Symbiodiniaceae in its tissues. However, although *E. singularis* had similar concentrations of symbionts and chlorophyll as many tropical soft corals and gorgonians (Rossi et al., 2018; Pupier et al., 2019b; Rossi et al., 2020), its C fixation rate was 10-fold lower except when compared to tropical sea rods (Rossi et al., 2018). This may be related to the polyp size of *E. singularis*, which is also comparable to sea rods, as a

TABLE 3 Recapitulative table of the effect of temperature and DOM concentration on all parameters measured, for each species.

	Parameter measured	<i>Eunicella singularis</i>		<i>Paramuricea clavata</i>	
		Temperature	DOM concentration	Temperature	DOM concentration
Physiology	Symbiont density	-			
	Total chlorophyll content	ns			
	Net photosynthesis	-			
	Gross photosynthesis	ns			
	P:R	-			
	Lipid content	ns		-	
	Protein content	ns		ns	
	Respiration rate	+		+	
Tissue	C content host	ns		-	
	C content symbionts	-			
	N content host	-		ns	
	N content symbionts	-			
	P content host	-		ns	
	P content symbionts	-			
	C:N host	+		-	
	C:N symbionts	+			
	C:P host	+		ns	
	C:P symbionts	+			
	N:P host	+		+	
	N:P symbionts	ns			
Nutrition	DOC uptake	+	+	ns	+
	DIC uptake host	+		ns	
	DIC uptake symbionts	-			
	DIC uptake holobiont	ns			
	DON uptake	ns	ns	-	ns
	DFAA uptake host	+		ns	
	DFAA uptake symbiont	ns			
	Urea uptake host	ns		-	
	Urea uptake symbionts	ns			
	NH4+ uptake host	ns		ns	
	NH4+ uptake symbionts	-			
	NO3- uptake host	ns		-	
	NO3- uptake symbionts	+			
	DFAA uptake holobiont	+			
	Urea uptake holobiont	ns			
	NH4+ uptake holobiont	ns			
NO3- uptake holobiont	+				

Parameters that significantly increased with temperature or DOM concentration are displayed with “+” and a green color, while parameters that significantly decreased are displayed with “-” and an orange color. On the contrary, parameters that were not significantly affected by temperature or DOM concentration are displayed as “ns” (not significant). Grey cells indicate a condition not tested for this parameter.



correlation between polyp size and primary productivity has been demonstrated in tropical gorgonians (Baker et al., 2015; Rossi et al., 2018). However, the amount of C supplied to *E. singularis* by photosynthesis under our experimental conditions ( $120 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , corresponding to the summer season) is lower than the amount of C obtained by heterotrophy (Ribes et al., 1999a; Ribes et al., 2003). These measurements confirm that Mediterranean gorgonians rely more on the uptake of particulate food, such as pico- and nanoplankton, than on the uptake of dissolved material.

The health of gorgonian species was significantly affected when the temperature was raised to 24°C for two weeks. A temperature of 24°C - 25°C is a threshold for many Mediterranean corals (Rodolfo-Metalpa et al., 2006; Tignat-Perrier et al., 2022), which was not often reached except at very shallow depths before 2011, but since then has been increasingly measured down to 40 m depth (Cerrano et al., 2000; Garrabou et al., 2009; Garrabou et al., 2022). After several weeks (5 to 8 weeks) at 25°C, gorgonians generally show tissue necrosis and microbial diseases (Tignat-Perrier et al., 2022). In this study, we did not aim to reach this final health state, but only to induce heat stress with reversible effects on gorgonian health. In *E. singularis*, bleaching and increased respiration rate resulted in a significant loss of cellular C, N and P content, as observed previously (Baker et al., 2018). In *P. clavata*, the increased respiration rates resulted in a significant decrease in lipid content and thus cellular C content, consistent with Tignat-Perrier et al. (2022). To compensate for the higher respiration rates and energy costs, *E. singularis* significantly increased its heterotrophic diet on DOC. Indeed, feeding on 3x and 6x [DOM<sub>nat</sub>] allowed *E. singularis* to meet more than 100% of its respiration requirements, consistent with previous findings that some corals can rely on DOC to compensate for loss of autotrophy (Levas et al., 2016). However, DOC uptake was not accompanied by major increase of DON or DIN uptake in either gorgonian. Overall, these changes resulted in a significant increase in the C:N ratio (and C:P ratio) of symbionts and host tissues of *E. singularis*, suggesting that this species may become N and P limited in the long term under heat stress. *Paramuricea clavata* also lost N in the form of DON. This release may be due to either increased release of mucoid exudates or increased release of coral cellular material (e.g., detachment of host cells) following heat stress (Tanaka et al., 2009). The last hypothesis seems to be the most likely, since it has indeed been observed that *P. clavata* is subjected to severe oxidative stress at high temperatures, leading to tissue necrosis after several weeks of stress (Tignat-Perrier et al., 2022). Overall, these observations strongly indicate that gorgonians are highly vulnerable to starvation during marine heat waves (MHWs). During the summer months, the stratification of the water column leads to a significant decline in zooplankton abundance in surface waters (Roemmich and McGowan, 1995), which directly impacts filter feeders like gorgonians by depriving them of a vital food source. Unlike tropical corals, Mediterranean gorgonians lack the ability to rely on the uptake of dissolved inorganic and organic nutrients, exacerbating their susceptibility to food scarcity.

## 5 Conclusion

The diet of Mediterranean gorgonians appears to rely more on the capture of plankton prey than on dissolved inorganic and organic nutrients. Previous studies has shown that *P. clavata* predominantly ingests particulate detritus (Ribes et al., 1999a) and captures a large number of nauplii and small prey items (< 200  $\mu\text{m}$ ) during periods of high prey abundance in autumn and spring (Coma et al., 1994). In *E. singularis*, symbionts can however bring a significant amount of C to the symbiosis, especially during summer, when light is maximal, and plankton abundance is minimal. We studied here the only symbiotic octocoral gorgonian of the Mediterranean Sea (*Eunicella singularis*) and one widespread asymbiotic gorgonian, *Paramuricea clavata*. As the response may differ across species, there may be other species-specific differences aside from symbiosis vs non-symbiosis that remains to be investigated. In addition, in tropical reefs, sponges are known to play a key role in processing DOM before returning it to corals in the form of particulate organic matter (the “sponge loop”, de Goeij et al., 2013). Given the high abundance of sponges in the coralligenous assemblages of the Mediterranean Sea, it would be valuable to investigate the potential importance of the sponge loop for gorgonian’s nutrition.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

## Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

## Author contributions

KL, SR and CF-P conceived and designed the experiment. KL performed the experiments. J-FM performed the mass spectrometry analyses. KL analyzed the data. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2023.1236164/full#supplementary-material>

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