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Warm-adapted sponges resist thermal stress by reallocating carbon and nitrogen resources from cell turnover to somatic growth

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Abstract

Ocean warming will affect the functioning of coral reef ecosystems with unknown cascading effects. Any perturbation in the ability of sponges to recycle the dissolved organic matter released by primary producers and make it available to higher trophic levels, might have unknown consequences for the reef trophic chain. Biogeochemical processes were measured in the sponge *Rhabdastrella globostellata* from the semi-enclosed lagoon of Bouraké, where temperatures reach 33.8°C and fluctuates by 6.5°C on a daily basis, and from a control reef (28°C). Using ^{13}C- and ^{15}N-labeled coral mucus, we experimentally investigated to what extent high temperature affected the carbon (C) and nitrogen (N) resources allocation in the sponge tissue and detritus. Sponges from Bouraké maintained at 32°C incorporated more ^{13}C- and ^{15}N-labeled coral mucus in the tissue and showed less detritus release when compared with sponges maintained at 28°C. In contrast, at 32°C control sponges showed lower ^{13}C- and ^{15}N-labeled coral mucus incorporation in tissue and higher release of detritus. Our results suggest that sponges adapted to extreme temperatures of Bouraké were able to reallocate C and N resources from cell turnover to somatic growth and reduce tissue damage. In contrast, non-adapted sponges at the control reef lack this mechanism and underwent tissue disintegration, highlighting the lethal effect of future warming. The change in C and N allocation in adapted sponges suggests a potential adaptation mechanism that allows *R. globostellata* to survive under thermal stress, but it could alter the availability of essential sources of energy with unknown consequences on the future reef trophic interactions.

One of the main effects of climate change is the increase in sea surface temperature causing ocean warming. An increase in seawater temperature will have a cascading effect on marine life and ecological processes worldwide. In coral reefs, sponges play a key role by recirculating carbon and nutrients produced by benthic primary producers, making these sources of energy available to the surrounding trophic chain (e.g., De Goeij et al. 2013; Rix et al. 2017, 2018). Although many studies have revealed the physiological stress caused by high temperature on sponges (e.g., Massaro et al. 2012; Bell et al. 2018; Strano et al. 2022), none of these studies have investigated its effect on the sponges ability to recycle carbon and nitrogen.

The negative effects of ocean warming on sponges have been largely demonstrated on the sponge reproduction (e.g., Abdul Wahab et al. 2014), photosynthetic rates (e.g., Bennett et al. 2017), pumping rates (e.g., Massaro et al. 2012), filtration efficiency, choanocyte chamber size and density (Massaro et al. 2012), and change in their associated microbial composition and functioning (e.g., Blanquer et al. 2016; Ramsby et al. 2018). De Goeij et al. (2009) suggested that cell turnover and shedding of choanocytes could be a mechanism used by sponges to prevent permanent damage under environmental stress, and Massaro et al. (2012) further hypothesized that thermal stress caused the reduction in choanocyte chamber size and

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density in *Rhopaloeides odorabile* exposed to 32°C. In addition, temperatures higher than 31°C increased the expression of heat shock proteins in some species (López-Legentil et al. 2008; Pantile and Webster 2011; Guzman and Conaco 2016). Although some studies suggested sponges to be more tolerant to ocean warming than other benthic organisms such as corals (Kelmo et al. 2013), many studies showed that both acute, short- and long-term exposure to high temperature has caused mass mortality events (e.g., Cebrian et al. 2011; Perkins et al. 2022; Bell et al. 2023). Likely, ocean warming effects on sponges' metabolism are species-specific, and depend on whether a particular species is already near or above its thermal optimum. For instance, species living in thermally variable environments cope better with heat stress when compared to species living in more stable environments (e.g., Oliver and Palumbi 2011; Rivest et al. 2017).

Sponge holobiont can assimilate the abundant dissolved organic carbon (DOC) in seawater, which can result in the release of cellular debris as particulate organic carbon (POC) and then as detritus (i.e., sponge loop pathway; De Goeij et al. 2013; Alexander et al. 2014; Maggioni et al. 2023*a*). An alternative pathway for the sponge loop has been hypothesized for sponges with massive growth, for which no significant release of detritus were found (e.g., Pawlik et al. 2016; McMurray et al. 2018). The authors hypothesized that assimilated DOM could be used mainly to produce biomass that, in turn, feeds sponge predators (e.g., Pawlik et al. 2016; McMurray et al. 2018). However, recent studies showed that both pathways could coexist in two deep-sea sponges species (Bart et al. 2021), and that significant detritus was released in the massive sponges *Aplysina archeri* (Kornder et al. 2022), and *R. globostellata* (Maggioni et al. 2023*a*). Although the study of these pathways should be better elucidated using more species, it is evident that sponges may play a crucial role as ecosystem engineers since they make available carbon (i.e., energy) to other organisms in the reef, and so link different trophic levels (e.g., De Goeij et al. 2013; Van Hoytema et al. 2023). Therefore, it becomes crucial to better understand to what extent ocean warming will affect the ability of sponge to recycle the organic matter in coral reef ecosystems.

In a previous field-based study (Maggioni et al. 2023*a*), we found that the sponge *R. globostellata* living in the semi-enclosed lagoon of Bouraké has developed mechanisms to cope with the extreme environmental conditions that characterize this special natural laboratory. This sponge species was already known to efficiently filter bacteria cells and remove DOM from surrounding seawater (Hildebrand et al. 2022). During the low tide, the environmental conditions at Bouraké ($pH_T = -0.8$ units; dissolved oxygen = −20%; temperature = + 2–3°C) could be claimed to be close to, or even worse than conditions predicted under the worst climate scenario. Although the harsh conditions, a healthy coral reef was found in Bouraké (Camp et al. 2017; Maggioni et al. 2021). The sponge *R. globostellata* forms massive colonies, with up to 40% of coverage, while elsewhere it is rare and forms only scattered, low-size colonies. By incubating sponges in situ at both high (i.e., normal conditions) and low tide (i.e., extreme conditions), we previously found that at the extreme condition tested (> 31°C), the sponge organic matter recycling changed from significant detritus release to no more detritus release in *R. globostellata* (Maggioni et al. 2023*a*). Because the observations were made in situ, where sponges experience a combination of factors, including warming, acidification and deoxygenation, we were unable to elucidate the driver of the observed change. Here, we tested if (1) this change was primarily due to the elevated temperature, (2) carbon (C) and nitrogen (N) resources were reallocated in sponge tissues (i.e., biomass) and detritus, and (3) the observed response was specific of sponges from Bouraké. These hypotheses were tested here by investigating the stable isotope tracing on warm-adapted *R. globostellata* from Bouraké (variable summer thermal history between 28°C and 33°C), and non-adapted ones from a control reef (constant temperature around 28°C). Enriched ^{13}C and ^{15}N incorporated by corals and released as mucus were used to track the ^{13}C and ^{15}N incorporations into the sponge tissue and their transfer as detritus at two temperature treatments (28°C and 32°C). Elemental fluxes changes (DOC, POC, PON, detritus) were also compared between sponges and temperature treatments.

Materials and methods

Samples collection and maintenance

On the 22 and 25 April 2022, fragments of *R. globostellata* of ca. 5–8 cm long were cut from 50 different mother colonies in Bouraké (west coast of New Caledonia), and at a control reef located in Tupeti Island (Thio, east coast of New Caledonia) (Fig. 1). While Bouraké is an extreme environment characterized by extreme and fluctuating conditions (Maggioni et al. 2021), Tupeti Island reef is a control site because environmental conditions were assumed to be at the ambient level. At both sites, fragments were collected by scuba diving and immediately fixed on a labeled PVC plate using a wire cable. Plates were then attached to a PVC structure fixed at 2–3 m depth on the reef (Fig. 1).

After 3 weeks of recovery in situ, sponges showed a complete tissue regeneration where the sponge was cut from the mother colony, and did not show signs of tissue necrosis and discoloration. After the recovery period, sponge fragments were collected at both sites and individually transported in sterile zip plastic bags. Plastic bags were fully filled with seawater and kept in an icebox during transport. At each site, a Hobo pendant temperature logger recorded each 10 min interval the temperature, which average of the period was used to set the start temperature in the acclimation tanks (26.3 ± 0.3°C).

In addition, in Bouraké, 60 fragments of 10–20 cm in diameter of the coral *Acropora tenuis* were collected at 2–4 m depth. Sponges and corals samples were kept in an icebox and transported within 3 h after collection to the "Aquarium des Lagons" in Nouméa (~ 90 km south from Bouraké, and

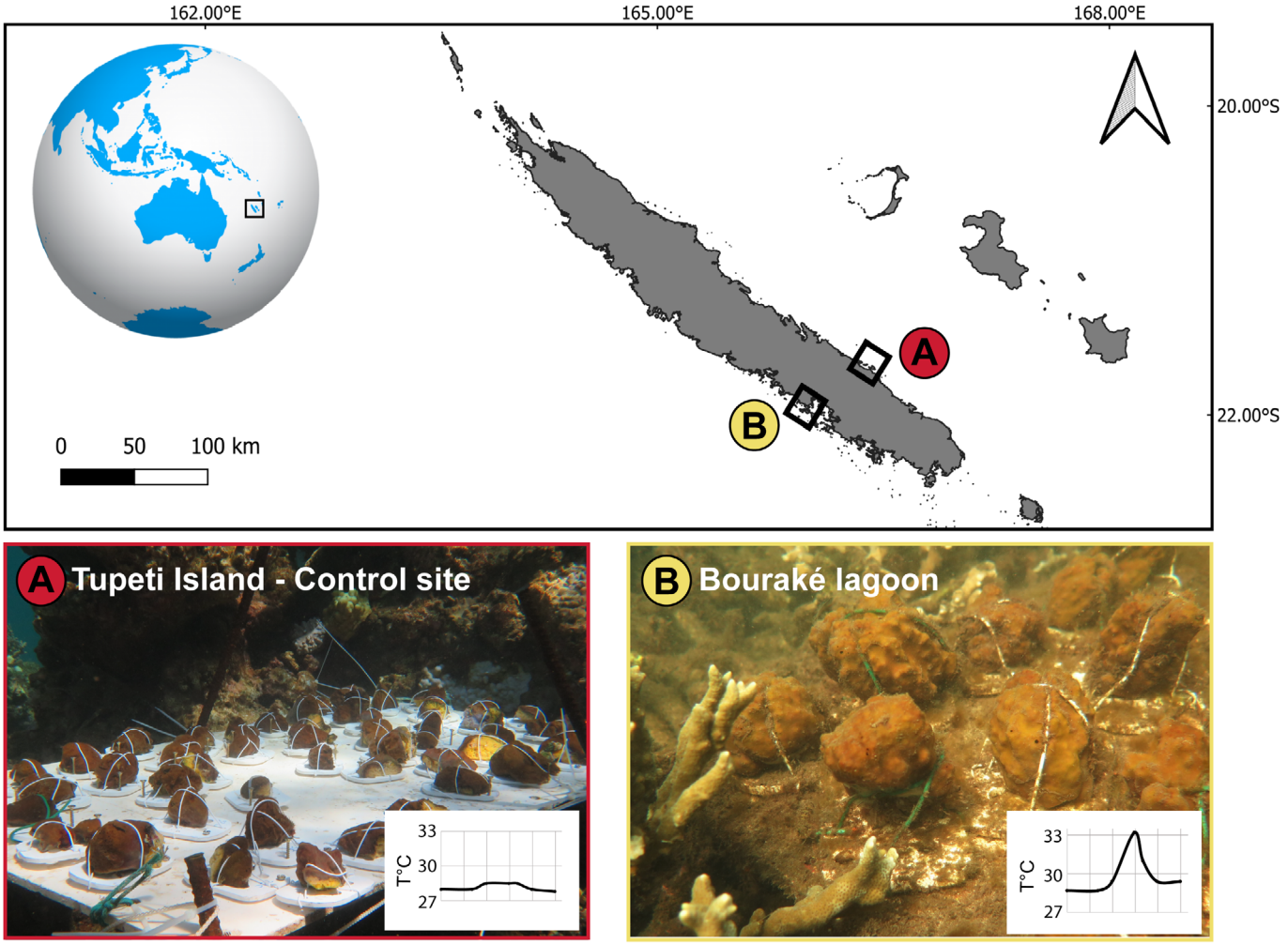

Fig. 1. Map of New Caledonia (top panel) with the two study sites and pictures of the fragments of *R. globostellata* freshly transplanted at the control site (Tupeti Island, **A**), and after 1 month in situ in the Buraké lagoon (**B**). Within panels A and B, a graph reports a 24 h diurnal cycle of a typical temperature variation measured during the summer at each study site. Georep New Caledonia database (https://georep.nc/, last access: November 2022) and Qgis software were used to build the figures.

144 km from the control site) where the experiment was run. For sponges, seawater inside the plastic bags was changed every 1 h to avoid stressful conditions during transport.

Acclimatization and experimental set-up

Two temperature conditions were used during the experimental set-up: (1) typical summer temperature of 28°C; and (2) fluctuating summer temperature ranging daily from 28.5°C to 33.2°C. These two temperature conditions mimic the in situ conditions measured during the summer 2020 when the temperature values were supposed to affect the *R. globostellata's* metabolism in Bouraké (Maggioni et al. 2023*a*).

Corals were acclimated and maintained in a unique 120 L tank (flow rates at 2 L min^{-1}) receiving ca. 300 μmol photons m^{-2} s^{-1} from two led light systems (Mitras LX6100, GHL Germany) on a 12 : 12 h light : dark cycle. Two submersible recirculating pumps (ON 1000, EHEIM, Germany) mixed seawater in the coral tank. The tank was supplied with unfiltered and non-stagnant seawater pumped at 3 m depth from the Baie des Citrons (Nouméa, New Caledonia). During the acclimation period (2 weeks), temperature in the coral tank was increased by 1°C each day from the value measured at the sampling sites (*ca* 26°C) until reaching 28°C, then maintained at 28°C during the whole experiment, and continuously checked using Neptune System connected to temperature probes.

Sponges were carefully cleaned of any epibionts and distributed into four tanks, two per site (25 sponges each tank of 48 L; flow rates at 800 mL min^{-1}) for acclimatization. Sponges were then gradually acclimated (during 1 week) to the two temperature conditions of 28°C and variable from 28.5°C to 33.2°C, with one independent tank per site (Supporting Information Fig. S1A). Each tank was independently supplied with unfiltered and non-stagnant seawater pumped at 3 m depth from the Baie des Citrons (Nouméa, New Caledonia)

ensuring at least one complete water renewal every hour. Seawater in the tanks were mixed using a submersible pump (micro-jet MC 320, Aquarium system, USA).

After acclimatization and coral labeling (see below), sponges were randomly divided among the 10 experimental tanks (20 L each) that were positioned below the coral tank to allow the labeled DOM from mucus to be filtered by the sponges. The experimental tanks received an ambient light of ca. 10 μmol photons m^{-2} s^{-1}, as measured using a NKE PAR with LI-193 spherical quantum sensor. Five tanks were setup at constant temperature (28°C) and contained each 4 sponges from Bouraké and 4 from Tupeti Island (Supporting Information Fig. S1B). Five more tanks were setup at variable temperature (28–33.2°C). For each temperature treatment, three tanks were positioned below the coral tank, which contained labeled corals (see below), and received directly its seawater with enriched DOM from corals mucus. The remaining four tanks (two for each temperature treatment) received coral mucus-free water (i.e., not from the coral tank) and were used as blanks in the labeling calculations.

Temperature was increased using heaters connected to two Apex controller (Neptune System, USA), which were set up to either maintain a temperature of 28°C (± 0.06°C), or to simulate the natural temperature fluctuation measured in Bouraké. During both acclimatization and experimental treatment, the seawater temperature was continuously measured by the Neptune System connected to temperature probes, while pH_T was measured two times a day in each tank using a portable pH-meter (913, Metrohm) calibrated with TRIS buffer (Dickson lab, batch #T28).

Coral labeling

Coral labeling procedure combined methods used by Naumann et al. (2010); Rix et al. (2018) and Campana et al. (2021). Corals were enriched with nutritive sources of labeled ^{13}C inorganic carbon and ^{15}N nitrogen by the addition of ^{13}C-$NaHCO_3$ and ^{15}N-$NaNO_3$ label compounds (Cambridge Isotope Laboratories, 99% ^{13}C and 98% ^{15}N) during 5 d. Each morning at 8:00, the inflow to the coral tank was stopped and 36 mg L^{-1} $NaH^{13}CO_3$ and 1 mg L^{-1} $Na^{15}NO_3$ were added. The effectiveness of this method was previously tested in a trial experiment (unpublished data). Aquaria pumps maintained water circulation and air exchange for the 8 h-labeling period after which the seawater flow-through was resumed. During this period, the inflows between the coral tank and the experimental tanks, which were positioned below the coral tank, were closed and experimental tanks received an independent inflows of water. Mucus were collected from 15 randomly coral colonies at day 1, 4, and 5 to verify the ^{13}C and ^{15}N incorporation. Corals were removed, rinsed in label-free seawater and air exposed for mucus production (2 min). The collected mucus was frozen at − 20°C, for $\delta^{13}C$ and $\delta^{15}N$ determinations.

Transfer and incorporation of labeled DOM to sponges

At the end of the 5 d of coral labeling, seawater from the coral tank containing isotopically-labeled *Acropora tenuis* was allowed to continuously feed the six 20 L experimental tanks containing *R. globostellata*. Thus, sponges filtered the labeled DOM from coral mucus for 4 d and 8 d. The flow through from the coral tank to the six tanks were regulated to allow 20 L h^{-1} of water to flow from labeled corals to sponges.

Sponge isotopic signature and elemental fluxes (six labeled tanks + four non-labeled tanks) were measured on two sponge specimens for each site of origin ($n = 2$), each tank ($n = 10$), and from each treatment ($n = 2$). A total of 20 sponges (12 from labeled tanks + 8 from non-labeled tanks) were incubated after 4 d, and the remaining 20 sponges (12 from labeled tanks + 8 from non-labeled tanks) were incubated after 8 d during which sponges exposed to the labeled treatment received labeled DOM seawater from the coral tank. The collected sponges were removed from the experimental tanks and rinsed in labeled-free flowing seawater during 10 min. Each sponge was then transferred into individual 2 L glass beakers filled with fresh labeled-free seawater and incubated for 3 h. Beakers were semi-submerged in a water bath ($n = 4$) containing each one 15-places submersible Variomag stirring plate. Each water bath contained a total of 13 incubation beakers (12 with sponges and one with only water that was used as a blank incubation) for labeled tanks, while 9 incubation beakers (8 with sponges and one as a blank incubation) for non-labeled tanks. As it was technically difficult to maintain the variation in the temperature during the incubation, sponges from the variable treatment were maintained at the highest temperature at which sponges were exposed. Therefore, sponges were incubated at two different temperatures according to their temperature treatment: (1) at 28°C, which is average control summer temperature, and (2) at 32°C, which is approximately the highest temperature to which sponges were exposed in Bouraké in 2020. The latter was also chosen since we previously hypothesized that 31°C is the threshold value triggering the break in the carbon recycling for this species (Maggioni et al. 2023*a*). Temperature in the water bath was verified by the temperature probes connected to the Apex systems. The same procedure was applied to both labeled and non-labeled sponges. The incubation was performed in the quasi-dark to avoid the contribution of photosymbionts into the C and N recycling. Of the 100 samples collected in situ, only 80 healthy individuals that showed actively seawater pumping were used for the experiment. Indeed, eight fragments collected at the control site showed signs of bleaching and tissue necrosis when we increased the experimental temperature in the tanks. In contrast, sponges from Bouraké did not show evident signs of stress.

The incorporation of labeled DOM from coral mucus (^{13}C and ^{15}N) in sponges' tissue and then in detritus were assessed on sponges exposed to labeled corals, while the change in concentrations of elemental fluxes parameters during incubations (i.e., DOC, POC, PON, and detritus fluxes), were assessed on sponges that were both labeled and non-labeled. For both analyses, we compared the responses between the two temperatures treatment (28°C and 32°C).

Elemental fluxes and seawater chemical measurement

Changes in DOC, POC, particulate organic nitrogen (PON), detritus, bacteria and phytoplankton were measured in each beaker as the difference in their concentrations in the incubation medium between the beginning and the end of each incubation. These changes in organic matter during incubations are considered as a flux resulting from the sponge metabolism. The DOC measurements were performed on two technical replicates for each temperature condition at the beginning (water from the inflow alimenting the tanks) and for each beaker at the end of the incubation (seawater medium on each incubation beaker). Sponges' samples were stored frozen in sterile zip bags at − 20°C for $\delta^{13}C$ and $\delta^{15}N$ analyses, and total C and N content determination. All glass bottles, beakers and vials used were precombusted, washed with HCl solutions (10%) and rinsed using milliQ water.

For particulate organic matter (PON and POC), beakers were shaken several times to ensure that the material potentially sticking to the walls and in the bottom were all filtered. The entire seawater in the chambers (1.9 L) was then filtered into precombusted (4 h at 450°C) GF/F filters. This method allowed us to collect all the detritus produced by the sponges from both incurrent and excurrent pores and those deposited at the bottom or in the walls of the beaker. Filters were then dried at 60°C for 24 h before ^{13}C and ^{15}N analyses and total C and N content determination (see below). Before analysis filters were acidified with 50 μL sulfuric acid (0.25 N) following the method of Raimbault et al. (2008).

For DOC, two precombusted (4 h at 450°C) glass ampoules were filled with water filtered using a glass syringe filtration system (SGE™) throughout two precombusted 25-mm GF/F filters. Samples were then acidified with 70% sulfuric acid (H_2SO_4), sealed, and stored at room temperature for DOC analysis by high-temperature catalytic oxidation (Sugimura and Suzuki 1988; Cauwet 1994) on a Shimadzu TOC-L analyzer. Typical analytical precision was ± 0.1–0.5 μM C (SD). Consensus reference materials (https://hansell-lab.earth.miami.edu/consensus-reference-material/index.html) were injected every 10 to 20 samples to ensure stable operating conditions.

Total bacteria abundance (BA) and phytoplankton abundance were measured on 1.49 mL of water fixed with 0.165 mL of paraformaldehyde in cryovials, first kept 10 min in the dark, and then stored at − 80°C. The different plankton and BA components were detected and counted using BD AccuriTM C6 cytometer. For that, cells were excited with 488 nm laser and green (530 nm), orange (575 nm), and red fluorescence (695 nm) emissions were measured. Phytoplankton were analyzed for 10 min at high flow rate (66 μL min^{-1}) and classified based on their characteristic flow cytometry signatures, while bacterioplankton were analyzed for 5 min at high flow rate (66 μL min^{-1}) and quantified by staining samples with Sybr Green-I, and relative cellular DNA quantified assuming stoichiometric dye binding. Life particulate organic carbon (LPOC) was then considered as the sum of phytoplankton (*Prochlorococcus* [Pro], *Synechococcus* [Syn], photosynthetic picoeukariote [Pico], nanoeukaryote [NanoEuk]), and bacterioplankton, all converted into their respective carbon contents. Carbon content of each type of phytoplankton and bacterioplankton was estimated using standard cell conversions: 53 fg C cell^{-1} for Pro (Morel et al. 1993), 470 fg C cell^{-1} for Syn (Campbell et al. 1994), 1496 fg C cell^{-1} for NanoEuk and Pico (Zubkov et al. 1998), and 20 fg C cell^{-1} for bacteria (Ducklow et al. 1993). Detrital carbon production/consumption during incubations was finally calculated as the portion of total POC not accounted for the LPOC (i.e., detritus = POC − LPOC) (Ribes et al. 1999; Hadas et al. 2009; McMurray et al. 2018). Bacteria and phytoplankton ingestion rates were also calculated according to the equations of Ribes et al. (1998) and Houlbrèque et al. (2004), which consider the growth of the prey during the incubations (detailed equation in the Supporting Information Data S1). Finally, bacteria and phytoplankton (LPOC) in μmol C h^{-1} g^{-1} ingested by sponges was used to evaluate their contribution in the C food sources.

Consumption (i.e., reduction; negative values) and/or production (i.e., increase; positive values) of each parameter measured during the incubations were calculated as the difference between their concentrations at the end and at the beginning of incubations. Then, data were corrected by the change in concentrations occurring in the blank chambers (only water), and normalized by the duration of incubation (h), the tank volume (L), and the sponge dry weight (g).

Isotopic analyses

Coral mucus and sponge tissue subsamples were lyophilized, shredded and homogenized by a mortar and pestle for both labeled and unlabeled samples. Subsamples of sponge tissues, coral mucus, and decalcified POM filters were then weighed and transferred into tin capsules for bulk $\delta^{13}C$ and $\delta^{15}N$ isotope analyses, and total C and N content. To remove any potential residual of inorganic carbon, POM filters were acidified with 50 μL sulfuric acid (0.25 N) following the method of Raimbault et al. (2008) before C and N stable isotope analyses.

Stable isotope and elemental analyses were conducted using elemental analyzer mass spectrometer Integra CN Sercon at the Mediterranean Institute of Oceanography (Marseille) (Raimbault et al. 2008; Lacoste et al. 2016). The carbon and nitrogen isotopic ratios are expressed in $\delta^{13}C$ and $\delta^{15}N$ notation (Vienna-PeeDee Belemnite limestone for carbon and atmospheric N_2 for nitrogen) as the deviation from standards in parts per mill, using the following equation:

$$\delta^{13}C \text{ and } \delta^{15}N = [(R\,\text{sample}/R\,\text{standard}) - 1] \times 1000$$

where R sample and R standard are the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$ in the sample and in the standard reference materials, respectively. R standard C = 0.011118 and R standard N = 0.00368 from Atmospheric N2. The precisions of replicate

analyses were 0.5‰ and 0.3‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively.

The incorporation of excess (i.e., background) ^{13}C and ^{15}N is expressed as the specific enrichment, that is, $\Delta\ \delta^{13}C$ and $\Delta\ \delta^{15}N$ values. This was calculated by subtracting the background $\delta^{13}C$ and $\delta^{15}N$ values of the control sponges/control POM filters from the values of the sponges exposed to labeled DOM from corals mucus. The fractional abundance (F) of the heavy isotope in the sample ($^{13}C/[^{13}C + ^{12}C]$ or $^{15}N/[^{15}N + ^{14}N]$) was calculated as F sample = R sample/(R sample + 1), and reported as atom percent (atm % = F × 100). The excess fractional abundance of heavy isotope (E) was calculated as the difference between the F sample and F background: E = F sample – F background.

Incorporation rates are the total uptake (I), calculated by multiplying E by the C or N content of the sponge tissue or detritus. To determine the total mucus-derived C ($^{12}C + ^{13}C$) and N ($^{14}N + ^{15}N$) incorporated, the total uptake (I) was divided by the fractional abundance of the labeled DOM from coral mucus supplied to the sponges. To determine the C : N ratios of coral mucus-derived organic matter incorporated into the sponge tissue and detritus, the total amount of coral mucus-derived C incorporated was divided by the total amount of coral mucus-derived N incorporated. The analytical error was less than 0.13 h for carbon and 0.61 h for nitrogen.

The enrichment in the sponge tissue was then graphically represented separately for day 4 and day 8. The incorporation of ^{13}C and ^{15}N into the sponge tissues was normalized by the weight of sponges (g^{-1}) and the number of days (day^{-1}). Similarly, the detritus produced during the 3 h incubations was normalized by the weight of sponges (g^{-1}) and hours (h^{-1}). Incorporation values in both sponges' tissues and detritus are expressed in μmol C or N $g^{-1}\ d^{-1}$ and μmol C or N $g^{-1}\ h^{-1}$, respectively. The specific fluxes of ^{13}C and ^{15}N are expressed in μmol C or N d^{-1} for sponge tissue and μmol C or N h^{-1} for detritus.

Statistical analyses

General linear model GLM analysis with "tank" as fixed factor was performed to test for a potential tank effect (three tanks per treatment). After verification of the absence of any tank effect, data between the replicated tanks were pooled, and the factor tank was excluded from subsequent models. Since we not found differences in the sponge elemental fluxes (i.e., organic matter recycling) of DOC, POC, PON, detritus, bacteria, and phytoplankton trend between day 4 and day 8 and between sponges exposed to labeled and non-labeled treatment, data were pooled. Whereas, we test for the effect of time in the incorporation of ^{13}C and ^{15}N in sponge tissue and detritus after 4 d and 8 d of exposure to labeled DOM. Significant differences in the elemental fluxes, enrichment, total flow and incorporation, were tested with two-way ANOVAs between temperature conditions (two levels, 32°C and 28°C), sponges origin (two levels, control and Bouraké), and their interaction. When the interaction was significant, a Tukey post hoc test was performed. ANOVAs were run after verification of normality and homoscedasticity of the data. Finally, to test the weather sponge elemental fluxes (i.e., organic matter recycling) of DOC, POC, PON, detritus, bacteria, and phytoplankton were statistically different from 0, a one-sample *t*-test was also performed. All analyses were conducted in R V4, using stats, factorminer, and vegan packages (R Core Team 2019).

Results

Environmental parameters

Seawater temperature variation during the experiment matched with the temperature variability measured in situ during the summer 2020 (Supporting Information Fig. S1). Temperatures varied from 28.5°C to 33.2°C in all the tanks for the variable treatment, and from 27.7°C to 28.3°C in all the tanks for the constant treatment, without substantial differences between tanks. Seawater pH_T were 8.1 ± 0.05 and 8.0 ± 0.07 at the variable and constant treatment, respectively.

Carbon and nitrogen consumption and production

DOC was significantly more consumed at 28°C than at 32°C for both control and Bouraké sponges (Fig. 2; Table 1; Supporting Information Table S1a), with a significant difference from 0 (one-sample *t*-test, $p < 0.05$). Although box plots showed some variability in the data, in general all sponges consumed DOC, except for the control sponges at 32°C, which released 0.08 ± 2.14 μmol C $g^{-1}\ h^{-1}$ (mean ± SD, Table S1a). Control sponges at 28°C consumed the highest amount of DOC with an average of -1.13 ± 1.67 μmol C $g^{-1}\ h^{-1}$, while Bouraké sponges showed an average consumption of -0.12 ± 1.32 μmol C $g^{-1}\ h^{-1}$ at 32°C and -1.00 ± 1.97 μmol C $g^{-1}\ h^{-1}$ at 28°C (Table S1a). During incubations, sponges consumed both phytoplankton and bacteria, while produced POC and PON, with the exception of Bouraké sponges that consumed both POC and PON at 32°C with average values of -0.34 ± 0.57 μmol C $g^{-1}\ h^{-1}$ and -0.05 ± 0.14 μmol C $g^{-1}\ h^{-1}$, respectively (Table S1a). Bouraké sponges also showed significant production of POC at 28°C and consumption at 32°C from 0 (one-sample *t*-test, $p < 0.05$). Average bacteria consumption was -0.10 ± 0.05 μmol C $g^{-1}\ h^{-1}$ and -0.09 ± 0.06 μmol C $g^{-1}\ h^{-1}$ at 28°C in control and Bouraké sponges, respectively, without significant differences in consumption between 28°C and 32°C (Table 1; Table S1a). In both sponges the consumption of bacteria at 28°C and 32°C was also significantly different from 0 (one-sample *t*-test, $p < 0.05$). Average phytoplankton consumption at 28°C was -0.11 ± 0.06 μmol C $g^{-1}\ h^{-1}$ and -0.09 ± 0.07 μmol C $g^{-1}\ h^{-1}$, while at 32°C it was 0.13 ± 0.06 μmol C $g^{-1}\ h^{-1}$ and -0.09 ± 0.04 μmol C $g^{-1}\ h^{-1}$ in control and Bouraké sponges, respectively (Table S1a). All phytoplankton consumption in both sponges and both temperatures was significantly different from

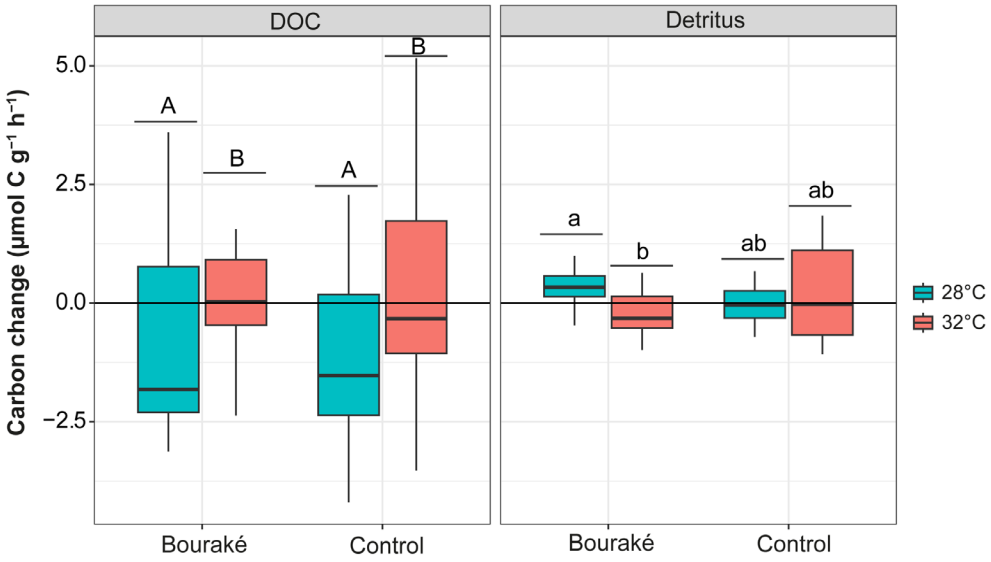

Fig. 2. Carbon change (production and consumption), as DOC and detritus, during the incubations at 28°C and 32°C for both sponges from Bouraké and Control site (Tupeti). Boxes are the interquartile range of data (25th and 75th percentiles); the horizontal line is the median, and the whiskers represent the data range (i.e., minimum and maximum). Capital letters referred to ANOVA two-way test, while small letters referred to Tukey post-hoc test. Unlike letters mean significant differences ($p < 0.05$).

0 (one-sample *t*-test, $p < 0.05$). A significant interaction between temperatures and origins was found for detritus with the highest detritus release (i.e., production) and the lowest detritus production (i.e., consumption) measured respectively at 28°C and 32°C for Bouraké sponges (Tables 1, S1a). Only Bouraké sponges showed significant release of detritus from 0 at 28°C (one-sample *t*-test, $p < 0.05$).

Sponge tissue and detritus enrichment

After 5 d of incubation with labeled ^{13}C and ^{15}N nutritive sources, coral mucus resulted to be enriched on average by 416.5 ± 62.5‰ for $\delta^{13}C$ and 900.6 ± 185.8‰ for $\delta^{15}N$ (data not showed). Isotopic signatures incrementally increased in the sponges' tissue between day 4 and day 8, suggesting gradual accumulations of labeled C and N over time, and finally their transfers in detritus (Fig. 3). Specifically, at day 4, control sponges tissue at 28°C and 32°C and Bouraké sponges tissue at 28°C had similar $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ values, while Bouraké sponges tissue at 32°C had the highest enrichment in ^{15}N (Fig. 3a; Table 1). The two sponges also showed significant differences in $\Delta\delta^{13}C$ based on their origin, with control sponges that had almost the double $\Delta\delta^{13}C$ than Bouraké sponges at 28°C (Fig. 3a, Table 1). Detritus enrichment showed high variability in both sponges and temperatures (Fig. 3a). Both Bouraké and control sponges detritus showed higher $\Delta\delta^{13}C$ enrichment at 32°C compared to 28°C (Fig. 3a). Detritus of Bouraké sponges showed, in average, an increase in both $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ at 32°C of 91‰ and 456‰, respectively. Whereas, control sponges detritus showed higher enrichment in $\Delta\delta^{13}C$ with significantly less $\Delta\delta^{15}N$ at 32°C compared to 28°C (Fig. 3a; Table 1). At day 8, $\Delta\delta^{13}C$ enrichment increased for all sponges tissue compared to day 4. Control sponges tissue at both 32°C and 28°C increased similarly their enrichment without significant differences between temperatures (Fig. 3b, Table 1). Whereas, in Bouraké sponges tissue both the $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ enrichment increased compared to day 4. At 32°C, the average enrichment of both the $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ further increased by 6.4‰ and 25.2‰, respectively, compared to 28°C (Fig. 3b, Table 1). In sponge detritus, the clustering was less evident than in day 4, with no clear temperature effect and no significant differences (Fig. 3b; Table 1). Control sponges detritus at 32°C showed a decrease in $\Delta\delta^{13}C$ and increase in $\Delta\delta^{15}N$, while Bouraké sponges detritus had a similar enrichment and a tendency like day 4 (Fig. 3b).

^{13}C and ^{15}N total fluxes and incorporation in the sponge tissue and detritus

Significant differences were found for ^{13}C and ^{15}N total fluxes in sponge tissue between temperatures and origins, while ^{15}N detritus only differed between temperatures in both day 4 and day 8 (Table S2). At day 4, control sponges tissue showed significantly higher ^{13}C total fluxes at 28°C compared to 32°C and Bouraké sponge tissue (Fig. S3; Table S2). Bouraké sponge tissue showed significantly higher ^{15}N total flux at 32°C compared to 28°C (Fig. S3; Table S2). At day 8, Bouraké sponges tissue always showed significant higher ^{13}C and ^{15}N total fluxes compared to 28°C, while control sponge tissue showed no more differences between 28°C and 32°C (Fig. S3; Table S2). For sponge detritus, we found differences between temperature at both day 4 and day 8, whit higher ^{13}C and ^{15}N total fluxes at 32°C compared to 28°C for both sponge (Fig. S3; Table S2).

Table 1. Summary of two-way ANOVAs between temperature conditions (two levels, 28°C and 32°C), origin (two levels, control and Bouraké), and their interaction. (**a**) The elemental fluxes measured during 3 h of incubation (days pooled); (**b**) ^{13}C and ^{15}N enrichments ($\Delta\delta^{13}C$ and $\Delta\delta^{15}N$) measured after 4 and 8 d of labeling, on both the sponge tissue and detritus; (**c**) ^{13}C and ^{15}N incorporation measured after 4 and 8 d of labeling, on both tissue and detritus; and (**d**) ^{13}C : ^{15}N ratio after 4 and 8 d of labeling. When the interaction was significant, a Tukey HSD test was applied. To summarize HSD results, Bouraké was indicated as B, and the control was indicated as C.

	Temp	**Origin**	**Temp × origin**	
	***F*-value (*p*)**	***F*-value (*p*)**	***F*-value (*p*)**	**HSD post hoc (*p*)**
(a) Elemental fluxes				
DOC	6.43 (< **0.05**)	0.006 (0.938)	0.164 (0.687)	
POC	1.865 (0.176)	0.030 (0.863)	4.369 (< **0.05**)	
PON	0.436 (0.511)	0.001 (0.977)	4.635 (< **0.05**)	
Detritus	0.577 (0.450)	0.177 (0.675)	8.566 (< **0.01**)	B.32°C < B.28°C (< **0.05**)
Bacteria	0.172 (0.680)	2.289 (0.135)	0.827 (0.366)	
Phytoplankton	0.454 (0.502)	3.72 (0.06)	0.428 (0.515)	
(b) ^{13}C and ^{15}N enrichment				
Day 4				
$^{13}C_{tissue}$	1.424 (0.248)	6.225 (< **0.05**)	1.684 (0.117)	
$^{15}N_{tissue}$	5.298 (< **0.05**)	4.646 (< **0.05**)	10.221 (< **0.01**)	B.32°C > B.28°C (< **0.01**); B.32°C > C.28°C (< **0.05**); B.32°C > C.32°C (< **0.05**)
$^{13}C_{detritus}$	20.422 (< **0.001**)	5.143 (< **0.05**)	2.264 (0.148)	
$^{15}N_{detritus}$	1.086 (0.311)	0.041 (0.841)	33.474 (< **0.001**)	B.32°C > B.28°C (< **0.001**); C.28°C > B.28°C (< **0.01**); B.32°C > C.32°C (< **0.01**) C.32°C < C.28°C (< **0.05**)
Day 8				
$^{13}C_{tissue}$	16.534 (< **0.001**)	7.247 (< **0.05**)	19.191 (< **0.001**)	B.32°C > B.28°C (< **0.001**); B.32°C > C.28°C (< **0.001**); B.32°C > C.32°C (< **0.001**)
$^{15}N_{tissue}$	24.67 (< **0.001**)	76.53 (< **0.001**)	22.60 (< **0.001**)	B.32°C > B.28°C (< **0.001**); B.28°C > C28°C (< **0.05**); B.32°C > C.32°C (< **0.05**) B.32°C > C.32°C (< **0.001**)
$^{13}C_{detritus}$	0.943 (0.344)	2.768 (0.113)	0.266 (0.612)	
$^{15}N_{detritus}$	0.661 (0.426)	0.278 (0.604)	1.076 (0.313)	
(c) ^{13}C and ^{15}N incorporation				
Day 4				
$^{13}C_{tissue}$	0.081 (0.779)	10.601 (< **0.05**)	5.963 (< **0.05**)	C.28°C > B.28°C (< **0.01**)
$^{15}N_{tissue}$	1.296 (0.269)	1.285 (0.271)	10.839 (< **0.001**)	B.32°C > B.28°C (< **0.05**); B.32°C > C.32°C (< **0.05**)
$^{13}C_{detritus}$	11.664 (< **0.01**)	4.980 (< **0.05**)	3.011 (0.099)	
$^{15}N_{detritus}$	4.678 (< **0.05**)	0.443 (0.513)	0.034 (0.855)	
Day 8				
$^{13}C_{tissue}$	14.650 (< **0.01**)	7.204 (< **0.05**)	28.039 (< **0.001**)	B.32°C > B.28°C (< **0.001**); B.32°C > C.28°C (< **0.001**); B.32°C > C.32 °C (< **0.001**)
$^{15}N_{tissue}$	22.10 (< **0.001**)	79.89 (< **0.001**)	32.22 (< **0.001**)	B.32°C > B.28°C (< **0.001**); B.32°C > C.28°C (< **0.001**); B.32°C > C.32 °C (< **0.001**)
$^{13}C_{detritus}$	3.362 (< **0.05**)	0.034 (0.856)	6.567 (< **0.05**)	C.32°C > C.28°C (< **0.05**)
$^{15}N_{detritus}$	6.258 (< **0.05**)	0.862 (0.364)	3.288 (0.08)	

(Continues)

Table 1. Continued

	Temp	Origin	Temp × origin	
	F-value (*p*)	*F*-value (*p*)	*F*-value (*p*)	HSD post hoc (*p*)
(d) $^{13}C : {}^{15}N$ ratio in sponge tissue and detritus				
Day 4				
$^{13}C : {}^{15}N_{tissue}$	1.274 (0.274)	19.620 (< **0.001**)	2.130 (0.161)	
$^{13}C : {}^{15}N_{detritus}$	21.218 (< **0.001**)	13.629 (< **0.01**)	7.534 (< **0.01**)	C.32°C > B.28°C (< **0.001**); C.32°C > B.32°C (< **0.001**); C.32°C > C.28°C (< **0.001**)
Day 8				
$^{13}C : {}^{15}N_{tissue}$	0.066 (0.800)	211.69 (< **0.001**)	1.232 (0.281)	
$^{13}C : {}^{15}N_{detritus}$	3.360 (0.082)	11.556 (< **0.01**)	1.609 (0.220)	

Bold indicates statistically significant *p*-value.

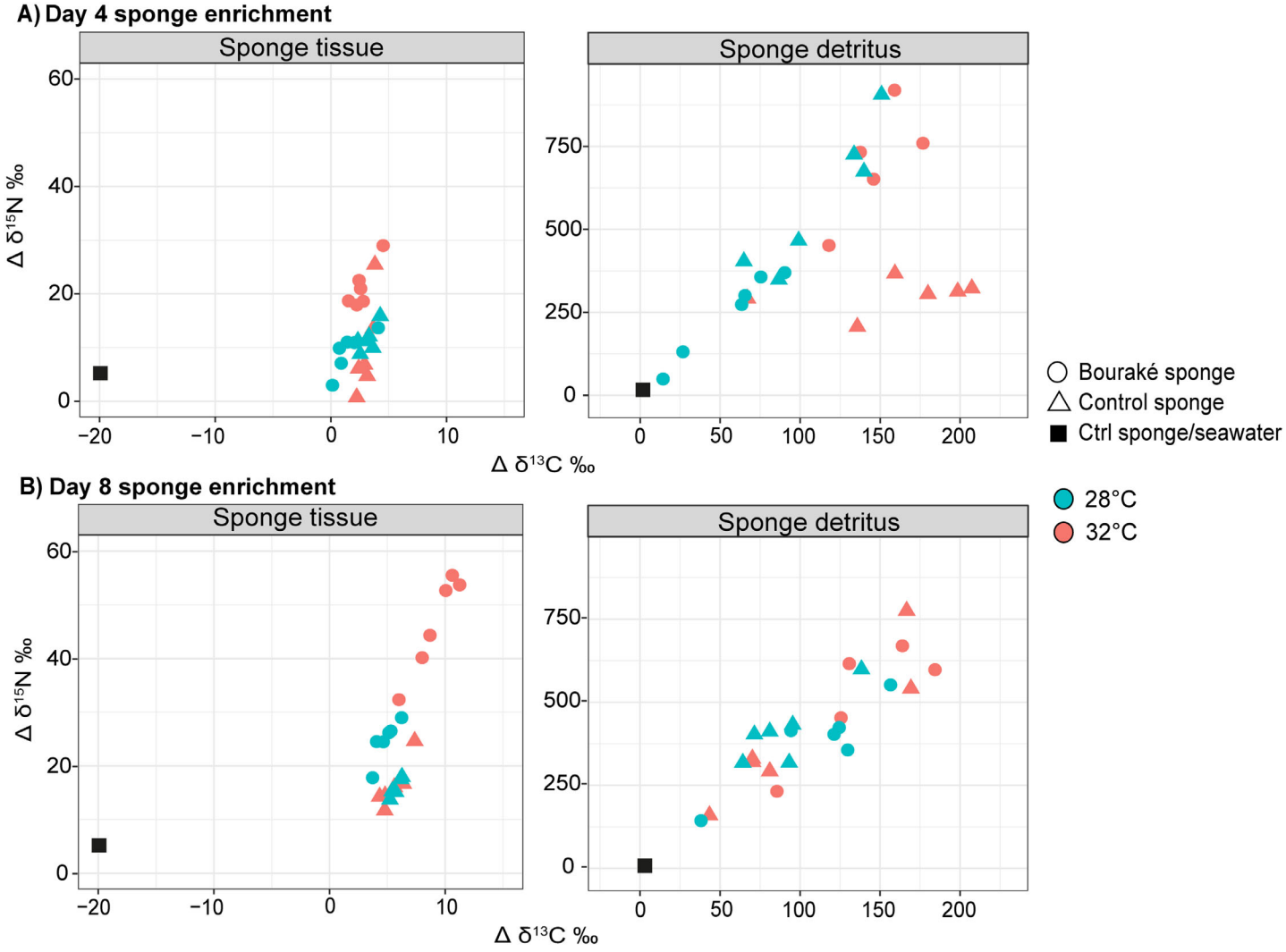

Fig. 3. Stable isotope enrichment of ^{13}C and ^{15}N in sponge tissue (left) and detritus (right). (**A**) Enrichment after 4 d, and (**B**) enrichment after 8 d of experiment. Different colors showed the two-temperature treatment at 28°C (blue) and 32°C (red) for both sponges from Bouraké (dots) and Control (triangles). The stable isotope signature of sponge tissue ($\delta^{13}C$ and $\delta^{15}N$), and the control enrichment of only seawater incubations for detritus are reported as ctrl (black square). Data for samples are expressed as specific enrichment $\Delta\delta^{13}C$ (‰) and $\Delta\delta^{15}N$ (‰).

When the fluxes of carbon and nitrogen were normalized to the weight of the sponge by obtaining the specific ^{13}C and ^{15}N incorporation, the responses of the sponge were confirmed (Table 1; Fig. 4). Significant differences in the interaction of temperatures and origins were found in sponges tissue at both day 4 and 8 and in ^{13}C detritus at day 8 (Table 1; Fig. 4a,b). The ^{15}N

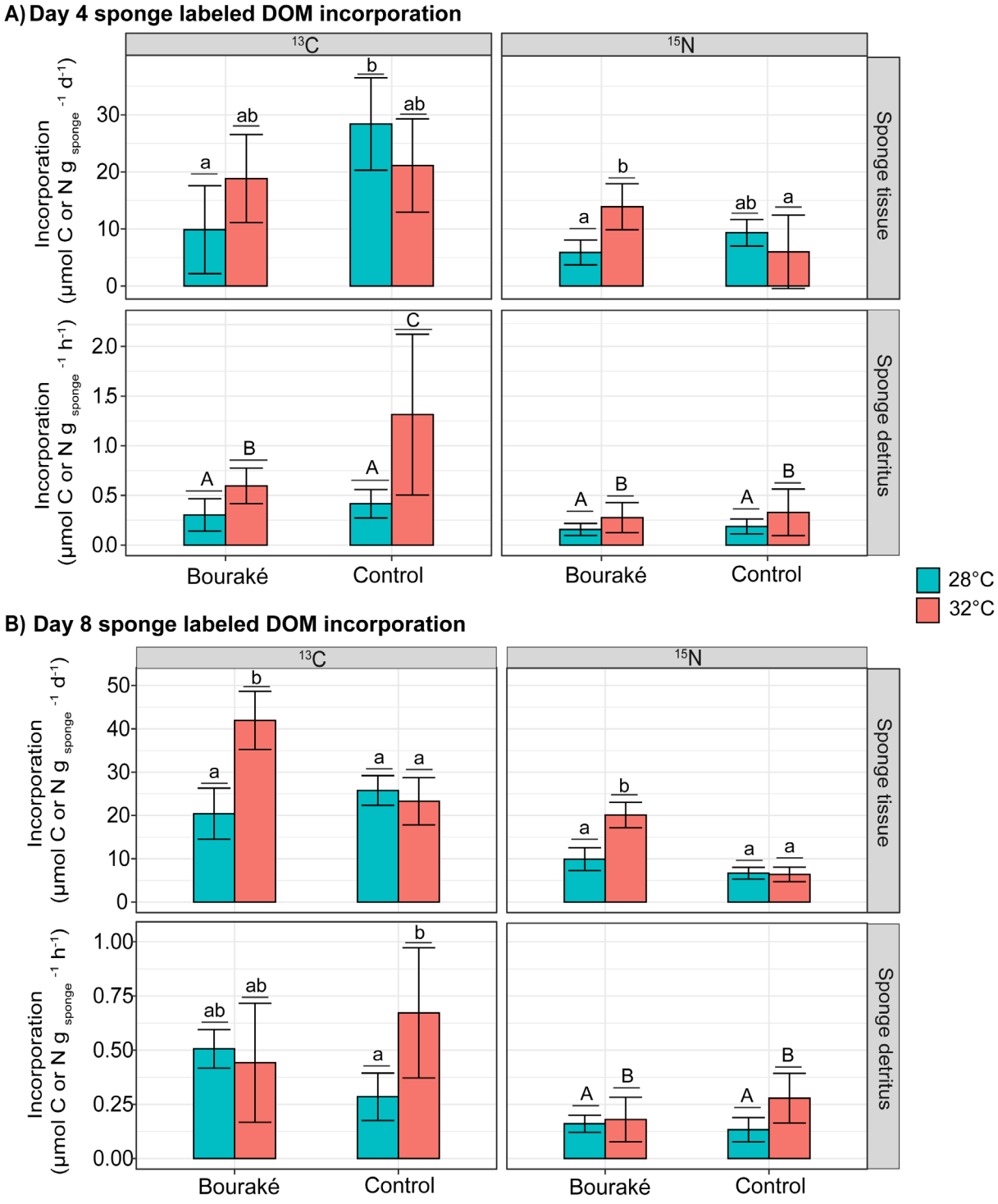

Fig. 4. Incorporation of labeled DOM in sponge tissue and detritus. (**A**) Labeled DOM incorporation after 4 d, and (**B**) labeled DOM incorporation after 8 d of experiment. Different colors showed the two temperatures treatment at 28°C (blue) and 32°C (red) for both sponges from Bouraké and control (Tupeti). Sponge tissue labeled DOM incorporation is normalized by day (d^{-1}), while sponge detritus labeled DOM incorporation by hour (h^{-1}). Bars represent the mean incorporation ± SD. Capital letters referred to ANOVA two-way test, while small letters referred to Tukey post-hoc test. Unlike letters mean significant differences ($p < 0.05$).

sponge detritus showed significant differences only between temperatures at both day 4 and 8 while ^{13}C sponge detritus showed differences in both temperatures and origins (Table 1; Fig. 4a,b). Specifically, at day 4, Bouraké sponges tissue showed two times higher average incorporation of both ^{13}C and ^{15}N at 32°C (18.8 ± 7.7 μmol C g^{-1} d^{-1}, and 13.9 ± 4.0 μmol N g^{-1} d^{-1}, respectively) compared to 28°C (9.9 ± 7.7 μmol C g^{-1} d^{-1}, and 5.9 ± 2.2 μmol N g^{-1} d^{-1}, respectively), with significant differences only for ^{15}N (Fig. 4a; Table 1). In addition, average incorporation of ^{15}N in Bouraké sponge tissue at 32°C was two times higher than in control sponge tissue at 32°C (Table 1; Fig. 4a). Control sponge tissue showed no significant differences in both ^{13}C (28.4 ± 8.1 μmol C g^{-1} d^{-1} at 28°C, and 21.1 ± 8.2 μmol C g^{-1} d^{-1} at 32°C) and ^{15}N (9.3 ± 2.3 μmol N g^{-1} d^{-1} at 28°C, and 6.0 ± 6.4 μmol N g^{-1} d^{-1} at 32°C) incorporation between temperatures, although reach the highest incorporation of 28.4 ± 8.1 μmol C g^{-1} d^{-1} ^{13}C at 28°C (Fig. 4a; Table 1). Bouraké sponge detritus showed higher significant incorporation of ^{15}N and ^{13}C at 32°C (0.28 ± 0.2 μmol N g^{-1} h^{-1}, and 0.60 ± 0.2 μmol C g^{-1} h^{-1}, respectively) compared to 28°C (0.16 ± 0.06 μmol N g^{-1} h^{-1}, and 0.30 ± 0.2 μmol C g^{-1} h^{-1}, respectively) (Fig. 4a, Table 1). Control sponge detritus showed the highest ^{13}C and ^{15}N incorporation at 32°C (1.31 ± 0.8 μmol C g^{-1} h^{-1}, and 0.33 ± 0.2 μmol N g^{-1} h^{-1}, respectively) with significant differences from 28°C (0.42 ± 0.1 μmol C g^{-1} h^{-1}, and 0.19 ± 0.07 μmol N g^{-1} h^{-1}, respectively) (Fig. 4a, Table 1).

At day 8, Bouraké sponge tissues showed more than two times higher average incorporation of both ^{13}C and ^{15}N at 32°C (42.0 ± 6.7 μmol C g^{-1} d^{-1}, and 20.1 ± 2.9 μmol N g^{-1} d^{-1}, respectively) compared to 28°C (21.3 ± 7.1 μmol C g^{-1} d^{-1}, and 9.9 ± 2.6 μmol N g^{-1} d^{-1}, respectively) (Fig. 4b; Table 1). As for day 4, control sponge tissue showed no significant differences in the average ^{13}C and ^{15}N incorporation between temperatures (Fig. 4b, Table 1). Bouraké sponge detritus did not show differences in the incorporation of ^{13}C between 28°C and 32°C (Fig. 4b, Table 1). Like day 4, control sponge detritus showed the highest ^{13}C and ^{15}N incorporation at 32°C (0.67 ± 0.3 μmol C g^{-1} h^{-1}, and 0.28 ± 0.1 μmol N g^{-1} h^{-1}, respectively) with significant differences from 28°C (0.28 ± 0.1 μmol C g^{-1} h^{-1}, and 0.13 ± 0.06 μmol N g^{-1} h^{-1}, respectively) (Fig. 4b, Table 1).

The ^{13}C : ^{15}N ratio of coral mucus-derived organic matter in sponge tissues at both day 4 and 8 showed significant differences between origins, with twice higher ratio in control sponges compared to Bouraké (Table 1; Fig. S4). The ^{13}C : ^{15}N ratio in sponge detritus showed significant interaction between origin and temperature at day 4, with control sponges at 32°C that had the highest ratio of 5.4 ± 1.9 (Table 1, Fig. S4). Sponge detritus ^{13}C : ^{15}N ratio showed significant differences in the origin at day 8 with higher values for Bouraké sponges compared to control sponges (Table 1, Fig. S4).

Discussion

In a previous study we measured in situ the ability of the sponge *R. globostellata* living in the lagoon of Bouraké to recycle nutrients and organic matter (Maggioni et al. 2023*a*). Data showed that the massive sponge consumed DOC and produced detritus. However, the process was stopped when sponges underwent very high temperatures (up to 31°C), that is, the sponge did not release detritus. Although it was possible to obtain these results because the measurements were made in situ and using a sponge species probably adapted to extreme conditions, it was not possible to identify whether temperature was the main cause of the change in resource allocation. Indeed, in experiments conducted in situ, multiple factors can act in combination and synergy. In addition, we only used sponges from Bouraké, lacking a control population living at "normal" reef conditions. Based on these preliminary findings, in the present study (1) the effect of temperature was tested by simulating in aquaria the thermal regime experienced at Bouraké and at a control reef; (2) enriched stable isotopes were used to follow the allocation of ^{13}C and ^{15}N resources into the sponge's tissue and detritus; (3) the responses of sponges living at the extreme reef at Bouraké were compared with a control reef.

Our study showed that sponges from Bouraké, incorporate almost double ^{13}C and ^{15}N derived from coral mucus into the tissue at 32°C than at 28°C. The control sponges had no differences in labeled DOM derived coral mucus incorporation in tissue between 32°C and 28°C. In contrast, Bouraké sponges showed slight differences in ^{13}C and ^{15}N incorporation in detritus among temperatures, while control sponges showed significant higher detritus ^{13}C and ^{15}N incorporation at 32°C compared to 28°C.

The ability of sponges to take up the DOC produced by the reef is well known (e.g., Mueller et al. 2014; Gantt et al. 2019; Maggioni et al. 2023*a*; Ribes et al. 2023), as well as the role of the associated microbes in the C and N cycles (e.g., Rix et al. 2020; Campana et al. 2021; Hudspith et al. 2021). The DOC can be recycled and resources are mainly used for cell turnover, which cause the release of detritus (i.e., sponge loop pathway, De Goeij et al. 2013), or mainly used for tissue growth and retained as biomass (e.g., Pawlik et al. 2016; McMurray et al. 2018). The factors triggering the two distinct pathways have not been fully clarified. However, it is likely that the differential reallocation of C and N resources from DOM via the two pathways is a sort of plasticity that some sponges can use in a changing environment and under stressed conditions (Alexander et al. 2015*a*,*b*; De Goeij et al. 2017). In agreement with our previous in situ measurements (Maggioni et al., 2023*a*), sponges from Bouraké incubated at 28°C recycled DOC and released significant amount of detritus during incubations (i.e., sponge loop pathway), while no more significant release of detritus was found at 32°C (Fig. 2). In contrast, control sponges did not produce/

consume significant quantity of detritus at both 28°C and 32°C. Although our elemental fluxes need to be considered carefully (i.e., we assumed a linear decrease in the respective fluxes during the 3 h incubation, and we may therefore underestimate them), the results on labeled DOM incorporation from coral mucus seem to go in the same direction (Fig. 4). At both days 4 and 8, Bouraké sponges showed double incorporation of ^{13}C and ^{15}N in tissue at 32°C than at 28°C, while control sponges showed an opposite response with higher incorporation at 28°C than 32°C. We hypothesized that at 28°C Bouraké sponges invest resources in cell turnover, while reallocate them to somatic growth (or other metabolic processes) at 32°C, resulting in higher tissue incorporation and enrichment at 32°C. Whereas, control sponges invest more resources in somatic growth (i.e., with less cell turnover) at 28°C and increase detritus release at 32°C. Looking to the ^{13}C and ^{15}N incorporation at day 4 into sponges detritus, Bouraké sponges detritus at 32°C was more enriched in ^{13}C, confirming the lower cell turnover. Interestingly, control sponges, despite seem to have higher cell turnover at 32°C (i.e., lower ^{13}C incorporation in tissue) showed higher detritus incorporation, suggesting that this detritus might be due to tissue degradation and not cell turnover. The ^{15}N incorporation showed the same trend even if the differences were smaller (Fig. 4a). These responses were also confirmed by the results on labeled DOM incorporation after 8 d of sponge exposure (Fig. 4b). In this case, the incorporation of ^{13}C and ^{15}N in Bouraké sponge tissue was higher and increased by almost twice compared to data obtained after 4 d of incubation. Similarly, control sponges continued to show higher detritus incorporation at 32°C than at 28°C. These results confirm that the two sponges have a different reallocation of ^{13}C and ^{15}N resources between somatic growth and cell turnover. This difference may depend, among other factors, on the environmental characteristics of the sponges' origin (i.e., their thermal history) and their degree of tolerance to high temperatures.

At 32°C, sponges adapted to lower temperature (control) may have increased cell turnover to maintain their tissue homeostasis, leading to an increased detritus release. Cell shedding is the result of the cell turnover that allows sponges to generate new choanocyte and maintain homeostasis (De Goeij et al. 2009). Alteration in choanocyte chambers size and density has already been reported on non-adapted sponges exposed to thermal stress (> 31°C) (Massaro et al. 2012). Massaro et al. (2012) also hypothesized that, under stress conditions, sponges may reduce the generation of new choanocyte and continually shed choanocyte cells, consequently producing detritus. Our findings on non-adapted sponges (control) differed to previous studies showing that sponges exposed to stress conditions cease to produce detritus (Alexander et al. 2015*a*,*b*; De Goeij et al. 2017). However, since the incubation time we used in this experiment unable us to distinguish the origin of detritus, we can only hypotheses that the detritus measured on control sponges exposed to 32°C could be due to a stress response to the high temperature that caused tissue degradation and disintegration. This hypothesis in control sponges was supported by (1) the absence of a difference in tissue ^{13}C and ^{15}N incorporation between 32°C and 28°C, (2) the higher incorporation of ^{13}C and ^{15}N in sponge detritus at 32°C than 28°C, (3) the higher detritus release at 32°C (0.55 ± 1.72 μmol C g^{-1} h^{-1}) than 28°C (0.01 ± 0.51 μmol C g^{-1} h^{-1}). As a confirmation, during the recovery period 8 control sponges died or showed signs of tissue necrosis, while Bouraké sponges did not show any evident sign of stress. Unfortunately, our short-term experiment did not enable us to follow the fate of sponges in the long term, and see if this stress at 32°C was followed by tissue necrosis and injury or even the sponge death. However, it has been reported that high temperature of more than 30°C induced mortality in sponges (e.g., López-Legentil et al. 2008; Bell et al. 2018; Ramsby et al. 2018). Further long-term experiments in aquaria and a reciprocal transplantation of this species between Bouraké and the control reef will help to shed light on its ability to cope with thermal stress.

The Bouraké sponge seems to better cope with a short-term exposure to high temperatures, likely because this population have already experienced chronical exposures to temperatures around 28°C with diurnal peaks at 33°C. Indeed, as we previously found, the respiration rates of *R. globostellata* from Bouraké was not significantly affected by an increase in temperature between 26°C and 32°C, suggesting a degree of adaptation (Maggioni et al. 2023*a*). In agreement, at the high and fluctuating temperatures tested during this experiment, labeled ^{13}C and ^{15}N were still more incorporated in the tissues at 32°C than at 28°C. We hypothesize that sponge adapted to high temperatures can reallocate resources from cell turnover (i.e., high detritus production, lower tissue incorporation) to somatic growth and tissue repair (i.e., lower detritus production, higher tissue incorporation). In contrast to what we found for control sponges, the response of the adapted sponges from Bouraké is in agreement with previous studies showing that sponges under food stress conditions reduce detritus production to reallocated resources for somatic growth (Alexander et al. 2015*a*,*b*; De Goeij et al. 2017). In addition, detritus of Bouraké sponges showed slight differences in ^{13}C and ^{15}N incorporation between 28°C and 32°C, suggesting that sponges could be able to maintain an equilibrium between cell proliferation and cell shedding (i.e., rejuvenate sponge tissue), and a basal tissue homeostasis also under thermal stress. We believe that *R. globostellata* have developed some adaptive mechanisms to avoid distress during the most extreme environmental conditions, fostering its somatic growth instead of cell turnover. Although we did not measure microbes associated with *R. globostellata*, it is known that the species has a high-microbial abundance (Moitinho-Silva et al. 2017), and their microbiome in Bouraké is dominate by the phylum Chloroflexi and their SAR202 clade (Maggioni et al. 2023*b*), which could be involved in the C cycling (Bayer et al. 2018) and in sponge C reallocation. The sponge

associated bacteria also play an important role in the sponge metabolism, especially with regard to N cycling (e.g., Taylor et al. 2007). Variable NO_x production and NH_4^+ recycling were already measured in *R. globostellata* under different tide conditions (Maggioni et al. 2023*a*). In this study, we found that Bouraké sponges at 32°C incorporated more N in the tissues than at 28°C, and likely used it for bacterial metabolic processes that could give them the surplus of energy necessary to support elevated temperatures. Another adaptive process could be the reduction of the cell turnover, favoring cell proliferation than cell shedding to repair tissue damage. In fact, Bouraké sponges decreased the release of detritus from 0.66 ± 1.05 μmol C g^{-1} h^{-1} at 28°C to an uptake of -0.33 ± 0.69 μmol C g^{-1} h^{-1} at 32°C, and increased the enrichment of sponge tissue at 32°C when compared to 28°C. This could allow sponges to save energy to maintain their health during thermal stress avoiding cells degradation (i.e., detritus production), a protection mechanism that control sponges seem not to have.

Although the C and N cycling in sponges is still a mechanism that needs further investigations, the present study experimentally confirms that the sponge *R. globostellata* take up and recycles DOM. However, recycling rates and incorporation of C and N differed between sponges' origin (Bouraké and control), likely due to their different ability to tolerate stressful temperature conditions. Adapted sponges showed a mechanism to cope with high temperatures by reallocating C and N resources from cell turnover to somatic growth, tissue repair and homeostasis. This mechanism could reduce damage and disintegration of sponge tissue under thermal stress conditions. However, this response involved a tradeoff that could cause a detrimental effect on the relative availability of C and N for other trophic levels. Future works should include *R. globostellata* cell kinetics, and the use of NanoSIMS technique to better understand the sponge utilization of C and N incorporated in tissues and released as detritus.

While at the normal summer temperature of 28°C both Bouraké and control sponges assimilated DOC and released it as detritus, at the highest temperature of 32°C the detritus was not released (i.e., negative value) by Bouraké sponges, and released (i.e., positive value) by control sponges (Table S1, Fig. 2), the later showing higher variability between samples, likely because some underwent metabolic stress and cells degradation (i.e., higher detritus release). We believe that this difference between the two sponge populations is due to their thermal history, that is, the level of their adaptation to thermal stress and ability to cope with high temperatures. In fact, several studies have suggested that reef organisms living in fluctuating temperature environments are more resistant to thermal stress (e.g., Oliver and Palumbi 2011; Rivest et al. 2017). Sponges in Bouraké are chronically subjected to fluctuating temperatures, which often exceeds 28°C. For example, in summer 2020, sponges experienced temperature higher than 28°C during 40% of the time, and fluctuations in daily temperature of up to 6.5°C (Maggioni et al. 2021). Furthermore, a study in sponge's transcriptome showed that a sub-lethal exposure at 32°C may promote tissue growth and protect sponge cells against extended stress conditions, therefore increasing the sponge ability to mitigate cellular damage (Guzman and Conaco 2016). However, it is important to note that our findings may only be true for Bouraké, where environmental conditions are (1) extremes, that is, the mean in the seawater temperature, pH and dissolved oxygen are different from the control reef, and (2) variables, that is, main parameters greatly varied, and although in a predictable range, reaching values well above the physiological thresholds for many species. The differences between the two sponges suggest that Bouraké sponges are adapted and probably better equipped to respond to rising ocean temperatures. However, we cannot know whether these sponges have acquired such competence over hundreds of years of evolution or whether such competence can be acquired rapidly, and thus be a possible response also for contemporary sponges in the coming decades. In fact, the topography of Bouraké, therefore the change in the environmental conditions, was formed likely over hundreds of years, so it is likely that organisms living there have had enough time to adapt. In addition, while there is a tendency to place greater emphasis on the most important parameters regarding climate change (i.e., temperature, pH, and dissolved oxygen), it is important to note that other parameters change in Bouraké, such as the quality and quantity of nutrients in the water. All these factors can favor or worsen the organism's physiological response. Therefore, in any experiment it becomes very important to accurately describe the variation of as many parameters as possible. Another aspect to consider is the variability of environmental parameters, which, although regular and predictable, in Bouraké may not necessarily reflect the reality of the future, and this although high variability is expected in coral reefs (Kwiatkowski and Orr 2018; Burger et al. 2020).

The present study corroborated our previous findings (Maggioni et al. 2023*a*), and experimentally demonstrates that (1) the reallocation of organic matter resources in *R. globostellata* was primarily triggered by the high temperature, (2) the observed responses were unique to Bouraké sponges, probably owing to their presumed ability to cope with extreme temperature variations. This suggests that while the Bouraké sponges exhibited a preventing mechanism to avoid cell degradation with lower cell turnover to reallocate resources for somatic growth and tissue repair, the control sponges likely experienced metabolic stress and high cell degradation. If we assume that contemporary sponges will adapt as they did in Bouraké, this study suggests that future coral reefs would be likely dominated by sponges that invest more energy in their individual growth, tissue repair and homeostasis. Less detritus will be available for sponges-associated detritivores, and less detritus will reach different trophic levels. Although it remains hypothetical and restricted to a specific case, our work

highlighted that adapted sponges have developed mechanism to cope with ocean warming, changing the way by which sponges reallocate C and N resources, therefore their quality and availability in the reef.

Data availability statement

The original contributions presented in the study are included in the Supplementary Material, further inquiries can be directed demanded to the corresponding author/s.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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