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Unravelling the versatile feeding and metabolic strategies of the cold-water ecosystem engineer *Spongosorites coralliophaga* (Stephens, 1915)

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Abstract

Sponges are often major players in the functioning of shallow-water ecosystems through their high biomass and high capacity in filter feeding. In comparison, little is known about the feeding and metabolic strategies of deep-sea sponges, although they can also form dense aggregations with high biomass. This situation hinders our understanding about how some sponge species thrive under the often food-limited conditions of the deep sea. In the present study we examined the feeding and metabolic strategies of 1) the massive demosponge *Spongosorites coralliophaga*, which was recently described as an important ecosystem engineer in cold-water coral reefs (CWCRs) and 2) the anthozoan *Parazoanthus anguicomus* and the ophiuroid *Ophiura ophiura*, i.e. two dominant epibionts on *S. coralliophaga*. All three benthic species have high density at CWCRs of the North-East Atlantic and knowing their feeding strategies facilitates future studies on carbon (C) and nitrogen (N) cycling at CWCRs. The on-board feeding experiments examined the processing of four isotopically-labelled food

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sources, namely ^{15}N-ammonium chloride, ^{13}C-glucose, ^{13}C/^{15}N-labelled microalgae, ^{13}C/^{15}N-labelled bacteria by *S. coralliophaga* and its symbiotic bacteria and the epibionts *P. anguicomus* and *O. ophiura* from the Mingulay reef complex and the Logachev mound (North-East Atlantic). There were no significant differences among the three species in terms of biomass-specific C and N assimilation rates; however, there were differences among *S. coralliophaga*, *P. anguicomus* and *O. ophiura* in how they processed the food sources and this is may be linked to interspecific variability in metabolic needs. *S. coralliophaga* preferentially assimilated particulate organic N (PON) over particulate organic C (POC) while this was not the case for *P. anguicomus* and *O. ophiura*. We did not detect the ^{15}N tracer in the bacterial biomarker D-Alanine suggesting that the preferential assimilation of N over C in *S. coralliophaga* was mediated by sponge cells instead of the bacterial symbionts. *S. coralliophaga* assimilated C and N from all four food sources and this versatile feeding strategy was accompanied by an ability for *de novo* synthesis of essential and non-essential hydrolysable amino acids (HAAs). We suggest that the recorded feeding and metabolic flexibility of *S. coralliophaga* plays an important role in the survival of this massive sponge under food-limited conditions in the deep sea.

Keywords: cold-water coral reefs (CWCRs); sponges; epibionts; essential amino acids; bentho-pelagic coupling; marine food webs

1. Introduction

Cold-water coral reefs (CWCRs) are important marine ecosystems found throughout the world's oceans down to 3,000 m water depth (Roberts et al., 2006). These are heterotrophic ecosystems that mainly depend on the supply of suspended particulate organic matter (POM) from the upper layers of the ocean (Duineveld et al., 2007; Soetaert et al., 2016). The suspended POM concentration decreases with depth. For example, in surface waters (0-150 m water depth) the average concentration of suspended

POM is about four times higher than between 150-4,000 m water depth (Copin-Montegut and Copin-Montegut, 1972; Fehling et al., 2012). In addition, the quality of organic matter decreases with increasing depth (e.g. Kiriakoulakis et al., 2007; Duineveld et al., 2012). Despite these decreases in the quantity and quality of POM with depth, CWCRs have been identified as hotspots of biomass, organic matter (OM) cycling (van Oevelen et al., 2009; White et al., 2012) and biodiversity, hosting more than 2500 species (Roberts and Cairns, 2014). At the CWCRs of the North-East Atlantic, sponges are among the most species-rich Phyla with hundreds of species being recorded (van Soest and Lavaleye, 2005; Roberts et al., 2009). However, available information about sponge biology in CWCRs is limited (van Duyl et al., 2008; Rix et al., 2016; see also Gatti et al., 2002; Thurber, 2007 for relevant studies in Antarctica) hindering our understanding about the mechanisms supporting the existence of all these sponge species under food-limited conditions as well as the role of sponges in the functioning of CWCRs. Studies on sponges from other deep-sea settings have shown that when these organisms form aggregations of high density/biomass they act as a nitrogen (N) sink (Hoffmann et al., 2009), a hot-spot for organic matter cycling (Cathalot et al., 2015) and they have an important impact on seawater microbial communities (Pile and Young, 2006; Yahel et al., 2007).

Studies on the feeding and metabolic strategies of sponges from shallow-water regions have shown that sponges and their symbiotic microorganisms (e.g. bacteria, Webster and Taylor, 2012 and references therein), effectively process a wide range of sources including dissolved organic carbon (DOC), particulate organic carbon (POC) and dissolved inorganic nitrogen (DIN) (Maldonado et al., 2012). This broad spectrum of utilizable sources, in combination with an often very high sponge density and biomass, can give sponges a key role in terms of pelagic-benthic coupling (Ribes et al., 2005), DIN fluxes (Jimenez and Ribes, 2007) and transfer of energy from lower to higher trophic levels in shallow-water systems (de Goeij et al., 2013).

Spongosorites coralliophaga (Stephens, 1915) is a massive demosponge that regularly occurs at CWCRs of the North-East Atlantic (van Soest and Lavaleye, 2005; Vad, 2013) and often hosts a species-rich community of epibionts composed mainly of cnidarians, ophiuroids, other sponges and bryozoans (Kazanidis et al., 2016). This community of epibionts hosted by *S. coralliophaga* had significantly higher species richness, diversity, density, and biomass than coral rubble and is dominated by the anthozoan *P. anguicomus* (Norman, 1868) and the ophiuroid *Ophiura ophiura* (Linnaeus, 1758) (Kazanidis et al., 2016). Based on this evidence, *S. coralliophaga* was characterized as an ecosystem engineer (sensu Jones et al., 1994). Despite its massive size and high density (up to 14 ind/m^2; Vad, 2013), there is no available information even for basic aspects of *S. coralliophaga*'s biology like its feeding and metabolic strategies as well as the role of symbiotic bacteria in the assimilation of food sources. Previous studies on the diet of cold-water sponges have shown that they feed on dissolved organic matter (DOM) (Rix et al., 2016), exclusively ingest particles smaller than 10μm (Witte et al., 1997), graze unselectively on ultraplankton (Pile and Young, 2006), remove up to 95% of bacteria and heterotrophic protists from the seawater (Yahel et al., 2007) and also they feed on fresh microalgae (Kazanidis and Witte, 2016). Apart from *S. coralliophaga*, information on the feeding biology of its epibionts *P. anguicomus* and *O. ophiura* is, also, absent. These two species dominate the epifaunal community of *S. coralliophaga* and studying their feeding can shed light on potential trophic niche overlap among the three species (i.e. the sponge, the anthozoan and the ophiuroid). Furthermore, *P. anguicomus* and *O. ophiura* are among the macrobenthic organisms with the highest density and biomass at Mingulay reef complex (MRC hereafter) in the North-East Atlantic (up to 2.4 and 2.6 ind. / sponge cm^3, respectively; Kazanidis et al., 2016; see also Vad, 2013); thus, knowledge on the assimilation of food sources by these two species can facilitate future studies focusing on C and N cycling at CWCRs (e.g. see van Oevelen et al., 2009).

Taking into account the wide range of food sources that can be used by sponges (see above), we tested the feeding and metabolism of *S. coralliophaga* by specifically selecting the following four

isotopically-labelled sources: ^{15}N-ammonium chloride (as a form of DIN), ^{13}C-glucose (as a form of DOC), and two forms of POM: ^{13}C/^{15}N- bacteria (an important component of many sponges' diet) and ^{13}C/^{15}N- microalgae (examining the ability of *S. coralliophaga* to feed on larger suspended particles). Isotope tracing experiments are a powerful tool in the examination -among others- of food processing (e.g. preferential assimilation of one element over another, e.g. Hunter et al., 2012) and synthesis of organic compounds [e.g. hydrolysable amino acids (HAAs), Middelburg et al., 2015)]. When combined with microbial biomarkers, isotope tracing experiments can shed light on the role of microorganisms in the processing and allocation of sources (Veuger et al., 2007; Middelburg et al., 2015). The latter is especially interesting in sponges since the biomass of symbiotic bacteria can be up to 40-50% of a sponge's biomass (Hentschel et al., 2003).

We hypothesized that *S. coralliophaga* has versatile feeding and metabolic strategies and specifically the ability to a) assimilate dissolved and particulate sources and b) utilize inorganic sources in the buildup of important organic components like the HAAs. In addition, we hypothesized that *S. coralliophaga*'s symbiotic bacteria fulfil an important role in the assimilation of the sources. Finally, we hypothesized that among the three species i.e. the sponge *S. coralliophaga*, the anthozoan *P. anguicomus* and the ophiuroid *O. ophiura* there would be differences in the assimilation rates and the processing of the sources.

Sponge specimens used in the ex-situ feeding experiments of the present study were collected from two CWCRs sites in the North-East Atlantic, namely the MRC and the Logachev mound (LM hereafter). Sponge specimens with the epibionts *P. anguicomus* and *O. ophiura* were collected only from MRC (see Table 1 below for details).

2 Materials and Methods

2.1 Collection of samples

Spongosorites coralliophaga specimens with and without epifauna were collected from MRC in the Outer Hebrides Sea and LM in the Rockall Bank, during the "Changing Oceans" expedition with Royal Research Ship "*James Cook"* (JC 073) in May and June 2012. The MRC and LM represent contrasting CWCRs in the North-East Atlantic. MRC is an inshore reef (~130-190 m depth) where bottom currents are up to 60 cm s^{-1} (Davies et al., 2009; Moreno Navas et al., 2014) while in the deeper (~500-1200 m depth) offshore LM the currents are up to 30 cm s^{-1} (Duineveld et al., 2007; Mohn et al., 2014). Sea-surface Chl-a concentrations (Fehling et al., 2012) and near-seabed polyunsaturated fatty acid (PUFAs) concentrations (Kiriakoulakis et al., 2007; Duineveld et al., 2012) indicate higher productivity at MRC than at LM. Finally, it has been shown that in MRC there is tight pelagic-benthic coupling i.e. food particles are transported from the ocean surface down to the seabed in less than an hour (Davies et al., 2009; Findlay et al., 2014) while in LM such a rapid vertical transport has not been reported (Duineveld et al., 2007; Mienis et al., 2007; Mohn et al., 2014; Soetaert et al., 2016).

S. coralliophaga specimens are large in size (see Fig. 1A) and yellow in color and were therefore easily spotted during surveys with the remotely operated vehicle (ROV) "*Holland I*". Specimens were collected using the vehicle's manipulator arm and placed in the vehicle's biobox, where they were kept until the return to the surface. Collection depth at MRC ranged from 122 to 131 m and from 683 to 800 m at LM. Due to a leakage in the vehicle's biobox, sponge specimens and their epifauna collected from MRC (i.e., those used in the MRC1 experiment, see below) were exposed to air for a few minutes after retrieval, before being transferred into the on-board acclimation tank (volume 500L, maximum flow of water in the pump was 3500L/h). In all the other cases (i.e. MRC2 and LM experiments) sponges were always kept underwater. Sponges and their epifauna from MRC and LM were kept in the same acclimation tank, which was filled with unfiltered seawater (collected with Niskin bottles attached to the CTD instrument) that was kept at 9^{o}C (using Aqua Medic chillers). This temperature is similar to bottom water values at MRC and LM (i.e. 9.0-9.9 ^{o}C). Every 2 to 3 days the seawater in the acclimation tank was

renewed with fresh unfiltered seawater. The maximum period that sponges were kept in the acclimation tank before being used in the feeding experiments was 72 h.

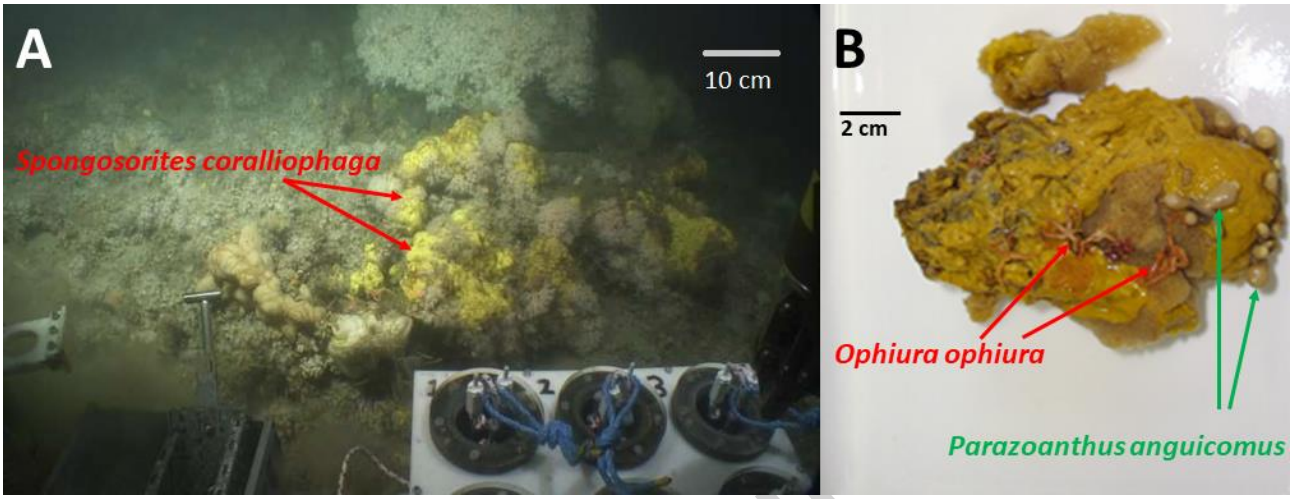

Fig. 1 (A) *Spongosorites coralliophaga* in Mingulay reef complex (MRC; Outer Hebrides Sea, North-East Atlantic). Image credit: "Changing Oceans" expedition (JC073). (B) *Spongosorites coralliophaga* (yellow sponge) with epibionts from MRC including the anthozoan *Parazoanthus anguicomus* and the ophiuroid *Ophiura ophiura*. Image credit: Georgios Kazanidis

2.2 Feeding experiments

2.2.1 Preparation of food sources (isotopically-labelled substrates)

Four isotopically-labelled substrates i.e. $^{15}NH_4Cl$ (ammonium chloride), ^{13}C-glucose, $^{13}C/^{15}N$-microalgae (the diatom *Thalassiosira rotula*) and $^{13}C/^{15}N$-bacteria were used. Ammonium chloride (Cambridge Isotope Laboratories, 99% ^{15}N labelling) and glucose (Cambridge Isotope Laboratories, 99% ^{13}C labelling) were dissolved before the research cruise in 0.2-μm-filtered seawater and samples were stored in 4^{o}C until use in the incubations. Microalgae and bacteria were dual-labelled with ^{13}C and ^{15}N stable isotopes. The diatom *T. rotula* was cultured in artificial seawater amended with *f*/2 medium (Guillard and Ryther,

1962; Guillard 1975) and enriched with 50% ^{13}C-bicarbonate ($NaH^{13}CO3$, Cambridge Isotope Laboratories) and 50% ^{15}N-sodium nitrate ($Na^{15}NO3$, Cambridge Isotope Laboratories). The diatom culture grew at 16^{o}C for a period of 21 days and with a light-dark cycle of 16:8 hours. The algae were harvested by centrifugation, rinsed in ultrapure water (milli-Q), lyophilized (-60^{o}C; -0.0001 mbar; 24 h) and stored at -20^{o}C. The produced detrital material was resuspended in glass fiber-filtered (GF/F) seawater before being used in the experiments. A natural community of bacteria was derived from a few mL of natural seawater, obtained from the Oosterschelde estuary (salinity 30) in the South-West of the Netherlands. The water sample was added to 1 L culture medium (M63) containing 0.02 mol L^{-1} glucose (10 atom% ^{13}C, Cambridge Isotopes) and 0.01 mol $^{L-}$1 ammonium chloride (10 atom% ^{15}N, Cambridge Isotopes). After 3 days of culturing in the dark, bacteria were concentrated by centrifugation (14 500 g) and rinsed with 0.2 μm filtered seawater to remove residual labelled substrates. Bacteria in the concentrate were kept frozen until use in the experiment.

2.2.2 Experimental set up

The condition of sponges was checked on the basis of their body coloration and signs of injuries. Specimens used in the feeding experiments had a natural yellow color and no injuries. The specimens of *S. coralliophaga* with epibionts (MRC1 experiment), due to their larger size (see Table 1 for details), were placed in 12-liter chambers and the *S. coralliophaga* specimens without epibionts (MRC2 and LM experiments) were placed in 6-liter chambers. The incubation period in the MRC1 experiments was 6 h and in MRC2 and LM experiments was 24h. Although the pumping activity of *S. coralliophaga* during the experiments was not measured, the good condition of sponges was also examined through measurements of oxygen consumption rates. Before conducting the measurements of oxygen consumption rate, the optodes (optical sensor devices) used were calibrated according to the AANDERA TD 218 operating manual using water samples of 0% and 100% oxygen saturation as calibration points.

Oxygen concentration (SE ±0.01 μmol L^{-1}) and temperature (SE ±0.01^{o}C) values were logged every 5 min at a control unit. Oxygen concentration inside chambers was not allowed to drop below 70% of the oxygen concentration at the beginning of the incubation (Hulth et al., 1994; Gontikaki et al., 2011). The duration of the incubation periods (Table 1) was also based on previous works with organisms from CWCRs (e.g. the cold-water sponges *Higginsia thielei* and *Rossella nodastrella* - van Duyl et al., 2008; the cold-water coral (CWC) *Lophelia pertusa* and the polychaete *Eunice norvegica* - Mueller et al., 2013, 2014). Due to limited availability of optodes, the oxygen concentration was measured in one/two out of three experimental chambers and in one control chamber (Table 1). Oxygen consumption was calculated from the decrease rate of oxygen concentration in the experimental and control chambers and it was expressed in μmol O_2 g dry weight of the specimens $(DW)^{-1}$ h^{-1} to facilitate comparisons with previous studies (Kutti et al., 2013; Cathalot et al., 2015). During the feeding experiments, the on-board cold room unfortunately experienced unexpected temperature fluctuations (Table 1). Because of these fluctuations, oxygen consumption was only inferred for time periods when the range of temperature variation was restricted to 1^{o}C (e.g., 7-8^{o}C or 8-9^{o}C). The effect of a relatively high temperature on oxygen consumption in the MRC1 experiment was corrected to 9^{o}C using a Q_{10} value of 8 (Dodds et al., 2007). Oxygen consumption rates for *S. coralliophaga* (i.e. 0.97±0.76 μmol O_2 g^{-1} sponge dry weight (DW) h^{-1} (Table 1) are in good agreement with values reported for the cold-water massive demosponge *Geodia barretti* (i.e., 1.5 μmol O_2 g^{-1} DW h^{-1}, Kutti et al., 2013, Cathalot et al., 2015), providing evidence about the good condition of *S. coralliophaga* during the feeding experiments. Evidence about the good condition of the *P. anguicomus* specimens was provided from observations on their tentacles, which were extended during the feeding experiments.

Table 1 Characteristics of the feeding experiments from Mingulay reef complex (MRC) and Logachev mound (LM). '*S. coralliophaga*' means '*Spongosorites coralliophaga*', '*P. anguicomus*' means '*Parazoanthus anguicomus*', and '*O. ophiura*' means '*Ophiura ophiura*'. Food sources: Glu:^{13}C-glucose; Amm:^{15}N-ammonium chloride; Microalgae: ^{13}C/^{15}N-microalgae; Bacteria: ^{13}C/^{15}N-bacteria; mg C: milligrams of carbon; mg N: milligrams of nitrogen. Oxygen consumption rates denoted with an asterisk (*) in MRC-1 have been corrected through the Q_{10} value [Q_{10}=8; see Dodds et al. (2007)] due to higher temperature in the incubation chambers than the *in-situ* temperature. The correction of oxygen consumption's rate was done using 9^{o}C as the reference value of temperature (i.e. temperature of seawater close to the bottom of MRC and LM during sample collection). Oxygen consumption rates have, also, been corrected for bacterial respiration in seawater. 'N/A' means 'not measured'.

Specimens used	Sampling region	Chamber size (L)	Incubation period (h)	Food sources	Number of chambers for recording oxygen consumption	Oxygen consumption rates of *S. coralliophaga* (μmol O_2 g DW^{-1} h^{-1})	Sponge Biomass (mg C) (mean±SD)	Sponge Biomass (mg N) (mean±SD)	Total C (mg) added in each chamber	Total N (mg) added in each chamber	Food source labelling C(%)	Food source labelling N(%)	Temperature range (^{o}C) in experimental chambers
S. coralliophaga with *P. anguicomus* and *O. ophiura*	MRC-1	12	8	Glu+Amm	1	1.50*	6974.6±2429.6	1621.9±559.6	15.6	0.04	99	99	13.4-14.2
		12	8	Microalgae	1	2.75* N/A	3710.6±1138.6	821.8±299.8	15.6	3.54	44	46	12.6-13.6
S. coralliophaga	MRC-2	6	24	Glu+Amm	N/A	N/A	977.9±263.6	222.3±71.1	7.8	0.02	99	99	7.3-9.2
		6	24	Microalgae	N/A	N/A	1534.4±566.5	334.2±130.7	7.8	1.77	44	46	6.6-9.2
		6	15	Bacteria	2	1.68	3063.6±347.2	707.1±83.7	7.8	2.28	47	44	6.9-9.4
S. coralliophaga	LM	6	24	Glu+Amm	2	0.76	2751.9±817.7	606.4±183.1	7.8	0.02	99	99	7.2-9.6
		6	24	Microalgae	2	0.32	2978.6±781.5	658.6±144.1	7.8	1.77	44	46	6.1-9.5
		6	24	Bacteria	2	0.60	2493.7±1137.5	566.6±272.0	7.8	2.28	47	44	5.7-8.6

Sponge specimens were placed in chambers filled with GF/F-filtered seawater (Table 1). The chambers were made from a transparent polycarbonate tube with an acetal plastic lid on top and they were supplied with a stirrer for the mixing of seawater and an optode (AANDERAA 3930) for measuring oxygen concentration and temperature inside chambers (see above for details on measurements of oxygen consumption rate). The feeding experiments were conducted in the dark.

One sponge specimen was placed in each chamber (Table 1). For each feeding experiment (i.e. MRC1, MRC2 and LM) three replicate incubations were run per food source (glucose/ammonium chloride, microalgae, bacteria). In the MRC1 experiment, we had also three replicates of *P. anguicomus* and three replicates of *O. ophiura* whereas in MRC2 and LM the specimens of *S. coralliophaga* had no epibionts. We had also three replicates used as a control (i.e. specimens that were not fed). The three replicate incubations were used also for measuring the respiration of the food sources into dissolved inorganic carbon (DIC). There was one chamber used as a control for the DIC measurements (i.e. chamber where only the food source was added and there were no specimens).

The food sources (i.e. isotopically-labelled substrates; see following section for details) were added in a concentration of 100 μmol L^{-1} C to approach the concentration of DOC close to the Rockall Bank seafloor values i.e. 51.0-73.0 μM (van Duyl et al., 2008). Ammonium chloride was added at 0.2 μM to approach the NH_4^+ ammonium close to the Rockall Bank seafloor values i.e. 0.14-0.30 μM (van Duyl et al., 2008). The total amount of C and N added in each chamber can be seen in Table 1.

2.3 Sample treatment and analyses

2.3.1 Processing of food sources by *Spongosorites coralliophaga*, *Parazoanthus anguicomus* and *Ophiura ophiura*

After the feeding experiments, sponge tissue was separated from the coral rubble using a scalpel and a pair of forceps, collected in plastic bags and stored in -20°C until analysis. Epifauna specimens were also stored in -20°C until analysis. In order to obtain values of the natural abundance of ^{13}C and ^{15}N values, three replicates of sponges and epifauna which were not used in the feeding experiments were preserved and stored in -20^{o}C for further analyses (see below).

The sponge and epifauna specimens frozen onboard were lyophilized (-60°C; -0.0001 mbar; 48 h) in the laboratory. After this, sponges and epifauna were ground using mortar and pestle, acidified through

the addition of HCl until effervescence ceased and then subsampled in tin cups (Mueller et al., 2014; Kazanidis & Witte, 2016). Organic C/N content of sponges and epifauna as well as stable isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) of bulk tissue were measured using a Thermo Electron FlashEA 1112 analyzer (EA) coupled to a Delta V isotope ratio mass spectrometer (IRMS) at Royal Netherlands Institute for Sea Research (NIOZ) in Yerseke.

Water samples were collected in triplicate from the chambers in order to study the respiration of the food sources into dissolved inorganic carbon (DIC). Specifically, water samples (5 mL) for $DI^{13}C$ analysis were taken immediately after the addition of the labelled food sources (t=0) and at the end of the experiment. Sampled water at t=0 was replaced with glass fiber-filtered seawater. The $DI^{13}C$ water samples were filtered through a 0.2 μm syringe filter and stored in 3.6 mL exetainers. Saturated $HgCl_2$ was added (0.2% v/v) to stop biological activity after which samples were stored at 4^{o}C until analysis. Background production of $DI^{13}C$ was estimated in control chambers to which only the isotopically-labelled food sources was added (i.e. no sponge or epifauna inside the chamber). This background production of $DI^{13}C$ was subtracted from production of $DI^{13}C$ measured in the experimental chambers. Respiration of the food sources was measured through the analysis of DIC concentration and C isotopic ratios of water samples taken at the beginning and the end of the incubations.

Stable isotope data are presented as a per mille (‰) deviation from a standard as δX (‰)=((R_{sample}-$R_{reference}$)-1) x 1000, where (X) is ^{13}C or ^{15}N, R_{sample} is the isotope ratio ($^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively) in the sample and $R_{reference}$ is the isotope ratio of the reference material ($R_{reference}$=0.0111797 for C and $R_{reference}$=0.0036765 for N). The atomic fraction (F) of ^{13}C and ^{15}N in specimens was calculated with F=R/(R+1). Excess values (E) are calculated as $E=F_{sample}–F_{background}$, so that positive excess values indicate uptake of the isotopically-labelled sources. Since the level of labelling was different among the food sources (Table 1 and Supplementary Table S1), excess values were normalized by dividing them with the enrichment of the respective food source (e.g., 0.44 for microalgae-derived C). Total C and N

assimilation was calculated as the product of excess (E) and the biomass of the specimen (Moodley et al. 2005). The biomass was expressed in C and N units and calculated by multiplying the specimen's dry weight (DW) with C and N content (as % of specimen's DW). The normalization of total C and total N assimilation to specimen's biomass was carried out through the division of total C and total N assimilation by biomass (in C or N units, respectively). Normalized C and N assimilation was expressed as ng C (or N) / mg C (or N).

2.3.2 Preferential assimilation of elements by *Spongosorites coralliophaga*, *Parazoanthus anguicomus* and *Ophiura ophiura*

The total assimilation C:N ratio for each food source was compared to the C:N ratio of each food source in order to examine the preferential assimilation of one element over the other (e.g., total assimilation C:N/ food source C:N ratio >1 shows preferential C assimilation as compared to their food while <1 shows preferential N assimilation). The C:N ratio of the assimilated food source was calculated as the ratio of C%:N% (w:w). This preferential assimilation was examined a) in the MRC1 experiment for *S. coralliophaga*, *P. anguicomus* and *O. ophiura* fed with microalgae, 2) in the MRC2 and LM experiments for *S. coralliophaga* fed with microalgae and bacteria.

2.3.3 Assimilation of C and N in the HAAs of *Spongosorites coralliophaga*

HAAs were extracted from sponge tissue and the food sources (i.e., microalgae, bacteria) and analyzed according to (Veuger et al., 2005, 2007). In short, HAAs were extracted following hydrolysis of 2.3 to 9.8 mg ground sponge material (carried out using 6 mol L^{-1} HCl, 110^{o}C, 20 h), followed by purification (cation-exchange chromatography, Dowex 50WX8) and derivatization. HAAs concentrations and isotopic ratios were measured using gas chromatography coupled to isotope ratio mass spectrometry through a combustion interface (GC-c-IRMS). Measured D-alanine concentrations were corrected for hydrolysis-

induced racemization as described in Veuger et al. (2005, 2007), using the factor 0.017 for the hydrolysis-induced racemization. The bacterial biomarker D-Alanine was used to examine the role of *S. coralliophaga*'s symbiotic bacteria in the assimilation of C and N (Veuger et al., 2005, 2007). Analyses of isotopic ratios of the HAAs were conducted at NIOZ. Due to logistical constraints we could not use the same subsamples for HAAs and bulk tissue analysis. Calculations regarding stable isotope composition have been carried out following the protocol detailed in section 2.3.1.

2.4 Statistical analysis

We tested for potential differences in 1) *Spongosorites coralliophaga*'s elemental assimilation among food treatments (i.e., among glucose, microalgae and bacteria for C and among ammonium chloride, microalgae and bacteria for N) at MRC1, MRC2 and LM experiments, 2) elemental assimilation among *S. coralliophaga*, *Parazoanthus anguicomus* and *Ophiura ophiura* fed with microalgae at the MRC1 experiment, 3) *Spongosorites coralliophaga*'s C assimilation and C respiration for glucose, microalgae and bacteria at MRC1, MRC2 and LM experiments, 4) *Spongosorites coralliophaga*'s total assimilation C:N/ food source C:N ratio between microalgae and bacteria at MRC2 and LM experiments, and 5) total assimilation C:N/ food source C:N ratio among *S. coralliophaga*, *Parazoanthus anguicomus* and *Ophiura ophiura* fed with microalgae at the MRC1 experiment, using functions available in R (R core team, 2013). First, the normality of the distributions was checked with the Shapiro-Wilk test and the equality of variances with the *F*-test (for two groups) or Bartlett test (for three groups). In the case of normal distribution and equal variances, the hypothesis that the groups have the same mean was tested either through the two-sample t test (two groups) or one-way ANOVA (three groups). In the case of normal distributions and unequal variances, the hypothesis that the groups have the same mean was tested either through the Welch's two sample t-test (two groups) or one-way analysis of means (not assuming equal variances) (three groups). Finally, in the case of the normal distribution criterion did not hold, the

hypothesis that the groups have the same median was tested either through a Wilcoxon rank sum test (two groups) or a Kruskal-Wallis rank sum test (three groups). All values in the text are presented as mean±standard deviation (SD).

3 Results

3.1 Processing of food sources by *Spongosorites coralliophaga*, *Parazoanthus anguicomus* and *Ophiura ophiura*

All *Spongosorites coralliophaga* specimens from MRC and LM regions assimilated glucose-derived C and microalgae-derived C, however, there was no bacteria-derived C assimilation in MRC2 sponge specimens, while only one specimen out of three at LM assimilated bacteria-derived C (Fig. 2). Comparison of C assimilation rates among treatments (glucose, microalgae, bacteria) revealed statistically-significant differences for the MRC1 specimens. Specifically, microalgae-C assimilation (1003±280 ng C mg C^{-1} d^{-1}) was higher than glucose-derived C (23±23 ng C mg C^{-1} d^{-1}) (Welch's two-sample t-test=6.04, p=0.02) (Fig. 2). In contrast to these findings from MRC1 about the assimilation of C, there were no significant differences among assimilation rates in the MRC2 and LM experiments.

Specimens of *S. coralliophaga* from MRC and LM assimilated N from all three sources (i.e., ammonium chloride, microalgae, bacteria) (Fig. 2). Comparison of N assimilation rates among treatments revealed significant differences only at MRC2 specimens (193±24.0, 34±18, 25±11 ng N mg N^{-1} mg N^{-1} added d^{-1} for ammonium chloride, microalgae, bacteria, respectively; oneway ANOVA F=79.81, p=0.00004755) (Fig. 2).

Comparison of the assimilation of microalgae-derived C among *S. coralliophaga*, *P. anguicomus* and *O. ophiura* in the MRC1 experiment did not reveal statistically significant differences (1003±280, 1946±2570 and 1159±375 ng C mg C^{-1} d^{-}1, respectively; one-way analysis of means, F=0.41, $p=0.69$). Similarly, the comparison of the assimilation of microalgae-derived N among *S. coralliophaga*,

P. anguicomus and *O. ophiura* in the MRC1 experiment did not reveal statistically significant differences (274±66, 526±750 and 160±20, respectively; one-way analysis of means, F=3.74, p=0.16).

Comparison between C assimilation and C respiration for each of the food sources revealed statistically significant differences for MRC1 specimens fed with microalgae as well as for MRC2 and LM specimens fed with glucose (Fig. 2). Specifically, there was higher assimilation than respiration at MRC1 (1003±280 vs 260±164 ng C mg C^{-1} d^{-1}; two sample t-test t=3.95, p=0.01), higher respiration than assimilation at MRC2 (571±182 vs 39±34 ng C mg C^{-1} d^{-1}; two sample t-test t=-4.96, p=0.01) and LM (174±66 vs 2.0±2.0 ng C mg C^{-1} d^{-1}; Welch two sample t-test t=-4.49, p=0.04).

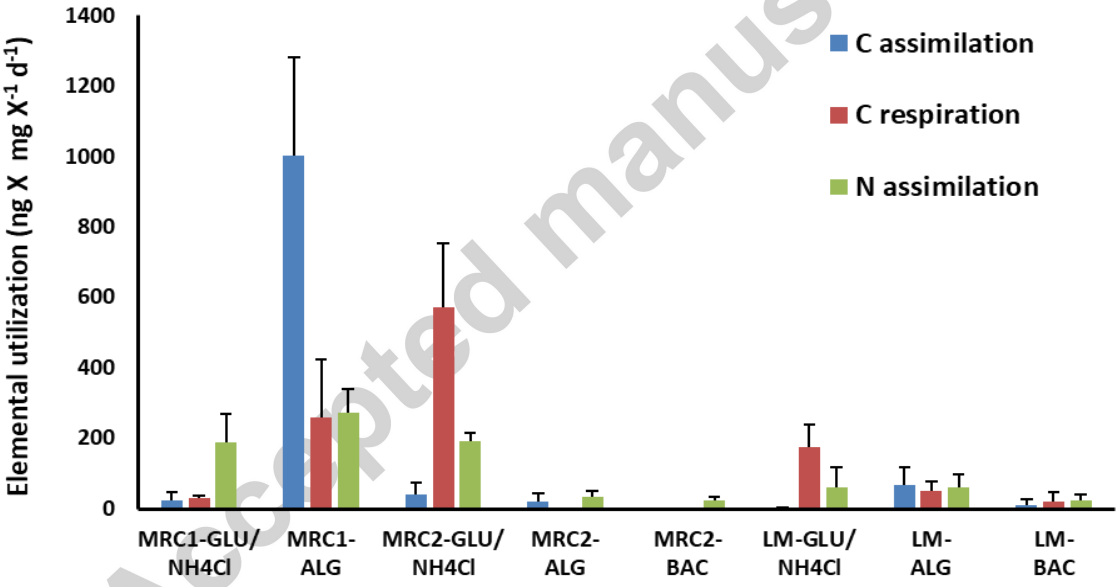

Fig. 2 C assimilation, C respiration and N assimilation in *Spongosorites coralliophaga* from MRC and LM, expressed as ng X mg X^{-1} d^{-1}, in which X represents C or N. Note that N assimilation has been normalized to the total amount of N added in each treatment (Table 1). GLU: glucose; NH_4Cl: ammonium chloride; ALG: microalgae; BAC: bacteria. The bars represent average±SD.

3.2 Preferential assimilation of elements by *Spongosorites coralliophaga*, *Parazoanthus anguicomus* and *Ophiura ophiura*

At the MRC2 and LM experiments the total assimilation C:N/ food source C:N ratio in *S. coralliophaga* was lower than 1.0 indicating a preferential assimilation of N over C (Table 2). This preferential assimilation of N over C was evident both in treatments with microalgae and bacteria. Comparison of the total assimilation C:N/ food source C:N ratio between treatments with microalgae and bacteria did not reveal statistically-significant differences neither at MRC2 (Wilcoxon rank sum test W=9, p=0.063) nor at LM (Wilcoxon rank sum test W=8, p=0.18). At the MRC1 experiment, the total assimilation C:N/food source C:N ratio in *S. coralliophaga*, *P. anguicomus* and *O. ophiura* was higher than 1.0, indicating a preferential assimilation of C over N (Table 2). Comparison of the total assimilation C:N/food source C:N ratio among the three species revealed statistically-significant differences (one-way ANOVA, F=18.74, p=0.0026).

Table 2 Food source C:N ratio, body C:N ratio, total assimilation C:N ratio and total assimilation/food source C:N ratio for MRC1, MRC2 and LM experiments. "NA" means not measured. Mean±standard deviation (SD) are given.

Species	Food source C:N ratio		Body C:N ratio		Total assimilation C:N ratio		Total assimilation C:N/ Food source C:N ratio	
	Microalgae	Bacteria	Specimens fed with microalgae	Specimens fed with bacteria	Specimens fed with microalgae	Specimens fed with bacteria	Specimens fed with microalgae	Specimens fed with bacteria
S. coralliophaga (MRC1)	4.44	NA	4.60±0.38	NA	4.72±0.43	NA	1.06±0.10	NA
S. coralliophaga (MRC2)	4.44	3.45	4.62±0.35	4.33±0.04	2.13±1.87	No uptake	0.48±0.42	No uptake
S. coralliophaga (LM)	4.43	3.45	4.49±0.27	4.42±0.10	2.50±1.15	0.47±0.81	0.56±0.26	0.14±0.23
P. anguicomus (MRC1)	4.44	NA	4.32±0.19	NA	5.01±0.53	NA	1.13±0.12	NA
O. ophiura (MRC1)	4.44	NA	4.12±0.36	NA	8.22±1.11	NA	1.85±0.25	NA

3.3 Profiles of HAAs in food sources (microalgae, bacteria) and in *Spongosorites coralliophaga*

Both for microalgae and bacteria, the highest contribution in the C pool of HAAs was from glutamine (22±2% and 20±1%, respectively) and the lowest from D-alanine (0.03±0.02% and 0.14±0.17%, respectively) (Fig. 3a). Similarly, in the N pool of HAAs the highest contribution was from glutamine (21±2% and 18±1%, respectively) and the lowest from D-alanine (0.04±0.02% and 0.21±0.25%, respectively) (Fig. 3b).

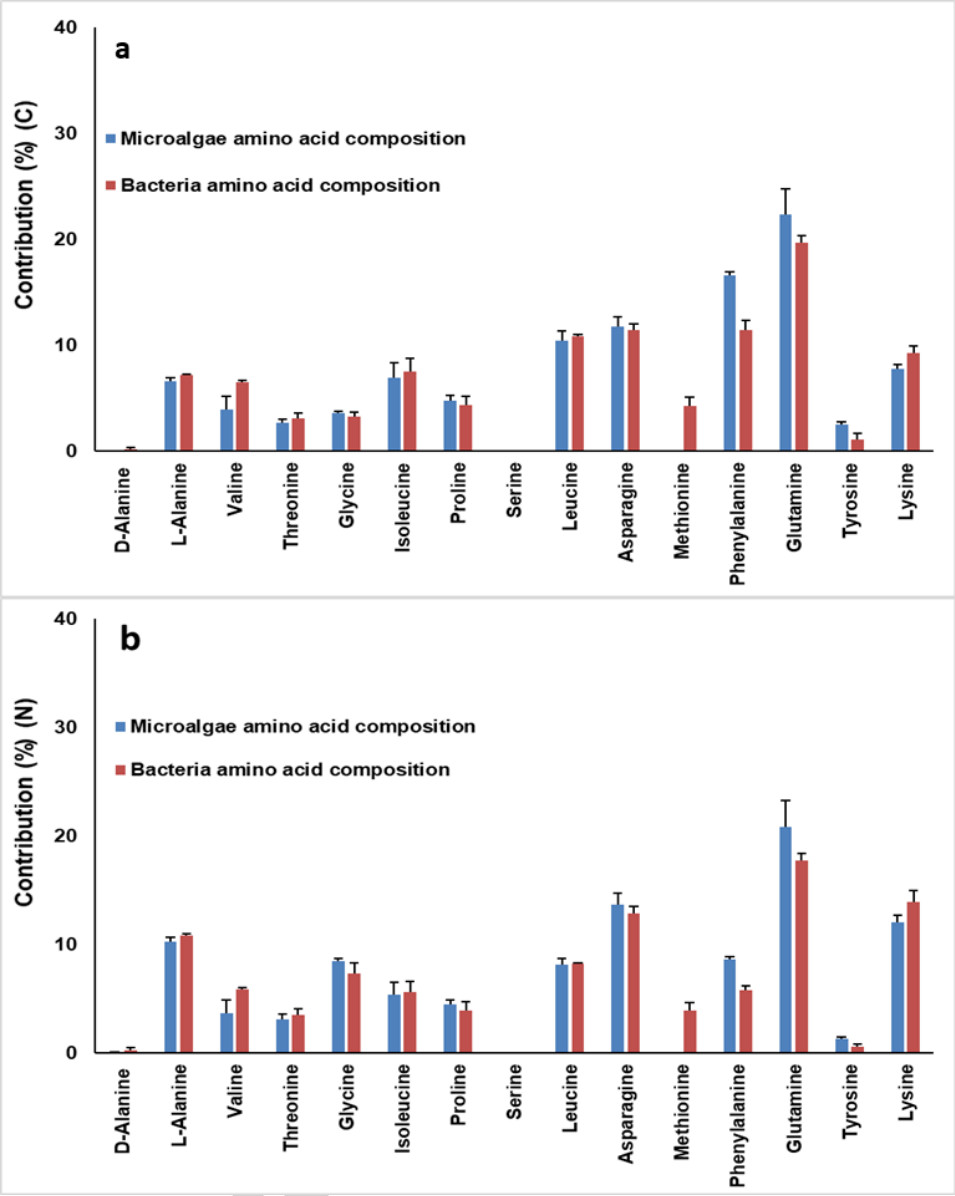

Fig. 3 Profiles of HAAs in terms of C (a) and N (b) for the food sources of microalgae and bacteria (% contribution to total concentration). The bars represent average±SD.

In *S. coralliophaga*, the HAAs comprised 31±8% and 36±8% of the total C and N content, respectively. The highest contribution in the HAAs C-pool was from glutamine (up to 16%) and asparagine (13%) and the lowest ones from D-alanine (0.6%) (Fig. 4a). For N, the highest contribution was from glycine (up to 18%) and asparagine (15%); the lowest contribution was from D-alanine (up to 1%) (Fig. 4b).

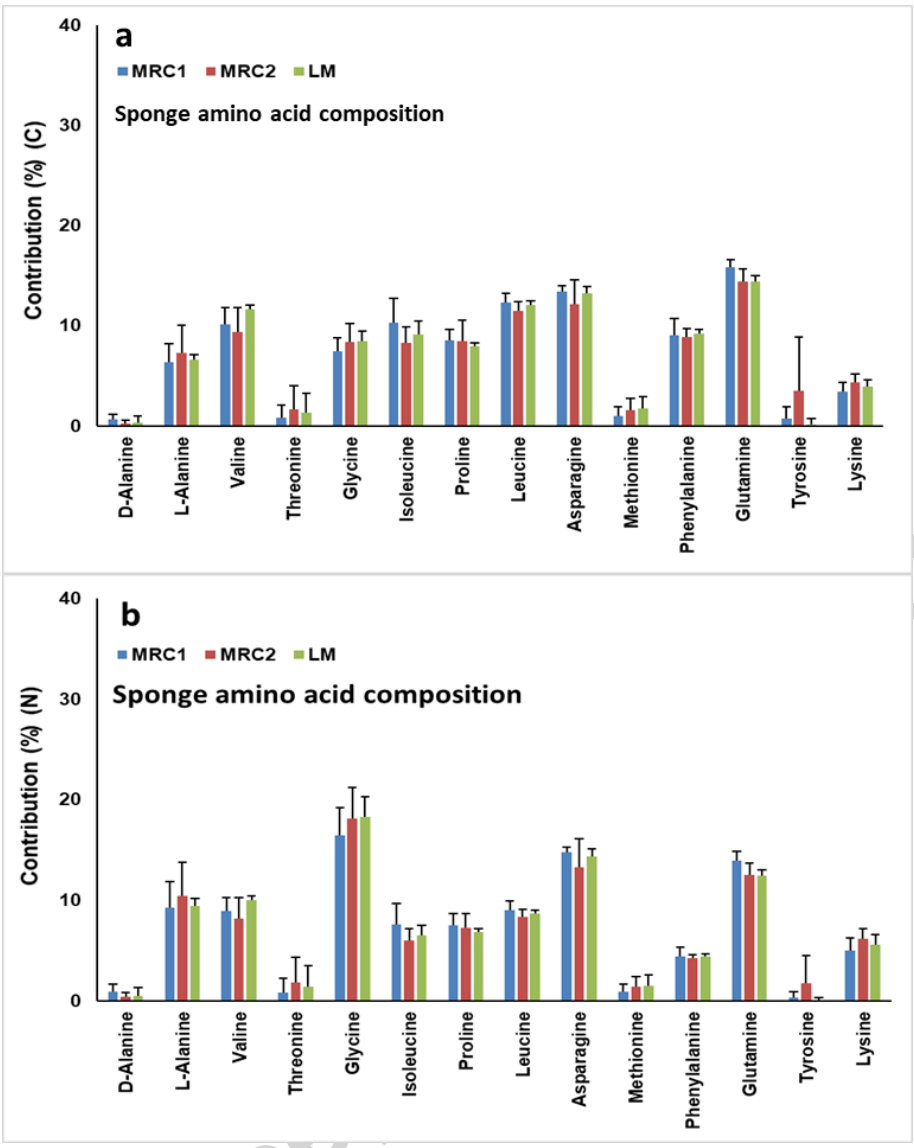

Fig. 4 HAAs in terms of C (a) and N (b) for the sponge *Spongosorites coralliophaga* in MRC1, MRC2 and LM. The bars represent average±SD.

3.4 Assimilation of C and N in HAAs of *Spongosorites coralliophaga*

At MRC1 and LM specimens, ~70% of glucose-derived C was incorporated in a single HAA (i.e., glutamine) (Fig. 5a), while microalgae- and bacteria-derived C was incorporated in a greater number of HAAs (mainly the non-essentials glutamine, phenylalanine, asparagine, and the essentials isoleucine and leucine) (Fig. 5 c, 5e). N from all sources (i.e. ammonium chloride, microalgae, bacteria) was incorporated in non-essenti al (e.g., L-alanine, glycine) and essential HAAs (e.g., threonine, leucine, lysine). Interestingly, there was no

tracer recovery in D-alanine (i.e., the bacterial biomarker) for any of the sources (low or no peaks in the c hromatogram) (Fig. 5b, 5d, 5f).

0

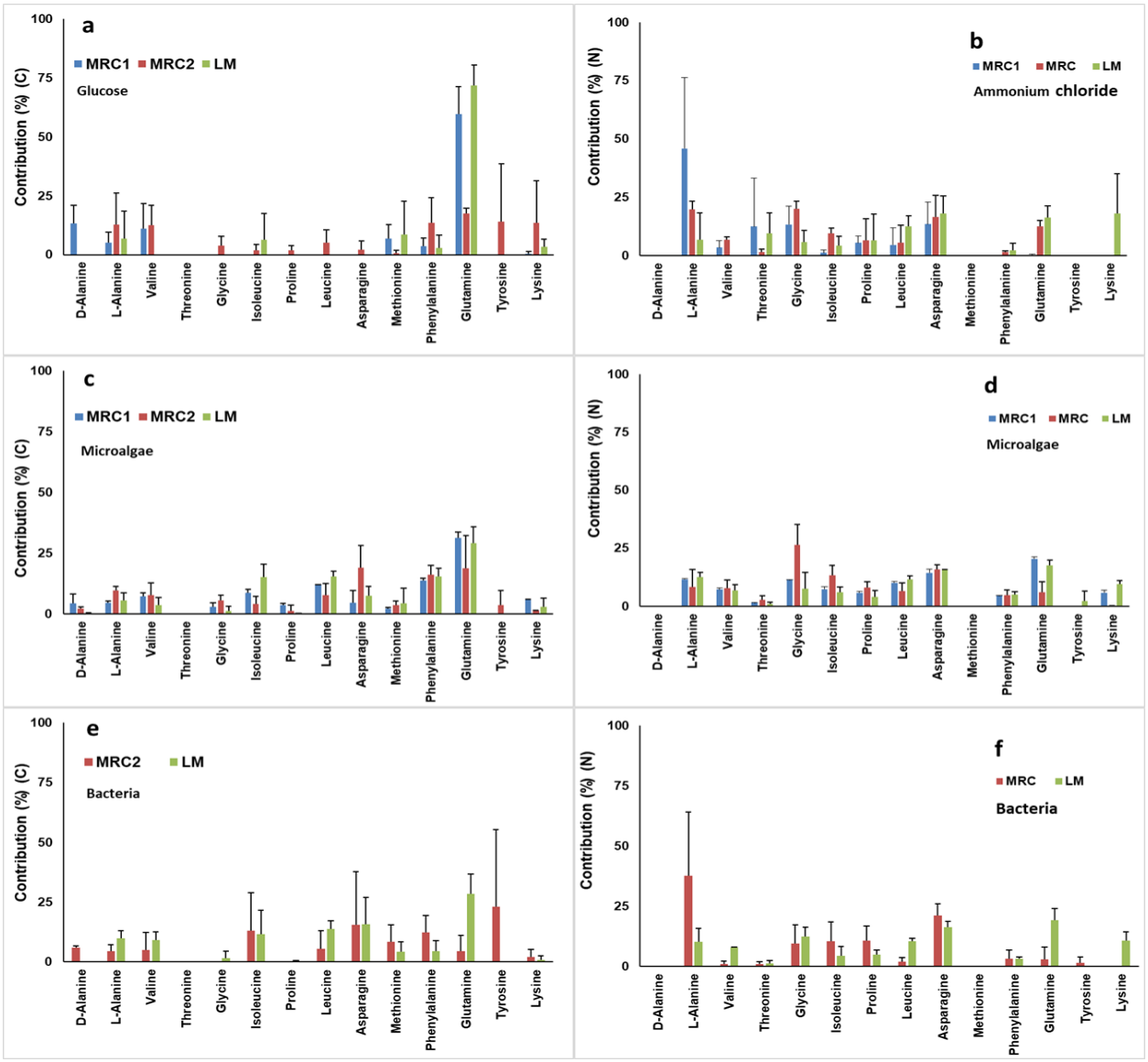

Fig. 5 Profiles of C and N assimilation [from glucose (a), ammonium chloride (b), microalgae (c, d), bacteria (e, f)] in *Spongosorites coralliophaga*'s HAAs (% contribution to total incorporation). The bars represent average±SD.

4 Discussion

4.1 Utilization of dissolved (DOM) and particulate organic matter (POM) by cold-water sponges

The massive sponge *S. coralliophaga* assimilated C from dissolved and particulate food sources, in agreement with our hypothesis. DOM is the largest source of organic matter in the water column (Libes, 2009) albeit largely of refractory nature (Burdige, 2002), thus, only a small fraction is accessible to marine organisms (Carlson, 2002; de Goeij et al., 2008; Gori et al., 2014). The uptake of glucose by *S. coralliophaga* shows that some forms of DOC may be an accessible C source for some deep-sea organisms like cold-water sponges. However, most of the glucose was respired and its contribution to the nutrition of *S. coralliophaga* is rather small; on the other hand, DOC can act as an important energy source for maintenance metabolism (Witte and Graf, 1996). Isotope tracing experiments conducted by Rix et al. (2016) showed that mucus produced by the CWC *Lophelia pertusa* was transferred into the cold-water sponge's *Hymedesmia coriacea* fatty acids (FA) (both in sponge- and bacteria-specific ones) demonstrating active processing of this form of DOC by the sponge and its bacterial symbionts. Findings from the present study and Rix et al. (2016) indicate that the structural complexity of DOC (e.g. simple forms like glucose used here *vs*. more complex forms like coral mucus used by Rix et al. (2016)), at least partly, determines its fate in the metabolic processing by deep-sea sponges.

Although a very small number of cold-water species have been studied in that respect, it seems that the abundance of symbiotic microorganisms in sponges plays an important role in the assimilation of DOC. Whereas *Aphrocallistes vastus*, which hosts no symbiotic microorganisms (Leys, 1999), does not take up DOC (Yahel et al., 2007), the high microbial abundance sponge *Geodia barretti* (Hoffmann et al., 2009) may meet more than 90% of its C budget from DOC and detritus (Leys et al., 2017). Up to now, no data

exist on the abundance and profile of symbiotic microorganisms hosted by *Spongosorites coralliophaga* and no conclusions can be drawn about the drivers shaping differences in C and N assimilation among MRC1, MRC2 and LM. However, taking into account findings about intraspecific variability of symbiotic microorganisms hosted by sponges (Weigel and Erwin, 2016; Marino et al., 2017), we suggest that differences found here among the MRC1, MRC2 and LM experiments might be related to intraspecific variability in the symbiotic microorganisms of *S*. *coralliophaga*. Furthermore, although direct evidence is not available, the differences found among the three experiments (e.g. higher assimilation of microalgae-C than glucose-C in the MRC1 experiment in contrast to MRC2 and LM findings about C assimilation) may also be related to differences in the incubation period (e.g. see in Roberts et al., 2016 and references therein about differences between short- and long-term incubation periods on respiration and calcification rates of the CWC *Lophelia pertusa*), and possible effects of elevated seawater temperature on cold-water sponge metabolism (e.g. see in Strand et al., 2017 about the effect of acute thermal stress on the ecophysiology of the boreal deep-sea sponge *Geodia barretti*). Up to now there is no available information about the role of incubation period and elevated temperature on the oxygen consumption of *S. coralliophaga* and thus in order to address that issue we used available information from work on other cold-water species (Dodds et al., 2007; Strand et al., 2017). Future experimental work could also shed light on the role of incubation period and elevated temperature on the feeding of cold-water sponges. The differences in the conditions among the three experiments (e.g. smaller incubation in MRC1 than MRC2 and LM experiments) have not enabled us to carry out a detailed comparison among the three experiments and we have mainly focused on the comparison of findings about the processing of the food sources within each of the experiments.

The assimilation of bacterial C in *S*. *coralliophaga* was smaller than of microalgae-derived C. However, previous studies on the feeding of shallow-water (e.g. Pile et al., 1997; Ribes et al., 1999, 2003; Thurber, 2007) and deep-sea sponges (Pile and Young, 2006; Yahel et al., 2007; see also Leys et al.,

2017) have shown that bacterial cells captured from ambient seawater are an important component of their diet. Comparisons between previous studies and the present one, however, should be done with caution since previous studies were based on measurements of capture or clearance rates of particles and not on direct assimilation of C added in chambers. Also, here we did not examine the natural diet of *S. coralliophaga* but we used bacteria that were cultured, freeze dried and resuspended in the chambers. It is possible that bacterial C plays a more important role in ecosystems with a relatively high amount of refractory organic matter (Hall and Meyer, 1998) while its role is limited in settings where fresh microalgae are supplied e.g., the CWCRs of present study (Duineveld et al., 2007; Davies et al., 2009; Kazanidis and Witte, 2016). In contrast to glucose and bacteria, microalgae-derived C was efficiently assimilated by *S. coralliophaga* and its epifauna (i.e., the anthozoan *P. anguicomus* and the ophiuroid *O. ophiura*) providing additional evidence for the important role of microalgae in the nutrition of CWCRs species (Duineveld et al., 2007, 2012; Davies et al., 2009; Kazanidis and Witte, 2016). Furthermore, this finding highlights the relevance of pelagic-benthic coupling as changes in the quantity and/or the quality of OM from upper ocean layers to the deep-sea (Sweetman et al., 2017) could affect the survival, condition and reproduction of deep-sea sponges (Witte, 1996; Billett et al., 2010; Robertson et al., 2017).

4.2 The versatile metabolic strategy of *Spongosorites coralliophaga*

The ability of *S. coralliophaga* to utilize various food sources is also highlighted by the *de novo* synthesis and alteration of dietary HAAs (Fig. 3 and 5). Interestingly, differences in the HAAs assimilation profiles were found between sponges fed with microalgae and bacteria, despite the almost identical patterns of HAAs composition in these two food sources and sponge specimens. The drivers behind this finding are not clear and perhaps are related to differences between microalgae and bacteria in terms of their cellular structure, biochemical composition (other than HAAs) and interactions with the metabolism of

other organic compounds (e.g. FA; de Goeij et al., 2008; Mueller et al., 2014; Rix et al., 2016). The ability of *de novo* synthesis of HAAs by *S. coralliophaga* is particularly evident through the incorporation of ammonium-derived N in non-essential (e.g., aspartic acid, glutamine) and essential HAAs (e.g., threonine, isoleucine, leucine, valine, phenylalanine and lysine). The efficiency of *S*. *coralliophaga* in the assimilation of DIN is also shown from the fact that the concentrations of ammonium used in present study were lower than those in previous studies (e.g., 30, 100 & 200 μM ammonium used in a study with *Aplysina aerophoba* collected from depths of 2-15m, Bayer et al., 2008). Interestingly, the N tracer was not recovered in the bacterial biomarker D-alanine, which suggests that the processing of ammonium in treatments using this compound may have been mediated by sponge cells and/or non-bacterial symbiotic microorganisms (e.g. Archaea). However, the role of each part (i.e. sponge cell *vs*. symbiotic microorganism), is unknown. Previous studies on N metabolism have revealed direct assimilation of ammonia by the host [e.g. in the sea anemones *Bartholomea annulate* and *Aiptasia pallida*, both of which host dinoflagellates) (Lipschultz and Cook, 2002) and in the hosting-chemoautotrophic bacteria coastal bivalve *Solemya velum* (Lee et al., 1999)] while in the symbiotic anemone *Aiptasia pulchella* it was shown that ammonia was principally incorporated into the glutamate and glutamine pools of the zooxanthellae (Swanson and Hoegh-Guldberg, 1998).

Metazoans are considered either incapable of synthesizing essential HAAs or synthesize them in smaller quantities than their metabolic needs (Fitzgerald and Szmant, 1997; Sherwood et al., 2011; Middelburg et al., 2015) and thus the putative essential HAAs must be supplied through food sources. Previous studies (e.g. works on shallow-water anthozoans; Wang and Douglas, 1999; Roberts et al., 2001) have provided evidence about the capacity of fauna to synthesize essential HAAs; to the best of our knowledge the present study is the first one that provides evidence about the ability of cold-water sponges to synthesize these important organic compounds. This evidence adds on recent findings on the reef-building CWC *Lophelia pertusa* by Middelburg et al. (2015). These authors showed that this

ecosystem engineer is capable -among others- of N fixation accompanied by the transfer of fixed N in bulk coral tissue and HAAs. Furthermore, the fact that ammonia is produced through the mineralization of particulate organic N (PON) (Maldonado et al., 2012) pinpoints to the possibility of a N-recycling system by *S. coralliophaga* and its symbiotic microorganisms (see also Middelburg et al., 2015). Comparison between the present findings and those from Middelburg et al. (2015) revealed also that in both species the assimilation of N from ammonium chloride in asparagine and glutamine was higher than other HAAs, which agrees with findings from the shallow-water anemone *Aiptasia pulchella* (Wang and Douglas, 1999; see also Swanson and Hoegh-Guldberg, 1998; Roberts et al., 1999). Interestingly, in *S. coralliophaga* high values of N assimilation from ammonium chloride were also found for L-Alanine and glycine while in *Lophelia pertusa* (Middelburg et al., 2015) and *Aiptasia pulchella* (Wang and Douglas, 1999) the assimilation of N in these two HAAs was among the lowest recorded values (see also Swanson and Hoegh-Guldberg, 1998).

The mechanism behind the preferential assimilation of N over C found for *S. coralliophaga* at MRC2 and LM experiments, is not clear. It is true that the loss of respired C could lower the assimilation C:N ratio; however, preferential assimilation of N over C was found even in specimens where respiration of C was low or not detected (Fig. 2). The absence of N tracer in the bacterial biomarker D-alanine, suggests that symbiotic bacteria did not have a role in the processing of PON. Hence, sponge cells and/or symbiotic microorganisms other than bacteria (e.g., Archaea) were involved in the assimilation of PON. Up to now the presence of Archaea in sponges has only been related to nitrification and denitrification (Radax et al., 2012) and thus we hypothesize that the preferential assimilation of PON over C is primarily mediated through sponge cells. In addition, it is highly likely that a part of the DIN (e.g., ammonia) released after the remineralization of POM is re-assimilated by the sponge's symbiotic microorganisms. Furthermore, sponges often act as a source of dissolved N compounds like nitrate [Corredor et al., 1988 (Caribbean coral reef sponges); Diaz and Ward, 1997 (Caribbean coral reef sponges); Jiménez and Ribes,

2007 (Mediterranean sublittoral rocky bottoms); Radax et al., 2012 Leys et al., 2017 (cold water sponges)], and it may be that preferential assimilation of N facilitates the conservation of precious N sources for sponge's survival and helps it to maintain stoichiometric homeostasis (Frost et al., 2002). The preferential assimilation of N over C to balance rapid excretions of DIN and thus retain stoichiometric homeostasis has also been mentioned in studies with macrobenthos from oxygen minimum zone sediments (Hunter et al., 2012).

4.3 Assimilation of food sources by the epibionts *P. anguicomus* and *O. ophiura*

In contrast to *S*. *coralliophaga*, its epibionts *P*. *anguicomus* and *O*. *ophiura* did not show a preferential assimilation of N over C and this is maybe linked to the metabolic needs of these two species and the relatively limited excretion of N compounds (e.g. nitrate) compared to sponges (Maldonado et al. 2012; Bourne et al. 2016). Interestingly, there were no significant differences among the sponge, the anthozoan and the ophiuroid for microalgae-C and microalgae-N assimilation rates. This finding suggests the potential for trophic competition among the three species for microalgae. However, considering the rapid downwelling in MRC that brings fresh microalgae from the ocean surface (up to 1 µg Chl *a* L^{-1}; Davies et al., 2009) to the seabed community in less than an hour we suggest that the supply of microalgae is not a limiting factor. Furthermore, limited trophic niche overlap among the three species is indicated by the versatile feeding and metabolic strategy of *S. coralliophaga* (sections 4.1 and 4.2) and previous works on the feeding of cold-water cnidarians and deep-sea ophiuroids. These studies have shown the ability of CWCs to feed on DOM, HAAs, bacteria, microalgae and zooplankton (Dodds et al. 2009; Duineveld et al. 2012; Gori et al., 2014; Mueller et al, 2014) and they have also described deep-sea ophiuroids as trophic generalists (Feder 1981; Pearson and Gage 1984; Fujita and Ohta 1988).

The present study recorded the versatile feeding and metabolic strategies of the cold-water ecosystem engineer *S. coralliophaga* including its capacity for *de novo* synthesis of essential and non-

essential HAAs using C and N both from dissolved (inorganic and organic) and particulate food sources. We suggest that this feeding and metabolic flexibility of *S. coralliophaga* facilitates its large body size under the food-limited conditions of CWCRs and its higher density than other demosponges in CWCRs (van Soest and Lavaleye, 2005; Vad, 2013). The absence of significant differences among *S. coralliophaga*, *P. anguicomus* and *O. ophiura* for microalgae-assimilation rates suggests the potential for trophic competition. However, using knowledge on food supply mechanisms at MRC and species feeding strategies (present study and previous ones), we suggest that the trophic niche overlap among *S. coralliophaga*, *P. anguicomus* and *O. ophiura* is limited. Interestingly, there was interspecific variability in the processing of microalgae-C and N, which maybe is linked to species' different metabolic needs. The three species studied here are among the benthic organisms with high density values in North-East Atlantic CWCRs (van Soest and Lavaleye, 2005; Vad, 2013) and knowledge on their feeding strategies facilitates future studies on C and N cycling in the important ecosystems of CWCRs (van Oevelen et al., 2009).

Data Declaration Statement

The data sets used in the present manuscript have been deposited to the British Oceanographic Data Centre (BODC) (www.bodc.ac.uk).

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Authors' contributions:

GK and UW designed the experiments. DvO provided labelled glucose, ammonium chloride/bacteria and UW provided labelled microalgae. GK collected samples and performed the on-board incubations. BV performed the amino acid extractions. GK carried out statistical analyses and wrote the manuscript with assistance from DvO and UW. All authors have approved the final article.

Competing financial interests:

We declare we have no competing interests.

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Highlights

- Cold-water ecosystem engineer sponge feeds on dissolved and particulate resources
- Preferential assimilation of nitrogen over carbon was not mediated by symbiotic bacteria
- Build-up of essential amino acids from inorganic compounds by the sponge holobiont