Metabolic variation in Caribbean giant barrel sponges: influence of age and sea-depth

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Metabolic variation in Caribbean giant barrel sponges: Influence of age and sea-depth

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ABSTRACT

The biochemical differentiation of widely distributed long-living marine organisms according to their age or the depth of waters in which they grow is an intriguing topic in marine biology. Especially sessile life forms, such as sponges, could be expected to actively regulate biological processes and interactions with their environment through chemical signals in a multidimensional manner. In recent years, the development of chemical profiling methods such as metabolomics provided an approach that has encouraged the investigation of the chemical interactions of these organisms. In this study, LC-MS based metabolomics followed by Feature-based molecular networking (FBMN) was used to explore the effects of both biotic and environmental factors on the metabolome of giant barrel sponges, chosen as model organisms as they are distributed throughout a wide range of sea-depths. This allowed the identification of differences in the metabolic composition of the sponges related to their age and depth.

1. Introduction

In recent years, coral reefs around the world have experienced substantial changes in their biodiversity as a response to changes in the environment resulting from both natural and anthropogenic phenomena (Hughes et al., 2007; Pandolfi et al., 2003). Global warming causes an increase in sea surface temperatures that leads to massive bleaching events of corals and are the primary driver of recent global reef decline (Hughes et al., 2017). The decay of corals has a profound influence on other organisms in the reef, including sponges and algae, which are taking over the substrate space freed by the coral decline (Bell et al., 2013, 2018; McManus and Polsenberg, 2004). Sponges, in particular, play a vital role in reef life, not only because they are the most abundant taxa in Caribbean reefs, but also because they are responsible for the transfer of nutrients along the trophic chain (De Goeij et al., 2013; Diaz and Rützler, 2001; Pawlik and McMurray, 2019; Rix et al., 2018).

Giant barrel sponges, which belong to the genus *Xestospongia* (order Haplosclerida), have been identified as an important reef player. Due to their large size and barrel-shaped appearance, these sponges provide a habitat for many other animals (Swierts et al., 2018a). In fact, the Caribbean giant barrel sponge, *Xestospongia muta*, can cover up to 9% of the available surface (Zea, 1993). Recent studies have shown that the population growth rate of *X. muta* has been rapidly increasing (McMurray et al., 2015), to the point that they are one of the most abundant organisms on Caribbean reefs (Loh and Pawlik, 2014). They also play an active part in the regulation of the nitrogen and carbon cycles in their habitat (Fiore et al., 2013; Southwell et al., 2008).

Xestospongia muta is characterized as a high microbial abundance (HMA) sponge with a slow growth rate and fluctuating chemical defenses (Gloeckner et al., 2014; Loh and Pawlik, 2014; Pawlik et al., 1995). The interaction of these sessile organisms with their environment, as well as their biological processes, must be mediated essentially, through chemical signals (Paul et al., 2006, 2019; Paul and Ritson-Williams, 2008). It can be anticipated, that changes in environmental conditions will be reflected at a metabolic level as an alteration of the metabolome (Hay, 2009; Noyer et al., 2011; Paul et al., 2006;

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Pawlik, 2011; Sacristan-Soriano et al., 2011). As expected, a wide range of metabolites has been reported in *Xestospongia* spp. sponges, most of which have shown significant responses to bioassays, confirming that they might have a role in various biological and physiological processes (Zhou et al., 2010). However, the biotic or abiotic factors driving the production of these compounds remain unclear mainly due to the lack of methods able to deal with their chemical complexity. The traditional single-targeted method has proved to be too limited to dereplicate such chemically complex situations, and it was not till the advent of a more holistic approach such as that provided by metabolomics, that these problems could be successfully revisited.

The use of "omics"-platforms to gain insight into the chemical ecology of marine sponges has changed the perspectives for their exploration (Paul et al., 2019). In particular metabolomics, defined as the study of all metabolites present in an organism under specific conditions (Viant, 2007), has been very useful for environmental studies (Bundy et al., 2008). Although targeted analyses have revealed significant information relating to changes in metabolites resulting from environmental factors (Noyer et al., 2011; Page et al., 2005), the use of metabolomics as an unbiased method enables a much broader approach that can lead to the identification of responses associated with a variety of previously unreported compounds (Greff et al., 2017; Reverter et al., 2016, 2018; Sogin et al., 2016).

Giant barrel sponges used in this study as model organisms have been considered as the "redwood of the reef" due to their unparalleled longevity. For instance, a specimen found in Curaçao was estimated to be up to 2300 years old (Van Soest et al., 2012). With the development of a growth model for *X. muta*, determining the age of these sponges has become relatively easy (McMurray et al., 2008). However, the effect that age might have on the lifecycle and physiological variation of the sponge is still far from being clearly understood. In particular, age could be expected to affect the metabolome, as has been reported in studies on most other organisms, such as several terrestrial plants (Lee et al., 2019; Yoon et al., 2019), humans (Jové et al., 2014; Yu et al., 2012) and marine sponge-associated bacteria (Ng et al., 2013).

Biological development of marine sponges could also very likely be greatly affected by another factor: sea-depth. Marine habitats can vary substantially depending on their distance from the sea surface due to great variations in environmental conditions, such as the availability of sunlight, pH, pressure, temperature and presence of predators. Therefore, giant barrel sponges, which can be found at depths ranging from less than 10 to beyond 100 m (Olson and Gao, 2013; Van Soest et al., 2014), should unavoidably be influenced by these environmental variations. Several studies on the influence of depth on the microbiome of this sponge suggested that depth gradients could cause great changes in some of the microorganisms associated to the sponge (Morrow et al., 2016; Olson and Gao, 2013; Villegas-Plazas et al., 2019). Considering that *X. muta* is an HMA sponge, changes in the microbiome could be expected to have a significant effect on the metabolome of the sponge. Notably, notwithstanding the importance of this relationship in the field of marine ecology, the studies on the effect of depth on the metabolic production of giant barrel sponges are still limited.

Recent studies have shown that the species previously known as *X. muta* is, in fact, a species complex that can be separated into three distinct genetic groups (cryptic species) according to their mitochondrial and nuclear DNA (Swierts et al., 2017). In the present study, samples belonging to *X. muta* of two different genetic groups, different ages and depth were collected in Curacao and chemically profiled in a holistic manner to detect possible variations in their metabolome. For this, liquid chromatography hyphenated to mass spectrometry (LC-MS) was used as an analytical platform, combined with multivariate data analysis (MVDA) and a molecular networking workflow. The high sensitivity of LC-MS, one of its outstanding features, allowed the detection of metabolites present in very low concentrations (Goulitquer et al., 2012). After applying MVDA to the obtained data, a molecular networking workflow allowed its interpretation from a broader perspective.

2. Materials and methods

2.1. Sample collection

Giant barrel sponges (*Xestospongia* spp) were collected by SCUBA diving in Curaçao from locations with different depths around the island during the months of February and March of 2017 (supporting information Table S1). Collected sponge samples were immediately stored on-site in 98% ethanol (w/w) at −20 °C. The samples were identified by DNA sequencing, using the I3-M11 partition of the CO1 mitochondrial gene according to (Swierts et al., 2013). The DNA analysis allowed the samples to be identified as belonging to three main genetic groups for giant barrel sponges in the Caribbean (groups 7 and 8) (Swierts et al., 2017). Although a few samples could be identified as belonging to group 9, these were not included in the study as their quantity was insufficient to carry out statistical analysis. The age of the specimens was determined through in-field measurements, of each sponge, height, osculum diameter and base circumference were measured using a measuring tape. These measurements were then upload on the website http://people.uncw.edu/pawlikj/xmuta_calc.html to calculate the sponge age with a growth model developed by McMurray et al. (2008). The depth at which each specimen was collected was also recorded and that value was used for the analyses.

2.2. Sample preparation for metabolomic analysis

The *Xestospongia* spp. samples were ground and extracted with ethanol and sonicated for 20 min three times, for the first cycle of extraction the ethanol used to preserve the samples in the field was used together with fresh ethanol. The three extracts were combined and dried using a Centrivap vacuum concentrator (Labconco, Kansas City, MO, USA) and were later desalted on 500 mg C-18 SPE cartridges of 45 μm particle size (Supelco, Bellefonte, PA, USA). For this, 50 mg of each dried extract was weighted and loaded onto the cartridge, that was previously preconditioned with 10 mL of methanol and 10 mL of water and eluted with 10 mL of water (F1), 10 mL of methanol (F2) and 10 mL of dichloromethane/methanol 1:1 (F3). The methanol fractions were analyzed by LC-MS.

2.3. Liquid chromatography- mass spectrometry for LC-MS and LC-MS/MS analysis

The methanol fractions obtained were dried using a Centrivap vacuum concentrator. In order to standardize sample concentration, the obtained residue was redissolved in 50% methanol (v/v) to a final concentration of 1 mg/mL. All samples were filtered with 0.2 μm Ministart RC15 hydrophilic non-sterile filters (Sartorius, Gottingen, Germany). Samples were injected in a randomized sequence within each genetic group, blanks were injected at the beginning and at the end of the sample sets and QC was injected at the beginning and after every 10 samples in the analysis. The reproducibility of the QC and Blanks was checked in PCA analysis (data not shown) where the blanks were separated from the samples and the QC were in the center of the samples in both cases the replicates appear overlapped showing the reproducibility between analysis. The analysis was carried out using a UHPLC-DAD-ESI-MS system consisting of a UPLC Acquity I-Class (Waters, Milford, MA, USA) hyphenated to a Bruker Impact HD MS spectrometer (Bremen, Germany) with electrospray ionization (ESI). The separation was performed on a Waters C18 (2.1 × 100 mm, 2.1 μm) column and eluted with a gradient of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) of 10%–100% B in 30 min, and 100% B for 5 min; 100%–10% B in 3 min and 2 min equilibration at 10% B. The flow rate was 0.3 mL/min, column temperature was 40 °C and the injection volume was 2 μL. The mass spectrometer parameters were set as follows:

1.5 bar of nebulizer gas, 6.0 L/min of drying gas, drying gas was 350 °C, capillary voltage was 4000 V, the collision energy was 8.0 eV, and collision Rf 600 Vpp. The function auto MS/MS was selected, and the top 5 features of each scan were chosen to perform MS/MS. The mass spectrometer was operated in positive mode in a range of 50–1200 m/z with a sample rate of 2 Hz. Collision energy values for MS/MS experiments were adjusted as follows: *m/z* 100, 12 eV; *m/z* 300, 20 eV; *m/z* 500, 30 eV; *m/z* 1000, 45 eV.

2.4. Statistical analysis

Data files obtained from the LC–MS/MS analyses were converted to mzXML format using Brucker Daltonics DataAnalysis (version 4.1, Bremen, Germany). The LC-MS/MS data was processed using MZMine2 (Pluskal et al., 2010). To build the feature matrix, mass detection was performed using centroid data. The noise level was set at 10,000 for MS and 100 for MS/MS. The chromatograms were built using the ADAP chromatogram builder (Myers et al., 2017) with a minimum number of scans of 3, group intensity threshold 1000, minimum highest intensity 10,000 and m/z tolerance of 0.05. Chromatograms were deconvoluted using a baseline cutoff algorithm with the following parameters: minimum peak height of 10,000, peak width range of 0.02–1.00 min, and baseline level of 1000. Chromatograms were deisotoped using an isotopic peak grouper algorithm with an *m/z* tolerance of 0.05 and retention time tolerance of 0.1 min. The features of each sample were aligned using a join alignment algorithm with the following parameters: 0.05 *m/z* tolerance and 0.1 min retention time tolerance. Using these parameters, three matrixes were created, one including all samples and one matrix with samples of each genetic group.

All the statistical analyses were performed using RStudio February 1, 1335 (RStudio team, 2018). The PERMANOVA (Permutational analysis of variance) analyses were performed using 'adonis ()' function in the 'vegan' package (Oksanen et al., 2019). This was applied to different age, sea-depth and genetic group samples, for which 9999 permutation and Bray-Curtis as dissimilarity measurement were used to investigate mutual interactions between metabolites and the factors. Constraint principal coordinate analyses were performed for the data matrixes obtained from LC-MS analysis and the factors found to be significant in the PERMANOVA using 'capscale ()' function with Bray-Curtis dissimilarity as a distance. The validity of the analysis was tested using the function 'anova.cca ()'; for both models $p > 0.05$ using 9999 permutations. Variation in the metabolome of genetic groups was assessed with a principal coordinate analysis (PCoA) using the 'cmdscale ()' function and the Bray-Curtis dissimilarity matrix as an input.

2.5. Molecular networking and dereplication

Feature base molecular networking (FBMN) workflow (Nothias et al., 2020) was carried out using the GNPS online platform (http://gnps.ucsd.edu) (Wang et al., 2016). Quantitative data matrix and MS/MS obtained from MZmine2 are shown in section 2.4. Files were uploaded to GNPS platform and the ions in the region between ±17 Da around the precursor *m/z* were removed to filter the data. Additionally, only the top six fragment ions of the MS/MS data were considered for further use to reduce data size. The mass tolerance for both precursor and the MS/MS fragment ions was set to 0.03 Da. With these tolerance and filter parameters, a molecular network was constructed, for which minimum cosine values for the edges and the matched fragments were set at 0.7 and 6, respectively. In addition, edges between two nodes were only included when both nodes were in common to their respective top 10 matching nodes. The maximum size of a molecular family was set to 100. The generated spectra in the network were then dereplicated using GNPS spectral libraries (Horai et al., 2010; Wang et al., 2016), and visualized using Cytoscape software (Shannon et al., 2003).To improve the annotation of the features in the MNs Network Annotation Propagation (NAP) an *in silico* tool was used (da Silva et al., 2018). The following NAP parameters were employed for the annotation: exact mass error for database search, 10 ppm; $[M + H]^+$ as adduct type:, cosine = 0.7 to select inside a cluster; 10 maximum candidate structures in the graph. The result from both FBMN and NAP were introduced in MolNetEnhancer workflow (https://ccms-ucsd.github.io/GNPSDocumentation/molnetenhancer/) (Ernst et al., 2019) to classify the features into chemical classes according to ClassyFire chemical ontology (Djoumbou Feunang et al., 2016). The results of the molecular networks for the matrixes with all the samples and the samples from genetic group 7 can be found in https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=cd2ffac11ce24c32ad6b8f84ce85240e and https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=48bd32ff37cc45a98575a78121bb33d2, respectively.

2.6. Isolation and identification of compounds

Using LC-MS profiling, target compounds were selected, corresponding to the most correlated features, both in positive and negative, to the CAP 1 from the constraint principal coordinate analysis that showed to be significantly correlated with depth using and spearman correlation test (supporting information Table S2). Two compounds were isolated from two of the ethanol extracts of the sponge samples collected at different depths, other compounds were not isolated due to the small amounts in which they were present in the samples. Two crude extracts FS (1.0 g) and FD (500 mg) were fractionated using 20-mL SPE LC-18 cartridges (Supelco) eluted successively with 100 mL of each of the following solvents: 100% water, 80% 60%, 40% 20% methanol/water mixtures, 100% methanol and methanol-dichloromethane (1:1, v/v). The cartridges were previously conditioned with 100 mL of methanol and 100 mL of water. The SPE fractionation yielded seven fractions labelled FS1-FS7 and FD1-FD7 for FS and FD, respectively. The target compounds selected previously were found in fractions in FS5 (51.2 mg) and FD5 (44.1 mg). Successive semi-preparative HPLC using an Agilent 1200 series system (Santa Clara, CA, USA) and a Luna 5 μm, C-18, 250 mm × 10 mm column (Phenomenex, Torrance, CA, USA) eluted at a flow rate of 3.5 mL/min allowed the isolation of compound **1** (2.8 mg) from FS5 and compound **2** (1.8 mg) from FD5.

The structure of these compounds was elucidated by NMR analyses at 25 °C with an AV-600 MHz NMR spectrometer (Bruker, Karlsruhe, Germany), operating at the ^{1}H NMR frequency of 600.13 MHz, equipped with a TCI cryoprobe and Z gradient system. Deuterated methanol was used as the internal lock. Their ^{1}H NMR and ^{13}C attached proton test (APT) spectra were initially recorded and 2D NMR techniques, correlated spectroscopy (COSY), heteronuclear single quantum coherence (HSQC) spectroscopy, and heteronuclear multiband correlation (HMBC) spectroscopy (supporting information Figs. S6–S14) were used to confirm the connectivity of the atoms in compound **1** and **2**.

3. Result and discussion

Sponges are, so far, one of the most studied sources of marine natural products (MNP). Among these, giant barrel sponges have been most extensively studied since their widespread distribution and relatively large size facilitates their identification and collection. Their chemical composition includes sterols, brominated fatty acids and terpenoids (Zhou et al., 2010). However, the qualitative and quantitative composition of their metabolome was found to vary depending on environmental conditions (Bayona et al., 2020; Villegas-Plazas et al., 2019). The correlations between changes in the metabolome of giant barrel sponges and two possible driving factors of its variation (age and depth) were investigated using LC-MS, given its capacity to detect very low concentrations of most metabolites. With the recent findings of Swierts et al. (2017) regarding the existence of three genetic groups among what had been previously considered to be only *X. muta* in mind, the collected samples were studied and classified accordingly. Thus, among the 69 samples collected, 41 belonged to genetic group 7 and 28 to genetic

group 8 (Swierts et al., 2017). Considering that these genetic groups could have different responses to biotic or abiotic factors such as age or depth, the interaction correlation between genetic groups and these two factors was also included in the study.

Among the factors evaluated with a PERMANOVA analysis, age and genetic group were found to significantly influence the variation of the sponge metabolome, whereas depth did not show a significant correlation (Table 1). The effect of the relationship between the studied factors was also calculated. In this case, no correspondence was found between metabolomic changes related to sample age and depth and/or genetic groups, that is, the changes in the metabolome caused by the ageing processes did not appear to be affected by the genetic group nor by the depth of the sample collection. On the other hand, while the effect of depth was not significant when considering the whole sample set, it did seem to vary according to the genetic group. This is supported by the fact that the interaction between the depth of collection and the genetic groups was found to be significant (Table 1).

In view of the significance of their interaction, the effect of depth on each genetic group was analyzed individually. The PERMANOVA results showed the metabolome of genetic group 7 to be largely affected by the depth ($F_{(1,40)} = 2.8171$, $R^2 = 0.067$, $p = 0.003$) and this was also confirmed by the results of a constraint principal coordinate analysis shown in Fig. 1 where the depth gradient along CAP 1 is observed with samples collected at higher depth being positively correlated with this axis, while samples collected at lower depth are negatively correlated (Fig. S2a). In contrast, the metabolome of genetic group 8 was not clearly correlated with the depth factor (PERMANOVA, $F_{(1,27)} = 1.457$, $R^2 = 0.053$, $p = 0.112$). This finding is in accordance with previous studies that identified depth could be one factor influencing the chemical composition of *X. muta* (Villegas-Plazas et al., 2019). According to the research of Villegas-Plazas et al. (2019) the effect of depth had seasonal variation could be related with variations on environmental conditions, such as temperature, light intensity, and temperature between seasons. The effect was observed only in the samples collected in autumn, while those of spring did not show any significant effect on the metabolome in relation to depth. In contrast, samples from the present study were collected during spring and although the effect of depth was observed, it was only significant for one of the genetic groups. This could mean that the divergences in the metabolome of different genetic groups can also be affected by the season, which could obscure the influence of depth on the metabolome during some seasons if the genetic factors are not considered. However, future research related with changes in the metabolome of different genetic groups depending on the season will be needed to confirm this hypothesis.

In the first place, the metabolome of the two phylogenetic groups can differ due to the genetic background of each group. Proof of this is the fact that the two phylogenic groups can be distinguished based on their chemical profile (Fig. S4). These chemical differences have also been reported for other sponge species that are closely related (Reverter et al., 2018). In addition, it was found that samples from group 7 have more unique features compared to group 8 (Fig. S5). The higher chemical richness of group 7 could be associated with higher potential for producing metabolites, that is, the sponges of group 7 might be more likely

Table 1
Permutational multivariate analysis of variance (PERMANOVA) testing the effect of depth of sample collection, age, and their genetic group of the samples on the metabolome. * indicates p-value<0.05.

	F-value	R^2	P value
Age	$F_{(1,69)} = 2.45$	0.024	0.014*
Depth	$F_{(1,69)} = 2.04$	0.020	0.050
Genetic group	$F_{(1,69)} = 30.16$	0.300	<0.001*
Age × Depth	$F_{(1,69)} = 0.78$	0.007	0.593
Age × Genetic Group	$F_{(1,69)} = 0.64$	0.006	0.781
Depth × Genetic Group	$F_{(1,69)} = 2.57$	0.026	0.020*
Age × Depth*Genetic group	$F_{(1,69)} = 0.79$	0.008	0.596

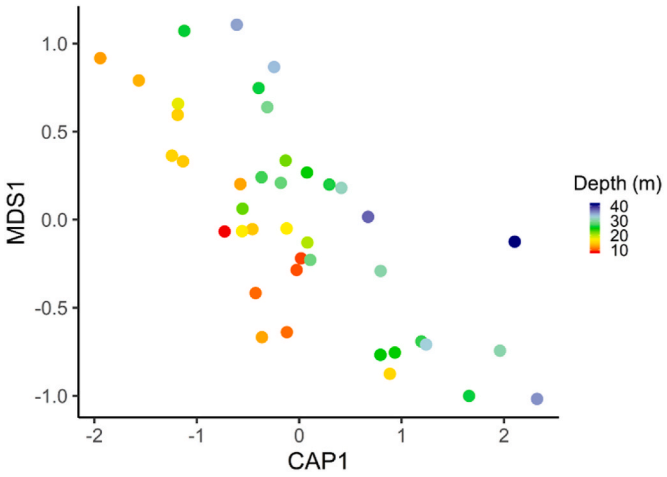

Fig. 1. Constraint principal coordinate analysis between depth (m) factor and metabolome of the genetic group 7. Constraint variable 1 (CAP 1) vs non constraint variable out of multidimensional scaling (MDS1).

to produce distinguished metabolites as a response to changes in environmental factors along a depth gradient. Furthermore, considering that *X. muta* is an HMA sponge, the variations observed in its metabolome could be associated to changes in the metabolism of the host itself (sponge), in the metabolism or the composition of its symbionts (microbiome) or changes in the metabolic interaction between the sponge and the symbionts. The alteration of the microbiome of giant barrel sponges in the Caribbean due to environmental factors related to differences in depth has been reported in earlier studies (Morrow et al., 2016; Villegas-Plazas et al., 2019), as has the influence of host phylogeny on the composition of the microbiome of *Xestospongia* spp (Swierts 2019; Swierts et al., 2018b). From this perspective, the changes in the metabolome due to depth may be attributed not only to different host responses according to the phylogenetic group but also to differences in their specific symbionts.

The LC-MS/MS data obtained from samples belonging to genetic group 7 was analyzed using the molecular networking workflow in the GNPS platform (Nothias et al., 2020; Wang et al., 2016) followed by *in silico* dereplication methods such as network annotation propagator (da Silva et al., 2018) and MolNetEnhancer (Ernst et al., 2019). This resulted in 40 molecular networks that contain three or more nodes, twenty of which could be classified into a molecular family according to the ClassyFire chemical ontology (Djoumbou Feunang et al., 2016). Most of the classified networks corresponded to lipids that include both fatty acids and glycerophospholipids or lipophilic compounds such as terpenoids and steroids while others were classified as polyketides and aromatic compounds such as benzoic acid derivates or quinones. To gain insight into the metabolites influenced by the depth gradient, the features contributing the most to each direction of the first axis in the constraint principal coordinate analysis (positive: deep, and negative: shallow) that also showed to be significantly correlated with depth, were selected for further investigation. As is shown in Fig. 2, most of the features related to samples collected at shallow depths were grouped in one cluster and classified as glycerophospholipids. A closer look at the MS spectra of these compounds revealed that they all contain a phosphatidylcholine moiety. In addition, considering their molecular weight, it was deduced that they only contain one fatty acid bonded to the glycerol unit, implying that they belong specifically to the *lyso*-phosphatidylcholine lipids (*lyso*-PC) family. Lastly, the isotopic pattern showed the presence of bromine atoms in some of the compounds.

Annotation of the selected features led to the identification of one brominated polyacetylene fatty acid and a *lyso*-phosphatidylcholine lipid that contains myristic acid as the acyl moiety. Further studies of the features of the glycerophospholipids network that showed different

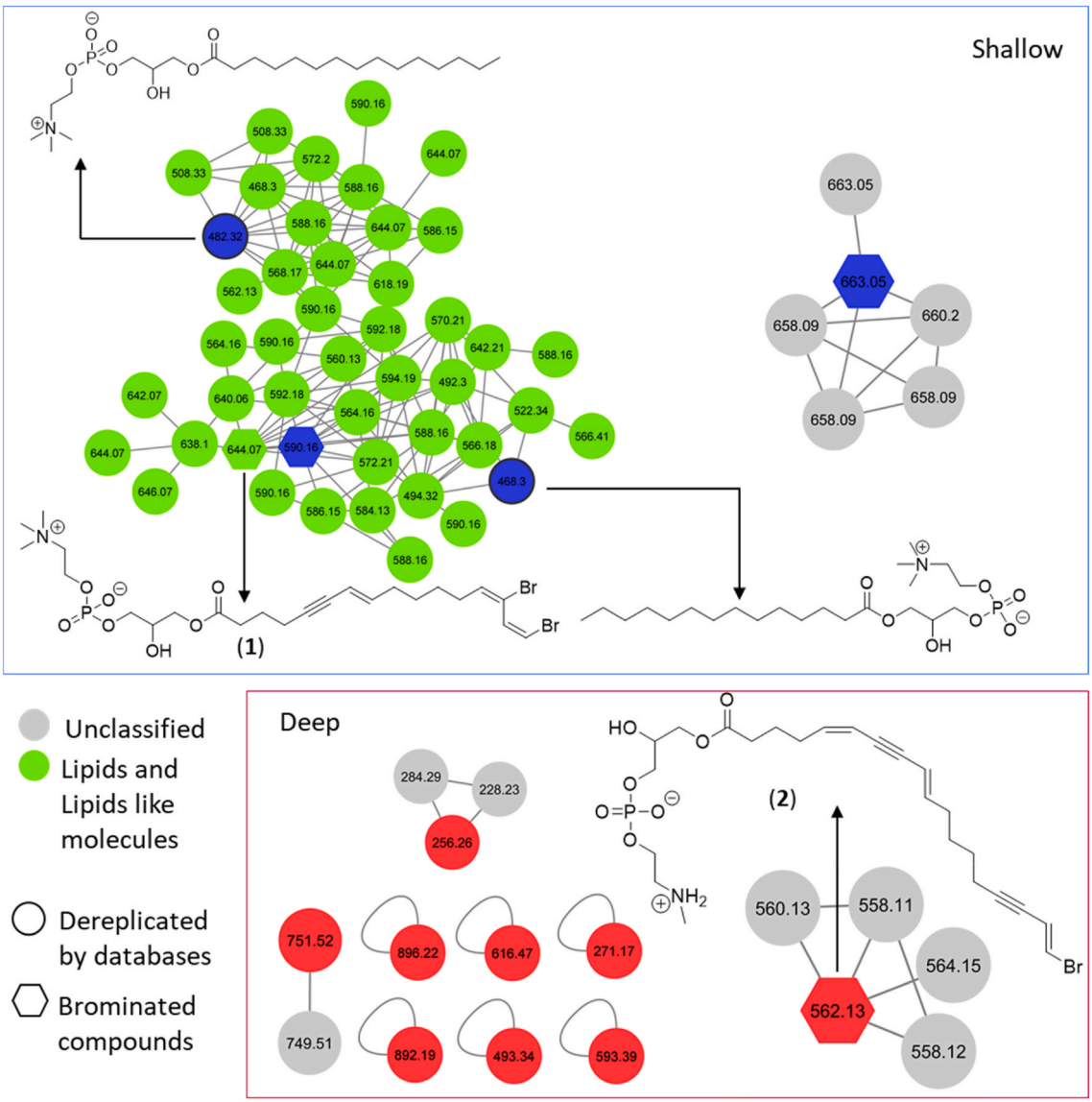

Fig. 2. Molecular network showing the clusters of the features identified to be changing due to depth. The nodes corresponding to important features obtained from the constraint analysis of principal coordinate are colored in blue (shallow) and red (deep). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

brominating patterns led to the identification of a previously unreported compound, 1-*O*-((7*E*,13*E*,15*Z*)-14,16-dibromohexadeca-7,13,15-trien-5-ynoyl)-sn-glycero-3-phosphocholine (Compound **1**) (Table 2). This compound was isolated as a white powder. The full elucidation of this compound was done using both NMR and HRMS. Its (+)-HRMS spectrum showed the proton adduct $[M+H]^+$ ions at *m/z* 642.0835, 644.0836, and 646.0803 corresponding to the molecular formula, $C_{24}H_{38}Br_2NO_7P^+$ (calc. 644.0810, 642.0831, 646.0790); the presence of the two bromine atoms was deduced from the isotopic pattern. The ^{13}C NMR spectrum showed the presence of a carbonyl carbon at δ_C 174.8 in addition to six sp^2 carbons and 2 *sp* carbons corresponding to three alkenes and one alkyne, respectively, indicating the presence of an acyl moiety. The carbons attached to heteroatoms (oxygen and nitrogen) in the region between δ_C 54 and 70 suggested the presence of one glycerol and one phosphatidylcholine moiety. The two bromine atoms observed in the mass spectra were found to be bonded to carbons involved in double bonds as shown by the shift in the chemical shift of these carbons. The full structure of compound **1** was determined using the COSY and HMBC spectra. The brominated fatty acid and phosphatidylcholine were found to be bonded to C1 and C3 of the glycerol molecule, respectively. Using the coupling constant, the stereochemistry of the double bonds was established as 7*E*,15*Z*. The stereochemistry of the double bond in C′-13 was determined to be *E* due to the correlation observed in the NOESY between protons in position 12′ and 15'.

Lyso-phosphatidylcholines *(lyso-PC)* account for only 0.3–4% of the total lipid content in sponge extracts (Genin et al., 2008), coinciding with studies conducted in animal cell membranes (Torkhovskaya et al., 2007). Lysophospholipids have been recognized as playing a role beyond being part of the membrane structure as molecular signaling molecules (Birgbauer and Chun, 2006; D'Arrigo and Servi, 2010; Torkhovskaya et al., 2007). These types of molecules have been related to different mechanisms of cell proliferation, such as the induction of growing factors synthesis or increasing the sensitivity of cells to growth factors (Torkhovskaya et al., 2007). In addition, similar molecules have been reported to be involved in the self-recognition and activation of immune systems though pro-inflammation signaling in sponges and corals (Müller and Müller, 2003; Quinn et al., 2016). Therefore, the increase in the production of *lyso*-PC in shallow depth sponges could be related to a defense mechanism developed as a response to higher predatory stress in shallower waters (Chanas and Pawlik, 1997). A

Table 2
^{1}H-NMR (600 MHz) and^{13}C-NMR (150 MHz) in CH_3OH-d_4 of compounds **1** and **2**.

	1			2		
	δ_H (ppm)	mult, *J* (Hz)	δ_C (ppm)	δ_H (ppm)	mult, *J* (Hz)	δ_C (ppm)
1'	–	–	174.8	–	–	175.1
2'	2.48	t, 7.4	33.8	2.39	t, 7.5	34.2
3'	1.81	quint, 7.2	25.2	1.74	quint, 7.4	25.1
4'	2.35	td, 7.0, 1.9	19.4	2.35	qd, 7.4, 1.1	30.5
5'	–	–	88.1	5.89	dt, 10.7, 7.5	142.6
6'	–	–	80.9	5.61	d, 10.7	111.3
7'	5.45	d, 15.8	111.3	–	–	85.6
8'	5.99	dt, 15.8, 7.1	144.0	–	–	93.7[a]
9'	2.08	m	33.6	5.66	dq, 15.8, 1.8	111.3
10'	1.40	m	29.5	6.10	dt, 15.8, 7.1	144.9
11'	1.44	m	29.1	2.17	qd, 7.0, 1.4	33.5
12'	2.04	m	32.0	1.53	m	29.1[b]
13'	6.07	td, 7.7, 1.4	137.4	1.53	m	28.9[b]
14'	–	–	114.8	2.30	m	19.8
15'	6.78	d, 7.6	132.3	–	–	93.6[a]
16'	6.56	d, 7.6	113.4	–	–	78.3
17'	–	–	–	6.24	dt, 14.0, 2.3	119.2
18'	–	–	–	6.70	d, 14.0	118.0
1	4.19, 4.12	dd, 11.4, 4.5, dd, 11.4, 6.2	66.3	4.17, 4.12	dd, 11.4, 4.7, dd, 11.4, 6.1	66.2
2	3.98	m	69.8	3.98	m	69.8
3	3.90	m	67.8	3.90	m	67.8
1''	4.30	m	60.4	4.10	m	61.6
2''	3.64	m	67.5	3.24	t, 4.8	50.9
N–Me	3.23	s	54.7	2.73	s	33.5

[a] Interchangeable carbons.
[b] Interchangeable carbons.

similar phenomenon has been observed in sponges of the genus *Oscarella* that displayed seasonal variation of two lysophospholipids of which increased production coincided with the embryogenesis and the larval development period (Ivanisevic et al., 2011). This shows that these types of molecules can perform a variety of roles in sponge development. However, the variation in their structures makes it difficult to establish specific roles for each compound individually. Lastly, even though lysophospholipids in general, and *lyso*-PC in particular, are universally present among metazoans, some chemical features in the acyl chain present in compounds from *Xestospongia*, such as bromine atoms and acetylenic groups, are characteristic of these specific organisms (Zhou et al. 2010, 2015). The fatty acids from *Xestospongia* have been reported to have a wide range of biological activities, suggesting that it is not only the lysophospholipid that has a biological role in this case, but the free fatty acid which could *per se* play an additional role in the sponge.

For samples collected at increasing depth, the features that were identified as important were not grouped in one single cluster. Instead, the significant compounds were found in small clusters within the molecular networking analysis, as shown in Fig. 2. This indicates that the environmental changes along the depth gradient do not cause a change in the production of a specific group of molecules, but rather, that the changes observed in the metabolome are compound specific. One of the reasons why the environmental changes along the depth gradient produce a response involving specific compounds could be that not all environmental and biotic factor variations are directly related to depth. While abiotic factors such as pressure and temperature are related to depth, other factors such as predatory stress can vary along the depth gradient. This can cause heterogenicity in the compounds whose production is altered at larger depths. Among the compounds that were identified as associated with greater depth, it was possible to isolate another previously unreported compound, 1-*O*-((5*Z*,9*E*,17*E*)-18-bromooctadeca-5,9,17-trien-7,15-diynoyl)-sn-glycero-3-*N*-Methylethanolamine (compound **2**).

Compound **2** was also isolated as a white powder. Its (+)-QTOF-ESI-MS spectrum showed the proton adduct $[M+H]^+$ ions at *m/z* 560.1394 and 562.1379, with a relative intensity of 1:1, indicating the presence of one bromine atom in the molecule. Consequently, the molecular formula was established as $C_{24}H_{36}BrNO_7P$ (calc. 560.1413 and 562.1392). Its NMR spectroscopic data (Table 2) was used to characterize the structure of this compound. The carbonyl carbon at δ_C 175.1 together with the *sp* and sp^2 carbons confirmed the presence of an acyl chain in the molecule. In addition, the absence of a terminal methyl or methylene group together with the unusual chemical shift of the carbons in terminal double bond at δ_c 119.2 and 118.0 confirmed the presence of a bromine atom in this position. The full structure of compound **2** was determined using the COSY and HMBC. The compound was determined to be formed by three moieties, a brominated unsaturated fatty acid, a glycerol molecule and *N*-methylethanolamine**.** The brominated fatty acid and *N*-methylethanolamine are bonded to C1 and C3 of the glycerol molecule, respectively. Using the coupling constant, the stereochemistry of the double bonds was established as 5*Z*,11*E*,17*E*.

Similarly, to the compounds found in shallower waters, compound **2** also belongs to the lysophospholipids family. However, the presence of a *N*-methylethanolamine moiety is very unusual. Although ethanolamine and choline are moieties commonly found in animal cells, *N*-methylethanolamine has been mostly reported to be present in the membrane of several by microorganisms (Dahal and Kim, 2017; Goldfine and Ellis, 1963; Schubotz et al., 2011). This suggests that compound **2** is produced totally or partially by an associated microorganism. As was discussed previously, the *Xestospongia* microbiome experiences changes due to depth. The fact that one of the metabolites associated with changes in depth contains a moiety produced by microorganisms is an example of the way in which changes in the microbiome can be reflected in changes in the metabolome of the holobiont.

In the second part of the study, the effect of age on the metabolome of the sponge was investigated. The metabolome of sponges proved to be affected by age, providing an important insight into their biological and chemical processes. From a biological perspective, this could imply that in sponges, the biological stage of development of a sponge is reflected by the metabolome as observed in other organisms (Yoon et al., 2019; Yu et al., 2012). For sponges in general, and particularly in the case of giant barrel sponges, the finding that the metabolome can be an indicator for the development of the sponge is important, as very little is known about the biological development of these sponges. Moreover,

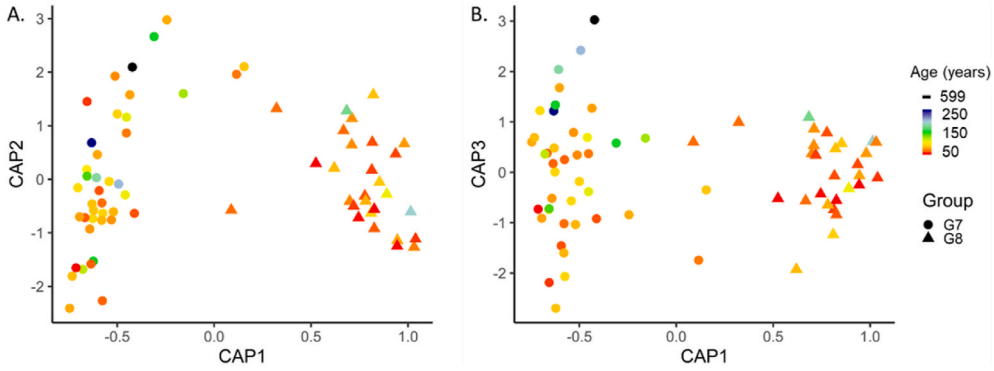

Fig. 3. Constraint principal coordinate analysis using age (years), depth (m) and genetic group as factors for samples of genetic group 7 and 8. A. CAP 1 vs 2 and B. CAP 1 vs 3.

these findings suggest that external factors could also affect the metabolome, as older sponges have had to adapt over time to more variations in the conditions of the water column, for example in the microbiome, the temperature or type and amount of nutrients. Therefore, it is possible that over the years, older sponges have, for example, recruited specific symbionts into their metabolome that afforded an adaptive advantage (Ribes et al., 2016; Turon et al., 2018).

The fact that the interaction between age and depth does not affect the metabolome is in agreement with the lack of any influence of depth on growth rate of *X. muta* (McMurray et al., 2008). Similarly, the interaction between age and genetic groups showed no significant effect on the metabolome, possibly because growth rates have been calculated for *X. muta,* but not for the individual genetic groups. Each genetic group could have different growth rates, as suggested by previous reports of differences in growth rate of *X. muta* and its sister species *X. testudinaria* (McGrath et al., 2018).

Considering that the effect of age was reflected in the metabolome, the second aim of this research was to identify those metabolites most affected by this process. The constraint principal coordinated analysis revealed the effect of age mainly CAP 2 and 3, while the separation due to genetic group can be observed along CAP 1 as shown in Fig. 3 and the detailed biplot is shown in Fig. S2b. The features that were found to be more correlated with these axes were selected as characteristic metabolites associated with age. Subsequently, a molecular network was built using the GNPS platform followed by *in silico* tools to increase the annotation of the features. This showed that most of the clusters in which the nodes corresponding to the compounds related to age were located were not classified. However, a closer look into the MS spectra of the selected compounds allowed us to draw some conclusions about the chemical structures of these compounds.

As shown in Fig. 4, most of the compounds that were identified to be related to older sponges displayed *m/z* values between 800 and 1000. This, together with their fragmentation pattern, indicated that the structure of these compounds corresponded to glycerophospholipids with one phosphatidylcholine and two acyl chains joined to the glycerol moiety. Further, the isotopic pattern of these compounds revealed the presence of 1–5 bromine atoms. A possible explanation for their presence could be that were cellular division increased in older sponges these compounds could also be increased as they are a constitutive element in membranes. Considering that it has been reported that the growth rate of giant barrel sponges decreases with the age of the sponge (McMurray et al., 2008), it is possible that the increment in the cellular division associated to this membrane lipids is related to higher cell turnover rates rather than a growing state (Alexander et al., 2014). Cell turnover rates have proven to be particularly fast in sponges compared to other animals and is believed to be a strategy to prevent cellular

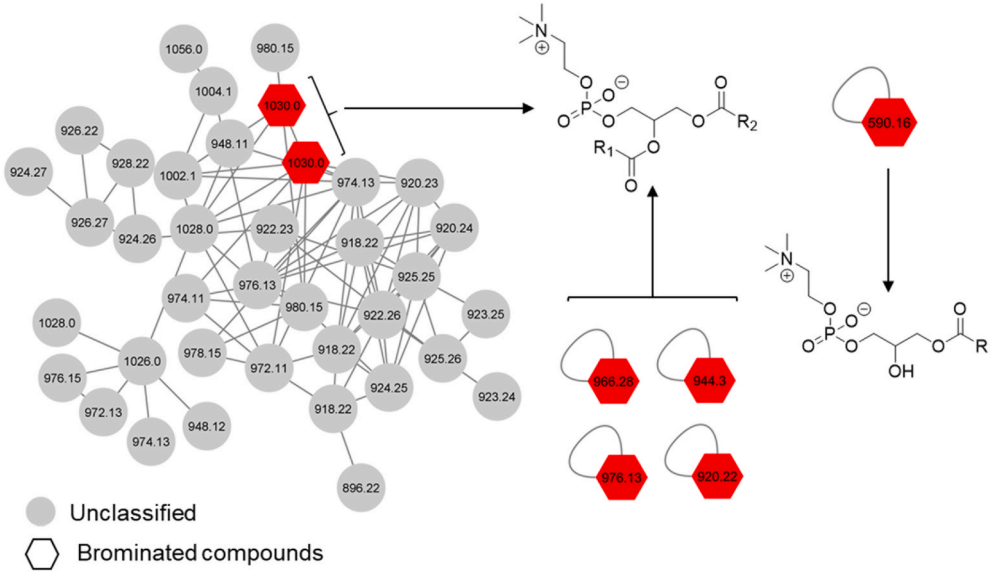

Fig. 4. Molecular network showing the clusters of the features identified to be changing depending on the age of the sponge. The nodes corresponding to features related with older sponges obtained from the constraint analysis of principal coordinate are colored in and red. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

damage (De Goeij et al., 2009). Another alternative could be that these molecules act as a sort of reservoir of the bioactive polybrominated fatty acids that are bonded to the glycerol. Polybrominated fatty acids have been reported to exhibit a wide range of biological activities, such as antiviral, antimicrobial and cytotoxic activity. Therefore, besides a merely functional role, the glycerophospholipids could act as a bank of these fatty acids that are involved in defense mechanisms and can be released in the event of situations that require their participation. However, further research about how the aging affects the chemistry and biochemistry of sponge cells is needed to prove this hypothesis.

4. Conclusions

The metabolome of giant barrel sponges belonging to two distinct genetic groups revealed their different response to changes along a depth gradient. Interestingly, only samples belonging to genetic group 7 displayed an effect on the metabolome in relation to the depth gradient. This shows that genetic variation of each genetic group can be reflected in the metabolic response that they have towards changes in their environments. The changes were related with a higher content of lysophosphatidylcholine lipids at shallower depths. These compounds have been reported to play several biological roles in animal cells indicating that environmental conditions such as light, temperature and predatory stress present in shallower waters might trigger the production of this family of compounds. Samples collected at greater depths did not reveal the overexpression of any specific group of compounds, but one of the compounds that was found to be related to these conditions contains a *N*-methylethoanolamine unit that has been reported only in bacteria, suggesting that it is the result of the interaction between the metabolisms of the sponge and their associated microorganisms. Apart from the depth, age was also found to affect the metabolome of giant barrel sponges with older specimens having increased amounts of glycerophospholipids. This is an important contribution as it is the first time that age has been evaluated as a factor for change in the metabolome of these sponges and could be indicative of an increase in cell replication in older sponges.

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CRediT author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

Alexander, B.E., Liebrand, K., Osinga, R., van der Geest, H.G., Admiraal, W., Cleutjens, J. P.M., Schutte, B., Verheyen, F., Ribes, M., van Loon, E., de Goeij, J.M., 2014. Cell turnover and detritus production in marine sponges from tropical and temperate benthic ecosystems. PLoS One 9, e109486. https://doi.org/10.1371/journal.pone.0109486.

Bayona, L.M., van Leeuwen, G., Erol, Ö., Swierts, T., van der Ent, E., de Voogd, N.J., Choi, Y.H., 2020. Influence of geographical location on the metabolic production of giant barrel sponges (*Xestospongia* spp.) revealed by metabolomics tools. ACS Omega 5, 12398–12408. https://doi.org/10.1021/acsomega.0c01151.

Bell, J.J., Davy, S.K., Jones, T., Taylor, M.W., Webster, N.S., 2013. Could some coral reefs become sponge reefs as our climate changes? Global Change Biol. 19, 2613–2624. https://doi.org/10.1111/gcb.12212.

Bell, J.J., Rovellini, A., Davy, S.K., Taylor, M.W., Fulton, E.A., Dunn, M.R., Bennett, H.M., Kandler, N.M., Luter, H.M., Webster, N.S., 2018. Climate change alterations to ecosystem dominance: how might sponge-dominated reefs function? Ecology 99, 1920–1931. https://doi.org/10.1002/ecy.2446.

Birgbauer, E., Chun, J., 2006. New developments in the biological functions of lysophospholipids. Cellular and Molecular Life Sciences CMLS 63, 2695–2701. https://doi.org/10.1007/s00018-006-6155-y.

Bundy, J.G., Davey, M.P., Viant, M.R., 2008. Environmental metabolomics: a critical review and future perspectives. Metabolomics 5, 3. https://doi.org/10.1007/s11306-008-0152-0.

Chanas, B., Pawlik, J.R., 1997. Variability in the chemical defense of the Caribbean reef sponge Xestospongia muta. In: Lessios, H.A., Macintyre, I.G. (Eds.), Proceedings of the 8th International Coral Reef Symposium. Smithsonian Tropical Research Institute, Balboa, pp. 1363–1368.

da Silva, R.R., Wang, M., Nothias, L.-F., van der Hooft, J.J.J., Caraballo-Rodríguez, A.M., Fox, E., Balunas, M.J., Klassen, J.L., Lopes, N.P., Dorrestein, P.C., 2018. Propagating annotations of molecular networks using in silico fragmentation. PLoS Comput. Biol. 14, e1006089 https://doi.org/10.1371/journal.pcbi.1006089.

Dahal, R.H., Kim, J., 2017. Rhodanobacter humi sp. nov., an acid-tolerant and alkalitolerant gammaproteobacterium isolated from forest soil. Int. J. Syst. Evol. Microbiol. 67, 1185–1190. https://doi.org/10.1099/ijsem.0.001786.

D'Arrigo, P., Servi, S., 2010. Synthesis of lysophospholipids. Molecules 15, 1354–1377. https://doi.org/10.3390/molecules15031354.

De Goeij, J.M., De Kluijver, A., Van Duyl, F.C., Vacelet, J., Wijffels, R.H., De Goeij, A.F.P. M., Cleutjens, J.P.M., Schutte, B., 2009. Cell kinetics of the marine sponge *Halisarca caerulea* reveal rapid cell turnover and shedding. J. Exp. Biol. 212, 3892–3900. https://doi.org/10.1242/jeb.034561.

De Goeij, J.M., van Oevelen, D., Vermeij, M.J.A., Osinga, R., Middelburg, J.J., De Goeij, A.F.P.M., Admiraal, W., 2013. Surviving in a marine desert: the sponge loop retains resources within coral reefs. Science 342, 108–110. https://doi.org/10.1126/science.1241981.

Diaz, M.C., Rützler, K., 2001. Sponges: an essential component of caribbean coral reefs. Bull. Mar. Sci. 62, 535–546.

Djoumbou Feunang, Y., Eisner, R., Knox, C., Chepelev, L., Hastings, J., Owen, G., Fahy, E., Steinbeck, C., Subramanian, S., Bolton, E., Greiner, R., Wishart, D.S., 2016. ClassyFire: automated chemical classification with a comprehensive, computable taxonomy. J. Cheminf. 8, 61. https://doi.org/10.1186/s13321-016-0174-y.

Ernst, M., Kang, B.K., Caraballo-Rodríguez, M.A., Nothias, L.-F., Wandy, J., Chen, C., Wang, M., Rogers, S., Medema, H.M., Dorrestein, C.P., van der Hooft, J.J.J., 2019. MolNetEnhancer: enhanced molecular networks by integrating metabolome mining and annotation tools. Metabolites 9, 144. https://doi.org/10.3390/metabo9070144.

Fiore, C.L., Baker, D.M., Lesser, M.P., 2013. Nitrogen biogeochemistry in the caribbean sponge, Xestospongia muta: a source or sink of dissolved inorganic nitrogen? PLoS One 8, e72961. https://doi.org/10.1371/journal.pone.0072961.

Genin, E., Wielgosz-Collin, G., Njinkoué, J.-M., Velosaotsy, N.E., Kornprobst, J.-M., Gouygou, J.-P., Vacelet, J., Barnathan, G., 2008. New trends in phospholipid class composition of marine sponges. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 150, 427–431. https://doi.org/10.1016/j.cbpb.2008.04.012.

Gloeckner, V., Wehrl, M., Moitinho-Silva, L., Gernert, C., Schupp, P., Pawlik, J.R., Lindquist, N.L., Erpenbeck, D., Wörheide, G., Hentschel, U., 2014. The HMA-LMA dichotomy revisited: an electron microscopical survey of 56 sponge species. Biol. Bull. 227, 78–88. https://doi.org/10.1086/BBLv227n1p78.

Goldfine, H., Ellis, M.E., 1963. N-methyl groups in bacterial lipids. J. Bacteriol. 87, 8–15.

Goulitquer, S., Potin, P., Tonon, T., 2012. Mass spectrometry-based metabolomics to elucidate functions in marine organisms and ecosystems. Mar. Drugs 10, 849–880. https://doi.org/10.3390/md10040849.

Greff, S., Aires, T., Serrão, E.A., Engelen, A.H., Thomas, O.P., Pérez, T., 2017. The interaction between the proliferating macroalga Asparagopsis taxiformis and the coral *Astroides calycularis* induces changes in microbiome and metabolomic fingerprints. Sci. Rep. 7, 42625. https://doi.org/10.1038/srep42625.

Hay, M.E., 2009. Marine chemical ecology: chemical signals and cues structure marine populations, communities, and ecosystems. Annual review of marine science 1, 193–212. https://doi.org/10.1146/annurev.marine.010908.163708.

Horai, H., Arita, M., Kanaya, S., Nihei, Y., Ikeda, T., Suwa, K., Ojima, Y., Tanaka, Kenichi, Tanaka, S., Aoshima, K., Oda, Y., Kakazu, Y., Kusano, M., Tohge, T., Matsuda, F., Sawada, Y., Hirai, M.Y., Nakanishi, H., Ikeda, K., Akimoto, N., Maoka, T., Takahashi, H., Ara, T., Sakurai, N., Suzuki, H., Shibata, D., Neumann, S., Iida, T., Tanaka, Ken, Funatsu, K., Matsuura, F., Soga, T., Taguchi, R., Saito, K., Nishioka, T., 2010. MassBank: a public repository for sharing mass spectral data for life sciences. J. Mass Spectrom. 45, 703–714. https://doi.org/10.1002/jms.1777.

Hughes, T.P., Kerry, J.T., Álvarez-Noriega, M., Álvarez-Romero, J.G., Anderson, K.D., Baird, A.H., Babcock, R.C., Beger, M., Bellwood, D.R., Berkelmans, R., Bridge, T.C., Butler, I.R., Byrne, M., Cantin, N.E., Comeau, S., Connolly, S.R., Cumming, G.S., Dalton, S.J., Diaz-Pulido, G., Eakin, C.M., Figueira, W.F., Gilmour, J.P., Harrison, H. B., Heron, S.F., Hoey, A.S., Hobbs, J.-P.A., Hoogenboom, M.O., Kennedy, E.V., Kuo, C., Lough, J.M., Lowe, R.J., Liu, G., McCulloch, M.T., Malcolm, H.A., McWilliam, M.J., Pandolfi, J.M., Pears, R.J., Pratchett, M.S., Schoepf, V., Simpson, T., Skirving, W.J., Sommer, B., Torda, G., Wachenfeld, D.R., Willis, B.L., Wilson, S.K., 2017. Global warming and recurrent mass bleaching of corals. Nature 543, 373–377. https://doi.org/10.1038/nature21707.

Hughes, T.P., Rodrigues, M.J., Bellwood, D.R., Ceccarelli, D., Hoegh-Guldberg, O., McCook, L., Moltschaniwskyj, N., Pratchett, M.S., Steneck, R.S., Willis, B., 2007. Phase shifts, herbivory, and the resilience of coral reefs to climate change. Curr. Biol. 17, 360–365. https://doi.org/10.1016/j.cub.2006.12.049.

Ivanisevic, J., Pérez, T., Ereskovsky, A.V., Barnathan, G., Thomas, O.P., 2011. Lysophospholipids in the mediterranean sponge Oscarella tuberculata: seasonal variability and putative biological role. J. Chem. Ecol. 37, 537. https://doi.org/10.1007/s10886-011-9943-2.

Jové, M., Portero-Otín, M., Naudí, A., Ferrer, I., Pamplona, R., 2014. Metabolomics of human brain aging and age-related neurodegenerative diseases. J. Neuropathol. Exp. Neurol. 73, 640–657. https://doi.org/10.1097/NEN.0000000000000091.

Lee, H.-J., Jeong, J., Alves, A.C., Han, S.-T., In, G., Kim, E.-H., Jeong, W.-S., Hong, Y.-S., 2019. Metabolomic understanding of intrinsic physiology in Panax ginseng during whole growing seasons. Journal of Ginseng Research 43, 654–665. https://doi.org/10.1016/j.jgr.2019.04.004.

Loh, T.-L., Pawlik, J.R., 2014. Chemical defenses and resource trade-offs structure sponge communities on Caribbean coral reefs. Proceeding of the National Academy of Sciences USA 111, 4151–4156. https://doi.org/10.1073/pnas.1321626111.

McGrath, E.C., Woods, L., Jompa, J., Haris, A., Bell, J.J., 2018. Growth and longevity in giant barrel sponges: redwoods of the reef or Pines in the Indo-Pacific? Sci. Rep. 8, 15317. https://doi.org/10.1038/s41598-018-33294-1.

McManus, J.W., Polsenberg, J.F., 2004. Coral–algal phase shifts on coral reefs: ecological and environmental aspects. Prog. Oceanogr. 60, 263–279. https://doi.org/10.1016/j.pocean.2004.02.014.

McMurray, S.E., Blum, J.E., Pawlik, J.R., 2008. Redwood of the reef: growth and age of the giant barrel sponge *Xestospongia muta* in the Florida Keys. Mar. Biol. 155, 159–171. https://doi.org/10.1007/s00227-008-1014-z.

McMurray, S.E., Finelli, C.M., Pawlik, J.R., 2015. Population dynamics of giant barrel sponges on Florida coral reefs. J. Exp. Mar. Biol. Ecol. 473, 73–80. https://doi.org/10.1016/j.jembe.2015.08.007.

Morrow, K.M., Fiore, C.L., Lesser, M.P., 2016. Environmental drivers of microbial community shifts in the giant barrel sponge, *Xestospongia muta*, over a shallow to mesophotic depth gradient. Environ. Microbiol. 18, 2025–2038. https://doi.org/10.1111/1462-2920.13226.

Müller, W.E.G., Müller, I.M., 2003. Origin of the metazoan immune system: identification of the molecules and their functions in sponges. Integr. Comp. Biol. 43, 281–292. https://doi.org/10.1093/icb/43.2.281.

Myers, O.D., Sumner, S.J., Li, S., Barnes, S., Du, X., 2017. One step forward for reducing false positive and false negative compound identifications from mass spectrometry metabolomics data: new algorithms for constructing extracted ion chromatograms and detecting chromatographic peaks. Anal. Chem. 89, 8696–8703. https://doi.org/10.1021/acs.analchem.7b00947.

Ng, Y.K., Hewavitharana, A.K., Webb, R., Shaw, P.N., Fuerst, J.A., 2013. Developmental cycle and pharmaceutically relevant compounds of Salinispora actinobacteria isolated from Great Barrier Reef marine sponges. Appl. Microbiol. Biotechnol. 97, 3097–3108. https://doi.org/10.1007/s00253-012-4479-0.

Nothias, L.-F., Petras, D., Schmid, R., Dührkop, K., Rainer, J., Sarvepalli, A., Protsyuk, I., Ernst, M., Tsugawa, H., Fleischauer, M., Aicheler, F., Aksenov, A.A., Alka, O., Allard, P.-M., Barsch, A., Cachet, X., Caraballo-Rodriguez, A.M., Da Silva, R.R., Dang, T., Garg, N., Gauglitz, J.M., Gurevich, A., Isaac, G., Jarmusch, A.K., Kameník, Z., Kang, K.B., Kessler, N., Koester, I., Korf, A., Le Gouellec, A., Ludwig, M., Martin, H.C., McCall, L.-I., McSayles, J., Meyer, S.W., Mohimani, H., Morsy, M., Moyne, O., Neumann, S., Neuweger, H., Nguyen, N.H., Nothias-Esposito, M., Paolini, J., Phelan, V.V., Pluskal, T., Quinn, R.A., Rogers, S., Shrestha, B., Tripathi, A., van der Hooft, J.J.J., Vargas, F., Weldon, K.C., Witting, M., Yang, H., Zhang, Z., Zubeil, F., Kohlbacher, O., Böcker, S., Alexandrov, T., Bandeira, N., Wang, M., Dorrestein, P.C., 2020. Feature-based molecular networking in the GNPS analysis environment. Nat. Methods 17, 905–908. https://doi.org/10.1038/s41592-020-0933-6.

Noyer, C., Thomas, O.P., Becerro, M.A., 2011. Patterns of chemical diversity in the mediterranean sponge *Spongia lamella*. PLoS One 6, e20844. https://doi.org/10.1371/journal.pone.0020844.

Oksanen, J., Blanchet, G., Friendly, M., Kindt, R., Legendre, P., McGlinn, P., 2019. Vegan: Community Ecology Package (Version 2.5-6), 2019.

Olson, J.B., Gao, X., 2013. Characterizing the bacterial associates of three Caribbean sponges along a gradient from shallow to mesophotic depths. FEMS Microbiol. Ecol. 85, 74–84. https://doi.org/10.1111/1574-6941.12099.

Page, M., West, L., Northcote, P., Battershill, C., Kelly, M., 2005. Spatial and temporal variability of cytotoxic metabolites in populations of the New Zealand sponge *Mycale hentscheli*. J. Chem. Ecol. 31, 1161–1174. https://doi.org/10.1007/s10886-005-4254-0.

Pandolfi, J.M., Bradbury, R.H., Sala, E., Hughes, T.P., Bjorndal, K.A., Cooke, R.G., McArdle, D., McClenachan, L., Newman, M.J.H., Paredes, G., Warner, R.R., Jackson, J.B.C., 2003. Global trajectories of the long-term decline of coral reef ecosystems. Science 301, 955. https://doi.org/10.1126/science.1085706.

Paul, V.J., Freeman, C.J., Agarwal, V., 2019. Chemical ecology of marine sponges: new opportunities through "-Omics.". Integr. Comp. Biol. https://doi.org/10.1093/icb/icz014.

Paul, V.J., Puglisi, M.P., Ritson-Williams, R., 2006. Marine chemical ecology. Nat. Prod. Rep. 23, 153–180. https://doi.org/10.1039/B404735B.

Paul, V.J., Ritson-Williams, R., 2008. Marine chemical ecology. Nat. Prod. Rep. 25, 662–695. https://doi.org/10.1039/B702742G.

Pawlik, J.R., 2011. The chemical ecology of sponges on caribbean reefs: natural products shape natural systems. Bioscience 61, 888–898. https://doi.org/10.1525/bio.2011.61.11.8.

Pawlik, J.R., Chanas, B., Toonen, R.J., Fenical, W., 1995. Defenses of Caribbean sponges against predatory reef fish. I. Chemical deterrency. Mar. Ecol. Prog. Ser. 127, 183–194.

Pawlik, J.R., McMurray, S.E., 2019. The emerging ecological and biogeochemical importance of sponges on coral reefs. Annual Review of Marine Science 12, 315–337. https://doi.org/10.1146/annurev-marine-010419-010807.

Pluskal, T., Castillo, S., Villar-Briones, A., Orešič, M., 2010. MZmine 2: modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. BMC Bioinf. 11, 395. https://doi.org/10.1186/1471-2105-11-395.

Quinn, R.A., Vermeij, M.J.A., Hartmann, A.C., Galtier d'Auriac, I., Benler, S., Haas, A., Quistad, S.D., Lim, Y.W., Little, M., Sandin, S., Smith, J.E., Dorrestein, P.C., Rohwer, F., 2016. Metabolomics of reef benthic interactions reveals a bioactive lipid involved in coral defence. Proc. Biol. Sci. 283, 20160469. https://doi.org/10.1098/rspb.2016.0469.

Reverter, M., Perez, T., Ereskovsky, A.V., Banaigs, B., 2016. Secondary metabolome variability and inducible chemical defenses in the mediterranean sponge *Aplysina cavernicola*. J. Chem. Ecol. 42, 60–70. https://doi.org/10.1007/s10886-015-0664-9.

Reverter, M., Tribalat, M.-A., Pérez, T., Thomas, O.P., 2018. Metabolome variability for two Mediterranean sponge species of the genus Haliclona: specificity, time, and space. Metabolomics 14, 114. https://doi.org/10.1007/s11306-018-1401-5.

Ribes, M., Calvo, E., Movilla, J., Logares, R., Coma, R., Pelejero, C., 2016. Restructuring of the sponge microbiome favors tolerance to ocean acidification. Environmental Microbiology Reports 8, 536–544. https://doi.org/10.1111/1758-2229.12430.

Rix, L., de Goeij, J.M., van Oevelen, D., Struck, U., Al-Horani, F.A., Wild, C., Naumann, M.S., 2018. Reef sponges facilitate the transfer of coral-derived organic matter to their associated fauna via the sponge loop. Mar. Ecol. Prog. Ser. 589, 85–96. https://doi.org/10.3354/meps12443.

Sacristan-Soriano, O., Banaigs, B., Becerro, M.A., 2011. Relevant spatial scales of chemical variation in *Aplysina aerophoba*. Mar. Drugs 9, 2499–2513. https://doi.org/10.3390/md9122499.

Schubotz, F., Lipp, J.S., Elvert, M., Hinrichs, K.-U., 2011. Stable carbon isotopic compositions of intact polar lipids reveal complex carbon flow patterns among hydrocarbon degrading microbial communities at the Chapopote asphalt volcano. Geochem. Cosmochim. Acta 75, 4399–4415. https://doi.org/10.1016/j.gca.2011.05.018.

Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., Ideker, T., 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 13, 2498–2504. https://doi.org/10.1101/gr.1239303.

Sogin, E.M., Putnam, H.M., Anderson, P.E., Gates, R.D., 2016. Metabolomic signatures of increases in temperature and ocean acidification from the reef-building coral, *Pocillopora damicornis*. Metabolomics 12, 71. https://doi.org/10.1007/s11306-016-0987-8.

Southwell, M.W., Weisz, J.B., Martens, C.S., Lindquist, N., 2008. In situ fluxes of dissolved inorganic nitrogen from the sponge community on Conch Reef, Key Largo, Florida. Limnol. Oceanogr. 53, 986–996. https://doi.org/10.4319/lo.2008.53.3.0986.

Swierts, T., 2019. Diversity in the Globally Intertwined Giant Barrel Sponge Species Complex. Leiden University, The Netherlands.

Swierts, T., Cleary, D.F.R., de Voogd, N.J., 2018a. Prokaryotic communities of Indo-Pacific giant barrel sponges are more strongly influenced by geography than host phylogeny. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 94, fiy194. https://doi.org/10.1093/femsec/fiy194.

Swierts, T., Huang, Y.M., de Voogd, N.J., 2018b. The giant barrel sponge facilitates the recovery of coral fragments after a tropical storm in Taiwan. Coral Reefs 37. https://doi.org/10.1007/s00338-018-1691-0, 675–675.

Swierts, T., Peijnenburg, K.T.C.A., de Leeuw, C.A., Breeuwer, J.A.J., Cleary, D.F.R., de Voogd, N.J., 2017. Globally intertwined evolutionary history of giant barrel sponges. Coral Reefs 36, 933–945. https://doi.org/10.1007/s00338-017-1585-6.

Swierts, T., Peijnenburg, K.T.C.A., de Leeuw, C., Cleary, D.F.R., Hörnlein, C., Setiawan, E., Wörheide, G., Erpenbeck, D., de Voogd, N.J., 2013. Lock, Stock and Two Different Barrels: Comparing the Genetic Composition of Morphotypes of the Indo-Pacific Sponge *Xestospongia testudinaria*. PLOS ONE 8, e74396. https://doi.org/10.1371/journal.pone.0074396.

Torkhovskaya, T.I., Ipatova, O.M., Zakharova, T.S., Kochetova, M.M., Khalilov, E.M., 2007. Lysophospholipid receptors in cell signaling. Biochemistry (Mosc.) 72, 125–131. https://doi.org/10.1134/S0006297907020010.

Turon, M., Cáliz, J., Garate, L., Casamayor, E.O., Uriz, M.J., 2018. Showcasing the role of seawater in bacteria recruitment and microbiome stability in sponges. Sci. Rep. 8, 15201. https://doi.org/10.1038/s41598-018-33545-1.

Van Soest, R.W.M., Boury-Esnault, N., Vacelet, J., Dohrmann, M., Erpenbeck, D., De Voogd, N.J., Santodomingo, N., Vanhoorne, B., Kelly, M., Hooper, J.N.A., 2012. Global diversity of sponges (Porifera). PLoS One 7, e35105. https://doi.org/10.1371/journal.pone.0035105.

Van Soest, R.W.M., Meesters, E.H.W.G., Becking, L.E., 2014. Deep-water sponges (Porifera) from bonaire and klein Curaçao, southern caribbean. Zootaxa 3878, 401–443. https://doi.org/10.11646/zootaxa.3878.5.1.

Viant, M.R., 2007. Metabolomics of aquatic organisms. Mar. Ecol. Prog. Ser. 332, 301–306. https://doi.org/10.3354/meps332301.

Villegas-Plazas, M., Wos-Oxley, M.L., Sanchez, J.A., Pieper, D.H., Thomas, O.P., Junca, H., 2019. Variations in microbial diversity and metabolite profiles of the tropical marine sponge *Xestospongia muta* with season and depth. Microb. Ecol. 78, 243–256. https://doi.org/10.1007/s00248-018-1285-y.

Wang, M., Carver, J.J., Phelan, V.V., Sanchez, L.M., Garg, N., Peng, Y., Nguyen, D.D., Watrous, J., Kapono, C.A., Luzzatto-Knaan, T., Porto, C., Bouslimani, A., Melnik, A. V., Meehan, M.J., Liu, W.-T., Crüsemann, M., Boudreau, P.D., Esquenazi, E., Sandoval-Calderón, M., Kersten, R.D., Pace, L.A., Quinn, R.A., Duncan, K.R., Hsu, C.-C., Floros, D.J., Gavilan, R.G., Kleigrewe, K., Northen, T., Dutton, R.J., Parrot, D., Carlson, E.E., Aigle, B., Michelsen, C.F., Jelsbak, L., Sohlenkamp, C., Pevzner, P., Edlund, A., McLean, J., Piel, J., Murphy, B.T., Gerwick, L., Liaw, C.-C., Yang, Y.-L., Humpf, H.-U., Maansson, M., Keyzers, R.A., Sims, A.C., Johnson, A.R., Sidebottom, A.M., Sedio, B.E., Klitgaard, A., Larson, C.B., Boya, P.C.A., Torres-Mendoza, D., Gonzalez, D.J., Silva, D.B., Marques, L.M., Demarque, D.P., Pociute, E., O'Neill, E.C., Briand, E., Helfrich, E.J.N., Granatosky, E.A., Glukhov, E., Ryffel, F., Houson, H., Mohimani, H., Kharbush, J.J., Zeng, Y., Vorholt, J.A., Kurita, K.L., Charusanti, P., McPhail, K.L., Nielsen, K.F., Vuong, L., Elfeki, M., Traxler, M.F., Engene, N., Koyama, N., Vining, O.B., Baric, R., Silva, R.R., Mascuch, S.J., Tomasi, S., Jenkins, S., Macherla, V., Hoffman, T., Agarwal, V., Williams, P.G., Dai, J., Neupane, R., Gurr, J., Rodríguez, A.M.C., Lamsa, A., Zhang, C., Dorrestein, K., Duggan, B.M., Almaliti, J., Allard, P.-M., Phapale, P., Nothias, L.-F., Alexandrov, T., Litaudon, M., Wolfender, J.-L., Kyle, J.E., Metz, T.O., Peryea, T., Nguyen, D.-T., VanLeer, D., Shinn, P., Jadhav, A., Müller, R., Waters, K.M., Shi, W., Liu, X., Zhang, L., Knight, R., Jensen, P.R., Palsson, B.Ø., Pogliano, K., Linington, R. G., Gutiérrez, M., Lopes, N.P., Gerwick, W.H., Moore, B.S., Dorrestein, P.C., Bandeira, N., 2016. Sharing and community curation of mass spectrometry data with global natural products social molecular networking. Nat. Biotechnol. 34, 828–837. https://doi.org/10.1038/nbt.3597.

Yoon, D., Choi, B.-R., Ma, S., Lee, W.J., Jo, I.-H., Lee, Y.-S., Kim, G.-S., Kim, S., Lee, Y.D., 2019. Metabolomics for age discrimination of ginseng using a multiplex approach to HR-MAS NMR spectroscopy, UPLC–QTOF/MS, and GC × GC–TOF/MS. Molecules 24. https://doi.org/10.3390/molecules24132381.

Yu, Z., Zhai, G., Singmann, P., He, Y., Xu, T., Prehn, C., Römisch-Margl, W., Lattka, E., Gieger, C., Soranzo, N., Heinrich, J., Standl, M., Thiering, E., Mittelstraß, K., Wichmann, H.-E., Peters, A., Suhre, K., Li, Y., Adamski, J., Spector, T.D., Illig, T., Wang-Sattler, R., 2012. Human serum metabolic profiles are age dependent. Aging Cell 11, 960–967. https://doi.org/10.1111/j.1474-9726.2012.00865.x.

Zea, S., 1993. Cover of sponges and other sessile organisms in rocky and coral reef habitats of Santa Marta, Colombian Caribbean Sea. Caribb. J. Sci. 29, 75–88.

Zhou, X., Xu, T., Yang, X.-W., Huang, R., Yang, B., Tang, L., Liu, Y., 2010. Chemical and biological aspects of marine sponges of the genus *Xestospongia*. Chem. Biodivers. 7, 2201–2227. https://doi.org/10.1002/cbdv.201000024.

Zhou, Z.-F., Menna, M., Cai, Y.-S., Guo, Y.-W., 2015. Polyacetylenes of marine origin: chemistry and bioactivity. Chem. Rev. 115, 1543–1596. https://doi.org/10.1021/cr4006507.