Received: 28 November 2017 | Revised: 10 February 2018 | Accepted: 12 February 2018

DOI: 10.1111/gcb.14116

PRIMARY RESEARCH ARTICLE

WILEY Global Change Biology

Elucidating the sponge stress response; lipids and fatty acids can facilitate survival under future climate scenarios

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Funding information
Royal Society of New Zealand, Grant/Award Number: Marsden Fund (VUW1505); Australian Research Council, Grant/Award Number: FT120100480; Australian Institute of Marine Science, Victoria University of Wellington, the PADI foundation; Deakin University; VUW Doctoral Scholarship

Abstract

Ocean warming (OW) and ocean acidification (OA) are threatening coral reef ecosystems, with a bleak future forecast for reef-building corals, which are already experiencing global declines in abundance. In contrast, many coral reef sponge species are able to tolerate climate change conditions projected for 2100. To increase our understanding of the mechanisms underpinning this tolerance, we explored the lipid and fatty acid (FA) composition of four sponge species with differing sensitivities to climate change, experimentally exposed to OW and OA levels predicted for 2100, under two CO_2 Representative Concentration Pathways. Sponges with greater concentrations of storage lipid, phospholipids, sterols and elevated concentrations of *n*-3 and *n*-6 long-chain polyunsaturated FA (LC PUFA), were more resistant to OW. Such biochemical constituents likely contribute to the ability of these sponges to maintain membrane function and cell homeostasis in the face of environmental change. Our results suggest that *n*-3 and *n*-6 LC PUFA are important components of the sponge stress response potentially via chain elongation and the eicosanoid stress-signalling pathways. The capacity for sponges to compositionally alter their membrane lipids in response to stress was also explored using a number of specific homeoviscous adaptation (HVA) indicators. This revealed a potential mechanism via which additional CO_2 could facilitate the resistance of phototrophic sponges to thermal stress through an increased synthesis of membrane-stabilizing sterols. Finally, OW induced an increase in FA unsaturation in phototrophic sponges but a decrease in heterotrophic species, providing support for a difference in the thermal response pathway between the sponge host and the associated photosymbionts. Here we have shown that sponge lipids and FA are likely to be an important component of the sponge stress response and may play a role in facilitating sponge survival under future climate conditions.

KEYWORDS
climate change, coral reef, fatty acid, heterotroph, lipid, mechanism, ocean acidification, ocean warming, phototroph, porifera

1 | INTRODUCTION

As the climate changes, ocean warming (OW) and ocean acidification (OA) pose a number of threats to coral reefs (Heron, Maynard, & Ruben Van Hooidonk, 2016; Hughes et al., 2017; Manzello, Eakin, & Glynn, 2017). Climate change conditions projected for 2,100 combined with ongoing degradation from local stressors, are expected to cause significant declines in coral cover and create space for other more tolerant organisms (Bell, Davy, Jones, Taylor, & Webster, 2013; Kroeker, Micheli, & Gambi, 2013; Norström, Nyström, Lokrantz, & Folke, 2009). Some coral reef sponges are able to tolerate elevated temperature and oceanic pCO_2, suggesting a capacity to proliferate on coral reefs as space is made available by declines in more sensitive reef species (Bell et al., 2013; Bennett et al., 2017; Duckworth & Peterson, 2013; Duckworth, West, Vansach, Stubler, & Hardt, 2012; Fang et al., 2013; Lesser, Fiore, Slattery, & Zaneveld, 2016; Stubler, Furman, & Peterson, 2015; Vicente, Silbiger, Beckley, Raczkowski, & Hill, 2015; Wisshak, Schönberg, Form, & Freiwald, 2013). To date, climate change research on marine sponges has focused primarily on the physiological response of these sessile organisms to predicted OW and OA. However, while physiological responses measure the systemic tolerance of sponges and reflect their ability to acclimate to environmental change, the mechanisms underpinning these responses remain unclear. Mechanistic understanding is required to assess the acclimatization and adaptation potential of this important phylum in the face of environmental change (Putnam, Barott, Ainsworth, & Gates, 2017). Furthermore, OW and OA are known to influence other cellular and molecular processes that may not be reflected by host physiology during experimental exposure (Pörtner, 2008), and it is likely that adjustments at the molecular or membrane level will ultimately define tolerance limits (Pörtner, 2002).

The lipid bilayer (cell membrane), which forms a permeable barrier for cells and subcellular organelles, is sensitive to environmental stressors (Hazel, 1995). This is particularly apparent in relation to temperature, where changes will alter cell membrane fluidity and cytotoxicity (Parrish, 2013; Vígh et al., 2007). At an organism's adapted temperature, lipids in this bilayer (membrane lipids) are in the liquid-crystalline phase, and enable vital cellular functions including the regulation of transmembrane activities (e.g. nutrient transport); the maintenance of solute gradients for energetic processes; and the facilitation of enzyme activity by providing a matrix where biochemical reactions can occur (Guillot, Obis, & Mistou, 2000; Hazel, 1995; Neidleman, 1987; Sinensky, 1974). As temperature increases, the movement of fatty acid (FA) acyl chains increases and membrane lipids assume a disordered inverted hexagonal phase. As such, beyond an organism's optimal temperature range, membranes become "hyperfluid", resulting in a loss of bilayer integrity, which subsequently compromises cell homeostasis and overall cell function (Hazel, 1995).

Ectothermic organisms, including sponges, can counteract the effects of increased temperature through the compositional alteration of membrane lipids in a process called homeoviscous adaptation (HVA) (Martin-Creuzburg & Elert, 2009; Nes, 1974; Volkman, 2003). HVA involves changes in the mechanical and chemical properties of the lipid bilayer to produce membranes with constant fluidity as temperature changes (Horváth et al., 2012; Parrish, 2013; Šajbidor, 1997; Sinensky, 1974; Vígh et al., 2007; Weirich & Reigh, 2001). Under thermal stress, organism-specific responses are employed to prevent membrane destabilization and to maintain the "ideal" functional state of the cell membrane (Guerzoni, Lanciotti, & Cocconcelli, 2001). Such adaptive mechanisms may involve: (i) shifts in the relative proportion of membrane lipids (phospholipids, glycolipids and sterols), where sterols are particularly important for maintaining membrane rigidity at super-optimal temperatures (Copeman & Parrish, 2004; Parrish, 2013); (ii) increasing FA chain length to reduce the fluidity of cell membranes (Guerzoni et al., 2001; Hochachka & Somero, 1984); (iii) increasing the proportion of saturated FA (SFA) in relation to polyunsaturated FA (PUFA) with temperature, as SFA are more resistant to lipid oxidation and facilitate greater membrane stability (Tchernov et al., 2004; Wada, Gombos, & Murata, 1994); and (iv) reducing the degree of FA saturation and increasing membrane fluidity, whereby unsaturated FA are less likely to pack together in the bilayer given their "kinked" structure (Hazel, 1995).

Sponge lipid composition is distinctive among marine organisms (Lawson, Stoilov, Thompson, & Djerassi, 1988), particularly in relation to their lipid bilayer, which contains an abundance of novel phospholipids and sterols, and a high diversity of FA. Sponges have an abundance of long-chain C_{22}-C_{30} FA with the presence of branched, odd-chain, or hydroxyl groups (Koopmans et al., 2015), commonly referred to as the "demospongic acids" despite more recent evidence confirming their biosynthesis is not specific to sponges (Bergé & Barnathan, 2005; Djerassi & Lam, 1991; Koopmans et al., 2015; Kornprobst & Barnathan, 2010; Rod'kina, 2005). Such long-chain FA are particularly important for environmental stress resistance, due to the role that they play in maintaining the fluidity of cell membranes and controlling cellular responses to external stimuli, including changing temperatures (Arts & Kohler, 2009; Bergé & Barnathan, 2005; Hixson & Arts, 2016; Parrish, 2013). These features of sponge lipid and FA composition may play at least some part in the persistence of sponges in unpredictable and variable environments (Djerassi & Lam, 1991; Genin et al., 2008; Lawson et al., 1988; Santalova et al., 2004). However, while it is understood that sponges can control the fluidity of their cell membranes with changing temperatures seasonally, e.g. by increasing the concentration of lipids that have a higher melting point in summer (Lawson et al., 1988), there is a lack of studies considering the effects of environmental change on sponge lipid and FA content (Arillo, Bavestrello, Burlando, & Sara, 1993).

Previous work in which four abundant Great Barrier Reef (GBR) sponge species—the phototrophic *Carteriospongia foliascens* and *Cymbastela coralliophila* and the heterotrophic *Rhopaloeides odorabile* and *Stylissa flabelliformis*—were experimentally exposed to OW and OA, revealed species-specific differences in tolerance to these stressors (Bennett et al., 2017), although the mechanisms underpinning the responses were not resolved. Elevated pCO_2 was also found to

provide the phototrophic sponges with protection from thermal stress; however, the pathways underlying this ameliorative effect also remained unclear (Bennett et al., 2017). Therefore, although we are beginning to understand sensitivity thresholds of sponges exposed to OW and OA, little is known about the mechanisms that enable them to cope with such environmental impacts. The physical properties of lipids within cell membranes, and the ability of organisms to regulate and adapt their cell lipid bilayers in response to thermal stress play a significant role in stress tolerance, and ultimately define an organism's thermal limits (Arts & Kohler, 2009; Geider, 1987; Hazel, 1995; Singh, Sinha, & Hader, 2002). Here, we assessed the lipid and FA composition of the phototrophic sponges *Carteriospongia foliascens* and *Cymbastela coralliophila*, and the heterotrophic sponges *Rhopaloeides odorabile* and *Stylissa flabelliformis*, before and after experimental exposure to OW and OA, and explored HVA mechanisms to ascertain whether more tolerant species are able to alter membranes to acclimate to OW through changes in lipid and FA composition.

2 | MATERIALS AND METHODS

2.1 | Experimental design

The experimental design is described in detail in Bennett et al. (2017). Briefly, sponges were exposed to nine combined temperature and pH treatments. Treatments were based on present day $CO_{2\ atm}$ levels (~400 ppm) and projected $CO_{2\ atm}$ increases for 2,100 under the IPCC "baseline" emission scenarios RCP6.0 (~800 ppm) and RCP8.5 (~1,200 ppm) (IPCC, 2014). The experiment was performed within the National Sea Simulator at the Australian Institute of Marine Science (AIMS). Target treatments for the experiment were 28.5, 30 and 31.5°C and pH (Total scale) 8.1, 7.8 and 7.6. All factors were fully crossed, resulting in nine experimental treatments and three replicates of each treatment.

Sponges collected from 10 to 15 m depth at Davies Reef on the Great Barrier Reef, Australia (18°82′S, 147°65′E). Due to the large size of adult sponges, for all species except *C. foliascens*, ~20 larger specimens were cut to form ~60 smaller clones. For *C. foliascens*, ~30 small individuals and 15 large individuals (each cut into 2–3 clones) were collected. Once healed, clones were treated as "individual" sponges and were randomly allocated to experimental tanks (maintained at 27°C and pH 8.1 = T0/ambient). A 12-week exposure period post-ramping was planned for all adult sponges, with experiments terminated on a species-by-species basis as lethal effects were observed (see Bennett et al., 2017 for more details).

2.2 | Tissue sampling

Six sponges of each species were sacrificed at time zero (T0) for initial tissue analysis. Tissue was then taken from experimental sponges at the final sampling point (n = 4 to 6 individuals per species per treatment, except where mortalities occurred (*C. foliascens* where n = 2 at 31.5°C/pH 8.1 and 31.5°C/pH 7.8; and *R. odorabile* where n = 2 at 31.5°C/pH 7.6). The final sampling point varied for each species, due to differing sensitivities to the RCP8.5 treatment conditions. The experiment was terminated after T = 2 weeks for the "sensitive" species, *C. foliascens* and *R. odorabile*, which were sensitive to RCP8.5 OW (*C. foliascens*) and the combined effects of OW and OA (*R. odorabile*). The more "tolerant" species, *S. flabelliformis* and *C. coralliophila*, resisted RCP8.5 conditions for significantly longer. The experiment was terminated after T = 8 weeks for *S. flabelliformis* following high levels of tissue necrosis at 31.5°C and *C. coralliophila* remained in the experiment for the full 12 week exposure, despite high levels of bleaching at 31.5°C. Sponge tissue was cryopreserved in liquid nitrogen in 1.5 ml vials for subsequent chlorophyll *a*, total lipid, lipid class and FA analysis.

2.3 | Chlorophyll *a* determination (phototrophic species only)

Chlorophyll *a* (Chl *a*) concentrations were determined for the two phototrophic species as a proxy for the presence of phototrophic symbionts, and therefore an index of sponge bleaching (Wilkinson, 1983), following the methods of Pineda, Duckworth, and Webster (2016). Chl *a* was extracted from approximately 50 mg of cryopreserved sponge tissue in 95% ethanol with a total of 1.4 ml pigment extract recovered from each sample. Triplicate 300 μl extracts of each sample were analysed on a Power Wave Microplate Scanning Spectrophotometer (BIO-TEKw Instruments Inc., Vermont, USA). Chl *a* concentrations were normalized to sponge wet weight as: Chl *a* (mg/ml) × extraction volume (ml)/wet weight (g).

2.4 | Total lipid

Lipids were extracted from 20 to 600 mg of freeze-dried and crushed sponge tissue, according to the method described by Folch, Less, and Sloane-Stanley (1957), following modifications by Conlan, Jones, Turchini, Hall, and Francis (2014). Samples were sonicated (Vibracell, Sonics and Materials, Newtown, USA), and then filtered into a scintillation vial. This process was repeated three times, resulting in ~9 ml of filtrate, to which 4.5 ml of a potassium chloride sample wash [KCl (0.44%) in H_2O (3)/CH_3OH (1)] was added. The mixture was incubated for 18 hr at room temperature, after which the bottom layer containing the extracted lipid was recovered and the solvent was evaporated under nitrogen. Total lipid content was weighed and standardized to dry weight and expressed in mg lipid per g freeze-dried sponge dry weight. Once lipid content was determined, the lipid fraction was resuspended in 1 ml dichloromethane for subsequent lipid class analysis.

2.5 | Lipid class analysis

Lipid class analysis followed the method described by Nichols, Mooney, and Elliot (2001), with modifications by Conlan et al. (2014). A 100 μl aliquot of the resuspended total lipid fraction was taken and analysed for lipid class composition using thin layer

chromatography and flame ionization detection (Iatroscan MK 6s, Mitsubishi Chemical Medience Tokyo Japan). Samples were spotted in duplicate onto silica gel S4-chromarods (5 μm particle size). Lipid separation followed a two-step sequence: (i) the elution of the phospholipids (PL), phosphatidylethanolamine (PE), phosphatidylserine-phosphatidylinositol (PS-PI) and phosphatidylcholine (PC) in a dichloromethane/methanol/water (50:20:2, by volume) solvent system; and (ii) after air drying, the elution of the acetone mobile polar lipids (AMPL), sterols (ST), sterol esters (WE), triacylglycerols (TAG), free fatty acids (FFA), and 1,3-diacylglycerol (DG) in a hexane/diethyl ether/formic acid (60:15:1.5, by volume) solvent system. The Iatroscan MK 6s was calibrated using known compound classes in the range of 0.1–10 μg (Sigma-Aldrich, Inc., St. Louis, MO, USA and from Nu-Chek Prep Inc., Elysian, MN, USA) and peaks were quantified using POWERCHROM version 2.6.15 (eDAQ Pty Ltd.). The contribution of each lipid class was standardized to mg lipid class per g lipid. Lipid classes were grouped as 'structural' and 'storage', depending on their primary functional roles. PL (PC, PS-PI, PE), AMPL and ST were combined as the structural lipid component, and WE, TAG, FFA and DG were combined as the storage lipids. The effects of OW and OA on individual lipid classes were also explored.

2.6 | Fatty acid analysis

FA were esterified into methyl esters using the acid-catalysed methylation method (Christie, Sébédio, & Juanéda, 2001) with modifications described by Conlan et al. (2014). 100 μl of internal standard (0.378 mg/ml, C23:0; Sigma-Aldrich, Inc., St. Louis, MO, USA) were added to a 100 μl aliquot of the total lipid fraction with 2.0 ml of the methylation catalyst, acetyl chloride: methanol (1:10). The resultant hexane supernatant, containing the FA extraction, was recovered into a gas chromatography (GC) vial for GC injection. Fatty acid methyl esters were isolated and identified using an Agilent Technologies 7890B GC System (Agilent Technologies, USA) equipped with a BPX70 capillary column (120 m × 0.25 mm internal diameter, 0.25 mm film thickness, SGE Analytical Science, Australia), a flame ionization detector (FID), an Agilent Technologies 7693 auto sampler, and a splitless injection system. The injection volume, injector and detector temperatures, and temperature programmes followed Conlan et al. (2014); the carrier gas was helium at 1.5 ml/min at a constant flow. Individual FA were then identified using known external standards (a series of mixed and individual standards from Sigma-Aldrich, Inc., St Louis, USA and Nu-Chek Prep Inc., USA), using the software GC CHEMSTATION (Rev B.04.03, Agilent Technologies). The resulting peaks were corrected by theoretical relative FID response factors (Ackman, 2002) and quantified relative to the internal standard C23:0.

FA content was standardized to weight of total lipid content for each sample and expressed as mg FA per g lipid. Total FA content and sums of major FA classes: saturated FA (SFA); monounsaturated FA (MUFA); polyunsaturated FA (PUFA); trans-unsaturated FA (TRANS-FA); omega-3 PUFA (*n*-3 PUFA); omega-6 PUFA (*n*-6 PUFA); omega-3 long-chain PUFA (*n*-3 LC PUFA) and omega-6 long-chain PUFA (*n*-6 LC PUFA) were calculated to explore how sponge FA content varied between species and how the FA profiles of sponges was affected by OW and OA.

2.7 | Homeoviscous adaptation mechanisms

The following calculations were also made to explore potential HVA mechanisms in response to the treatments:

(1) The ratio of sterol to phospholipid (sterol:phospholipid)
(2) The ratio of structural to storage lipid (structural:storage)
(3) Mean chain length (MCL) following Guerzoni et al. (2001):

$$\mathrm{MCL} = \Sigma\,(\text{mg FA g lipid}^{-1} \times C)/\text{total mg FA g lipid}^{-1}$$

(4) The ratio of SFA to PUFA (SFA:PUFA)
(5) The degree of FA unsaturation (DoU) following Guerzoni et al. (2001):

$$\begin{aligned}\mathrm{DoU} = [&\Sigma\,\text{monoenes} + 2 * (\Sigma\,\text{dienes}) + 3 * (\Sigma\,\text{trienes}) \\ &+ 4 * (\Sigma\,\text{tetraenes}) + 5 * (\Sigma\,\text{pentaene}) \\ &+ 6 * (\Sigma\,\text{hexaenes})]/\text{total FA}\end{aligned}$$

2.8 | Data analysis

Data analyses were performed with PRIMER-E (PRIMER version 6.0, PERMANOVA+, Plymouth Marine Laboratory, Plymouth, UK). All graphs were generated using GRAPHPAD PRISM (GRAPHPAD Software, version 6.00 for Windows, La Jolla California USA). For all analyses, Euclidean distances were used to generate a resemblance matrix. All multivariate data were standardized prior to generating the resemblance matrix. Permutational post hoc comparisons were used to determine which treatments (species, temperature, pH) differed significantly. A 5% significance level was used for all tests.

2.8.1 | Univariate analysis

To test the effect of temperature and pH on chlorophyll *a* concentration and HVA mechanisms, a two-way Permutational Multivariate Analysis of Variance (PERMANOVA) was employed, with temperature and pH as fixed factors.

2.8.2 | Multivariate analysis

To determine whether lipid and FA composition were significantly different between species at time zero, a one-way PERMANOVA was conducted on a matrix of total lipid and standardized lipid class data, total FA, FA classes and individual FA data, with species as a fixed factor. To test and visualize the effect of temperature and pH on sponge lipid and FA profiles a two-way PERMANOVA was conducted on a matrix of total lipid and standardized lipid class data

(lipid profiles) and a matrix of total FA, FA classes and individual FA data (FA profiles) for each species following exposure to the treatments, with temperature and pH as fixed factors. CAP was used to display significant differences in lipid and FA profiles between species and treatments. SIMPER analysis identified the individual FA contributing to differences in sponge FA profiles between treatments.

3 | RESULTS

3.1 | The lipid and FA composition of four sponge holobionts

PERMANOVA revealed significant differentiation in the lipid and FA composition between the four sponge species (Pseudo-F(3, 20) = 14.05, p = .001), which was also clearly evident in the CAP ordination (Figure 1). The profiles of the phototrophic sponges was similar (p = .066), both species had low total lipid content (50 to 54 mg lipid g sponge^{-1} for *C. foliascens* and *C. coralliophila*, respectively). In contrast, the heterotrophic sponges had a higher concentration of total lipid (141 and 159 mg lipid g sponge^{-1} for *R. odorabile* and *S. flabelliformis* respectively). Interestingly, the profiles of the two sensitive species, *C. foliascens* and *R. odorabile*, were similar (p = .073) due to a significant contribution of AMPL to their total lipid concentration (Figures 1 and 2a). Furthermore, although the overall profiles of the two more tolerant species, *C. coralliophila* and *S. flabelliformis*, were significantly different (p = .003), their profiles were both characterized by higher concentrations of storage lipids, phospholipids and sterols (Figures 1 and 2a).

The two phototrophic sponges and *S. flabelliformis* had similar concentrations of total FA (70, 68 and 71 mg g lipid^{-1} for *C. foliascens*, *C. coralliophila*, and *S. flabelliformis* respectively), almost double that of the heterotrophic *R. odorabile* (45 mg g lipid^{-1}). Both phototrophic sponges had an abundance of SFA (16:0 in particular), as well as a high concentration of the MUFA 16:1*n*-7 and 18:1*n*-9 (Figures 1 and 2b; Table S1), whereas the heterotrophic sponge FA profiles were significantly different from each other (p = .003). The profile of *R. odorabile* was distinguished by greater concentrations of trans FA (Figures 1 and 2b), whereas *S. flabelliformis* was characterized by a high concentration (6.5 mg g lipid^{-1}) of arachidonic acid (ARA; 20:4*n*-6) and 26:2*n*-17 (32 mg g lipid^{-1}; Table S1). The FA profiles of the two more tolerant species, *C. coralliophila* and *S. flabelliformis* (*S. flabelliformis* in particular) were characterized by higher bioactive *n*-3 and *n*-6 LC PUFA (Figure 1). *C. coralliophila* had the lowest PUFA concentration (Figure 2b), and proportionately the highest contribution of bioactive *n*-3 and *n*-6 PUFA (Figure 2c).

3.2 | Chl *a*—bleaching index

Chl *a* concentrations in the two phototrophic sponges declined with increasing temperature (Figure 3a-b). *C. foliascens* Chl *a* concentrations were significantly lower at 31.5°C compared to 28.5°C

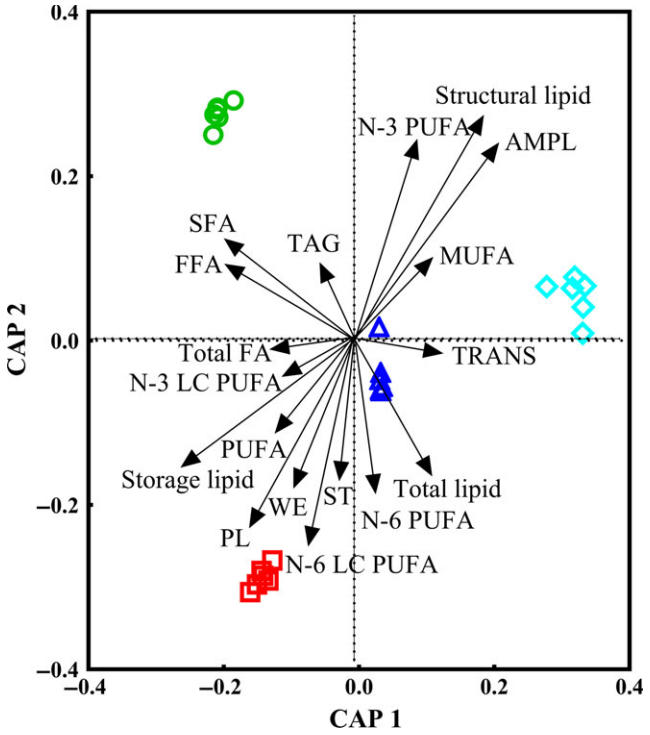

FIGURE 1 Canonical analysis of principal coordinates (CAP) ordination showing the lipid and FA composition of four GBR sponge species prior to exposure to experimental treatments. One-way PERMANOVA and *post hoc* pairwise comparisons identified significant differences in lipid and FA composition between the four species. Each point on the CAP represents an individual sponge where ○ = *Carteriospongia foliascens*; △ = *Cymbastela coralliophila*; ◇ = *Rhopaloeides odorabile*; and □ = *Stylissa flabelliformis*. The lipid and FA classes correlated with the differences between groups are overlaid. WE = wax ester, TAG = triacylglycerol, FFA free fatty acids, ST = sterol, AMPL = acetone mobile polar lipid, PL = phospholipid (PS + PC + PI + PE), SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; and TRANS = trans fatty acid, LC = long chain [Colour figure can be viewed at wileyonlinelibrary.com]

(p = .011), and *C. coralliophila* Chl *a* concentrations declined significantly at both 30°C (p = .004) and 31.5°C (p < .001).

3.3 | Sponge lipid and FA profiles under OW and OA

There was no significant effect of pH or temperature on the lipid class profiles of the four sponge species (Table 1). However, temperature had a significant effect on the FA profile of all species, except *S. flabelliformis* (Table 1). The FA profile of bleached *C. foliascens* (as assessed by reduced Chl *a* concentrations above) exposed to 31.5°C differed significantly from that of sponges in the lower temperature treatments (28.5°C: p = .015; 30°C: p = .029). Sponges at 31.5°C had a lower total FA content (32.5 mg g lipid^{-1}) than sponges at 28.5°C (33.1 mg g lipid^{-1}) and 30°C (33.4 mg g lipid^{-1}), primarily due to a reduction in SFA and MUFA, including SFA 16:0; MUFA 16:1 *n*-7; and the demospongic acids 5,12-Me 18:2n 9, 5,9-Me

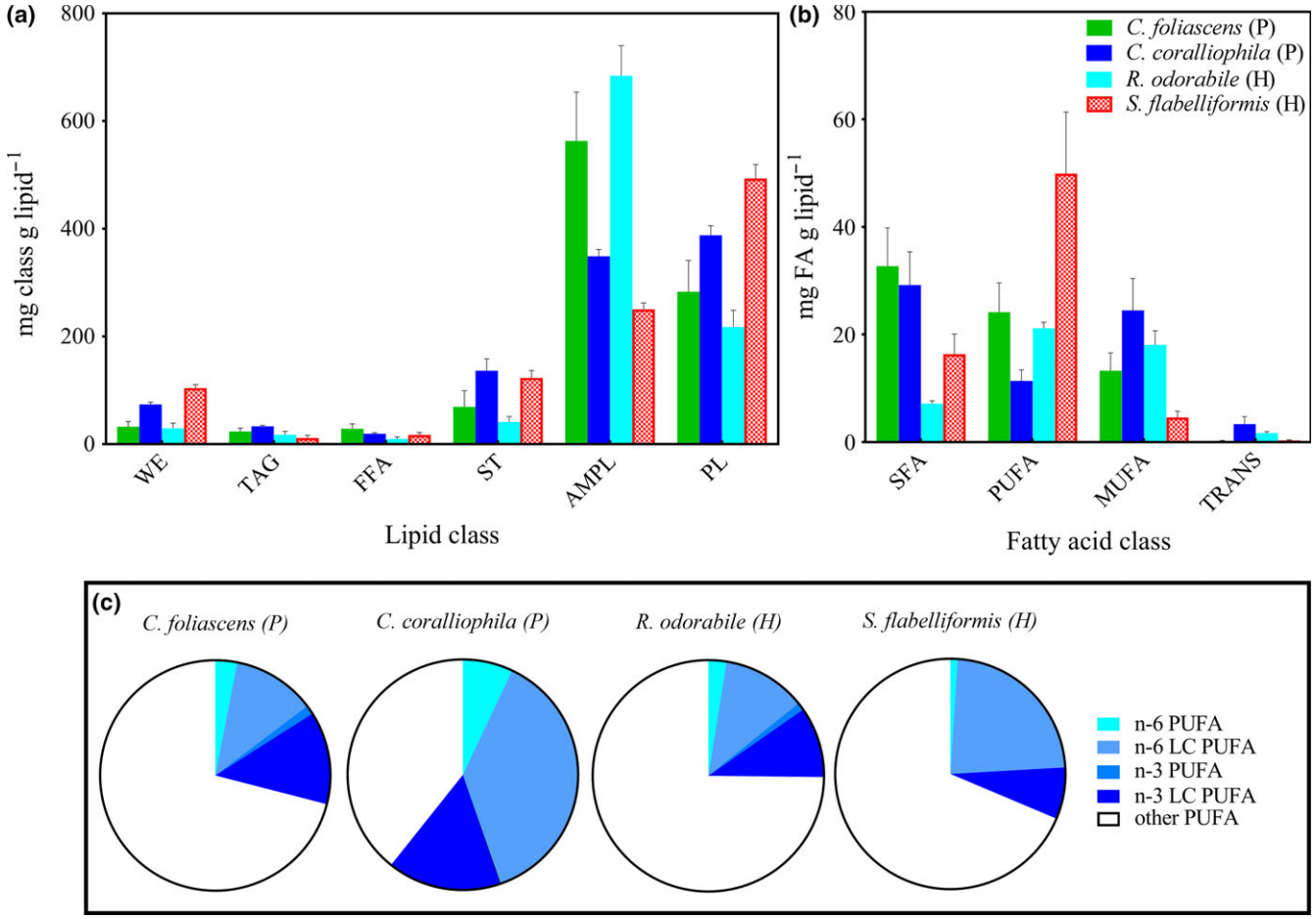

FIGURE 2 Time zero (T0) lipid and FA class composition of each species presented as (a) Average (+*SE*) mg lipid *per* gram of sponge (mg lipid sponge^{-1}). Lipid classes are coded as follows: (i) storage lipid: WE = wax ester, TAG = triacylglycerol, FFA = free fatty acids, (note 1,3-diacylglycerol (DG) was detectable in trace amounts in *C. coralliophila* only at T0 and is not presented here); and (ii) structural lipid: ST = sterol, AMPL = acetone mobile polar lipid, PL = phospholipid (PS + PC + PI + PE). (b) Average (+*SE*) FA *per* mg of lipid (FA mg g lipid^{-1}). Major FA classes are coded as follows: SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; and TRANS = trans fatty acid). (c) PUFA composition, presented as the relative proportion (%) of each PUFA type (*n*-6, *n*-3 *n*-6 long chain and *n*-3 long chain, and other PUFA) to the total PUFA content for each species (*n* = 6 to 9 sponges *per* species). P = phototrophic species and H = heterotrophic species [Colour figure can be viewed at wileyonlinelibrary.com]

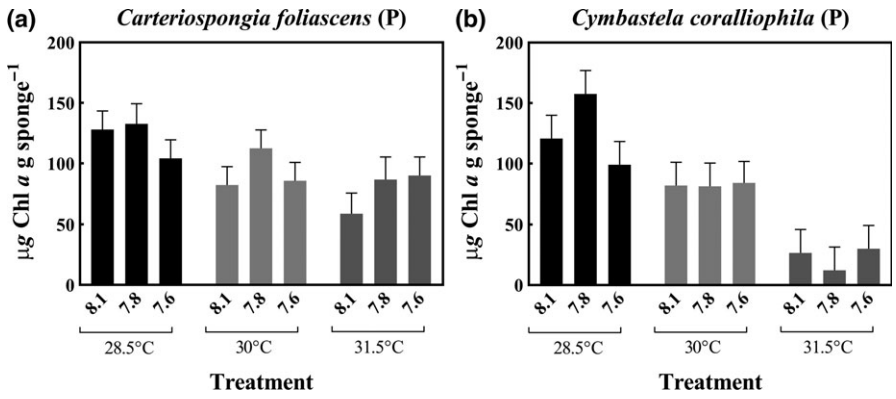

FIGURE 3 Chlorophyll *a* concentration for (a) *Carteriospongia foliascens* and (b) *Cymbastela coralliophila*. Values are mean μg chlorophyll *a* per gram of sponge wet weight (*n* = 4 to 6 for *C. foliascens*, and *n* = 6 for *C. coralliophila*) and mean P:R ratio (*n* = 6 *per* treatment—except where mortalities occurred) ± *SE* for each treatment. P = phototrophic species

TABLE 1 Results of 2-factor PERMANOVA testing the effects of pH and temperature of sponge lipid and FA profiles

	C. foliascens			*R. odorabile*			*S. flabelliformis*			*C. coralliophila*		
	df	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
Lipid												
Temp	2, 27	2.38	.118	2, 42	0.35	.908	2, 27	0.56	.703	2, 38	0.78	.607
pH	2, 27	2.35	.095	2, 42	0.62	.680	2, 27	0.75	.524	2, 38	0.71	.675
Temp*pH	4, 27	2.44	.067	4, 42	0.86	.545	4, 27	0.40	.944	4, 38	0.68	.812
Fatty acid												
Temp	2, 27	2.90	**.012**	2, 42	3.28	**.047**	2. 27	0.86	.519	2, 38	5.14	**.001**
pH	2, 27	1.46	.180	2, 42	0.86	.431	2. 27	1.24	.317	2, 38	1.15	.322
Temp*pH	4, 27	1.79	.060	4, 42	0.22	.973	4. 27	0.62	.849	2, 38	0.95	.516

Significant *p*-values ($p < .5$) are bolded.

18:2n 9 and 5,9-Me 24:5 *n*-3 (Figure 4a). The FA profile of sponges at 31.5°C was further distinguished from those in the lower temperature treatments by a higher concentration of the SFA 6:0 and 22:0, as well as the *n*-6 PUFA 18:3 *n*-6, and *n*-3 LC PUFA including 22:5 *n*-3 (DPA; Figure 4a). It is interesting to note here that, although the concentration of DPA increased in sponges at 31.5°C, the concentration of other FA, including 20:4*n*-6 (ARA) and 20:5 *n*-3 (EPA), declined by almost half relative to the concentration at 28.5°C.

The FA profile of *C. coralliophila* at 28.5°C differed from the FA profile of bleached sponges at 31.5°C ($p = .001$). As with *C. foliascens*, this difference was characterized by a reduction in SFA and MUFA content in bleached sponges, driven primarily by declines in SFA 16:0 and the MUFA 14:1 *n*-5, 16:1 *n*-7 and 18:1 *n*-9 (Figure 4b), although 17-Me 26:1 *n*-9 increased in these sponges. Meanwhile, the concentration of SFA 6:0 and 14:0 increased in sponges at 31.5°C (Figure 4b). Similar to bleached *C. foliascens*, the PUFA content of *C. coralliophila* increased in sponges exposed to 31.5°C, with 22:2 *n*-6 and 22:4 *n*-6 contributing significantly to this difference (Figure 4b). Interestingly, the FA profile of *C. coralliophila* at 28.5°C also differed from sponges exposed to 30°C ($p = .004$) as a result of an increase in SFA 3, 7, 11, 15-tetra-Me 16:0 and a higher PUFA content in these sponges, with the *n*-6 LC PUFA 22:2 *n*-6 and 22:5 *n*-6 making a significant contribution to this increase (Figure 4b).

The FA profile of *R. odorabile* at 28.5°C differed significantly from that of sponges at 31.5°C ($p = .019$) due to an increase in MUFA, as despite a number of MUFA declining at 31.5°C (14:1 *n*-5, 17:1 *n*-7 and 22:1 isomers), 17-Me 26:1 *n*-9 increased by more than 50% in thermally stressed sponges (Figure 4c). Further to this, trans FA 18:1 *n*-9t declined at 31.5°C, as did three PUFA: 16:3 *n*-4; 23-Me 5, 9 24:2 *n*-17; and 26:2 *n*-17 (Figure 4c). Meanwhile, and similar to the thermally sensitive *C. foliascens*, the concentration of 20:4 *n*-6 (ARA) declined by almost half compared to that in sponges at 28.5°C. There was no significant treatment effect on the FA profile of *S. flabelliformis*.

3.4 | Sponge homeoviscous adaptation mechanisms

There was no significant effect of OW or OA on the ratio of structural to storage lipids across all species (Table 2; Figure 5b,c). There was, however, a significant pH effect on the ratio of ST: PL in *C. foliascens* (Table 2; Figure 5a). This ratio increased significantly when sponges were exposed to reduced pH (Figure 5a). There was a significant effect of temperature on mean chain length (MCL) for the heterotrophic sponge *R. odorabile* (Table 2; Figure 5c). MCL of FA increased with exposure to increased temperature, with sponges at 31.5°C having significantly higher MCLs than those at 28.5°C. The degree of FA unsaturation (DoU) increased significantly with temperature for both phototrophic species, *C. foliascens* and *C. coralliophila* (Table 2; Figure 5d). In contrast, DoU decreased significantly for the heterotrophic species *R. odorabile* with OW, and although nonsignificant, *S. flabelliformis* DoU followed a downward trend at 31.5°C/pH 7.6 (Table 2; Figure 5d). The ratio of SFA to PUFA subsequently decreased significantly with temperature for *C. coralliophila* (Table 2; Figure 5d) and was lowest under ambient pH in the highest temperature treatment for *C. foliascens* (Table 2; Figure 5d), however, this was not significant.

FIGURE 4 Canonical analysis of principal coordinates (CAP) ordination (left) of sponge FA profiles following exposure to OW and OA, and bar graph (right) showing main FA contributing to differences in sponge FA profiles between treatments. Two-way PERMANOVA and *post hoc* pairwise comparisons identified significant differences in FA composition for (a) *Carteriospongia foliascens* (b) *Cymbastela coralliophila* and (c) *Rhopaloeides odorabile*. Each point on the CAP represents an individual sponge exposed to a different OW/OA treatment where ▲ = 28.5°C/pH 8.1, ● = 28.5°C/pH 7.8, ■ = 28.5°C/pH 7.6, ▲ = 30°C/pH 8.1, ● = 30°C/pH 7.8, ■ = 30°C/pH 7.6, ▲ = 31.5°C/pH 8.1, ● = 31.5°C/pH 7.8, ■ = 31.5°C/pH 8.6 ($n = 3$ to 6 individuals *per* species *per* treatment, except where mortalities occurred for *R. odorabile* where $n = 2$ at 31.5°C/pH 7.6). As there was no significant treatment effect on the *S. flabelliformis* FA profile, a CAP ordination is not presented [Colour figure can be viewed at wileyonlinelibrary.com]

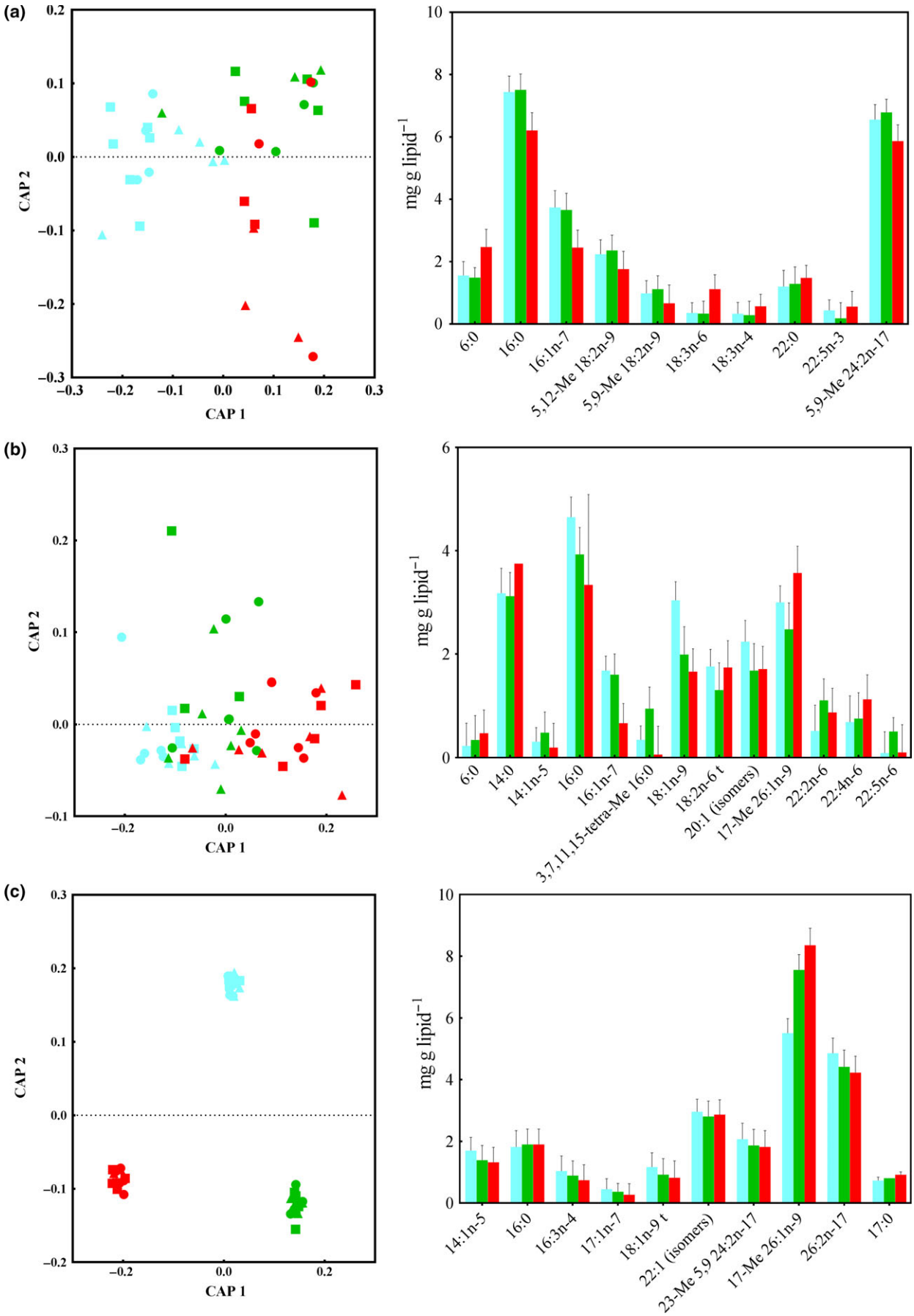
(a)
CAP 2
CAP 1
mg g lipid−1
6:0
16:0
16:1n-7
5,12-Me 18:2n-9
5,9-Me 18:2n-9
18:3n-6
18:3n-4
22:0
22:5n-3
5,9-Me 24:2n-17
(b)
CAP 2
CAP 1
mg g lipid−1
6:0
14:0
14:1n-5
16:0
16:1n-7
3,7,11,15-tetra-Me 16:0
18:1n-9
18:2n-6 t
20:1 (isomers)
17-Me 26:1n-9
22:2n-6
22:4n-6
22:5n-6
(c)
CAP 2
CAP 1
mg g lipid−1
14:1n-5
16:0
16:3n-4
17:1n-7
18:1n-9 t
22:1 (isomers)
23-Me 5,9 24:2n-17
17-Me 26:1n-9
26:2n-17
17:0

TABLE 2 Results of 2-factor PERMANOVA testing the effect of pH and temperature on sponge HVA mechanisms

	C. foliascens			*R. odorabile*			*S. flabelliformis*			*C. coralliophila*		
	df	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
ST: PL												
Temp	2, 27	0.60	.596	2, 42	1.14	.30	2, 27	1.19	.305	2, 38	0.43	.636
pH	2, 27	5.01	**.012**	2, 42	1.17	.31	2, 27	0.42	.672	2, 38	0.98	.396
Temp*pH	4, 27	1.20	.341	4, 42	0.77	.59	4, 27	0.55	.716	4, 38	1.10	.355
Struc: Store												
Temp	2, 27	0.36	.696	2, 42	0.11	.89	2, 27	0.70	.493	2, 38	1.45	.255
pH	2, 27	2.55	.084	2, 42	0.12	.88	2, 27	0.34	.724	2, 38	<0.01	.927
Temp*pH	4, 27	1.72	.173	4, 42	0.45	.77	4, 27	0.18	.955	4, 38	0.16	.959
MCL												
Temp	2, 27	0.51	.599	2, 42	4.32	**.019**	2, 27	1.22	.322	2, 38	1.56	.210
pH	2, 27	1.12	.343	2, 42	0.44	.622	2, 27	1.75	.162	2, 38	0.69	.485
Temp*pH	4, 27	1.03	.405	4, 42	0.22	.937	4, 27	0.53	.826	4, 38	1.06	.398
DoU												
Temp	2, 27	3.29	**.046**	2, 42	3.53	**.035**	2, 27	0.80	.492	2, 38	4.35	**.022**
pH	2, 27	0.38	.679	2, 42	0.88	.443	2, 27	0.16	.890	2, 38	2.16	.117
Temp*pH	4, 27	2.08	.112	4, 42	0.19	.940	4, 27	0.68	.696	4, 38	2.43	.054
SFA: PUFA												
Temp	2, 27	0.72	.519	2, 42	3.01	.059	2. 27	1.01	.443	2, 38	4.52	**.023**
pH	2, 27	1.20	.306	2, 42	0.07	.924	2. 27	1.05	.430	2, 38	0.49	.490
Temp*pH	4, 27	2.82	.052	4, 42	0.33	.851	4. 27	0.73	.725	2, 38	1.35	.268

Significant *p*-values ($p < .5$) are bolded.

4 | DISCUSSION

Exploration of sponge lipid and FA composition revealed indicators of intrinsic sponge tolerance to OW and OA, provided insight into the types of sponges that will survive in a warmer, high CO_2 ocean, and identified potential mechanisms of climate change acclimation in sponges. While it is well established that lipids and FA play an important role in stress resistance, and the ability of an organism to maintain appropriate membrane function in the face of environmental change is intimately linked to tolerance, this is the first time that these responses have been demonstrated in sponges.

4.1 | Innate sponge tolerance

The two phototrophic sponges had similar lipid and FA profiles due to an abundance of FFA, triacylglycerides and SFA. While this is likely reflective of their specific mode of nutrition, future work exploring the FA profiles of the sponge host and associated symbionts in isolation will be needed to confirm this (Bergé & Barnathan, 2005; Wada & Murata, 1998). What is of particular interest here, however, are the different lipid and FA profiles that separate the sensitive species, *C. foliascens* and *R. odorabile*, from the more tolerant species, *C. coralliophila* and *S. flabelliformis*. The thermally resistant sponges had a high storage lipid content, due to a greater contribution of WE. This storage lipid may facilitate resistance of these species to OW and OA by providing energy during periods of stress (Anthony, Hoogenboom, Maynard, Grottoli, & Middlebrook, 2009; Kattner & Hagen, 2009). Wax esters may also function as structural elements, providing cell membrane support and possibly serving as FA carriers in the biosynthesis of structural lipids (Marsden, 1975; Nevenzel, 1970; Parrish, 1988); such features likely also assist resistance to environmental stress. Meanwhile, the more sensitive species had a high concentration of AMPL. This group of lipids contains pigments, glycolipids and monoacylglycerols (Murata & Siegenthaler, 2006; Parrish, 2013), which are found in abundance in bacterial lipids (Shaw, 1974), reflecting the higher microbial content of these sponge species (Luter et al., 2015; Moitinho-Silva et al., 2017). Glycolipids in particular are important for membrane stability (Hölzl & Dörmann, 2007) and may play a role in facilitating bacterial survival within the sponge host, where environmental conditions can be variable (Thomas et al., 2010).

The more thermally resistant species also had a higher concentration of sterols and phospholipids, the primary constituents of the lipid bilayer. These structural lipids are fundamental for cell support and protection, and help to maintain membrane fluidity under stressful conditions (Lawson et al., 1988; Murata & Siegenthaler, 2006; Paulucci, Medeot, Dardanelli, & De Lema, 2011); they also likely provide a key indicator of environmental stress tolerance in these sponges (Tchernov et al., 2004). Furthermore, the more thermally resistant heterotroph, *S. flabelliformis*, had a high concentration of

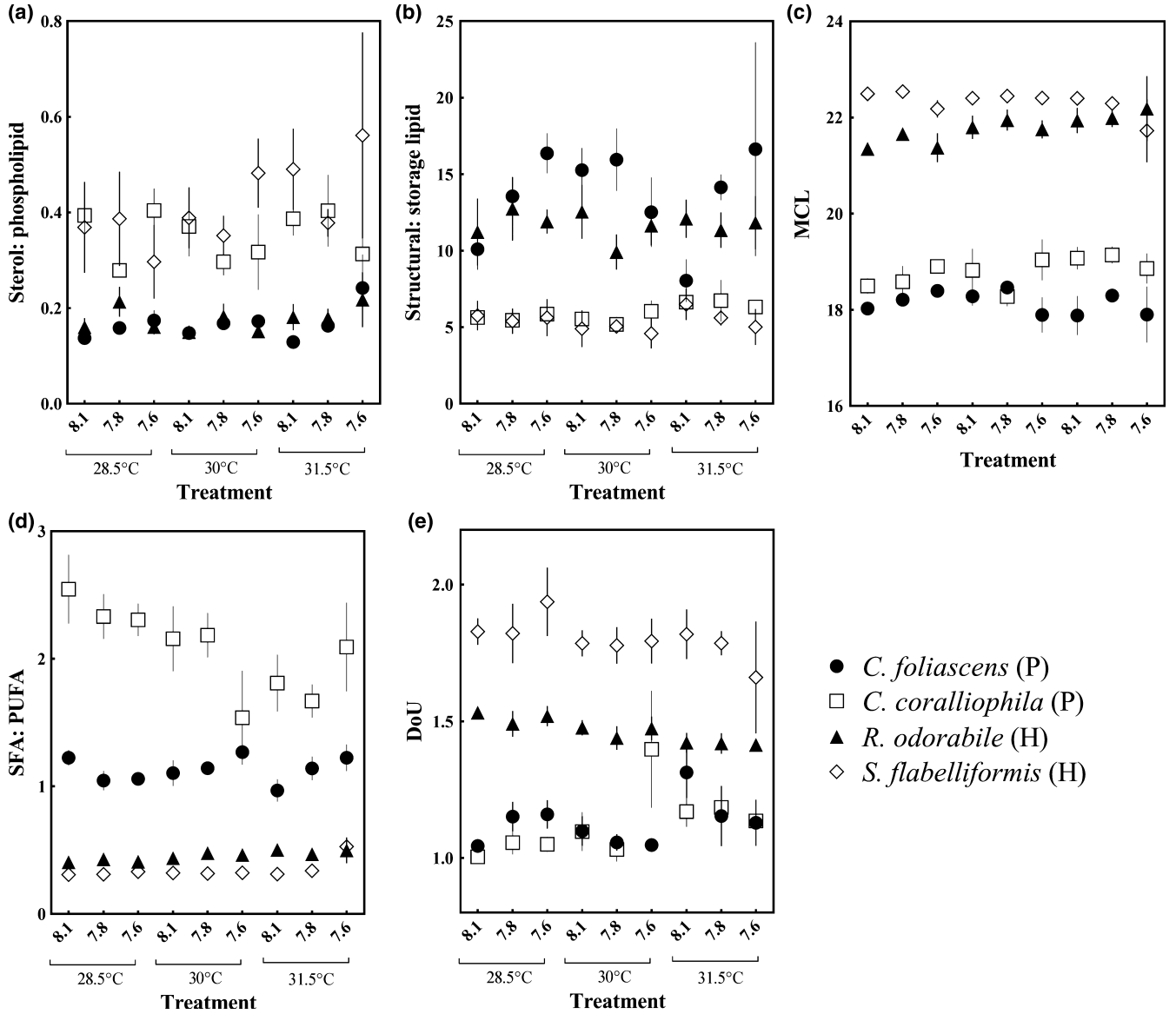

FIGURE 5 Exploration of potential HVA methods in sponge holobionts. The effects of OW and OA on: (a) the ratio of sterol to phospholipid; (b) the ratio of structural to storage lipid; (c) Mean chain length (MCL); (d) the ratio of SFA to PUFA; and (e) the degree of FA unsaturation (DoU) for each species exposed to OW and OA (n = 3 to 6 individuals *per* species *per* treatment, except where mortalities occurred for *Rhopaloeides odorabile* where n = 2 at 31.5°C/pH 7.6). P = phototrophic species and H = heterotrophic species

n-3 and *n*-6 LC PUFA. Similarly, while the more thermally resistant phototrophic *C. coralliophila* had a low PUFA content, over half of these PUFA were bioactive *n*-3 and *n*-6 LC PUFA. This class includes biologically important FA, which are significant constituents of phospholipids in sponge cellular membranes. A high abundance of these may reflect physiological differences that could facilitate the resistance of these species to environmental stress (Koopmans et al., 2015; Lawson et al., 1988; Mueller-Navarra, 1995; Tocher, 2003). The presence of *n*-3 and *n*-6 LC PUFA is consistently associated with increased stress resistance across a range of taxa, with deficiencies correlated with reduced growth, as well as increased mortality and susceptibility to stressors and disease (Bachok, Mfilinge, & Tsuchiya, 2006; Immanuel, Palavesam, & Petermarian, 2001; Koven et al., 2001; Müller-Navarra, Brett, Liston, & Goldman, 2000; Parrish, 2013; Pernet & Tremblay, 2004). An abundance of bioactive PUFA would provide buffering capacity for sponges under stressful conditions, and thus contribute to the ability of these sponges to maintain membrane function and subsequent cell homeostasis in the face of environmental change. *S. flabelliformis* had a particularly high content of ARA, a FA involved in eicosanoid synthesis. Eicosanoids are metabolites that play a role in cellular regulation of processes such as the fluid and electrolyte fluxes that are important in the regulation of membranes, e.g. during a thermal stress response (Koven et al., 2001).

The low overall PUFA content of *C. coralliophila* may also explain why this species is better able to tolerate increased temperature,

compared to the sensitive phototrophic *C. foliascens*. While *n*-3 and *n*-6 LC PUFA are important for stress resistance, PUFA of thylakoid membranes in photosynthetic organisms are particularly sensitive to temperature and are the primary targets of lipid oxidation (Botić et al., 2015; Wada & Murata, 1998; Wada et al., 1994). Therefore high PUFA concentrations may put *C. foliascens* at an elevated risk of lipid oxidation, which may, at least partially, explain its sensitivity to OW. Further work exploring lipid oxidation in these species upon exposure to OW would help elucidate how a high PUFA content impacts the ability of sponges to tolerate thermal stress.

4.2 | Sponge lipid and FA profiles in a high CO_2 world

While OW and OA did not significantly impact sponge lipid profiles, the FA profile of all species, except *S. flabelliformis*, shifted significantly with increasing temperature. Bleached phototrophic sponges (characterized by reduced chlorophyll *a* content) experienced significant declines in their dominant SFA (16:0), whereas the concentration of the second most abundant SFA in these two species (22:0 for *C. foliascens* and 14:0 for *C. coralliophila*) increased with temperature. The decline in 16:0 in thermally stressed phototrophic sponges is either due to loss of symbionts or reflects a breakdown in symbiont FA biosynthesis, and the subsequent reduced translocation of this FA to the sponge host (Figueiredo et al., 2012; Hillyer, Tumanov, Villas-Bôas, & Davy, 2016; Imbs & Yakovleva, 2012). Meanwhile, the increase in other abundant SFA either reflects a switch in diet, e.g. to acquiring carbon heterotrophically from the water column to compensate for reduced photosynthate translocation during bleaching (Grottoli, Rodrigues, & Juarez, 2004; Grottoli, Rodrigues, & Palardy, 2006; Hoadley et al., 2015), or a different metabolic response by the host to replace lost 16:0. The concentration of the short chain SFA 6:0 also increased with temperature for both species. Short chain SFA tend to be of bacterial origin (Bergé & Barnathan, 2005), suggesting an increase in microbial abundance, and associated short chain SFA neogenesis, in bleached sponges. The MUFA characteristic of healthy sponges declined in bleached phototrophic sponges. MUFA are a readily catabolized energy source (Tocher, 2003) and it is possible that bleached sponges break down these MUFA to produce energy as compensation for the energy deficit that occurs with photosynthetic dysfunction.

Likewise, MUFA characteristic of T0 sponges declined with temperature in the sensitive heterotrophic *R. odorabile*, again suggesting catabolism of these MUFA by the sponge host. *R. odorabile* has an abundance of nonphotosynthetic microbes (Webster & Hill, 2001; Webster, Wilson, Blackall, & Hill, 2001) and temperature-related mortality has previously been correlated with a breakdown in the relationship between the host and its associated symbionts (Fan, Liu, Simister, Webster, & Thomas, 2013; Webste, Cobb, & Negri, 2008) which, like phototrophic symbionts, play important metabolic roles in the symbiosis. *R. odorabile* feeding efficiency is also reduced under thermal stress (Massaro, Weisz, Hill, & Webster, 2012), and this, combined with symbiosis breakdown, suggests that this species also catabolizes MUFA to generate energy due to its typical energy source being compromised. Interestingly, however, the overall *R. odorabile* MUFA concentration increased due to a doubling of 17-Me 26:1 *n*-9 (a "demospongic acid"). The relative proportion of such FA in sponges has been shown to vary with season, and is suggested to play a role in the maintenance of sponge membrane fluidity with changes in temperature (Hahn et al., 1988). An increase in this FA may act to reduce the fluidity of thermally-perturbed cell membranes, possibly reflecting a thermal stress response by these sponges (Guerzoni et al., 2001; Hochachka & Somero, 1984).

Sponge PUFA content increased in both phototrophic species with increasing temperature. For the sensitive *C. foliascens*, this increase occurred alongside a significant shift in LC PUFA concentration. While the concentration of DPA (22:5 *n*-3) increased, that of EPA (20:5 *n*-3) declined by almost half compared to sponges exposed to 28.5°C, indicating that EPA is elongated to DPA, which is particularly important for the structural integrity of cell membranes (Anholt, 2004). The concentration of another important FA, ARA (20:4*n*-6), declined by almost half when both thermally sensitive species were exposed to 31.5°C, compared to sponges exposed to 28.5°C. It is possible that this decline reflects ARA entering the eicosanoid pathway, a stress-signalling cascade activated by exposure of cells to oxidative stress (Hillyer et al., 2016; Lõhelaid, Teder, & Samel, 2015). Meanwhile, the more thermally tolerant species experienced no change in LC PUFA concentration. The stable PUFA content of these sponges under elevated temperature reflects the tolerance of these sponges to thermal stress, and indicates either selective retention of LC PUFA under stress or simply an absence of stress associated with such conditions for these species. Regardless, depletion of these important PUFA, as observed in the sensitive sponge species, is commonly associated with a deterioration in organism health, as evidenced in the bleached coral *Pavona frondifera* for which a reduction in *n*-3 and *n*-6 PUFA increased susceptibility to disease and mortality (Bachok et al., 2006).

4.3 | Homeoviscous adaptation

We explored the ability of sponges to compositionally alter their membrane lipids to prevent membrane destabilization in response to environmental stress using a number of different HVA indicators. The ratio of sterols to phospholipids increased significantly at reduced pH for the thermally sensitive *C. foliascens*, with the greatest increase occurring in the high OW/OA treatment. Sterols and phospholipids are the primary constituents of the lipid bilayer, and sterols are particularly important for maintaining membrane rigidity with changing environmental conditions (Los & Murata, 2004; Parent, Pernet, Tremblay, Sevigny, & Ouellette, 2008; Presti, 1985); for instance bivalve species living in variable environments, such as surface waters, alter the sterol concentration of their cell membranes in response to seasonally varying temperatures (Copeman & Parrish, 2004; Parrish, 2013). Additional inorganic carbon available under OA may stimulate symbiont photosynthetic rates, resulting in an increased translocation of photosynthetically derived carbon to

C. foliascens (Fu, Warner, Zhang, Feng, & Hutchins, 2007; Morrow et al., 2015). It appears that *C. foliascens* is able to utilize this additional carbon to increase sterol biosynthesis, likely reflecting a HVA mechanism and providing a putative pathway via which elevated CO_2 facilitates resistance to thermal stress.

The average FA chain length increased significantly with temperature for *R. odorabile* (primarily through an increase in the concentration of the demospongic acid 17-Me 26:1 *n*-9), likely reflecting an attempt at membrane stabilization by increasing the proportion of FA with higher melting points (Suutari & Laakso, 1994).

The degree of FA unsaturation (DoU) increased significantly with temperature for both phototrophic species, yet decreased significantly for the heterotrophic *R. odorabile* with OW, and although nonsignificant, the DoU in *S. flabelliformis* was lowest in the high OW/OA treatment. A decrease in DoU suggests either an attempt by the heterotrophic sponge to mitigate peroxidation, or more likely, increased host metabolism in response to stress (Hillyer et al., 2016). An increase in the proportion of unsaturated FA, on the other hand, has been observed for a number of photosynthetic microorganisms in response to super-optimal temperatures (Guerzoni, Ferruzzi, Sinigaglia, & Criscuoli, 1997; Guerzoni et al., 2001; Guillot et al., 2000; Wada et al., 1994). This is due to the activation of the oxygen-dependent desaturase system, which not only introduces double bonds into SFA to increase DoU, but in turn protects cells from oxidative and thermal stress by consuming additional oxygen and reactive oxygen species accumulated at high temperatures (Guerzoni et al., 1997, 2001). This is supported by observed declines in the ratio of SFA to PUFA for both phototrophic sponges with increased temperature (although nonsignificant for *C. foliascens*) and an understanding of the cellular oxidation levels experienced by these species following exposure to OW would help confirm this stress response pathway. It is important to note that the lipid and FA profiles presented in this study are for the sponge holobiont; i.e. the sponge animal and its associated symbionts in their entirety. It is probable that the difference in response between these two nutritional types is the culmination of both the animal and microbial components of the sponge holobiont responding differently to thermal stress. Future work exploring the sponge host and associated symbiont responses in isolation is therefore required to better understand the mechanisms underlying these observed responses. Nevertheless, activating an oxygen-consuming desaturase system would be particularly important for phototrophic organisms, due to the excessive production of reactive oxygen species as a result of the inactivation of the oxygen-evolving capability of PSII at stressful/photoinhibiting temperatures (Wada & Murata, 1998).

5 | CONCLUSION

Through the exploration of sponge lipid and FA composition we reveal previously uncharacterized components in the sponge stress response, providing insight into potential mechanisms contributing to the resilience of this ecologically important phylum during environmental change. Sponges with a greater content of storage lipids, as well as a higher proportion of phospholipids and sterols, and higher concentrations of *n*-3 and *n*-6 PUFA exhibited the greatest resistance to OW and OA. These lipids are the primary constituents of the lipid bilayer of cell membranes and likely enable sponges to maintain membrane function and cell homeostasis in the face of environmental change (Geider, 1987; Guillot et al., 2000; Hazel, 1995; Singh et al., 2002), including OW and OA as demonstrated here. We also reveal that sponges can respond to thermal perturbations with a diversity of lipid and FA alterations, including shifting the proportion of membrane lipids, and changing the degree of FA unsaturation and FA elongation. Such mechanisms likely contribute to the acclimatization potential of these species under climate change, although direct measurements of membrane fluidity would further substantiate this stress response pathway. Finally, we discovered distinct differences in the responses of phototrophic and heterotrophic sponges to thermal stress, suggesting that associated photosymbionts and the sponge host respond differently.

ACKNOWLEDGEMENTS

This project was funded by the Australian Institute of Marine Science, Victoria University of Wellington, the PADI foundation, the Royal Society of New Zealand Marsden Fund VUW1505 and Deakin University. NSW was funded through an Australian Research Council Future Fellowship FT120100480, HMB was funded by a VUW Doctoral Scholarship. Staff of the Australian Institute of Marine Science, particularly SeaSim staff, Christine Altenrath, Florita Flores, Andrew Negri, Stephen Boyle and Sarah Sutcliffe (JCU student volunteer) are acknowledged for their invaluable assistance in running the experiment in the Sea Simulator. Jessica Conlan and Julia Strahl are acknowledged for sharing their expertise.

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How to cite this article: Bennett H, Bell JJ, Davy SK, Webster NS, Francis DS. Elucidating the sponge stress response; lipids and fatty acids can facilitate survival under future climate scenarios. *Glob Change Biol*. 2018;24:3130–3144. https://doi.org/10.1111/gcb.14116