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Altered acrylic acid concentrations in hard and soft corals exposed to deteriorating water conditions

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Abstract

A reliable marker of early coral response to environmental stressors can help guide decision-making to mitigate global coral reef decline by detecting problems before the development of clinically observable disease. We document the accumulation of acrylic acid in two divergent coral taxa, stony small polyp coral (*Acropora* sp.) and soft coral (*Lobophytum* sp.), in response to deteriorating water quality characterized by moderately increased ammonia (0.25 ppm) and phosphate (0.15 ppm) concentrations and decreased calcium (360 ppm) concentration, using nuclear magnetic resonance spectroscopy (NMR)-based metabolomic techniques. Changes in acrylic acid concentration in polyp tissues free of zooxanthellae suggest that acrylic acid could be a product of animal metabolism and not exclusively a metabolic by-product of the osmolyte dimethylsulfoniopropionate (DMSP) in marine algae or bacteria. Our findings build on previously documented depletions of acrylic acid in wild coral potentially correlated to temperature stress and provide additional insight into approaches to further characterize the nature of the metabolic accumulation of acrylic acid under controlled experimental conditions.

Key words: water quality deterioration, hard and soft coral metabolomics, *Acropora* sp., *Lobophytum* sp., nuclear magnetic resonance spectroscopy, acrylic acid concentration

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Introduction

Traditional health evaluation methods (e.g., culture, cytology, and histopathology) are increasingly employed to evaluate coral health (Stoskopf 2011). However, key early markers of coral health decline are needed for effective early preventative health management decisions to protect coral communities. The use of advanced metabolomic techniques to determine baseline physiologic function in corals and study of their responses to disease and environmental stressors can help identify early diagnostic markers of coral health status (Viant et al. 2002, 2003; Viant 2007; Tjeerdema 2008). Use of emerging NMR-based metabolomic techniques in soft corals from different geographic locations has provided early evidence of the broad usefulness of these methods (He et al. 2014).

The purpose of the work we report here was to investigate changes in the metabolite profiles of two taxonomically widely separated corals, a stony small polyp coral (*Acropora* sp.) and a soft coral (*Lobophytum* sp.), during their responses to an unplanned water quality deterioration in the system housing them. This opportunity arose during an untargeted study of coral baseline metabolomes where we were evaluating metabolomics methods for sample collection, storage, and extraction

protocols for coral samples, and performing metabolic fingerprinting using ^{1}H-NMR spectroscopy on colonies maintained under good water quality conditions that had supported vigorous coral growth.

Materials and methods

Animal management, water quality monitoring, and biopsy sampling

The corals examined in this study were housed in a long established (>10 year) 180 gallon glass aquarium equipped with 14 000 k metal halide (Plusrite, Ontario, California, USA) and VHO actinic (UV Lighting Company International, Brook Park, Ohio, USA) lights, protein skimmer, and ReeFlo Dart pump (ReeFlo Pumps, Colorado Springs, Colorado, USA). The aquarium community comprised various species of hard corals (*Acropora* sp., *Caulastrea* sp., *Euphyllia* sp., *Galaxea* sp., *Hydnophora* sp., *Montipora* sp., *Seriatopora* sp.), soft corals (*Cladiella* sp., *Discosoma* sp., *Lobophytum* sp., *Plexaurella* sp., *Protopalythoa* sp., *Ricordea* sp., *Sarcophyton* sp., *Sinularia* sp., *Zoanthus* sp.), and fishes (*Zebrasoma velifer*, *Zebrasoma flavescens*, *Centropyge loriculus*, *Amphiprion frenatus*, *Chromis viridis*). Fishes and corals were fed a mixed diet of green and red algae (Ocean Nutrition, Newark, California, USA) and commercial frozen fish/coral food (San Francisco Bay Brand, Inc., Newark, California, USA). Water quality parameters such as temperature, salinity by specific gravity, pH, ammonia (salicylate test), nitrite, nitrate, calcium (precipitation/titration test), phosphate (orthophosphate test), and alkalinity were routinely monitored on a weekly basis, as well as at the times of sample collection for metabolomic assessment using commercial colorimetric test kits commonly used in the management of artificial sea water systems (API, Chalfont, Pennsylvania, USA). Sudden changes in three water quality parameters taken in aggregate characterized a decline in water quality that was recognized as needing immediate remediation to minimize impending impacts on coral health (Delbeek and Sprung 1994; Borneman 2001). The accuracy of colorimetric rapid test kits relative to standard validated bench chemistry methods varies substantially by test, but they generally provide sufficient precision to support water management decisions in aquaculture, and their use is a common practice in small- to medium-sized systems to support water quality management decisions made by biologists and farmers (Naigaga et al. 2016). To optimize accuracy and precision, it is important to use kits that are in date and properly stored, and to follow the directions carefully (Naigaga et al. 2016), as was done for the system in this study. Quality control of API commercial test kits to assure agreement with laboratory prepared standards is conducted by lot prior to distribution to consumers by the manufacturer, Mars Fishcare (GJ. Jones, personal communication, 2017). A limited number of individuals conducted the routine water testing of the aquarium system we examined, using in-date kits properly stored away from light and heat. Unexpected changes in test results were repeated with a fresh kit by multiple aquarists. The sudden simultaneous aggregate change in readings of three separate water quality parameters based on different underlying chemical reactions further strengthened our confidence that a change in water quality had occurred. The limits of detection for the three water parameter test kits that showed changes due to water quality decline are 0 mg/L for ammonia, 20 mg/L for calcium, and 0 mg/L for phosphate. After the collection of coral samples used in this study to represent corals in declining water quality, subsequent monitoring of the system water and coral health confirmed our interpretation. The three water quality parameters continued to worsen, as did other parameters, reaching conditions where frank coral loss occurred before the extensive mitigation efforts of water changes and system reconstruction returned the system to stability. Ammonia in a healthy reef system is rapidly removed through bacterial reduction to nitrite, and concentrations detectable with colorimetric tests provide warning of an inability of biofilter organisms to sufficiently reduce bio-wastes. Detectable inorganic phosphate concentrations can indicate an unexpected phosphate source or be an indication of protein skimmer inefficiency or loss of phosphate fixing organisms in biofilters. Elevated phosphates can support excessive algal growth and the depletion of water calcium concentrations necessary for coral skeletal growth. Water calcium concentrations are monitored

Table 1. Routine water quality test results for the five months prior to the sampling of corals under good water quality conditions for ^{1}H-NMR analysis.

Water quality parameter	Mean	Range	Mode	% of total readings
Temperature (°C)	25	24–27	24	50
Specific gravity	1.024	1.023–1.025	1.024	50
pH	8.2	8.1–8.4	8.2	67
Ammonia (mg/L)	0	0–0	0	100
Nitrite (mg/L)	0	0–0	0	100
Nitrate (mg/L)	0	0–0	0	100
Calcium (mg/L)	430	400–500	400	50
Phosphate (mg/L)	0	0–0	0	100

Note: All water quality test kits used were in date and stored properly. Water quality testing was routinely completed by a small number of individuals.

for depletions of the mineral critical for coral skeleton growth. Parameters considered optimal for this aquarium were 0 ppm ammonia, 0 ppm phosphate, and ≥420 ppm calcium. A summary of water quality testing over the period of the metabolomics investigations is provided in Table 1, showing the stability of the system prior to the sudden water quality deterioration event due to unknown cause(s).

Coral samples were collected at two time points. Multiple small samples (ranging from 219.8 to 453.7 mg) were collected from the distal tips of a colony of stony small polyp coral (*Acropora* sp.) and soft coral (*Lobophytum* sp.) maintained under water quality conditions that had supported vigorous coral growth. One sample was processed for metabolomics analysis as described below, and the rest were stored at −80 °C for future work.

The same coral colonies were sampled for the second time 445 d later in a similar manner, 11 d after an unplanned acute aquarium water quality decline characterized by moderately increased ammonia (0.25 ppm) and phosphate (0.15 ppm) and moderately decreased calcium (360 ppm). At this time, the sampled corals showed clinical signs of distress with bleaching and algal growth where corals had previously exhibited active growth. Coral samples obtained from each collection were immediately frozen at −79 °C to quench enzymatic activity and minimize the oxidative destruction of susceptible metabolites. This was done by placing tissue samples directly in ~1 cm diameter × ~2.5 cm deep wells drilled into a dry ice block. Snap frozen coral samples were transferred to individual cryogenic tubes and then to a −80 °C freezer for storage prior to extraction with coral from deteriorated water quality conditions. Flash-frozen deteriorated water quality coral samples were processed without storage within 30 min of collection. Individual, similarly sized coral samples were selected from each deep frozen cohort for each type of NMR experiment and for the zooxanthellae-dissociated polyp experiments.

Sample processing and NMR data acquisition

All samples were kept on wet ice throughout processing. Each sample was weighed, then 2:1 (*v*/*w*) of amphibian Ringer's (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) was added. A 1.4 mm stainless steel bead blend (Next Advance, Inc., Averill Park, New York, USA) was added at a ratio of 1:1 (*v*/*v*), and the samples were placed in a Bullet Blender (Next Advance, Inc., Averill Park, New York, USA) on speed 8 for 3 min. The homogenized samples were centrifuged at

13 000*g* for 25 min. The supernatants of all samples were collected and re-frozen at −80 °C and stored for 17 d prior to NMR analysis.

Extracts were thawed on wet ice and pipetted into a 96-well MultiScreen filter plate with Ultracel-10 membrane (Millipore Ltd., Carrigtwohill, Ireland) that had previously been prepared by soaking and multiple washes with ultrapure water. The plate was centrifuged at 3000*g* for 60 min at 21 °C, then 100 µL of Milli-Q water (Merck KGaA, Darmstadt, Germany) was added to each well, and centrifugation was repeated. The resulting filtrates were lyophilized (Savant ISS110 SpeedVac Concentrator, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA), resuspended in 50 µL 100% deuterium oxide with 20 mmol/L phosphate buffer, 0.1 mmol/L TSP, and 1 mmol/L formate (Sigma-Aldrich, St. Louis, Missouri, USA), and placed in 5 mm NMR tubes (Wilmad-LabGlass, Vineland, New Jersey, USA). ^{1}H spectra were acquired on a 14.1 T Varian INOVA 600 MHz spectrometer at 25 °C. A one-pulse sequence with pre-saturation of the water resonance using a 90° flip angle and a total repetition time of 12.65 s was used to obtain the ^{1}H spectra.

A combination of homonuclear ^{1}H, heteronuclear ^{1}H, and ^{13}C correlated NMR methods were applied to confirm the structure and identity of acrylic acid. These 1D and 2D pulsed field NMR experiments were performed on a Bruker AVANCE 700 MHz spectrometer with cryoprobe CP10K at ^{1}H frequency 700.17 MHz and ^{13}C frequency 176.05 MHz at 25 °C using Bruker Topspin 3.1 software (Bruker, Karlsruhe, Germany).

Data processing

Data were processed with ACD Labs 12.0 1D NMR Processor (Advanced Chemistry Development, Toronto, Ontario, Canada). Data processing included zero filling to 16 000 points, Fourier transformation, phase correction, baseline correction, and alignment of the reference TSP signal. For quantitative metabolite analysis, regions containing residual water and those upfield of TSP or downfield of 9.0 ppm were excluded. Intelligent bucket integration was used with a bin width of 0.04 ppm. The common integrals were normalized by dividing each bin by the sum of all the integrals per sample. To refine quantitative comparison of spectra across water conditions, for each compound of interest, the metabolite concentrations (the area under the curve of the summation of major peaks uncomplicated by peak overlap) were adjusted by dividing by the gross weight of the original coral sample. Chenomx NMR Suite 7.63 (Chenomx, Edmonton, Alberta, Canada), the Human Metabolome Database (Wishart et al. 2013), the Yeast Metabolome Database (Jewison et al. 2012), and the Spectral Database for Organic Compounds (National Institute of Advanced Industrial Science and Technology 2016) were used for peak identification.

Studies of coral tissue dissociated from zooxanthellae

The identification of acrylic acid as a key potential marker of coral response to water quality deterioration raised the question of whether this was a coral response or a response of the symbiotic zooxanthellae. To assess this, we isolated coral polyp tissues from zooxanthellae using methods modified from Banaszak (2007). Frozen samples of *Acropora* sp. and *Lobophytum* sp. were weighed (*Acropora* sp. 791.3 mg and *Lobophytum* sp. 207.8 mg). Coral tissue was scraped off the underlying structure with a sterile scalpel blade and placed in an Eppendorf tube with 1 mL amphibian Ringer's solution. Tissue was homogenized at 20 000 rpm for 20 s with an Omni Prep Multi-Sample Homogenizer (OMNI International: The Homogenizer Company, Kennesaw, Georgia, USA). Coral tissue homogenates were centrifuged at 16 000*g* for 10 min; the coral supernatant was saved, and the zooxanthellae pellet was discarded. The supernatant was centrifuged twice more at 16 000*g* for 10 min, discarding the zooxanthellae pellet each time. Ten microliters of the final isolated coral animal tissue supernatant was placed in a Neubauer improved hemocytometer

(INCYTO, Chungnam-do, Korea) to count zooxanthellae cells and assess for contamination. Zooxanthellae contamination of coral animal tissue isolates was further tested with evaluation for chlorophyll a absorbance spectrophotometrically (wavelengths 430 and 662 nm) (Nanodrop 1000, Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA). Coral tissue isolate samples were frozen at −80 °C, lyophilized at −85 °C (FreeZone Plus 2.5, Labconco, Kansas City, Missouri, USA), and rehydrated with 400 µL 10% deuterium oxide containing 0.1 mmol/L TSP. ^{1}H spectra were acquired on a Bruker AVANCE 500 MHz spectrometer with Oxford Narrow Bore Magnet, HP XW 4200 Host Workstation, using Topspin 3.1 software version (Bruker, Karlsruhe, Germany) to test for the presence of acrylic acid in coral tissue isolates.

Results

The quality of the ^{1}H-NMR spectra obtained supported metabolomic analysis. Minimal line broadening, between 1.09 and 2.85 Hz, indicated minimal interaction between the reference compound TSP and protein components of coral extracts, supporting its use in lieu of formate as an internal reference standard (Kriat et al. 1992). Metabolites identified in *Acropora* sp. and *Lobophytum* sp. are provided in Table 2. Spectra from *Acropora* sp. in good water quality and deteriorating water conditions are provided in Fig. 1. Similar data for *Lobophytum* sp. are provided in Fig. 2.

Table 2. List of metabolites identified in the ^{1}H-NMR spectra of both coral species (*Acropora* sp. and *Lobophytum* sp.) from good and deteriorated water quality conditions.

Metabolites	Reference chemical shift (ppm) and peak shape (br s, d, dd, ddd, dq, m, q, or s)
Alcohols	
Methanol	3.34 (s)
Alkaloids	
Trigonelline	4.428 (s), 8.072 (m), 8.826 (m), 9.114 (s)
Amino acids	
Alanine	1.47 (d), 3.76 (q)
Betaine	3.25 (s), 3.89 (s)
Carnitine	2.43 (dd), 3.21 (s), 3.42 (m), 4.56 (br s)
Glycine	3.54 (s)
Valine	0.976 (d), 1.029 (d), 2.261 (m), 3.601 (d)
Amino acid derivatives	
N,N-Dimethylglycine	2.91 (s), 3.71 (s)
Alkylamines	
Dimethylamine	2.7 (s)
Trimethylamine	2.89 (s)
Fatty acids	
Acetate	1.91 (s)
Acrylic acid[a]	5.89 (dd), 6.14 (q), 6.40 (dd), 12.08 (s)

(*continued*)

Table 2. (*concluded*)

Metabolites	Reference chemical shift (ppm) and peak shape (br s, d, dd, ddd, dq, m, q, or s)
Isovalerate	0.9 (d), 1.94 (dq), 2.05 (d)
Guanidines	
Methylguanidine[b]	2.833 (s), 3.366 (s)
Organic acids	
Formate[c]	8.44 (s)
Lactate	1.32 (d), 4.1 (q)
Malonate	3.11 (s)
Purine bases	
Adenine	8.11 (s), 8.12 (s)
Pyrimidine bases	
Thymine	1.86 (s), 7.37 (s)
Quaternary ammonium	
Choline	3.189 (s), 3.507 (dd), 4.056 (ddd)
Sulfonyls	
Dimethyl sulfone[b]	3.138 (s)

Note: Metabolites were identified using Chenomx NMR Suite 7.63 (Chenomx, Edmonton, Alberta, Canada), the Human Metabolome Database (Wishart et al. 2013), the Yeast Metabolome Database (Jewison et al. 2012), the Spectral Database for Organic Compounds (National Institute of Advanced Industrial Science and Technology 2016), and reference chemical shifts predicted from ACD Labs 12.0 1D NMR Processor (Advanced Chemistry Development, Toronto, Ontario, Canada). br s, broad singlet; d, doublet; dd, doublet of doublets; ddd, doublet of doublet of doublets; dq, doublet of quartets; m, multiplet; q, quartet; s, singlet.

[a]Actual chemical shifts identified on coral sample spectra for acrylic acid were 5.66 ppm (dd), 6.02 ppm (dd), and 6.13 ppm (q).

[b]Only found in *Lobophytum* sp.

[c]Formate was added as a secondary reference standard, but was present in the pilot and feasibility study samples (which did not have exogenous formate).

The majority of identified metabolites in the polar metabolomes of the two species were parallel and included amino acids such as alanine, glycine betaine, glycine, and valine, organic acids such as formate (also added as a second internal standard), lactate, and malonate, as well as metabolites from fatty acid metabolism such as choline and acetate. Alkylamines, including dimethylamine and trimethylamine, and the pyrimidine base thymine, were also identified in both coral species. Other common metabolites of both coral species included trigonelline, methanol, and acrylic acid. Differences of note between the polar metabolomes of the two coral species were that methylguanidine and dimethyl sulfone were found in *Lobophytum* sp. but not *Acropora* sp. Spectra from *Acropora* sp. also contained more distinct peaks at chemical shifts consistent with carnitine and isovalerate.

When corals were exposed to deteriorating water quality, the most dramatic changes in abundant metabolite concentrations observed were the nearly twofold increases in acrylic acid and acetate

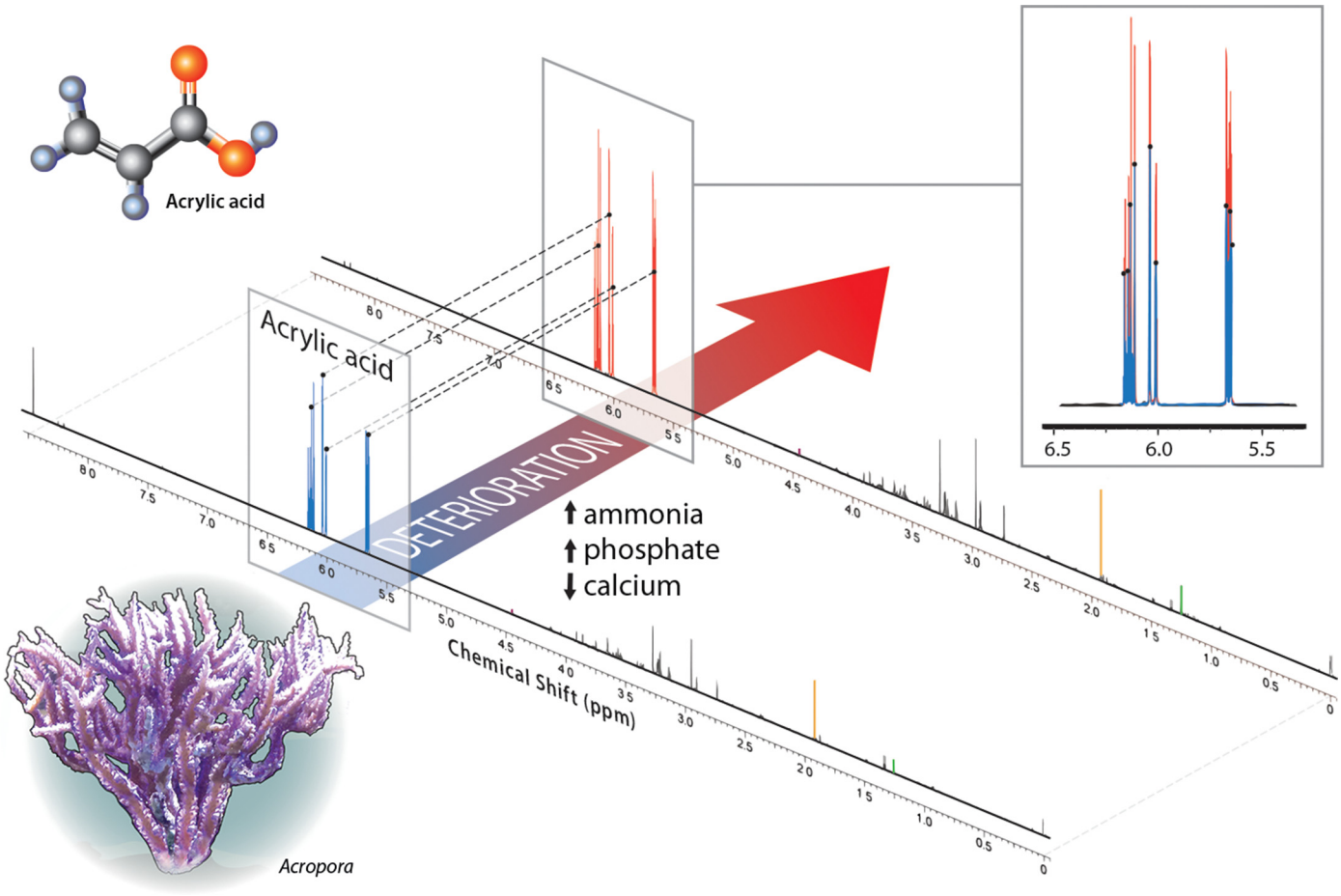

Fig. 1. A representative comparison of ^{1}H-NMR spectra of *Acropora* sp. living in good water conditions and later exposed to an unplanned deterioration of water conditions suggests that increased concentrations of acrylic acid may be a useful marker of coral distress because of reduced availability of environmental calcium and increased ammonia and phosphate. Acrylic acid (5.66 ppm (dd), 6.02 ppm (dd), and 6.13 ppm (q)) concentrations in deteriorated water (red) are 1.8 times those found in corals living in good water (blue). Other metabolites with altered concentrations (acetate (orange) 1.91 ppm (s); lactate (green) 1.32 ppm (d) and 4.1 ppm (q); trigonelline (purple) 4.4 ppm (s), 9.1 ppm (s), 8.07 ppm (m), and 8.8 ppm (m); thymine (too small to indicate in the figure) 1.86 ppm (s) and 7.37 ppm (s)) were of much lower concentrations, making their quantitation less reliable. There is no *y*-axis in the figure because peak heights are schematic. d, doublet; dd, doublet of doublets; m, multiplet; q, quartet; s, singlet.

concentrations in both *Acropora* sp. and *Lobophytum* sp. Of particular note, acrylic acid was confirmed in isolated zooxanthellae-free polyp tissues from both coral species, suggesting animal tissue metabolic origin. The much lower concentrations of trigonelline also increased in both coral species exposed to deteriorated water, whereas tissue concentrations of lactate and thymine decreased. Metabolite concentration percent changes adjusted for coral sample weight are listed in **Table 3**.

Discussion

Metabolic profiles based on polar metabolites were obtained from very small samples using a non-toxic, readily obtained extraction solvent, amphibian Ringer's solution (Hurley-Sanders et al. 2016) that produced repeatable results, and may have some advantages over the use of pure water (Gordon et al. 2013) and other extraction methods. Arabinose and glucose, common monosaccharide components of *Acropora* sp. mucus (Wild et al. 2005), were not identified in our spectra suggesting

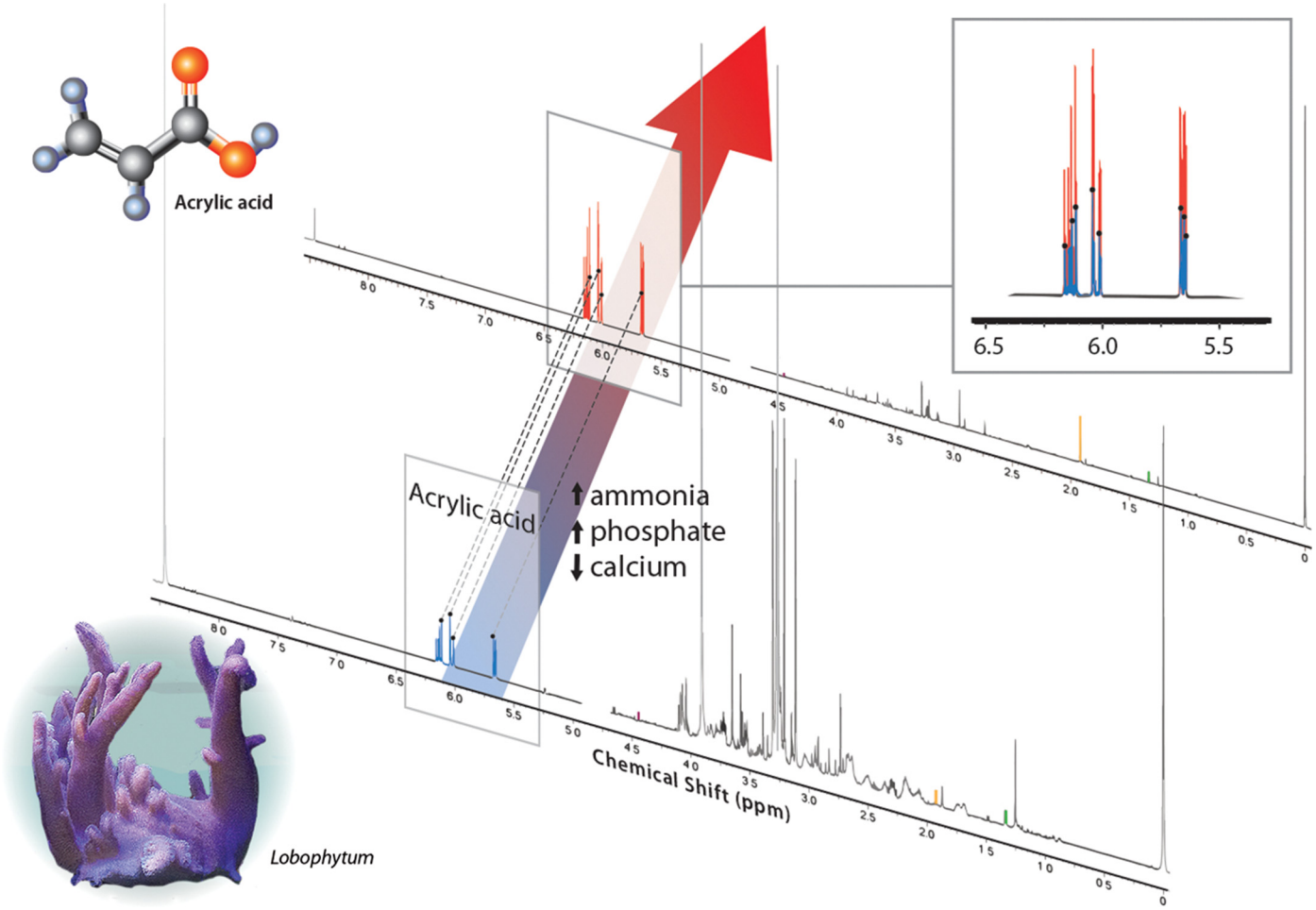

Fig. 2. A representative comparison of ^{1}H-NMR spectra of *Lobophytum* sp. living in long-established good water conditions and of the same colony subjected to an unplanned deterioration of water conditions suggests that increased concentrations of acrylic acid may be a useful marker of coral stress because of reduced availability of environmental calcium and increased ammonia and phosphate. Acrylic acid (5.66 ppm (dd), 6.02 ppm (dd), and 6.13 ppm (q)) concentrations in deteriorated water (red) are 1.9 times those found in corals living in good water (blue). Other metabolites with altered concentrations from exposure to deteriorated water conditions (acetate (orange) 1.91 (s) ppm; lactate (green) 1.32 ppm (d) and 4.1 ppm (q); trigonelline (purple) 4.4 ppm (s), 9.1 ppm (s), 8.07 ppm (m), and 8.8 ppm (m); thymine (too small to indicate in the figure) 1.86 ppm (s) and 7.37 ppm (s)) had much lower concentrations in coral tissues making their quantitation more amenable to peak integration techniques. There is no *y*-axis in the figure because peak heights are schematic. d, doublet; dd, doublet of doublets; m, multiplet; q, quartet; s, singlet.

that mucus was a minimal component of our coral samples. The lack of DMSP and DMS in our spectra is of particular interest. Both compounds have received recent attention in coral metabolic studies, particularly in responses to thermal stress (Broadbent et al. 2002; Raina et al. 2013). Acrylic acid has been considered a breakdown product of DMSP, which is considered an osmolyte of algae and a major carbon source for marine bacteria (Reisch et al. 2013). It is possible that our extraction and homogenization method produced a sample that de-emphasizes metabolites of zooxanthellae. A focused effort to process coral tissues avoiding zooxanthellae rupture or inclusion yielded similar results to our standard protocol, supporting this conjecture.

The metabolomes of the two coral genera from different orders were very similar. Metabolites identified from both coral genera included amino acids, pyrimidines, alkylamines, organic acids,

Table 3. Area under curve quantitation for key metabolites from *Acropora* sp. and *Lobophytum* sp. from good and deteriorated water quality conditions.

	Percent change (deteriorated/good)	Good water (area under curve)	Deteriorated water (area under curve)
***Acropora* sp.**			
Acrylic acid	180	0.2133	0.3287
Acetate	180	0.0108	0.0164
Lactate	0	0.0042	0.0033
Thymine	−10	0.0008	0.0006
Trigonelline	250	0.0004	0.0009
Sample weight (g)	—	0.430	0.363
***Lobophytum* sp.**			
Acrylic acid	190	0.0532	0.2134
Acetate	180	0.0029	0.0108
Lactate	−70	0.0059	0.0042
Thymine	−90	0.0043	0.0008
Trigonelline	0	0.0002	0.0004
Sample weight (g)	—	0.220	0.454

Note: The sum of areas under the curve for assigned ^{1}H-NMR spectral peaks after normalization of the spectrum and adjustment based on the TSP internal standard for each compound of interest are presented. Percent changes are calculated for each compound for a given coral species by dividing the area under the curve for deteriorated water coral samples by the area under the curve for coral samples from good water and then multiplying by 100 times the ratio of the weights of the good over the deteriorated water coral samples originally processed for spectroscopy.

alcohols, and chemical species involved in fatty acid metabolism. *Acropora* sp. and *Lobophytum* sp. shared all but two of the identified metabolites. Samples from *Lobophytum* sp. contained methylguanidine and dimethyl sulfone, which were not found in *Acropora* sp. The overall similarity suggests the potential for the development of markers that are useful across broad coral taxonomic groups.

Metabolites did not appear de novo or disappear in either coral species in response to deteriorating water quality; however, concentrations of both acetate and acrylic acid increased. Acetate is important for the metabolism of fats, carbohydrates, and synthesis of acetyl-CoA, and is critical to TCA cycle production of energy through aerobic metabolism (Daintith 2008; Rosenthal and Glew 2009). Acetate may be formed from the hydrolysis of acetyl-CoA to facilitate the distribution of a carbon substrate in an organism experiencing anaerobic conditions (Knowles et al. 1974). Evidence also supports the metabolism and detoxification of acrylic acid to carbon dioxide and acetyl-CoA destined for the citric acid cycle via the pathway for the metabolism of propionate not dependent on vitamin B_{12} (Finch and Frederick 1992). Therefore, an increase in acetate concentration in corals exposed to deteriorating water conditions may represent a shift to anaerobic metabolism and (or) an increase in catabolism of elevated concentrations of acrylic acid.

Acrylic acid concentrations rose dramatically in corals exposed to deteriorating water quality. Acrylic acid can be produced by multiple species of coral-associated bacteria, in addition to being present in

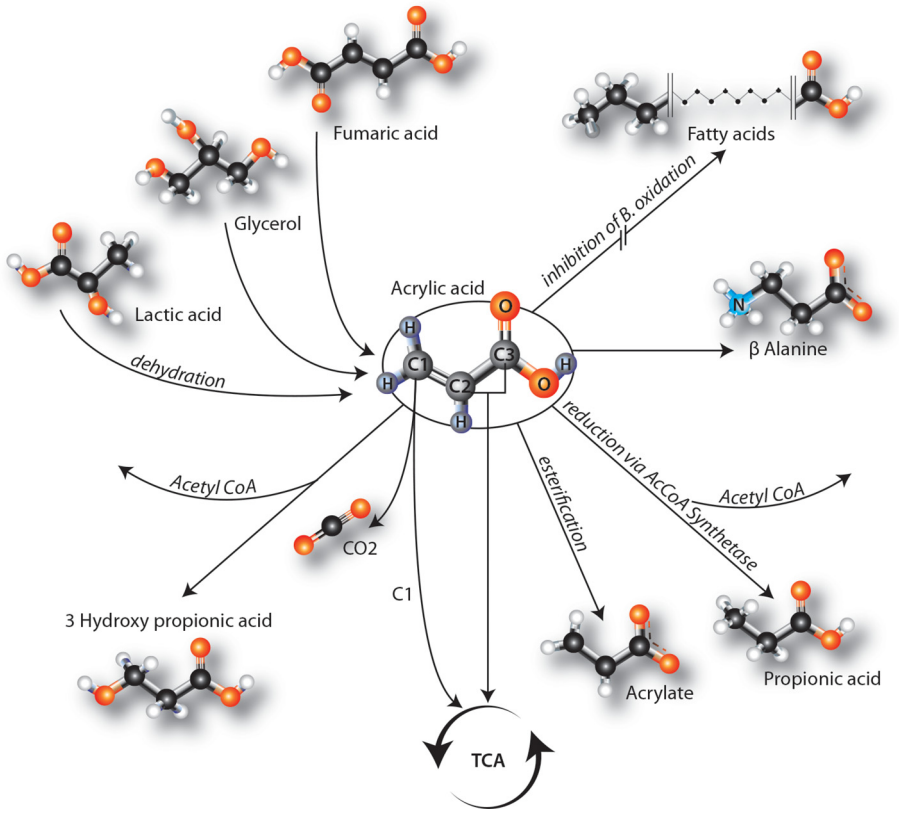

Fig. 3. Acrylic acid in coral tissues would be anticipated to have multiple metabolic interactions, as shown here. Acrylic acid would be expected to inhibit beta-oxidation of fatty acids and to drive the TCA acid cycle through two different pathways, with one generating carbon dioxide. In addition to serving as a precursor to acrylate through esterification, increasing acrylic acid concentrations would be expected to drive the formation of acetyl-CoA in the production of 3-hydroxypropionic acid and propionic acid. Fumaric acid, glycerol, and lactic acid are among the potential precursors of coral-derived acrylic acid. TCA, tricarboxylic acid; acetyl-CoA, acetyl coenzyme-A.

coral tissue and coral mucus (Broadbent and Jones 2004; Raina et al. 2009). It has been implicated as both a bacteriostatic antibacterial agent and a carbon source for certain bacteria (Sieburth 1960; Slezak et al. 1994; Ansede et al. 2001; Raina et al. 2009). Quantitative bacterial assays were not part of our study design, but no evidence of dramatic shifts in bacterial loads of samples was observed. We cannot rule out the possibility that a bacterial metabolic response may contribute to increased acrylic acid concentrations observed in coral polyp tissues.

Acrylic acid has previously been identified in numerous species of hard coral, including *Acropora* sp. (Tapiolas et al. 2010, 2013), and reference has been made to a "carbon of methyl acrylate" from the soft coral *Sinularia abrupta* (Missakian et al. 1975, p. 2514). Our findings of increased acrylic acid concentrations contrast with reports of decreased acrylic acid content in wild corals exposed to thermal stress. Another study of marine algal cultures speculated that carbon dioxide or iron limitation, excess copper, and (or) hydrogen peroxide exposure might cause alterations in DMSP metabolism, and secondarily deplete acrylic acid pools (Sunda et al. 2002). Within the scope of our investigations, we can only speculate why we saw accumulation rather than depletion of acrylic acid in the corals we studied. It is reasonable to postulate that metabolic response to thermal stress could be distinct from

responses to chemical stressors reported in our study. Thermal stress and potentially each of the individual chemical stressors are likely to have different effects on the anabolism and catabolism of both the coral animals themselves and their zooxanthellae, as well as the greater coral-associated biome of non-zooxanthellae algae, fungi, protozoa, and bacteria. The decrease in acrylic acid during thermal stress may represent an aggregate depletion of a key metabolite in a thermally stimulated growth phase of a coral or its associated biome, in the absence of other resource limitations, whereas the increase in acrylic acid we report may represent the accumulation of a metabolite that is either normally not as well used in either growth/reproductive inhibition or occurs in a shift to anaerobic metabolism.

We propose, however, that acrylic acid accumulation during exposure to deteriorated water quality could be a primary protective response to oxidative stress caused by excess ammonia rather than being related to the retardation of coral growth due to reduced calcification, and that this accumulation could occur through several metabolic pathways (**Fig. 3**) (Stoskopf 2011). Acrylic acid remains charged in aqueous environments at physiologic pH and has previously been proposed as a key component of the coral antioxidant system. It readily scavenges hydroxyl radicals and is more effective at scavenging reactive oxygen species than DMSP (Sunda et al. 2002). These properties would be particularly beneficial in coral tissues experiencing oxidative stress due to exposure to excess ammonia.

Our studies illustrate the usefulness of captive coral models as a research tool and ^{1}H-NMR spectroscopy for the evaluation of the health and physiologic status of taxonomically divergent coral species. Conserved aspects of metabolomes, with specific metabolites serving as markers of stress, can inform better approaches to mitigation and preventative management of both captive and wild coral reefs.

List of abbreviations

NMR	nuclear magnetic resonance spectroscopy
^{1}H-NMR	proton nuclear magnetic resonance spectroscopy
^{13}C-NMR	carbon nuclear magnetic resonance spectroscopy
Acetyl-CoA	acetyl coenzyme-A
DMS	dimethylsulfide
DMSP	dimethylsulfoniopropionate
TCA	tricarboxylic acid
TSP	trimethylsilylpropanoic acid

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Author contributions

Conceived and designed the study: LSHW, JNN, MKS. Performed the experiments/collected the data: LSHW, JNN, HSG. Analyzed and interpreted the data: LSHW, JNN, HSG, MKS. Drafted or revised the manuscript: LSHW, JNN, HSG, MKS.

Competing interests

The authors have declared that no competing interests exist.

Data accessibility statement

All relevant data are within the paper and maintained by the authors in the data repository of the NCSU Center for Marine Sciences and Technology Marine Magnetic Resonance Facility (emc.ncsu.edu/research/facilities/marine-magnetic-resonance/mmrf-data-repository/).

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