Metabolomic signatures of coral bleaching history

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Coral bleaching has a profound impact on the health and function of reef ecosystems, but the metabolomic effects of coral bleaching are largely uncharacterized. Here, untargeted metabolomics was used to analyse pairs of adjacent Montipora capitata corals that had contrasting bleaching phenotypes during a severe bleaching event in 2015. When these same corals were sampled four years later while visually healthy, there was a strong metabolomic signature of bleaching history. This was primarily driven by betaine lipids from the symbiont, where corals that did not bleach were enriched in saturated lyso-betaine lipids. Immune modulator molecules were also altered by bleaching history in both the coral host and the algal symbiont, suggesting a shared role in partner choice and bleaching response. Metabolomics from a separate set of validation corals was able to predict the bleaching phenotype with 100% accuracy. Experimental temperature stress induced phenotype-specific responses, which magnified differences between historical bleaching phenotypes. These findings indicate that natural bleaching susceptibility is manifested in the biochemistry of both the coral animal and its algal symbiont. This metabolome difference is stable through time and results in different physiological responses to temperature stress. This work provides insight into the biochemical mechanisms of coral bleaching and presents a valuable new tool for resilience-based reef restoration.

Results

Metabolomic signatures of historical bleaching phenotype. The liquid chromatography with tandem mass spectrometry (LC–MS–MS) metabolomic data produced 3,574 unique metabolite features, of which 138 had a spectral match to a known compound in the Global Natural Product Social Molecular Networking (GNPS) libraries (metabolomics standards initiative level two42). Richness, entropy and evenness were significantly higher in historically non-bleached corals (Wilcoxon test richness \( P = 0.021 \), entropy \( P = 0.010 \), evenness \( P = 0.013 \); Extended Data Fig. 2). The total metabolome showed strong differentiation between historically bleached and non-bleached corals (permutational multivariate analysis of variance (PERMANOVA) \( F = 11.03, R^2 = 0.19, P < 0.001 \)). We sampled a set of 12 additional, in situ coral colonies with known bleaching history that were not included in the original dataset to validate our findings (hereafter ‘validation corals’). These validation corals also had a strong metabolomic signature of bleaching history (PERMANOVA \( F = 4.18, R^2 = 0.29, P < 0.001 \)). Principal component plots (Fig. 1a,b) showed clear clustering of the historical phenotypes with significant sub-clustering of genotypes within phenotypes (PERMANOVA \( F = 2.50, R^2 = 0.051, P = 0.029 \)). Furthermore, neutral net classification analysis predicted the historical bleaching phenotype with 100% accuracy in both the initial laboratory corals and the in situ validation corals (Supplementary Table 1).

Supervised random forests classification analysis was used to identify metabolites that most strongly distinguished the two phenotypes. Except for a glutamate-phenylalanine dipeptide, the 100

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Betaine lipids distinguish bleaching history. The strongest metabolite drivers of coral bleaching history from the random forests were betaine lipids. The most abundant betaine lipid was a fully saturated diacylglycerylcarboxyhydroxymethylcholine (DGCC 16:0/0:0, m/z 562.3685 DGCC 22:6/0:0, m/z 822.5840 DGCC 20:5/0:0; Fig. 1c and Extended Data Fig. 3). This molecule was highly enriched in historically non-bleached corals in the lab (P<0.001; Fig. 2b,c) and in the in situ validation set (P<0.002; Extended Data Fig. 4). Further analysis of this molecular network revealed that other lyso-betaine lipids with fully saturated fatty acid chains (DGCCs 14:0/0:0, 17:0/0:0, 20:0/0:0) were also strong classifiers (ranked in the top 0.08% of VIP predictors) and enriched in non-bleached corals (Fig. 2a and Supplementary Fig. 2 and Supplementary Table 2). A collective analysis showed that the DGCC unsaturation index (calculated

strongest classifiers were all unannotated molecules. Thus, we used network annotation propagation from GNPS to classify unknowns into putative molecular families (Fig. 1c,d). This analysis revealed that small peptides, betaine lipids, triterpenoids, microbial natural products, fatty acids, phosphoethanolamines and steroids were the most important classes for distinguishing historical bleaching phenotypes (Fig. 1d).

Fig. 1 | Metabolomes of coral reflect bleaching history. a, PCA of lab corals using all compounds. Points (n = 5 per genotype) represent individual samples coloured by genotype (n = 10). Fill represents historical bleaching phenotype (grey, non-bleached; white, bleached). b, PCA of in situ validation set using all compounds. Points represent single samples from individual genotypes in the validation set that were not evaluated in a (n = 12). Fill represents historical bleaching phenotype (grey, non-bleached; white, bleached). c, Top 500 most important compounds for discriminating between historical bleaching phenotypes, ranked by descending variable importance. Colours represent molecular families assigned to individual molecules. Labels denote molecules of biological interest and the most informative molecule for discriminating between phenotypes, a betaine lipid. d, Relative variable importance of molecular families in the top 500 most important compounds, arranged by ascending median rank (lower is more informative). Individual points correspond to ranked molecules in c, with horizontal bars showing the first and third quartiles of each molecular family. Boxes represent the interquartile range (IQR). Colours correspond to molecular families in c.
according to ref. 21 was lower in non-bleached corals (Extended Data Fig. 4). The effect of bleaching history on the lipid saturation of these compounds was even found between two betaine lipids that differed only by a single fatty acid desaturation (Fig. 2f,g). The DGCC lipid drivers prompted a search for related diacylglycerolyltrimethylhomoserine (DGTS) lipids26, another family of phosphorous-free algal lipids. These compounds did not exhibit the stark differential abundance based on bleaching history like the DGCC lipids (Extended Data Fig. 5 and Supplementary Fig. 3, validated at a level one match22).

Relationships between algal symbionts and betaine lipids. We investigated the relationships between saturated and unsaturated betaine lipids and the ratio of Durusdinium and Cladocopium coral symbionts (Extended Data Figs. 6 and 7). There was a significant positive correlation between the abundance of a long, unsaturated betaine lipid (m/z 562.3685, DGCC 22:6/0:0) and the density of Cladocopium algal symbionts (P < 0.001, R² = 0.325), and a significantly negative correlation between a shorter, fully saturated version of the lipid (DGCC 16:0/0:0, m/z 490.3688, P = 0.005, R² = 0.167; Extended Data Fig. 7a,b). There was no significant correlation between Durusdinium algal density and these lipids (Extended Data Fig. 7c,d).

Analysis of known compounds. Among known compounds, a glutamate-phenylalanine dipeptide was the most important for predicting phenotype (Extended Data Fig. 8). This peptide was significantly enriched in historically non-bleached corals (P < 0.0001; Extended Data Fig. 8c,d and 9a; and Supplementary Fig. 4). A majority of the important predictors were phosphocholine derivatives and monoyacylglycerides (Extended Data Fig. 8). Several classes of bioactive lipids, including steroids and prostaglandins, also demonstrated high predictive power. Platelet activating factor (PAF), a bioactive lipid, was significantly enriched in historically bleached corals (P < 0.0001; Extended Data Fig. 9b), while its inactive precursor, lyso-PAF, was significantly higher in non-bleached corals (P < 0.0001; Extended Data Fig. 9c and Supplementary Fig. 5). Pheophorbide A, an algal-produced chlorophyll breakdown product, was significantly enriched in historically non-bleached corals (P < 0.0001; Extended Data Fig. 9d), as was the photosynthetic compound fucxanthin (P = 0.0008; Extended Data Fig. 9e). In the in situ validation corals, all compounds of interest (glutamate-phenylalanine, lyso-PAF, pheophorbide A and fucxanthin), except PAF, followed the same trend as the lab corals (Extended Data Fig. 9f–j).

Metabolite source mapping. We generated LC–MS–MS data separately on bleached corals and symbiont isolates to identify the putative source of metabolites. After mapping the metabolome into three components (that is, coral host, algal symbiont, or shared; Fig. 3a inset), we assessed the effect of bleaching history on the metabolome profiles of each compartment (Fig. 3).

The ‘shared metabolome’ (n = 1,447 features) strongly reflected bleaching history (Fig. 3a; PERMANOVA F = 12.974, R² = 0.22, P < 0.0001). PAF and lyso-PAF were shared metabolites driving the bleaching history phenotype, as were many of the DGCC lipids. Many of the most abundant DGCC lipids were shared between coral and algal metabolomes; however, the distinctive DGCC 16:0 m/z 490.3688 was 25 times more abundant in samples prior to bleaching than those collected after, and 63-fold more abundant in the symbiont isolates than in bleached corals.
Metabolites putatively of coral host origin (n=660 features) also showed a phenotypic difference based on bleaching history (Fig. 3b; PERMANOVA F=72.22, R²=0.247, P<0.001) and genotype (Fig. 3b; PERMANOVA F=22.59, R²=0.619, P<0.001). A random forests classification verified this strong data structure based on bleaching history (out-of-bag error 2%, n=24 bleached, n=25 non-bleached) and identified the glutamate-phenylalanine dipeptide as the top coral predictor of historical bleaching phenotype. We also identified other host-derived metabolic drivers including tryptamine and pantothenic acid, both of which were more abundant in the historically non-bleached corals (Extended Data Fig. 9k,l; P<0.001, P<0.001, respectively).

The symbiont metabolome (n=1,783 features) also strongly reflected bleaching history (Fig. 3c; PERMANOVA F=137.53, R²=0.494, P<0.001). DGCC lipids were again major contributors to the bleaching history phenotype with DGCC 14:0 (m/z 462.3424) being the strongest classifier and DGCC 16:1/0 (m/z 488.3581) the third. Interestingly, the metabolome of the algal symbionts also showed significant sub-clustering by host genotype (Fig. 3c; PERMANOVA F=12.71, R²=0.369, P<0.001). We note that the fully bleached coral host tissue and the algal isolates also clustered by historical bleaching phenotype (Extended Data Fig. 10a,b).

Experimental temperature stress. Lab corals were sampled before and after a six-day heat stress (31.4±0.08°C; mean ±1 standard deviation) or ambient control (28.4±0.15°C) temperature exposure. Heat-stressed corals underwent a significantly larger decline (sixfold higher magnitude change) in photochemical efficiency (Fv/Fm) than control corals (F=54.140, P<0.001; Supplementary Fig. 6). There was a significant positive correlation between the normalized heat response (NHR; Supplementary Methods) of the two phenotypes (P<0.001) with substantial explanatory power (R²=0.2409) and a slope significantly less than 1 (m=0.565, P<0.001). Variance in the non-bleached NHR was 32.5% higher than in bleached NHR, but was not significantly different (P=0.161). Mean NHR was significantly larger in non-bleached corals (P=0.005).

There was a significantly different metabolic response to heat stress between historical bleaching phenotypes (Fig. 4a; interaction P=0.018), which contributed to a larger metabolic difference between phenotypes after thermal stress. Symbiont and host compounds changed significantly over time in both phenotypes (Fig. 4bc; P<0.027). Although the phenotypes were significantly different at both timepoints (P<0.001), they undergo broadly similar changes in both host (Fig. 4b) and symbiont compartments (Fig. 4c).

Membrane lipids demonstrated a significant effect of historical phenotype (P<0.002; Extended Data Fig. 10c) and all historically bleached corals converged. Bioactive molecules were also significantly different between phenotypes (P<0.001; Extended Data Fig. 10d) and had a nearly orthogonal response to heat stress between phenotypes. Putative microbial products were significantly different between phenotypes (P<0.001; Extended Data Fig. 10e) and displayed a highly variable response to temperature stress based on phenotype.

Using NHR of all molecules, 1,014 compounds (28.4%) had outlier magnitudes of change, including 34 of the 100 top compounds for discriminating between historical bleaching phenotypes in the pre-stress lab corals (Fig. 5a). Thus, 66% of the compounds responsible for distinguishing between the initial bleaching history phenotypes did not change substantially under heat stress (Fig. 5b). Most of the top discriminatory compounds that responded to heat stress (24 of 34) only changed in a single phenotype (that is, these molecules only changed in the historically bleached (n=12) or non-bleached (n=12) under stress). Three molecules decreased abundance in both phenotypes and seven molecules had antagonistic changes in abundance, all of which increased in the non-bleached phenotype and decreased in the bleached phenotype.

Five molecular families had significant bleached-specific NHRs (Fig. 5c; nucleotides, steroids, xanthins, fatty acids and monoacylglycerides; P<0.027), and two families had significant changes in both phenotypes (carnitines and phosphoethanolamines; P<0.037; Supplementary Table 3). Steroids, monoacylglycerides, endocannabinoids, indoles and chlorophylls had significantly larger magnitude NHR than the average molecule (P<0.046; Supplementary Table 4). Betaines, chlorophyll, indoles, monoacylglycerides and steroids were significantly enriched (P<0.024) in molecules with the top 5% magnitude of change (Supplementary Table 5).

Discussion

Our data demonstrate a strong metabolomic signature of bleaching history in the coral host and symbiotic algae years after exposure to thermal stress. When subjected to experimental temperature stress, a majority of the compounds that distinguished historical bleaching phenotypes did not change substantially, and if they did, typically changed only in one phenotype or the other, yielding increasingly discrete overall metabolomes. The main difference between bleach-
Thermally resistant corals may have fundamentally different functions for the DGCC lipid class, as diacyl lipids are more likely to form bilayers, whereas lyso-lipids move more freely in cells. Although the exact location of these DGCC lipids in the algae is unknown, they may contribute directly to the thermal stability of the plastids, a known requirement for thermal tolerance\(^2\), or other membranes of the symbiont. It is also possible that their role in thermal tolerance is through regulatory mechanisms, similar to other bioactive lipids\(^{\text{10,11}}\), or through their chaperone properties as suggested in ref. \(^{\text{25}}\). Importantly, the related phosphate-free DGTS betaine lipids did not exhibit the same historical bleaching response, emphasizing the role of the DGCC forms. The absence of phosphorus in betaine lipids suggests this element is probably important for the bleaching response in corals, and accordingly, phosphate deficiency has been shown to affect the symbiotic algae and promote bleaching experimentally\(^{\text{22}}\). Regardless of their organelle source or mechanism of function, the enrichment of saturated lyso-DGCC lipids in resilient corals on a live reef provides potential for their use as biomarkers of coral resilience and identifies betaine lipid saturation as an important biological mechanism behind coral bleaching.

The bioactive lipid PAF and its precursor lyso-PAF, both found in the coral host and the algal symbiont, were also significantly different between historical bleaching phenotypes. These molecules are known to play a role in coral physiology, immune response and self/non-self recognition\(^{\text{12,13}}\), with increased PAF in corals undergoing stress\(^{\text{14}}\). Interestingly, the activated form of the molecule (PAF) was higher in historically bleached corals, whereas the inactive form (lyso-PAF) was higher in non-bleached corals even under ambient conditions. This finding indicates that immune modulation of both the coral host and the algal symbiont may contribute to the vastly different algal symbiont communities in adjacent, conspecific coral colonies.

Historical bleaching signatures were also found in the metabolites from the coral host, including glutamate-phenylalanine, tryptamine and pantothentic acid. Pantothentic acid is a B-vitamin serving an essential role in fatty acid metabolism and the tricarboxylic acid cycle, suggesting that central carbon metabolism may play a role in determining bleaching phenotype. Changes in corals’ central carbon metabolism occur during temperature stress\(^{\text{15}}\), but historical imprinting after recovery has not been shown. Dipetptides, such as glutamate-phenylalanine, also play a role in corals’ response to temperature stress, where they were proposed to represent increased proteolysis\(^{\text{16}}\), but their exact role has yet to be elucidated. Tryptamine is a monoamine alkaloid with potential neuromodulatory activity that modulates the microbiome in other organisms\(^{\text{36,37}}\), suggesting a role in cross-talk between different components of the holobiont.

Other metabolites from the symbiotic algae important for bleaching phenotype included the chlorophyll breakdown product phophorhphide \(\text{a}\) and the accessory pigment fucoxanthin, both significantly enriched in non-bleached corals. This trend was observed in the pre-stress corals but became even stronger during heat stress with a \(>1,000\)-fold increase in both compounds for the non-bleached corals. This suggests that corals that do not bleach during temperature stress may be degrading chlorophyll and producing more photoprotective photosynthetic pigments such as fucoxanthin and other carotenoids. The breakdown of the light harvesting complex in chlorophyll \(\text{a}\) has previously been linked to bleaching resistance\(^{\text{38}}\) as has the production of photoprotective xanthophylls\(^{\text{16}}\). However, to our knowledge, the stability of this pattern under non-thermally stressful conditions has not been shown before.

It is important to note that ‘metabolite source mapping’ cannot definitively identify the source of molecules because it is nearly
impossible to rid corals completely of symbiont cells. Nevertheless, it is useful to identify the putative origin of molecules in untargeted metabolomics data from coral holobionts, and is akin to metabolite mapping of germ-free and colonized mice, which has been used to track metabolites from the microbiome component of other holobionts\(^{39,42}\). Along with the signatures of the coral symbiont, our data also show significant genotypic influence nested within phenotypes for the total, host, symbiont and shared metabolome. Interestingly, the significant host genotype effect in the symbiont metabolome suggests host effects on symbiont biochemistry in hospite. Furthermore, some compounds that were significantly different by phenotype do not correlate with quantitative polymerase chain reaction (qPCR) quantification of the relative C:N ratio of symbionts in coral hosts, supporting concurrent signatures of both the host and the symbiont in the bleaching history response.

Many of the molecules that distinguish historical bleaching phenotypes did not respond to heat stress, suggesting that latent or causative effects of historical bleaching are partially decoupled from the active short-term heat response, similar to gene expression profiles\(^{43}\). These differences are overlaid on broad phenotypic patterns, where non-bleached coral molecules tend to have a higher magnitude change in abundance. Using a multivariate approach, we identified changes during heat stress in both host- and symbiont-derived compounds. The total metabolome exhibited a significant interaction between phenotype and time, amplifying the separation of phenotypes from their pre-stressed states. This indicates that thermally tolerant corals do not become more biochemically similar to their sensitive conspecifics during heat stress, supporting broadly different pathways in each phenotype. Although it is not a direct comparison, the finding of metabolome changes in both phenotypes is somewhat in contrast to the findings of ref.\(^{25}\), which found that Durusdinium did not respond to heat stress.

Historically, marine conservation has focused on passive habitat protection to mitigate the effects of stressors on coral reefs\(^{44}\). However, the increasing frequency and severity of coral bleaching events necessitates active approaches to coral reef restoration, including asexual propagation, transplantation, larval enhancement and assisted evolution. As mass bleaching events become more common, it is important to ensure that reefs are restored using resilient coral stock\(^{39,42}\); without this consideration, the success rates of these efforts will likely be low\(^{46}\). Thus, the identification of resilient corals for conservation and management has become critically important for the future of coral reefs, but can be time-consuming as it often
depends on natural bleaching outcomes or experimental results. Our metabolomics approach presents an opportunity for fast and cost-effective screening for resilient corals without thermal stress. By screening for metabolite biomarkers such as saturated betaine lipids, managers can test and select resilient coral stocks for restoration when corals are healthy. These selective restoration strategies are needed to create resilience during future bleaching events\(^2\), supporting durable, climate-wise coral ecosystems.

This study demonstrates a metabolomic signature associated with past bleaching in several components of the coral holobiont. This difference between phenotypes is largely driven by the saturation state of betaine lipids associated with the algal symbionts and immune response molecules. Additionally, sublethal heat stress does not seem to alter this metabolomic signature of the two historical bleaching phenotypes, but rather amplifies the distinction. This work provides insight into the biochemical and physiological mechanisms involved in coral bleaching and symbioses ecology, and provides a novel tool for restoring resilient coral reefs in the face of global climate change.

**Methods**

**Ethics statement.** Coral collections were made under Hawai‘i Department of Land and Natural Resources permit SAP 2020-25 issued to the Hawai‘i Institute of Marine Biology.

**Coral collection, stress test and metabolomic sampling.** Individual *Montipora capitata* colonies were tagged during the 2015 bleaching event in Kāne‘ohe Bay, Hawai‘i\(^4\). These tagged corals represent pairs of colonies immediately adjacent to each other where one completely bleached while the other remained visibly healthy (Supplementary Fig. 1b). These tags have been actively maintained in situ, forming each other where one completely bleached while the other remained visibly healthy (Supplementary Fig. 1c). These genotypes were evenly split between five historically bleached colonies and five historically non-bleached colonies selected from the pairs mentioned above. We returned the coral fragments to the Hawai‘i Institute of Marine Biology, mounted them on aragonite plugs and acclimated them for four weeks in indoor mesocosms (hereafter ‘lab corals’). After acclimation, we subjected the lab corals to a five-day temperature stress at 31.4°C or a control (ambient) temperature of 28.4°C. We sampled each fragment for metabolomics analysis before and after six days of heat stress with a 5-mm-diameter dermal curette. Each metabolomics sample was extracted in 500 µl of 70% methanol, kept on ice for 30 min and then stored at −80°C until shipping. Methanol extractions were shipped on dry ice to Michigan State University for untargeted metabolomics analysis. To examine whether the phenotypic differences resolved in the pre-stress lab samples were consistent with coral samples taken directly from the reef, we returned to the original reef in October 2019 and collected a fragment from an additional 12 coral colonies with known bleaching histories that were not included in the original dataset (hereafter ‘validation corals’).

To validate the fractionation of metabolites from the symbionts and the host, we bleached three fragments of a historically bleached (colony 19) and non-bleached (colony 20) coral by raising the temperature to 33 °C over five days until visibly white before sampling with a 5 mm dermal curette. We collected healthy fragments of these colonies, airbrushed them with 1 µm filtered seawater to remove tissue and used centrifugation to produce Symbiodiniaceae cell isolates. Experimentally bleached corals (primarily host tissue) and symbiont isolates were extracted in methanol as described above.

**Mass spectrometry data collection and processing.** Methanolic extracts were analysed on a Thermo Q Exactive high-performance liquid chromatography system. The mobile phase was 0.1% formic acid in Milli-Q water (channel A) and acetonitrile (channel B; chromatographic and mass spectrometry details available in Supplementary Methods). All files were processed with MZmine 2.53 software, GNPS molecular networking and SIRIUS\(^{1,2}\) (Supplementary Methods, data are publically available at gnps.ucsd.edu under MzML/ID).

**qPCR.** DNA was extracted from tissue samples taken from lab and validation corals with a CTAB-chloroform protocol (https://doi.org/10.17504/protocols.io.dqy7y). To determine the concentrations of different symbiont types and host cells in these samples, we used qPCR on an Applied Biosystems StepOnePlus system with two technical replicates. Actin assays were used to quantify symbionts of the genera *Cladocodium* and *Durusdinium*, and *Pax-C* assays were used to quantify the coral host cells\(^1,3\). Cycle threshold (*Ct*) values were corrected for fluorescence and copy number using the equations of Cunning, Ritson-Williams and Gates\(^4\). Samples were re-extracted and/or re-analysed, if *Ct* replicate standard deviation was greater than one between replicates or if only a single replicate amplified.

**Statistical analyses.** Metabolite data were used to predict historical bleaching phenotype in lab corals using neural network classification (JMP 14 Pro, SAS Software) with a holdback proportion of 0.333, a learning rate of 0.1, a squared penalty method and no random seed. We then filtered the dataset to only known compounds and reclassified the data again. To identify which compounds were most important for distinguishing the historical bleaching phenotypes we used supervised random forests classification analysis (randomForest R package) with 5,000 trees to generate VIPs of all the compounds and the known compounds separately.

We examined the abundance of all molecules in the study using principal component analysis (PCA) (JMP 14 Pro, SAS Software) and then compared the distribution of genotypes and phenotypes using PERMANOVA in the R package vegan using 999 permutations. PCAs and PERMANOVAs were conducted independently for initial and validation sets using (1) all compounds, (2) known compounds, (3) compounds shared between symbionts and the host, (4) symbiont mapped compounds and (5) host mapped compounds. We compared the abundance of metabolites of interest and *H*\(_2\) gain (from mass change analysis) using *t*-tests when assumptions of normality and homoscedasticity were met, otherwise we used non-parametric Wilcoxon tests. We examined the abundance of highly informative lipids thought to be associated with symbiont genera\(^1\) using linear models. We calculated the NHR from the heat stress time series as the difference between final timepoints in metabolite abundance in the high temperature and control treatments less the initial difference using the formula below, where *Ab* is abundance (further details of the NHR statistical analysis are available in the Supplementary Methods):

\[
\text{NHR} = (\text{Ab}_{\text{high, final}} - \text{Ab}_{\text{control, final}}) - (\text{Ab}_{\text{high, initial}} - \text{Ab}_{\text{control, initial}})
\]

To examine the holobiont-wide response to thermal stress, we conducted a PCA on all experimental stress samples. To calculate change due to thermal stress, we used a two-way PERMANOVA with historical bleaching phenotype and timepoint (initial, final) as factors. We also analysed changes by timepoint within each phenotype when there were significant differences between phenotypes but not an interaction with time. We analysed all molecules and then used the same framework to select specific components to reanalyse all molecules, host mapped molecules and symbiont mapped molecules.

**Metabolite source mapping.** To better understand the origin of metabolites from different components of the holobiont (that is, coral host or algal cells), we applied a method we term ‘metabolite source mapping.’ We took advantage of our ability to experimentally bleach corals and largely rid them of their algal symbionts and to isolate algal symbionts (see ‘Coral collection, stress test and metabolomic sampling’ for a description of the experimental bleaching and algal isolation). Thus, we can generate LC–MS–MS metabolomics data on separate samples of bleached corals and symbiont isolates using the same analytical methods described above.

The LC–MS–MS data from bleached corals, symbiont cells and the original bleached coral experimental data were processed together using MZmine and feature-based molecular networking as described above. The combined feature table generated was then divided into three different sub-metabolomes: (1) a ‘coral host metabolome’, comprising molecules detected only in a fully bleached coral and not in the symbiont isolates; (2) a ‘symbiont metabolome’, consisting of molecules found only in isolated symbiont cells not in a bleached coral; and (3) a ‘shared metabolome’, those molecules found in both the symbiont isolates and bleached coral.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

Feature-based molecular networking is available at: https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=b9b9b61261118c14ba1881df483560012d3 and raw files are available at massive.ucsd.edu under MassI/VE IDs MSV000085272 and MSV000085925. Ecological and metabolomic data and analysis scripts are available at https://github.com/druryc/mcap_metabolomics. 

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Author contributions
T.N.F.R. and D.C. conceived the experiment. T.N.F.R., J.D., R.A.Q. and D.C. collected data. All authors analysed data, wrote the manuscript and approved the final version.
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The authors declare no competing interests.

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Extended Data Fig. 1 | Kāne’ohe and coral bleaching history. Corals were collected from Reef 13 in Kāne’ohe Bay, O‘ahu, Hawai‘i (a). A representative pair of bleached and non-bleached corals at Reef 13 during the 2015 bleaching event (b). Overall sampling schematic (c). Abbreviations in panel C are as follows: NB=Non-Bleached and B=Bleached.
Extended Data Fig. 2 | Metabolome diversity metrics. Diversity metric for the metabolomes of bleached (B) and non-bleached (NB) corals including Shannon’s entropy (a), richness (b), and evenness (c). Boxplots are median with quartiles and whiskers extending 1.5 IQR beyond quartiles.
Extended Data Fig. 3 | DGCC lipid MS/MS. Extracted ion chromatogram and MS/MS spectra of betaine lipid DGCC 16:0/0:0 described in this manuscript in positive (a) and negative (b) modes. The proposed annotation of each selected fragment ion is highlighted by a red number.
Extended Data Fig. 4 | DGCC lipid abundances and saturation index. Box and whisker plots of (a) the most abundant fully saturated betaine lipid for the \textit{in situ} validation corals and (b) the most important molecule for distinguishing between phenotypes from the random forests variable importance plot. Boxplots are median with quartiles and whiskers extending 1.5 IQR beyond quartiles. c, DGCC unsaturation index (calculated according to Rosette et al. 2019) from experimental bleached and non-bleached corals. d, Log total abundance of lyso-DGCC lipids from experimental corals. Boxplots are median with quartiles and whiskers extending 1.5 IQR beyond quartiles.
Extended Data Fig. 5 | DGTS lipid abundances. DGTS lipid abundances in corals based on HBP. These betaine lipids do not exhibit substantial differences between HBPs like the related DGCCs. Boxplots are median with quartiles and whiskers extending 1.5 IQR beyond quartiles. auc= area under curve.
Extended Data Fig. 6 | Symbiont genotypes in corals from this study. Relative symbiont abundance of genotypes in the study in 2019 (a) and for a larger set that includes validation samples from 2018 (b). *Cladocopium* vs *Durusdinium* dominance is stable over time.
Extended Data Fig. 7 | Betaine lipids and algae abundances. Linear regression of saturated (A,C) and unsaturated (B,D) betaine lipids versus Cladocopium (A,B) and Durisdinium (C,D) algal symbionts. Shaded areas represent 95% confidence intervals.
Extended Data Fig. 8 | Known metabolite relationships with bleaching history.  

**a**, PCA of lab corals using only known compounds. Points (n = 5 per genotype) represent individual samples colored by genotype (n = 10). Fill represents HBP (gray = non-bleached, white = bleached).  

**b**, PCA of field validation set using only known compounds. Points represent single samples from individual genotypes in the validation set which were not evaluated in panel A (n = 12). Fill represents HBP (gray = non-bleached, white = bleached).  

**c**, All known compounds ranked by descending variable importance for discriminating between HBPs. Colors represent molecular families assigned to individual molecules corresponding to Fig. 1.  

**d**, Relative variable importance of molecular families in all known compounds, arranged by ascending median rank (lower is more informative). Individual points correspond to ranked molecules in panel C, with horizontal bars showing first and third quartiles of each molecular family. Colors represent molecular families as in panel C.
Extended Data Fig. 9 | Abundances of known compounds in bleached and non-bleached corals. Box and whisker plots of biologically interesting known metabolites in the ‘lab corals’ (a-e) and the in situ ‘validation corals’ (f-j). Coral host-derived metabolites pantothenic acid (k) and tryptamine (l). Boxplots are median with quartiles and whiskers extending 1.5 IQR beyond quartiles. y-axis is the area under curve abundance.
Extended Data Fig. 10 | Symbiont and bleached metabolome relationships with bleaching history and molecular family responses to heat stress. PCA of all metabolites found in replicate symbiont pellets (a) and all metabolites found in replicate bleached host fragments (b). These samples were collected from colony 19 and 20, which are included in the larger lab dataset and represent a bleached and non-bleached colony, respectively. Fill represents HBp (gray = non-bleached, white = bleached). Multivariate response to heat stress for (c) Membrane Lipids and (d) Bioactive Molecules (e) Putative Microbial Products. Fill represents HBp (gray = non-bleached, white = bleached). Color represents initial and heat stress timepoints (black = initial, red = heat stress). Gray ellipses represent 95% confidence interval of phenotypes. Gray bars connect paired before-after samples.
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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection No code was used to collect data.

Data analysis The following software and packages were used to analyze data:

- GNPS, MZMine 2.53, SIRIUS, MeMSChem, JMP Pro 14, R 3.5, Rstudio 1.1.453
- R packages: Tidyverse, readxl, cowplot, janitor, viridis, vegan, car, magicfor, factoextra, egg

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Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Study description |
|--------------------|
| This study uses adult corals from 22 colonies, pooled by historical bleaching phenotype. Metabolomic samples from historical bleaching phenotypes were collected for all colonies. A subset of 10 colonies were exposed to sublethal thermal stress and a control (n=5 replicates x 2 tanks per genotype) and sampled before and after 5 days of thermal stress. |

| Research sample |
|-----------------|
| This study uses 22 known Montipora capitata adult colonies with historical bleaching performance data. Samples were chosen based on the design to segregate by historical bleaching phenotype. The sample represents Montipora capitata in Kaneohe Bay, Hawaii. |

| Sampling strategy |
|-------------------|
| Samples were chosen based on the design to segregate by historical bleaching phenotype. 22 samples is an average-to-large sample size for molecular work in reef-building corals. |

| Data collection |
|-----------------|
| CD, TR, JD collected samples and physical data (fv/fm). Samples were collected using dermal curettes, fv/fm data was collected using a Walz Diving PAM. |

| Timing and spatial scale |
|--------------------------|
| Samples were collected between June (initial) and September (validation) 2019. All samples came from a single reef (reef 13) in Kaneohe Bay. |

| Data exclusions |
|-----------------|
| No data were excluded. |

| Reproducibility |
|-----------------|
| The validation set was used to compare the segregation of historical bleaching phenotype in metabolomic data with separate collections, which reproduced the main finding. |

| Randomization |
|---------------|
| Not relevant, groups were created by historical bleaching phenotype. |

| Blinding |
|---------|
| Metabolomics data processing and acquisition was conducted blind to historical bleaching phenotype. Photochemical efficiency data was collected using a diving PAM for all corals at two timepoints, blinded to phenotype but not to temperature treatment. |

| Did the study involve field work? |
|----------------------------------|
| Yes | No |

Field work, collection and transport

| Field conditions |
|------------------|
| Shallow patch reef, Kaneohe Bay, Oahu, HI |

| Location |
|---------|
| Reef 13, shallow patch reef in Kaneohe Bay, Oahu, HI (21.451, -157.796) |

| Access & import/export |
|------------------------|
| The site was accessed by boat and did not interfere with the environment. Collections were made under Hawaii Department of Land and Natural Resources permit SAP 2020-25 issued to the Hawaii Institute of Marine Biology. |

| Disturbance |
|-------------|
| The study did not cause any disturbance to the reef beyond fragment collections, which are a negligible fraction of colony biomass. |

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Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

| Laboratory animals | This study did not involve laboratory animals. |
|--------------------|---------------------------------------------|
| Wild animals       | Fragments from 22 adult Montipora coral colonies (hermaphrodites) were collected. Adult colonies were not removed and sampling was minimally invasive, representing a small fraction of biomass of each colony. |
| Field-collected samples | Validation corals were transported to HIMB in ambient seawater and sampled 1 hour after collection. For the initial lab corals, we returned fragments to the Hawai‘i Institute of Marine Biology, mounted them on aragonite plugs and acclimated them for four weeks in indoor mesocosms (hereafter ‘lab corals’) at ambient temperature conditions and a maximum midday irradiance of ~400 μmol/sec. |
| Ethics oversight   | No ethical oversight is required. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.