1  ***Resilience in a time of stress: revealing the molecular***
2  ***underpinnings of coral survival following thermal bleaching events***

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## Abstract

24    Coral bleaching events from thermal stress are increasing globally in duration,
25  frequency, and intensity. Bleaching occurs when a coral's algal symbionts are expelled,
26  resulting in a loss of color. Some coral colonies survive bleaching, reacquire their symbionts and
27  recover. In this study, we experimentally bleached *Montipora capitata* colonies to examine
28  molecular and physiological signatures of intrinsic differences between corals that recover
29  (resilient) compared to those that die (susceptible). All colonies were collected from the same
30  bay and monitored for eight months post-bleaching to identify specific colonies exhibiting long-
31  term resilience and survival. Using an integrated systems-biology approach that included
32  quantitative mass spectrometry-based proteomics, 16S rRNA of the microbiome, total lipids,
33  symbiont density and diversity, we explored molecular-level mechanisms of tolerance in pre-
34  and post-bleached colonies and found biomarkers of resilience that can confidently identify
35  resilient and susceptible corals before thermal-induced bleaching events. Prior to thermal
36  stress, resilient corals were characterized by a more diverse microbiome and increased
37  abundances of proteins involved in multiple carbon and nitrogen acquisition strategies, symbiont
38  retention and acquisition, and pathogen resistance. Susceptible corals had early signs of
39  symbiont rejection and had resorted to utilizing urea uptake pathways for carbon and nitrogen.
40  Further, molecular signatures identified prior to bleaching were amplified after bleaching,
41  suggesting these pathways may be deterministic in a colony's fate. Our results have important
42  implications for the future of reefs, revealing molecular factors necessary for survival through
43  thermally-induced bleaching events and providing diagnostic biomarkers for coral reef
44  management.
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## Significance statement

47  Corals are being negatively impacted by the increase in the number and duration of thermal-
48  induced bleaching events. There are, however, some individuals within a single species that will
49  bleach and, after time, reacquire symbionts and physiologically recover while neighboring
50  colonies will die. Here, we used a multidisciplinary approach to understand the biochemical
51  details of the physiological changes of resilient and susceptible *Montipora capitata* to thermal-
52  induced bleaching.  Resilient corals were characterized by their use of multiple carbon and
53  nitrogen acquisition strategies, metabolically active symbiont relationships, abundant antiviral
54  proteins, and a diverse microbiome. We reveal a multi-factor molecular-level approach for
55  confidently identifying resilient and susceptible coral colonies so that environmental managers
56  can rapidly select quality candidates for propagation while in the field.
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## Introduction

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60  Coral reefs are one of the most diverse and structurally complex ecosystems on Earth,
61  providing shelter and habitat for many organisms (1). Tens of millions of people in more than one
62  hundred countries have coastlines adjacent to coral reefs and depend on them for their livelihoods
63  (2). Unfortunately, coral reefs are declining rapidly throughout the world due to pollution, coastal
64  development, overexploitation (3, 4), and effects associated with climate change (5, 6). As global
65  seawater surface temperatures increase, large-scale thermal-induced coral bleaching events
66  (loss of distinctive coral coloration due to expelling of algal symbionts) are becoming
67  increasingly common worldwide (7-9).
68  When water temperatures surpass a thermal threshold for a given coral species it leads to a
69  breakdown in the association between the coral host and the symbiotic algae, Symbiodiniaceae.
70  This breakdown results in symbiont expulsion and is termed "coral bleaching" due to the loss of
71  the pigmented symbiont (3). In addition to the distinct colors, Symbiodiniaceae provide much of
72  the energetic requirements for the host in the form of organic carbon and nitrogen
73  photosynthetic byproducts (10). The expulsion of Symbiodiniaceae during bleaching events
74  metabolically compromises the host (11), leading to reduced physiological performance,
75  reduced reproductive capacity and can lead to widespread mortality.
76  A coral colony is a holobiont – a collection of organisms that includes the coral, its symbionts,
77  and the microbial microbiome that exists on and in coral tissue. The diversity of the microbiome
78  on corals frequently decreases with thermal-induced bleaching (12, 13). While some of these
79  changes may be associated with the loss of Symbiodiniaceae, other changes in microbiome
80  diversity appear to be driven by thermal stress directly (14). If cascading microbiome changes
81  induced by bleaching disrupt relationships with bacteria or archaea that benefit coral host
82  metabolism or pathogen defense, then those microbiome changes may also contribute to post-
83  bleaching coral mortality.
84  Coral microbiomes demonstrate host specificity, bolstering the hypothesis that bleaching-
85  induced microbiome disruptions play a significant role in host mortality (15-19). However,
86  disentangling relationships between corals and their microbiomes is challenging without
87  gnotobiotic models. Moreover, although genomic evidence and correlations between taxonomic
88  abundance and disease provide some hints, it is still unclear which specific microbiome
89  changes associated with bleaching or other stressors are helpful or harmful for the survival of
90  the coral host. Indeed, while a rich literature documents alterations to microbiome structure and
91  stability by many specific stressors and coral diseases — including changes to beta-diversity
92  (20, 21), richness (22), and the abundance of particular taxa — far fewer data are available on
93  which of these diverse microbiome changes best predict the future survival of coral hosts. Thus,
94  data linking microbiome change with subsequent coral survival could be vital to interpreting the
95  ecological consequences of shifts in bleaching-induced microbiome structure for corals.
96  Coral death due to the interconnected physiological and microbiological consequences of
97  bleaching events can lead to total community collapse if mortality is widespread. However,
98  some coral species, and individuals within species, are resilient to the effects of bleaching and
99  appear to return to pre-bleaching status. Corals resilient to thermal stress can reacquire
100  symbionts, fully recover physiological performance and yield viable gametes (e.g., 23), though
101  the timescale for post-bleaching recovery can vary from weeks to a year (24-27). Post-bleaching
102  recovery times may be shorter in corals that have previously been exposed to multiple annual

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103  bleaching events (28), perhaps by priming the physiological responses necessary to survive
104  bleaching.
105     There are several possible contributing factors that may provide greater coral holobiont
106  resilience to bleaching events: host- and/or symbiont- species (29-32), host genotype (33-38),
107  or the microbial constituents of the holobiont's microbiome (39, 40). At the center of this
108  complex equation is the host's metabolic capacity before and after bleaching (i.e., how is the
109  coral acquiring nutrients to sustain growth and immune function?). Without adequate carbon
110  and nitrogen, the coral cannot maintain systems of cellular and tissue repair, retain, or reacquire
111  symbionts, or fight off pathogens (e.g.,41, 42). This suggests that the pre-bleaching molecular
112  physiology of the coral host may be the most important factor in determining resilience to
113  bleaching stress and mortality.
114     The interactions between an organism and its environment are complex and depend upon
115  ecological and evolutionary history. Corals in Hawai'i, Kāneo'he Bay, O'ahu have endured an
116  increased frequency of bleaching events. Results from bleaching surveys in the bay have
117  shown a decrease in the proportion of coral colonies bleached over time (62% in 1996 to 30% in
118  2015) but an increase in the number of colonies dying (<1% in 1996 to 22% in 2015). Kāneo'he
119  Bay is inhabited by the reef-building Scleractinian coral *Montipora capitata* (43). These corals
120  are typically found in tropical waters, living within 1-2°C of their upper thermal limit (44, 45). This
121  suggests that projected future water temperatures will specifically threaten this species (e.g.,
122  46). *Montipora capitata* has been shown to have higher thermal tolerance than other Hawaiian
123  coral species (47) and is thereby an ideal candidate to study the long-term effects of thermal
124  acclimatization or adaptation to reveal the underlying molecular physiology that supports
125  bleaching resilience.
126     In this study, we report the results of a joint coral physiology, proteomic, lipid, and microbiome
127  analysis comparing the features of *M. capitata* coral colonies that recovered *in situ* from
128  experimental bleaching against the features of those that died. Colonies of visually healthy *M.*
129  *capitata* were exposed to 30°C water in experimental tanks for three weeks to simulate thermal
130  stress and induce bleaching. Samples for proteomics, microbiome diversity, total lipids,
131  Symbiodiniaceae density and clade diversity were collected before thermal stress ($T_1$) and three
132  weeks after bleaching occurred ($T_2$) (Fig.1). A control set of the same colonies did not undergo
133  thermal-induced bleaching. After the three weeks, corals were outplanted to the field and
134  monitored for eight months to identify colonies that recovered from the thermal-induced
135  bleaching event and those that died. These outcomes were then retroactively used to label the
136  previously collected samples as deriving from *resilient* or *susceptible* colonies. The proteomic,
137  microbial, and physiological differences uncovered in this study thus describe intraspecific
138  differences associated with bleaching resilience in the field. Because coral colonies were
139  collected from and outplanted to the same location at Moku O Lo'e island in Kāneo'he Bay, they
140  experienced the same water and thermal conditions throughout the study.
141     Comparison of resilient vs. susceptible *M. capitata* using semi-quantitative proteomics allowed
142  the generation of metabolic maps of abundant enzymes and outlined coral's energetic priorities
143  that may confer resilience in a changing climate. Several proteins significantly differed between
144  resilient and susceptible colonies prior to experimental thermal stress, contributing to bleaching
145  resilience. These resilience-associated proteins reveal dominant nutritional and metabolic
146  strategies underpinning the ability to survive bleaching. The proteome also revealed evidence

4

147     for symbiont rejection, antiviral activity, enhanced immune response to pathogens, and carbon
148     and nitrogen pathways exclusive to resilient colonies. Additional molecular-level metrics of the
149     holobionts were monitored, including total lipids, microbiome diversity, and symbiont density and
150     diversity, allowing us to evaluate and establish whole-organism biomarkers of resilience. These
151     molecular-level signatures could be used to predict coral resilience or susceptibility prior to a
152     bleaching event. Last, we report a multi-factor approach to identify the corals that will survive future
153     bleaching events, the linchpin to coral management, propagation efforts, and restoration success.

## Results

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155     In September 2017 seventy-four colonies of *Montipora capitata* were tagged (with an ID) and
156     collected, acclimated in tanks for two weeks, sampled ($T_1$), gradually exposed to increasing
157     water temperatures to reach 30°C, and held at 30°C for three weeks to simulate a thermal
158     bleaching event (Fig. 1). Water temperatures were returned to ambient temperatures over 4
159     days, after 24 hrs at that temperature corals were sampled ($T_2$) and then were monitored for
160     long-term survival and recovery for eight months (Figs. 1 and S1). After three months
161     (December), 22 colonies died; these colonies will be referred to as "susceptible". Fifty-two
162     colonies recovered and reacquired symbionts; these colonies will be referred to as "resilient".
163     After December, no coral mortality was observed. By May, all the colonies that survived reached
164     pre-bleaching coloration (Fig. 1B). At $T_1$ and $T_2$, we obtained coral samples to examine
165     physiological performance and recovery. Six of the 52 resilient colonies and six of the 22
166     susceptible colonies were randomly selected for the study and frozen sub-samples at both
167     timepoints were used for mass spectrometry-based proteomics, total lipid content,
168     Symbiodiniaceae density and diversity, and bacterial community composition.
169     At $T_1$, no significant difference was measured in symbiont density between the resilient and
170     susceptible colonies (Fig. 1B, Dataset S1A). During the thermal event, the symbiont density
171     decreased in both cohorts at the same rate; within 3 months after $T_2$, the resilient cohort
172     returned to pre-bleaching Symbiodiniaceae density. Additionally, there was no significant impact
173     of bleaching, time point, bleaching tolerance, or colony of origin on symbiont clade abundances
174     (Fig. S1B; Dataset S1B).
175     A total of 2,193 coral proteins were identified at $T_1$, 2,161 coral proteins were identified at $T_2$,
176     and 1,424 coral proteins were shared between those timepoints, indicating constitutive
177     expression (Fig. S2, Dataset S2A-G). Analysis of the resilient and susceptible colonies
178     independent of timepoint revealed 2,276 proteins detected in the resilient cohort and 2,066
179     proteins in susceptible colonies.
180

181     **Biological enrichment analysis.** Enrichment analysis of Gene Ontology (GO) terms was
182     completed using MetaGOmics (48), an unbiased method to identify significantly different
183     metabolic processes represented in proteomes of the resilient vs. susceptible cohorts at both
184     timepoints using log-fold change (base 2; LFC) of GO term assignments (Dataset S3A-B).
185     Resilient colonies at $T_1$ were characterized by multiple cellular responses to external signals,
186     receptor activity, and monosaccharide binding. Sterol esterase activity was the most enriched
187     term (LFC=5.0; Fig. 2A). Six GO terms were significantly enriched in susceptible colonies prior
188     to bleaching ($T_1S$) and included proteins involved in urea and amide catabolism, nickel binding,
189     and the removal of superoxides (Fig. 2A). After thermal bleaching, 51 GO terms were enriched

5

190    in resilient corals, 33 of which were unique (Fig. 2B). Proteomes of resilient colonies post-
191    bleaching ($T_2R$) were enriched in regulation of phagocytosis and meiotic cell cycle, vesicle-
192    mediated transport, hormone regulation, and cardiac muscle processes. Although some of the
193    labels for these processes may not seem to apply to corals, proteins associated with the
194    "cardiac muscle process" GO term are involved in sodium and calcium exchange while
195    "regulation of systemic arterial blood pressure" proteins include sodium-driven chloride
196    bicarbonate exchange proteins involved in pH regulation. Many of the identified GO terms in
197    susceptible colonies post-bleaching involved the cellular processing of metabolites, including
198    sterols, methionine, betaine, sarcosine, lipids, and glutamate. Additionally, several terms were
199    related to DNA or RNA processing.
200
201    **Immune system responses.** To elucidate complete metabolic pathways being preferentially
202    utilized by either the resilient or susceptible colonies at the two timepoints, significant differential
203    abundances of proteins were calculated (Fig. 2C, D; significance only reported when $p \leq 0.05$;
204    Dataset S2G-H). Comparisons of resilient and susceptible colony proteomes before the
205    simulated thermal bleaching event ($T_1$), revealed that resilient colonies had 39 proteins at
206    significantly higher abundances than the susceptible cohort, whereas susceptible colonies
207    possessed 56 proteins at significantly higher abundances (Fig. 2C). In $T_1R$ colonies, signal
208    peptidase (LepB) yielded the highest differential abundance, followed by F-type H+-transporting
209    ATPase subunit alpha (ATPeF1A), and a membrane attack complex component/perforin
210    (MACPF) domain-containing protein known to lyse virus-infected and pathogenic bacterial cells.
211    Susceptible corals before bleaching ($T_1$) significantly increased the abundance of Fibropellin-1
212    (EGF1), a component of the apical lamina, the surface glycoprotein melanoma-associated
213    antigen p97 (MFI2), an enzyme involved in glycosylating proteins, alpha 1,2-
214    mannosyltransferase (KTR1_3), and the urea degrading enzyme urease (URE1).
215     After the colonies were bleached ($T_2$), the resilient cohort was characterized by 108 proteins
216    that significantly increased in abundance while the susceptible cohort significantly increased the
217    abundance of 63 proteins (Fig. 2D). A CyanoVirin-N domain-containing protein (CVNH) was
218    identified to have the most consistent expression across the 12 colonies tested (i.e., lowest $p$-
219    value), a high LFC in the $T_1R$ proteome, and the highest LFC in $T_2R$ (Fig. 2D). Further analysis
220    of this protein sequence against the conserved domain database revealed that it contains four
221    CyanoVirin-N conserved domains with viricidal activity that interact with the glycoproteins on the
222    viral envelope (49). Conversely, chitinase (CHIC), an enzyme capable of degrading chitin,
223    exhibited the highest LFC in the susceptible corals post-bleaching (Fig. 2D), another possible
224    indication of symbiont degradation.
225     Cluster analysis of proteins that were identified across all experiments to be significantly
226    abundant in at least one treatment (LFC $\geq |1|$, $p$-value$<0.01$) are represented in a heatmap that
227    spans fifteen metabolic pathways (Fig. 3). Resilient and susceptible coral proteomes are most
228    similar at $T_1$ (clusters 1-3, 9-12) compared to the proteomes at $T_2$ (Fig. 3). Furthermore, resilient
229    corals prior to bleaching exhibited higher abundances of several proteins involved in anti-viral
230    activity, immune response, and symbiosome maintenance (clusters 4-7) than susceptible corals.
231    After thermal bleaching, the resilient coral colonies maintain a significantly higher abundance of
232    six enzymes, including CNVH (cluster 1), compared to susceptible corals. The $T_2R$ cohort
233    uniquely increased the abundance of 37 additional proteins (cluster 3) involved in nitrogen

6

234    metabolism, immune response, endosome/symbiosome activity, and DNA translation, among
235    others. Pre-bleaching susceptible corals ($T_1S$), despite exhibiting somewhat similar proteomic
236    trends to $T_1R$, revealed one unique cluster (cluster 8) of 9 proteins that were significantly
237    increased in abundance. These proteins play a role in structures and functions such as the
238    extracellular matrix, and immune response, or are associated with lysosome/phagosome
239    activity. Post-bleaching susceptible corals ($T_2S$) had the most distinct proteomic response, with
240    depletion of nearly all proteins represented by clusters 1-8 and enrichment in a unique suite of
241    proteins in carbon, nitrogen and lipid metabolism, the biosynthesis of secondary metabolites,
242    the extracellular matrix, and the immune system (clusters 11-12).

243    **Resilient corals retain lipids through the thermal bleaching event.** Previous investigations
244    on recovery from thermal bleaching events revealed that *M. capitata,* unlike other corals, has
245    the unique ability to replenish energy reserves within 1-2 months after the bleaching event,
246    making it one of the coral species with the fastest recovery rates (50). Pre-bleaching, resilient
247    corals had significantly greater abundances of enzymes involved in lipid degradation compared
248    to the susceptible cohort (*e.g.,* PSAP and LIP, Fig. 2A). To determine if pre-bleaching lipid
249    biomass (*i.e.,* $T_1$) is a significant and predictable metric to identify *M. capitata* colonies that will
250    recover from thermal bleaching events, total lipids were measured. No significant difference in
251    coral lipid content was found before the simulated thermal event (*i.e.,* between $T_1R$ and $T_1S$
252    colonies; Dataset S1D-E). Coral lipid content varied significantly by bleaching status at $T_2$ ($T_2B$
253    vs. $T_2NB$: $p=0.00079$; Fig. 2F) and long-term tolerance to bleaching ($T_2R$ vs. $T_2S$: $p=0.00095$;
254    Fig. 2E). Interaction of the two variables was also significant ($p=0.044$): susceptible corals
255    experienced a decrease in mean lipid content by 44% after exposure to thermal stress ($T_1S$ and
256    $T_2S$), while resilient colonies decreased by only 16% ($T_1R$ and $T_2R$).

257    **Resilient corals have more diverse bacterial communities.** Alpha diversity of the
258    microbiome based on the 16S rRNA V4 variable region was quantified using Faith's
259    phylogenetic diversity (Faith's PD), a measure of microbiome richness that accounts for
260    phylogeny. Faith's PD significantly differed among groups defined by each combination of
261    timepoint, bleaching resilience, and bleaching status (Kruskal Wallis $p=0.0045$). Prior to
262    bleaching ($T_1$), resilient *M. capitata* had higher alpha diversity compared to susceptible corals
263    (Kruskal Wallis $p=0.016$; Fig. 4A). However, this difference could be attributable to multiple
264    comparisons (false discovery rate (FDR) q=0.061). After bleaching, previously low-diversity
265    susceptible corals exhibited significantly increased alpha diversity ($p=0.0065$, FDR q=0.048),
266    while previously high-diversity resilient corals did not ($p=0.52$, FDR q=0.6). Thus, resilient corals
267    showed smaller microbiome changes during bleaching and over time than susceptible corals,
268    consistent with greater stability in microbiome richness.
269      Beta diversity was also quantified to determine the similarities of the bacterial communities
270    between cohorts. Across combinations of timepoint, resilience, and bleaching status, microbial
271    community composition differed qualitatively (Unweighted UniFrac PERMANOVA; $p=0.002$) and
272    quantitatively (Weighted UniFrac PERMANOVA; $p=0.041$). These differences were not
273    attributable to differences in microbiome dispersion (Weighted and Unweighted UniFrac
274    $p>0.05$).
275      The main taxonomic drivers of community differences revealed that Gammaproteobacteria
276    were well represented in the six resilient colonies and nearly absent in susceptible colonies (Fig.

277     4), consistent with the overall community differences detected in beta-diversity analysis. At the
278     family level, multiple microbial families showed striking differences between the resilient and
279     susceptible cohorts. The clearest of these differences was seen in *Moraxellaceae*, a family in
280     class Gammaproteobacteria that was only found in resilient corals at $T_1$. Microbiome
281     Multivariate Association with Linear Models (51), a statistical analysis method that identifies
282     multivariable association between microbial features and metadata (i.e., time, resilience,
283     bleaching), confirmed that *Moraxellaceae* significantly correlated with both time and resilience
284     (MaAsLin2$_{TIME}$ *p*=0.0001; MaAsLin2$_{RvS}$ *p*=0.0007; Table S1). Additionally, the *Caulobacteraceae*
285     microbial family—in the phylum Proteobacteria—was present at elevated abundance in resilient
286     corals, and lower abundance in susceptible ones (irrespective of timepoint or bleaching status
287     (MaAsLin2$_{RvS}$ *p*=0.0006).

## Discussion

289     Although multiple Cnidarian species have had their proteomes analyzed (*e.g., Eunicea*
290     *calyculata* (52); *Amphistegina gibbosa* (53); *Acropora microphthalma* (54); *Acropora millepora*
291     (55), *Montipora capitata* (56, 57)), to date, no studies have exhaustively explored pre-bleaching
292     protein-level physiology in combination with multiple other molecular-level factors to determine if
293     there are traits predictive of resilience to bleaching events. Despite previous research showing
294     that symbiont clade D can lead to reduced levels of bleaching in multiple coral species or
295     improve thermal tolerance (29, 36), these coral colonies show no significant differences in clade
296     abundances or distributions across bleaching status, time point collected, or tolerance to
297     bleaching (Dataset S1A, B; Fig S1B). In general, more proteins were detected in all corals at $T_2$
298     post-bleaching relative to $T_1$ pre-bleaching, a common response to exogenous stressors (e.g.,
299     58-60). Simultaneous activation of multiple metabolic pathways provides bleached *M. capitata*
300     with new carbon and nitrogen acquisition strategies as symbiont-delivered photosynthate is
301     diminished or absent. Our primary hypothesis is that molecular phenotypic differences -
302     resulting from genetics or epigenetics - will enhance the ability of some individual corals to
303     mitigate the effects of bleaching. Before thermal stress occurred ($T_1$), the only significant
304     differences were identified in protein abundances and in the microbiome diversity between
305     susceptible and resilient colonies. Examination of the significantly changing metabolic pathways
306     in the resilient and susceptible cohorts reveals for the first time how nutritional strategies,
307     antiviral mechanisms, and microbiome diversity pre-and post-bleaching dictate survival.
308
309     **Phenotypic advantages in resilient corals prior to bleaching.** Several proteins in metabolic
310     pathways associated with maintenance of a functional symbiont- host relationship were present
311     at increased abundance in the resilient coral proteome prior to the bleaching event. The primary
312     active pathways that were enhanced in resilient corals pre-bleaching include sterol and lipid
313     degradation, cellular respiration, oxidative phosphorylation, and carbon metabolism (Fig. 2A).
314     Total lipid biomass in the resilient corals had a broader range of values and a higher average
315     than the susceptible cohort (Fig. 2E).
316     Prior to bleaching, resilient corals utilize heterotrophic feeding and symbiont photosynthate.
317     The GO term sterol esterase was the most significantly increased term in resilient *M. capitata*
318     colonies. Analysis of proteins contributing to this GO term revealed the dominating contributors
319     to the enrichment analyses included a gastric triacylglycerol lipase-like protein (LIP) (Fig. 3;

320    cluster 4) and the lipid-specific degradation enzyme saposin (PSAP; Fig. 2C). These enzymes
321    are typically involved in digestion and likely reside in the coral gastric cavity, suggesting that at
322    $T_1$, pre-bleaching, the resilient corals are using a heterotrophic feeding strategy in addition to
323    photosynthate from *Symbiodiniaceae*. This was not unexpected as *M. capitata* has been
324    previously observed to utilize heterotrophy when not bleached (61). $T_1$ resilient corals also
325    possessed higher abundance of early endosome antigen 1 (EEA1), an essential protein in
326    symbiosis establishment (i.e., 62-64), providing resilient corals with an advantage for
327    maintaining symbiont relationships compared to the susceptible corals. Utilization of diverse
328    feeding strategies would provide a distinct advantage to the resilient corals as the excess
329    carbon can be shuttled into lipid storage vesicles (61, 65).
330    Resilient corals present evidence of more active cellular respiration pathways at $T_1$ prior to the
331    bleaching event. Although many enzymes in the carbon metabolism pathway are constitutively
332    expressed in resilient and susceptible corals, the increased abundance of isocitrate lyase (ICL;
333    Fig. 2C) provides resilient corals utilization of the glyoxylate shunt, a TCA-cycle bypass that
334    allows cells to complete anabolic reactions with 2-carbon units without losing carbon as $CO_2$
335    (the opposite of what is observed in susceptible corals at $T_1$; Fig. S3A). Further, increased
336    abundances of glycine hydroxymethyltransferase (GLYA; Fig. 2C) provides single carbon (1C)
337    units to the cell, fueling the glyoxylate shunt for the generation of larger carbon-storage
338    molecules (*i.e.,* lipids) from the excess 1-2 carbon unit small molecules, again bypassing the
339    generation/loss of $CO_2$ (66, 67). Additional evidence, such as increased abundance of pyruvate
340    dehydrogenase (PDHa; Fig. 2C) and multiple acetyl-CoA transferases support increased
341    cellular respiration and the potential to store excess energy in resilient corals.
342    Prior to bleaching, the resilient corals appear to prime anti-viral activity and exhibited a
343    significantly more diverse microbiome, which likely supported their immune response. Previous
344    demonstrations of "frontloading" immune response or pathogen-fighting enzymes have been
345    shown to increase survival in corals (68). One of the most differentially abundant proteins that
346    was elevated in resistant colonies at $T_1$ was a cyanovirin-like protein (CVNH; Figs. 2C), an
347    evolutionarily conserved protein that binds to viruses and blocks entry into the cell (69). CVNH
348    was also detected in higher abundances in the resilient cohort through $T_2$, post-bleaching (Figs.
349    2D and 3, cluster 1). To determine if there were identifiable viral proteins in the whole-coral
350    protein extractions and mass spectrometry analyses conducted, the data was analyzed using a
351    larger database that included 5 coral-associated viral proteomes (Table S2). The number of
352    confident peptides associated with the viral proteins detected were not statistically different
353    between resilient and susceptible colonies at $T_1$, yet their presence in whole-holobiont protein
354    extract does corroborate the need for corals to produce antiviral proteins. Additionally, the $T_1R$
355    colonies hosted a significantly more diverse microbiome (Fig. 4A), which has a positive effect on
356    host health (70). The resilient microbiome included the *Moraxellaceae* bacterial family, which
357    was only found in resilient coral colonies. *Moraxellaceae* are associated with local wastewater
358    and they are known to have high numbers of antibiotic resistance genes (ARGs) (71, 72). As the
359    coral host's immune system is activated against pathogenic bacteria and releases antimicrobial
360    defenses, the *Moraxellaceae* bacterial family's high number of ARGs may provide them with an
361    advantage for long-term residence on the host. As *Moraxellaceae* has been found to be a
362    common component of many shallow water coral microbiomes, these bacteria may be important
363    in shaping a healthy coral holobiont (73). The functional role of this bacterial family's unique

9

364    genome in conferring resilience against bleaching to coral colonies is unknown, but the close
365    association of *Moraxellaceae* on resilient *M. capitata* colonies merits further research.
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367    **Molecular signs of stress before thermal induced bleaching in susceptible corals.** A
368    detailed proteomics analysis revealed the metabolic processes identified in susceptible corals
369    prior to bleaching, including urea and amide catabolism, nickel binding, and urease activity (Fig.
370    2A). Redirected nitrogen and carbon uptake pathways in susceptible corals suggest a decrease
371    in symbiont-derived photosynthate at $T_1$. Previous molecular-level investigations of symbiont-
372    host relationships have demonstrated that the majority of nitrogen assimilation occurs via the
373    symbiont-directed GS/GOGAT (glutamine synthase/glutamine oxoglutarate aminotransferase)
374    activity or the coral host-directed glutamine synthetase or glutamate dehydrogenase activity
375    (e.g., 74). Prior literature suggests that the majority of nitrogen uptake is from symbiont-
376    transferred metabolites resulting from their utilization of free ammonia in the water column (e.g.,
377    75), although it has been suggested that the assimilation of nitrogen by the host itself is
378    underestimated (76). Here, there is evidence that the susceptible coral colonies utilized urea as
379    their primary nitrogen source at $T_1$ (Fig. S3).
380      We propose that high levels of the urease enzyme may be a biomarker of a dysfunctional
381    metabolic relationship between the coral host and its algal symbiont. Urea, a soluble nitrogen-
382    rich molecule, is degraded intracellularly to yield ammonia and carbon dioxide ($CO_2$) via urease
383    enzyme (URE1, Fig. 2A,C and S3). At $T_1$ in susceptible corals, GO terms for nickel-binding
384    activity and carbonic anhydrase activity are enriched (Fig. 2A). Proteins associated with these
385    functions provide the required nickel cofactor for urease (77) and increased abundances of
386    carbonic anhydrase enzyme (CA) rapidly converted $CO_2$, byproducts of the reaction, into
387    carbonic acid (or bicarbonate). It has been suggested that coral cells rely on this pathway to
388    acquire additional nitrogen when under stress (78, 79). For example, in corals lacking
389    Symbiodiniaceae, urease activity increased to compensate for the lack of Symbiodiniaceae-
390    provided nitrogen (79). Previous experiments on corals revealed that urea- and nickel-
391    enrichments increased photosynthesis and calcification rates, suggesting that these molecules
392    support coral growth in adverse environmental conditions (77). Isocitrate dehydrogenase
393    (ICDH1; FC: 0.57)), an enzyme that is increased under nitrogen starvation, is slightly increased
394    in $T_1$ susceptible (compared to $T_1R$) corals. ICDH1 links the carbon metabolism (TCA cycle) and
395    nitrogen cycle together to generate glutamate (via GS-GOGAT; Fig. S3A). The increased
396    presence of enzymes involved in these alternate routes of nitrogen and carbon acquisition
397    provide molecular evidence for their use as potential biomarkers of environmental stress and/or
398    the beginnings of dysfunctional symbiosis.
399      After thermal bleaching ($T_2$), the abundance of urease (URE1) continues to increase and is
400    consistently more abundant in susceptible corals at both timepoints (Fig. 3, cluster 9).
401    Therefore, urea-dominated nitrogen acquisition strategy in the host increases as the host-
402    symbiont relationship becomes compromised in susceptible coral colonies responding to
403    thermal stress. Although it has been proposed that the prokaryotic host-associated microbiome
404    could provide bioavailable nitrogen via nitrogen fixation when the host is stressed, the 16S
405    rRNA does not provide species-level resolution that would definitively reveal if any of the noted
406    bacterial families in $T_2S$ microbiome were nitrogen-fixers.

10

407    Importantly, the susceptible corals displayed early evidence for the rejection and degradation
408    of symbiosomes in before the thermal stress starts. The coral's symbionts reside in specific
409    phagosomes called symbiosomes; corals therefore have specific enzymatic and signaling
410    pathways to disrupt the standard phagosome recycling mechanisms, ensuring the symbiont's
411    residence. Typical phagosome recycling via hydrolytic enzymes is directed by Rab11
412    expression in coral hosts (80). Established, healthy symbiotic relationships therefore inhibit the
413    Rab11 pathway, resulting in a decrease in Rab11 abundance (e.g., 80). Susceptible corals at $T_1$
414    displayed significantly increased abundance of Rab11 (Fig. 2C), an early indication of a
415    dysfunctional symbiotic relationship and potential host-directed degradation of the symbiosome,
416    or symbiophagy (81). This host-symbiont disequilibrium hypothesis in $T_1S$ corals is further
417    supported by increased abundance of Tubulin alpha (TUBA), a phagocytosis protein recognized
418    to be active in symbiont degradation (82), among other functions. Three enzymes detected at
419    significantly increased abundances were involved in glycan degradation, in particular the
420    mechanism involved in cleaving mannose-based oligosaccharides: alpha-L-fucosidase
421    (FUCA1), mannose-receptor (MRC1), and mannosyl-oligosaccharide alpha-1,3-glucosidase
422    (GANAB) (Fig. 3, cluster 8). As mannose is recognized by lectin proteins in corals to identify
423    pathogens and symbionts, the degradation of these mannose-based oligosaccharides would
424    weaken physical associations of the host with symbionts and its microbiome (83, 84). Further,
425    the degradation of these oligosaccharides would specifically provide easily accessed glucose
426    monomers for supporting the energy-demands of the susceptible coral colonies at $T_1$. Increased
427    presence of mucin proteins (*i.e.,* MUC4, Fig. 2C), a noted deterrent to pathogen colonization
428    (85), and lectins, pathogen recognition proteins (e.g., TLEC2; Fig. 3, cluster 8) suggest that $T_1$
429    susceptible corals are being challenged by pathogens, further weakening their immune system
430    prior to the bleaching event. The $T_1S$ microbiome was less diverse than the $T_1R$ colonies'
431    microbiomes and more variable across the entire susceptible cohort (*i.e.,* each $T_1S$ colony had a
432    different taxonomic composition). Quantification of the panel of proteins listed here linked to
433    symbiont rejection could provide coral managers with a rapid biomarker test for identifying which
434    corals are stressed and may not be suitable for propagation, even under optimal environmental
435    conditions.
436
437    **Divergent metabolic strategies in resistant and susceptible corals post-bleaching.**
438    Constitutive post-bleaching ($T_2$) response across all *M. capitata* included many components of
439    the phagocytic and endocytic pathways, indicating that active symbiotic expulsion (86) during
440    thermal stress-induced bleaching was occurring regardless of whether the corals were resilient
441    or susceptible. In particular, Rab11, the inhibitor of symbiosome degradation observed in $T_1S$,
442    was detected at increased abundances in both coral groups at $T_2$ relative to the $T_1$ samples.
443    Other constitutively expressed immune response proteins detected at higher abundances at $T_2$
444    included NOD, MAPK, WNT, and TOLL-like receptors. All of these signaling pathways have
445    been previously observed in corals (87) and their detection suggests that during bleaching the
446    innate immune system is activated. Here, we detected increased abundances of the protein
447    responsible for the irreversible step in gluconeogenesis in susceptible corals, and increased
448    abundances of two irreversible steps in glycolysis in the resilient corals (Fig. S3B). This
449    proteomic evidence suggests that after bleaching, the resilient corals have a more accessible
450    glucose source, whereas the susceptible corals are catabolizing non-carbohydrate sources,

451 such as lipids and proteins. These enzymatic pathway analyses also provide a molecular
452 foundation for the observed 49% decrease in lipid biomass in the susceptible colonies and the
453 insignificant change in lipids in the resilient corals between pre- and post-bleaching (Fig. 2F).
454

455 **Resilient corals diversify metabolic pathway utilization to recover from thermal**
456 **bleaching.** After bleaching, several GO terms were enriched in resilient *M. capitata*: amino acid
457 synthesis (methionine, and proline), sulfur amino acid metabolic process, immune response, cell
458 signaling/oxidoreductase, endoplasmic reticulum (ER) organization, oxoacid metabolism, and
459 ribosome assembly (Fig. 2B). Sulfur amino acids, such as methionine, are antioxidants and
460 therefore capable of providing oxidative protection to cells (88). Increased levels of these amino
461 acids in resilient corals could be indicative of increased need for protection against oxidative
462 stress resulting from the heat. Sulfurtransferase enzymes are present in both susceptible and
463 resilient corals, however they are significantly increased in resilient corals (Fig. 3, clusters 1 and
464 2). Heat stress has been found to also induce an increase in endoplasmic reticulum transcripts
465 in *Acropora hyacinthus* (89), mirroring our findings of increased abundance of ER proteins.
466 Increases in cell signaling and ribosome assembly proteins are likely indicative of more normal
467 cellular trafficking in healthy host tissue, enabling recovery from thermal bleaching. Several
468 metabolic pathways are discussed below that support the resilient metabolism through thermal
469 stress compared to the susceptible coral cohort.

470 Resilient corals activate endocytic uptake pathways and heterotrophic feeding after bleaching
471 event to aid in nutritional recovery. Multiple enzymes involved in endocytosis are increased in
472 the $T_2R$ cohort providing a heterotrophic avenue for carbon and nitrogen acquisition (Fig. 3,
473 cluster 3). Increased abundance of 2 tubulin alpha proteins (TUBA), vacuolar sorting endocytic
474 protein (VPS4), dynamines and coatamers (COPG, COPB2) imply increased endocytic activity
475 of particles, such as pathogens or food (82, 90). Significantly increased abundance of lysosome
476 associated membrane protein (LAMP) may indicate that symbiont engulfment and degradation
477 is an additional potential source of nutrition for resilient corals post bleaching (81). Significantly
478 increased peptide degradation enzymes in the $T_2R$ cohort included glutamyl amino peptidase
479 (ENPEP, (Fig. 3, cluster 3), which cleaves acidic amino acids from the N-terminus of peptides
480 for subsequent degradation to enhance cellular growth. $T_2R$ also significantly increased a
481 vitellogenic carboxypeptidase (CPVL), a protein involved in the degradation of yolk proteins.
482 This may be a sign of a physiological switch to sacrifice reproductive potential to increase the
483 chances of bleaching recovery and short-term survival. Abundant protease/peptidases (Fig. 3,
484 cluster 3) and lipases (*e.g.*, triacylglycerol lipase PNLIP; Fig. 2D) in resilient colonies can break
485 the bonds of macromolecular complexes to generate mobile small molecules that can be
486 recycled or further degraded for energy. In support of these findings suggesting adequate
487 nutritional resources were available in the resilient cohort post-bleaching, the increased
488 abundance of transketolase (TKT) in $T_2R$ may indicate a higher abundance of thiamine (vitamin
489 B1) compared to $T_2S$ (91, 92).

490 To further aid in recovery, resistant corals post bleaching appear to utilize several new
491 pathways to aid in cellular nitrogen and carbon demands. Although the urea degrading enzyme
492 URE1 is detected in both $T_2S$ and $T_2R$ corals, $T_2R$ displayed increased abundance of polyamine
493 oxidase (MPAO; Fig. 3 cluster 3) which may allow resilient colonies to access polyamine-
494 nitrogen as needed and produce beta-alanine. Beta-alanine (aminoproponoic acid) is a
495 degradation product of the nucleotide uracil and is a precursor to acetyl-CoA. Notably, it has

12

496   been found to increase cellular oxygen consumption and respiration rates (93). Detecting
497   multiple enzymes involved in these pathways to be at significantly higher abundance in the $T_2R$
498   colonies provides a molecular explanation for hypothesized improved energy production
499   compared to the $T_2S$ colonies when symbiont derived photosynthate is diminished. Further, this
500   energy may have provided resilient colonies the ability to significantly increase multiple
501   enzymes responsible for DNA transcription and translational processes (Fig. 3, cluster 3).

502    $T_2R$ also launched an antiviral campaign during thermal stress to assist the immune system.
503   Cyanovirin protein (CVNH) was detected in resilient corals at $T_1$ and $T_2$ at three-fold higher
504   abundance, compared to susceptible colonies (Fig. 3, cluster 1). Although we do not believe this
505   to be the only mode of protection for the resilient cohort, high production of this protein could
506   increase the resilience of these corals after bleaching events when they are simultaneoulsy
507   coping with multiple stresses.

508   **Catastrophic metabolic choices in $T_2S$ corals.** GO term enrichment analysis revealed a
509   greater abundance of proteins participating in peptide degradation and protein transport in
510   susceptible *M. capitata* post-bleaching (Fig. 2B,D; Fig. 3 cluster 12). Since Symbiont-derived
511   photosynthate nutrition is absent in the bleached corals, susceptible colonies may have
512   increased mobilization and degradation of proteins and peptides to provide the needed energy
513   for cellular maintenance. Decreases in the free amino acids pool resulting from protein
514   degradation in thermally bleached *Acropora aspera* suggests that these amino acids are being
515   metabolically leveraged to provide energy during low photosynthate yield (94).
516    Specifically, susceptible hosts post-bleaching express an abundance of enzymes that suggest
517   host-directed catabolism of remaining symbionts. Several lysosomal-targeted
518   peptidases/degradation enzymes were significantly increased in the susceptible corals in
519   response to bleaching (e.g., cathepsin CATL, galactosidase GLB, and Niemann Pick C2 protein
520   NPC2, Fig. 3, clusters 7, 12). The early signs of a weakened host-symbiont relationship for $T_1S$
521   corals (discussed earlier) appears to have progressed further in the susceptible cohort by $T_2$. At
522   $T_2,$ health-compromised/dead symbionts may be leaking organic substrates that are degraded
523   by lysosomal and intracellular peptidases and hydrolase enzymes (Fig. 3, cluster 12). NPC2
524   enzymes are concentrated in the symbiosome and participate in the direct sterol transfer from
525   symbionts (95). Evidence of increased host-directed transfer of sterols from the symbiont
526   combined with increased lysosomal-catabolic processes indicate that after bleaching, the
527   symbiosome and its contents are targeted for rapid degradation in susceptible colonies (81).
528    Further, the decrease in symbiont-derived photosynthate in susceptible corals leads to
529   activated gluconeogenesis and the degradation of glycine betaine. Glucose is one of the
530   primary carbon molecules transferred to holobionts in cnidarian dinoflagellate symbiosis (96,
531   97). The increased abundance of the irreversible enzymes pyruvate carboxylase (PC) and
532   phosphoenolpyruvate carboxykinase (PCK1) in susceptible corals reveals that gluconeogenesis
533   (*i.e.*, the generation of glucose from pyruvate) is more active than glycolysis (*i.e.*, the
534   degradation of glucose; Fig. S3B). Increased abundance of these enzymes suggests that
535   susceptible corals are more glucose-limited compared to the resilient corals after bleaching
536   events. Gluconeogenesis depends on the catabolism of amino acids, glycine betaine, and lipids
537   (Fig. 3, cluster 12). Lipid degradation is evidenced by the significant decrease in total lipid
538   biomass between $T_1S$ and $T_2S$ colonies (Fig. 2E-F). Only recently were glycine betaines
539   recognized to be a major reservoir of nitrogen for corals and the near-complete glycine betaine

540 catabolic and biosynthesis pathways have been uncovered in several genomes of cnidarians
541 (refs within:, 98). $T_2S$ corals increased abundance of the betaine-degrading enzymes betaine-
542 homocysteine S-methyltransferase (BHMT), glycine N-methyltransferase (GNMT), and
543 sarcosine dehydrogenase (SARDH). Ngugi et al., (98) suggest that glycine betaines are
544 abundant nitrogen reservoirs that are easily degraded into other nitrogen compounds such as
545 amino acids.
546  Susceptible coral proteomes post bleaching also revealed a trend of potential decreased
547 immune function at $T_2$. Immune pathways results indicate that the NOD, MAPK, and TOLL-like
548 signaling pathways are suppressed in $T_2S$ corals ($p<0.10$; Dataset S2H). The suppression or
549 inactivation of these important immune pathways may make the corals vulnerable to disease
550 and colony mortality. The suppressed beta diversity in $T_2S$ corals reveals a shift to a less
551 diverse symbiotic bacterial community, which may be an indicator of the onset of infection (refs
552 within:, 99). Susceptible *M. capitata* colonies also increased tyrosinase (TYR), an indicator of
553 immune response to an infection (100) or neutralization of reactive oxygen species (101)
554 demonstrating that $T_2S$ corals are being challenged.

## Coral management and restoration applications

556 As the ultimate goal for coral management is to be able to predict resilient coral phenotypes
557 before investing time and money in restoration, a rapid assay to determine health status is
558 needed. Here we presented three significant differences in the resilient and susceptible coral
559 colonies before the thermal bleaching to forecast long-term health through thermal events:
560 proteins (Fig 2A,C), lipids (Fig. 2E), and microbiome diversity (Fig. 4A). Previous work on corals
561 has revealed that decreases in lipid content and in microbiome diversity can be associated with
562 a range of environmental responses and are not exclusively associated with susceptibility to
563 bleaching stress. Here we propose a protein-based assay to predict resilience and capture more
564 informative results on the molecular-level health of *M. capitata.* We have identified seven
565 proteins that could be quantified in corals before bleaching events as a resilience-based assay
566 to select colonies for propagation or other management strategies (Fig. 5). If using mass
567 spectrometry, quantifying five peptides through the detection of $\geq 5$ diagnostic fragment ions
568 from each of these proteins would provide the user with high confidence on both positive and
569 negative signals of pre-bleaching resilience. This short list could also be expanded to generate
570 a 60 minute assay with up to 250 peptides that are simultaneously monitored, providing further
571 information on heterotrophic feeding/lipid degradation (*i.e.*, LIP, PSAP), antiviral activity (*i.e.,*
572 CNVH), symbiophagy (*i.e.,* RAB11, TUBA), pathogen recognition (*i.e.*, TLEC), mucin proteins
573 (*i.e.,*MUC4), urea degradation (*i.e.,*URE1), and mannose degradation (*i.e.,* FUCA1, MRC1,
574 GANAB). Alternatively, selected proteins identified here as resilience biomarkers could be
575 developed into a hand-held rapid antigen test with multiple test and control lines that could be
576 assessed in the field on rice-grained size coral tissue samples.

## Concluding Remarks

578 This is the first study to use an analytical toolbox that included mass spectrometry-based
579 proteomics, 16S rRNA analyses of the microbiome, total lipids, and symbiont density and
580 diversity to identify intrinsic differences that confer recovery and survival in corals before thermal

14

581  bleaching events. This study is also unique in that the *Montipora capitata* coral colonies had
582  unexpected, yet vastly different, outcomes from the thermal bleaching event despite identical
583  environmental histories and, to the best of our knowledge, coral genotypes. Our intent was to
584  reveal a multi-factor molecular-level approach for confidently identifying resilient and susceptible
585  coral colonies so that environmental managers could rapidly select quality candidates for
586  propagation while in the field. Despite monitoring a range of physiological and molecular
587  metrics, very few significant differences were discovered in the study that could positively
588  identify resilient colonies before a thermal bleaching event. Despite lipids being a tractable
589  metric in the field, the differences in the resilient vs. susceptible coral lipids were only present
590  *after* the thermal bleaching event, making lipids ineligible as a biomarker for long term survival
591  *prior* to thermal stress. Additionally, lipids are non-specific biomarkers since their levels are
592  often influenced by a variety of physiological factors (*i.e.,* infection, reproduction, tissue
593  thickness, etc.). Promising and distinct differences observed in the 16S rRNA analysis of
594  bacterial diversity revealed that the resilient cohort hosted a significantly more diverse
595  microbiome before the thermal event. Although microbiome diversity could aid as a metric for
596  selecting healthy, robust coral colonies, microbiome stability and diversity can be linked to water
597  chemistry (102), temperature (14), and short or long term diseases (103, 104). Additional
598  research needs to be conducted to determine if specific microbial clades are significantly
599  correlated to coral recovery and resilience through thermal induced bleaching and what
600  functional roles they play. Quantitative proteomics analyses identified proteins that were
601  significantly different in the two cohorts before the bleaching event, that could 1) allow confident
602  predictions in selection of resilient over susceptible colonies and 2) reveal specific molecular
603  advantages in the form of active pathways and primed immune responses that allow resilient *M.*
604  *capitata* corals to survive the thermal stress despite the expulsion of *Symbiodiniaceae.* Resilient
605  corals have a significantly higher abundance of antiviral proteins and express multiple enzymes
606  involved in a diverse range of carbon and nitrogen acquisition such as lipid degradation,
607  heterotrophic feeding, and respiration. Conversely, colonies that did not survive thermal
608  bleaching had pre-bleaching molecular markers at elevated abundances that play an active role
609  in symbiont rejection, pathogen recognition, and mannose and urea degradation. The proteins
610  represented in each of these pathways and cellular mechanisms can be fully developed into
611  rapid molecular assays to help assess corals and guide mitigation strategies deployed by reef
612  management.

## METHODS

614  **Coral Colony Collection and Experiment.** Seventy-four coral colonies of *Montipora capitata*
615  (approximately 30 cm in diameter) were collected from Moku O Lo'e island (patch reef) located
616  in Kāne'ohe Bay, O'ahu, Hawai'i (21.428°N, 157.792°W) in August 2017. Colonies were brought
617  to shore and acclimated in flow-through outdoor tanks at the Hawaii Institute of Marine Biology
618  (HIMB) for two weeks. At the time of collection, colonies were divided in two pieces to compare
619  physiological performance for the same genotypes with/without exposure to thermal stress (Fig.
620  1). In September, one half of each coral colony was exposed to warmer water temperatures to
621  simulate a natural thermal bleaching event (56). To reach the 30°C temperature goal for the
622  bleaching treatment, experimental tank temperatures were increased 2°C per day (1°C every 12
623  hours) for four days. The colonies were rotated once a week between tanks to minimize tank

624  effects. For the bleaching experiment, *M. capitata* colonies were kept at this elevated
625  temperature for three weeks to induce complete coral bleaching in all the colonies. After
626  bleaching occurred, the tank temperature was lowered, following the previously described rate,
627  back to ambient temperature (22°C) and subsamples of coral were taken. The coral halves that
628  were not exposed to thermal stress remained at 25°C and were also rotated within the tank to
629  minimize tank effects. Then, all corals (bleached and not bleached) were placed on racks off
630  HIMB to monitor survival and physiological recovery *in situ* for eight months. Bleaching
631  assessments were conducted on all colonies every week using the Coral Watch Card (The
632  University of Queensland, Australia), along with assessments of mortality (Fig. S1A). Colonies
633  that bleached and recovered were deemed to be part of the resilient cohort while colonies that
634  bleached and died were deemed susceptible to bleaching. All resulting proteomic search
635  results, protein accession numbers and annotation files, lipid data, symbiont density and clade
636  data, chlorophyll data and R code for plot generation and data analysis have been deposited in
637  GitHub (https://github.com/Nunn-Lab/Publication-coral-resilience).
638  Branches from twelve *M. capitata* colonies were collected at two time points: 1) in September
639  after temperature acclimation in the tanks but before colonies were bleached ($T_1$) and 2) in late
640  September, 24 hours after bleached colonies were gradually returned to ambient temperature
641  ($T_2$). All coral samples were collected 1 cm from the tip of a branch and snap frozen immediately
642  in liquid nitrogen. Frozen samples were shipped to the University of Washington on dry ice and
643  stored at -80°C. Samples for protein extraction consisted of 2 mm thin cross-sections of the
644  branches, encompassing both tissue and skeletal matrix.
645
646  **Symbiont and Chlorophyll analyses.** Chlorophyll a concentrations and dinoflagellate symbiont
647  (Symbiodiniaceae) densities from each of the colonies were investigated (Dataset S1A). Briefly,
648  chlorophyll a was extracted with 100% acetone and absorbance was measured with a light
649  spectrophotometer (Dataset S1B). Symbionts were separated from triplicate ground coral tissue
650  by centrifugation and symbiont pellets were homogenized prior to being counted using a
651  hemocytometer. Chlorophyll a and symbiont densities were standardized to grams of ash-free
652  dry weight (gdw) of coral tissue (Dataset S1A,C). In order to assess the ratio of
653  Symbiodiniaceae C and D clades a 4mm piece of frozen *Montipora capitata* was crushed using
654  a frozen mortar and pestle and total DNA was extracted and Quantitative Real Time PCR
655  (qPCR) assay of the symbionts' actin genes was used to determine the ratio of
656  Symbiodiniaceae C and D clades (Dataset S1B, Fig. S1B). Further details found in SI Methods.
657
658  **Lipid Analyses.** Total lipids were analyzed on each sample following the methods of Rodrigues
659  and Grottoli (50). Briefly, whole fragments (tissue plus skeleton) were crushed and digested in a
660  2:1 chloroform:methanol solution, sequentially washed in a 0.88% KCl solution, dried under
661  grade 5.0 $N_2$ gas to a constant weight (Dataset S1D-E)
662
663  **Proteomics.** Six colonies from the resilient cohort and six colonies from the susceptible cohort
664  were randomly selected as bioreplicates to track phenotypic differences in protein abundance
665  through time (*i.e.,* $T_1$ and $T_2$). Details can be found in SI Methods. Briefly, proteins were
666  extracted from whole coral fragments (4 mm diameter x 1 mm thick, tissue plus skeletal matrix)
667  and resulting protein concetrations were determined with bicinchoninic acid (BCA) Protein

668    microplate assay. Protein lysates (50µg per coral sample) were reduced, alkylated, and
669    digested Trypsin (modified porcine sequencing grade trypsin; Promega; 1:20 enzyme:coral
670    protein). Each digested peptide sample was amended with Peptide Retention Time Calibration
671    Mixture (PRTC; Pierce) such that 50 fmol of PRTC was analyzed with 1 µg of coral peptides for
672    each mass spectrometry experiment.
673     *M. capitata* samples were analyzed using liquid chromatography coupled to tandem mass
674    spectrometry (LC–MS/MS) on a Q–Exactive–HF (Thermo Scientific) in Data Dependent
675    Acquisition (DDA) Top 20 mode. Samples were separated using a heated (50°C) 40 cm long
676    analytical column packed with C18 beads (Dr. Maisch HPLC, Germany, 0.3 µm, 120Å).
677    Peptides were chromatographically separated on a Waters nanoAcquity UPLC using an
678    acidified (0.01% formic acid) acetonitrile:water gradient of 2–45% over 120 minutes.  Internal
679    and external standards were monitored to ensure peptide peak area correlation variances were
680    <10% through the duration of the analyses. Data was searched against a translated *M. capitata*
681    transcriptome (105) GSE97888_Montiporacapitata_transcriptome.fasta). Protein identifications
682    from the whole-cell lysates are reported if two or more peptides were identified, at least one
683    terminus was tryptic, and the false discovery rate <0.01) (Dataset S2A-E). Differential relative
684    protein abundances for resilient vs. susceptible corals were determined for each timepoint ($T_1$
685    and $T_2$) using the QPROT-QSPEC package (106)(Dataset S2G-H). Differential abundances of
686    proteins are reported with the following *p*-value cutoff rules: 1) *p*<0.10 if several proteins within a
687    pathway, 2) *p*<0.05 if significance of an individual protein, or 3) *p*<0.01 if identifying a potential
688    biomarker.
689    
690    **MetaGOmics Biological Enrichment Analysis.** To determine if categories of proteins were
691    enriched in the resilient vs. susceptible coral cohorts at the two timepoints, a biological
692    enrichment strategy that analyzes Gene Ontology (GO) categorical terms was used to compare
693    sets of detected proteins (48). Top results are reported with a cutoff E-value <1E-10. A fasta file
694    of all *M. capitata* protein sequences confidently identified in these experiments (File S3) was
695    analyzed with MetaGOmics v.0.1.1. Although MetaGOmics was designed to analyze
696    microbiomes, the use of the software was modified to work with a single organism by ignoring
697    the taxonomic enrichment analysis to instead examine functions that are significantly enriched
698    or depleted in pairwise comparisons of coral cohorts. Additional details found in SI Methods.
699    
700    **Microbiome 16S rRNA Analyses.** Total DNA was extracted from the corals selected for this
701    study (n=12) using the Qiagen DNA extraction kit. All 16S rRNA gene amplicon sequence data,
702    processing steps and code for quality control on the microbiome data and analysis are available
703    on GitHub (https://github.com/tanyabrown9/Resilient_vs_Susceptible_Mcapitata). Sequences
704    are deposited in NCBI as bioproject PRJNA933787. Initial sequencing resulted in a collection of
705    2,472,819 total reads, with an average read depth of 68,689 (± 28,107 SD) sequences per
706    sample (Dataset S4A-B). Amplicon sequence data were processed using the QIIME2 software
707    package (107). Alpha diversity was assessed using the number of unique observed ASVs in
708    rarefied samples by the Simpson's Evenness and Shannon's Diversity Indexes. Overall
709    differences in alpha diversity across susceptibility and time points were tested using Kruskal-
710    Wallis tests. Post-hoc comparisons were performed within each group as well as combined
711    comparisons with *p*-values for pairwise tests between treatments adjusted for multiple

712    comparisons using Bonferroni correction. Beta diversity was assessed between samples using

713    Weighted UniFrac distances and Bray-Curtis dissimilarities. The significance of differences in

714    beta-diversity between susceptibility and time was tested using PERMANOVA (108). The top 10

715    bacterial families in each sample type were selected for taxonomic analysis. Significant

716    differences between bacterial families, susceptibility, and timepoint were carried out using a

717    nested ANOVA. Multivariate Association with Linear Models was performed on the 16S data

718    using the R package MaAsLin2 (51). Additional details can be found in SI Methods.

## 719    Data, Materials, and Software Availability

720    All raw MS proteomic data and protein FASTA files used for searching can be accessed at

721    PRIDE accession PXD021262 (UsernameXXXXX). All resulting proteomic search results,

722    protein accession numbers and annotation files, lipid data, symbiont density and clade data,

723    chlorophyll data, and R code for plot generation and data analysis have been deposited in

724    GitHub (https://github.com/Nunn-Lab/Publication-coral-resilience). All 16S rRNA gene amplicon

725    sequence data, processing steps, and code for quality control on the microbiome data and

726    analysis are available on GitHub as well

727    (https://github.com/tanyabrown9/Resilient_vs_Susceptible_Mcapitata). All study data are

728    included in the article and/or as SI Datasets. All sequences used in this study are publicly

729    available through NCBI GenBank and ProteomeXchange PRIDE. Accession numbers and

730    annotations are provided as supplementary files (Datasets S1-3).

731

732    **Figures**

733    **Fig. 1A:** Total cell counts (x$10^9$) for Symbiodiniaceae per gdw$^{-1}$ ($n$=6 for each point) illustrating the

734    experimental design to track effects of thermally induced bleaching on 12 *Montipora capitata* colonies that

735    were monitored for 8 months. After 8 months, each colony was retroactively labeled and 6 colonies that

736    reacquired symbionts and recovered (green) and 6 colonies that were susceptible to thermal stress

737    (purple) were characterized. Symbiodiniaceae density for the (**A**) control cohort maintained at ambient

738    (25$^o$C) temperature and (**B**) experimental cohort that underwent thermally induced bleaching (30$^o$C) for 4

739    weeks; light green: ($n$=6) resilient colonies reacquired symbionts and recovered post-bleaching; light

740    purple: ($n$=6) colonies susceptible to thermally induced bleaching that did not recover. Coral sub-samples

741    were collected before (T$_1$) and after exposure to thermal stress (T$_2$) to assess host performance and

742    symbiont and microbial composition in corals. Note that resilient and susceptible colonies were identified

743    three months later (December); after this period no additional mortality was observed.

744  **Fig. 2.** Quantitative molecular data on proteins A-D and lipids E-F, completed on the same 12 colonies
745  used throughout the study (*n=6* susceptible*, n=6* resilient*)*. A and B) Volcano plots depicting -Log (p-
746  value) vs the Laplace corrected Log Fold Change (LFC) for protein-associated Gene Ontology terms.
747  Colored dots signify GO terms that were statistically significantly different between resilient and
748  susceptible corals (Laplace corrected Log fold ≤-0.5 or ≥0.5 and *p*-value ≤0.01). A) $T_1$ with negative LFC
749  indicating proteins more abundant in resilient corals ($T_1R$) while positive values correspond to proteins
750  that were at higher abundance in susceptible corals ($T_1S$). B) $T_2$ where negative LFC indicates greater
751  abundance in resilient ($T_2R$) *M. capitata* while GO terms with positive values are higher in susceptible
752  corals ($T_2S$). C and D). Volcano Plots of individual protein abundances. LFC $\leq$ -0.5 are proteins that were
753  detected in significantly higher abundance in the resilient coral cohorts (green) at C) $T_1$ and D) $T_2$. LFC $\geq$
754  0.5 are proteins that were detected in higher abundance in the susceptible coral cohorts (purple) before
755  thermal-stress-induced bleaching (C: $T_1$) and after (D: $T_2$). E and F) Average lipid biomass (g/g dry
756  weight) measurements on all resistant (green) and susceptible (purple) samples from the different cohorts
757  at E) $T_1$ before bleaching and F) $T_2$ after bleaching (grey boxes) and non-bleached controls maintained at
758  $25^oC$ (light green and purple "NB" boxes).

759  **Fig. 3.** Clustered heatmap of the subset of proteins identified to have a log fold change $\geq$1 or $\leq$-1.0 (*p*
760  <0.05) in time point comparisons: $T_1R$ vs. $T_1S$ or $T_2R$ vs. $T_2S$. Heatmap shades of blue indicate averaged
761  NSAF values for bioreplicates per condition, normalized by the row mean. Rows are clustered using a
762  correlation algorithm and a dendrogram was set to cut 12 distinct clusters (indicated by #s 1-12 black).
763  Right panel dot-matrix indicates metabolic categories identified through KEGG, UniProt and GO (D) viral
764  defense or (R) reproduction, $CO_2$ or as a substrate ($CO_2^-$) or product ($CO_2^+$), or Ca- binding domain (i.e.,
765  Ca-binding).
766
767  **Fig. 4.** Phylum-level distribution of bacteria identified in resilient and susceptible colonies based on 16S
768  rRNA sequencing data for A) $T_1$ ($T_1R$: dk green, $T_1S$: dk purple), B) $T_2$ after thermal stress ($T_2R$: grey with
769  green outline, $T_1S$: white with dk purple outline), and C) $T_2$ control (NB) samples not exposed to thermal
770  stress ($NBT_2R$: lt green, $NBT_2S$: lt purple). Colony ID numbers are listed on the x-axis. These same 12
771  colony IDs were used for all analyses presented. Size of the dot represents the log transformation of the
772  phylum-level counts.
773
774  **Fig. 5.** Heatmap of significantly different proteins (p<.01) identified between resilient and susceptible
775  corals at $T_1$, pre-bleaching event. Colors depict NSAF values for each of the twelve coral bioreplicates,
776  normalized by row mean. Clustered dendrograms were completed with the correlation algorithm on the x
777  and y axis to generate groups for significantly increased or decreased abundances in the resilient vs.
778  susceptible cohorts.
779


780  Supplemental Information

781  **Fig. S1**. A. Bleaching assessments were completed on all colonies every week using the Coral
782  Watch Card where 1 indicates bleached, but not dead and 6 indicates the highest symbiont
783  density.  Noted times of T1 and T2 indicate when samples were collected for this study. B.
784  Proportion of clade C (blue) and D (yellow) identified from Resilient (greens; *n=6*) and
785  Susceptible (Purple; *n=6*) coral colonies at timepoint 1 (pre-bleaching) and timepoint 2 (NB-
786  nonbleached cohort, B- thermally bleached cohort). No statistical differences were identified
787  using ANOVA related to bleaching status, bleaching tolerance, or timepoint.
788

789 **Fig. S2.** Venn Diagram of proteins confidently Identified in resilient (*n=6*) and susceptible coral
790 colonies (*n=6*) investigated at A. timepoint 1 before thermally bleached, B. timepoint 2 after
791 thermal bleaching, and C. the overlap of all 4 treatments and timepoints.
792
793 **Fig. S3.** Illustration of the proteins identified to be significantly increased in abundance in
794 resilient (greens) or susceptible (reds) coral cohorts involved in the interconnected biochemical
795 pathways of glycolysis/gluconeogenesis, the TCA cycle, urea degradation, and the glutamine
796 synthase/glutamine oxoglutarate aminotransferase (GS/GOGAT) pathway at A. timepoint 1
797 before thermally bleached and B. timepoint 2 after thermal bleaching. Image was made using
798 BioRender.
799
800 **Fig. S4.** Graphical illustration of a multiple sequence alignments of protein
801 lcl|c238733_g2_i2|m.24867, noted to be significantly more abundant in the resilient colonies.
802 The top query sequence in red (Query_59870) represents the input sequence mentioned. The
803 next two sequences are from *Acropora millepora* and *Mucilaginbacter sp.* MYSH2 (both in red).
804 Multiple alignment results revealed four highly conserved CyanoVirin-N domains (CVNH: 95%
805 Query coverage; e-value 2e-30) depicted in grey.
806
807 **Dataset 1.** Physiological metrics on coral colonies from the study including: a readme file,
808 symbiont density, symbiont clade distributions, chlorophyll a concentrations, lipid raw data, and
809 lipid biomass data.
810 **Dataset 2.** Processed proteomic data on each experiment in a range of formats: a readme file,
811 proteins identified per experiment, Normalized Spectral Abundance Factors (NSAF) on all valid
812 proteins identified, an accession number-based annotation file, QSPEC results for timepoints 1
813 and 2, ABACUS output file before processing.
814 **Dataset 3.** MetaGOmics output files including: a readme file, MetaGOmics results from analysis of
815 resistant vs. susceptible coral proteins identified at timepoint 1, MetaGOmics results from analysis of
816 resistant vs. susceptible coral proteins identified at timepoint 2.
817 **Dataset 4.** Microbiome sequence counts, metadata, and mapping information.
818
819 **File S1.** Fasta files of protein sequences predicted from transcriptome for Montipora capitata plus
820 contaminant protein database.
821 **File S2.** Fasta files of protein sequences predicted from transcriptome for Montipora capitata plus
822 contaminant protein database and 5 viral proteomes (see Table S1).
823 **File S3.** Fasta file of all identified protein sequences from these experiments that were used as input for
824 MetaGOmics analysis.
825
826 **Table S1.** Resulting table from MaAsLin2 analysis.
827 **Table S2.** Table of coral viral proteomes selected, location the database was found, and the number of
828 proteins downloaded.
829

830 ## Acknowledgments

839

840

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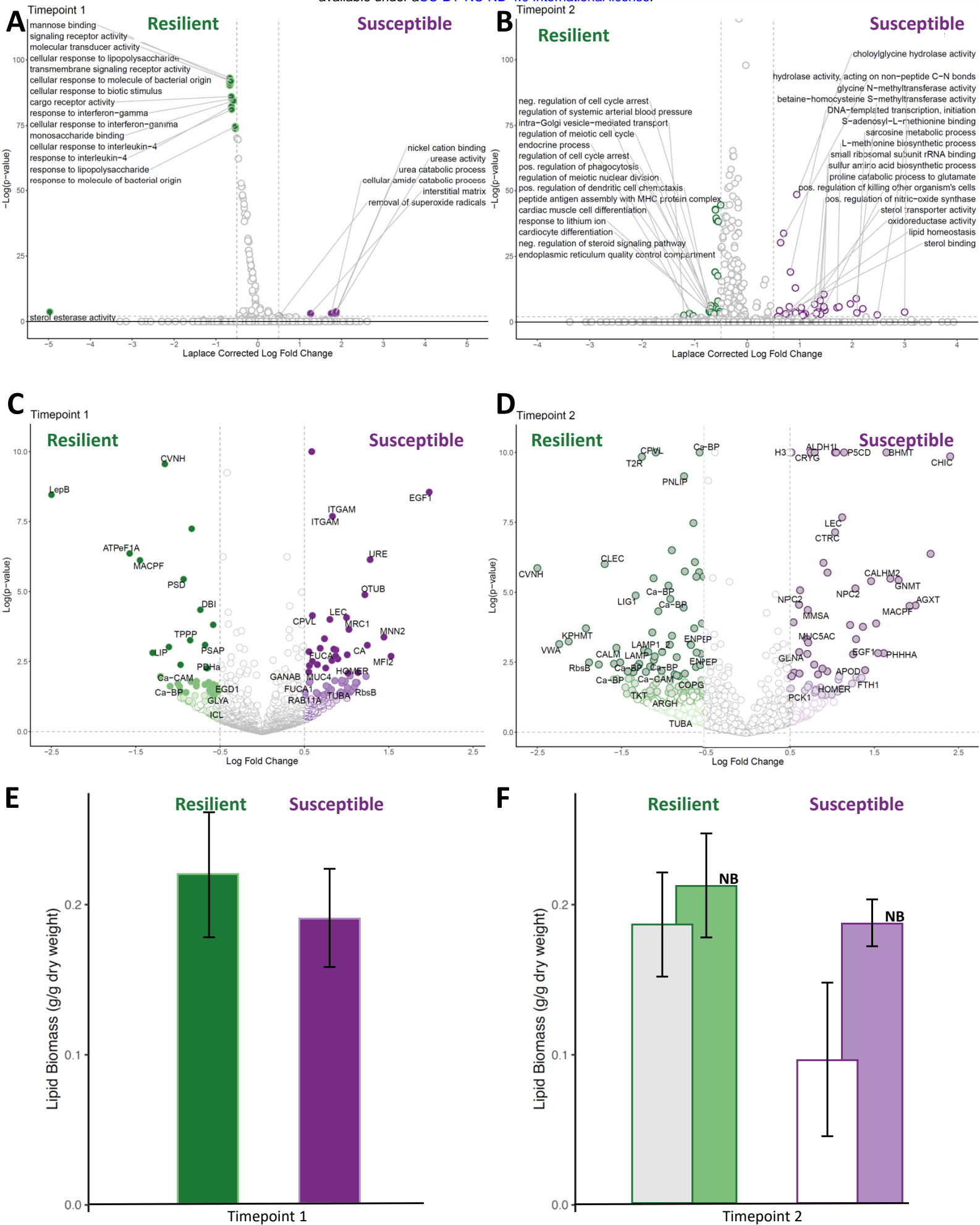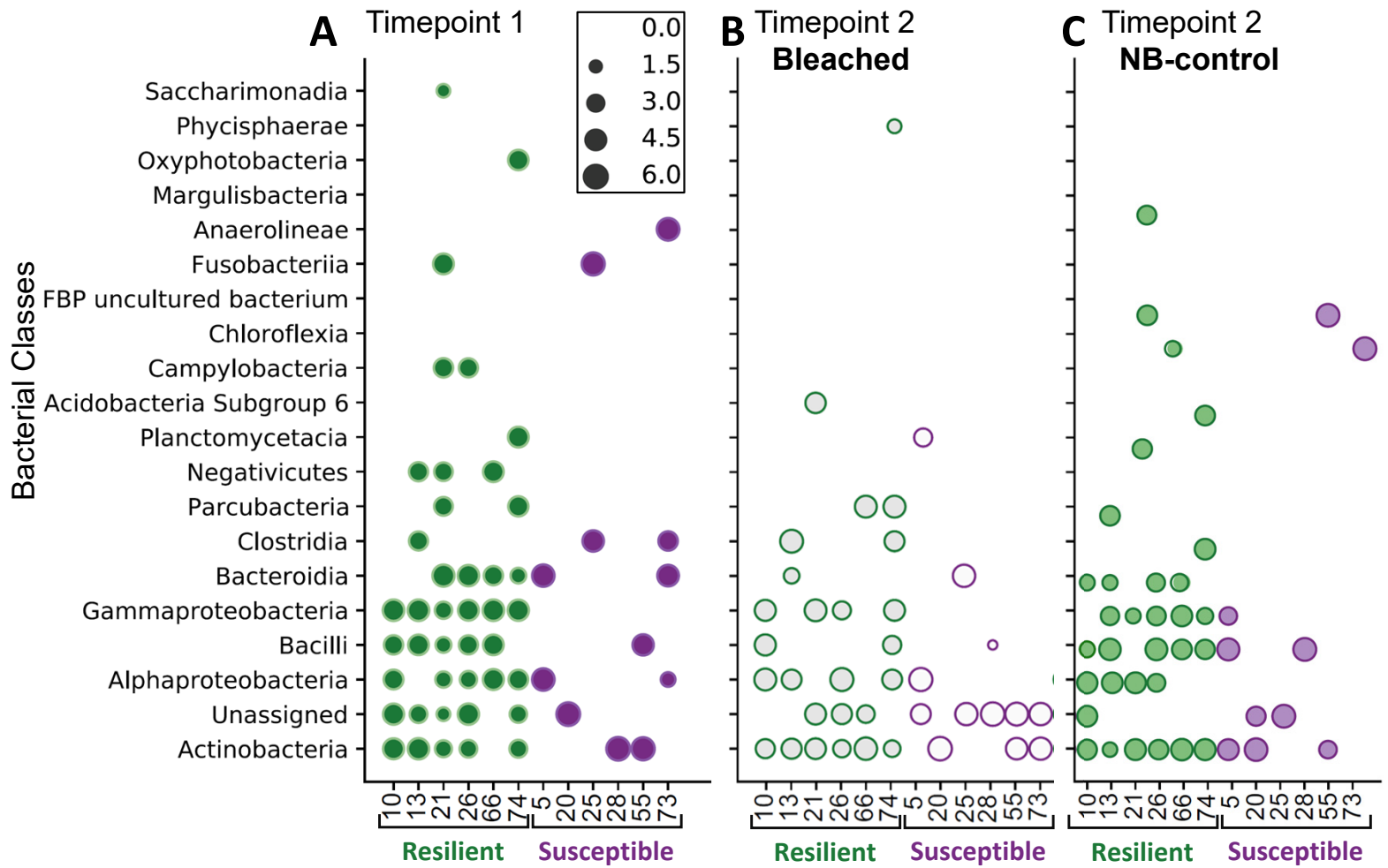1108       *Austral ecology* 26(1):32-46.

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# Figure 1
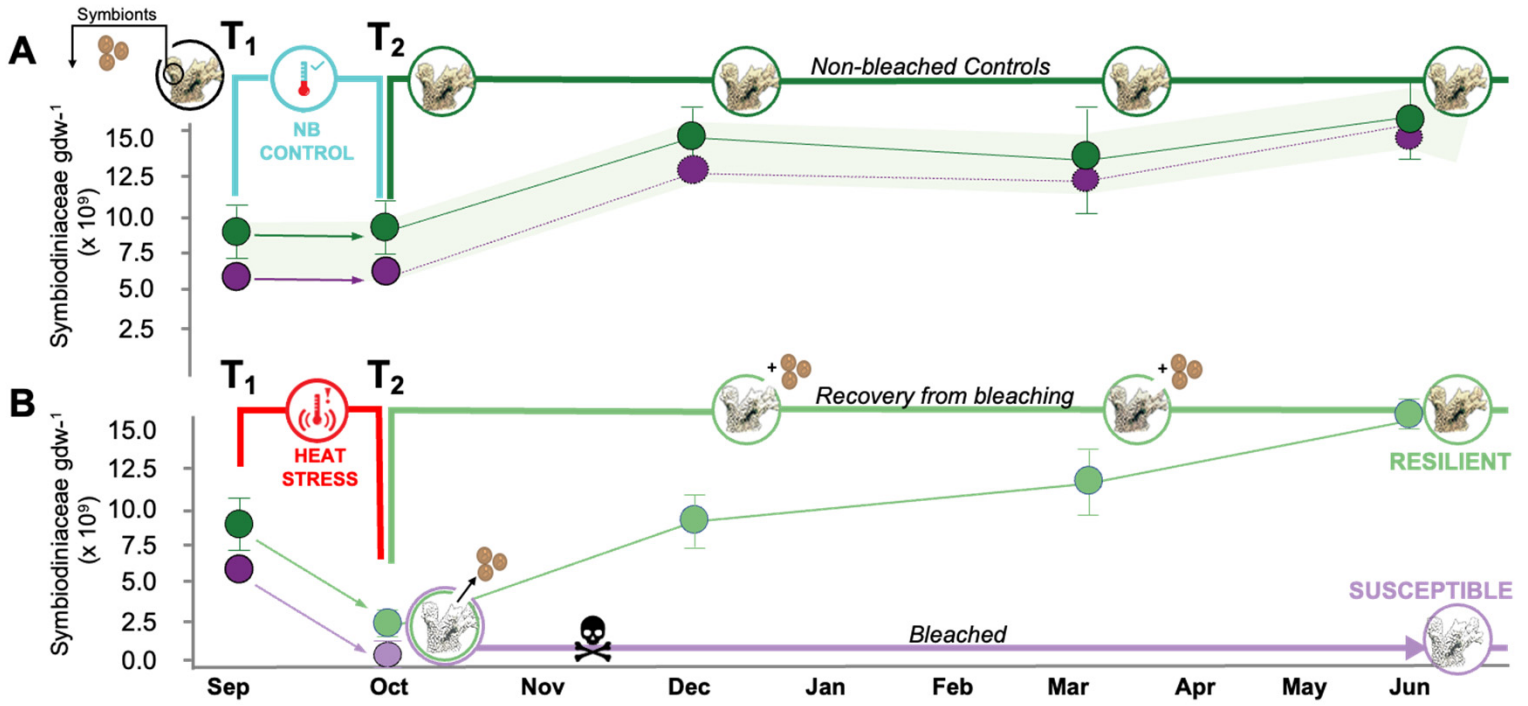
# Figure 2

# Figure 3

# Figure 4

# Figure 5