Mayfield & Lin *Applied Sciences* Supplemental File S1: online supplemental material

Online supplemental methods (OSM)

OSM1: the lab experiment. The *Orbicella faveolata* elevated temperature study is described in detail in Aguilar et al. [1] and Mayfield et al. [2], and details of the proteomic analysis can be found in a prior work [3]; this supplement namely aims to include key details of these prior publications to aid those interested in taking a similar approach. In brief, 20 colonies from "Little Conch" (UKO2; offshore; Figure 1), "The Rocks" (UKI2; inshore), and "Cheeca Rocks" (UKI1; inshore) were tagged and genotyped in July 2017 [4]. A subset of 5-6 were cored into 10-20 fragments (~4.4-cm diameter) with a Nemo V2 electric waterproof drill (USA). Half of the 360 fragments were used in an inshore-offshore reciprocal transplant (not discussed herein), while the remaining 180 were allowed to recover in the field for two days prior to transport in Yeti coolers (USA) filled with seawater to the University of Miami's Rosenstiel School of Marine and Atmospheric Sciences (RSMAS). Once at the RSMAS "Experimental Reef Laboratory," the cores were placed randomly in seawater tables, where they were allowed to recover from July 20 to 27.

Fragments were randomly exposed to 1) short-term (5-day) ambient temperatures (30ºC; see this website for real-time temperature data for Cheeca Rocks.), 2) long-term (31-day) ambient temperatures, 3) short-term (5-day) very high temperatures (33ºC), or 4) long-term (31-day) high-temperature (32ºC; n=3 tanks/treatment & time) such that a spectrum of bleaching responses would be elicited (Table 1), as defined by the following "fragment health designations" (FHD): healthy controls (HC; either 30ºC treatment), sub-lethally stressed (SLS; 32ºC for 31 days or 33ºC for 5 days for certain genotypes), or actively bleaching (AB; either high-temperature treatment for certain genotypes). Then, based on a genotype's response to the thermal challenge, it was given a "colony health designation" (CHD): bleaching-resistant ("BLR;" failure to pale at either of the high temperatures) or bleaching-susceptible ("BLS;" paling at either high-temperature treatment [see main text for details.]). Unlike for the field samples (described in the main text), no corals were deemed "intermediately" bleaching-susceptible ("INT") in the laboratory experiment.

OSM2: temperature data. As the mean monthly maximum (MMM) at the field sites is approximately 31ºC (August), NOAA's Coral Reef Watch's (CRW) algorithms would predict that corals of these reefs would bleach after 4–8 weeks of exposure to MMM+1ºC=32ºC (i.e., 4–8 degree-heating weeks [DHWs]). However, prior observations [5] have found that, by assuming thermal stress to only accumulate at temperatures >32ºC, bleaching likelihood and severity are both underestimated. Field data show that the temperature above which corals begin to become thermally stressed is closer to 31.3ºC [5]. CRW's models, then, do not accurately predict timing of onset of bleaching, nor bleaching severity, at any of the four field sites (including the "test" reef discussed elsewhere). The "very high" (33ºC; "V" in the multivariate plots), high (32ºC; "H"), and control (30ºC; "C") treatments in the laboratory experiment consequently correspond to DHWs of 1.2 (1.7ºC x 0.7 weeks), 3.1 (0.7ºC x 4.4 weeks), and 0, respectively; degree-heating days (DHDs) of 8.5, 21.7, and 0, respectively; and degree-heating hours (DHHs) of 204, 521, and 0, respectively.

As a comparison, the colonies from which plugs were made were exposed to 2.4 DHWs, 16.8 DHDs, and 396 DHHs *in situ* over the period in which the experiment was conducted (August 2017). Note that the latter does not simply equate to DHD x 24 since temperature was logged every three hours; on many days, the temperature only rose above 31.3ºC ephemerally. In these cases, a full DHD would be logged, while the DHH might be as low as three. Bleaching occurred in the lab and *in situ* at around 400 DHHs in 2017. Similarly, the field test colonies monitored in 2019 bleached between 2 and 5 DHWs (based on thermal stress accruing above 31.3ºC; see Figure 1.).

OSM3: sample designations-details. Because the goal of this work was to develop protein-based models for predicting the susceptibility of corals to bleaching, we first characterized the proteomes of a subset of 21 laboratory samples representing diverse FHD: 11 HC, 5 high-

temperature-acclimating (HTA; failed to have bleached while at either of the two high-temperature treatments; note that for most analyses, these samples were pooled with the 11 HC.), 3 SLS, and 2 AB. Since each coral "puck" (fragments that had been cut with the drill & mounted on ceramic tiles with epoxy) was sacrificed in its entirety, the fate of an individual fragment was not tracked over the duration of the experiment; the justification for this is because sub-sampling each puck multiple times would cause stress, thereby biasing the interpretation of the high-temperature response. Instead, it was hypothesized that clonemates derived from the same source colony would behave similarly with respect to high-temperature exposure; if, for instance, a fragment was resistant to bleaching at 33ºC for five days, but a clonemate exposed to 33ºC began bleaching by day 10, the 5-day-sacrificed sample would be deemed SLS. On the other hand, if a sample sacrificed after five days of exposure to 33ºC did not demonstrate bleaching, nor did a clonemate exposed to this temperature for a longer period, the sacrificed fragment would be assumed to be HTA. This explains why a repeated measures design was not undertaken; in contrast, the same field colonies were sampled repeatedly (discussed in the main text) since at least several weeks transpired among sampling events, and it was hypothesized that this would be sufficient time for wound healing.

OSM4: protein extractions. A subset of 20 laboratory samples was chosen for analyses: 5, 10, and 5 fragments from 2, 5, and 2 colonies from Little Conch, Cheeca Rocks, and The Rocks, respectively, which represented 2, 3, and 1 genotypes, respectively (Table 1). All fragments from The Rocks were of the same genotype (skyblue). Of these, 4, 5, 6, and 5 were from the short-term control, short-term high, long-term control, and long-term high temperature treatments, respectively. Pucks were pulverized in liquid nitrogen (LN_2) by a hydraulic press (Baileigh Industrial, USA) into a wet, sand-like consistency and frozen at -80ºC. Later, ~100 mg of ground tissue+skeleton were transferred into a tube containing 1.2 ml of TRIzol™ (Invitrogen, USA) and further homogenized with a mortar and pestle in a fume hood for 5-10 min (or until no pieces of corals were visible to the naked eye & the solution was a uniform, translucent pink). Then, 1 ml of TRIzol+coral tissue homogenate was transferred to a new tube, and RNAs, DNAs, and proteins were extracted as in a prior work [6], though with several modifications. Briefly, upon resuspending the proteins in the final 1 ml of buffer PWII (95% ethanol+2.5% glycerol), 500 µl of the proteins in PWII were frozen at -80ºC to serve as a backup, with the remainder transported on dry ice to the Miami Integrative Metabolomics Research Center at the University of Miami's Miller School of Medicine. Proteins were then dried in a speed vacuum ("speed-vac;" Labconco, USA), and the pellet was resuspended in 100 µl of 0.5 M triethyl ammonium bicarbonate (TEAB; Thermo-Fisher Scientific [TFS], USA) with 0.067% SDS (hereafter "TEAB-SDS"). It is worth mentioning that the LN_2+TRIzol dual-homogenization method was critical for ensuring that the durable cell walls of the Symbiodiniaceae cells *in hospite* were effectively ruptured. The use of gentler extraction procedures are adequate for isolating anthozoan host proteins but not those of their dinoflagellate endosymbionts (i.e., resulting in an overly high, inaccurate host:endosymbiont protein ratio [7]).

OSM5: protein quantification & quality assessment (QC). Upon dissolving the proteins in TEAB-SDS via vigorous vortexing (Vortex Genie, USA), a 5-µl aliquot was diluted 10-fold in water and quantified with a BCA assay from Pierce (USA); this dilution step was critical since both TEAB and SDS interfere with the BCA assay at higher concentrations. A second, 1-2-µl aliquot of protein was mixed with 2X Laemmli sample buffer (BioRad, USA), boiled at 95% for 5 min, and loaded into a PHASTgel gradient 4-15 polyacrylamide gel from GE Healthcare (USA). The gel was then loaded into the PHast System (GE Healthcare) after inserting two PhastGel SDS buffer strips. Proteins were run alongside 1 µg/µl of BSA standard and 1 µl of Plus2 pre-stained protein standard (TFS) under separation method 3. After ~2 hr, the gel was washed thrice with water and then stained with 10-20 ml of SimplySafe Blue Stain (Invitrogen) for 1 hr at room temperature (RT). The stained gel was then washed repeatedly with water until bands could be visualized with the naked eye (typically overnight). Please see Supplemental File S2 for an image of a representative gel.

OSM6: proteome profiling justification. Mayfield et al. [2] employed a shotgun proteomics approach in which proteins were sequenced in direct proportion to their cellular abundance without the use of labels; this was done because we were interested in proteins exclusively maintained by corals of certain treatments (e.g., high temperature only). Herein an "isobaric tags for relative and absolute (protein) quantification" (iTRAQ) approach was instead taken because it was hypothesized that the use of iTRAQ labels would remove bias associated with the null results generated in the prior shotgun proteomic analysis; briefly, when using mass spectrometry (MS) to profile proteomes, it can be difficult to know whether failure to sequence a protein reflects absence of that protein in the sample (a true negative) or simply failure of the MS to sequence that protein (a technological artifact). Because we were interested in generating models capable of predicting laboratory and test field coral samples, we biased the analysis to those proteins instead found in all samples analyzed.

Since there are only eight iTRAQ labels (113-119 & 121; SCIEX, USA) and 20 lab samples to be analyzed, three iTRAQ runs were required for the lab samples. We hypothesized that batch-to-batch variation could be a concern across the three runs and so created a normalizing sample (hereafter "normalizer") that was run with each batch of seven samples. This master sample was made by mixing 1.2 µl of protein from each of the 21 coral samples to be analyzed (including sample B5-7, which was compromised & later excluded from analysis). This normalizer was diluted to 66 µg in 90 µl such that it would be at the same concentration as the target samples, labeled with the 113 iTRAQ label in all three runs, and used as the denominator in the calculation of the ratios to control for batch-to-batch-variation.

OSM7: iTRAQ. To the 20 coral samples and 3 normalizers (22 µg each [3]), we added 1 µl of tris-2-carboxyethyl-phosphine (TCEP; Sigma-Aldrich, USA) to reduce the dissolved proteins' disulfide bonds. Samples (n=23) were then vortexed, centrifuged at 15,000 RPM for 5 min (hereafter simply referred to as "spun"), and incubated at 60ºC for 1 hr. Samples were spun and alkylated with 1 µl of freshly prepared 84 mM iodoacetamide (Sigma-Aldrich), vortexed, spun again, and incubated in the dark at RT for 30 min. Samples were once again spun and then mixed with 10 µl of 0.1 µg/µl sequencing grade modified trypsin (Promega, USA) for 3 hr at 37ºC. Then, an additional 1 µl of trypsin was added, and proteins were digested overnight at 37ºC.

After spinning, samples (~43 µl) were dried in a speed-vac, resuspended in 0.5 M TEAB, and mixed with 50 µl of isopropanol and 17-22 µl of the appropriate iTRAQ reagent (SCIEX iTRAQ Reagent-8plex 25 U kit) according to the manufacturer's recommendations (lot#A7012): 18, 18, 22, 18, 17, 18, 20, and 22 µl for labels 113, 114, 115, 116, 117, 118, 119, and 121, respectively (Table 1). Samples were then vortexed, spun, and incubated at RT for 2 hr. Reactions were quenched with 100 µl of water for 30 min and dried to 10-20 µl in the speed-vac. Then, samples from each batch of 7-8 (the normalizer [113] plus the 6-7 target samples) were combined into the same tube and dried to completion. The three pellets were washed thrice with water, drying to completion after each wash except for the last, in which 30 µl were left to be later mixed with 30 µl of 2.5% trifluoroacetic acid (TFA; Sigma-Aldrich). Acidified proteins (pH=~2.2) were then purified with Pierce graphite spin columns (TFS; manufacturer's recommendations) to remove any residual buffers, enzymes, and/or insoluble material. iTRAQ-labeled samples were resuspended in 2% acetonitrile with 0.1% formic acid (both from Sigma-Aldrich) prior to nano-liquid chromatography (nano-LC) on an Easy nano-LC 1000 (TFS) as described in Desoubeaux et al. [8], and peptide eluates from a 2-98% acetonitrile gradient were individually run on a Q Exactive™ Orbitrap LTQ mass spectrometer (TFS) as in Musada et al. [9].

The 36 Florida Keys National Marine Sanctuary field biopsies, whose proteomes were analyzed to validate the predictive capacity of the neural net (NN) and other predictive models derived from the 20 experimental samples (as well as for use in field sample-exclusive predictive models as described in the main text), were analyzed similarly, though with the following exceptions. First, only seven samples were analyzed in a batch; iTRAQ label 121 was not used except in one instance resulting from the manufacturer sending an empty tube of label 117 (Table 2). Secondly, 58 and 70 µg of protein were analyzed for the batch normalizers and coral samples,

respectively. The former was made my mixing 6.5 µl (1.9 µg/µl) from each of the 36 samples and served to control for batch-to-batch variation. Finally, unlike for the experimental samples, proteins were not randomized across iTRAQ batches (A-F in Table 2); this is because, even when using a normalizing control sample in each batch (label 113), there was still such extensive variation among batches (see Supplemental File S2.) that it was difficult to separate biological from batch effects. Therefore, one representative sample from each CHD- BLR, BLS, and INT-was analyzed for each of two sites (one inshore & one offshore) for one of the three sampling dates (July [pre-bleaching], August [during-bleaching], or December [control] 2019) in a batch (Table 2). Samples from July, August, and December were therefore analyzed in batches A-B, C-D, and E-F, respectively. Accession numbers for the field coral imagery and MS data are found in the main text.

OSM8: MS data processing. RAW data files from the instrument were loaded into Proteome Discoverer® (ver. 2.2, TFS), and the default conditions were used to query each of the two fasta mRNA sequence libraries described below. These conditions included the Q Exactive, a Fourier transform mass spectrometer, being operated in MS2 mode with HCD activation. We used a peak integration tolerance of 20 ppm, and the peak integration method was based on the most confident centroid algorithm. Precursor and fragment mass tolerances were 10 ppm and 0.02 Da, respectively, and up to two missed cleavages were permitted. The collision energy was allowed to span from 0 to 1,000, and the precursor mass was confined to 350-5,000 Da. Under these conditions, both *O. faveolata* and Symbiodiniaceae dinoflagellate (*Breviolum+Durusdinium* hybrid assembly) assembled contig fasta files from Aguilar et al. [1] were queried (described in detail below). The two fasta sequence database, three RAW MS (TFS), three MZML (open-access MS peaks), and six MZID (open-access MS results) files are publicly available on UCSD's MassIVE repository (accession: MSV000086530; cross-listed with Proteome Xchange [accession: PXD022796]), and the RAW files can be accessed on MassIVE as well as the National Center for Environmental Information database (accession: 0242879; cross-listed with NOAA's Coral Reef Information System database). In addition to a minimum peptide length of 6 amino acids (aa), 144 aa was set as the maximum. For both host and dinoflagellate fasta library querying, decoy and contaminant databases were queried simultaneously such that false discovery rates (FDRs) could be calculated. Only proteins whose confidence scores (as *q*-values) fell below the FDR-adjusted alpha of 0.01 were included. Of these proteins, we only considered those with an iTRAQ label; note that the remaining, untagged proteins could be used for future presence/absence analyses (*sensu* [2]).

As an additional QC criterion, we required *a priori* that we would only include proteins sequenced in all three batches of lab samples. This is because, despite having 1) randomly allocated corals from different genotypes and treatments to each of the three lab coral iTRAQ batches and 2) run the identical, normalizing sample in all three batches, it is nevertheless possible that batch effects could lead to type I or II statistical errors. For instance, if a peptide was only sequenced in batch 1 but not in batches 2-3, we did not assign 0 concentrations to this protein in the latter two batches but instead omitted it. Of the high-confidence proteins found in each batch with iTRAQ labels, we required that two map to the same conceptually translated contig so that we would have greater confidence in the protein identity and compartment of origin. A similar approach was taken with the field samples except that, in certain cases (e.g., when only July & August samples were analyzed), we considered proteins found within the subset of samples used in the particular model being tested, even if they were not quantified in samples excluded from that model; this is why 28 proteins could be used for July-August models vs. only 16 when all three months were considered (i.e., 12 proteins were measured in July & August samples but not in December 2019 ones.).

OSM9: fasta databases queried. The fasta files were derived from an RNA-Seq analysis of >70 *O. faveolata* transcriptomes, including those 21 experimental samples (Table 1) from which proteins were extracted. In other words, both RNAs and proteins were extracted from the same sample, with the RNA analyzed by RNA-Seq (Aguilar et al. [1]) and the proteins analyzed herein (an additional 50 samples from Aguilar et al. [1] were analyzed by RNA-Seq & *not* by proteomics.).

From the same 70 transcriptomes, a Symbiodiniaceae dinoflagellate fasta mRNA sequence database was also assembled, and this was used for the querying of the same RAW files from the MS. Each of the RAW files was queried twice, once against the host transcriptome and once against the endosymbiont one. It is worth noting that, although genomes are available for these species, we opted to instead query sequences derived directly from the study organisms given that genomic sequences were obtained from different genotypes. Because the 6-7 coral samples in each iTRAQ batch represented a mix of host genotypes, we did not query host transcriptomes in a genotype-by-genotype fashion (e.g., querying all proteins against the skyblue genotype, then querying all against the lightyellow genotype, etc.). Having queried MS spectral data from a mix of genotypes against a composite transcriptome database likely signifies that the software only considered peptides that were identical across all samples in each batch (so as not to consider sequence bias in labeling efficiency, for instance). This issue would also affect the endosymbiont analysis since, like the host corals, a mix of lineages was present across samples.

Unlike for nucleic acid analyses, in which contigs can be confidently mapped to individual Symbiodiniaceae lineages, tryptic peptides are too short (6-10 aa) to do so with confidence. Indeed, in many cases sequenced peptides could not be confidently ascribed even to host or endosymbiont. For these reasons, and because *O. faveolata* hosts a large diversity of dinoflagellates in the Florida Keys [4], we did not resolve sequences into exact Symbiodiniaceae genera. In preliminary analyses, both host and dinoflagellate endosymbiont fasta libraries were actually queried simultaneously with the same RAW file, though it was found that only a small number of proteins (dozens) passed the FDR threshold discussed below; this is because, unlike BLAST, MS algorithms do not use exact protein sequences, but instead MS peaks that are used to infer aa molecular weights. For highly conserved proteins, the software is unlikely to assign large numbers of peptides to the correct compartment of origin with statistical confidence (based on peptide score *q*-values). When each compartment's transcriptome/genome is queried in isolation, however, a greater proportion of proteins (typically hundreds) can be confidently assigned. Because we were concerned with assigning sequenced peptides to the incorrect compartment of origin, we enacted the additional rule mentioned above that two peptides mapped to the same protein. It is possible that increasing the mapping stringency even further (e.g., 3-4 peptides/reference protein) could ultimately lead to an elucidation of the exact Symbiodiniaceae species from which the protein emerged, though this would result in far reduced number of proteins (<10).

OSM10: statistical analysis-details. Because entire coral plugs were sacrificed at each sampling time for the laboratory samples, fragment was nested within genotype x temperature x time to ensure that intra-genotypic variability was accommodated. In the cases when the fragment[genotype x temperature x time] term was not statistically significant, we assumed that a fragment from one genotype sacrificed after five days of laboratory treatment exposure was equivalent to an unsacrificed clonemate in the same treatment. An extensive array of statistical analyses, both univariate and multivariate, was undertaken with the 46 coral and 40 dinoflagellate proteins from the 20 laboratory samples that passed all QC; please see a prior work for details [3]. This included a response screen to search for differentially concentrated proteins (DCPs) and a stepwise discriminant analysis (SDA) to partition differing FHD and CHD by their proteomes; the latter is discussed for both laboratory and field samples in the main text. Additionally, principal components analysis (PCA), multi-dimensional scaling (MDS), and non-parametric multivariate ANOVA (NP-MANOVA) were undertaken with the laboratory and field coral proteomes to explore multivariate trends across temperatures, sites, genotypes, and phenotypes.

OSM11: proteomic predictive modeling-overview. There were two proteomic predictive modeling goals. In the first, we sought to simply train the artificial intelligence (AI) to discern corals of the various FHD of the 20 laboratory (Figure 4a) and 36 field (Figure 4c) samples. Although these models are of little use in conservation since an AB sample can be observed by eye by SCUBA divers, it was important to ensure that there was sufficient proteomic variation among

HC, SLS, HTA, and AB colonies to develop more sophisticated predictive models. Because it was unknown whether HC at temperatures <MMM would behave similarly as HTA samples, these groups were kept separate for certain analysis. Discriminant analysis and, in some cases, SDA (in which proteins are added in stepwise fashion) were generally capable of resolving differences among the FHD using a subset of iTRAQ proteins (discussed in the main text). However, distinguishing the CHD, which represents an intrinsic, entrained property of the colony or genotype, is more difficult because, in the case of a bleaching-susceptible coral, the model would be trained with data from HC, SLS, HTA, and AB biopsies. Distinguishing an HC biopsy from an AB one is theoretically easy, but distinguishing a HC biopsy from a BLS coral vs. one from a BLR colony is more difficult; in many cases, they may be very similar, but the resulting model would only have real-world utility if it could correctly classify a BLS coral *before* that colony was stressed. For this reason, more complex modeling types, namely machine-learning, was required to correctly classify the CHD from the proteomic biopsies. In both the FHD and CHD predictive models, the FHD/CHD was the model Y, and the proteins were the predictors. Because we hypothesized that the genotypes would demonstrate variable bleaching susceptibility based on *in situ* observations (Figure 1 & Supplemental File S2), we did not treat site or genotype as a random effect.

In certain cases, the same colony was used in the laboratory study (Table 1) *and* sampled during the 2019 field experiment. Although these *in situ* colonies could nevertheless be used as "test" samples, it would be preferable to use data from those that were in no way featured in initial model training as true "field-test" samples. For this reason, we made sure include data from samples of the "test reef," Crocker Reef, in most of the models described in the main text (as well as Table S2 & Supplemental File S2). Corals from this offshore reef have suffered greatly from climate change and disease; in the future, then, it would be optimal to also have a true test reef from a more resilient inshore site, as well (assuming any still exist beyond the two from which samples were analyzed herein: Cheeca Rocks & The Rocks).

OSM12: predictive modeling details. As discussed in the main text, JMP® Pro 17's "model screening" platform was run with the 86 proteins as predictors (X) for the laboratory samples and the following two coral phenotypes as Y: FHD (the actual status of the analyzed biopsy/fragment; HC, HTA, SLS, or AB) and CHD (the ultimate resilience of the colony as a whole [assessed over time]; BLR or BLS). In the initial analysis, the following modeling types were tested with 14 training (sample D6-6 was omitted since the CHD could not be determined) and 5 validation samples (ensuring that at least one of each of the 2-4 health designations was represented in the validation set): bootstrap forest, discriminant analysis, generalized multivariate regression (gen-reg), k-nearest neighbors, naïve Bayes, NN, partial least squares (PLS), stepwise regression, XGBoost, and support vector machines. The scripts (JSL) for all models that passed QC (accuracy>80%) have been made publicly available on JMP Public. Please note that, in these preliminary trials, test sample data were not considered since the field samples were later used to field-test the models that passed QC.

For each model, the following fit parameters were calculated: root mean square error (RMSE), Akaike's information criterion (adjusted to the total number of model parameters, i.e., AICc), training sample classification accuracy (how well the model fit the training data), validation sample classification accuracy (how well the model fit the validation or test data), and the overall accuracy (1–misclassification rate). Among these parameters, we sought the model that had the highest chance of correctly determining the bleaching status (FHD) or susceptibility (CHD) of a coral. When two models were characterized by the same accuracy, the difference between the training and validation sample accuracy was the second criterion for model ranking; all else being equal, the model whose training and validation accuracies were most similar was prioritized. Because NNs were oftentimes the superior modeling type, a large number of NN were built with an automated GUI as described in the main text with either validation column (holdback) data alone used for validation, validation *and* test column data used for validation, or random holdback data (either 10, 20, or 30% of data held back from the training model & then used for validation). Kfold

validation was used in some instances, though in general this approach was avoided since the data were difficult to reproduce given the random nature of the folds. Note that, because different proteins were sequenced from the lab and field samples, the "lab➔field" (i.e., lab-derived data used to make predictions of field coral phenotype) predictive models (Tables 3 & S2) generally featured only 2-5 proteins. In contrast, "lab➔lab" and "field➔field" models could include 86 and 16-28 proteins, respectively.

Online supplemental results (OSR)

OSR1: supplemental tables

Table S1. Non-parametric multivariate ANOVA (NP-MANOVA) of field coral proteomic data. The coordinates from the first three, four, and four multi-dimensional scaling dimensions for the 5 endosymbiont, 11 host coral, and all 16 proteins, respectively, were used (stress=0.08, 0.07, & 0.09, respectively) as the model Y's. The approximate (approx.) and Exact *F* statistics have been shown for factors with >2 and 2 categories, respectively, and statistically significant differences are highlighted in **bold** ($p<0.01$). In the "Multiple comparisons" column, lowercase letters denote statistically significant inter-mean *post-hoc* differences ($p<0.01$). AB=actively bleaching. HC=healthy control. HTA=high-temperature-acclimating. SLS=sub-lethally stressed.

Factor	df	Approx. or Exact *F*	*p*	Multiple comparisons
Endosymbiont proteins only (n=5)				
Site	3	1.26	0.28	
Shelf	1	2.04	0.13	
Genotype	9	0.54	0.96	
Date	2	3.15	0.01	
Colony health designation	2	0.23	0.97	
Fragment health designation	3	0.99	0.48	
Host coral proteins only (n=11)				
Site	3	2.05	0.03	
Shelf	1	1.66	0.19	
Genotype	9	0.91	0.62	
Date	2	6.29	**<0.01**	July(ab)=December(b)≠August(c)
Colony health designation	2	0.90	0.52	
Fragment health designation	3	2.66	**<0.01**	HC(a)>AB(ab)>SLS(b)=HTA(b)
Host coral+endosymbiont proteins (n=16)				
Site	3	2.28	0.02	
Shelf	1	1.67	0.19	
Genotype	9	0.96	0.55	
Date	2	4.24	**<0.01**	August(a)>July(ab)>December(b)
Colony health designation	2	0.36	0.94	
Fragment health designation	3	1.34	0.22	

Table S2. Models for predicting field coral bleaching susceptibility (as colony health designation [CHD]) from field & lab coral data. Note that there were five proteins in common between the lab and July 2019 (pre-bleaching) coral datasets, of which one was *not* also found in the August (bleaching) samples (i.e., only four proteins were incorporated when August data were included in model training.). When a modeling approach could accept missing data (e.g., bootstrap forest), all five proteins were considered, even in the instances in which these proteins were absent from certain sample datasets; for modeling approaches that *could not* accommodate missing data (e.g., neural networks [NN]), only those proteins found in all samples were considered. Three additional models (d, h, & i) can be found in Supplemental File S2. When only one model type passed quality control (QC; accuracy>80%), the most important protein is instead shown in the bottom row. NA=not applicable. SVM=support vector machines.

Model abbreviation	**CHD-lab+field➔ CHD-lab+field(a)**	**CHD-lab+ field➔ CHD-lab+field(b)**	**CHD-lab+field➔ CHD-lab+field(c)**	**CHD-lab+field➔ CHD-lab+field(e)**	**CHD-lab+field➔ CHD-lab+field(f)**	**CHD-lab+field➔ CHD-lab+field(g)**
Model short name	July only (a)	July only (b)	30% holdback (HB)	Excluding Dec.	Excluding Aug.	December holdback
Sample size	Lab(19)+field(12)	Lab(19)+field(12)	28	Lab (19)+field (24)	Lab (19)+field (18)	Lab (19)+field (30)
Validation column name	23-6-2 (a-c)	22-6-3	NA	30-10-3	25-9-3	NA: used 6 Dec. samples as HB
Training data (#)	Lab(15)+field(8)	Lab(13)+field(9)	Random (15)	Lab (16)+field (14)	Lab (11)+field (14)	Lab (19)+field (24)
Training data-months	Lab & Jul.	Lab & Jul.	Lab+all field	Lab, Jul., & Aug.	Lab, Jul., & Dec.	Lab, Jul., & Dec.
Validation data (#)	Lab(3)+field(3)	Lab(4)+field(2)	NA	Lab (1)+field (9)	Lab (7)+field (2)	Field (6)
Validation data-months	Lab & Jul.	Lab & Jul.	Lab, Jul., & Aug.	Lab, Jul., & Aug.	Lab, Jul., & Dec.	Dec.
Test data (# samples)	Lab(1)+field(1)	Lab(2)+field(1)	NA	Lab (2)+field (1)	Lab (1)+field (2)	NA
Test data-months	Lab & Jul.	Lab & Jul.	NA	Lab, Jul., & Aug.	Lab, Jul., & Dec.	NA
Training proteins (#)	5	5	4	4	2	2
Model type#1 (accuracy)	NN: 95±10% (n=20)	NN: 94±10% (n=20)	SVM: 82±3.5% (n=20)	SVM (86%)	Bootstrap forest (90%)	NN: 98±5% (n=20)
Model type#2 (accuracy) or most important protein	SVM (87%)	OFAVBQ_DN207472_c1_g1_i1	NN: 82±12% (n=20)	OFAVBQ_ DN222422_c2_g1_i4	OFAVBQ_DN222422_ c2_g1_i4	OFAVBQ_DN222422_ c2_g1_i4

Online supplemental references

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