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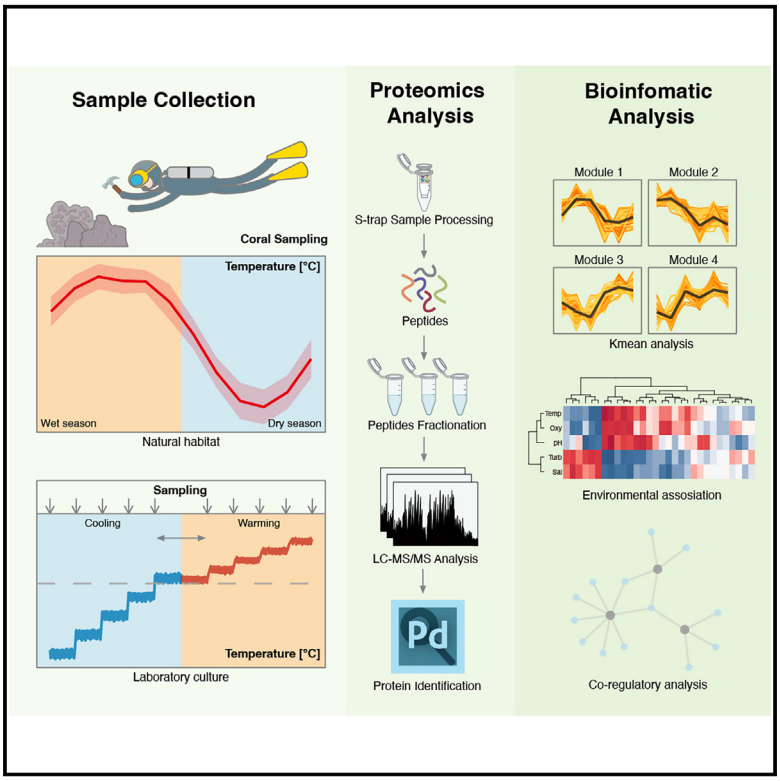
Proteomic insights into the environmental adaptation of the subtropical brain coral host *Platygyra carnosa*

Graphical abstract



Highlights

- Integrated sample processing enabled label-free quantitation of >5,000 coral host proteins
- Differential wet/dry seasonal proteomes indicated active heterotrophy and growth processes
- Co-regulation analysis revealed protein modules in adaptation to environmental factors
- Lab tests validated temperature-adaptation hubs underlying stress response and proteostasis

Authors

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In brief

Evolutionary biology; Nature conservation; Proteomics

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Proteomic insights into the environmental adaptation of the subtropical brain coral host *Platygyra carnosa*

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SUMMARY

Despite the rapid coral reef decline from climate change, the molecular dynamics underlying coral environmental responses remain elusive. Filling this gap is vital to reef conservation. Here, we investigated the seasonal proteomes of *Platygyra carnosa*, a stress-tolerant subtropical brain coral, using natural samples across wet and dry seasons with distinct environmental conditions. Over 5,000 coral host proteins were profiled, revealing co-regulated modules related to temperature, pH, dissolved oxygen, salinity, and turbidity. Importantly, these modules formed scale-free networks coordinated by hub proteins that are strongly correlated with environmental drivers, suggesting their key roles in environmental adaptation. Laboratory validation confirmed the temperature-responsive hub proteins, including HSP90B1 and HSPA5 that modulate stress response and protein homeostasis. Our study characterized the brain coral host proteome with unprecedented depth, revealing co-regulated modules underlying environmental adaptation. It sets the stage for proteome-based approaches in promoting coral resilience, leading to more informed conservation and restoration efforts.

INTRODUCTION

The unprecedented rate of environmental changes due to anthropogenic pressures constitutes an extreme threat to coral reefs, which are of indispensable social, ecological, and economic value.[1] Hermatypic scleractinian corals (reef-building or stony corals) are the fundamental elements of the coral reef ecosystem, and are highly sensitive to environmental drivers.[2] For example, elevated ocean temperatures have recently caused mass coral bleaching and mortality worldwide.[3] Moreover, corals have limited metabolic tolerance to other environmental fluctuations, such as deoxygenation, suspended sediments, low salinity, and eutrophication.[4–7] Therefore, mitigating climate and environmental changes are concluded as the sustainable solution to conserving and restoring coral reefs.[8]

In addition to the conservation efforts, the future of reef-building corals largely depends on their capacity to respond adaptively to environmental fluctuations.[9] From the first genomic mapping in 2011 to the single-cell transcriptomics in 2021, our knowledge about the molecular biology of coral adaptation advanced greatly in many fronts.[10,11] These include the dynamics of genes that are responsive to elevated temperature,[12,13] acidification,[14,15] deoxygenation,[4,16] suspended sediments,[17] low salinity,[5] etc. These understandings are largely based on transcriptomic studies, yet proteins are the primary working force to determine the organism's phenotype. It is generally noted that there is little concordance between the RNA transcripts and the corresponding protein levels in a cell's response to perturbations.[18] Therefore, in addition to the genome and transcriptome, the coral proteome can provide more functional insights into the vibrancy of cells and their adaptation to drivers.[19,20]

The advancing quantitative proteomics has been applied to understand the effects of changes in seawater quality on coral

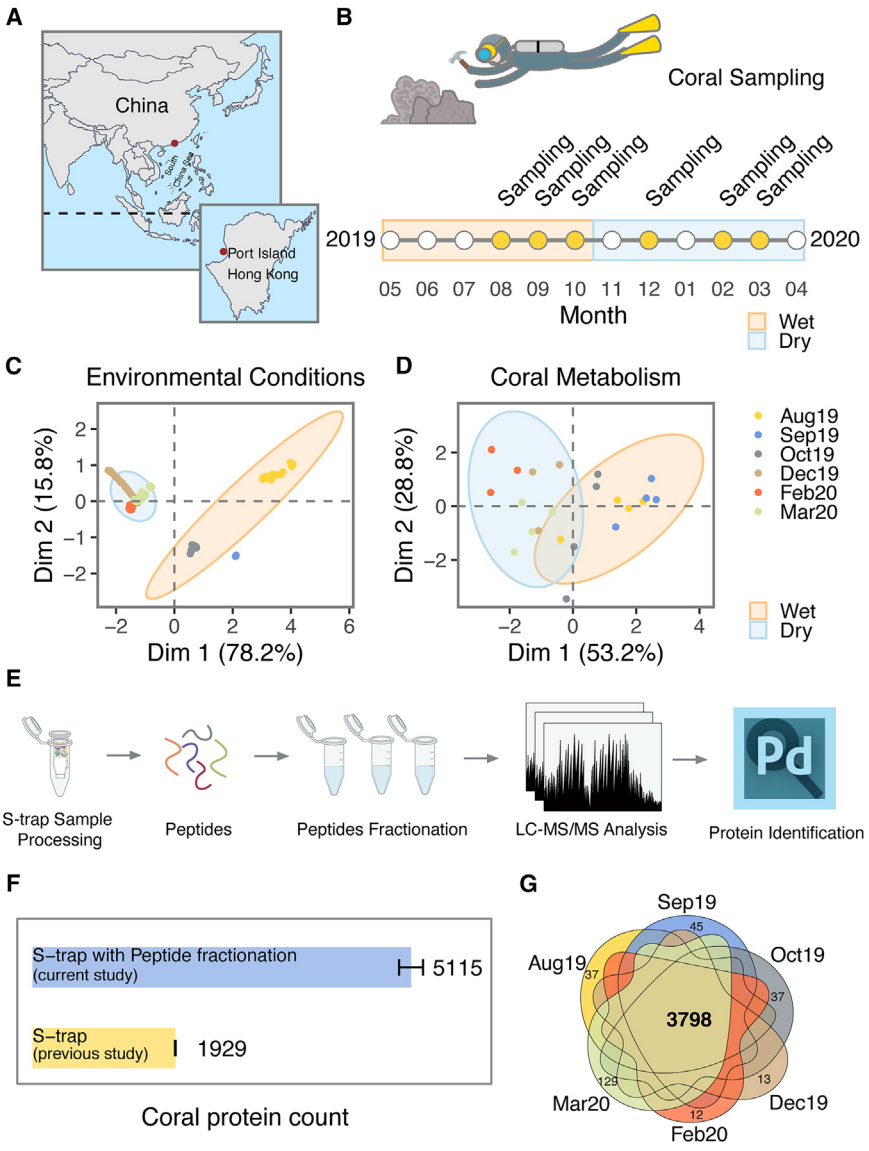

Figure 1. Coral sampling and sample processing

(A) Map showing the location of sampling site.

(B) Timeline exhibiting time points of coral sampling using SCUBA diving.

(C) Principal-component analysis (PCA) of *in-situ* environmental conditions. Ellipses represent 90% CI and colors indicate seasons or months.

(D) PCA of *in-situ* metabolic data.

(E) Schematic of the sample process and analysis workflow using LC-MS/MS.

(F) Bar graph comparing two methods, S-trap with Peptide fractionation and S-trap only, in recovery of coral host proteins. Data are mean ± SD.

(G) Venn diagram of coral host proteins identified from samples collected in different months.

health in Taiwan and the Caribbean region,[21,22] as well as empowering investigations into stress responses,[21,23–28] skeleton formation,[29] disease,[30] and symbiosis establishment[31–33] of coral hosts and their model animals (cnidarian *Apitasia* or *Nematostella vectensis*). For example, a study recovered 1,230 unique proteins (774 from the coral host and 456 from the symbionts) and revealed that bilateral nutrient exchange is the key to symbiosis balance under elevated temperature.[26] Due to technological limitations, coral proteomics research often identified relatively low number of proteins, ranging from hundreds to less than three thousand. Such suboptimal proteome coverage may impose inherent constraints and potential biases on data interpretation.

In this study, we integrated the high pH reverse-phase fractionation with S-trap method to enhance the proteome depth in environmental adaptation research on reef-building coral, *Platygyra carnosa*, in subtropical Hong Kong waters. In this region, *P. carnosa* is a common stony brain coral species with high resistance to fluctuating environmental conditions. Laboratory heat stress experiment revealed that *P. carnosa* could survive under increasing temperature from 25°C to 32°C,[34] even though its primary endosymbionts (>75%) were thermal sensitive species *Cladocopium* C1.[35] In addition, stable isotope analysis showed that host and symbiont isotopic niche overlap of *P. carnosa* was lower than 10%,[36] indicating heterotrophic nutrition was dominant. Based on these findings, we hypothesized that host factors play a dominant role in the environmental resistance of *P. carnosa*. Besides, mounting evidence indicated that environmental experience dictates coral proteomic changes and greatly affects the acquisition and maintenance of stress tolerance.[37–39] Therefore, we further hypothesized that the environmental adaptation of the host proteome underlies the high resistance of *P. carnosa*. To address these, we profiled the dynamics of *P. carnosa* coral host proteome in subtropical habitats with high environmental variability.

RESULTS

In-depth profiling of seasonal *P. carnosa* host proteome in a natural habitat

Port Island is located in the Great Bay Area of the South China Sea (Figure 1A), and it is a habitat having various coral species.[40] Over the past 20 years, the environmental conditions (temperature, pH, dissolved oxygen, salinity, and turbidity) of this coral habitat displayed seasonal fluctuations (Figure S1A–S1E). Principal-component analysis (PCA) using these environmental data showed that the months can be categorized into two phases, which are termed as the wet (May to October) and dry (November to April) seasons (Figure S1F). This is in line with

Table 1. Summary of *in-situ* environmental conditions

	Temperature (C)		Oxygen saturation (%)		pH		Turbidity (FNU)		Salinity (PSU)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Aug19	28.35	0.32	128.91	3.58	8.06	0.01	0.07	0.04	32.61	0.27
Sep19	28.81	0.01	105.91	0.27	8.10	0	0	0	35.93	0.02
Oct19	28.15	0.09	104.46	0.78	7.97	0	0.20	0.03	37.36	0.08
Dec19	19.75	0.02	101.99	0.64	7.90	0	1.00	0.22	37.95	0.02
Feb20	18.32	0.02	98.91	0.36	7.91	0.01	0.56	0.03	37.92	0.01
Mar20	20.05	0.03	105.17	3.38	7.87	0.01	0.51	0.02	37.28	0.06

the biseasonal pattern of the subtropical coral habitats.[41] Despite the fluctuating environmental conditions, *P. carnosa* persists well as a common coral here and in the South China Sea.[42] Therefore, we chose to investigate the adaptive proteomic dynamics of *P. carnosa* in the Port Island area.

We collected coral samples at six different time points spanning from 2019 (August, September, October, and December) to 2020 (February and March) (Figure 1B). At the same time, we recorded the environmental data (Figures S2A and S2B; Table 1) and physiological parameters (Figures S2C–S2F) using *in-situ* coral monitoring techniques.[43,44] PCA analysis showed that the environmental conditions and the physiological parameters were both divided into distinct wet- (August, September, and October) and dry-season (December, February, and March) phases accordingly (Figure 1C and D). Among the coral physiological parameters, respiration rate (R) and net photosynthesis rate (P_{net}) were higher in the wet-season months. Yet, the P:R ratio (ratio of gross photosynthesis rate and respiration rate), representing the productivity of coral holobiont, was higher in the dry-season months (Figures S2C–S2E). Notably, the Fv/Fm value, which reflects the PSII photochemical efficiency of the microalgae and the fitness of the coral endosymbionts system remained stable across six time points. In parallel, the whiteness level remained low (Figure S2F). These results indicate that, despite the changes in certain physiological parameters, the brain corals adapted well to the seasonal fluctuations in the habitat and maintained good fitness. It is of high interest to understand whether and how the dynamic proteome mediates the coral adaptation.

We have established an S-trap sample processing method[45] that could recover thousands of stony coral proteins in quantitative mass spectrometry analysis. In addition, the offline high pH peptide fractionation strategy is known to extend the dynamic range and coverage of liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) analysis.[46] To maximize the depth of analysis, we integrated the S-trap and peptides fractionation strategy to process the collected coral samples, resulting in 46 fractions per sample before the nanoLC-MS/MS (Figure 1E). The integrative S-trap/fractionation method identified an average of 5,115 coral host proteins from individual samples, which is a 165% increase from our previous study using S-trap without peptide fractionation (Figure 1F).[45] In total, 3,798 proteins existed ubiquitously in all samples from the six months (Figure 1G). Functional annotation shows that the recovered coral proteins were involved in the regulation of various cellular processes and metabolic processes (Figure S3A). The most representative cellular components covered intracellular structure, membrane, and extracellular region (Figure S3B). In terms of molecular function, they were associated with protein binding, compound binding, and hydrolase activity (Figure S3C). Therefore, our novel workflow recovered the host proteome of natural stony corals in an unprecedented depth, providing us with a comprehensive dataset to examine coral adaptation mechanisms at the proteome level.

Dynamics of *P. carnosa* host proteome reflects seasonal nutrient strategy

Next, we compared the proteomes of wet and dry season samples. In total, 4,391 and 4,222 proteins were identified ubiquitously from wet and dry season samples, respectively (Figure 2A). As mentioned previously, 3,798 proteins were commonly identified in all 6 months of both seasons, and we termed them as the "core proteome" of the two seasons. In addition, 593 and 424 proteins were stably present only in wet season and dry season samples, respectively. We termed these proteins as "wet- and dry-stable proteins", and their seasonal persistency suggests potential roles in environmental adaptation. Indeed, functional annotation revealed that the wet-stable proteins are enriched with activities in cilium assembly and movement, including IFT20, TCTN1, and CFAP206 (Figure 2B, left panel). The cilia are critical in coral homeostasis and stress response by facilitating the heterotrophic mode of nutrient acquisition and exchange with the environment.[47–49] Therefore, the wet-season persistency of cilia proteins may reflect their important functions in obtaining nutrients and migrating the heat. In comparison, a cluster of dry-stable genes were enriched with rRNA processing and RNA metabolisms, including IMP3, NOP2, and GAR1 (Figure 2B, right panel; Table S1), indicating active biosynthesis powered by sufficient nutrients.[50]

For the 3,798 core proteins shared between wet season and dry season, we examined their differential expression. This revealed 128 and 107 core proteins with significantly higher levels in wet and dry season, respectively (Figure 2C). Functional enrichment analysis showed that the proteins with higher levels in wet season (wet-high) are enriched in the responses to heat and elevated temperature, chaperone-mediated protein folding, as well as regulators of immune response (Figure 2D, left panel; Table S2). Therefore, in addition to heat shock response and maintaining protein homeostasis, the temperature adaptation may involve more interactions with the surrounding microorganisms in the wet season. In comparison, a significant proportion of the dry-high proteins are enriched in the fatty acid metabolism

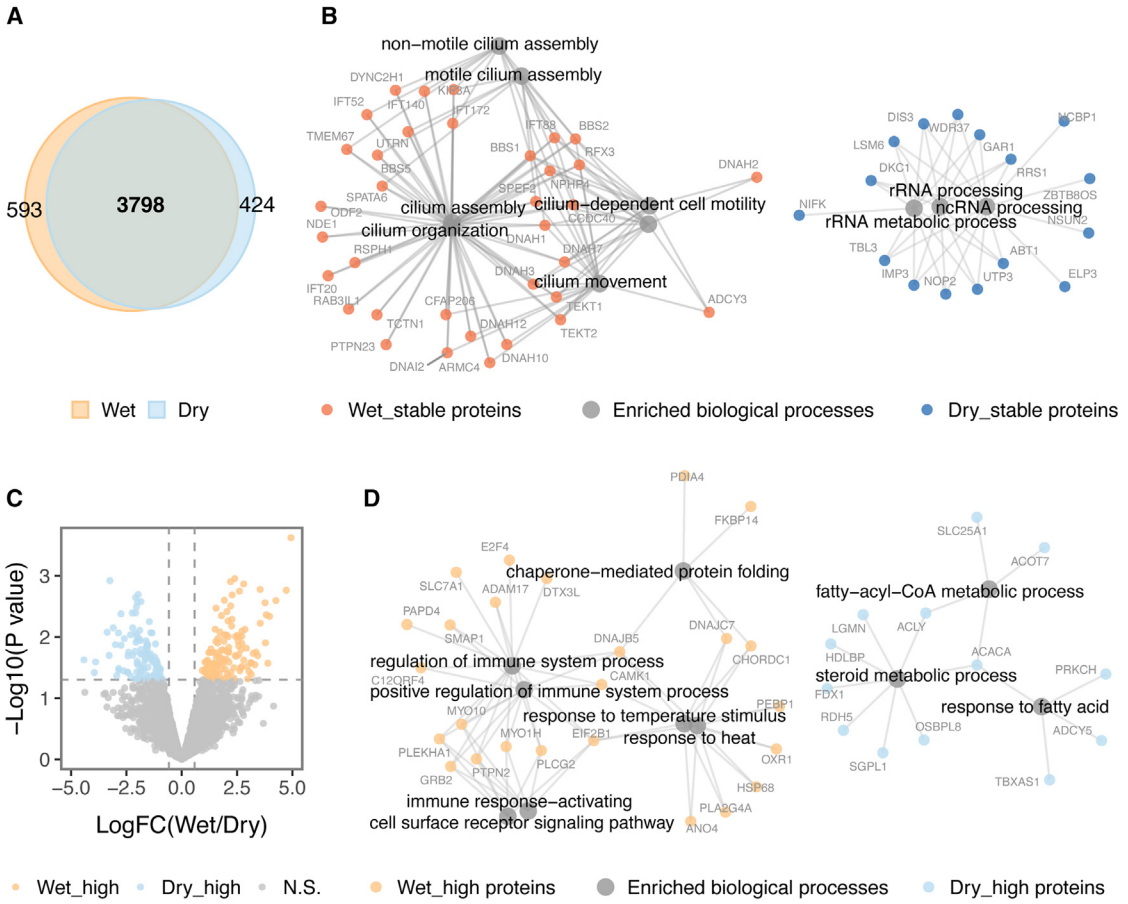

Figure 2. Seasonal fluctuations affect *Platygyra carnosa* host proteome

(A) Venn diagram of coral host proteins identified from wet season samples and dry season samples.

(B) Enriched biological processes of proteins that stably presented in wet season or dry season.

(C) Differential expression analysis of 3,798 coral host proteins that ubiquitously existed in six months.

(D) Enriched biological processes of proteins that had highly levels in wet season or dry season.

pathways and the steroid metabolic process (Figure 2D, right panel; Table S2), including HDLBP, FDX1, RDH5, etc. It is known that corals acquire sterols (precursors of steroid) either from their endosymbionts or dietary sources,[51] thus the upregulated steroid metabolic process suggests elevated nutrient assimilation in the dry season. Indeed, the physiological parameters support this point with higher P:R ratio (ratio of gross photosynthesis rate and respiration rate) in the dry season, indicating enhanced nutrient assimilation (Figure S2E). Taken together, the dynamics of *P. carnosa* host proteome reflects that its adaptation to the seasonal changes is underlined by alterations in the nutrient strategy.

Endosymbionts play an important role in coral holobionts and understanding their proteome dynamics could provide additional insights. However, it is challenging to fully dissect the proteome of endosymbionts due to the complexity and lack of genomic annotations. Here, we attempted to search the raw MS files against the proteome database of *Cladocopium* C1, a dominant species of endosymbionts community identified in coral *P. carnosa* from Hong Kong waters.[35] This revealed 837 endosymbionts proteins and 473 of them were detected in both wet and dry season, while only 29 of these core proteins were differentially regulated during seasonal change (Figures S4A and S4B). Among 29 differentially regulated proteins, chlorophyll A-B binding protein and heat shock proteins (HSP60 and HSP70) had higher levels in wet season (Figure S4C), indicating endosymbionts enhanced photosynthetic efficiency to sustain energy production, coupled with activation of proteostasis mechanisms to counteract protein denaturation and preserve cellular homeostasis under high temperature and strong light intensity during the wet season.[52,53] In contrast, elevated concentrations of chloroplast ATP synthase and pyruvate orthophosphate dikinase (PPDK) suggest a coordinated adaptation to the dry season (Figure S4C), where ATP synthase optimizes photophosphorylation efficiency to sustain energy flux, while PPDK redirects carbon metabolism toward lipid/starch biosynthesis, enhancing photosynthetic nutrient transfer to the coral host.[54] These findings align with proteome dynamics of coral host, and further support a flexible nutrient strategy evolved by the brain coral host *P. carnosa*.

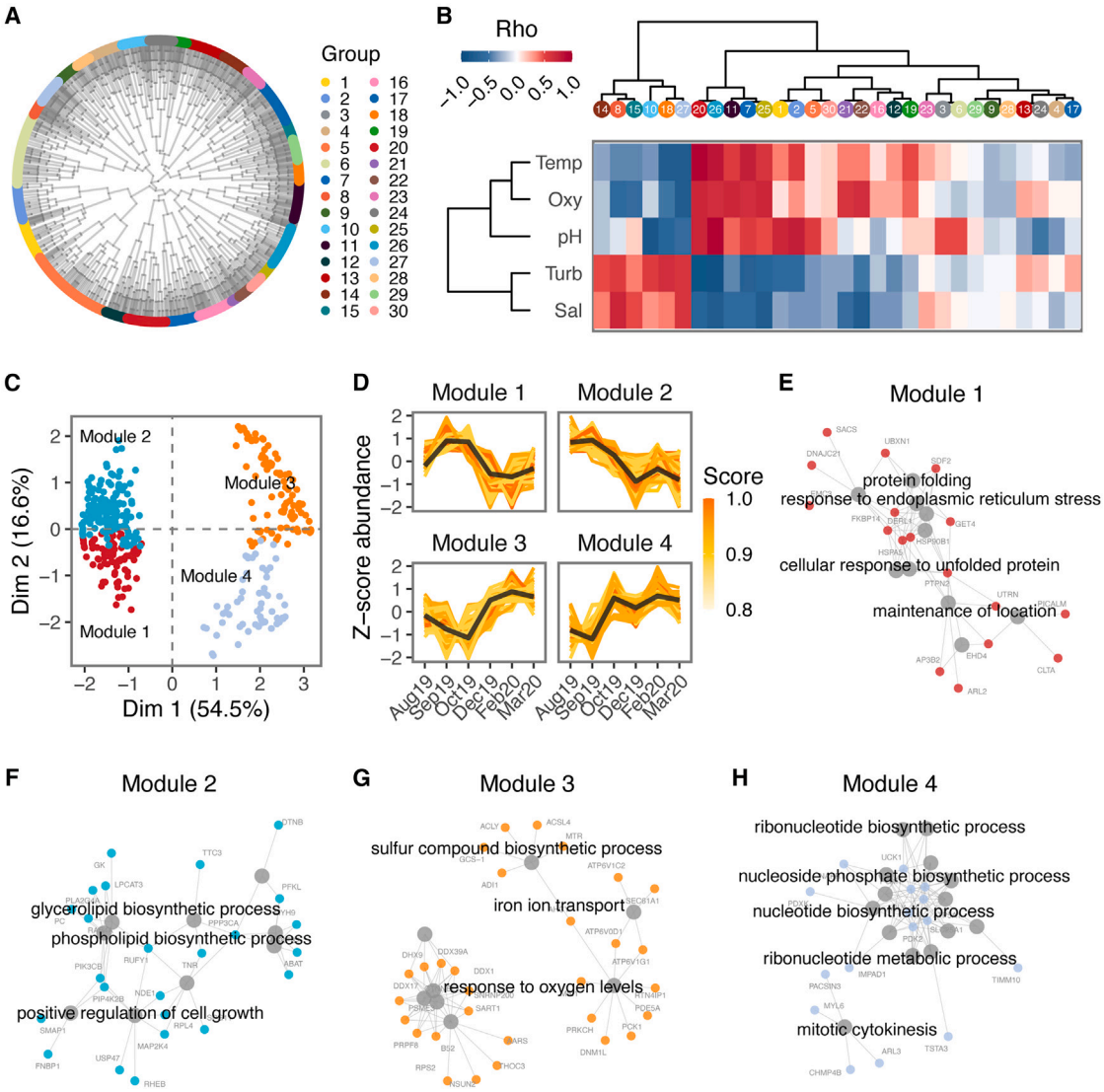

Figure 3. Co-regulatory identified protein modules associated with environmental conditions

(A) Hierarchical clustering of coral host proteins existed in at least three months.

(B) Spearman's coefficient heatmap of the representative protein from each group and environmental parameters.

(C–H) (C) Principal-component analysis, (D) line plots of regulatory patterns, and (E–H) enriched biological processes of four protein modules that are highly associated with environmental parameters.

Linking *P. carnosa* host protein modules to environmental drivers

Proteins involved in the same biological processes often show temporal coordination in their levels.[55] Accordingly, co-regulatory analysis can infer functional protein modules and provide a systemic understanding of responses to environmental changes. We performed a temporal co-regulatory analysis of coral proteins that are detected in at least three sampling months (5,476 proteins). Based on Spearman correlation of regulatory patterns,[56,57] we established 30 co-regulatory groups that are highly structured with sizes ranging from 54 to 537 proteins (Figure 3A; Table S3, rho: 0.71–0.89).

To identify the protein groups with potential functions in response to individual environmental factors, we examined the correlation between the protein dynamics and the fluctuating environmental parameters recorded at the natural habitat, namely temperature ("Temp"), pH, dissolved oxygen ("Oxy"), turbidity ("Turb"), and salinity ("Sal") (Figure 3B). Interestingly, the environmental factors showed a coordinated pattern in their associations with coral proteins. Temp, pH, and Oxy form a cluster with similar protein-correlation patterns that are generally opposite to the other cluster of Turb and Sal. This suggests synergistic effects of environmental factors in driving the proteomic dynamics of brain corals. Orthogonally, we observed that several coral protein groups displayed strong associations with all five

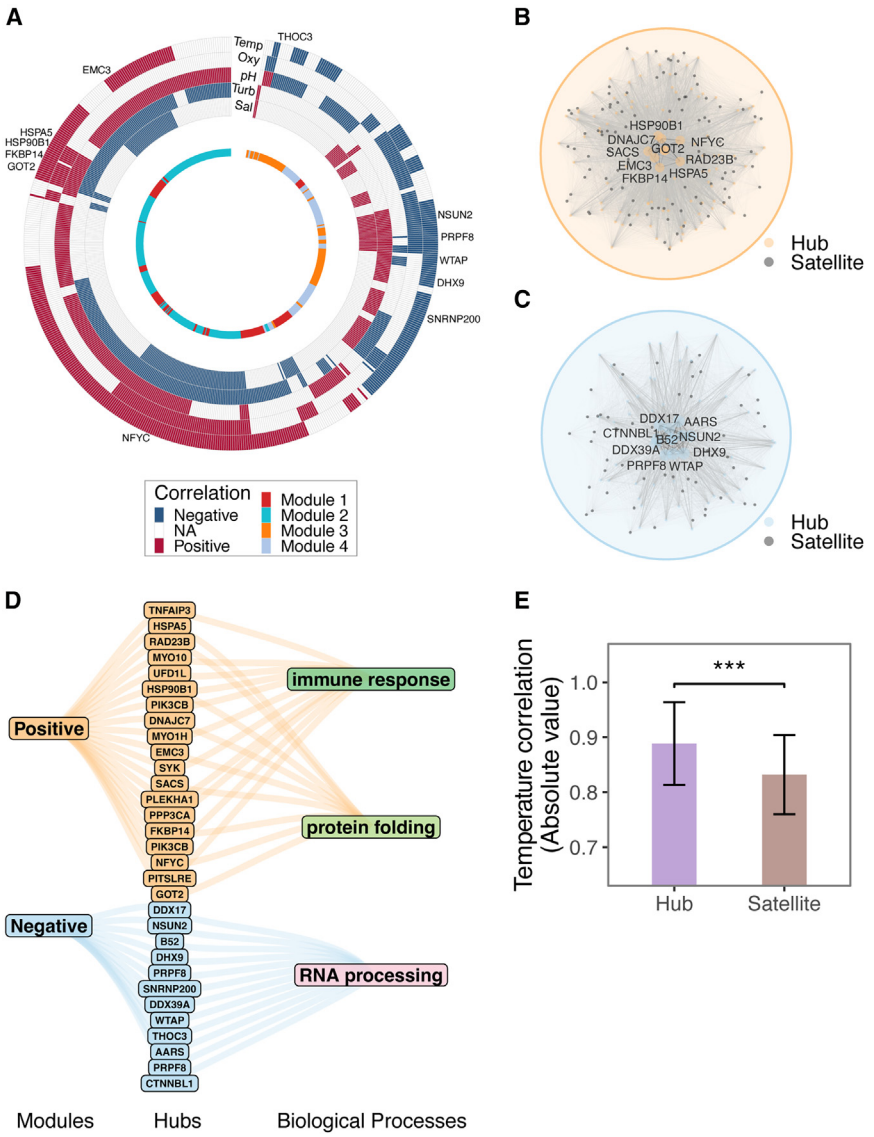

Figure 4. Network analysis of environmental-related protein modules

(A) The Spearman's coefficient heatmap of environmental-related protein modules.

(B and C) Scale-free network of positive and negative protein modules. Size of node indicated the role of proteins in the network.

(D) Enriched biological processes of protein hubs in positive and negative modules.

(E) The comparison of temperature correlation (absolute value) between hub and satellite proteins. Data are mean ± SD and ***p value <0.001.

environmental factors. For example, group 20 and 26 have strong positive correlations with Temp, pH, and Oxy, and strong negative correlations with Turb and Sal. In contrast, group 18 and 27 display strong negative correlations with Temp, pH, and Oxy and strong positive correlations with Turb and Sal (Figure 3B). The strong environmental correlations in different directions suggest that these protein groups might have essential functions in response to seasonal fluctuations of these factors. Therefore, we chose to focus on these four groups as potential functional modules. We termed group 20 and 26 as module 1 and 2, group 18 and 27 as module 3 and 4, respectively.

PCA analysis of the protein dynamics showed that modules 1 and 2 proteins are well separated from proteins of modules 3 and 4 (Figure 3C). The seasonal trajectories of proteins in individual modules typically showed two phases that fit with the wet-dry seasonal distinction (Figure 3D), in line with their tight association with different environmental factors (Figure 3B). Biological process enrichment analysis (Table S4) revealed that proteins of the modules are enriched in protein folding and ER stress (module 1, Figure 3E), lipid biosynthesis (module 2, Figure 3F), sulfur compound biosynthetic process and response to oxygen levels (module 3, Figure 3G), and nucleotide biosynthetic process (module 4, Figure 3H). These results suggest that the protein and metabolic homeostasis are closely coordinated at the proteome level in adapting to seasonal changes.

Next, we further examined the four modules for the correlation of individual proteins with the five environmental drivers (Figure 4A). Similar to the observations at the modular level (Figure 3B), proteins of strong positive correlations with Temp/pH/Oxy tend to have strong negative correlations with Turb/Sal. In view of the similar patterns of correlation with the environmental factors at the modular (Figure 3C) and protein (Figure 4A) level, we combined modules 1 and 2 as the positive module (positively related to Temp/pH/Oxy), while modules 3 and 4 were merged as the negative module (negatively related to Temp/pH/Oxy). Generally, the positive module proteins have high levels in the wet season and the negative module proteins have high levels in the dry season (Figure 3D).

We then further examined the protein connectivity within the correlation networks of these two seasonal modules. Biological networks are typically scale-free with densely connected hub proteins of essential functions in addition to peripheral satellite proteins.[58] In line with this, we observed that the seasonal modules of the coral proteins were organized around hub proteins with high intragroup connectivity ($K_i \geq$ median, see method for calculation of K_i) (Figure 4B). Annotations of the hub proteins highlighted their key functions in coral adaptation to the seasonal fluctuations of environmental drivers. Specifically, the Positive module hubs include HSP90B1, HSPA5, PIK3CB, PLEKHA1, etc. that are implicated in immune response and protein folding (Figures 4B and 4D). For example, HSP90B1 and

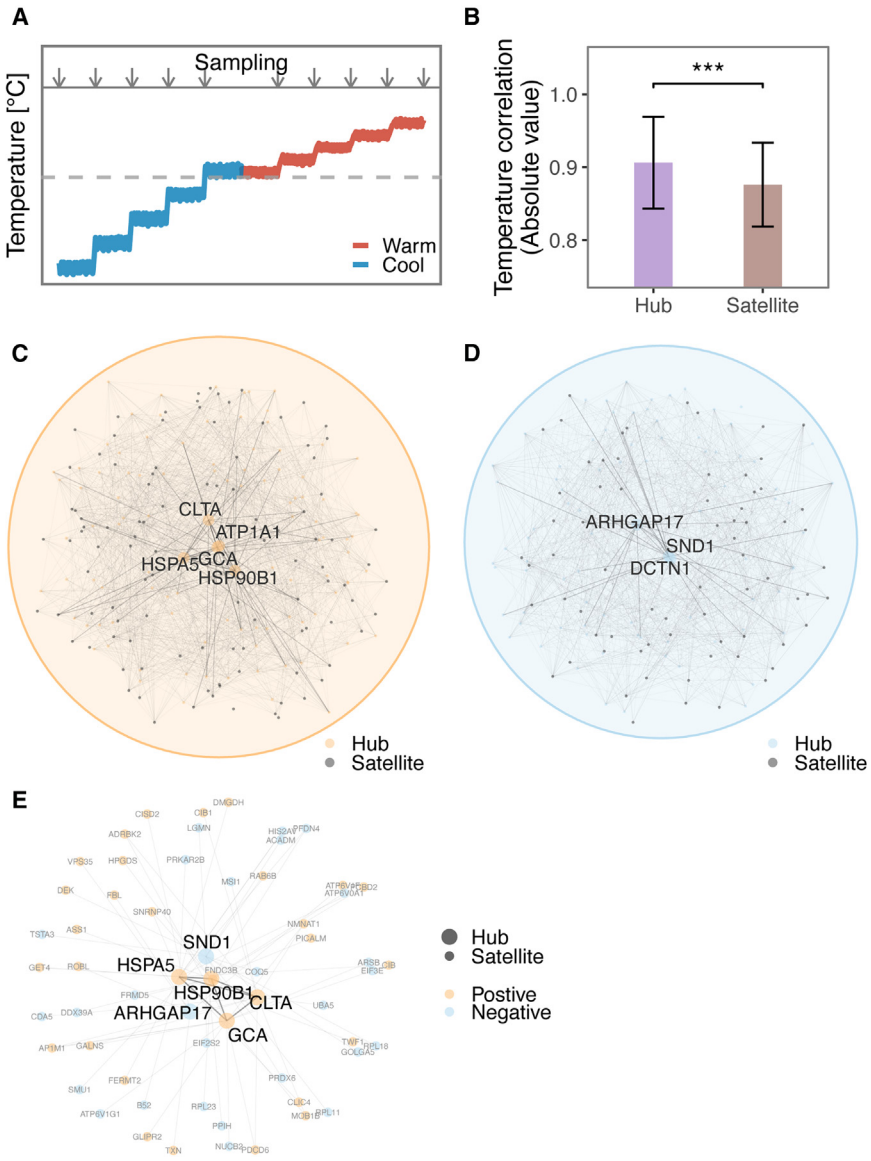

Figure 5. Co-regulatory recognized temperature-responsive protein modules

(A) Experimental design of laboratory temperature gradient culture.

(B) The comparison of temperature correlation (absolute value) between hub and satellite proteins. Data are mean ± SD, and ***p value <0.001.

(C and D) Scale-free networks of temperature-positive and -negative modules.

(E) Scale-free network of overlapping temperature-responsive protein modules between natural and laboratory-cultured corals.

Delineating temperature-responsive hub proteins

Increasing ocean temperature is the primary contributor of coral bleaching worldwide.[60] Previous studies suggested that seasonal temperature variation is the main driver faced with *P. carnosa*.[34] In line with this, the seasonal hub proteins include ones that function in heat responses (Figure 4B). To delineate the key responsive proteins that adapt to temperature fluctuation as the single driver, we carried out a temperature gradient experiment in the laboratory. From the regular culture condition (24°C), coral specimens were treated with changing temperatures that cover the annual range (16°C–28C) at Port Island (Figure 5A; more details in method). Quantitative mass spectrometry identified 2646 coral proteins from the laboratory samples. By applying the same co-regulatory workflow and connectivity analysis, we identified two scale-free networks that are positively or negatively correlated with the temperature changes, respectively (Figures 5C and 5D; Figure S6). Consistent with the findings using natural samples, the hub proteins have stronger correlation with the temperature changes than the satellite proteins (Figure 5B).

HSPA5 belong to the heat shock protein family that consists of ATP-dependent molecular chaperones in the endoplasmic reticulum. In eukaryotic cells, HSP90B1 and HSPA5 chaperones have important functions in many cellular processes, which contain and are not limited to stabilizing and folding proteins DNA repair, development, and the immune response.[53,59] In contrast, the negative module hubs proteins included PRPF8, DDX17, DHX9, etc that are enriched with RNA processing functions (Figures 4C and 4D; Table S5). Functionally, these hub proteins may play a central role in regulating the adaptation to environmental drivers. Indeed, the hub proteins displayed significantly stronger correlation with individual drivers than the satellite proteins (Figure 4E; Figure S5). Understanding the physiological functions of the hub proteins is key to delineating the coral responses to the seasonal fluctuation of the environmental drivers.

Comparing the protein modules of the natural habitat and the temperature gradient, we identified an overlapping network of 60 coral proteins (Figure 5E; Table S6). Of the network hubs, four proteins (HSP90B1, HSPA5, CLTA, and GCA) were positively correlated with the temperature changes and two proteins (SND1 and ARHGAP17) demonstrated negative correlation, suggesting their essential functions in adapting to temperature fluctuation. As discussed previously, HSP90B1 and HSPA5 regulate the unfolded protein response, which is key to survival during various stresses (e.g., heating) that cause protein misfolding.[53] In addition, ARHGAP17, CLTA, and GCA are implicated in membrane trafficking and immune responses, and SND1 has functions in RNA

processing. Overall, these results reinforce the importance of these hub proteins and biological processes in coral host adaptation to temperature changes.

DISCUSSION

Over the past decades, the coral reefs have experienced a large-scale degradation, which is projected to persist for decades.[61] Many studies focused on the mechanisms to enhance thermal resistance and concluded that the heat resistance of coral was underpinned by host-endosymbiont specificity, host transcriptomic plasticity, and diverse endosymbiotic association.[62] Despite the insights, these studies mainly investigated branch corals, including *Acropora*,[63–65] *Pocillopora*,[62,66,67] *Stylophora*,[11,68,69] and so on, with limited investigations of brain corals. Growing evidence indicates that branch and brain corals have different physiological characterizations. For example, branch corals, like *Acropora*, are more likely to rely on nutrients provided by their endosymbionts, while brain corals, like *Platygyra*, obtain nutrients mainly by heterotrophic feeding.[36] Therefore, insights from branch corals may not explain the performances of brain corals.

Here, we present a deep proteome profiling of the brain coral host *P. carnosa*, and revealed the seasonal dynamics and modularity of its protein network. The specimens were collected from the natural habitat with precise recordings of the *in-situ* environmental and physiological parameters. The wet-dry season comparison revealed enrichments of different protein machineries and pathways in acquiring nutrients, suggesting the strategies of brain coral host in adapting to the changes of environmental resources. In addition, growing evidence indicated that nutrient strategy affects the thermal tolerance of stony corals.[36,70] Notably, a recent study reported that nutrient is the most understudied area in coral research.[71] Our study may inform on the mechanisms that underlie stony coral's nutrient strategy in environmental adaptation.

Structural features of the coral host proteome revealed coregulation networks and hub proteins that were closely correlated with the seasonal fluctuations of various environmental drivers. Utilizing laboratory cultures, we validated a series of temperature-correlated hub proteins of important functions in energetic metabolisms, unfolded protein response and immunity. In line with this, studies of bleached tissues of other coral species reported significant proteomic changes of metabolism, heat stress, and immunity.[24,28] Notably, heat shock protein 90 beta and NADH ubiquinone oxidoreductase were also identified as the core proteins associated with coral's environmental responses.[22] Future studies are warranted to investigate the alterations of the protein network during the disruption of coral adaptation that leads to bleaching.

Overall, the present study explored the brain coral host proteome with unprecedented depth and breadth, laying the foundations for a protein-level molecular understanding of brain corals, even other heterotrophic stony corals. The dissection of environment-responsive protein modules will empower the interpretation of studies on how environmental drivers linked to global change alter the physiologic functions of stony coral hosts.

Limitations of the study

It is well known that the coral-*Symbiodiniaceae* symbiosis plays a central role in adapting to environmental fluctuations. Due to the challenges in delineating the endosymbionts proteome, we should cautiously interpret the *Symbiodiniaceae* results (Figure S4). We hence focused on the *P. carnosa* coral host proteins in this work. Deciphering the genetic and proteomic basis of environmental adaptation in *Symbiodiniaceae* and the mechanisms underlying its breakdown are important next steps for developing methods that maintain and restore healthy coral reefs.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to the lead contact, Liang Zhang (liangzhang.28@cityu.edu.hk).

Materials availability

This study did not generate new unique reagents.

Data and code availability

Proteomics data have been deposited into the iProX (ProteomeXchange ID: PXD060745). Raw proteomics datasheets used for analysis could be found in Tables S7 and Table S8. This paper does not report original code, but code used for data analysis and visualization could be found at https://github.com/EveeeeMa/iScience_coral. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization, H.M., L.L.C., and L.Z.; methodology, H.M., Y.P., L.L.C., and L.Z.; investigation, H.M., W.D., and J.T.H.C.; visualization, H.M., G.L., and L.Z.; funding acquisition, H.M., W.D., L.L.C., and L.Z.; project administration, J.W., L.L.C., and L.Z.; supervision, L.L.C. and L.Z.; writing – original draft, H.M. and L.Z.; writing – review and editing, H.M., W.D., J.T.H.C., L.L.C., and L.Z.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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  - Protein extraction and digestion using S-Trap
  - Offline high pH reverse phase fractionation of peptides
  - LC/MS-MS analysis
- QUANTIFICATION AND STATISTICAL ANALYSIS

- Database searching and protein quantification
- Bioinformatic analysis and statistical analysis
- Data visualization

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2025.112287.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
National Bureau of Standards buffer	Xylem Ltd	
Sodium Dodecyl Sulfate (SDS)	Thermo Scientific	Cat#28312
Triethylammonium bicarbonate (TEAB)	Thermo Scientific	Cat#90114
Dithiothreitol (DTT)	Thermo Scientific	Cat#R0861
Iodoacetic Acid (IAA)	Thermo Scientific	Cat#35603
Trypsin Protease	Thermo Scientific	Cat#90057
Formic acid	Thermo Scientific	Cat#28905
Phosphoric acid	Merck	Cat#100573
Methanol	Merck	Cat#106035
Acetonitrile (ACN)	Merck	Cat#100030
Ammonium formate	Merck	Cat#70221
Critical commercial assays		
S-Trap micro kit	Protifi	Cat#NC1828286
Protein Assay Kit (Lowry assay)	Bio-Rad	Cat#5000111
Software and algorithms		
Proteome Discoverer (version 2.2)	Thermo Scientific	
R studio (R version 4.2.1)	Posit	https://posit.co/download/rstudio-desktop/
eggNOG-mapper	Cantalapiedra et al.[72]	https://github.com/eggnogdb/eggnog-mapper
Limma	Ritchie et al.[73]	https://bioinf.wehi.edu.au/limma/
clusterProfiler	Wu et al.[74]	https://github.com/YuLab-SMU/clusterProfiler
ggplot2	Wickham, Hadley[75]	https://ggplot2.tidyverse.org/
Hmisc	Frank E Harrell Jr	https://hbiostat.org/r/hmisc/
Deposited data		
Raw proteomics data of all coral samples	This study	ProteomeXchange ID: PXD060745
Environmental data	Environmental Protection Department of Hong Kong SAR, China	https://cd.epic.epd.gov.hk/EPICRIVER/vicmarineannual/result/
In-situ metabolic rates of coral *Platygyra carnosa*	Dellisanti et al.[76]	https://doi.pangaea.de/10.1594/PANGAEA.937210
Water quality data	Dellisanti et al.[77]	https://doi.pangaea.de/10.1594/PANGAEA.937214
Other		
Platygyra carnosa proteome	Ma et al.[45]	https://doi.org/10.1021/acs.jproteome.0c00812
Cladocopium proteome	Chen et al.[78]	https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_947184155.2/

METHOD DETAILS

Coral fragments collection and laboratory culture

Coral fragments of *P. carnosa* were collected using SCUBA diving in a shallow area (2−4 m depth) at Port Island (Hong Kong, 22°50′ N, 114°35′ E). In this study, three colonies of *P. carnosa* with healthy appearance were randomly selected, and one fragment per colony (about 20 cm^2) was collected using a hammer and chisel (Figure 1B). After collection, coral fragments were kept in a zip-lock bag filled with seawater and transported to the laboratory within two hours. All fragments were stored at −80°C immediately. The in-situ environmental parameters of each sampling were measured as below: Water column oceanographic parameters (temperature, salinity, pH, dissolved oxygen, and turbidity) were monitored with a YSI multiparameter sonde (YSI Exo2 Water Sonde) calibrated in the laboratory prior to each sampling day. The pH was calibrated with National Bureau of Standards (NBS) buffers (pH 4.01,

7.00, 10.06) at 25°C provided by Xylem Ltd. The accuracy of measurements was provided by Xylem Ltd.: temperature (± 0.01), salinity (± 0.01), pH (± 0.1), dissolved oxygen (± 0.1 mg/L), and turbidity (± 0.3 FNU).

Coral fragments collected for laboratory culture were from a single colony of coral *P. carnosa* with a healthy appearance in October 2020. All fragments were kept in a zip-lock bag filled with seawater and transported to the laboratory within two hours, and were cut into small pieces of 9 cm^2 before culture. All fragments were kept at 24°C with natural light period (14:10) for two weeks acclimation (culture medium was refreshed once per week; here we used artificial seawater made with Coral Aquarium Sea Salt as culture medium) before the start of the experiment. After acclimation, 40 coral fragments were randomly separated into two groups and placed in two experimental tanks, cooling tank and warming tank. Temperature setting of each tank was changed once a day at 9:00 a.m., and temperature stabilized within 30 mins (Cooling tank: 24 °C to 16°C, Warming tank: 24°C to 28°C). Two coral fragments were randomly sampled from each tank before the temperature adjustment and kept at –80°C freezer immediately.

Protein extraction and digestion using S-Trap

For protein extraction, coral fragments were grounded into powder in liquid nitrogen using sterile pestles and mortars. The micro S-Trap kit was used according to previous research.[45] Briefly, 0.2 g of coral powders were homogenized in lysis buffer (5% SDS and 50 mM TEAB, pH 7.55). The protein concentration of the lysate was determined using the Lowry assay. Lysates with 50 μg of protein were reduced using 30 mM DTT (final concentrations) at 55°C for 15 min, and alkylated with 25 mM IAA in the dark at room temperature for 30 min. Next, a final concentration of 1.2% phosphoric acid was added to the samples and then mixed with 6.6 volumes of protein binding buffer (90% aqueous methanol, 100 mM TEAB, pH 7.1). The processed samples were loaded into the S-Trap microcolumn in a 1.75 mL collecting tube and centrifuged at 4,000g for 5 min (repeated at least twice). Then, the column was washed three times with 150 μL binding buffer before 150 μL of digestion buffer (50 mM TEAB, pH 8.0) containing trypsin (protein: trypsin = 25:1) was added to the column and incubation at 37°C overnight. The resulting peptides were sequentially eluted by 50 μL of 50 mM TEAB, 0.1% formic acid in water, and 0.1% formic acid in 80% acetonitrile. Three fractions were combined and dried at 45°C. The resulting peptides were resuspended with 50 μL 0.1% formic acid in water.

Offline high pH reverse phase fractionation of peptides

Resuspended peptide sample peptides were fractionated using a Waters XBridge C18 3.5 μm 4.6×250 mm column on a 2695 Separations Module coupled with 2998 Photodiode Array Detector (Water, USA) operating at a flow rate of 0.8 mL/min with three buffer lines: Buffer A consisting of water, buffer B of ACN and Buffer C of 10 mM ammonium formate (pH 8.0). Peptides were separated by a linear gradient from 5% B to 35% B in 62 min followed by a linear increase to 60% B in 5 min and ramped to 70% B in 3 min. Buffer C was constantly introduced throughout the gradient at 10%. Fractions were collected at 90s intervals to a total of 46 fractions. Samples were acidified with formic acid to a final concentration of approximately 0.1% prior to concentration using vacuum centrifugation. 10 μL of buffer A was added to resuspend the peptides, and centrifuged at 21,000 g at 4°C for 5 min. The supernatant was transported into a sample vial and stored at –20°C for LC/MS-MS analysis.

LC/MS-MS analysis

Samples were analyzed using liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) with an Q Exactive HF hybrid quadrupole-Orbitrap mass spectrometer coupled to an EASY-nLC™ 1200 system (Thermo Fisher Scientific, Waltham, MA, USA). Next, 6 μL of each sample was injected, and separation was performed with an analytical C18 column (250 mm, 75 μm, 3 μm; PepSep, Denmark) at a flow rate of 350 nL/min for 34 min. The mobile phase was a mixture of buffer A and buffer B. Elution gradient parameters were set up as follows, using buffer B: 0–25 min in 10–30%; 25–30 min in 25–45%; 30–34 min in 45–80%. Full MS was recorded in positive mode (350 to 1800 m/z) with a mass resolution of 120,000. The positive ion mode was employed with the spray voltage of the mass spectrometer at 2000 V, and a spray temperature of 320°C for peptides. Normalized collision energy was set to 35% and the stripped collision energy was 5%. Automatic gain control settings for Fourier transform mass spectrometry (FTMS) survey scans were 500,000 and for FT MS/MS scans 5000. Maximum injection time was 50 ms for survey scans and 60 ms for MS/MS scans.

QUANTIFICATION AND STATISTICAL ANALYSIS

Database searching and protein quantification

P. carnosa database generated from transcriptomics data[45,79] was used to perform Label-free Quantitation analysis on Proteome Discoverer (version 2.2, Thermo Fisher Scientific, USA). 46 raw files per sample were merged into one for analysis. The parameters used were as follows: enzyme = trypsin, max missed cleavages = 2, static modifications = carbamidomethyl/+57.021 Da (C), dynamic modifications = oxidation/+15.995 Da (M) and phosphorylation/+79.966 (S, T, Y), precursor mass tolerance = 10 ppm, and fragment mass tolerance = 0.02 Da. All protein sequences identified from samples were annotated by eggNOG-mapper v2[72] using default setting. Sequences annotated as metazoan were regarded as coral host proteins. The normalized abundance of proteins determined by Proteome Discoverer was used for downstream analysis.

Bioinformatic analysis and statistical analysis

Bioinformatic analysis was proceeded on R using R studio (R version 4.2.1). Limma[73] package was used for differential expression analysis, and clusterProfiler[74] was applied for biological process enrichment analysis. The co-regulatory network was built on the pairwise spearmen correlation coefficient of the regulatory patterns of protein pair. The pairwise spearmen correlation coefficient and statistical test were calculated and done by using the rcorr function of Hmisc package. The protein modules were clustered by applying hclust function of R stats package on protein correlation matrix. The intragroup connectivity was calculated by $K_i = k_i / k_{max}$ (ki is connection number of protein, and k_{max} is the maximum connection number of protein in the network). The detail of each statistical test is provided in each figure legend. *P* values were significant and reported in the text when $p < 0.05$ (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

Data visualization

Data visualizations were proceeded on R using R studio (R version 4.2.1), and ggplot2[75] package was mainly used. More details could be found in Data and Code under resource availability Section.