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Proteomic profiling of healthy and diseased hybrid soft corals *Sinularia maxima* × *S. polydactyla*

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ABSTRACT: Emerging diseases of marine invertebrates have been implicated as one of the major causes of the continuing decline in coral reefs worldwide. To date, most of the focus on marine diseases has been aimed at hard (scleractinian) corals, which are the main reef builders worldwide. However, soft (alcyonacean) corals are also essential components of tropical reefs, representing food, habitat and the 'glue' that consolidates reefs, and they are subject to the same stressors as hard corals. *Sinularia maxima* and *S. polydactyla* are the dominant soft corals on the shallow reefs of Guam, where they hybridize. In addition to both parent species, the hybrid soft coral population in Guam is particularly affected by *Sinularia* tissue loss disease. Using label-free shotgun proteomics, we identified differences in protein expression between healthy and diseased colonies of the hybrid *S. maxima* × *S. polydactyla*. This study provided qualitative and quantitative data on specific proteins that were differentially expressed under the stress of disease. In particular, metabolic proteins were down-regulated, whereas proteins related to stress and to symbiont photosynthesis were up-regulated in the diseased soft corals. These results indicate that soft corals are responding to pathogenesis at the level of the proteome, and that this label-free approach can be used to identify and quantify protein biomarkers of sub-lethal stress in studies of marine disease.

KEY WORDS: Soft coral · Coral disease · Shotgun proteomics · Hybrid · *Sinularia maxima* · *S. polydactyla*

INTRODUCTION

The global decline in coral reefs, including a 50 and 80% decrease in hard coral cover in the Indo-Pacific and Caribbean regions, respectively (Gardner et al. 2003, Bruno & Selig 2007), has been attributed to a diversity of factors, which include both natural and anthropogenic stressors (Pandolfi et al. 2003, Harvell et al. 2007, Jackson et al. 2014). Disease is one of the primary stressors affecting the loss of reef-building corals, and coral diseases have increased worldwide over the past 3 decades due to interactions with other stressors, including climate change and reduced water quality. These have reduced host immunity and increased pathogen abundance and virulence, contributing to the widespread prevalence of disease in marine organisms (Martin et al. 2010, Ruiz-Moreno et al. 2012, Altizer et al. 2013, Burge et al. 2014).

Among marine diseases, those affecting scleractinian corals are reported with increasing frequency and often on a global scale (Ruiz-Moreno et al. 2012). However, in spite of their prominence in the literature and on the reef, diseases are not confined to reef-building corals, and diseases of soft corals are also increasingly common. One of the most well-characterized marine diseases is aspergillosis, which caused widespread mortality of the Caribbean gorgonian soft coral *Gorgonia ventalina* in the 1990s (Kim & Harvell 2004, Burge et al. 2013a). Other recent disease outbreaks have occurred in the Mediterranean gorgonians *Paramuricea clavata* (Cerrano

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& Bavestrello 2008, Cupido et al. 2008), *Eunicella cavolinii* and *E. singularis* (Carella et al. 2014). In contrast, diseases of alcyonacean soft corals have been rarely reported. To date, these include lesions in *Sinularia* sp. and *Lobophytum* sp. from Palmyra Atoll (Williams et al. 2011), ulcerated yellow spot syndrome in *Sarcophyton ehrenbergi* from Indonesia (Cervino et al. 2012) and *Sinularia* tissue loss disease (STLD) from *Sinularia* spp. from Guam (Slattery et al. 2013). Given the dominance of alcyonacean soft corals on Indo-Pacific reefs, this relative absence of disease reports could be due to these soft corals being less vulnerable to disease or to the fact that few researchers study this group.

Identifying the impacts of disease based on population level characteristics and physiological traits is difficult, as most corals reveal a limited range of visible signs, and the affected organisms may become locally extinct by the time the disease is identified. With the widespread prevalence of coral diseases leading to rapid coral reef decline, there is a pressing need to utilize novel approaches to understand sublethal effects of disease in affected corals and how they adapt to changing environmental conditions (Pollock et al. 2011, Mydlarz et al. in press). Molecular techniques, such as transcriptomics and microarrays, have recently been used to study changes in gene expression relative to abiotic stressors, particularly thermal stress, in several scleractinian corals (e.g. Edge et al. 2005, DeSalvo et al. 2008, Bellantuono et al. 2012, Barshis et al. 2013, Edge et al. 2013, Pinzón et al. 2015). However, fewer studies have evaluated the impacts of disease on gene expression. Transcriptomic approaches have found that *Acropora cervicornis* affected by white band disease has higher expression of genes associated with immune function than healthy colonies (Libro et al. 2013), and expression of the gene for an antimicrobial peptide was up-regulated on exposure to the coral pathogen *Vibrio coralliilyticus* in *Pocillopora damicornis* (Vidal Dupiol et al. 2011). Transcriptomic studies of soft corals are rare (Lopez et al. 2011, Woo et al. 2012), but one study of *G. ventalina* identified differential expression of many genes in response to microbial pathogenesis (Burge et al. 2013b). Likewise, many coral genes have demonstrated differential expression in response to exposure to lipopolysaccharide and other pathogen-associated molecular patterns, which are often used as proxies for pathogen challenge (Kvennefors et al. 2010, Weiss et al. 2013). Unlike the transcriptome, which provides information on changes in RNA production but gives no information about the proteins transcribed or post-translational modifications, the proteome represents the functional phenotype that rapidly responds to environmental changes. To date, studies on changes in levels or activity of individual proteins in response to stressors, including disease, have focused largely on immunoblotting techniques or assays that measure relative activity levels of specific enzymes (Downs et al. 2000, Barneah et al. 2006, Bromage et al. 2009, Mydlarz & Palmer 2011). Weston et al. (2012) appear to be the first to have used quantitative high-throughput proteomics to assess changes in coral protein expression in response to thermal stress. Recently, proteomics has gained importance as an emerging field in the study of human diseases, where it has been used to identify mechanisms associated with the onset and progression of the disease (reviewed in Hanash 2003). In the case of marine organisms, proteomics has become a powerful tool for monitoring disease in the aquaculture industry (Rodrigues et al. 2012). Specifically, expression proteomics, or proteomic profiling, provides information about the differentially expressed proteins between 2 conditions under consideration (e.g. healthy and diseased), while functional proteomics deals with the study of the biological functions of proteins and their relationships to disease processes (reviewed in Slattery et al. 2012).

Soft corals belonging to the genus *Sinularia* represent a major constituent of Indo-Pacific coral reef ecosystems, where they provide food, habitat and the 'glue' that consolidates the reef. *S. maxima* and *S. polydactyla* are the 2 most abundant species of soft corals on the shallow back-reefs surrounding Guam and the islands of Micronesia (Slattery et al. 2001). Slattery et al. (2008) discovered the presence of a hybrid soft coral *S. maxima* × *S. polydactyla* at Piti Bomb Holes, Guam, which surpassed both of the parent species in its resistance to predation and sediment stress. However, the hybrids were observed to be more susceptible the newly emerging STLD than either of the parent species (Slattery et al. 2013). In Guam, STLD was first noted in *S. polydactyla* in 2001, and it subsequently spread to the hybrid and to *S. maxima*. Disease prevalence in the hybrid population ranged from 9 to 12% from 2003 to 2012, compared with 2 to 3% in the parent species (Slattery et al. 2013). STLD has also recently been observed in *S. polydactyla* on reef flats in West Papua, Indonesia, and at multiple sites in the Philippines (M. Slattery & D. J. Gochfeld pers. obs.), indicating that this disease is widespread throughout the western Indo-Pacific. To date, the causative agent for STLD remains unknown, although histological studies of diseased tis-

sue samples revealed the presence of foreign eukaryotic cells in the intermesogleal vacuoles (Slattery et al. 2013). STLD causes gradual tissue degradation and ultimately necrosis (Slattery et al. 2013). The current study uses proteomics as a tool to identify differences in protein expression between healthy and diseased hybrid soft corals, as a preliminary approach to understand the sub-lethal impacts of this disease on soft coral colonies. Other than a recent paper testing high-throughput (i.e. shotgun) proteomics in a scleractinian coral exposed to thermal stress (Weston et al. 2012), this is the first paper to utilize proteomics in cnidarians relative to disease.

MATERIALS AND METHODS

Sample collection and preparation

Small (<1 cm) samples from 'branches' of the hybrid soft coral *Sinularia maxima* × *S. polydactyla* were collected from healthy colonies (n = 3) and from diseased tissues on colonies affected by STLD (n = 3). STLD progress has been characterized in 3 stages (see Fig. 1 in Slattery et al. 2013): wrinkled branches (early stage), heavily wrinkled branches (advanced stage) and tissue loss lesions ultimately resulting in partial or complete colony mortality (late stage). For the current study, the diseased samples were collected from a region of heavily wrinkled branches to ensure that the disease was well advanced but that the tissue was still intact, with no evidence of necrosis. Only the hybrid was used in this preliminary study due to its greater prevalence of STLD relative to the parent species. The coral holobiont (i.e. the coral tissue, along with its symbiotic zooxanthellae and microbiota) was examined as a whole. Samples were collected from <1 m depth in Piti Bomb Holes Marine Preserve, Guam, under a scientific collecting license from the Guam Department of Agriculture. Samples were maintained in seawater for the brief transit to shore and then immediately transferred to cryovials containing urea extraction buffer and placed in liquid nitrogen. Samples were transported to the University of Mississippi on dry ice.

The healthy and diseased hybrid soft coral samples were ground in liquid nitrogen. The ground sample was transferred to 1.5 ml low binding centrifuge tubes (LB CT) to which 0.5 ml urea extraction buffer containing 6 M urea, 2 M thiourea, 40 mM Tris-HCl, 60 mM dithiothreitol (DTT) and 1× Halt protease inhibitor cocktail (PI-78442; Fisher Scientific) was added. The samples were then sonicated for 30 s, 3 times, at 4°C. The samples were incubated by shaking at 4°C for 30 min and then centrifuged at 10 000 × *g* for 16 min at 4°C. The extraction was repeated twice and the pooled supernatants were transferred to 15 ml centrifuge tubes. Acetone, at –20°C, was added in the ratio 1:5 and the samples were incubated overnight at –20°C. Samples were centrifuged at 10 000 × *g* for 60 min at 4°C and the supernatant was decanted. The pellet was air-dried and then redissolved in 700 µl solubilization buffer (6 M urea, 2 M thiourea, 100 mM ammonium bicarbonate) and transferred into 1.5 ml LB CT. The samples were vortexed at 4°C in order to dissolve the pellet completely. The protein concentration was determined using the Bradford assay (Bio-Rad) using a bovine serum albumin (BSA) standard. Sample aliquots of 20 µg were prepared and stored at –80°C until further use.

Reduction, alkylation and digestion

Following the protein assay, the sample (20 µg) was reduced using 100 mM DTT (1 h, at room temperature [RT]) and 300 mM iodoacetamide (IAA) (1/2 h at RT in the dark). Protein samples were digested using trypsin (Promega) 25:1 ratio (protein:enzyme) overnight at RT while vortexing. The reaction was stopped by incubating with trifluoroacetic acid (TFA) to a final concentration of 1% at RT and then centrifuged at 14 000 × *g* for 16 min at RT. The supernatant was transferred to a new LB CT and then lyophilized.

Purification of the sample and nano-ultra performance liquid chromatography-mass spectrometry (UPLC MS^E) analysis

The digested sample (5 µg) was desalted using a C_{18} ZipTip (Millipore) and eluted with 1:1 ratio 0.1% TFA in acetonitrile and water. The sample was lyophilized and then redissolved in 0.1% formic acid (FA) in water. The tryptic digest of *Saccharomyces cerevisiae* enolase (Waters, part no. 186002325) was added to samples as an internal standard. The samples were analyzed on a nanoACQUITY UPLC system (Waters) using MassLynx 4.1, equipped with NanoLockSpray-nanoESI source (Waters) coupled to a SYNAPT first generation mass spectrometer (Waters). The nano-LC separation was performed by loading the sample with 0.1% FA in water onto a trap column (Symmetry C_{18} 5 µm, 180 µm × 20 mm) at a flow rate of 15 µl min^{-1} for 1 min. The binary solvent system of 100% water with

0.1% FA (solvent A), 100% acetonitrile with 0.1% FA (solvent B) was used. The LC separation was performed on a 100 µm × 100 mm, 1.7 µm BEH130 C_{18} analytical column at 400 nl^{-1} min using a gradient of 1 to 40% B over 120 min at 35°C. A concentration of 200 fmol $µl^{-1}$ [Glu^1]-fibrinopeptide B human (GFP) was infused as a calibrant peptide into the NanoLock-Spray ion source at 600 nl min^{-1} and acquired every 30 s. The mass accuracy was calibrated using GFP mass 785.8426 $[M + 2H]^{+2}$. The mass spectrometer was operated in positive V-mode with alternate low- and high-energy modes of acquisition. The specific mass to charge ratio (*m/z*) scan range was from 50 to 1990, with an acquisition scan time of 1 s, constant collision energy of 6 eV in low-energy mode and in the elevated mode the collision energy was increased from 15 to 40 eV. Each sample was analyzed in triplicate by loading 1 µg of digested protein onto the nanoACQUITY UPLC system using the enolase digest as an internal standard at a final concentration of 100 fmol $µl^{-1}$.

Data processing and ProteinLynx Global SERVER search for protein identification

Low energy and elevated energy modes of acquisition (MS^E) raw data files were processed for deisotoping and deconvolution using ProteinLynx Global SERVER (PLGS) v.2.5 (Waters). Exact mass retention time (EMRT) peptide clusters were identified using PLGS software. Protein identifications were obtained using an ion accounting algorithm within PLGS by searching against an in-house-generated non-redundant database (21 488 entries) and against a *Symbiodinium* database (588 entries) downloaded from the Swiss-Prot database (http://www.uniprot.org) to which the enolase sequence was appended. The following parameters were used in the PLGS search: peptide and fragment tolerance was set to automatic; minimum fragment ion matches per peptide (3); minimum number of product ion matches per protein (7); minimum peptide matches per protein (2); carbamidomethyl C and oxidation M as fixed and variable modifications, respectively; maximum number of missed tryptic cleavages (1); and false positive rate of the identification algorithm was set to 4%. Label-free quantification and changes in the abundance of proteins between healthy and diseased samples was performed using Waters differential protein expression analysis software, which is part of PLGS. Normalization of the data was done using the internal standard enolase (P00924). The results in Table 1 include only proteins that were identified with a confidence level >95%, although proteins that were identified with a confidence level of 50 to 95% are included in Table 2 (Xu et al. 2008). The up- and down-regulation of the proteins in the diseased soft corals, relative to the healthy soft corals, was identified using the following probability criteria: $0 \leq p \leq 0.05$ for up-regulation and $0.95 \leq p \leq 1$ for down-regulation, respectively.

RESULTS

The proteins identified with a confidence level of >95% are included in Table 1. Of the 18 proteins identified with this level of confidence, 16 proteins exhibited significant quantitative variability (i.e. significantly increased or decreased expression) between healthy and diseased soft corals. Of these, 8 proteins (5 metabolic, 1 stress and 2 chaperone proteins) were down-regulated and 8 proteins (5 stress, 1 metabolic and 2 photosynthetic proteins) were up-regulated in the diseased samples relative to the healthy samples. One additional protein identified with >95% confidence was found to be unique to the healthy soft corals, and one was found to be unique to the diseased soft corals. In addition, 4 proteins identified with a confidence level of 50 to 95% were also up-regulated in the diseased soft corals (Table 2), while 3 proteins identified at this level of confidence in the diseased soft corals were not found in healthy soft corals (Table 2).

DISCUSSION

Although coral diseases are an ongoing global phenomenon that has negatively influenced the stability and structural features of the reef, relatively little is known about their sub-lethal physiological impacts. To our knowledge, this is the first study to use quantitative proteomics as a starting point to understand the effects of soft coral pathogenesis on various cellular processes. Data from this study identified differences in protein expression in healthy and diseased hybrid soft corals. Within the diseased soft corals, there was significant down-regulation of several metabolic proteins and significant up-regulation of several stress proteins. These results suggest that the stress of disease carries a physiological cost manifested in reduced metabolic activity compared to the healthy soft corals, which is likely due to the production of stress proteins integral to cellular repair pro-

Table 1. Proteins significantly up- or down-regulated in healthy vs. diseased hybrid soft coral samples, based on protein identification with a confidence limit of >95%. H:D represents the protein ratio in healthy/diseased soft corals: >1 down-regulated in diseased soft corals, <1 up-regulated in diseased soft corals. $0.95 \leq p \leq 1$ for down-regulation in diseased soft corals; $0 \leq p \leq 0.05$ for up-regulation in diseased soft corals

Protein identification (source species)	Ratio (H:D)	p-value	SwissProt accession number	Function
Proteins down-regulated in diseased hybrid soft corals				
Heat shock cognate 71 kDa protein (*Danio rerio*)	1.27	>0.99	Q90473	Stress
Glyceraldehyde-3-phosphate dehydrogenase (fragment) (*Symbiodinium* sp.)	1.45	>0.99	Q76EI4	Metabolism
Proliferating cell nuclear antigen (fragment) (*Symbiodinium goreaui*)	2.03	>0.99	A5X6J9	Metabolism
78 kDa glucose-regulated protein homolog (*Schizosaccharomyces pombe*)	1.25	>0.99	P36604	Metabolism
Poly [ADP-ribose] polymerase 4 (*Crassostrea gigas*)	1.35	>0.99	K1PUC2	Metabolism
Ankyrin domain family member E (*Homo sapiens*)	1.27	>0.99	Q6S8J3	Metabolism
Ubiquitin (*Ostrea edulis*)	1.14	0.95	Q0R0E9	Chaperone
Polyubiquitin (*Pongo pygmaeus*)	1.14	>0.99	Q39256	Chaperone
Proteins up-regulated in diseased hybrid soft corals				
Heat shock protein Hsp70 (fragment) (*Chondrosia reniformis*)	0.89	0.02	O44350	Stress
Spring green fluorescence emitting protein (*Sarcophyton* sp.)	0.88	0.01	B5BRC3	Stress
GFP-like fluorescent chromoprotein cFP484 (*Clavularia* sp.)	0.79	<0.0001	Q9U6Y3	Stress
Green fluorescent GFP-like protein (*Sarcophyton* sp.)	0.66	0.03	B6CTZ6	Stress
Cyan fluorescent protein (fragment) (*Montastraea cavernosa*)	0.61	<0.0001	Q95UA7	Stress
Peridinin–chlorophyll *a*-binding protein apoprotein (*Symbiodinium* sp.)	0.91	0.03	Q8H1J6	Photosynthesis
Light-harvesting protein (fragment) (*Symbiodinium* sp. C3)	0.68	<0.0001	E0WED4	Photosynthesis
Neural alfa2 tubulin (*Paracentrotus lividus*)	0.75	<0.0001	Q6TS36	Metabolism
Unique proteins found in either healthy or diseased hybrid soft corals				
Azami-Green (*Galaxea fascicularis*)	Diseased		Q60I25	Stress
Triosephosphate isomerase (fragment) (*Obelia* sp.)	Healthy		Q6PTK3	Metabolism

Table 2. Proteins significantly up-regulated in diseased hybrid soft coral samples and unique proteins found in either healthy or diseased samples, based on protein identification with a confidence limit of 50 to 95%. H:D represents the protein ratio in healthy/diseased soft corals: <1 up-regulated in diseased soft corals. $0 \leq p \leq 0.05$ for up-regulation in diseased soft corals

Protein identification (source species)	Ratio (H:D)	p-value	SwissProt accession number	Function
Proteins up-regulated in diseased hybrid soft corals				
Heat shock protein 90 1 (fragment) (*Symbiodinium* sp. C3)	0.38	0.01	Q0R0F7	Stress
Ribulose-1, 5-bisphosphate carboxylase/oxygenase large subunit (*Symbiodinium* sp. clade C)	0.61	<0.0001	A9YEC5	Photosynthesis
Malate dehydrogenase chloroplastic (*Arabidopsis thaliana*)	0.84	0.04	Q9SN86	Metabolism
Zinc finger CCCH domain-containing protein 13 (*Homo sapiens*)	0.53	<0.0001	Q5T200	Transcription
Unique proteins found in either healthy or diseased hybrid soft corals				
Cytochrome P450 61 (*Schizosaccharomyces pombe*)	Diseased		O13820	Steroid biosynthesis
Ascorbate peroxidase (*Symbiodinium* sp. clade C)	Diseased		E2FJQ6	Stress
Putative catalase peroxidase 61 (*Symbiodinium microadriaticum*)	Diseased		W6ABZ1	Stress
Superoxide dismutase (*Symbiodinium* sp.)	Healthy		A3KLM4	Stress

cesses in diseased soft corals. In addition, this study observed differential expression of several functional proteins that would likely have significant immunological and physiological effects and would ultimately impact the fitness of these hybrids on coral reefs.

Protein expression analysis of the healthy and diseased soft corals indicated that several fluorescent proteins (FPs) were significantly up-regulated in diseased samples, and Azami-Green (Q60I25), from the hard coral *Galaxea fascicularis* (Karasawa et al. 2003), was found only in diseased samples. FPs serve

a number of roles that protect the coral holobiont from stress, including photoprotection of the coral's dinoflagellate symbionts from potentially damaging solar irradiation (Salih et al. 2000). They also serve as part of the immune response, in which FPs scavenge reactive oxygen species, often produced during phagocytosis of microbial pathogens, thereby contributing to the coral's antioxidant response (Palmer et al. 2009a). Up-regulation of FPs in scleractinian corals is often stimulated by injury or pathogenesis and accompanied by increased hydrogen peroxide scavenging and a change in the coral's pigmentation (Palmer et al. 2009b, D'Angelo et al. 2012). Although increased expression of FPs in diseased soft corals was not associated with a pigmentation response, the observed up-regulation likely represents a stimulation of the antioxidant immune defense in diseased corals in response to STLD, which could enhance their ability to provide photoprotection to their zooxanthellae in order to maintain this symbiosis under stress. Owing to their activities in photoprotection and immunity, FPs may serve as useful markers for identifying physiological stress associated with disease and bleaching in soft corals

Also up-regulated in the diseased soft corals were photosynthetic proteins associated with the symbiont, including Peridinin–chlorophyll *a*-binding protein (PCP, Q8H1J6; Table 1), ribulose-1,5-biphosphate carboxylase/oxygenase (RuBisCo, A9YEC5; Table 2) and a light-harvesting protein (E0WED4; Table 1). When subjected to various stressors, the coral holobiont may respond by altering metabolic and photosynthetic activity, thus affecting the coral–zooxanthellae symbiosis. In conjunction with increased expression of stress genes in the host coral *Montastraea cavernosa* exposed to abiotic stressors, Edge et al. (2013) found increased expression of symbiont genes related to metabolism, carbon dioxide fixation (including RuBisCo) and growth, but decreased expression of genes related to photosynthesis and photoprotection (including PCP). However, Shearer et al. (2012) found up-regulation of PCP genes in zooxanthellae of *Acropora millepora* while undergoing allelopathy with various species of algae. Unlike some coral diseases (e.g. yellow band disease; Cervino et al. 2008) in which zooxanthellae are specifically damaged, the symbionts do not appear to be directly targeted by STLD, at least as observed via histology (D. A. Renegar pers. comm.). However, our results suggest that photosynthetic function within the soft coral *Sinularia maxima* × *S. polydactyla* is either directly or indirectly impacted by STLD and may be involved in protection of the already immunocompromised soft coral from further deleterious consequences that might affect the soft coral–algal symbiosis.

The results of our protein expression analysis also indicated up-regulation of the heat shock proteins HSP70 (Table 1) and HSP90 (Table 2) in the diseased soft corals. HSPs are produced by both corals and their symbiotic zooxanthellae in response to various types of stressor, including temperature changes and increased exposure to UV light (Robbart et al. 2004, DeSalvo et al. 2010). They play critical roles as molecular chaperones, facilitate the repair of stress-associated misfolded proteins and prevent their aggregation, which could otherwise be harmful to the coral (Lanneau et al. 2008). Thus, HSPs serve important roles in cellular protection and repair processes, and they are commonly used as biomarkers of various types of stress in corals (Sharp et al. 1997, Downs et al. 2005, Shearer et al. 2012, Kenkel et al. 2014). For example, in the soft coral *Dendronephthya klunzingeri*, HSP90 was found to be up-regulated in response to thermal stress and PCBs (Wiens et al. 2000), and both HSP70 and HSP90 were up-regulated in the soft coral *Scleronephthya gracillimum* when exposed to benzo[a]pyrene (Woo et al. 2012), whereas the hard coral *A. millepora* exhibited increased expression of HSP genes as an immune response to microbial challenge in the absence of heat stress (Brown et al. 2013). Increased levels of expression of HSPs in our diseased samples may similarly represent an immune response to STLD stress. Interestingly, the protein Q90473 (Table 1), also belonging to the HSP70 family, was down-regulated in our diseased samples. This constitutively expressed HSP, also known as heat shock cognate protein, is a molecular chaperone protein that performs conventional cellular functions (Moon et al. 2001). HSC70 occurs as an integral part of the cells and facilitates proper folding of proteins, while providing protection against oxidative stress, pH fluctuations and exposure to UV light in oyster oocytes (Corporeau et al. 2012), and it has been shown to be up-regulated in response to thermal stress in corals (Robbart et al. 2004).

A variety of proteins associated with cell repair and proliferation, as well as metabolism, were also differentially expressed in healthy and diseased hybrid soft corals. Ubiquitin (Q0R0E9; Table 1), which serves multiple roles in the degradation and repair of damaged or misfolded proteins (Downs et al. 2000), was down-regulated in diseased soft corals. Whereas up-regulation of ubiquitin has been frequently reported in corals in response to heat or hyposalinity

stress (Downs et al. 2000, 2009, DeSalvo et al. 2010), other ubiquitin-like proteins were down-regulated in coral larvae in response to heat stress (DeSalvo et al. 2008, Meyer et al. 2011) and differentially expressed in *A. millepora* transplanted from sites throughout the Great Barrier Reef to a common garden site at Heron Island (Granados-Cifuentes et al. 2013). Proliferating cell nuclear antigen (PCNA, A5X6J9; Table 1), which is involved in DNA replication (Moldovan et al. 2007), was down-regulated in the diseased soft corals, likely due to reduced capacity for growth or tissue regeneration in STLD-affected soft corals. In contrast, PCNA was up-regulated in experimentally wounded samples of the reef-building coral *Porites lobata*, which indicated locally accelerated growth as a component of the repair mechanism (D'Angelo et al. 2012), while the oyster *Crassostrea gigas* exhibited lower levels of PCNA, which in turn affected reproductive capacity, in animals stressed by reduced food (Jouaux et al. 2013). Various proteins involved in the metabolic pathways were either up- or down-regulated in the diseased soft corals. For example, GAPDH (Q76EI4; Table 1), which is involved in the production of glucose for energy and carbon via glycolysis, was down-regulated. However, GAPDH may also be involved in non-metabolic processes, including transcription activation and initiation of apoptosis (Krasnov et al. 2013). A poly [ADP-ribose] polymerase 4 (PARP; Table 1) was down-regulated during exposure to benzo[a]pyrene in the soft coral *S. gracillimum* (Woo et al. 2012), as well as in our diseased soft corals. In contrast, malate dehydrogenase (MDH; Table 2), which is a catalyst involved in the Krebs cycle metabolic pathway (Minárik et al. 2002), was up-regulated in the diseased soft corals. Thus, unsurprisingly, metabolic function in the soft coral *S. maxima* × *S. polydactyla* appears to be affected by disease. Several other proteins, including glutathione peroxidase (which is an oxidative stress response protein), chaperone proteins and proteins involved in metabolism and biosynthesis were also identified in the soft coral samples, but these did not exhibit significant up- or down-regulation in the diseased soft corals. This may be an artefact of the limited number of annotated cnidarian proteins within existing proteomic databases and represents an interpretative challenge for researchers working with non-model marine organisms (Slattery et al. 2012, Weston et al. 2012).

In summary, STLD in the soft coral *S. maxima* × *S. polydactyla* resulted in significant changes to the soft coral holobiont proteome, including proteins involved in repair, photosynthesis, stress responses and metabolic pathways. Given the diverse functional roles of these proteins, and the likely cost of their expression (Palmer et al. 2010, Tomanek & Zuzow 2010), our results indicate that STLD will have significant physiological, ecological and evolutionary consequences for these important constituents of Pacific coral reef communities. Furthermore, these results indicate that shotgun proteomics can be utilized to identify the functional differences in proteins expressed by healthy and diseased individuals, providing a greater understanding of stress responses relative to data acquired in the more commonly used transcriptomic approaches (e.g. Hoover et al. 2007). The data presented here represent a pilot-scale study that demonstrates the value of proteomics in research on marine diseases; future studies will focus on differential susceptibility to STLD between the parents and the hybrid, and how their proteomes mediate these processes.

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