Resilience to climate change in an octocoral involves the transcriptional decoupling of the calcification and stress response toolkits.

Sergio Vargas[1,*], Thorsten Zimmer[1], Nicola Conci[1], Martin Lehmann[2], Gert Wörheide[1,3,4]

Author affiliations:

1. Department of Earth and Environmental Sciences, Paleontology & Geobiology, Ludwig-Maximilians-Universität München, Richard-Wagner-Str. 10, 80333 München, Germany.
2. Biozentrum der LMU München, Department of Biology I–Botany, Grosshaderner Strasse 2–4, Planegg-Martinsried, Germany.
3. GeoBio-Center, Ludwig-Maximilians-Universität München, Richard-Wagner-Str. 10, 80333 München, Germany.
4. SNSB – Bayerische Staatssammlung für Paläontologie und Geologie, Richard-Wagner-Str. 10, 80333 München, Germany.

* Corresponding author: sergio.vargas@lmu.de

Abstract

Up to one-third of all described marine species occur in coral reefs, but the future of these hyperdiverse ecosystems is insecure due to local and global threats, such as overfishing, eutrophication, ocean warming and acidification. Although these impacts are expected to have a net detrimental effect on reefs, it has been shown that some organisms like octocorals may remain unaffected, or benefit from, anthropogenically induced environmental change, replacing stony corals in future reefs. Despite their importance in future shallow-water environments, the molecular mechanisms leading to the resilience to anthropogenic-induced stress observed in octocorals remain unknown. Here, we use manipulative experiments, proteomics, and transcriptomics to show that the molecular toolkit used by *Pinnigorgia flava*, a common Indo-Pacific gorgonian octocoral, to deposit its calcium-carbonate skeleton is resilient under simulated climate change. Sublethal, simulated global warming triggered a stress response in *P. flava* but did not affect the expression of 27 transcripts that encode Skeletal Organic Matrix (SOM) proteins present in this species' skeleton. Exposure to simulated ocean acidification did not cause a stress response but triggered the downregulation of many transcripts, including an osteonidogen homolog present in the SOM. The observed transcriptional decoupling of the skeletogenic and stress-response toolkits provides a mechanistic explanation for the resilience to anthropogenically-driven environmental change observed in octocorals.

Introduction

As a consequence of anthropogenic-induced global climate change, extreme climatic events, like the heat waves affecting the Great Barrier Reef in 2016, 2017, and 2020, are likely to increase in frequency impacting marine communities in unprecedented ways (Hughes et al., 2017, 2018, 2019). In coral reefs, these phenomena can lead to the displacement of stony corals as the dominant reef-building taxon by other benthic organisms and cause community phase shifts with ecosystem-level effects (Done, 1992). To date, both theoretical (Fung, Seymour, & Johnson, 2011) and empirical (Schmitt, Holbrook, Davis, Brooks, & Adam, 2019) studies predict and support the occurrence of phase shifts in coral reefs to non-coral dominated systems at a global scale, shifting to communities where groups as diverse as algae, sponges, and other cnidarians such as corallimorpharians and octocorals are the key players (see Norström, Nyström, Lokrantz, & Folke, 2009 for a review).

In many marine ecosystems, octocorals (*e.g.*, soft-corals, gorgonians) are common and important community members. They increase the spatial complexity of the habitats in which they occur (Quattrini et al., 2014) and provide refuge to numerous invertebrate species (Buhl-Mortensen & Mortensen, 2005; Cúrdia et al., 2015) . In shallow water ecosystems, such as coral reefs, octocorals can outgrow stony corals and become dominant after extreme climatic events, like anomalously strong El Niño events (Ruzicka et al., 2013), or under extreme environmental conditions, like those prevailing in volcanic-seep acidified waters (Inoue, Kayanne, Yamamoto, & Kurihara, 2013a). For instance, in Florida, octocorals increased their abundance over 11 years after the 1997/98 El Niño event, becoming the most abundant taxon in some localities and dominating shallow fore-reefs (Ruzicka et al., 2013). Similarly, the soft coral genus *Rhytisma* became dominant after a significant coral bleaching event in the Aldabra Atoll (Indian ocean) in 1998 (Spencer et al., 2005), and the "blue coral," *Heliopora coerulea*, a species of the reef-building zooxanthellate octocoral clade Helioporacea, increased its abundance to over 50% over a decade in the Bolinao Reef Complex in the northern Philippines (Atrigenio, Aliño, & Conaco, 2017). Like scleractinians, helioporacean octocorals produce a rigid skeleton made of aragonite (a polymorph of calcium carbonate) (Colgan, 1984), but their calcification machinery appears to be more resilient to environmental stressors than that of most scleractinians (Kayanne, Harii, Ide, & Akimoto, 2002; Shaish, Levy, Katzir, & Rinkevich, 2010). Thus, octocorals may be possible winners in future global climate change scenarios, potentially replacing scleractinians as the main reef framework-building organisms (Inoue, Kayanne, Yamamoto, & Kurihara, 2013b).

Studies on scleractinian corals indicate that the response of these organisms to anthropogenic-driven climate change depends on the stress source and differs among species and ontogenetic stages (Davies, Marchetti, Ries, & Castillo, 2016; Moya et al., 2012; Thomas et al., 2018). In *Acropora hyacinthus*, heat stress triggers a large and dynamic transcriptomic response characterized by the modulation of different metabolic and cell cycle processes in the early stress response, and of spliceosome activity, RNA- and DNA-related metabolic processes, cell stress and cell metabolism before bleaching onset (Seneca & Palumbi, 2015). In contrast, ocean acidification does not appear to trigger a pronounced transcriptional response in adult corals colonies (Davies et al., 2016; González-Pech, Vargas, Francis, & Wörheide, 2017) but elicit the modulation of several calcification-related genes in the primary polyps of *Acropora millepora* (Moya et al., 2012). Despite the observed tolerance to climate change-induced stress of octocorals, their resilience mechanisms to tolerate climate change-driven stress that enables outgrowing and outcompeting less resilient organisms, like stony corals, remain unknown.

However, since growth in octocorals requires the deposition of new calcium-carbonate skeletal elements (calcite sclerites, or aragonite fibers in case of the blue corals) to support the colony structurally (Lewis & Vonwallis, 1991), resilience to climate change in this group must be linked to their ability to sustain calcification under challenging abnormal environmental conditions (Gabay, Benayahu, & Fine, 2013; Gabay, Fine, Barkay, & Benayahu, 2014; Gómez et al., 2015; Inoue et al., 2013a). Indeed, with the exception of the Helioporacea, the octocoral tissues effectively isolate sclerocytes from the surrounding seawater (Gabay et al., 2014), putatively allowing these cells to sustain the expression of transcripts involved in calcification under situations of environmental stress, and the colonies to outgrow and outcompete less resilient organisms. To test this hypothesis, we characterized the skeletal proteome of the common Indo-Pacific gorgonian octocoral *Pinnigorgia*

flava and use manipulative experiments and transcriptomics to assess the effect of simulated global warming and ocean acidification on the expression of transcripts involved in calcification in this species. Together, our data provide new insights into the mechanisms of resilience used by octocorals to cope with anthropogenically driven climate change.

Materials and Methods

Experimental model and subject details

We used clonal explants of a colony of *Pinnigorgia flava* (Nutting, 1910) kept at 25 °C and a 12:12 h light cycle in a 642 L marine aquarium system at the Department of Earth- and Environmental Sciences, Paleontology and Geobiology, Ludwig-Maximilians Universität München (see below). *P. flava* is a colonial, zooxanthellate soft coral (Octocorallia) endemic to the Great Barrier Reef and sporadically found in the coral triangle in SE Asia. The colony under study is cultured in our research aquaria since more than a decade. It forms non-anastomosing, pinnated light purple colonies with brownish polyps. The sclerites are white to yellow bent spindles, capstans, and rods. Currently, *P. flava* belongs to the family Gorgoniidae.

Determination of calcification hotspots along the body axis of P. flava

We used calcein, a calcium-binding fluorescent dye that permanently incorporates into the skeleton, to investigate the distribution of calcification sites along the body axis of *P. flava* (Holcomb, Cohen, & McCorkle, 2013). We incubated three colonies of *P. flava* for 72 h in a glass container with 500ml of a 50µg/ml calcein disodium salt (Sigma-Aldrich) in 0.2 µm filtered artificial seawater (Suppl. Fig. 1). We exchanged the seawater with fresh seawater+calcein every 24 h. After staining, we fixed the colonies in 80% EtOH and stored them at ~5 °C until further processing.

To assess whether calcification preferentially occurs on the tip of the colonies or, on the contrary, the calcification hotspots occur along the colony body axis, we cut the colonies from top to bottom every five millimeter using a sterile scalpel and placed each piece in 1.5 ml microcentrifuge tubes containing one ml of sodium hypochlorite (NaOCl 10%; Fluka). After a three hour incubation in bleach, we rinsed the sedimented sclerites six times with distilled water and stored them in 80% ethanol. We then placed a sample of sclerites onto glass slides and embedded them in Eukitt quick-hardening mounting medium (Fluka Analytical) before covering the sample with a glass coverslip.

We observed the sclerites under epifluorescence (excitation band-pass filter 420-490 nm, barrier long-pass filter 515 nm) on a Leica DMLB microscope coupled to a Leica DFC 480 camera and an I3 filter set. We exposed the stained sclerites for ten seconds and acquired pictures using Leica Application Software “LAS V4.5”. To determine the number of stained sclerites per colony region, we sampled the top and bottom fragments of the colonies and counted stained and total sclerites at a 100X magnification along one horizontal transect crossing the slide from left to right.

Proteomic analysis of the skeletal organic matrix of P. flava's *sclerites*

To determine the skeletal proteome of *P. flava*, we sampled four colonies of about four centimeter in length and incubated them in sodium hypochlorite (5%, Fluka) for 72 h under moderate shaking (30 rpm; IKA Rocker 3D digital). We then rinsed the sedimented sclerites six times with Milli-Q water and dried them at 37°C for 24 h. This procedure yielded approximately 0.75 g of dry sclerites, which we ground with a mortar and pestle before incubating again in sodium hypochlorite (2%) for four hours under moderate mixing (30 rpm). After bleaching, we rinsed the powder six times with Milli-Q water and dried it overnight at 37°C. To dissolve the calcitic mineral, we incubated the dry powder in 10% acetic acid overnight under moderate mixing (20 rpm). We centrifuged the resulting solution at 13,500 rpm for 30 min to separate the acetic-insoluble matrix (AIM) from the acetic-soluble matrix (ASM). To isolate the ASM, we centrifuged (4600 rpm for 70 min at 16°C) the supernatant through 15 mL Amicon ultrafiltration devices with a 3 kDa cutoff membrane and added four volumes of methanol, one volume of chloroform and three volumes of Milli-Q water to one volume of desalted solution to precipitate the proteins by centrifugation at 5,500 rpm for 15 min (Wessel & Flügge, 1984). After discarding the upper phase, adding three volumes of methanol and centrifuging the sample at 5,500 rpm for 15 min, we air-dried the resulting ASM pellet and resuspended both the ASM and AIM fractions in 95% Laemmli buffer + 5% β-mercaptoethanol. We used a 1-dimensional sodium dodecyl sulfate-polyacrylamide (SDS-PAGE) gel (Mini-PROTEAN Tetra System, Bio-Rad, USA) to separate electrophoretically the skeletal organic matrix proteins before mass spectrometry. To visualize the extracted SOM protein fractions, we ran an SDS-PAGE for 90 min at 80V, increasing the voltage to 100V after the gel front passed the boundary between the stacking and the resolving gel. We used the Precision Plus Protein Dual Xtra Standard (Bio Rad,12 band, 2kD-250kD) as a size standard and stained the gel after fixing for 20 min in a fixation solution (50% ethanol, 40% Milli-Q and 10% acetic acid), washing in 30% ethanol for ten minutes, and in Milli-Q water for ten minutes, with silver nitrate using the Proteo Silver Plus Silver Stain Kit (Sigma-Aldrich, USA). For this, we incubated the gel in sensitizer solution for ten minutes, washed it as described above, and equilibrated it for ten minutes in the silver solution. Before developing, we washed the stained gel for one minute in Milli-Q water and submerged it in a developing solution for five minutes. After stopping the development reaction, we washed the gel for 15 min in Milli-Q water. We used an orbital shaker at 60 rpm for all steps described above. For mass spectrometry, we ran the SDS-PAGE with the six technical replicates of the extracted SOM protein fractions for 40 min at 80V until the protein extracts passed the boundary between the stacking and the resolving gel, and manually excised the bands with a sterile scalpel. We then subjected the isolated proteins to alkylation, reduction, and tryptic digestion (0.1 µg/µl trypsin at 37°C, overnight). We used an LTQ Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) coupled with a Rheos Allegro liquid chromatograph (Flux Instruments GmbH, Basel, Switzerland) to analyze three µl of the digested sample after separation using a self-made column of 75 µm diameter, 15 cm length, C18 particles of 2 µm diameter and 100 Å pore size (Dr. Maisch GmbH, Ammerbruch-Entringen, Germany). We prepared the MS grade mobile phases as follows A) water containing 10% acetonitrile (ACN), B) ACN containing 10% water, each combined with 0.1% formic acid. We used the following gradient: 40 min (0-23% B), 40 min (23-85% B), five minutes (85-100% B), 25 min (100% B), three minutes (100-0% B) and 20 min (0 % B) for re-equilibration, and a constant 40 µl/min flow at RT (22°C). We used the programs Xcalibur 2.0 (Thermo Fisher Scientific Inc., 30 Waltham, USA) and MaxQuant Version 1.5.2.8 (Cox and Mann 2008, doi: 10.1038/nbt.1511) to acquire and analyze the

MS/MS data, respectively. To estimate protein abundances, we used the MaxLFQ algorithm (Cox et al., 2014) and calculated the relative iBAQ value (Schwanhäusser et al., 2011) of the detected SOM proteins. We filtered the LC-MS/MS results to remove hits from known conventional contamination sources using the common Repository Adventitious Proteins (cRAP) database before mapping the peptides against a transcriptome reference of *P. flava* (Conci, Wörheide, & Vargas, 2019). We translated the transcripts matching proteins present in the SOM fractions and annotated them against the UniProtKB database using blastp (https://blast.ncbi.nlm.nih.gov/), and SignalP 4.0 (Petersen, Brunak, von Heijne, & Nielsen, 2011) to predict the presence of signal peptides, transmembrane regions, and GPI anchors within the predicted protein sequences. Additionally, to assess the distribution of the detected *P. flava* SOM proteins among cnidarians, we screened a database composed of 120 transcriptomes from representatives of this phylum using *P. flava's* SOM proteins as queries for the search. We deposited the mass-spectrometer *RAW* files in the LMU Open Data repository, https://data.ub.uni-muenchen.de/189/.

In vivo experiments:

We used a 360 L marine aquarium under a 12h day, 12h night cycle controlled by GHL Mitras LX 6200-HV LED lights that yielded ten kLux at the water surface. Based on hourly measurements over one year (2017), average water temperature and pH in the system are 24.92 ± 0.24 °C and 8.30 ± 0.14, respectively. Based on weakly measurements over one year (2017), the average PO_4^{-3}, NO_2^- and NO_3^- concentrations in the water are 0.092 ± 0.071 mg/L, 0.014 ± 0.072 mg/L, 2.681 ± 3.882 mg/L; the concentration of NH_3/NH_4^+ in the water was consistently below detection (i.e. < 0.05 mg/L). Weekly or daily monitoring in subsequent years revealed that these values are stable and can be taken as a baseline for the system.

To assess the effect of global warming on *P. flava*, we randomly assigned nubbins (n = 18) to six ten liter aquaria filled with ca. six liter artificial seawater and partially immersed in the 360 L aquarium described above. Water evaporation in the ten liter thanks was compensated every day with water filtered by reverse osmosis. To provide adequate water mixing in each tank, we used a submersible water pump (300 L/h; Eheim, Germany). For the temperature experiment, after an acclimation period of four days, we randomly selected three tanks and gradually increased the water temperature to 29-30°C for five days (~1°C per day) using 50 W water heaters (Eheim, Germany). We then kept the *P. flava* colonies at 29-30°C for three days. Afterward, we cut octocorals in two lower and upper sections using sterile scissors and flash-frozen them in liquid nitrogen before storing them at -80°C until further processing. During the experiment, we monitored the water temperature every minute using PCE-PHD 1 dataloggers (PCE Instruments, Germany) and, additionally, we manually measured the temperature of all aquaria twice a day (in the morning and the evening) using a regular thermometer (TFA, accuracy ± 0.5°C). We monitored water conductivity, pH, density, redox potential, and nutrient profile in the ten liter tanks every other day. The observed values were similar to those of the main tank.

We assessed the effect of ocean acidification on twelve colonies of *P. flava* distributed in two 30 L tanks (control and treatment) connected to a 320 L salt-water "mother" tank. To lower (0.1 pH units drop per day) the seawater pH, we pumped CO_2 into the treatment tank (see details in González-

Pech et al., 2017) to achieve a pH of 7.8 (from a starting value of 8.2). We kept the pH stable for three days and lowered it again to 7.6 over three days. After reaching pH = 7.6, we allowed the octocorals to acclimate for three days before decreasing the pH to its final value of 7.3. We maintained this pH for a period of ca. two months using an automatic pH computer and monitored it throughout the whole experiment using a PCE-PHD 1 datalogger (PCE Instruments, Germany). The pH of the "mother" and control tank was 8.2 throughout the experiment. The temperature and nutrient levels in the "mother" tank were as detailed above. At the end of the experiment, we cut the octocorals at the base using sterile scalpels and then flash-frozen them in liquid nitrogen before storing them at -80°C until further processing.

Analysis of differential gene expression

We extracted total RNA from the upper section of the octocorals (see above) using the Direct-zol RNA MiniPrep kit (Zymo Research) following the manufacturer's protocol. We assessed the purity and integrity of the RNA extracts using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA) and a Bioanalyzer 2100 (Agilent Inc., USA). In general, all samples showed high 260/230 and 260/280 ratios and RIN values above 8.5. We used Lexogen's SENSE Total RNA-Seq Library Prep Kit according to the manufacturer's instructions to generate Illumina-ready, stranded transcriptome libraries for all extracted samples. We sequenced the libraries (50 PE) in an Illumina HiSeq2000, quality controlled the resulting reads with the program *bl-filter-illumina* from the BioLite suite (Howison, Sinnott-Armstrong, & Dunn, 2012) and mapped them to an available *P. flava* reference transcriptome (Conci, Wörheide, et al., 2019) using Salmon (Patro, Duggal, Love, Irizarry, & Kingsford, 2017). We analyzed the resulting count matrix using DESeq2 (Love, Huber, & Anders, 2014) and used the obtained list of differentially expressed genes for GO-term enrichment analyses using TopGO (Alexa & Rahnenfuhrer, 2016), REVIGO (Supek, Bošnjak, Škunca, & Šmuc, 2011) and CirGO (Kuznetsova, Lugmayr, Siira, Rackham, & Filipovska, 2019). We deposited the reads in the European Nucleotide Archive under the project PRJEB38757, accession numbers ERS4652944-ERS4652972.

Results

Calcification in P. flava *occurs along its entire body axis*

Visual inspection of stained sclerites in the top vs. bottom parts of *P. flava* colonies revealed that this species produces new sclerites along its entire colony axis with a slight but significant increase in the number of stained sclerites toward the tips of the colony. Examination of whole colonies sections revealed that calcification usually occurs at the polyps (Fig. 1). Despite the increased number of stained sclerites at the top of the colony, we could not detect any evident calcification hotspots along the colony axis.

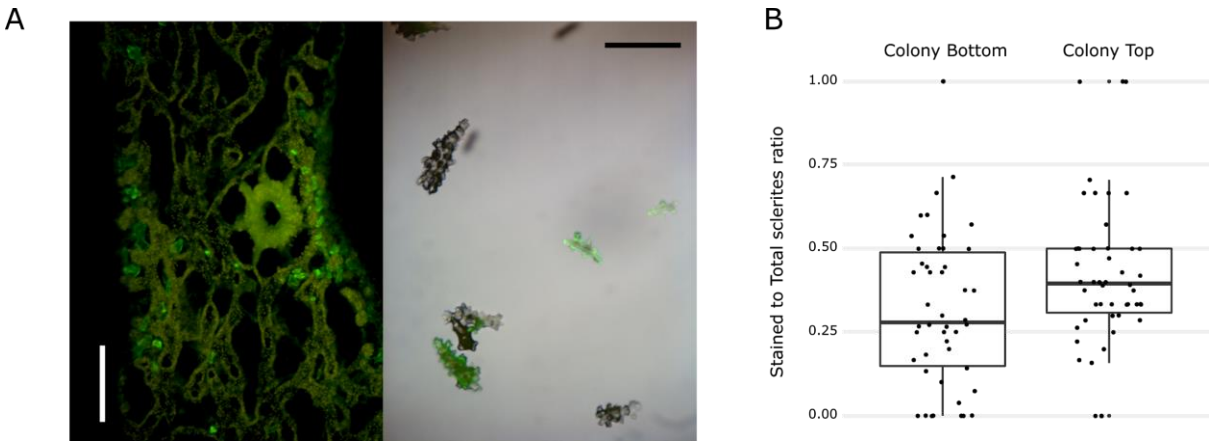

Figure 1. Calcification in *Pinnigorgia flava*. **A:** *In situ* and isolated calcein stained sclerites, note the distribution of newly deposited $CaCO_3$ (lighter green dots) around the polyp. **B:** ratio of calcein stained to total number of sclerites in bottom vs. top colony parts. Welch two sample t=-2.17, df=92.531, p=0.034.

Taxonomically widespread and restricted proteins form the skeletal proteome of P. flava

After filtering potential known contaminants (e.g., keratins, trypsin), we identified a total of 27 transcripts as the skeletal organic matrix (SOM) proteome of *P. flava*. Label-free quantification (LFQ) revealed 14 proteins with a higher abundance in the acetic-insoluble matrix (AIM), seven proteins exclusive to the acetic-soluble matrix (ASM), and five proteins shared by both fractions (Fig. 2).

Biochemically, seven of the detected SOM proteins are membrane-bound, containing either transmembrane domains (n = 3), GPI anchors (n = 3), or both (n = 1). About 46% (n = 13) of the SOM proteins detected contain signal peptide motifs, indicating that these proteins are secretion targets (Fig. 2). Using similarity-based searches, we found the SOM proteome to contain several components of the extracellular matrix, like collagens and laminin, glycoproteins with calcium-binding domains, like nidogen 2 (osteonidogen) and agrin, and proteins likely involved in cell adhesion, like otoancorin, hemicentin and several proteins bearing von Willebrand factor type A (VWA) domains. Finally, we also found a protein similar to galaxin, two proteins enriched in aspartic-acid residues (acidic, pI ~ 3.4) with no significant UniProt blast hits, and several other proteins with diverse functionalities (e.g., disulfide isomerase or protease activity). According to their iBAQ, six proteins had abundances >5% in the skeletal proteome of *P. flava*. The two acidic proteins ranked first and third, osteonidogen and collagen were the second and fourth most abundant proteins, and galaxin and agrin ranked fifth and sixth in abundance. These six proteins account for 90% of the total iBAQ-derived skeletal protein abundance (Fig. 2). On average, the remaining proteins accounted for only 0.5% (±0.6%) of the total iBAQ-derived abundance.

In terms of their taxonomic distribution (Fig. 2), similarity searches against other cnidarians revealed that the skeletal proteome of *P. flava* includes proteins with a widespread distribution among cnidarians, such as all detected collagens, agrin, osteonidogen, galaxin, and several enzymes, among others. Other proteins, like hemicentin or the protein kinase Nell 1, displayed a patchier occupancy

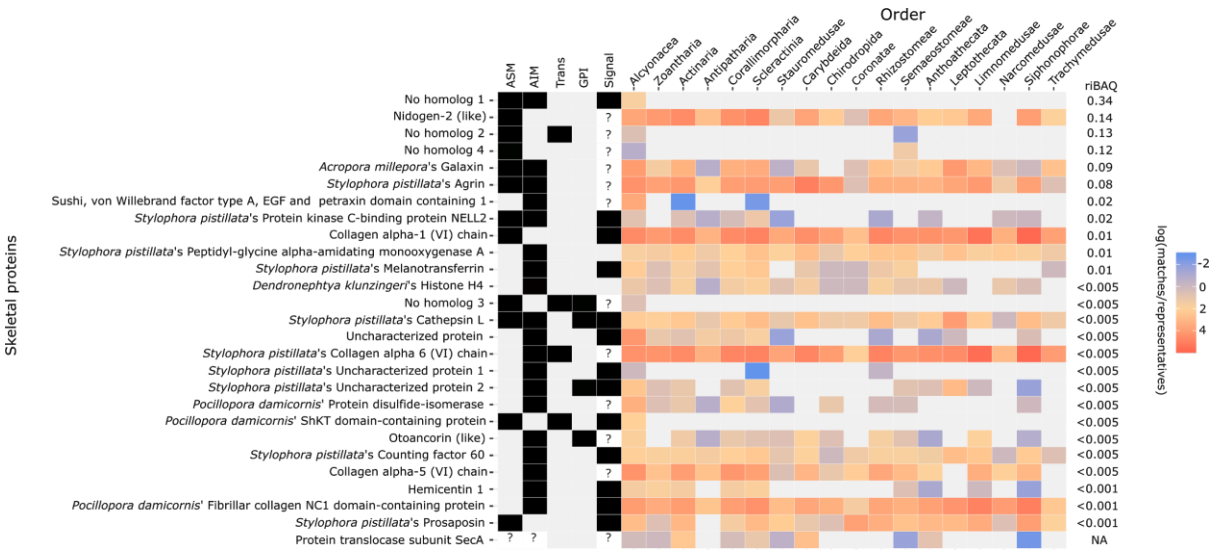

Figure 2. Presence/absence (black/gray rectangles, respectively) in the acid soluble (ASM) and insoluble (AIM) matrix, biochemical properties, distribution among cnidarians and abundance in the skeletal proteome of 27 proteins present in the skeleton of *P. flava*.

and were found mostly in other anthozoans. Finally, only a few SOM components, namely the two acidic proteins found, a serine protein-kinase receptor and a protein similar to laminin, had a restricted taxon occupancy with significantly similar proteins found almost exclusively in other octocorals (i.e., Order Alcyonacea).

Climate change-driven stress does not modulate the calcification toolkit of P. flava

Exposure to sublethal, high-temperature (~29-30°C, ~5°C above long-term average in the aquaria) seawater resulted in the modulation of 751 transcripts (Benjamini-Hochberg corrected $p < 0.05$), with 221 and 108 transcripts down (log2 fold change ≤ -1) or upregulated (log2 fold change ≥ 1) in heat-treated colonies. GO-term enrichment analyses revealed that heat-stressed colonies actively modulated transcripts involved in redox homeostasis and protein folding, including two representatives of the heat-shock protein 70 family, and processes like cell death and immune response (Fig. 3 and Suppl. Table 1 and Suppl. Table 2). Heat stress caused the significant downregulation of one carbonic anhydrase and one galaxin, not affecting the remaining 25 calcification-related proteins previously identified in octocorals (Conci, Wörheide, et al., 2019). Also, heat stress did not significantly change in the expression of any of the 27 transcripts encoding SOM proteins in *P. flava*.

In contrast to heat stress, ocean acidification did not trigger a stark stress response in *P. flava*. Colonies of this species exposed to simulated ocean acidification significantly ($p < 0.05$) downregulated a set of 70 transcripts involved in vacuolar transport and transmembrane signaling, among others, and including skeletogenesis-related GO-terms such as the “Regulation of bone mineralization” or “Bone trabercula formation” (Fig. 4 and Suppl. Table 3). Downregulated transcripts included one calcification-related, uncharacterized skeletal matrix protein, and two SOM-encoding transcripts, namely osteonidogen --the second more abundant SOM protein-- and a prosaposin-homolog.

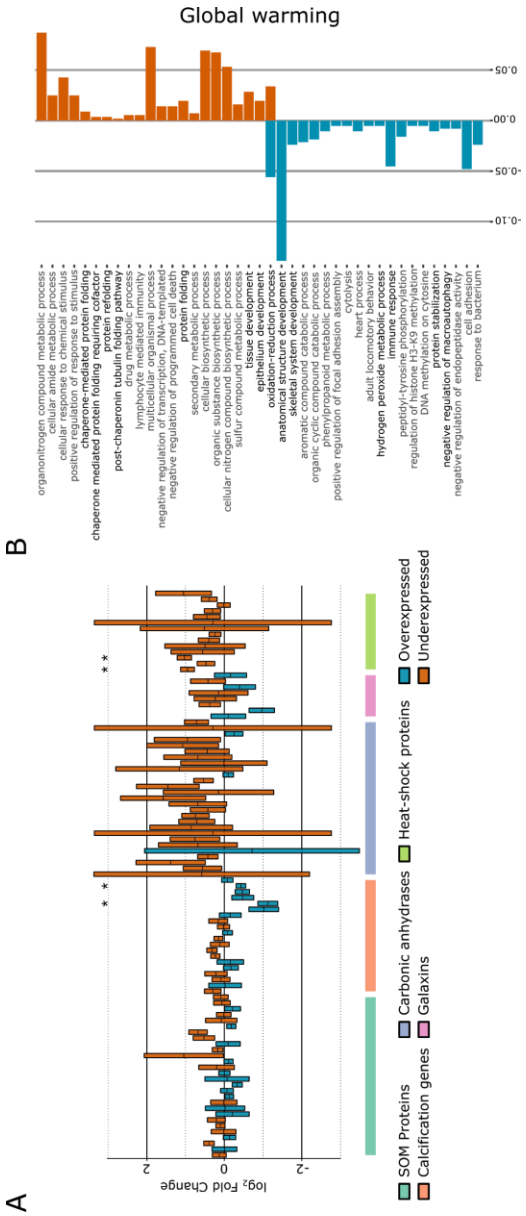

Figure 3. A: Change in gene expression of 27 SOM proteins, calcification related proteins *sensu* Conci et al. (2019), carbonic anhydrases, galaxins and heat-shock proteins in *P. flava* colonies exposed to simulated sea surface warming. **B:** Enriched GO-terms in the set of over or underexpressed genes under simulated sea surface warming.

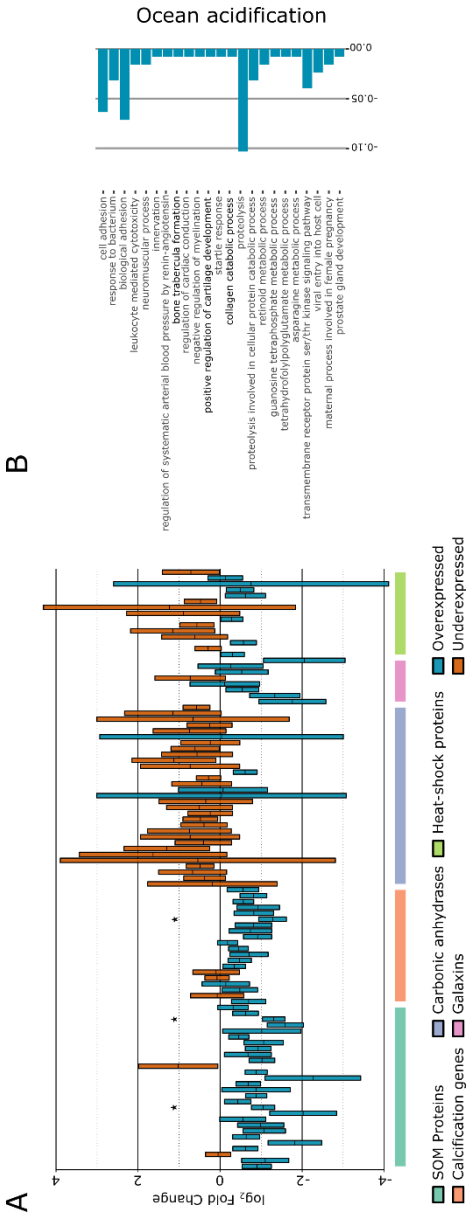

Figure 4. A: Change in gene expression of 27 SOM proteins, calcification related proteins *sensu* Conci et al. (2019), carbonic anhydrases, galaxins and heat-shock proteins in *P. flava* colonies exposed to simulated ocean acidification. **B:** Enriched GO-terms in the set of over or underexpressed genes under simulated ocean acidification.

Discussion

Sclerite growth in octocorals involves the synthesis by sclerocytes of skeletal organic matrix (SOM) proteins and their transport to a sclerite-forming vacuole where primordia mostly made of irregularly shaped $CaCO_3$ crystals form (Kingsley, 1984). Sclerite primordia continue growing by the deposition of more regular crystals in an extracellular space created by multiple sclerocytes (Goldberg & Benayahu, 1987). In *P. flava*, our results indicate that the deposition of new sclerites occurs throughout the colony axis, increasing toward the colony tips. This pattern of calcification is similar to that of *Leptogorgia virgulata*, one of the few other octocoral species where data on calcification dynamics exist (Kingsley & Watabe, 1989). It indicates that active sclerocytes intersperse along the colony axis and that under normal conditions, we should not expect spatial differences in the expression of SOM-encoding transcripts in this species. Hence, the exposure of colonies of *P. flava* to stress factors affecting the expression of SOM encoding transcripts should impair the deposition of new sclerites at the colony level. The collapse of the calcification machinery, in turn, would result in a reduced ability of octocorals to sustain growth and outcompete other reef organisms, like stony corals, in stressful environments.

The skeletal proteome of *P. flava* is similar to other previously characterized proteomes in octo- and scleractinian corals (Conci, Lehmann, Vargas, & Wörheide, 2019; Drake et al., 2013; Ramos-Silva et al., 2013) and contains a mixture of taxonomically restricted and widespread elements as reported in other octocorals (Conci, Lehmann, et al., 2019). This trend is particularly evident among the most abundant proteins in the SOM. Among those, galaxin and the two acidic proteins detected had a very narrow taxonomic distribution. Galaxins mostly occur within Cnidaria (Conci, Wörheide, et al., 2019), acidic proteins appear to be rapidly evolving, and species-specific proteins are generally difficult to assign to cnidarian orthology groups using similarity searches (Conci, Lehmann, et al., 2019). Our results are consistent with that observation, as the two acidic proteins found do not show significant similarity to other proteins deposited in databases such as SwissProt, and only matched proteins found in other octocorals. Among the group of taxonomically widespread proteins, we found typical components of animal basement membranes (Erickson & Couchman, 2000) with a broad distribution within Cnidaria and generally within Metazoa. Indeed, three of the six most abundant proteins, namely agrin, collagen, and osteonidogen are glycoproteins involved in modulating the interactions between the extracellular matrix and the cells (Erickson & Couchman, 2000). Among vertebrates, agrin participates in synaptogenesis (Kröger & Schröder, 2002). Specifically, this protein coordinates the development of neuromuscular junctions, stabilizing, and aligning the pre- and postsynaptic apparatuses of neurons and muscle fibers, respectively. It also triggers the differentiation of neuron growth cones into presynaptic terminals capable of calcium-dependent neurotransmission (Kröger & Schröder, 2002; Ruegg & Bixby, 1998). Its presence in the SOM of sclerites may imply a similar role in calcification, aligning the sclerocytes around sclerite primordia and triggering membrane differentiation to allow for calcium secretion into the calcifying space. In the case of osteonidogen, mammalian osteocytes and osteoclasts overexpress this protein (Bechtel et al., 2012), suggesting a direct role in calcification in that group and a plausible involvement in calcification in octocorals.

To respond more rapidly to and survive episodes of environmental stress, resilient stony corals constitutively upregulate components of the coral cell death and immune pathways, and genes

involved in response to stress, like heat-shock proteins (Barshis et al., 2013). The concomitant downregulation of genes involved in calcification observed during environmental stress in these organisms (Barshis et al., 2013; Ramos-Silva et al., 2013) suggests that the transcriptional frontloading of the stress response toolkit comes at the expense of the coral calcification machinery and could lead to its collapse. Accordingly, colonies of *Siderastrea siderea* exposed to ocean acidification and warming show a parabolic response of calcification, mostly driven by the abrupt drop in calcification rates under more extreme environmental regimes (Castillo, Ries, Bruno, & Westfield, 2014). In contrast, our results indicate that octocorals mechanistically decouple the transcriptional regulation of the calcification and stress-response toolkits and sustain the production of all the molecules necessary for the formation of new sclerites during events of climate change-driven environmental stress. In this regard, we detected only a mild effect of environmental stress on the calcification toolkit of *P. flava*, with simulated ocean warming affecting the expression of a galaxin and a carbonic anhydrase and ocean acidification modulating the expression of osteonidogen and a prosaposin homolog. Galaxins, carbonic anhydrases, and osteonidogen are all calcification related genes in corals and other organisms, and mutations in human prosaposin cause Guacher's disease, a disorder characterized by the deterioration of the skeleton (Vaccaro et al., 2010). The reported linear response of octocoral calcification rates under conditions of ocean acidification (Gómez et al., 2015) is consistent with the hypothesis of the decoupling between the calcification and stress-response toolkits of octocorals, as it indicates that the observed drop in calcification is mostly driven by the environmental setting, not by the response of the octocorals. Under long-term adverse conditions, decoupling the physiological response to stress from calcification may give octocorals a competitive advantage over other species, like stony corals, adapted to respond better to episodic stress and lead to the community shifts observed in many reef locations (Inoue et al., 2013a; Ruzicka et al., 2013).

In summary, our results provide mechanistic insights into octocoral resilience to climate change. They indicate that the fundamental differences in the way in which the calcification and stress-response toolkits of soft and stony corals interplay lie at the base of the different responses to climate change observed in these groups. Anthropogenic-induced global climate change will undoubtedly impact future marine communities in unprecedented ways (Hughes et al., 2019). Processes such as acclimation and adaptation (Palumbi, Barshis, Traylor-Knowles, & Bay, 2014), acting at organismal- and population-levels, and phenomena affecting the community, like ecological memory (Hughes et al., 2019), shape the response of coral reefs to these environmental pressures. Our results show that, compared to stony corals, octocorals use different gene regulation strategies to face climate change. Thus, understanding the diversity of molecular mechanisms involved in resilience, as well as their regulation in different reef organisms, is pivotal to predicting the future of the world's coral reefs.

Acknowledgments

We thank Gabriele Büttner for support during laboratory work and Dr. Peter Naumann for supporting the aquarium facilities of the Section of Geobiology and Paleobiology of the Dept. of Earth & Environmental Sciences. We would also like to thank René Neumaier for the design, administration, and support of our High-Performance Computing system; our work would be impossible without his careful and detailed work. The Deutsche Forschungs Gesellschaft funded this

study through the grants Va1146-2/1 and Wo896-18/1. GW acknowledges funding through the LMU Munich's Institutional Strategy LMUexcellent within the framework of the German Excellence Initiative. SV thanks N. Villalobos T., M. Vargas V., S. Vargas V. and S. Vargas V. for their support.

References

Alexa, A., & Rahnenfuhrer, J. (2016). *topGO: Enrichment Analysis for Gene Ontology* [R Package version 2.30.1].

Atrigenio, M., Aliño, P., & Conaco, C. (2017). Influence of the blue coral *Heliopora coerulea* on scleractinian coral larval recruitment. *Journal of Marine Biology*, *2017*. doi: 10.1155/2017/6015143

Barshis, D. J., Ladner, J. T., Oliver, T. A., Seneca, F. O., Traylor-Knowles, N., & Palumbi, S. R. (2013). Genomic basis for coral resilience to climate change. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(4), 1387–1392.

Bechtel, M., Keller, M. V., Bloch, W., Sasaki, T., Boukamp, P., Zaucke, F., … Nischt, R. (2012). Different domains in nidogen-1 and nidogen-2 drive basement membrane formation in skin organotypic cocultures. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*, *26*(9), 3637–3648.

Buhl-Mortensen, L., & Mortensen, P. B. (2005). Distribution and diversity of species associated with deep-sea gorgonian corals off Atlantic Canada. In A. Freiwald & J. M. Roberts (Eds.), *Cold-Water Corals and Ecosystems* (pp. 849–879). Berlin, Heidelberg: Springer Berlin Heidelberg.

Castillo, K. D., Ries, J. B., Bruno, J. F., & Westfield, I. T. (2014). The reef-building coral *Siderastrea siderea* exhibits parabolic responses to ocean acidification and warming. *Proceedings of the Royal Society B*, *281*(1797). doi: 10.1098/rspb.2014.1856

Colgan, M. W. (1984). The cretaceous coral *Heliopora* (Octocorallia, Coenothecalia)—a common Indo-Pacific reef builder. In N. Eldredge & S. M. Stanley (Eds.), *Living Fossils* (pp. 266–271). New York, NY: Springer New York.

Conci, N., Lehmann, M., Vargas, S., & Wörheide, G. (2019). Comparative proteomics of octocoral and scleractinian skeletomes and the evolution of coral calcification. *bioRxiv* doi: 10.1101/2019.12.30.891028

Conci, N., Wörheide, G., & Vargas, S. (2019). New non-bilaterian transcriptomes provide novel insights into the evolution of coral skeletomes. *Genome Biology and Evolution*, *11*(11), 3068–3081.

Cox, J., Hein, M. Y., Luber, C. A., Paron, I., Nagaraj, N., & Mann, M. (2014). Accurate proteome-wide label-free quantification by delayed normalization and maximal peptide ratio extraction, termed MaxLFQ. *Molecular & Cellular Proteomics: MCP*, *13*(9), 2513–2526.

Cúrdia, J., Carvalho, S., Pereira, F., Guerra-García, J. M., Santos, M. N., & Cunha, M. R. (2015). Diversity and abundance of invertebrate epifaunal assemblages associated with gorgonians are driven by colony attributes. *Coral Reefs* , *34*(2), 611–624.

Davies, S. W., Marchetti, A., Ries, J. B., & Castillo, K. D. (2016). Thermal and pCO2 stress elicit divergent transcriptomic responses in a resilient coral. *Frontiers in Marine Science*, *3*, 112.

Done, T. J. (1992). Phase shifts in coral reef communities and their ecological significance. *Hydrobiologia*, *247*(1), 121–132.

Drake, J. L., Mass, T., Haramaty, L., Zelzion, E., Bhattacharya, D., & Falkowski, P. G. (2013). Proteomic analysis of skeletal organic matrix from the stony coral *Stylophora pistillata* . *Proceedings of the National Academy of Sciences of the United States of America*, *110*(10), 3788–3793.

Erickson, A. C., & Couchman, J. R. (2000). Still more complexity in mammalian basement membranes. *The Journal of Histochemistry and Cytochemistry: Official Journal of the Histochemistry Society*, *48*(10), 1291–1306.

Fung, T., Seymour, R. M., & Johnson, C. R. (2011). Alternative stable states and phase shifts in coral

reefs under anthropogenic stress. *Ecology*, *92*(4), 967–982.

Gabay, Y., Benayahu, Y., & Fine, M. (2013). Does elevated pCO2 affect reef octocorals? *Ecology and Evolution*, *3*(3), 465–473.

Gabay, Y., Fine, M., Barkay, Z., & Benayahu, Y. (2014). Octocoral tissue provides protection from declining oceanic pH. *PloS One*, *9*(4), e91553.

Goldberg, W. M., & Benayahu, Y. (1987). Spicule formation in the gorgonian coral *Pseudoplexaura flagellosa*. 1: Demonstration of intracellular and extracellular growth and the effect of ruthenium red during decalcification. *Bulletin of Marine Science*, *40*(2), 287–303.

Gómez, C. E., Paul, V. J., Ritson-Williams, R., Muehllehner, N., Langdon, C., & Sánchez, J. A. (2015). Responses of the tropical gorgonian coral *Eunicea fusca* to ocean acidification conditions. *Coral Reefs* , *34*(2), 451–460.

González-Pech, R. A., Vargas, S., Francis, W. R., & Wörheide, G. (2017). Transcriptomic resilience of the *Montipora digitata* holobiont to low pH. *Frontiers in Marine Science*, *4*, 403.

Holcomb, M., Cohen, A. L., & McCorkle, D. C. (2013). An evaluation of staining techniques for marking daily growth in scleractinian corals. *Journal of Experimental Marine Biology and Ecology*, *440*, 126–131.

Howison, M., Sinnott-Armstrong, N. A., & Dunn, C. W. (2012). BioLite, a lightweight bioinformatics framework with automated tracking of diagnostics and provenance. *TaPP*. Retrieved from https://www.usenix.org/system/files/conference/tapp12/tapp12-final5.pdf

Hughes, T. P., Barnes, M. L., Bellwood, D. R., Cinner, J. E., Cumming, G. S., Jackson, J. B. C., … Scheffer, M. (2017). Coral reefs in the Anthropocene. *Nature*, *546*(7656), 82–90.

Hughes, T. P., Kerry, J. T., Baird, A. H., Connolly, S. R., Dietzel, A., Eakin, C. M., … Torda, G. (2018). Global warming transforms coral reef assemblages. *Nature*, *556*(7702), 492–496.

Hughes, T. P., Kerry, J. T., Connolly, S. R., Baird, A. H., Eakin, C. M., Heron, S. F., … Torda, G. (2019). Ecological memory modifies the cumulative impact of recurrent climate extremes. *Nature Climate Change*, *9*(1), 40–43.

Inoue, S., Kayanne, H., Yamamoto, S., & Kurihara, H. (2013). Spatial community shift from hard to soft corals in acidified water. *Nature Climate Change*, *3*(7), 683–687.

Kayanne, H., Harii, S., Ide, Y., & Akimoto, F. (2002). Recovery of coral populations after the 1998 bleaching on Shiraho Reef, in the southern Ryukyus, NW Pacific. *Marine Ecology Progress Series*, Vol. 239, pp. 93–103. doi: 10.3354/meps239093

Kingsley, R. J. (1984). Spicule Formation in the Invertebrates with Special Reference to the Gorgonian *Leptogorgia virgulata*. *Integrative and Comparative Biology*, *24*(4), 883–891.

Kingsley, R. J., & Watabe, N. (1989). The dynamics of spicule calcification in whole colonies of the gorgonian *Leptogorgia virgulata* (Lamarck) (Coelenterata: Gorgonacea). *Journal of Experimental Marine Biology and Ecology*, *133*(1), 57–65.

Kröger, S., & Schröder, J. E. (2002). Agrin in the developing CNS: new roles for a synapse organizer. *News in Physiological Sciences: An International Journal of Physiology Produced Jointly by the International Union of Physiological Sciences and the American Physiological Society*, *17*, 207–212.

Kuznetsova, I., Lugmayr, A., Siira, S. J., Rackham, O., & Filipovska, A. (2019). CirGO: an alternative circular way of visualising gene ontology terms. *BMC Bioinformatics*, *20*(1), 84.

Lewis, J. C., & Vonwallis, E. (1991). The function of surface sclerites in gorgonians (Coelenterata, Octocorallia). *The Biological Bulletin*, *181*(2), 275–288.

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*(12), 550.

Moya, A., Huisman, L., Ball, E. E., Hayward, D. C., Grasso, L. C., Chua, C. M., … Miller, D. J. (2012). Whole transcriptome analysis of the coral *Acropora millepora* reveals complex responses to CO2-driven acidification during the initiation of calcification. *Molecular Ecology*, *21*(10), 2440–2454.

Norström, A. V., Nyström, M., Lokrantz, J., & Folke, C. (2009). Alternative states on coral reefs:

beyond coral–macroalgal phase shifts. *Marine Ecology Progress Series*, *376*, 295–306.

Palumbi, S. R., Barshis, D. J., Traylor-Knowles, N., & Bay, R. A. (2014). Mechanisms of reef coral resistance to future climate change. *Science*, *344*(6186), 895–898.

Patro, R., Duggal, G., Love, M. I., Irizarry, R. A., & Kingsford, C. (2017). Salmon provides fast and bias-aware quantification of transcript expression. *Nature Methods*, *14*(4), 417–419.

Petersen, T. N., Brunak, S., von Heijne, G., & Nielsen, H. (2011). SignalP 4.0: discriminating signal peptides from transmembrane regions. *Nature Methods*, *8*(10), 785–786.

Quattrini, A. M., Etnoyer, P. J., Doughty, C., English, L., Falco, R., Remon, N., … Cordes, E. E. (2014). A phylogenetic approach to octocoral community structure in the deep Gulf of Mexico. *Deep-Sea Research. Part II, Topical Studies in Oceanography*, *99*, 92–102.

Ramos-Silva, P., Kaandorp, J., Huisman, L., Marie, B., Zanella-Cléon, I., Guichard, N., … Marin, F. (2013). The skeletal proteome of the coral *Acropora millepora*: the evolution of calcification by co-option and domain shuffling. *Molecular Biology and Evolution*, *30*(9), 2099–2112.

Ruegg, M. A., & Bixby, J. L. (1998). Agrin orchestrates synaptic differentiation at the vertebrate neuromuscular junction. *Trends in Neurosciences*, *21*(1), 22–27.

Ruzicka, R. R., Colella, M. A., Porter, J. W., Morrison, J. M., Kidney, J. A., Brinkhuis, V., … Colee, J. (2013). Temporal changes in benthic assemblages on Florida Keys reefs 11 years after the 1997/1998 El Niño. *Marine Ecology Progress Series*, *489*, 125–141.

Schmitt, R. J., Holbrook, S. J., Davis, S. L., Brooks, A. J., & Adam, T. C. (2019). Experimental support for alternative attractors on coral reefs. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(10), 4372–4381.

Schwanhäusser, B., Busse, D., Li, N., Dittmar, G., Schuchhardt, J., Wolf, J., … Selbach, M. (2011). Global quantification of mammalian gene expression control. *Nature*, *473*(7347), 337–342.

Seneca, F. O., & Palumbi, S. R. (2015). The role of transcriptome resilience in resistance of corals to bleaching. *Molecular Ecology*, *24*(7), 1467–1484.

Shaish, L., Levy, G., Katzir, G., & Rinkevich, B. (2010). Coral Reef restoration (Bolinao, Philippines) in the face of frequent natural catastrophes. *Restoration Ecology*, *18*(3), 285–299.

Spencer, T., Laughton, A. S., Flemming, N. C., Stobart, B., Teleki, K., Buckley, R., … Callow, M. (2005). Coral recovery at Aldabra Atoll, Seychelles: five years after the 1998 bleaching event. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, *363*(1826), 251–255.

Supek, F., Bošnjak, M., Škunca, N., & Šmuc, T. (2011). REVIGO summarizes and visualizes long lists of gene ontology terms. *PloS One*, *6*(7), e21800.

Thomas, L., Rose, N. H., Bay, R. A., López, E. H., Morikawa, M. K., Ruiz-Jones, L., & Palumbi, S. R. (2018). Mechanisms of thermal tolerance in reef-building corals across a fine-grained environmental mosaic: Lessons from Ofu, American Samoa. *Frontiers in Marine Science*, *4*, 434.

Vaccaro, A. M., Motta, M., Tatti, M., Scarpa, S., Masuelli, L., Bhat, M., … Salvioli, R. (2010). Saposin C mutations in Gaucher disease patients resulting in lysosomal lipid accumulation, saposin C deficiency, but normal prosaposin processing and sorting. *Human Molecular Genetics*, *19*(15), 2987–2997.

Wessel, D., & Flügge, U. I. (1984). A method for the quantitative recovery of protein in dilute solution in the presence of detergents and lipids. *Analytical Biochemistry*, *138*(1), 141–143.

Data availability statement:

- Raw sequence reads: European Nucleotide Archive project PRJEB38757, accession numbers ERS4652944-ERS4652972.
- Raw MS/MS data files: https://data.ub.uni-muenchen.de/189/
- Transcriptome assembly, proteome annotations, count matrix, scripts and other data files used in this study: https://gitlab.lrz.de/palmuc/pinnigorgia_resilience

Author Contributions:

SV Conceptualization, Methodology, Software, Validation, Formal Analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Visualization, Supervision, Funding Acquisition. **TZ** Investigation, Formal Analysis. **NC** Investigation, Formal Analysis, Writing - Review & Editing. **ML** Investigation. **GW** Resources, Writing - Review & Editing, Funding Acquisition.