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The molecular ecophysiology of closely related pocilloporid corals of New Caledonia

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Abstract

Given the severe threats against Earth's coral reef ecosystems, there is an urgent need to collect baseline data such that the condition of these endangered habitats can be tracked over the coming decades. Many of the most spectacular coral reefs are found in remote locations that are only accessible by research ships; therefore, little is known about the vast majority of the planet's reefs. In order to fill in a critical knowledge dearth in an ecologically important section of Melanesia, the Khaled bin Sultan's Living Oceans Foundation carried out coral reef surveys in both the far southern and far northern regions of New Caledonia as part of their "Global Reef Expedition." There was a particular emphasis on monitoring the health of the resident pocilloporid corals, which, like all reef-building scleractinians, are engaged in a mutualistic relationship with intracellular dinoflagellates of the genus *Symbiodinium*. Of the 120 *Pocillopora* spp. colonies analyzed in detail, 10 were found to be outliers based on a series of multivariate statistical approaches. These aberrantly behaving colonies differed from statistically normal ones in demonstrating higher expression levels of two host coral and four *Symbiodinium* mRNAs. Furthermore, all 10 colonies were found in the relatively more densely populated and mined south; no outliers were uncovered in the northern atolls, which encompass some of the most pristine and aesthetically appealing reef ecosystems in the world. That being said,

mRNA expression of nearly all stress-sensitive biomarkers was higher in these New Caledonian corals than in closely related pocilloporids from other regions of the South Pacific. This suggests that even the remote reefs and atolls of the country's far northern reaches have not been completely spared from anthropogenic harm.

Key words: coral reefs, dinoflagellate, ecology, molecular biology, New Caledonia, stress

Introduction

Earth's coral reefs are threatened by a barrage of anthropogenic insults, including global climate change (GCC; Hoegh-Guldberg et al., 2007; Mayfield & Gates, 2007) and water pollution (Kabricius, 2005; Huang et al., 2011). Unfortunately, little is known about the vast majority of the planet's coral reefs. For instance, reefs of the southernmost regions of French Polynesia were not surveyed for the first time until early 2013 (Mayfield et al., 2015). Similarly, the most remote regions of Fiji (Lau Province) and Tonga (the Ha'apai and Vava'u archipelagos) also went un-surveyed until well into 2013 (Mayfield et al., 2017b and 2017a, respectively). Coral reefs of New Caledonia (Figure 1) have also received little scientific focus (but see Andrefouet et al., 2009), yet the remoteness and governmental protection of the atolls in the northernmost part of this Melanesian nation might have conferred the resident corals with an added degree of resilience to GCC given the absence of local-scale anthropogenic pressures (e.g., overfishing). In other words, we hypothesize that reefs far from human population centers are healthier than those abutting cities (e.g., reefs of Southern Taiwan, which are within 50 km of one of the biggest cities in Asia, Kaohsiung [Liu et al., 2012]). Even within New Caledonia, a gradient of anthropogenic impact could be hypothesized to exist. In the southern reefs (SR), 1) nickel mining around Prony Bay and 2) tourism and relatively higher population densities in the Ile des Pins Archipelago could have resulted in decreases in water quality that may be detrimentally impacting the resident corals. No such industry or tourism exists in the northern reefs and atolls (NRA); only scientists holding government permits may visit this region.

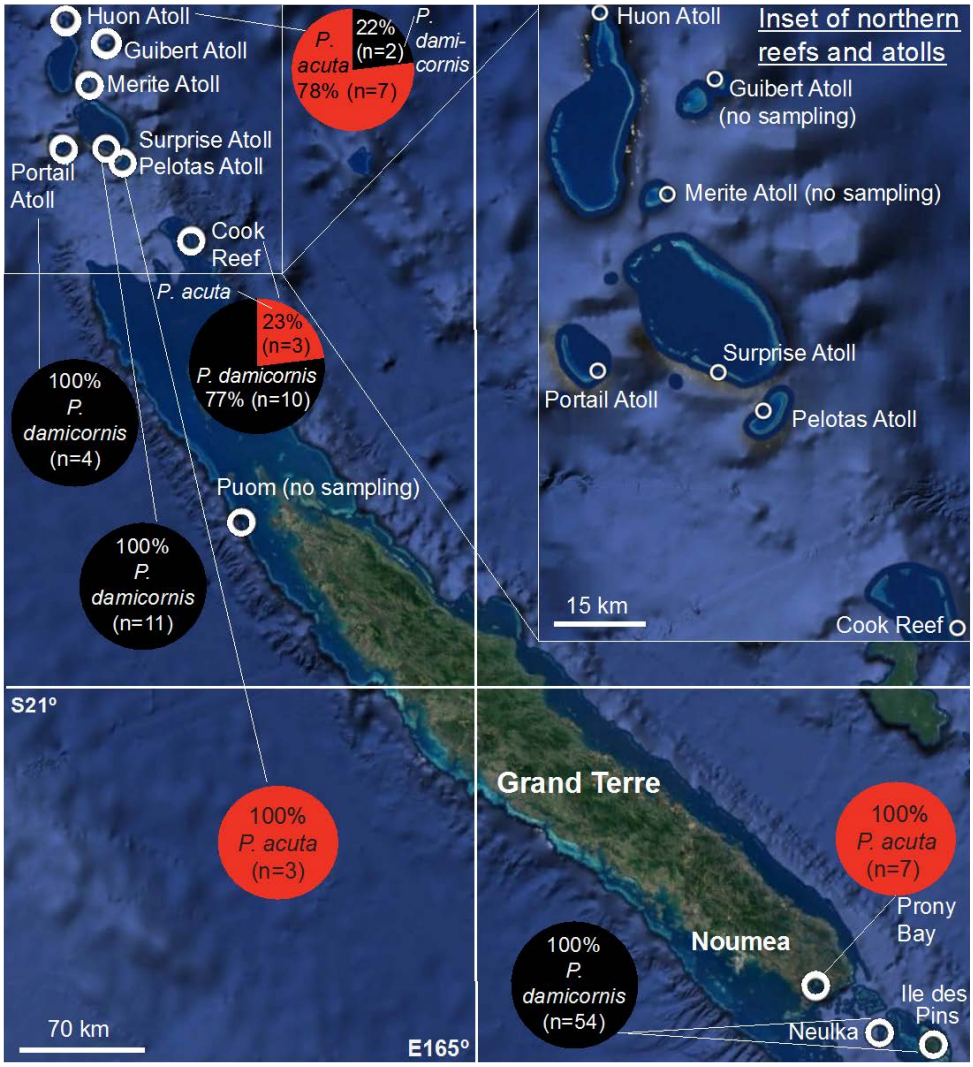

Fig. 1. Map of New Caledonia showing genetic breakdown of the sampled *Pocillopora* spp. colonies. Due to the proximity of the northern reefs and atolls, they have been additionally displayed in a 5-fold magnified inset. The six genotyped colonies from Neulka have been grouped with the 48 genotyped colonies from Ile des Pins into the same pie graph. In this and all remaining figures, *Pocillopora acuta* and *P. damicornis* are presented as red and black icons/wedges, respectively.

Molecular biology-driven approaches have dramatically aided in our understanding of both the basic biology (Mayfield et al., 2010; Peng et al., 2011; Chen et al., 2012; Mayfield et al., 2012b; Wang et al., 2013; Mayfield et al., 2014c; Chen et al., 2015, 2016, 2017) and environmental physiology (Mayfield et al., 2011, 2012a, 2013a-d, 2014a-b, d; Putnam et al., 2013; Mayfield, 2016; Mayfield et al., 2016b) of reef-building corals and sea anemones engaged in endosymbiotic relationships with dinoflagellates of the genus *Symbiodinium*. For nearly 20 years, scientists have attempted to use molecular biomarkers to confer a level of health to both experimental (Downs et al., 2000) and field (Downs et al., 2005) coral colonies. However, these endeavors have been fraught with methodological hurdles; for instance, these works by Downs and colleagues failed to consider that their antibodies likely bound protein antigens of both host corals and their intracellular dinoflagellates. The data generated are therefore inherently biased. Such a failure to consider both compartments of the coral-*Symbiodinium* mutualism has also led to the production of unreliable transcriptome-scale datasets (e.g., Barshis et al., 2013). Therefore, in 2009 we generated the first protocol for quantifying gene expression in an endosymbiotic organism (Mayfield et al., 2009). Since then, we have used these real-time PCR (qPCR)-based protocols to assess expression of gene mRNA biomarkers in samples of the model reef coral *Pocillopora damicornis* (Traylor-Knowles et al., 2011) from across the Indo-Pacific (e.g., Mayfield et al., 2016a). Specifically, we have focused on expression of genes encoding proteins involved in certain cellular processes known to be temperature-sensitive in corals, such as *Symbiodinium* photoinhibition and metabolism (Jones et al., 1998; Table 1) and host coral detoxification of the reactive oxygen species (ROS) produced by photoinhibited *Symbiodinium* (Lesser, 1996, 1997, 2006), including copper-zinc superoxide dismutase (*cu-zn-sod*).

Although gene expression levels cannot be used to make inferences about reef-building coral physiology and cell biology due to the lack of congruency between gene and protein expression in coral anthozoans and *Symbiodinium* (Mayfield et al., 2016c), they can nevertheless be used to identify corals behaving in a statistically aberrant manner (Mayfield et al., 2017a). For instance, molecular biomarker data from Fijian corals were analyzed within a multivariate statistical framework, and a number of outliers were uncovered (Mayfield et al., 2017b). These corals were characterized not only by lower *Symbiodinium* densities, but also by elevated expression levels of genes known to encode proteins involved in the cellular stress response (Table 1),

Table 1. Response variables. Although 14 response variables were assessed for each of 120 of the 140 total pocilloporid colonies sampled in New Caledonia, only the 10 molecular-scale response variables (MSRV) were hypothesized to be useful in assigning a level of aberrancy to the sampled colonies (i.e., the color [scored as described in the text though excluding green, purple, and blue colonies], polyp expansion, and size [two parameters] data were excluded from the outlier assignment exercise.). The Fiji and Tonga results were presented in Mayfield et al. (2017b and 2017a, respectively). Statistically significant intra-country outlier vs. non-outlier differences (student's *t*-test, $p<0.05$) have been underlined, and only the *Symbiodinium* zinc-induced facilitator-like 1-like and host coral copper-zinc superoxide dismutase mRNAs were expressed at higher levels by outliers than non-outliers in all three countries (both highlighted in green). In contrast, *lectin* was not expressed at significantly different levels between outliers and non-outliers for any of the three countries (highlighted in yellow). For gene expression data, the ranges and means have been expressed as non-normalized threshold cycle (Ct) values. When 1-way ANOVA detected a significant country effect ($p<0.01$), Tukey's *post-hoc* tests were performed, and letters adjacent to standard deviations (std. dev.) in these columns only reflect Tukey's honestly significant differences ($p<0.05$). Lower-case letters in the left-most column instead correspond to the references (as footnotes) that first described the response variables. For the normalized MSRV data, please see Figure S3.

Response variable	Abbreviation	Proxy/ function	New Caledonia results	New Caledonia range (mean± std. dev.)	Tonga results	Tonga range (mean± std. dev.)	Fiji results	Fiji range (mean± std. dev.)
Physiological response variables								
maximum colony length (cm)	max. length	size	outlier=non-outlier	8-44 (21±6.6)[a]	outlier=non-outlier	5-38 (16±9.0)[b]	outlier=non-outlier	6-61 (18±12)[b]
planar surface area (cm^2)	planar SA	size	outlier=non-outlier	49-809 (220±150)[a]	outlier=non-outlier	17-720 (160±170)[b]	outlier=non-outlier	22-1600 (202±303)[b]
color	color	color	outlier=non-outlier	1-4 (1.5±0.9)[b]	outlier=non-outlier	1-4 (1.9±1.2)[a]	outlier=non-outlier	1-4 (1.4±0.8)[b]
polyp expansion	poly expan.	feeding	outlier=non-outlier	Yes:No= 2:1	outlier=non-outlier	Yes:No= 4:1	not reported for most samples	

Response variable	Abbreviation	Proxy/ function	New Caledonia results	New Caledonia range (mean± std. dev.)	Tonga results	Tonga range (mean± std. dev.)	Fiji results	Fiji range (mean± std. dev.)
Biological composition response variables (MSRV#1-2)								
RNA/DNA ratio[a]	RNA/DNA	total transcription	outlier=non-outlier	0.1-6.9 (1.6±1.1)[ab]	outlier>non-outlier	0.1-4.6 (0.8±0.9)[b]	outlier=non-outlier	0.1-36 (3.3±7.7)[a]
Symbiodinium genome copy proportion[b]	Sym GCP	*Symbiodinium* density	outlier=non-outlier	0.3-24 (5.2±5.3)[c]	outlier=non-outlier	6-80 (33±16)[a]	outlier<non-outlier	3.5-67 (27±14)[b]
***Symbiodinium* mRNA expression (MSRV#3-6)**								
ribulose-1,5 bisphosphate carboxylase/oxygenase[c]	Sym *rbcL*	photosynthesis	outlier>non-outlier	17-25 (19±1.5)[a]	outlier=non-outlier	18-32 (21±2.5)[b]	outlier>non-outlier	18-32 (22±2.4)[b]
zinc-induced facilitator-like 1-like[d]	Sym *zifl11*	zinc transport	outlier>non-outlier	19-29 (24±1.9)[b]	outlier>non-outlier	18-31 (23±2.7)[a]	outlier>non-outlier	20-34 (25±2.9)[b]
heat shock protein 90[d]	Sym *hsp90*	stress response	outlier>non-outlier	18-28 (22±1.6)[a]	outlier=non-outlier	21-30 (25±1.7)[b]	outlier>non-outlier	21-31 (24±1.6)[b]
ubiquitin ligase[d]	Sym *ubiq-lig*	stress response	outlier>non-outlier	21-32 (24±1.6)[a]	outlier=non-outlier	23-34 (26±2.4)[b]	outlier>non-outlier	26-36 (29±1.9)[c]
Host coral mRNA expression (MSRV#7-10)								
carbonic anhydrase[d]	host *ca*	carbon metabolism	outlier>non-outlier	15-25 (19±2.3)[b]	outlier>non-outlier	15-27 (18±2.2)[a]	outlier=non-outlier	17-30 (22±2.5)[c]
lectin[e]	host *lectin*	cell adhesion	outlier=non-outlier	18-27 (23±1.7)[a]	outlier=non-outlier	20-30 (23±2.2)[a]	outlier=non-outlier	22-30 (26±1.9)[b]
copper-zinc superoxide dismutase[d]	host *cu-zn-sod*	stress response	outlier>non-outlier	20-28 (23±1.8)[a]	outlier>non-outlier	20-31 (23±1.8)[a]	outlier>non-outlier	22-32 (26±1.8)[b]
green fluorescent protein-like chromoprotein[f]	host *gfp-cp*	light absorption	outlier=non-outlier	14-27 (18±2.3)[a]	outlier>non-outlier	16-32 (24±2.8)[b]	outlier>non-outlier	18-30 (22±2.3)[b]

[a]Mayfield et al. (2011). [b]Mayfield et al. (2009). [c]Mayfield et al. (2012a). [d]Mayfield et al. (2014d). [e]Mayfield et al. (2013d). [f]Mayfield et al. (2014a).

such as heat shock protein 90 (*hsp90*) and ubiquitin ligase (*ubiq-lig*; Welchman et al., 2005). Curiously, outliers were *not* more frequently sampled from marginal environments, such as reefs with low average live coral cover (ALCC); their distribution was effectively random, though it is possible that environmental parameters (EP) which were not measured, such as nutrient levels, were instead responsible for eliciting aberrant behavior in these coral colonies.

As mentioned above, New Caledonia is hypothetically characterized by a gradient of anthropogenic influence, from the relatively more densely populated and intensively mined south, to the atolls of the north that are dozens of kilometers from human habitations and are protected within a series of federally governed marine parks. We therefore sought herein to employ the same molecular biomarker profiling+multivariate statistical analysis (MSA) approach used to assess coral health elsewhere in the South Pacific to determine whether corals displaying aberrant behavior were more common in the southern, potentially more impacted reefs. We hypothesized that not only would this be the case but also that, more generally, expression of stress biomarker mRNAs would be lower in *P. damicornis* colonies sampled from the more remote reefs of the NRA relative to the SR. Finally, we hypothesized that stress biomarker levels would be lower in corals of the NRA than at reefs surveyed in Fiji (Mayfield et al., 2017b) and Tonga (Mayfield et al., 2017a) given their remote nature and protected status.

Materials and Methods

Sample and environmental data collection. In November 2013, the Khaled bin Sultan Living Oceans Foundation's (LOF) research vessel *M. Y. Golden Shadow* traversed two major regions of New Caledonia (Figure 1): the SR (Ile des Pins [Figure 2], Neulka [Figure 2], and Prony Bay [Figure 3a]; 26 sites) and the NRA (Figure 3b-f; 53 sites). For details of the coral reef surveys, readers are referred to our prior works given that the identical survey methods were used herein (Mayfield et al., 2015, 2017b). Briefly, habitat maps were made as in Saul and Purkis (2015), and point-intercept surveys were conducted by multiple divers at depths typically spanning 5-30 m (in 5-m increments) to characterize the benthos. ALCC was calculated at each site, as it was hypothesized that a greater number of corals displaying aberrant behavior (described in more detail below) would be sampled from marginalized reefs characterized by low ALCC or, more generally, a diminished level of health (as inferred from ecosystem-scale parameters such as algal cover and disease prevalence).

Unlike in our prior surveys in the Austral Islands (French Polynesia), Cook

Islands, Fiji, and Tonga, a series of night dives were conducted (see Tables 2-3.). Night dive sites were chosen earlier in the day, and colonies were tagged with neon glow sticks so that they could be easily re-located at night. In general, 10 colonies were selected at 10 m±1 within a ~100 m^2 area at each site (n=4 night dive sites). The same characteristics as described below for all sampled colonies were measured, except that for night dive colonies, biopsies (50 mg) were removed at around 12:00 (±1 hr) and again after sunset (18:30±30 min). By sampling the same colony during daylight and darkness, we hoped to gain not only greater insight into the molecular pathways exploited by corals to recover from high ultraviolet (UV) radiation exposure and high pO_2 *in vivo*, but also to better gauge which colonies were of comprised resilience; those colonies with wider fluctuations in molecular physiology between day and night could be hypothesized to be less physiologically robust since such behavior may coincide with significant deviations from homeostasis. In the context of this manuscript, we seek to mainly present these light-dark data, without focusing on the diel differences and their physiological implications, which will be presented in a future work (Mayfield et al., in prep.).

The model reef coral *P. damicornis*, or, alternatively, its closely related sister species *P. acuta* (Schmidt-Roach et al., 2014), was sampled from 11 (Table 2) and 18 (Table 3) of the survey sites in the SR and NRA, respectively. Colonies were sampled at a range of depths, from typically 5 to 30 m (the same depths at which surveys were conducted), and there was always at least a 10-m distance between colonies except for those of the night dive sites. Prior to removing a small biopsy with bone-cutting pliers as described in Mayfield et al. (2015), colony polyp expansion behavior was noted. If even a small fraction of a colony's polyps were outside of their coralites, colonies were scored as "polyps expanded." Then, colonies were photographed. Maximum (max.) colony length (i.e., the distance spanning the two most distant points of the colony; cm) and planar surface area (SA; cm^2) were measured from images with ImageJ (National Institutes of Health, USA) by scaling to an object of known size included next to each colony when it was photographed. Color was determined subjectively for each of the sampled colonies, and each was scored as normal (1), pale (2), very pale (3), bleached (4), green, purple, or blue (the latter three were rare and not given numeric scores.). Max. length, planar SA, polyp expansion, and color were deemed "physiological response variables" (PRV; Table 1).

A number of EP were assessed at the survey sites, including 1) region (SR vs. NRA), 2) island (n=8; see Figures 1-3 and

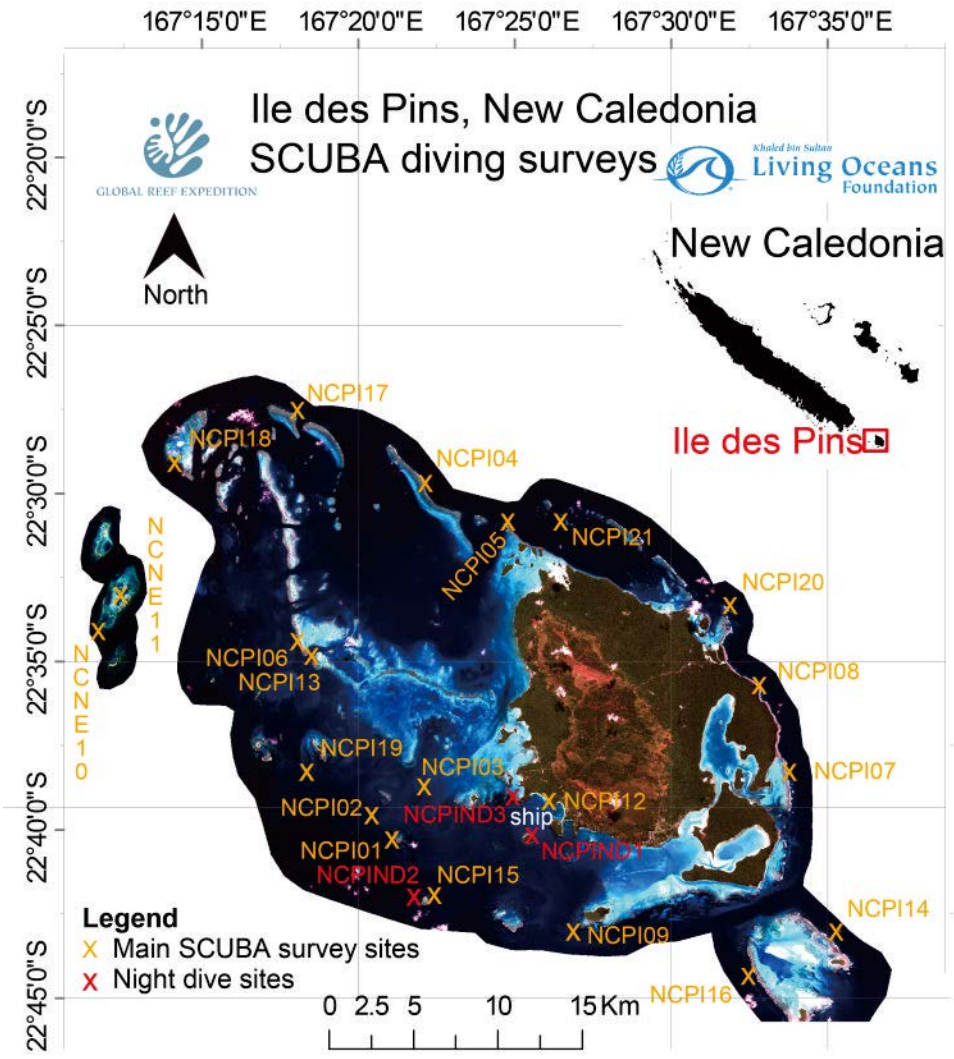

Fig. 2. Southern New Caledonia dive sites: Ile des Pins and Neulka.

Table 2. Southern New Caledonia site information. The target coral species *Pocillopora damicornis*, or, alternatively, its closely related sister species *P. acuta*, was observed at and sampled from 11 of the 26 sites surveyed across Ile des Pins (NCPI; n=19 survey sites + 2 night dive [ND] sites; Figure 2), Neulka (NCNE; n=2 sites; Figure 2), and Prony Bay (NCPB; n=3 sites; Figure 3a), and the data in this table are from these 11 sites only. In total 91 samples were obtained from 62 pocilloporid coral colonies (ND colonies were sampled twice.), and all but one of the uniquely sampled colonies were genotyped (excluding colony #33 from Neulka). Of these 91 samples, 80 (67, 6, and 7 from NCPI, NCNE, and NCPB, respectively) were analyzed in detail and are the focus of this work. For the following 15 sites, *P. damicornis* may have been present, but it was not sampled: NCPI03, NCPI06-09, NCPI12-14, NCPI16-21, and NCPB24. For the environmental data for all 26 surveyed sites, please see the online supplemental data file. The average temperatures (temp.), salinities, and live coral cover (ALCC) percentages reflect the means across the 11 sites from which corals were sampled (±std. dev.), not all 26 surveyed sites. "." = missing data.

Site	Exposure	Reef type	Reef zone	Latitude-	Longitude	Date (2013)	Temp. (ºC)	Sal-inity	ALCC (%)	#Corals analyzed/ collected (sample#)	*P. damicornis* (PD)/*P. acuta* (PA) present
NCPI01	Protected	Patch	Lagoon	22.6651	167.3512	28-10	24.1	35.3	43	9/9 (#1-9)	PD only (n=9)
NCPI02	Protected	Patch	Lagoon	-22.6522	167.3525	28-10	24.5	35.3	26	2/2 (#10-11)	PD only (n=2)
NCPI04	Exposed	Barrier	Forereef	-22.4963	167.3709	29-10	24.8	35.2	20	6/6 (#12-17)	PD only (n=6)
NCPI05	Exposed	Fringing	Forereef	-22.5147	167.4135	29-10	24.9	35.2	23	2/2 (#18-19)	PD only (n=2)
NCPIND1[a]	Protected	Fringing	Lagoon	-22.6718	167.4287	30-10	.	.	.	Light: 10/10 (#20-29) Dark: 9/10 (#21D-29D)	PD only (n=10)
NCNE10	Protected	Patch	Forereef	-22.5694	167.1961	31-10	23.9	35.2	28	5/6 (#30-32, 34-35)	PD only (n=5)[b]
NCNE11	Protected	Patch	Forereef	-22.5594	167.2079	31-10	24.0	35.3	31	1/1 (#36)	PD only (n=1)
NCPI15[a]	Protected	Barrier	Forereef	-22.7006	167.3740	01-11	24.2	35.3	25	Light: 5/10 (#37, 43-46) Dark: 9/10 (#37D-45D)	PD only (n=10)
NCPIND3[a]	Protected	Fringing	Lagoon	-22.65355	167.4186	02-11	.	.	.	Light: 7/9 (#47-50, 53-55) Dark: 8/9 (#47D-53D, 55D)	PD only (n=9)
NCPB22	Protected	Fringing	Bay	-22.31393	166.8439	04-11	24.7	34.4	59	3/3 (#56-58)	PA only (n=3)
NCPB23	Protected	Fringing	Bay	-22.36591	166.8904	04-11	24.3	35.3	63	4/4 (#59-62)	PA only (n=4)
			Southern New Caledonia avg.±std. dev.				24.4 ±0.4	35.2 ±0.3	35±16	80 samples across 62 unique colonies (61 genotyped)	54 PD (88.5%) 7 PA (11.5%)

[a]Night dive; colonies were sampled in both light and dark periods. [b]Certain samples at site were not genotyped, so *P. acuta* may have been sampled.

Table 3. Northern New Caledonia site information. Of the 53 sites surveyed in the northern reefs and atolls (NRA), the target coral species *Pocillopora damicornis*, or, alternatively, its closely related sister species *P. acuta*, was sampled from 18, and the data in this table are from these sites only. In total 49 samples were taken from 42 unique pocilloporid coral colonies from Pelotas Atoll (NCPE; n=3 colonies; Figure 3b), Cook Reef (NCCR; n=14; Figure 3c), Portail Atoll (NCPO; n=4; Figure 3d), Huon Atoll (NCHU; n=9; Figure 3e), and Surprise Atoll (NCSU; n=12; Figure 3f); 40 of these 49 samples are the focus of this work. For the following 35 sites, *P. damicornis/P. acuta* may have been present, but no colonies were sampled: NCPE25, NCCR28-29, NCCR32, NCCR35, NCCR38, NCPO41, NC42 (a remote reef in the Coral Sea near the town of Puom; Figure 1), NCHU48-54, NCGU55-56 (Guibert Atoll; Figure 1), NCGU58-59, NCHU60, NCSU62-63, NCME64-65 (Merite Atoll; Figure 1), NCSU66-71, NCPO72-73, NCSU74, and NCPE75-76. For the environmental data for all 53 surveyed sites, please see the online supplemental data file. The average temperatures (temp.), salinities, and live coral cover (ALCC) percentages reflect the means across the 18 sites from which corals were sampled (±std. dev.), and the average seawater temperature (temp.) at the NRA (25.8ºC) was significantly higher than in the southern reefs (SR; 24.4ºC; student's *t*-test, $p<0.05$). ALCC did not vary between regions ($p>0.05$). The New Caledonia averages presented in the final row reflect the means across all 79 survey sites (±std. dev.), not just those from which corals were sampled. "." = missing data.

Site	Exposure	Reef type	Reef zone	Latitude	Longitude	Date (2013)	Temp. (ºC)	Salinity	ALCC (%)	#Corals analyzed/ collected (sample#)	*P. damicornis* (PD)/*P. acuta* (PA) present
NCPE26	Intermediate	Atoll	Forereef	-18.57095	163.2118	06-11	25.1	35.2	40	3/3 (#63-65)	PA only (n=3)
NCCR27	Exposed	Barrier	Forereef	-18.9424	163.5725	07-11	25.7	35.2	25	3/4 (#66-68)	PD only (n=4)
NCCR30	Intermediate	Barrier	Forereef	-19.0986	163.5593	08-11	25.4	35.2	27	1/2 (#70)	PA only (n=1)[a]
NCCR31	Intermediate	Barrier	Backreef	-18.9855	163.5052	08-11	25.5	35.2	24	2/2 (#72-73)	PD only (n=2)
NCCR33	Exposed	Barrier	Forereef	-19.0532	163.6834	09-11	25.3	35.2	15	2/2 (#74-75)	PD only (n=2)
NCCR34	Protected	Barrier	Channel	-19.06056	163.6304	09-11	25.3	35.3	30	2/2 (#76-77)	PD (n=1) & PA (n=1)
NCCR36	Exposed	Barrier	Forereef	-18.8493	163.5307	10-11	25.6	35.1	26	1/1 (#78)	PD only (n=1)
NCCR37	Intermediate	Barrier	Backreef	-18.8486	163.4469	10-11	25.7	35.2	30	1/1 (#79)	PA only (n=1)
NCPO39	Exposed	Atoll	Backreef	-18.50812	162.9083	11-11	25.5	35.2	21	1/2 (#80)	PD only (n=2)

Site	Exposure	Reef type	Reef zone	Latitude	Longitude	Date (2013)	Temp. (ºC)	Salin-ity	ALCC (%)	#Corals analyzed/ collected (sample#)	*P. damicornis* (PD)/*P. acuta* (PA) present
NCPO40	Protected	Atoll	Backreef	-18.45819	162.8889	11-11	25.6	35.1	32	2/2 (#82-83)	PD only (n=2)
NCHU43	Exposed	Atoll	Forereef	-17.88725	162.8975	13-11	26.1	35.1	27	0/1	PD only (n=1)[b]
NCHU44	Intermediate	Atoll	Forereef	-17.93625	162.8920	13-11	25.9	35.1	30	1/1 (#85)	PA only (n=1)
NCHU45	Protected	Atoll	Lagoon	-17.99775	162.9063	13-11	26.1	35.1	32	1/1 (#86)	PA only (n=1)
NCHU46	Intermediate	Atoll	Forereef	-18.06129	162.8282	14-11	26.0	34.9	18	0/1	PD only (n=1)
NCHU47	Intermediate	Atoll	Forereef	-17.97818	162.8960	14-11	26.3	35.1	23	2/2 (#88-89)	PA only (n=2)
NCHU57	Intermediate	Atoll	Backreef	-17.96940	162.9171	17-11	26.3	35.1	26	3/3 (#90-92)	PA only (n=3)
NCSU61	Intermediate	Atol	Forereef	-18.50630	163.1276	19-11	25.8	35.2	41	5/5 (#93-97)	PD only (n=4)[a,c]
NCSUND4[d]	Protected	Atoll	Lagoon	.	.	20-11	.	.	.	Light: 5/7 (#98, 100, 102-104) Dark: 5/7 (#99D-100D, 102D-104D)	PD only (n=7)
					North New Caledonia avg.±std. dev.		25.7±0.4	35.1±0.1	27±6.8		26 PD (67%) 13 PA (33%)
					Total # analyzed for molecular-scale response variables/total # genotyped (North New Caledonia)					40/40	
					New Caledonia avg.± std. dev.		25.7±0.8	35.7±0.2	30±11		
					Total # analyzed for molecular-scale response variables/total # unique colonies genotyped (SR+NRA)					120/101	80 PD (79%)+ 21 PA (21%)

[a]Certain samples at site were not genotyped, so other pocilloporid species may have been present. [b]certain sample(s) was/were genotyped but not processed for other molecular response variables. [c]Colony 94 was not genotyped and is therefore not found in the heat map (Figure 4). [d]Night dive; colonies were sampled in both light and dark periods.

Tables 2-3.), 3) site (n=29; see Figures 2-3 and Tables 2-3.), 4) reef exposure (n=3 classifications: exposed, intermediate, or protected [see Mayfield et al., 2017b for details].), 5) reef zone (n=5: forereef, backreef, lagoon, channel, or bay), 6) reef type (n=4: fringing reef, patch reef, barrier reef, or atoll), 7) sampling date (n=18 sampling days), 8) sampling time (n=4 categorical groupings: <10:00, 10:00-14:00, 14:00-18:00, or 18:00-22:00), 9) colony depth (n=6 categorical groupings: 5-10, 10-15, 15-20, 20-25, 25-30, or >30 m), 10) site temperature (n=4 categorical groupings: 23-24, 24-25, 25-26, or >26°C), 11) site salinity (n=4 categorical groupings: <35, 35.1, 35.2, or 35.3), 12) colony photosynthetically active radiation (PAR) level (n=2 categorical groupings: 0 or >0 μmol m^{-2} s^{-1}), 13) site ALCC (n=4 categorical groupings: 15-30, 30-45, 45-60, or >60%), 14) host (n=2: *P. damicornis* or *P. acuta*), 15) color (n=7; see classifications above.), and 16) *Symbiodinium* assemblage (n=2 categorical groupings: clade C only or clades A+C). Although the latter three parameters are not strictly "environmental," they were nevertheless hypothesized to influence coral physiology.

Molecular-scale response variables (MSRV). RNAs and DNAs were extracted from the 140 pocilloporid samples, which had been preserved in RNALater® (Life Technologies) and transported under a CITES permit from New Caledonia to Taiwan's National Museum of Marine Biology and Aquarium (NMMBA). Upon extracting the nucleic acids as in Mayfield et al. (2015), an RNA/DNA ratio was calculated (MSRV#1) to serve as a proxy for total gene transcription. The *Symbiodinium* genome copy proportion (GCP; Mayfield et al., 2009; MSRV#2) was calculated from 20 ng DNA as described previously (Putnam et al., 2013) in order to estimate the density of *Symbiodinium* within the sampled tissues. It was hypothesized that abnormally high and low RNA/DNA and *Symbiodinium* GCP, respectively, would be indicative or stress; regarding the latter parameter, corals require high *Symbiodinium* densities (10^6 cells cm^{-2}) in order to calcify and reproduce (Gates, 1990; Brown, 1997; Furla et al., 2005). In addition to these two biological composition response variables, expression of four and four gene mRNAs was measured in the host coral and *Symbiodinium* compartments, respectively (Table 1), as described in Mayfield et al. (2017a-b). Briefly, the eight genes encode proteins involved in a number of cellular processes previously hypothesized to be affected by environmental change, such as the stress response (*hsp90*, *ubig-lig*, and *cu-zn-sod*) and metabolism (zinc-induced facilitator-like 1-like [*zifl1l*] and carbonic anhydrase [*ca*]). Specific justification for

the incorporation of these biomarkers into this "coral aberrancy test" can be found in Mayfield et al. (2017b).

In addition to these 14 response variables (the 10 MSRV+colony color, size [2 parameters], and polyp expansion behavior), two additional parameters were measured from the same DNAs from which the *Symbiodinium* GCP and RNA/DNA ratio values were calculated. First, the host corals were genotyped with PCR followed by sequencing (two directions) as in Mayfield et al. (2015). Secondly, the *Symbiodinium* assemblage was determined to clade level using the qPCR assays of Correa et al. (2009). Although host coral genotype and *Symbiodinium* assemblage are technically properties of the sampled organisms, they were generally considered to be EP for the suite of statistical analyses employed (discussed in greater detail below).

Overview of statistical analyses. Data analysis was near-identical to that of our prior works (Mayfield et al., 2017a-b). Briefly, a suite of statistical approaches was used to model the relationship between coral physiology and environment. MSA were then exploited to identify outliers in the dataset. Univariate and MSA were next utilized to determine whether outlier frequency varied across environmental gradients and whether the response variables that delineated outliers from normally behaving colonies were the same for the two predominant coral species sampled: *P. damicornis* and *P. acuta*. Unlike in our prior works, the effect of country (Fiji, Tonga, and New Caledonia) was also tested herein for each of the 14 response variables using 1-way ANOVA. The Austral and Cooks Islands were excluded from this analysis since, in general, different MSRV were assessed in those pocilloporid coral samples (Mayfield et al., 2016a). JMP (ver. 12.0.1) was used for all statistical analyses outlined below except for multi-dimensional scaling (MDS) and analysis of similarity (ANOSIM), which were carried out with PRIMER (ver. 5).

Although 140 coral samples were taken from 104 unique *Pocillopora* spp. colonies (the night dive colonies were sampled twice.), 119 such samples are the focus of the remaining statistical analyses (the 120 listed in Tables 2-3 minus colony 94, which was not genotyped and so was therefore excluded from most statistical analyses). Please see the online supplemental data file for reasons why the additional 20 colonies were excluded from the analysis. In general, this stemmed from poor extraction efficiency (low RNA or DNA yield), though in other cases, the biopsies were never extracted at all since it was envisioned that they might serve as backups were something to happen to the extracted RNAs, DNAs, and proteins.

Univariate statistical approaches, primarily ANOVA, were first used to test

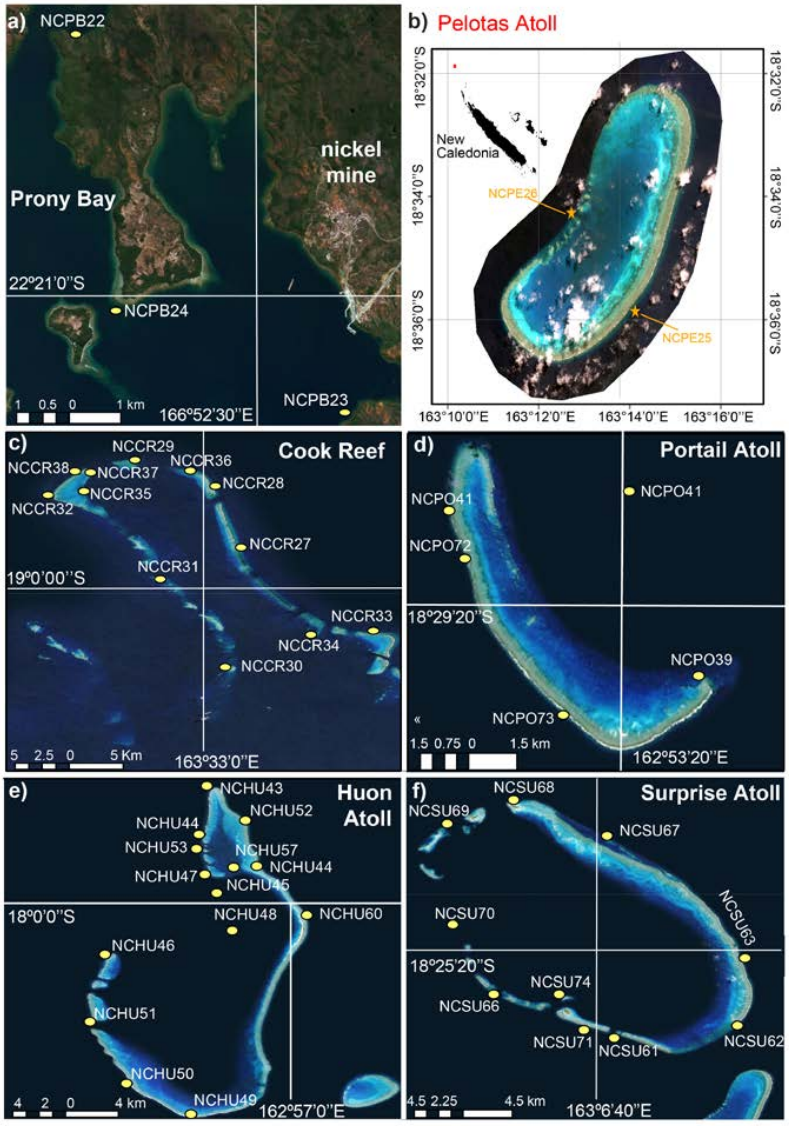

Fig. 3. Prony Bay and Northern New Caledonia dive sites. In (b), Pelotas Atoll has additionally been plotted as a red dot in an inset of the entire nation of New Caledonia to emphasize the far northerly position of the northern reefs and atolls (b-f). The fourth night dive site of the mission, which took place at Surprise Atoll (f), was not plotted, as GPS coordinates were not taken. However, the site was a sheltered, lagoonal patch reef. Maps of Guibert and Merite Atolls have not been included since pocilloporid corals were not sampled from sites surveyed within these.

for the effects of the EP listed above on the response variables quantified in the coral biopsies. Multivariate ANOVAs (MANOVAs), which featured a canonical correlation analysis (CCA) algorithm, were also used to model the effects of environment on coral molecular physiology. Then, principal components analysis (PCA) and MDS were used to uncover variation in the dataset and similarity between samples, respectively. Outliers were identified using the dual-calculation approach described in our prior works (Mayfield et al., 2017a-b). First, the Mahalanobis distance was calculated for each sample across the 10 MSRV, and, if it was greater than the upper control limit (UCL) calculated by JMP (4.2), a sampled was considered to be a potential outlier. Then, for those samples whose Mahalanobis distances were above 4.2, we required that at least one MSRV was over three standard deviations above the mean (z-score>3). For each response variable whose z-score was >3, the sample was given a "heat map score" of 1. In other words, a sample was only considered an outlier when its Mahalanobis distance was >4.2 and its heat map score was ≥1. Finally, we looked to check that these outliers were the most distant and distinct points in the PCA and MDS plots. Although these ordination techniques use completely different algorithms, they tended to support the strictly quantitative outlier detection assignments described above in our prior works (Mayfield et al., 2016a, 2017a-b). This system of statistical "checks and balances" ideally ensures that statistically normal samples are not inadvertently misclassified as outliers. Following the outlier assignment exercise, we used a series of approaches to determine which response variables were most important in partitioning outliers from non-aberrantly behaving coral colonies. All such statistical methods are discussed in greater detail below.

Univariate statistical analyses. As a preliminary step, we tested the effect of environment (the 16 EP described above) on 1) coral molecular physiology (12 response variables; using ANOVA) and 2) host coral, outlier, and colony polyp extension frequency (using X^2 tests). Since host frequency, outlier frequency, and polyp expansion frequency were assessed across 15, 16, and 16 EP, respectively (Table 4), Bonferroni adjustments of 3.9, 4.0, and 4.0, respectively, were made to *a priori*-chosen α level of 0.05, resulting in a modified α level of 0.013. All other response variables, including max. colony length, planar SA, and the 10 MSRV (Table 1; *Symbiodinium* GCP, the RNA/DNA ratio, and expression of the eight genes; n=12 response variables in total [color was considered as an EP in this analysis.]) were analyzed across the

16 EP with 1-way ANOVAs after confirming both normality (Shapiro-Wilk W test $p>0.05$) and homogeneity of variance (Levene's test $p>0.05$) of the data. Transformations were conducted when the latter two conditions were not met. Because 192 ANOVAs were performed, a Bonferroni adjustment of 13.9 was made to the *a priori*-chosen α level of 0.05, resulting in a modified α of 0.004. Similarly, a 15 EP (the same as above, though excluding host) x 12 response variable 1-way ANOVA matrix was also generated individually for each of the two species sampled: *P. damicorni*s and *P. acuta*. For these species-specific comparisons, the *a priori*-chosen α level of 0.05 was instead divided by the square root of 180 to generate the Bonferroni-adjusted α level (also 0.004). For each of the 12 response variables, the coefficient of variation was calculated in addition to the mean and standard deviation to quantify the overall variation between the sampled colonies.

MSA. MSA can 1) uncover relationships amongst response variables at a lower false positive error rate than univariate ANOVA and 2) identify combinations of response variables that best partition samples within a multi-dimensional dataspace. Several MSA were taken herein to understand the relationship between environment and coral physiology; all data were converted to *z*-scores prior to MSA to account for the response variables having different units and scales. First, MANOVA was performed to determine the effect of each of the 16 EP on the multivariate mean centroid calculated across the 10 MSRV. The two size-based parameters were excluded from this analysis (and all those henceforth). Briefly, MANOVA tests whether vectors of means (rather than simply individual means) of different samples are from the same distribution. JMP's CCA algorithm was used in conjunction with MANOVA to model environmental effects. Separate MANOVAs were then performed for each species across the 15 EP (the 16 aforementioned minus "host") since it was hypothesized that these closely related species may demonstrate subtle differences in their molecular physiology.

PCA was next performed with the 10 MSRV to determine the combinations of parameters that best accounted for variation in the dataset (*sensu* Chen et al., 2015). As an alternate, ordination-based means of visualizing the dataset in multiple dimensions, PRIMER was used to construct a Bray-Curtis similarity matrix (online supplemental data file), and an MDS plot based off of this matrix was created using data from the 119 samples analyzed for all MSRV. PRIMER's ANOSIM function was used to test for the effects of the 16 EP on separation of samples within the dataspace generated. For a more detailed explanation of

MANOVA+PCA and MDS+ANOSIM, readers are referred to Sokal & Rohlf (1995) and Clarke & Warwick (1994), respectively. It was hypothesized that both PCA and MDS could be used corroborate the outliers identified (described below).

Outlier analysis. Mahalanobis distances were calculated using the 10 MSRV only. Samples characterized by distance values above the UCL of 4.2 were considered to be "Mahalanobis distance outliers." Then, a heat map was constructed, and samples with *z*-scores >3 for a certain response variable were given a score of 1. When a sample was considered a Mahalanobis distance outlier *and* had a heat map score of 1 or greater, it was considered an outlier. Several measures were taken to understand the response variable(s) that contributed most to a sample being deemed an outlier. First, JMP's predictor screening function was used to rank the 10 MSRV in order of their contribution to the cumulative difference between the 10 outliers uncovered and all other samples (n=109). Then, a "variability index" was calculated by taking the standard deviation across the *z*-scores of all 10 MSRV for each sample. It was hypothesized that those samples with high Mahalanobis distances (i.e., the outliers) would also demonstrate more variability between the individual response variables assessed, particularly gene expression. This variability index was regressed against the Mahalanobis distance, and the significance of the correlation was tested with a linear regression *t*-test.

Differences in certain response variables between outliers and non-outliers were compared across both *P. damicornis* and *P. acuta* with 2-way ANOVA (outlier status vs. species), and individual mean differences between the four interaction groups were tested with Tukey's honestly significant difference (HSD) tests ($p<0.05$). Next, MANOVA+CCA was performed using JMP's "discriminant analysis" function to determine the response variables that best led to the separation of samples characterized by different heat map scores (which ranged from 0 [non-outliers] to 4 [extreme outliers]). An α level of 0.05 was established *a priori* for all such MSA, though a Bonferroni adjustment of 3.9 was made to the α levels of the MANOVAs of Tables 4-5 since 15-16 comparisons were made; this resulted in an adjusted MANOVA α of 0.013.

Results and Discussion

Data accessibility. For high-resolution habitat maps of the survey sites, including drop-cam video footage, please consult the following, open-access website: **http://maps.lof.org/lof**. Ideally, maps will be available in 2018. For images of the 1) surveyed reefs, 2) colonies sampled (*in situ*), and 3) "macro" photographs of these colonies' polyps,

please see the "New Caledonia" sub-heading of coralreefdiagnostics.com. This website also includes all data presented herein. Additionally, the data have been consolidated into an online supplemental data (Excel) file ("New Caledonia manuscript data file"), which can be downloaded from the *Platax* website. This spreadsheet includes all environmental data from the 79 sites, all molecular+physiological data from 119-120 of the 140 sampled colonies, *z*-score-transformed data, results of the PCA (eigenvectors and eigenvalues) and certain CCA (e.g., effect of the heat map score), a Bray-Curtis similarity matrix (used to build the MDS plot), and a separate worksheet on the light vs. dark data (which will be the focus of a forthcoming, sister work [Mayfield et al., in prep.]).

Ecological data. Reefs of Ile des Pins (Figure 2) were largely dominated by algae and were therefore characterized by low coral cover (online supplemental data file). *Asparagopsis* sp. was the most common red algae species found at depths ranging from 15 to 20 m. The reefs had generally been overgrown by both macro and turf algae, and there was a high density of damselfish and their algal lawns. *Leptoria* sp. was the most abundant coral, possibly because this species is known to prefer cooler waters (the average temperature of the 19 sites was only 24.4±0.5ºC std. dev.).

Prony Bay's reefs (Figure 3a) were characterized by a very high diversity of corals (the highest of all 79 surveyed sites), and communities ranged from plating *Leptoseris* spp. areas to dense thickets of bottlebrush acroporids. There were also medium-sized to large table *Acropora* spp. colonies. Several hard coral species that were not observed at Ile des Pins were observed, such as *Cantharellus jebbi*, *Madracis kirbyi, Cyphastrea japonica,* and *Leptoseris gardineri*. In the shallows (3-5 m), extensive thickets or bushes of *Anacropora* spp. were present, and these patches shifted to staghorn thickets and branching *Porites* spp. meadows at greater depths. The sites were characterized by relatively poor visibility, with substantial particulate organic matter in the water column. This could be due to the intensive mining efforts nearby (see Figure 3a.).

At Cook Reef (Figure 3c), the reef framework appeared to be from *Porites lobata*, though large, burrowing bivalves had eroded extensive sections of the reef; this resulted in brittle and incongruent substrate in many areas. Several unique species of corals were observed, including *Favites chinensis, Zoopilus* sp., *Hydnophora grandis,* and *Pocillopora kelleri*. Thick stands of *Acropora robusta* had 30-60% partial mortality due to gardening by damselfish. Indeed, as at Ile des Pins, there were large numbers of damselfish present amongst the dead

staghorn coral matrix. Furthermore, numerous crown of thorns seastars (COTS) were collected, and an extensive amount of coral had been consumed at the time of surveying. Storm damage was also prevalent, as evidenced by large rubble fields. Soft corals were colonizing a large proportion of the open substrate, which might account for the low number of hard coral recruits observed. Finally, large, calcified *Halimeda* sp. flakes were concentrated in small pockets or mounds. This could potentially be an indication of a high carbonate budget on the forereef slope of the reef system.

The following observations were made elsewhere in the NRA (inc. Merite Atoll, Surprise Atoll, Huon Atoll, Guibert Atoll, Portail Atoll, and Pelotas Atoll). First, many survey sites were characterized by relatively high coral cover, with mixed communities of plating *Echinophyllia*, bottlebrush acroporids, and *Pavona maldivensis*. The lagoon dives consisted mainly of *Porites* spp. framework, with smaller coral recruits present. Large stands of spindle acroporids and foliaceous *Turbinaria* spp. were also common. Monospecific stands of *Porites cylindrica* were also noted. Large mats of encrusting leather corals covered substantial portions of the tops of the reef pinnacles. Extensive COTS damage was documented at Guibert and Huon Atolls. This is unusual as these atolls are remote (far from emergent landmasses) and unaffected by nutrient runoff. Finally, disease was seen on the large table acroporids of Surprise Atoll.

Coral cover (Tables 2-3 and online supplemental data file). ALCC averaged 30% across the 79 surveyed sites (Table 3), and it was similar in the NRA (31±8.5% [std. dev. for this and all error terms from hence forth]) and the potentially more heavily impacted SR (29±16%; student's *t*-test, $p>0.05$). It did vary significantly across the 11 islands surveyed (1-way ANOVA effect of island, $p<0.001$); this was mainly driven by the cover at Prony Bay (60±2.3%) being significantly higher than that of all other islands except for Pelotas Atoll (45±6.9%) and Puom (31%; n=1 site only). Although coral cover did not differ significantly across exposure categories or reef types (1-way ANOVA, $p>0.05$), it did vary across reef zones ($p<0.001$); reefs within the sheltered bay (Prony Bay; the only bay reef system surveyed; 60±2.3%) were characterized by higher coral cover than were forereefs (30±9.5%), backreefs (28±5.8%), and lagoons (27±12%).

Genotype breakdown (Figure 1 and Tables 2-3). The genetic breakdown of 101 of the sampled colonies can be seen in Figure 1. All but 3 of the 104 uniquely sampled colonies were genotyped (excluding Neulka colony 33, Cook Reef colony 71, and Surprise Atoll colony 94). Briefly, all sampled colonies from Ile des Pins were genotyped as *P. damicornis*

(Table 2), as were the majority of the genotyped colonies across the country as a whole; of the 101 unique colonies genotyped, 80 (79%) and 21 (21%) were *P. damicornis* and *P. acuta*, respectively (Table 3). The first *P. acuta* colonies of the research mission, which began at Iles de Pins (Figure 2), were observed at Prony Bay, an extremely sheltered embayment lined with nickel mines (see site description above for details of the strange marine habitats abutting these mines.). No *P. damicornis* colonies were observed at, or sampled from, Prony Bay (Figure 1).

The sampled colonies of the NRA (Figure 3), in contrast, featured a mix of *P. damicornis* (67%) and *P. acuta* (33%; Figure 1 and Table 3). The host genotype frequency did not differ significantly between the SR and NRA (Table 4), though it did vary significantly across all eight islands/reefs/atolls from which pocilloporid corals were sampled (Table 4). Host genotype frequency also varied across reef exposure, type, and zone. Regarding reef type, *P. acuta* was not found on patch reefs, whereas *P. damicornis* was found on all reef types (atolls, barrier reefs, fringing reefs, and patch reefs). Similarly, *P. acuta* was rarely sampled from the interior regions of the lagoons (though they were found in the backreef region). *P. acuta* was only found at intermediate or protected sites; only *P. damicornis* was sampled from exposed reefs. Indeed, *P. acuta* preferred sites of intermediate exposure (65% of the colonies at sites characterized by intermediate exposure were genotyped as *P. acuta*.). Physiological differences between *P. acuta* colonies of the protected vs. the intermediately exposed sites are discussed in greater detail below.

Univariate environmental differences in coral molecular physiology (Figure S1 and Tables 4-5). When controlling for the large number of ANOVAs performed by Bonferroni-adjusting the α level, there were relatively few significant effects of environment on coral physiology when the data were analyzed across both host species sampled (Table 4). The host genotype frequency, which did vary across environments, is discussed above. Coral size (max. length and planar SA) varied across reef zones and reef types. Corals were larger in the lagoon (mean max. length=23 cm) than on the forereef (18 cm; Tukey's HSD, $p<0.05$), and corals were larger on fringing reefs (24 cm) than all other reef types (~17-20 cm; Tukey's HSD, $p<0.05$). These findings were driven mainly by *P. damicornis* (Table 5); colony size did not vary across environments for *P. acuta* (Table 5). This may mean that the former species is more phenotypically and morphologically plastic than the latter, as is also evidenced by numerous other findings outlined below (e.g., diversity of colony color).

There were several interesting, species-specific differences in coral physiology. First, there was a significant effect of host on colony color (Table 4); only *P. damicornis* colonies were found to be fluorescent blue, purple, or green. In contrast, *P. acuta* tended to be the normal brown-yellow color, or variations thereof (see images on coralreefdiagnostics.com.). This may be due to color-related differences in host coral *gfp-cp* expression; expression of this gene mRNA was significantly higher in purple fluorescent colonies than those of normal coloration (Figure S1). *P. acuta* colonies possessed over 2-fold higher *Symbiodinium* densities than *P. damicornis* (i.e., Sym GCP in Table 4), and this could also have contributed to color differences between colonies of the two pocilloporid species sampled herein.

The MSRV that varied most across environments was the *Symbiodinium hsp90* mRNA (Table 4). Expression of this gene, which encodes a molecular chaperone involved in the cellular stress response (Feder 1996), varied over both space and time. The region effect is discussed in more detail below. With respect to islands, it was expressed at significantly higher (~30-fold) levels by colonies from Ile des Pins than those of Pelotas Atoll (Tukey's HSD, $p<0.05$). This was due to the behavior of *Symbiodinium* populations within *P. damicornis* (Table 5), as *P. acuta* was not found at Ile des Pins (Figure 1 and Table 2). As another statistically significant spatial effect, *Symbiodinium* within coral colonies found on fringing reefs expressed significantly higher levels (~4-fold) of *hsp90* than those within corals found on atolls (Table 4). This difference was also driven by *P. damicornis* (Table 5) and not *P. acuta*. Indeed, virtually no environmental effects were documented across the response variables for *P. acuta*, though this may be due to the 4-fold lower sample size of this species relative to *P. damicornis* (Figure 1).

Regarding temporal effects, *Symbiodinium hsp90* mRNA expression was 4-fold higher at night than it was in the morning (Tukey's HSD $p<0.05$; Table 4), a difference detected only for *Symbiodinium* within *P. damicornis* (Table 5). Unsurprisingly, then, its expression was ~50% higher at PAR levels of 0 μmol m^{-2} s^{-1} than at positive PAR values (Table 4). Host coral *lectin* mRNA expression was also significantly, 2-fold higher in the dark than in the light (Table 4). Such light vs. dark differences in molecular biomarker expression levels are the subject of a sister work (Mayfield et al., in prep.) and will not be discussed at length herein.

Although there were multiple environmental effects on the multivariate means for both species analyzed together (Table 4) and for *P. damicornis* analyzed separately (Table 5), in most cases, there

Table 4. Univariate statistical analysis of the New Caledonia dataset-I: host species analyzed together. The values below the environmental parameters (EP; top row) represent the number of categorical groupings, and when analysis of similarity found an EP to significantly affect coral physiology ($p<0.013$), the EP name has been underlined. X^2 tests and 1-way ANOVAs were used to analyze the frequency (freq.) and molecular+physiological data, respectively. Comparisons that were statistically significant at the Bonferroni-adjusted α levels of 0.013, 0.013, 0.013, 0.004, and 0.013 for the host freq. X^2 tests, outlier freq. X^2 tests, polyp expansion (expan.) freq. X^2 tests, molecular-scale response variable (MSRV) ANOVAs, and multivariate ANOVAs (MANOVA; Wilks' lambda was calculated.), respectively, are highlighted in green. Colony 94, which was not genotyped, was excluded from the analysis. Underlined *p*-values are discussed in the Results section, and, in general, the time and photosynthetically active radiation (PAR) results will be discussed in another work (Mayfield et al., in prep.). *Symbiodinium* (Sym) assemblage was tested as an EP, though since it did not significantly affect any of the response variables, it has been excluded from the table. ALCC=average live coral cover. NS=not statistically significant. NA=not applicable.

EP/ MSRV	region (n=2)	island (n=8)	site (n=26)	expo-sure (n=3)	reef zone (n=5)	reef type (n=4)	date (n=18)	time (n=4)	depth (n=6)	temp. (n=4)	salinity (n=4)	PAR (n=2)	ALCC (n=5)	host (n=2)	color (n=7)
host freq.	NS	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.004$	$p<0.001$	$p<0.001$	NS	$p<0.001$[a]	$p<0.01$	$p<0.001$	$p<0.001$	NA	$p<0.001$
outlier freq.	$p<0.01$	NS	NS	NS	NS	NS	NS	NS	$p<0.013$	NS	NS	NS	NS	NS	NS
polyp expan. freq.	NS	NS	NS	NS	NS	NS	NS	$p<0.001$	NS	NS	$p<0.013$	NA[b]	NS	NS	NS
max. length[c]	NS	NS	$p<0.002$	NS	NS	$p<0.001$	NS	NS	NS	$p<0.002$[a]	NS	NS	$p<0.002$	NS	NS
planar Sa[c]	$p<0.004$	$p<0.004$	$p<0.001$	NS	$p<0.002$	$p<0.001$	$p<0.001$	NS	NS	$p<0.002$[a]	NS	NS	$p<0.002$	NS	$p<0.001$
RNA/DNA[d]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sym GCP[e]	NS	$p<0.002$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	$p<0.001$	NS
Sym *rbcL*[d]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sym *zifl1f*	NS	NS	NS	NS	NS	NS	NS	$p<0.001$	NS	NS	NS	NS	NS	NS	NS

EP/ MSRV	region (n=2)	island (n=8)	site (n=26)	expo-sure (n=3)	reef zone (n=5)	reef type (n=4)	date (n=18)	time (n=4)	depth (n=6)	temp. (n=4)	salinity (n=4)	PAR (n=2)	ALCC (n=5)	host (n=2)	color (n=7)
Sym *hsp90*[e]	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	NS	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.003$	$p<0.001$[a]		$p<0.001$	$p<0.002$	NS	NS
Sym *ubiq-lig*[e]	NS	$p=0.002$	$p<0.004$	$p<0.001$	NS	NS	$p<0.001$	NS	NS	$p<0.002$[a]	NS	NS	NS	NS	NS
host *ca*[d]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
host *lectin*[c]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	$p=0.004$	NS	NS	NS
host *cu-zn-sod*[d]	$p<0.004$	NS	$p<0.002$	$p<0.001$	NS	$p<0.003$	$p<0.001$	NS	$p<0.001$	$p<0.003$[a]	NS	NS	$p<0.002$	NS	NS
host *gfp-cp*[c]	NS	NS	$p<0.001$	NS	NS	$p<0.002$	$p<0.001$	NS	NS	$p<0.003$[a]	NS	NS	$p<0.001$	NS	$p<0.001$[c]
multivariate mean[f]	$p<0.001$	$p<0.001$	NS	$p<0.004$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	NS	$p<0.002$[a]	NS	$p=0.002$	$p<0.001$	$p<0.001$	$p<0.001$

[a]temperature was not recorded at a number of sites; interpret finding cautiously. [b]This parameter was not assessed at night. [c]log-transformed data. [d]rank-transformed data. [e]See Figure S1. [f]*z*-scores.

Table 5. Univariate statistical analysis of the New Caledonia dataset-II: host species analyzed separately. The values below the environmental parameters (EP; top row) represent the number of categorical groupings, and when analysis of similarity found an EP to significantly affect coral physiology (p<0.013), the EP name has been underlined. X^2 tests and 1-way ANOVAs were used to analyze the frequency (freq.) and molecular+physiological data, respectively. Comparisons that were statistically significant at the Bonferroni-adjusted α levels of 0.013, 0.013, 0.004, and 0.013 for the outlier freq. X^2 tests, polyp expansion (expan.) X^2 tests, molecular-scale response variable (MSRV) ANOVAs, and multivariate ANOVAs (MANOVA; Wilks' lambda was calculated.), respectively, are highlighted in green. Underlined p-values are discussed in the Results section, and, in general, the time and photosynthetically active radiation (PAR) results are discussed in another work (Mayfield et al., in prep.). ALCC=average live coral cover. Sym=*Symbiodinium*. NS=not statistically significant. NA=not applicable.

***Pocillopora damicornis* (n=99)**

EP/MSRV	region (n=2)	island (n=8)	site (n=26)	expo-sure (n=3)	reef zone (n=5)	reef type (n=4)	date (n=18)	time (n=4)	depth (n=6)	temp. (n=4)	sal-inity (n=4)	PAR (n=2)	ALCC (n=5)	color (n=6)	Sym assem-blage (n=2)
outlier freq.	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
polyp expan. freq.	NS	NS	NS	NS	NS	NS	NS	p<0.001	NS	NS	NS	Na[a]	NS	NS	NS
max. length[b]	NS	NS	p<0.001	NS	p<0.003	p<0.001	p=0.004	NS	NS	NS	NS	NS	p<0.005	NS	NS
planar Sa[b]	NS	NS	p<0.001	NS	p<0.001	p<0.001	p=0.001	NS	NS	NS	NS	NS	p<0.002	NS	NS
Sym GCP[b]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
RNA/DNA[c]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sym *rbcL*[c]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sym *zifl1l*[b]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sym *hsp90*[b]	p<0.001	p<0.002	p<0.001	NS	NS	p<0.003	p<0.001	p<0.002	p<0.001	p<0.001[d]	NS	p<0.002	NS	NS	NS
Sym *ubiq-lig*[b]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
host *ca*[c]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
host *lectin*[b]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
host *cuznsod*[c]	p<0.002	NS	p<0.001	p<0.002	NS	NS	p<0.001	NS	p<0.001	p<0.001[d]	NS	NS	p<0.001	NS	NS
host *gfp-cp*[c]	NS	NS	p<0.001	NS	p<0.003	p<0.003	p<0.001	NS	NS	NS	NS	NS	p<0.001	p<0.001	NS
multivariate mean[e]	p<0.001	NS	p<0.01	NS	NS	p<0.001	p<0.01	p<0.01	NS	p<0.01[d]	NS	p<0.01	NS	p<0.001	NS

***Pocillopora acuta* (n=20)**

EP/MSRV	region (n=2)	island (n=8)	site (n=26)	exposure (n=3)	reef zone (n=5)	reef type (n=4)	date (n=18)	time (n=4)	depth (n=6)	temp. (n=4)	salinity (n=4)	PAR (n=2)	ALCC (n=5)	color (n=6)	Sym assemblage (n=2)
outlier freq.	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NA[F]	NS	NS	NS
polyp expan. freq.	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NA[f]	NS	NS	NS
max. length[b]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NA[f]	NS	NS	NS
planar Sa[b]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NA[f]	NS	NS	NS
Sym GCP[b]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NA[f]	NS	NS	NS
RNA/DNA[c]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NA[f]	NS	NS	NS
Sym *rbcL*[c]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NA[f]	NS	NS	NS
Sym *zif11f*[b]	NS	NS	NS	p=0.003	NS	NS	NS	NS	NS	NS	NS	NA[f]	NS	NS	NS
Sym *hsp90*[b]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NA[f]	NS	NS	NS
Sym *ubiq-lig*[b]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NA[f]	NS	NS	NS
host *ca*[c]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NA[f]	NS	NS	NS
host *lectin*[b]	NS	NS	NS	NS	NS	NS	NS	NS	p<0.002	NS	NS	NA[f]	NS	NS	NS
host *cuznsod*[c]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NA[f]	NS	NS	NS
host *gfp-cp*[c]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NA[f]	NS	NS	NS
multivariate mean[e]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NA[f]	NS	NS	NS

[a]Polyp expansion was monitored in the day only. [b]log-transformed data. [c]rank-transformed data. [d]temperature was not recorded at a large number of sites; interpret finding cautiously. [e]*z*-scores. [f]*P. acuta* was sampled only during the light.

was no clear distinction between centroids in the CCA plots (data not shown); furthermore, samples did not tend to cluster near the centers of their respective centroids. This likely has to do with the extensive spread in the individual response variables (Table 1). For instance, the average coefficient of variation for the 10 *P. damicornis* MSRV was nearly 130%, meaning that the standard deviation was typically higher than the mean. Such was also the case for *P. acuta* (mean coefficient of variation=105%). Extensive variation between samples, even those of the same species from the same sampling site, also characterized the Austral-Cooks (Mayfield et al., 2016a), Fiji (Mayfield et al., 2017b), and Tonga (Mayfield et al., 2017a) datasets. In fact, the average coefficient of variation across all 10 MSRV was also 130% in the latter country's dataset. Given such variation, the statistically significant MANOVA findings are not discussed at great length herein except for the MANOVA+CCA of the heat map scores, which is discussed in the context of the outlier analysis below.

Regional differences in molecular physiology (Figure 4 and Tables 4-5). Upon looking at the MSRV data in the form of a heat map (Figure 4), it was evident that *P. damicornis* colonies of the SR tended to have higher expression of stress biomarker mRNAs than conspecifics in the NRA (Figure 4a); this is in alignment with our hypothesis. Specifically, a larger number of green cells (low *z*-scores) can be seen in Figure 4b, which corresponds to *P. damicornis* colonies of the NRA. In fact, the following two mRNAs were expressed at significantly higher levels in the southern, presumably more anthropogenically impacted part of the country, in comparison to the relatively more pristine NRA (Table 4): *Symbiodinium hsp90* (5-fold difference) and host coral *cu-zn-sod* (2-fold difference). Both such differences were driven by *P. damicornis* (Table 5); neither gene differed in expression across regions for *P. acuta*. Furthermore, a large number of green cells (low *z*-scores) was *not* seen in the *P. acuta* samples from the NRA (Figure 4c), meaning that *P. acuta* behaved more similarly across the two regions than did *P. damicornis*.

Outlier analysis (Figures 5-6 and S2 and Tables 4-6). Only 10 of the 119 samples analyzed in detail for all 10 MSRV fulfilled the two outlier criteria outlined above (8.5% of all samples; Table 6), and all 10 outliers were found in the SR (10/80=13%); this represents a significantly higher percentage than in the NRA (0/39=0%; X^2 test, $p<0.01$). Outlier frequency was affected by only one other environmental parameter (Table 4): depth. This is due to the fact that outliers were only sampled across three of the seven depth categories: 5-10 m (n=4 outliers out of 11 total colonies sampled from these

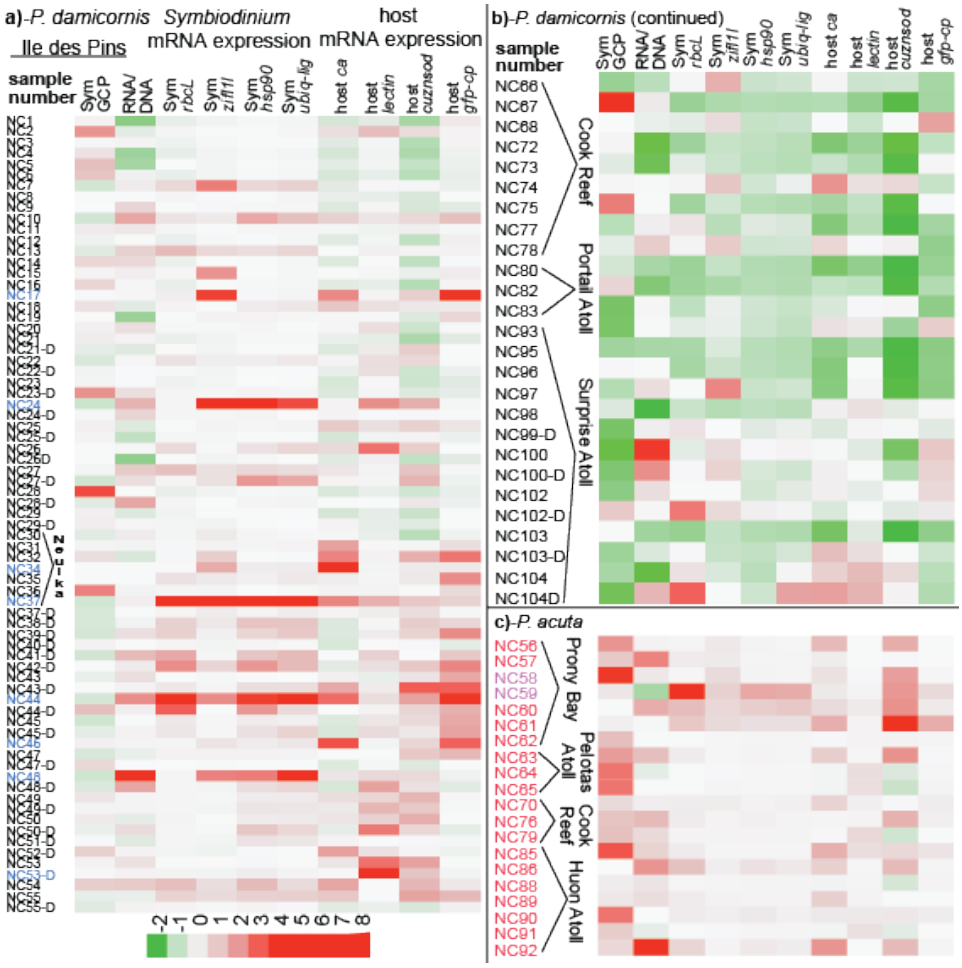

Fig. 4. Heat maps depicting standardized (*z*-score) molecular physiological data for the 99 *Pocillopora damicornis* and 20 *Pocillopora acuta* colonies sampled across both Southern and Northern New Caledonia. Only *P. damicornis* was found at Ile des Pins (a), and outliers are highlighted in blue. Both *P. damicornis* (b) and *P. acuta* (c) were found in the northern reefs and atolls, and outliers are highlighted in blue and purple, respectively. The legend for all three panels is found below (a). A *z*-score >3 for any response variable was associated with a heat map score of 1 (i.e., if two response variables had *z*-scores >3 for one sample, that sample's heat map score would be 2.).

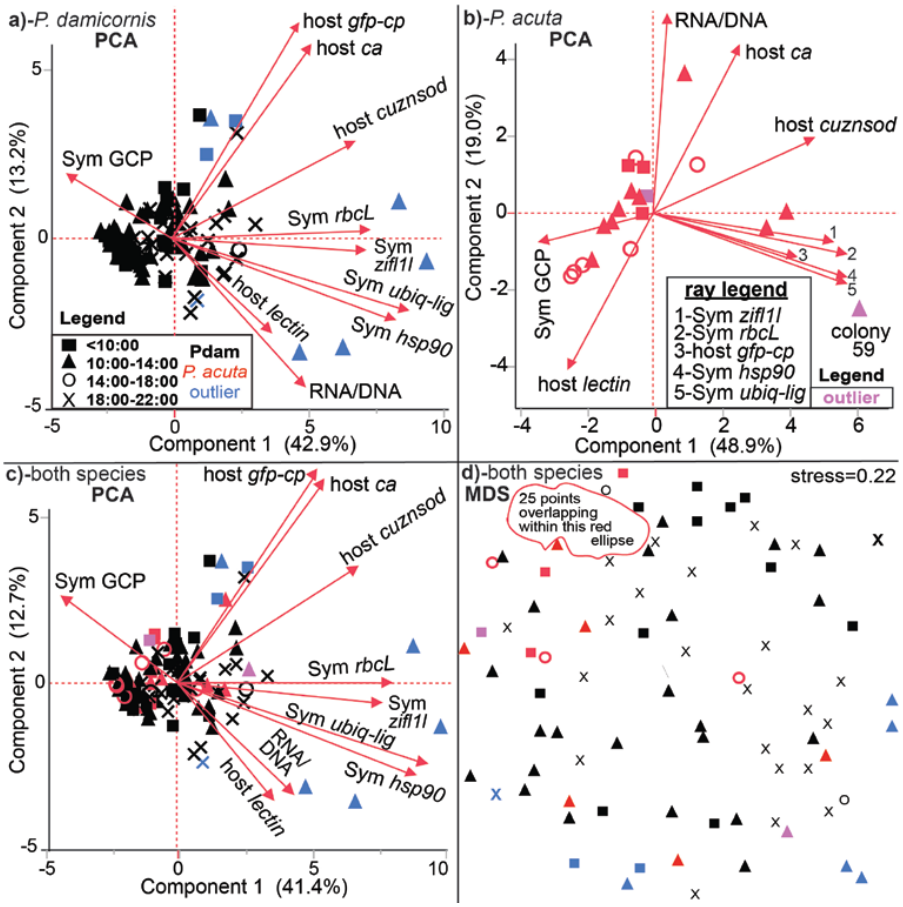

Fig. 5. Principal components analysis (PCA) and multi-dimensional scaling (MDS). PCA was performed for the *Pocillopora damicornis* dataset in isolation (a), the *P. acuta* dataset in isolation (b), and both datasets analyzed in tandem (c). As another ordination approach, PRIMER was then used to create an MDS plot based off of a Bray-Curtis similarity matrix for both species (d). The legends for all panels appear in the lower left-hand corner of (a) and the lower right-hand corner of (b), and the *P. damicornis* (n=8) and *P. acuta* (n=2) outliers are highlighted in blue and purple, respectively. Twenty-five data points in the MDS plot overlapped extensively and have been instead depicted by a hand-drawn ellipse outlined in red.

depths; 36%), 10-15 m (n=5 outliers out of 64 total; 8%), and 20-25 m (n=1 outlier out of 17 total; 6%). Outlier frequency did not vary across environment when data were analyzed separately for each species (Table 5). In contrast to our hypothesis, outliers were not more frequent on reefs characterized by low ALCC levels (Table 4).

Seven of the eight *P. damicornis* outliers were found to be the most distant and distinct points in the PCA plot (Figure 5a). The four blue triangles to the right-most region of the plot (Figure 5a) represent colonies 24, 37, 44, and 48; all four colonies were from Ile des Pins, and all four were outliers (Table 6). These four colonies were differentiated from the "core" region of the plot along principal component (PC) axis 1, which was dominated by the four *Symbiodinium* gene mRNAs in terms of loading scores (see the online supplemental data file for eigenvalues and eigenvectors.). In contrast, three other outliers were differentiated from the core sample region along PC2: colonies 17, 34, and 46. The MSRV contributing most to PC2 were instead host coral genes: *ca* and *gfp-cp*. Only one of the eight *P. damicornis* outliers, colony 53-D (dark), was not well differentiated from the non-outlier samples with PCA.

Only one of the two *P. acuta* outliers, in contrast, was well differentiated by PCA when analyzing the *P. acuta* dataset in isolation (Figure 5b); colony 59 diverged markedly along PC1, in which the four *Symbiodinium* genes contributed the highest positive loading scores (as was also the case for PC1 of the *P. damicornis*-only plot [Figure 5a]). The second *P. acuta* outlier, colony 58, which was also from Prony Bay, appears near the center of the PCA biplot (Figure 5b). When analyzing the data from both species together (Figure 5c), the biplot appears much like that of the *P. damicornis*-only biplot; this is due to the fact that nearly 80% of the sampled colonies were genotyped as *P. damicornis*. In this case, the seven *P. damicornis* outliers that were distinguished by PCA in Figure 5a again partitioned away from the central region of the plot. In contrast, neither of the two *P. acuta* outliers appeared to be distinct. This is likely due to the fact that the *P. damicornis* dataset was characterized by more variation than that of *P. acuta* (see the discussion on coefficients of variation above.). Furthermore, the PCA biplots of *P. damicornis* and *P. acuta* encompassed ~55% and ~68% of the variation, respectively, and this disparity may have led to the differential ability to distinguish outliers for each species.

When using a completely different calculation known as Bray-Curtis similarity, it was clear that the majority of the 10 outliers were the most dissimilar samples in the dataset (Figure 5d; the distance between two points is inversely

Island/ sample	species	Collection time	Colony depth (m)	Color	Max. length (cm)	Planar SA (cm^2)	Sym GCP x 100	RNA/ DNA	*Symbiodinium* genes (n=4)				Host coral genes (n=4)					
									rbcL	*zifl11*	*hsp90*	*ubiq-lig*	*ca*	*lectin*	*cu-zn-sod*	*gfp-cp*	Ma Dis	Heat map score
Ile de Pins, Neulka, and Prony Bay (10/80 samples[a] were outliers; 13%)																		
NCPI04 (1/6 samples analyzed were outliers; 17%)																		
17	<u>*P. damicornis*</u>	9:38	9.7	purple	16	120	3.1[b]	1.3	2890	**976**	208	54.3	12400	147	287	**53100**	6.0	2
NCPIND1 (1/19 samples analyzed were outliers; 5%)																		
24	*P. damicornis*	11:50	11.4	pale	23	280	0.3	3.3	.	**131**	**757**	**70.1**	.	1046	523	2700	8.9[c]	3
NCNE10 (1/2 analyzed samples were outliers; 50%)																		
34	<u>*P. damicornis*</u>	9:22	14.5	normal	14	80	2.5[b]	1.2	999	499	523	96.8	**24222**	47	208	76.9	5.8	1
NCPI15 (night dive 2; 3/14 analyzed samples were outliers; 21%)																		
37[d]	<u>*P. damicornis*</u>	11:30	10.5	very pale	19	137	0.52[b]	1.6	**3730**	**173**	**757**	**137**	9610	466	238	13900	7.4	4
44[d]	*P. damicornis*	12:05	9.1	normal	18	131	0.72	4.1	**5034**	157	**999**	**208**	14200	315	435	**53100**	5.9	4
46	<u>*P. damicornis*</u>	12:10	9.2	normal	11	60	4.5[b]	1.4	.	300	850	189	**15300**	38.0	256	43200	4.5[c]	1
NCPIND3 (2/15 analyzed sampled were outliers; 13%)																		
48[d]	<u>*P. damicornis*</u>	11:45	10.5	normal	31	270	0.41[b]	**5.8**	406	154	706	**177**	6640	574	406	2350	7.8	2
53D	*P. damicornis*	18:50	10.3	green	22	234	3.3	1.5	3730	7.0	999	104	2960	**3480**	511	18000	8.0	1
NCPB22 (1/3 analyzed samples were outliers; 33%)																		
58	<u>*P. acuta*</u>	9:05	5.0	very pale	13	91	**21**[b]	1.5	4920	1514	523	90.3	3170	31	361	1820	4.3	1
NCPB23 (1/4 analyzed samples were outliers; 25%)																		
59	59*P. acuta*	10:40	20.8	very pale	22	166	4.5	0.22	**4380**	61	499	76.9	3170	80	511	14600	4.8	1

Island/ sample	species	Coll-ection time	Colony depth (m)	Color	Max. length (cm)	Planar SA (cm^2)	Sym GCP x 100	RNA/ DNA	*Symbiodinium* genes (n=4)					Host coral genes (n=4)				
									rbcL	*zifl11*	*hsp90*	*ubiq-lig*	*ca*	*lec-tin*	*cu-zn-sod*	*gfp-cp*	Ma Dis	Heat map score
Outlier avg. (normalized data; n=10)					19	157	4.1	2.2	$3x10^{7}$	$2x10^{5}$	8602	10^{5}	10^{5}	5007	3530	$2x10^{5}$	6.4	2.0
					±	±	±	±	±	±	±	±	±	±	±	±	±	±
					5.9	79	1.7	1.7	$4x10^{7}$	$2x10^{5}$	8950	10^{5}	$8x10^{4}$	7250	883	$2x10^{5}$	1.6	1.2
Non-outlier avg. (normalized data; n=109)					21	229	5.4	1.6	$9x10^{7}$	$4x10^{5}$	1560	$3x10^{4}$	$3x10^{4}$	1930	1740	$9x10^{4}$	2.4	0.1
					±	±	±	±	±	±	±	±	±	±	±	±	±	±
					6.7[e]	156[e]	0.5[e]	1.0[f]	10^{7}[f]	$5x10^{5}$[f]	2180[f]	$3x10^{4}$[f]	$3x10^{4}$[f]	2013[e]	1510[f]	10^{5}[f]	1.0	0.3
***P. damicornis* outlier average (normalized data; n=8)**					19	164	1.9	2.5	$3x10^{6}$	$2x10^{5}$	9830	$2x10^{4}$	10^{5}	6130	3340	$2.5x10^{5}$	6.8	2.3
					±	±	±	±	±	±	±	±	±	±	±	±	±	±
					6.2	86	1.6	1.7	$4x10^{6}$	$2x10^{5}$	9530	$2x10^{4}$	$8x10^{4}$	7770	883	$2.5x10^{5}$	1.5	1.3
***P. damicornis* non-outlier average (normalized data; n=91)**					21	243	4.6	1.5	10^{6}	$4x10^{5}$	1720	$3x10^{4}$	$3x10^{4}$	2066	1670	10^{5}	2.4	0.1
					±	±	±	±	±	±	±	±	±	±	±	±	±	±
					6.9[e]	163[e]	4.8[e]	0.87[f]	10^{6}[f]	$5x10^{5}$[f]	2320[f]	$3x10^{4}$[f]	$3x10^{4}$[f]	2140[f]	1400[f]	10^{5}[f]	1.0	0.2
***P. acuta* outlier average (normalized data; n=2)**					18	129	13	0.86	$3x10^{6}$	$8x10^{4}$	3680	$6x10^{4}$	$3x10^{4}$	530	4320	$7x10^{4}$	4.5	1.0
					±	±	±	±	±	±	±	±	±	±	±	±	±	±
					6.4	53	12	0.91	$4x10^{6}$	$2x10^{4}$	4880	$7x10^{4}$	$7x10^{3}$	234	140	$7x10^{4}$	0.32	0.0
***P. acuta* non-outlier average (normalized data; n=18)**					18	163	9.3	2.1	$8x10^{5}$	$2x10^{4}$	809	10^{4}	$4x10^{4}$	1220	2206	$4x10^{4}$	2.6	0.1
					±	±	±	±	±	±	±	±	±	±	±	±	±	±
					5.2[e]	98[e]	5.6[e]	1.6[f]	$8x10^{5}$[f]	$2x10^{4}$[e]	1083[e]	$2x10^{4}$[e]	$3x10^{4}$[f]	937[e]	1990[f]	$6x10^{4}$[f]	1.2	0.3
***P. damicornis* average (normalized data; n=99)**					21	235	4.4	1.6	10^{6}	$6x10^{4}$	2380	$3.9x10^{4}$	$4x10^{4}$	2390	1804	$1.1x10^{5}$	2.8	0.24
					±	±	±	±	±	±	±	±	±	±	±	±	±	±
					6.8	158	4.7	1.0	$2x10^{6}$	$9x10^{4}$	4048	$6.4x10^{4}$	$4x10^{4}$	3120	1440	$1.4x10^{5}$	0.15	0.73
***P. acuta* average (normalized data; n=20)**					18	159	9.6	2.0	10^{6}	$3x10^{4}$	1096	$1.7x10^{4}$	$4x10^{4}$	1150	2430	$4.7x10^{4}$	2.8	0.20
					±	±	±	±	±	±	±	±	±	±	±	±	±	±
					5.2[e]	97.7[e]	6.0[f]	1.5[f]	10^{6}[f]	$3x10^{4}$[e]	1750[e]	$3x10^{4}$[e]	$3x10^{4}$[f]	912[e]	1990[f]	$6x10^{4}$[f]	0.34	0.41

[a]The latter number represents the total number of samples analyzed for all response variables, not the total number of colonies sampled (which may be greater). [b]Possesses background levels of clade A *Symbiodinium*. [c]Imputed data were used to calculate the Mahalanobis distance. [d]colony was also sampled at night (at which point gene expression signatures were not aberrant). [e]log-transformed data. [f]rank-transformed data.

proportional to their similarity or directly proportion to their dissimilarity.). With the exception of one of the two *P. acuta* outliers (see purple triangle in Figure 5d corresponding to colony 59 from Prony Bay.), all other outliers essentially formed the perimeter of the MDS plot. Please see the online supplemental data file for exact similarity values between all pairs of samples. It should be noted that the stress of the MDS plot was greater than 0.2, meaning that these findings should be interpreted with caution.

When looking at the similarity data (Figure 5d) in more detail, several EP were revealed by ANOSIM to significantly influence coral molecular physiology (see underlined EP in Table 4.). In our experience, though, ANOSIM is more likely to detect a significant difference when there are a large number of categorical groupings (>10; e.g., date and site), regardless of whether or not such findings are 1) visually compelling in the MDS plot and 2) biologically meaningful. Therefore, the date and site effects documented by ANOSIM are not discussed further herein. However, another EP with only four categorical groupings, reef type, was found by ANOSIM to significantly affect coral molecular physiology (Table 4), so a separate MDS plot featuring data points labeled by reef type was created to determine whether such a reef type difference was visually compelling (data not shown); such was not found to be the case, and samples from all four reef types appeared intermixed and not well distinguished from each other in the MDS plot. It is worth noting that, when MANOVA determined an EP to significantly affect coral molecular physiology, ANOSIM typically did, as well (one exception being site, which was found to significantly affect coral molecular physiology by ANOSIM but not by MANOVA [Table 4].).

When looking at ANOSIM findings for the *P. acuta* dataset (Table 5), a significant effect of reef exposure on coral molecular physiology was documented. Upon creating an MDS plot (Figure S2), this finding appeared visually convincing; the protected coral colonies generally clustered towards the right side of the plot, with the corals sampled from reefs of intermediate exposure generally clustering on the left. It is also clear that one of the two outliers, colony 59 (a *P. acuta* colony from Prony Bay) is the most dissimilar point in the plot, though, curiously, the second outlier (also a *P. acuta* colony from Prony Bay) is near the MDS plot's center. This indicates that its behavior is more similar to that of colonies from intermediately exposed reefs than it is to those of protected reefs. That being said, a number of protected reef colonies partitioned with those of the intermediate reef colonies. This is evidence of the far greater spread/diversity in physiology of

protected reef corals compared to intermediate reef corals, which tended to be more similar to each other in their molecular physiology.

An exploration of what drives the greater variability in the molecular physiology of corals from protected environments relative to those inhabiting intermediately exposed reefs is a worthwhile topic for future research and may be related to the fact sheltered waters may be more stagnant; this would mean the corals would have to rely more strongly on autotrophy as food may not be readily swept past their extended polyps. In other words, variability in their nutritional strategies could drive sub-cellular differences in expression of genes involved in, for instance, metabolism (e.g., *Symbiodinium zifl1l*). In contrast, murky waters with low visibility (e.g., Prony Bay) may actually necessitate that coral hosts rely *more* on heterotrophy unless they have adaptations for low light levels, such as high chlorophyll a content. Although *Symbiodinium* densities were not abnormally high in corals of Prony Bay, it is possible that their pigment concentrations were higher than those *Symbiodinium* from corals of high light areas (e.g., most of the high-visibility, clear NRA), and that such high chlorophyll a levels could aid them in living in low light environments.

As it stands, the reasons underlying the physiological differences between corals of protected and intermediately exposed reefs remain to be elucidated. Perhaps upon increasing the size of the collective LOF GRE pocilloporid coral health dataset, only half of which has been analyzed to date, we will begin to be more thoroughly able to tease apart drivers of physiological variation in corals sampled across an array of different environmental states. For instance, a larger dataset may allow us to further explore not only the differences in the molecular physiology of *P. acuta* across reefs of differing exposure mentioned above but also across ALCC levels (Table 5); although these data have not been depicted, the molecular physiology of corals of the lower ALCC grouping (15-30%) was well separated (i.e., highly different) from that of corals of the highest ALCC grouping (>60%). This lack of congruency between coral health and reef health (i.e., coral cover) is discussed in more detail below.

When looking at the differences between the 10 outliers and the 109 non-outlier samples (Table 6), the expression of all four *Symbiodinium* genes and two of the four host coral genes (*ca* and *cu-zn-sod*) were significantly higher in outliers. Specifically, *Symbiodinium rbcL*, *zifl1l*, *hsp90*, and *ubiq-lig* were expressed at 3-, 5-, 5-, and 3-fold higher levels, respectively, by outliers over non-outliers, and host coral *ca* and *cu-zn-sod* were expressed at 3- and 2-fold higher levels, respectively, by outliers

over non-outliers. However, when looking at outlier vs. non-outlier differences individually for the two coral species sampled, host coral *lectin* differed significantly for *P. damicornis*, though *Symbiodinium rbcL* did not. Furthermore, expression of no gene mRNAs differed between the two *P. acuta* outliers and the 18 non-outlier *P. acuta* samples (Table 6), though this is likely due to the small number of *P. acuta* outliers and the associated high variation (and therefore inability to detect a significant difference in the student's *t*-tests).

JMP's predictor screening function was used to determine which MSRV contributed most significantly to the global difference between outliers and non-outliers (Figure 6a), and results tended to corroborate those of the student's *t*-tests of Table 6; *Symbiodinium zifl1* and host coral *cu-zn-sod* mRNA expression contributed most significantly to the overall difference. Similarly, those MSRV that did not differ significantly between outliers and non-outliers in the student's *t*-tests of Table 6, such as host coral *gfp-cp* mRNA expression, contributed small portions to the overall variance. Curiously, some MSRV that *did* differ significantly between outliers and non-outliers, such as *Symbiodinium rbcL* mRNA expression, did not contribute significantly to the cumulative difference between outliers and non-outliers in the predictor screening analysis, despite being highly expressed genes. According to JMP's website, predictor screening uses a "bootstrap forest partitioning model" to evaluate the "contribution of predictors" (i.e., the MSRV) on the response (i.e., difference between outliers and non-outliers). Therefore, the relative proportional contribution calculated by the predictor screening model does not necessarily scale with the relative magnitude of the difference of a particular response variable between outliers and non-outliers, though it has tended to in our prior works (e.g., Mayfield et al., 2017a-b).

As a further means of gaining insight into the differences between outliers and non-outliers, the variability index was calculated for each sample (i.e., the standard deviation calculated across all 10 MSRV *z*-scores). The mean variability index for the 10 outliers of 1.79 was nearly 3-fold higher than that of the non-outliers (0.65; student's *t*-test $p<0.001$), though it did not vary across environments (data not shown). As hypothesized, the variability index did correlate significantly, positively, and linearly with the Mahalanobis distance (Figure 6b); those samples whose multivariate mean deviated most from the global mean (i.e., high Mahalanobis distances) tended to be those with the widest variation between response variables (i.e., high variability indices). This is unsurprising given that the Mahalanobis distance flags samples that fall outside of the correlation structure of the dataset.

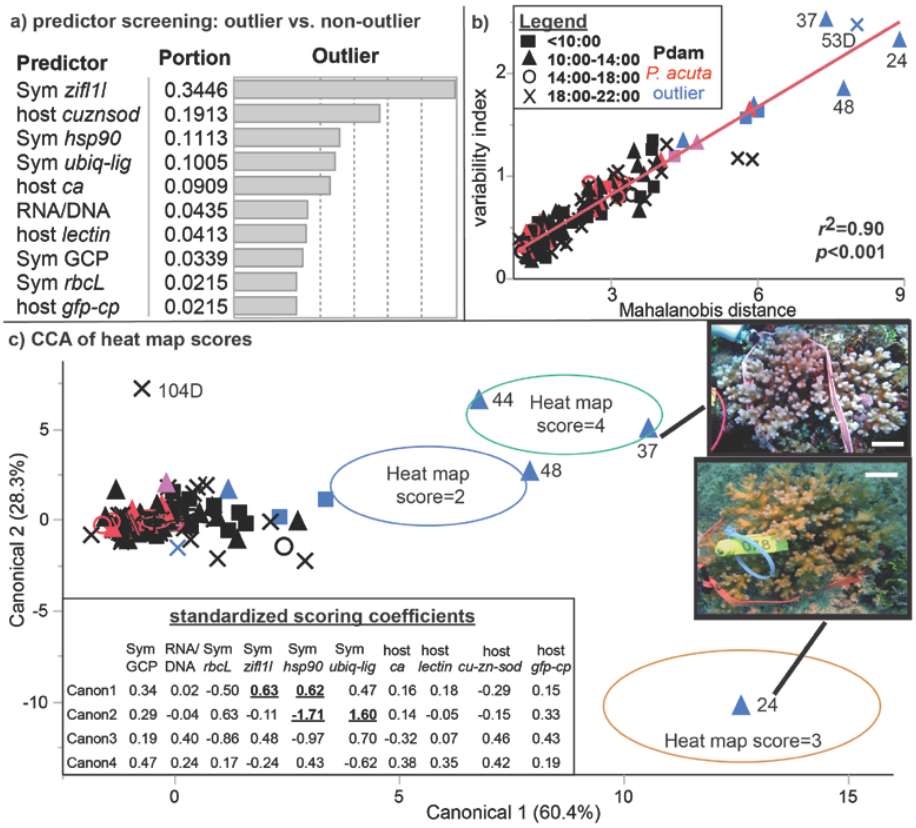

Fig. 6. Outlier analysis. JMP's predictor screening algorithm was used to rank the 10 molecular-scale response variables (MSRV) in terms of their contribution to the overall difference between the 10 outliers and the 109 non-outliers (a). Linear regression analysis was used to assess the degree of correlation between the Mahalanobis distance and the variability index (b), and several outliers (blue and purple for *Pocillopora damicornis* and *P. acuta*, respectively) have been labeled. The legend for (b-c) is found within (b). Canonical correlation analysis (CCA) was used to model differences between samples with heat map scores of 0-1 (non-outliers) and 2-4 (outliers). The 95% confidence centroids for the heat map score groups of 0 and 1 are masked beneath the densest assemblage of data points to the left of the plot. The standardized scoring coefficients have been included in an inset, as have the images of two prominent outliers (scale bars=5 cm).

As a final means of modeling differences between outliers and non-outliers, a CCA plot was generated after testing for the effect of heat map score on the multivariate mean. In general, samples with heat map scores of 0 and 1 were not outliers, whereas all samples with heat map scores of 2 or more were. In some cases, samples with heat map scores of 1 were outliers. Using this approach (Figure 6c), five outliers identified by other means appear distinct from the core region of the plot along canonical axis (CA) 1: colonies 17 and 48 (heat map score=2), colony 24 (heat map score=3; pictured in bottom-most inset), and colonies 37 (pictured in top-most inset) and 44 (heat map score=4). These samples were distinguished from the core region of the plot by virtue of their *Symbiodinium zifl1l* and *hsp90* mRNA expression levels (see standardized scoring coefficients in inset of Figure 6c.). Interestingly, one sample not found to be an outlier by other approaches, colony 104-D (dark), appears relatively distinct from the other points along CA2. Although the variation encompassed by this axis is only half that of CA1, this result is unsurprising given the relatively high Mahalanobis distance (5.6) of this sample. This represents the highest distance for a sample that was not ultimately considered to be an outlier (due to the fact that no MSRV possessed a *z*-score>3).

Country-wide differences in the MSRV (Table 1 and Figure S3). As the identical protocols were used to measure the same 10 MSRV in pocilloporid corals sampled from Fiji and Tonga, we compared data across the three countries with 1-way ANOVA. The non-normalized threshold cycle (Ct) values are shown in Table 1, and the normalized data have been plotted in Figure S3; it is evident that, in most cases, the normalized and non-normalized gene expression data show very different trends. For instance, *Symbiodinium hsp90* mRNA expression was highest for samples of New Caledonia when the data were left un-normalized (Table 1). However, when normalizing to recovery of the exogenous Solaris® RNA spike and the *Symbiodinium* GCP (Figure S3c), expression of this gene was actually highest in samples of Fiji. This was the only gene, in fact, whose expression was not highest in corals of New Caledonia; all other genes, including the stress genes *ubiq-lig* (*Symbiodinium*; Figure S3f) and *cu-zn-sod* (coral host; Figure S3i), were expressed at significantly higher levels by samples of New Caledonia relative to both Fiji and Tonga. Furthermore, *Symbiodinium* densities were 5-6-fold lower in New Caledonian corals than in those of Fiji and Tonga (Figure S3b). Regarding outlier vs. non-outlier differences for each country analyzed individually, only two genes, *Symbiodinium*

zifl1l and host coral *cu-zn-sod*, differed significantly between outliers and non-outliers in the datasets of all three countries (Table 1). In contrast, host coral *lectin* did not differ between aberrantly behaving corals and statistically normal ones in any country (Table 1). In future projects, then, this gene may be excluded from the biomarker panel.

The observation that expression of all stress-targeted mRNA biomarkers, excluding *Symbiodinium hsp90*, was higher in pocilloporid corals of New Caledonia than in those of the two other surveyed nations is a curious finding, especially given that the NRA of New Caledonia, in particular, were amongst the healthiest surveyed during the GRE, at least from an ecological perspective (high fish biomass, high coral cover and diversity, low disease prevalence, etc.). When comparing the MSRV data from these NRA only against the Fiji and Tonga datasets, all such differences were maintained (data not shown). This demonstrates that those reef ecosystems that appeared the healthiest to the naked eye (i.e., the NRA) were not necessarily characterized by the healthiest corals. Such a lack of congruency between coral health and coral reef health was actually proposed several years ago by Wooldridge (2014) and is hypothetically related to the fact that the corals on reefs characterized by high coral cover tend to be growing quickly; this is associated with higher respiration rates, which, in turn, reduce future capacity for autotrophy. If corals become malnourished through such diminished autotrophy, they may become more susceptible to environmental changes since, for instance, they may have consumed their cellular energy stores prematurely. This could also explain why the molecular physiology of *P. acuta* differed significantly between low (15-30%) and high (>60%) coral cover reefs (Table 5 [the MDS plot has not been shown.]); whereas no outliers were uncovered from reefs of coral cover lower than 45%, both of the *P. acuta* outliers (colonies 58 and 59) were from reefs of Prony Bay with incredibly high coral cover (59 and 63%, respectively). In short, such high ALCC reefs may not necessarily be associated with healthier corals than those reefs characterized by relatively lower hard coral cover. As a further, more extreme example of a situation in which coral reef and reef coral health may be incongruent, the few surviving colonies on reefs that have undergone a high-temperature-induced, mass coral bleaching event are likely to be more robust than conspecifics on a high coral cover reef that have not yet been exposed to such bleaching-inducing temperatures.

Although we incorrectly hypothesized that corals of New Caledonia would demonstrate lower stress gene expression levels than pocilloporids from elsewhere

in the South Pacific, our hypothesis that a north-south gradient in molecular biomarker expression would exist was confirmed; as mentioned above, corals of the SR tended to express higher levels of stress biomarker mRNAs, such as host coral *cu-zn-sod*, than conspecifics from the more remote NRA. Furthermore, all 10 outliers in the 119-sample New Caledonia dataset were from the SR (8 and 2 at Ile des Pins and Prony Bay, respectively). Despite the existence of such a gradient, and in alignment with the prior treatise on inter-country differences in molecular biomarker expression, the overall gene expression levels were high for all 119 samples analyzed in detail. This includes genes such as *hsp90*, which should only be synthesized by individuals undergoing a cellular stress response (Hochachka & Somero, 2002); such was also documented in the highly remote Austral and Cook Islands (Mayfield et al., 2016a). These data seem to suggest that, in contrast to our hypothesis, even corals far from large human population centers have not been spared from the effects of global-scale stressors, such as GCC. Although some have hypothesized that it may benefit corals to continually engage their cellular stress responses, as, for instance, doing so could better prepare them for future environmental changes (Barshis et al., 2013), the significant energetic expense required for sustaining such a stress response over long-term timescales may eventually detract from processes such as growth and reproduction. In closing, whether or not such constitutively high stress biomarker mRNA expression levels documented in corals of New Caledonia and elsewhere in the South Pacific are diagnostic of future decreases in physiological performance remains to be determined. This topic should be addressed urgently, though since, if the molecular biomarker panel+MSA unveiled herein *does* prove to have diagnostic capacity, it could be used for proactive monitoring of coral reef ecosystems around the Indo-Pacific.

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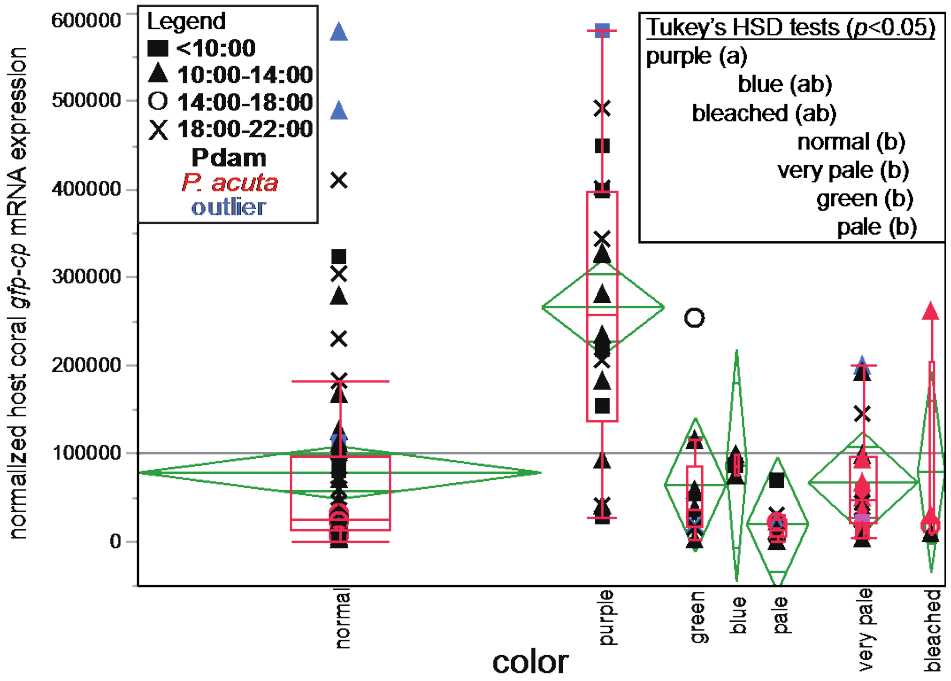

Fig. S1. Host coral green fluorescent protein-like chromoprotein (*gfp-cp*) mRNA expression across coral colonies of different colors. The global mean across all samples (~100,000) has been plotted as a gray, horizontal line, and data points have been plotted within both mean diamonds (green; the width is proportional to the sample size.) and normal quantile box plots (red). Tukey's honestly significant difference (HSD) *post-hoc* tests were used to determine individual mean differences (p<0.05), and results can be found in an inset in the upper right of the figure. The *Pocillopora acuta* outliers (purple; n=2) are generally not visible.

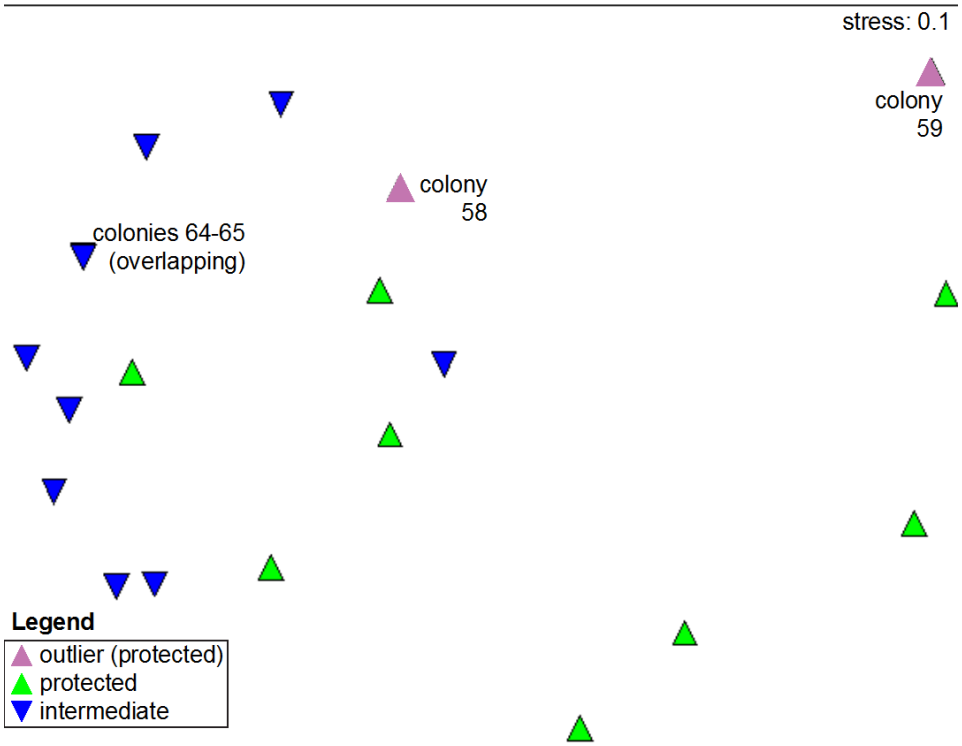

Fig. S2. Multi-dimensional scaling (MDS) plot demonstrating the statistically significant (ANOSIM Global *R*, $p<0.01$) effect of exposure (intermediate vs. protected) on the molecular physiology of *Pocillopora acuta* (n=20). Both *P. acuta* outliers from Prony Bay (protected) have been labeled (purple icons). Only 19 of the 20 colonies are visible since colonies 64 and 65 overlapped.

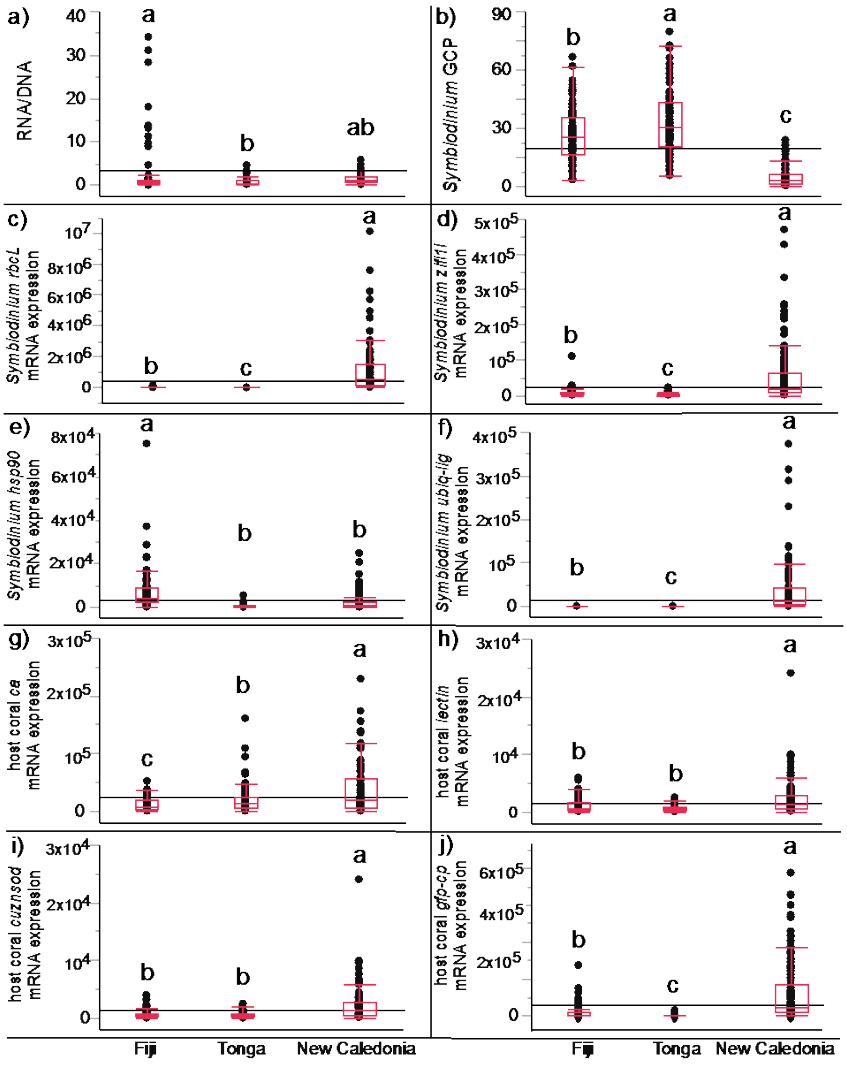

Fig. S3. Country-wide differences in the 10 molecular-scale response variables (MSRV). All coral sample data, as well as normal quantile box plots, have been plotted for the Fiji (June, 2013), Tonga (September, 2013), and New Caledonia (November, 2013) research missions (the x-axis labels for all panels are found at the bottom of the figure.). In certain cases, the box plots are so compressed that they appear are horizontal lines. Significant effects of country were detected in the 1-way ANOVA (p<0.05) for all MSRV. Tukey's honestly significant difference tests were then performed, and lowercase letters signify differences between individual country means (p<0.05). For the non-normalized threshold cycle (Ct) values, please see Table 1.