TRANSCRIPTOMIC SIGNATURES OF ACCLIMATION IN 'SUSCEPTIBLE' *ACROPORA SURCULOSA* AND 'RESISTANT' *PORITES RUS*

BY

CONSTANCE SARTOR

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SUPERVISORY COMMITTEE
Dr. Sarah Lemer
Dr. Laurie Raymundo
Dr. Peter Houk
Dr. Kate Quigley

UNIVERSITY OF GUAM

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Title: Transcriptomic signatures of acclimation in 'susceptible' *Acropora surculosa* and 'resistant' *Porites rus*

As ocean temperatures rise globally, the search for individual coral genotypes that are currently able to survive environmental extremes becomes more critical. Certain corals in Guam inhabit more thermally variable shallow reef flats than their conspecifics in other locations and may provide insight into whether some coral individuals can acclimate to climate change. We reciprocally transplanted stress 'resistant' *Porites rus* and stress 'susceptible' *Acropora surculosa* between two depths with more and less variable thermal and irradiance regimes. Throughout the ten-week transplant we collected tissue color values, bleaching rates, Symbiodiniaceae measurements, and transcriptomic samples. In the natural populations, both coral species expressed distinct transcriptomic patterns based on depth. When transplanted from the more variable shallow depth to the more stable deep depth, both coral species showed 'transcriptional dampening', indicating acclimation. When transplanted to the more variable shallow site, however, *A. surculosa* exhibited a more pronounced 'Type B' environmental stress response (ESR) while *P. rus* showed more transcriptomic plasticity. After the ten-week transplant, there was still evidence of transplantation stress closely resembling a 'Type A' ESR in both coral species at both depths that could make it difficult to differentiate an environmental stress response from a transplantation stress response. With an enhanced understanding of how more 'susceptible' and 'resistant' coral species respond to thermal and irradiance stress, we can better predict how these corals will acclimate in the future as climate change progresses.

Keywords: coral, reciprocal transplant, transcriptomics, acclimation

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Chapter 1. Introduction

Coral bleaching and species susceptibility to stress

At the end of the 20th century, our oceans began to experience "large-scale" coral bleaching. Rather than small, localized events, there was an increase in both the frequency and intensity of coral bleaching throughout the Caribbean, Pacific Ocean, and Indian Ocean (Hughes et al., 2017). The first mass bleaching event in tropical reefs occurred in 1998 and by 2010, 48% of the world's reefs had been impacted by bleaching; by 2015 we experienced record high sea surface temperatures (Hughes et al., 2017). There is evidence that the most geographically extensive record of coral bleaching occurred from 2015 to 2016, in response to elevated sea surface temperatures (NOAA Coral Reef Watch 2015; Bahr et al., 2017; Hughes et al., 2018). As of 2019, marine heatwaves were three times longer than they were in the 1980's (Smale et al., 2019). Hence, there is an urgent need to understand what is driving bleaching events and how to prevent them.

Bleaching occurs when the coral host loses its algal endosymbionts (Family: Symbiodiniaceae) (Glynn, 1984) and is caused by various stressful environmental factors; temperature and irradiance are thought to be the most prominent (Brown, 1997). In fact, elevated irradiance and temperature are often linked; bleaching is typically response to a combination of high solar radiation and temperature stress (Gleason and Wellington, 1993). While temperature inhibits the Photosystem II (PSII) repair processes of the Symbiodiniaceae, irradiance causes photodamage to PSII (Brown and Dunne, 2016). As many corals are already living at or near their upper temperature threshold (Coles et al., 1978), they are vulnerable to small increases in sea surface temperature (Cossins and Brown, 2011). Prolonged thermal stress usually leads to

widespread bleaching events. For example, elevated temperatures from El Niño events can strengthen the likelihood of mass coral bleaching events (Lough et al., 2018). In contrast, both short- and long-term periods of high irradiance exposure can cause bleaching in corals, although bleaching is typically more geographically localized when caused by irradiance stress compared to thermal stress (Gleason and Wellington, 1993). Increased temperatures can also make the algal symbionts more susceptible to photoinhibition from irradiance, catalyzing their expulsion from the coral host (Elstner, 1988). Climate change is predicted to increase the incidences of elevated irradiation and sea surface temperature and to consequently increase the risk of coral bleaching as well (Laffoley et al., 2016).

Coral bleaching may have various consequences for corals, aside from their outright mortality, and their surrounding ecosystems in both the short and long term. During and shortly after bleaching, corals often experience shifts in their Symbiodiniaceae, compromised photosynthetic efficiency, reduced skeletal growth (Baird et al., 2008), and greater susceptibility to disease and predation (Lesser et al., 2007). In the long term, corals may exhibit increased risk of partial and full mortality and decreased fecundity and growth (Ward et al., 2000). As a reef recovers from a bleaching event, algae may begin to take over and fish communities may shift, potentially reducing fishery productivity (Graham et al., 2007). Some reefs recover more quickly than others (Koester et al., 2020), but with climate change, it becomes increasingly difficult for heat-sensitive animals to survive.

Acclimation stress and susceptibility to stress

Based on their physiology and life histories, some coral species are more susceptible than others to bleaching and environmental stressors. Studies have pointed out broad physical characteristics that can be used to classify certain ‘susceptible’ coral taxa (Baird and Marshall,

2002; Pratchett et al., 2013). Deemed one of the most bleaching susceptible coral genera, *Acropora* is fast-growing and exhibits a diversity of growth forms. Other genera including *Montipora,* are more likely to bleach as well (Pratchett et al., 2013). Raymundo et al. (2019) provided metrics on how coral species in Guam responded to bleaching, pointing out that certain *Acropora* and *Montipora* corals exhibited more intense population declines. In contrast, the structurally hardier and slower-growing genera such as *Platygyra, Porites,* and *Favia* resist bleaching related conditions for longer periods of time (Baird and Marshall, 2002).

The historical environmental conditions experienced by a coral can also influence its ability to resist bleaching (Grimsditch and Salm, 2006, Coles and Brown, 2003). Here, "acclimation" will refer to the short-term (days to weeks) physical or physiological response of an individual coral. In our rapidly changing climate, some corals are constantly being exposed to near-lethal temperatures (Reigl et al., 2011). Long-term exposure to warmer temperatures has resulted in thermal acclimation of some corals, including *Pocillopora damicornis* and *Platygyra verweyi* (Coles and Brown, 2003, Somero, 2010). *Acropora nana* colonies were able to quickly acclimate in as little as seven days (Bay and Palumbi, 2015), whereas other species including *Goniastrea aspera* take longer (Brown and Cossins, 2011). Just as with physical susceptibility to bleaching, acclimation is more prevalent in certain taxa. In a meta-analysis of corals in the Great Barrier Reef, Marshall and Baird (2000), found that *Acropora* and *Pocilloporidae* corals were more susceptible to bleaching episodes and less likely to acclimate. In addition to between-species acclimation ability, there is also variation in the acclimation ability within a coral species. For example, certain colonies of *Porites* species can still be found thriving in areas with irradiance levels beyond what has been documented to trigger bleaching (Smith and Birkeland, 2007).

Crucial to the survival of most shallow water scleractinians, Symbiodiniaceae also aid in acclimation ability of the coral host. Corals host up to four different genera of Symbiodiniaceae with varying susceptibility to stress, including *Symbiodinium, Breviolum, Cladocopium* and *Durusdinium.* Fabricius et al. (2004) found that bleaching-resistant *Porites* corals contained higher densities of Symbiodiniaceae genus *Durusdinium* after a thermal stress event. In a controlled acclimation study, small shifts in Symbiodiniaceae genera occurred when *Porites* corals were transplanted between thermally extreme and stable environments (Barshis et al. 2010). In a similar study on *Acropora* corals, Palumbi et al. (2014) also found insignificant proportions of *Durusdinium* shifts in *Cladocopium*-dominated corals. The density of Symbiodiniaceae cells also changes in response to thermal (Piggot et al., 2009) and solar radiation (Wicks et al., 2010) stress. In some coral species, Symbiodiniaceae may acclimate faster than the host. For example, Cohen and Dubinsky's (2015) six-month reciprocal transplant of *Stylophora pistillata* in the Red Sea focused simply on the abundance of Symbiodiniaceae and found faster acclimation in the symbionts. Higher Symbiodiniaceae densities during thermal stress may also indicate susceptibility to bleaching (Cunning and Baker, 2013). Looking at fluctuations in Symbiodiniaceae density and shifts in community composition provides insight into how well a coral host responds to environmental changes.

Corals mostly obtain nutrients autotrophically through their Symbiodiniaceae, but they can also heterotrophically feed on plankton. The ability to switch between the two feeding mechanisms, or 'heterotrophic plasticity', can play a role in coral acclimation. In fact, relying on autotrophy alone can be limiting, and some corals can increase their heterotrophy rates when experiencing environmental stress (Hughes and Grottoli, 2013). For example, *Porites rus* colonies in Moorea, French Polynesia showed increased heterotrophy when transplanted to a

more variable environment (Padilla-Gamiño et al., 2012). In a similar study by Grottoli et al. (2006), *Montipora capitata* corals with greater heterotrophic plasticity than *Porites compressa* exhibited increased resilience to bleaching events in the long term. Heterotrophic plasticity may improve corals' chances of survival in the variable environments associated with climate change.

Stress response and acclimation at a cellular level

Transcriptomic analysis can provide insight into how corals respond to stress by linking underlying cellular processes to environmental factors (Bay and Palumbi, 2016). Increased numbers of differentially expressed genes can indicate a response to environmental stress (Seneca and Palumbi, 2015). Some corals exhibit more plasticity in their gene expression response than others (Kenkel and Matz, 2016), which may help with their acclimation ability (Davies et al., 2016). Through gene annotations and functional enrichment analyses, we can get closer to understanding the cellular processes that occur when a coral is undergoing environmental stress. Under thermal stress, corals produce reactive oxygen species (ROS) (Lesser, 2006), which can inflict damage upon lipids, DNA, and proteins (DeSalvo et al., 2008). To mitigate the damage caused by ROS, the coral produces various antioxidants including ferritin and superoxide dismutase (Downs et al., 2000). This oxidative stress also impacts the endoplasmic reticulum (ER) and can cause an unfolded protein response (Ruiz-Jones and Palumbi, 2017). The endoplasmic reticulum (ER) stores most calcium ions within the cell, so when the ER homeostasis is disrupted, calcium ions are released, calcium-binding decreases, and overall calcium homeostasis is disrupted as well (DeSalvo et al., 2008). When calcium homeostasis is thrown off, there is often a reorganization of the cytoskeleton (Mayfield and Gates, 2007). Under constant oxidative stress, the final response is often apoptosis (Tiwari et al., 2002).

Within the coral host, a variety of categories of genes are associated with this stress response (Table 1). Under thermal stress, corals experience a disruption in homeostasis including skeletal structure (DeSalvo et al., 2008), protein breakdown (Brown et al., 2002) and reduction of calcium ion binding (Davies et al., 2016). Under increased solar radiation, corals also experience reduction in calcium ion binding (Aranda et al., 2011), cellular damage (Lesser and Farrell, 2004), and production of reactive oxygen species (ROS) (Lesser, 2006). Recently, Lock (2021) reported that transplantation of *Porites* corals caused a disruption in calcium homeostasis, endoplasmic reticulum (ER) stress, and protein turnover, all of which are indicative of a stress response.

Table 1. List of gene functional categories involved in coral stress response, obtained from a literature review of 18 scientific papers published from 2002 to 2021.

Environmental stressor	Gene function group	Description	Coral species	Reference
Heat	Calcium homeostasis	Ca2+ ion binding	*Siderastrea siderea; Orbicella faveolata*	Davies et al. 2016; DeSalvo et al. 2008
	Skeletal homeostasis		*Orbicella faveolata*	Desalvo et al 2008
	ER homeostasis		*Siderastrea siderea*	Davies et al. 2016
	ER homeostasis	Ubiquitin-like protein ligase	*Goniastrea aspera*	Brown et al. 2002
	ER homeostasis	Ubiquitin-like protein ligase	*Pocillopora acuta*	Mayfield et al. 2018
	ER homeostasis	Unfolded protein response	*Acropora hyacinthus*	Ruiz-Jones and Palumbi 2017
	Oxidative stress	oxidoreductase	*Pocillopora damicornis; Orbicella faveolata*	Selmoni et al. 2020; Desalvo et al. 2008
	Cellular transport	Ion transport	*Acropora millepora*	Bernadet et al. 2019; Meyer et al. 2011; Dixon et al. 2015
	Cell signaling	Signaling receptor	*Pocillopora damicornis*	Selmoni et al. 2020
	ER homeostasis	Protein heterodimerization		Tonione et al. 2020
	ER homeostasis	protein synthesis	*Acropora palmata*	Portune et al. 2010
	Metabolism		*Acropora palmata; Acropora millepora; Pocillopora damicornis*	Portune et al. 2010; Meyer et al. 2011; Vidal-Dupiol et al. 2014

	extracellular	ECM structural constituent		Dixon et al. 2015
Irradiation	Ca2+ homeostasis	Ca2+ ion binding	*Orbicella faveolata*	Aranda et al. 2011
	Cell damage		*Orbicella faveolata*	Lesser and Farrell 2004
	Endoplasmic reticulum		*Orbicella faveolata*	Aranda et al. 2011
	Oxidative stress	production of ROS		Lesser 2006
	DNA repair		*Orbicella faveolata*	Lesser and Farrell 2004
Transplantation	Cellular transport	Ion transport	*Porites lobata*	Lock 2021
	Ca2+ homeostasis		*Porites lobata*	Lock 2021
	Structural homeostasis	ECM structural constituent	*Porites lobata*	Lock 2021
Bleaching		Structural constituent of ribosome	*Acropora cervicornis*	Drury 2019

Reciprocal transplant experiments (RTE) are a viable method for performing controlled acclimation studies on organisms (Palumbi, 1984). By exchanging individuals within and between different locations and measuring a component of fitness, one can determine if the individuals have the potential to acclimate to a different environment (Lee-Yaw et al., 2016). RTEs have been performed on corals to determine whether they can acclimate to more variable (Barshis et al., 2010; Bay and Palumbi, 2017), brighter (Bongaerts et al. 2011), warmer (Cohen and Dubinsky, 2015) and nutrient poor (Tamir et al., 2020) environments. These RTEs have provided information on how corals acclimate through growth (Raymundo, 2001), Symbiodiniaceae density and composition (Bongaerts et al. 2011; Cohen and Dubinsky, 2015; Tamir et al. 2020), gene expression (Bay and Palumbi, 2017), photophysiology (Bongaerts et al., 2011), microbiome (Ziegler et al. 2016), protein expression (Barshis et al., 2010), calcification (Cohen and Dubinsky, 2015) and gene body methylation (Dixon et al., 2018). Most of these RTE studies involve gene expression and indicate key responses to environmental stressors. While corals in higher-quality environments upregulate housekeeping genes, corals in lower-quality

habitats upregulate environmental response genes (Dixon et al., 2018). The response, however, is often influenced by the coral's original habitat (Barshis et al., 2010) and there are tradeoffs between growth and survival in corals that can tolerate more stressful environments (Bay and Palumbi, 2017). Reciprocal transplantation will be used in the present study to examine the impacts of variable thermal and irradiance regimes on coral acclimation. This differs from previous coral reciprocal transplant studies in that it compares baseline, control, and transplant treatment groups and assesses two contrasting coral genera (*Acropora* and *Porites*).

Study Species

With regard to bleaching and stressor susceptibility, *Acropora* and *Porites* are at opposite ends of the spectrum (Colgan et al., 1987; Palmer et al., 2010; Raymundo et al., 2019). In Guam, there are representatives of both species, including *Acropora surculosa* and *Porites rus* (Figure 1, Raymundo et al., 2019). *Acropora* and *Porites* corals are widespread, found in the Atlantic, Pacific, and Indian Oceans, but *A. surculosa* and *P. rus* are only found in the Indo-Pacific (Forsskål, 1775; Dana, 1846). *A. surculosa* is fast-growing, high temperature-susceptible, branching, and a broadcast spawner (Kuffner and Paul, 2004). In Pago Bay, Guam, *A. surculosa* colonies found between 2 m and 8 m depths mainly host Symbiodiniaceae of the genus *Cladocopium* (Moscato, 2020). In contrast, *Porites rus* is a slow-growing, plating, columnar coral and reproduces via gonochoric brooding and release of larvae (Penland et al., 2004). *Porites rus* also has deeper depths limits than *Acropora surculosa,* reaching beyond 15 m in depth. In Guam, *Porites lobata* corals primarily host *Cladocopium* (Lock, 2021), but we do not currently have information on the Symbiodiniaceae genus composition hosted by *P. rus*. Due to its thicker tissue, *Porites rus* is suggested to be more resistant to thermal stress than other stony

corals (Loya et al., 2001). By studying both *Acropora* and *Porites* genera, we are able to compare acclimation ability in two corals with distinct life history strategies and susceptibilities.

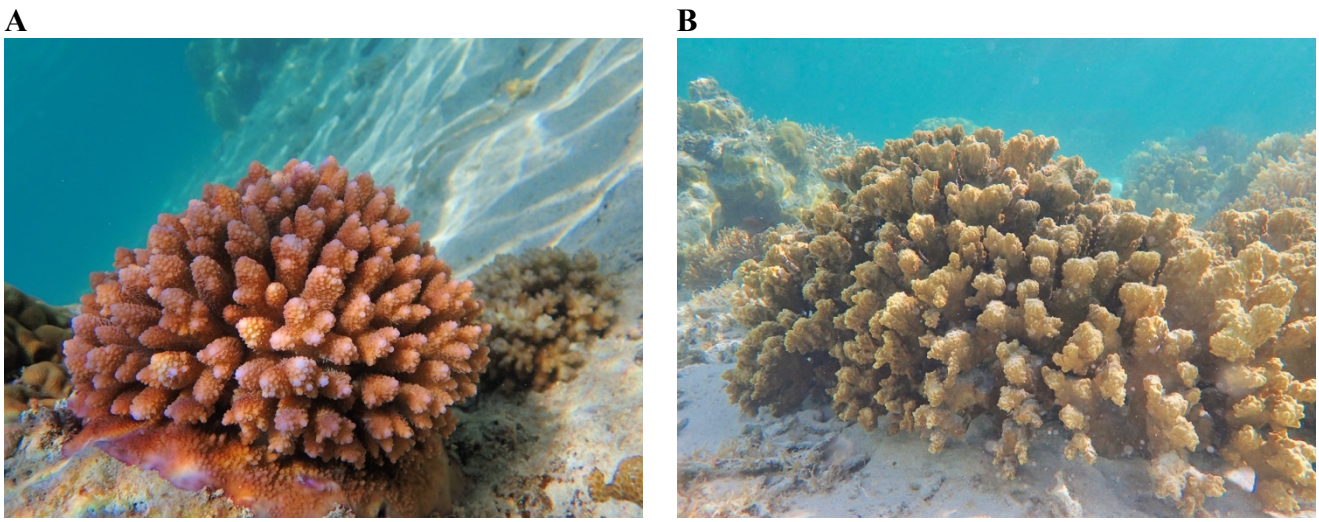


Figure 1. *Acropora surculosa* (A) and *Porites rus* (B)

Objectives

Although there have been transcriptomic studies on reciprocally transplanted corals, the present study is the first to compare two species with contrasting bleaching susceptibilities simultaneously: *Acropora surculosa* and *Porites rus.* By conducting a reciprocal transplant between two depths (2 m and 10 m) of both *Acropora surculosa* and *Porites rus*, this study examined how the two coral species acclimated to the exact same temperature and light regimes. To quantify the acclimation ability of the corals, we made physical observations, assessed changes in gene expression, and characterized the Symbiodiniaceae communities as the corals were transplanted.

With this work I address three main questions:

1. Are coral colonies living in more variable (2 m deep) versus more stable (10 m deep) habitats acclimated to their environments?

2. Can coral colonies living in a more variable habitat acclimate to a more stable habitat and vice versa?

3. Does the molecular stress response differ between the two coral species?

My tested hypotheses were the following:

H_{01}: Corals living in more variable habitat cannot acclimate to more stable habitat.

H_{A1}: Corals living in more variable habitat can acclimate to more stable habitat.

H_{02}: Corals living in more stable habitat cannot acclimate to more variable habitat.

H_{A2}: Corals living in more stable habitat can acclimate to more variable habitat.

H_{03}: *A. surculosa* exhibits a stronger acclimation response than *P. rus.*

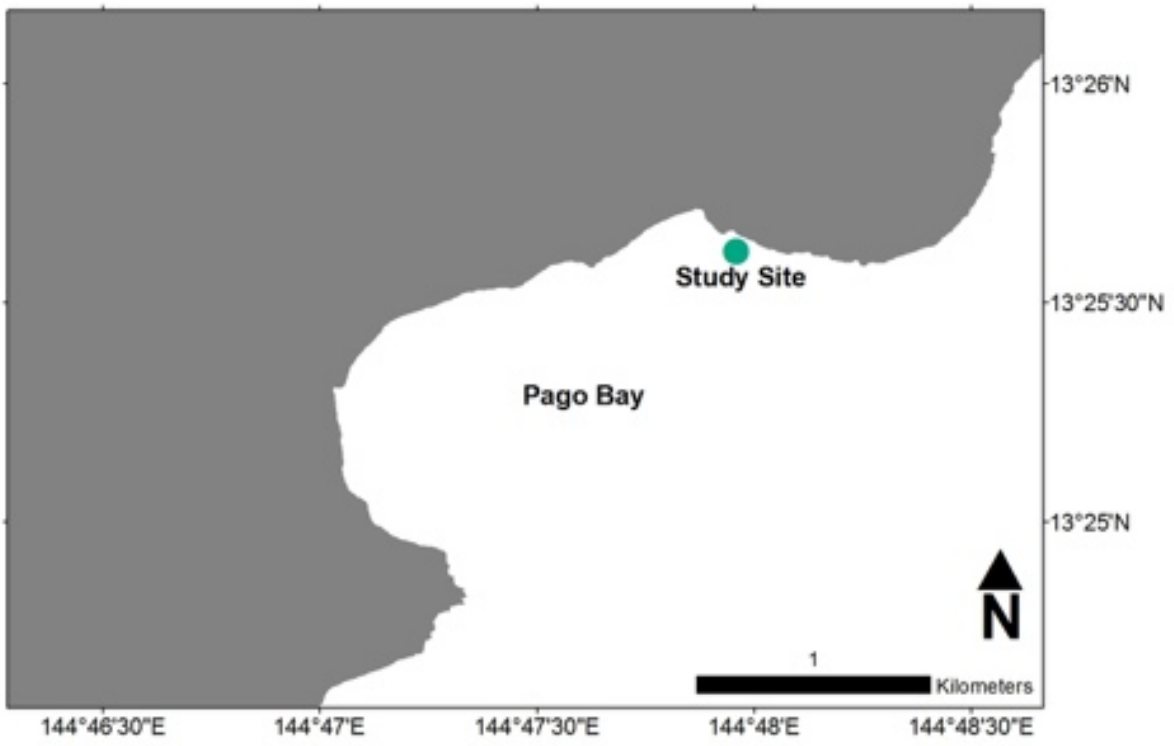
H_{A3}: *P. rus* exhibits a stronger acclimation response than *A. surculosa.*

Chapter 2. Methods

Sampling and reciprocal transplant design

Acropora surculosa samples were collected from Pago Bay (N13.426885, E144.798821, Figure 2A) where colonies are scattered along a gradual slope from 2 m to 10 m depth over 100 m (lateral extent). *Porites rus* samples were collected from Finger Reef in Apra Harbor (N13.443973, E144.638381, Figure 2B) where colonies blanketed a steeper slope from 2 m to greater than 10 m in depth over 50 m (lateral extent). Sampled *A. surculosa* and *P. rus* colonies were typically <0.25 m and >1 m in diameter, respectively. The 2 m depth environment was selected because of its high variability in terms of temperature and irradiance throughout the day and months, whereas the 10 m depth environment exhibits less variable temperature and more constant irradiance regimes (see results section). Forty parent coral colonies, ten *A. surculosa* and ten *P. rus* at 2 m and 10 m depths, were tagged in December 2018 and monitored once a month through photographs and the CoralWatch Color Card (Siebeck et al., 2006) to assess tissue color changes over time. The coral tissue was visually matched to a color value on the CoralWatch Color Card, with higher values corresponding to darker tissue colors.

A.



B.

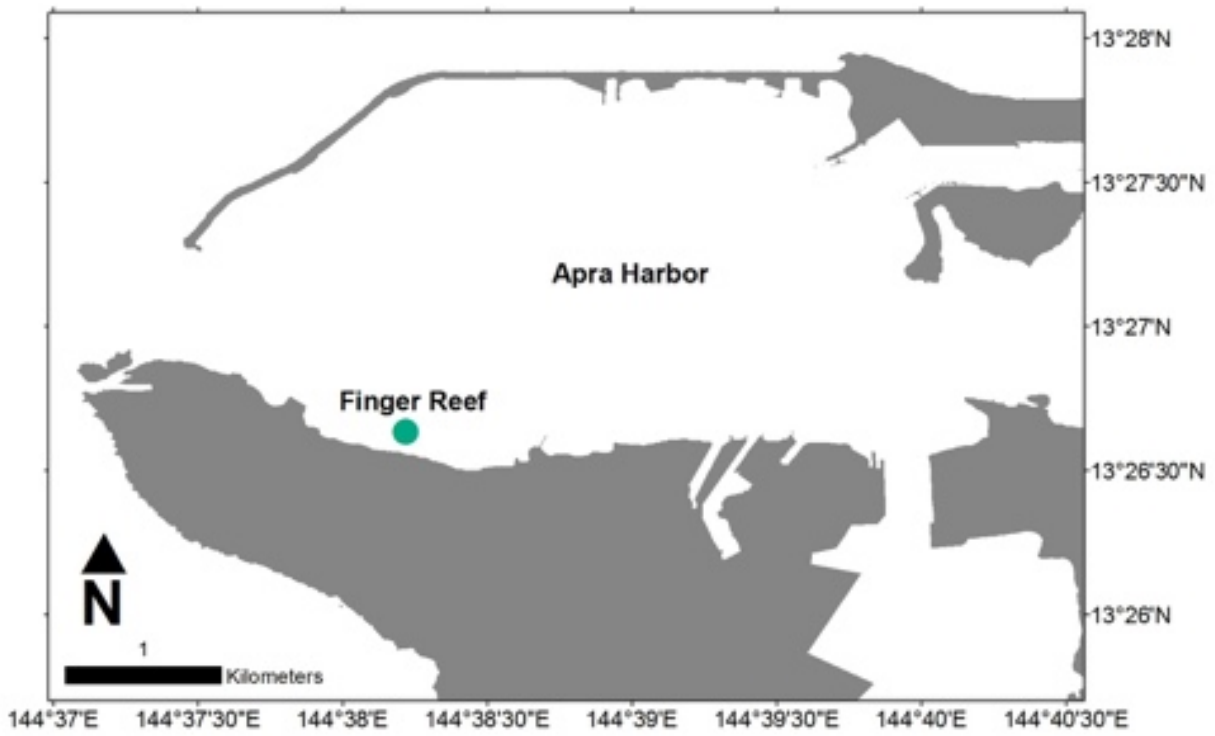


Figure 2. Site map of Pago Bay (A) and Apra Harbor (B)

Prior to transplantation, four platforms made of fiberglass boards, metal rods, and PVC rounds were installed at both 2 m and 10 m sites. The reciprocal transplant (Figure 3) was initiated on 29 May and 11 June 2019 in Apra Harbor and Pago Bay, respectively. We conducted the transplant experiment during the warmest part of the year with the aim of observing a more pronounced stress response. Prior to the transplant, we collected one fragment (10-15 cm long for *P. rus* and 5-10 cm long for *A. surculosa*) from each parent colony at each depth (2 m and 10 m) and immediately placed it in RNAlater, to establish baseline gene expression (40 total: 20 *A. surculosa,* 20 *P. rus*). A total of 240 coral fragments (120 *A. surculosa,* 120 *P. rus*) were then reciprocally transplanted between the deep and shallow sites. Using a clonal design, we transplanted three 'control' fragments from each parent colony to their original depth ("SS" shallow control, "DD" deep control), to assess the impact of transplantation stress (i.e. cutting coral and adhering it to transplant board). To assess the impact of transplantation to a different environment, we relocated three 'transplant' fragments from each parent colony to the alternate depth ("SD" shallow transplanted to deep, "DS" deep transplanted to shallow). All samples were randomly adhered to one of two transplant boards with marine epoxy. Fragments were then monitored weekly through photographs and visual observations for tissue color change with ColorWatch measures, bleaching and survival rates. Eight weeks after the transplant started, one coral fragment from each donor colony (18 *A. surculosa,* 3 replicates from each transplant group; 22 *P. rus,* 3 replicates from S/SS/SD/DS, 4 replicates from D/DD) was randomly collected from the transplant boards at both sites and depths and stored in RNAlater

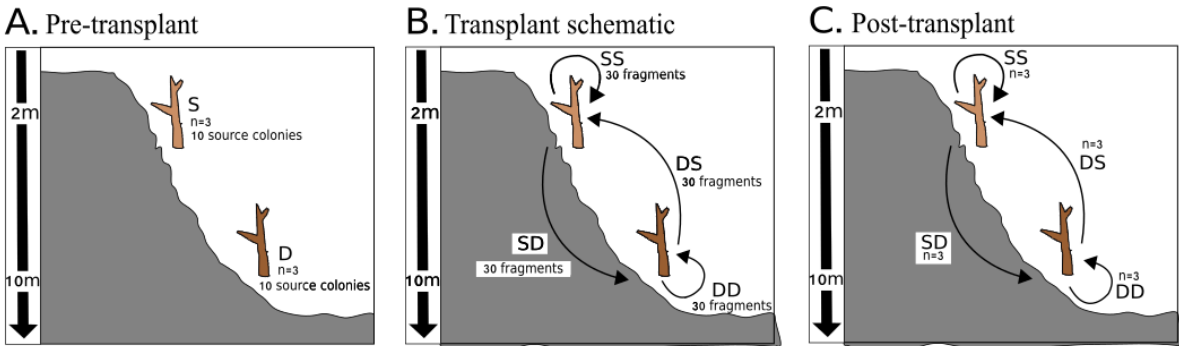


Figure 3. Example of *Acropora surculosa* sampling design. Before transplantation, 3 samples were collected from shallow 'S' and deep 'D' source colonies (A). The experiment consisted of transplanting 30 fragments (3 replicates from each source colony) from deep to shallow 'DS', shallow to deep 'SD', deep control 'DD', and shallow control 'SS' (B). After the 10-week transplant experiment, 3 samples were collected from each transplant group (C).

Biotic and abiotic data collection and analyses

To track irradiance and temperature variations, two HOBO Pendants® were deployed at each depth at both sites from January to October 2019. The light sensors of the HOBO Pendants® (Onset) were cleaned monthly to control biofouling and ensure accurate data readings. Temperature and irradiance data were recorded every five minutes and collected once a month before being analyzed with RStudio (R Core Team 2021). To assess the difference in temperature and irradiance values between depths and sites, effect sizes were calculated using Cohen's d in the 'effsize' package in RStudio (Torchiano, 2017). To determine differences in daily variance, the 'var()' function from the 'stats' package (R Core Team 2021) was used. Note that not all of the irradiance data is used in this analysis. Due to biofouling on the sensor, only the first week of data post-cleaning was used.

To visualize survival rates of the coral fragments throughout the transplant experiment, Kaplan-Meier survival curves were generated using the 'survival' package in R (Therneau,

2021). To analyze changes in tissue color throughout the transplant, ColorWatch values were compared using Shapiro-Wilks test for normality followed by the Welch two-sample t-test.

RNA extraction and library preparation

RNA extraction for the *A. surculosa* samples was conducted using the Qiagen RNeasy extraction kit (Hildenheim, Germany) on a QIACube automated extraction robot following the manufacturer's instructions. Due to higher levels of mucopolysaccharides in *P. rus* compared to those in *A. surculosa,* a different RNA extraction method was used for *P. rus.* RNA was extracted from the *P. rus* samples using the Omega E.Z.N.A. Mollusc RNA Kit (Omega Bio-Tek, Inc.) protocol. After extraction, total RNA was quantified using a Qubit HSRNA kit (Life Technologies, Carlsbad, CA) and qualified using a picoRNA assay in a BioAnalyzer (Agilent Technologies, Santa Clara, CA).

RNA sequencing was used to identify genes involved in response to thermal, irradiance, and transplantation stress. cDNA libraries for 40 samples (18 *A. surculosa:* 3 replicates from each transplant group; 22 *P. rus,* 3 replicates from S/SS/SD/DS, 4 replicates from D/DD, note differences in number of replicates due to availability of sequencer) were prepared using the NEBNext® Ultra™ II RNA Library Prep Kit for Illumina® (New England Biolabs) protocol. Libraries were barcoded with individual indices from NEBNext Multiplex Oligos for Illumina (New England Biolabs). The final concentrations for each library were quantified using a Qubit HSDNA kit (Life Technologies, Carlsbad, CA) and qualified using a BioAnalyzer assay (Agilent Technologies, Santa Clara, CA). The 18 *A. surculosa* and 22 *P. rus* libraries were pooled and sequenced on an Illumina NextSeq 500 (San Diego, CA) in two separate runs on site.

Transcriptome assembly and annotation

Reads from each sample were demultiplexed and trimmed of low-quality fragments and sequencing adapters using Trim Galore (Martin, 2011). Reads shorter than 25 bp or with an average quality score less than 30 were discarded. The quality of reads was then assessed using FastQC (Andrews, 2010). rRNA and Symbiodiniaceae sequences were then filtered out of the dataset using Bowtie v2.1.0 (Langmead and Salzberg, 2012) to align the reads to SILVA LSU and SSU rRNA databases (https://www.arb-silva.de/) and three concatenated symbiont genomes (*Symbiodinium kawagutii*, *Symbiodinium microadriaticum,* and *Breviolum minutum*, reads available under Genbank accession numbers GCA_001939145.1, GCA_003297005.1, and GCA_009767595.1, respectively).

Presence of potential clones was assessed by generating identity-by-state (IBS) matrices in ANGSD (Korneliussen et al., 2014) with filters -minMapQ 20, -minQ 20, -doIBS 1. Using the IBS covariance matrices, relatedness dendrograms were plotted in R v4.0.2 (R Development Core Team, 2021) to visualize sample relatedness. A threshold line was added to the dendrogram above the nodes of biological replicates (i.e., samples from the same parent colony, but different transplant groups) and used to detect clones.

All cleaned *A. surculosa* reads from the 18 sequenced samples were aligned to the *Acropora digitifera* genome (Shinzato et al., 2011) while cleaned *P. rus* reads from the 22 sequenced samples were aligned to the *Porites rus* genome (Wibberg, 2018) using Bowtie v2.1.0 (Langmead and Salzberg, 2012) to perform a Trinity genome-guided assembly (Haas et al., 2013). The resulting BAM files were used to generate a reference transcriptome for each species with the default Trinity parameters. The quality of the resulting reference transcriptomes was assessed using Trinity's Transcriptome Assembly Quality Assessment. Initially, both reference

transcriptomes appeared to be too large with 499,020 total *A. surculosa* transcripts and 508,820 *P. rus* transcripts. Excessive amounts of transcripts often occur when assembling transcriptomes from many samples and do not reflect a biological reality but rather the presence of multiple copies of homologous transcripts with small sequence variations. The isoforms and highly homologous sequences in both transcriptomes were filtered out using cd-hit-est (Fu et al., 2012) to reduce sequence redundancy and improve performance of downstream analyses. The resulting transcriptome sizes were 298,618 *A. surculosa* transcripts and 156,977 *P. rus* transcripts. Finally, a Benchmarking Universal Single-Copy Orthologs (BUSCO) (Simão et al., 2015) assessment was then conducted to determine the completeness of the transcriptomes using the Metazoa lineage with a BLAST e-value cutoff of 1e-03. The assembled reference transcriptomes were then annotated by performing BLASTX searches of reads against cnidarian sequences from the Uniprot database (www.uniprot.org) with an e-value cutoff of 1e-5.

Gene expression analyses and gene ontology

Reads from each *A. surculosa* sample were mapped against the annotated *Acropora surculosa* reference transcriptome and reads from each *P. rus* sample were mapped against the annotated *P. rus* transcriptome using Bowtie v2.1.0. Read counts per gene were generated with RSEM (Li & Dewey, 2011) using the Trinity script align_and_estimate.py (Grabherr et al., 2011; Haas et al., 2013) with default parameters. Gene counts were imported into R v4.0.2 (R Development Core Team, 2021) using the tximport package (Soneson et al., 2015). For all downstream analyses, a minimum of ten counts across all samples was used as the threshold for retaining a gene. Significant differentially expressed genes (DEGs) across multiple comparisons were determined

with a cut-off of log fold change > 2 and an adjusted p value (padj) < 0.01 using the Deseq2 package (Love et al., 2014).

A principal component analysis of variance stabilized gene expression was performed using the 'plotPCA()' function from the 'DESeq2' package in R to visualize clustering of data for significant DEGs (padj < 0.01, log fold change >2). A discriminant analysis of principal components (DAPC) was also used on variance stabilized gene expression of significant DEGs (padj< 0.01, log fold change >2) to visualize clustering of treatment groups along a discriminant function using the 'adegenet' package (Jombart and Ahmed, 2011).

Gene ontology (GO) enrichment analyses were used to examine gene function differences between coral transplant groups. Following the GO-MWU pipeline (Wright et al., 2015; (https://github.com/z0on/GO_MWU), these analyses were performed using adaptive clustering of GO categories and Mann-Whitney U (MWU) tests based on ranking of signed log p-values. For each of the comparisons (D vs. S, DD vs. D, SS vs. S, SD vs. SS, DS vs. DD), an individual enrichment test was performed for two GO domains: Biological Process and Molecular Function, with a false discovery rate (FDR) set to 10%.

Symbiont density and taxonomy

The Symbiodiniaceae cell density protocol was adapted from Fitt et al. (2000). Coral tissue was removed using an airbrush with filtered seawater and the coral skeleton was dried. To isolate the zooxanthellae, the water sample was vortexed for 30 s, centrifuged for five minutes at 5000 rpm, before removing the supernatant; this process was repeated three times. The zooxanthellae fraction was then resuspended with filtered seawater and transferred to a haemocytometer. Zooxanthellae were counted under a compound microscope (Leica CME, 100X), conducting

three replicates per sample. The surface area of the coral skeleton was determined using a paraffin wax dipping method (Stimson and Kinzie, 1991) and symbiont density per sample was calibrated accordingly. To determine differences in symbiont densities between transplant groups, a Shapiro-Wilks test for normality was used, followed by a one-way ANOVA test.

Methods for determining Symbiodiniaceae genus identification were adopted from Barfield et al (2018). Symbiont sequences separated from our coral sequences (as described above) were aligned with Bowtie v2.1.0 to transcriptomes of Symbiodiniaceae genera *Symbiodinium* and *Breviolum* from Bayer et al. (2012) and genera *Cladocopium* and *Durusdinium* from Ladner et al. (2012), simultaneously. The resulting SAM files were used to count relative proportions of reads producing highly unique matches, determined by a mapping quality of 40 or higher, to each Symbiodiniaceae transcriptome, using a custom perl script zooxType.pl (https://github.com/z0on/).

Chapter 3. Results

Baseline characterization of habitats and bleaching rates

Overall, both temperature and irradiance were higher and more variable at a depth of 2 m than at 10 m in both Pago Bay and Apra Harbor (Figure 4, Supplemental Table 1). Between February and October 2019, the 2 m site in Pago Bay was warmer *(d* = -0.302) and brighter *(d* = -0.253*)* than the 10 m site (Supplemental Table 1). Between January and November 2019, the 2 m site in Apra Harbor was also warmer *(d* = -0.319*)* and brighter *(d* = -0.380*)* than the 10 m site (Supp. Table 1). The 2 m site was also more variable with regard to temperature (Pago Bay: $p < 0.001$, Apra Harbor: $p < 0.001$) and irradiance (Pago Bay: $p < 0.001$, Apra Harbor: $p < 0.001$) throughout the eight-month monitoring period. Pago Bay was warmer than Apra Harbor at both depths (2 m: $d = 0.553$, 10 m*:* $d = 0.746$*),* but not brighter (2 m: $d = -0.039$, 10 m: $d = 0.032$) at either depth (Supplemental Figure 1).

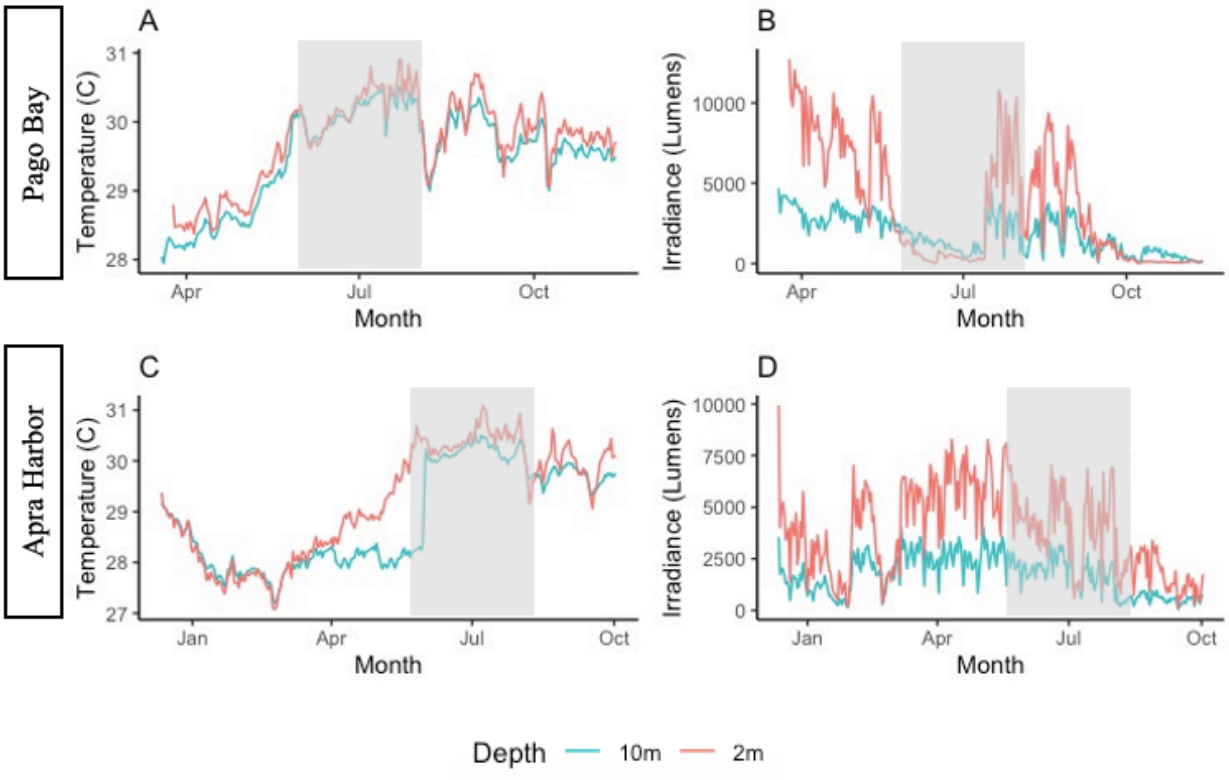


Figure 4. Average daily temperature (°C) (A) and irradiance (lumens) (B) in Pago Bay (Feb-Nov 2019) and temperature (C) and irradiance (D) in Apra Harbor (Jan-Nov 2019) using HOBO Pendants (Onset). The red line indicates average values at the 2 m depth and the blue line indicates the average values at the 10 m depth. The grey rectangle indicates the duration of the transplantation experiment.

Prior to the transplant experiment (Pago Bay: Feb-May 2019, Apra Harbor: Dec-May 2019), average coral tissue color values were significantly darker at the 10 m depth than at the 2 m depth for both sites (*A. surculosa*: $p < 0.001$, *P. rus*: $p < 0.001$, Supplemental Table 2). Color values also tended to decrease over time as Guam transitioned from the cool dry season to the warm wet pre-bleaching season. During the transplant (May-Aug 2019), on average, corals transplanted to the 2 m site decreased in color value and corals transplanted to the 10 m site increased in color, however, the difference was insignificant (Figure 5a, b).

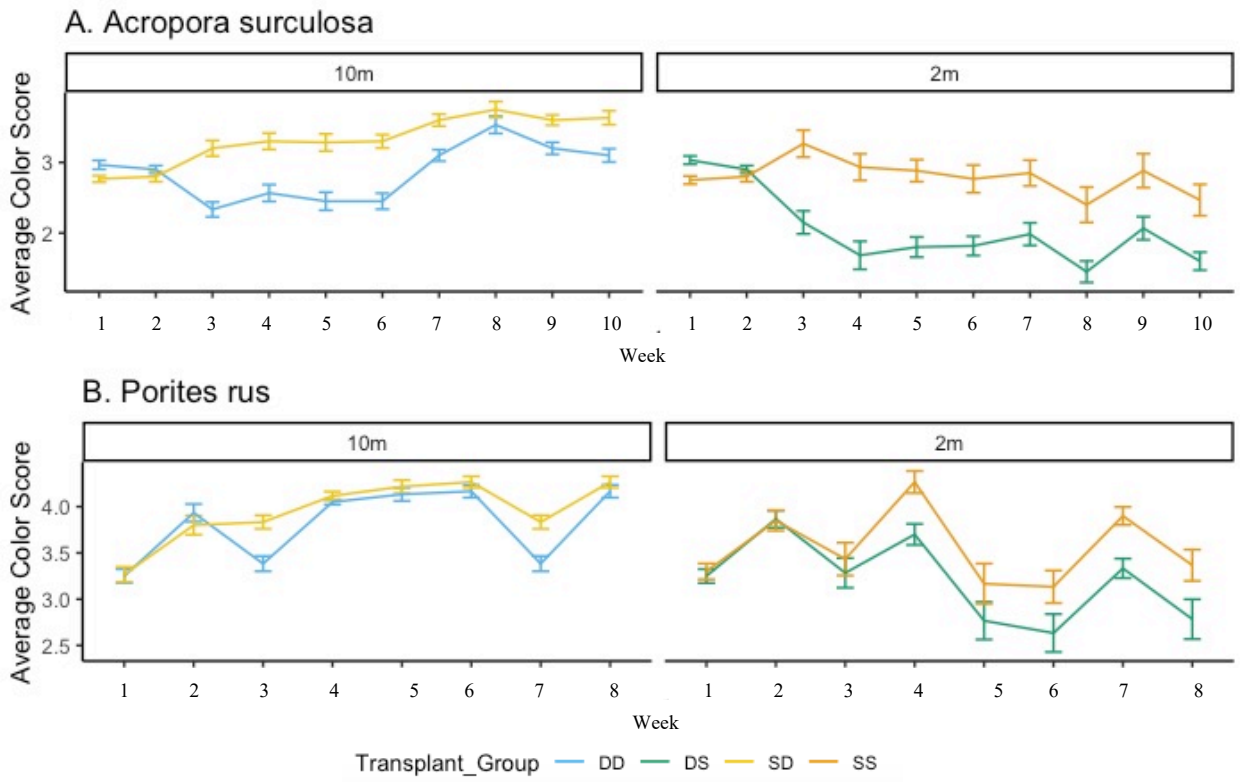


Figure 5. Transplanted (A) *Acropora surculosa* and (B) *Porites rus* corals color chart data. Points represent average (±standard error) color value over three replicate coral fragments. Treatment groups are as follows: shallow control (SS), shallow transplanted to deep (SD), deep control (DD), deep transplanted to shallow (DS).

A total of 240 coral fragments (120 *A. surculosa,* 120 *P. rus*) were transplanted between the deep and shallow sites. One of the two *P. rus* shallow site transplant platforms was colonized by a farming damselfish, eliminating 30 coral fragments from the experiment. By the end of the ten-week transplant, eight *A. surculosa* and two *P. rus* fragments had died, all from the shallow control (SS) or deep to shallow (DS) transplant group (Figure 6).

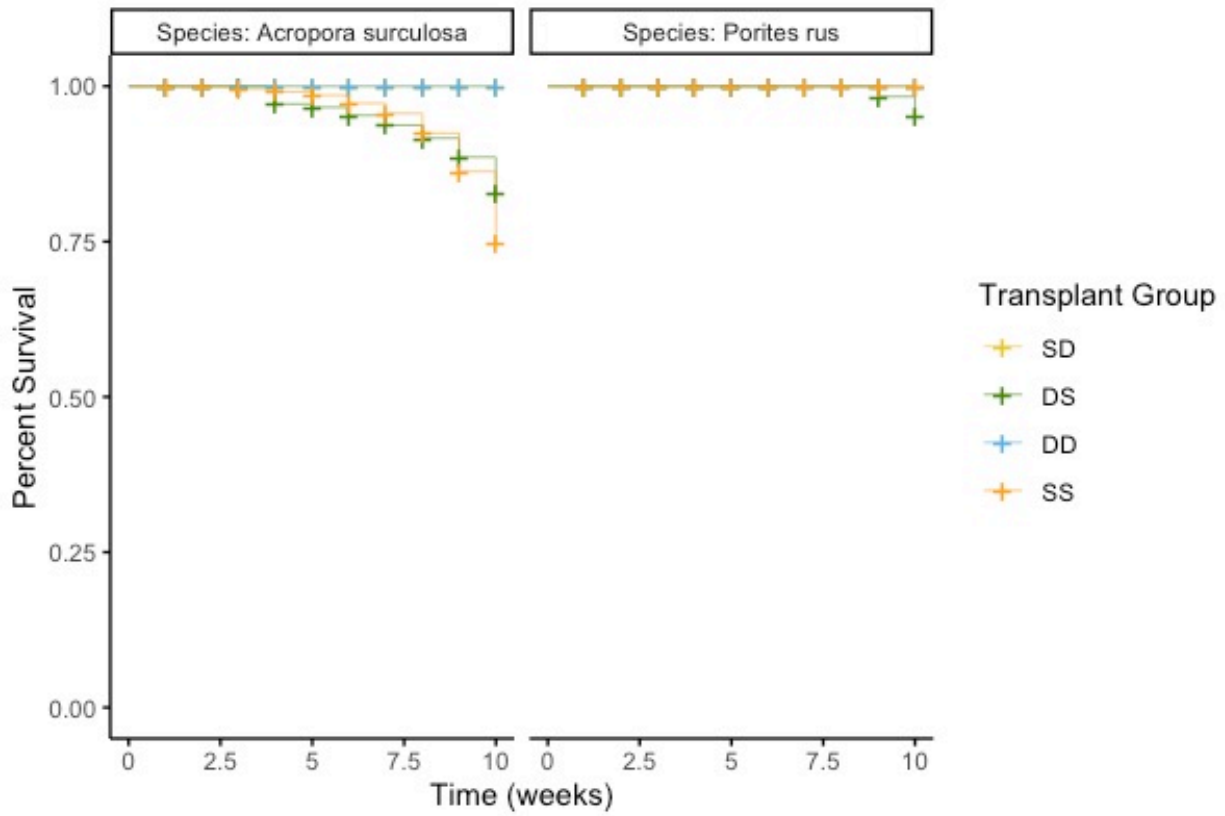


Figure 6. Kaplan-Meier survival curves for transplanted *Acropora surculosa* and *Porites rus* fragments. Treatment groups are as follows: shallow control (SS), shallow transplanted to deep (SD), deep control (DD), deep transplanted to shallow (DS).

Symbiont characterization

All *A. surculosa* samples predominantly hosted Symbiodiniaceae genus *Cladocopium* (>90%), followed by *Breviolum* (<10%) (Figure 7A). Similarly, *P. rus* samples mainly hosted *Cladocopium* (>50%), followed by *Breviolum* (<30%), and *Symbiodinium* (< 30%) (Figure 7B). There was no significant shift in the Symbiodiniaceae community structure after transplantation. With a symbiont density range from 89,401 cells per cm² to 604,928 cells per cm², there were significantly less ($p < 0.0001$) Symbiodiniaceae cells in the *A. surculosa* samples than in the *P. rus* samples, which ranged from 154,421 cells per cm² to 2,421,976 cells per cm². Neither *P. rus*

nor *A. surculosa* Symbiodiniaceae densities (Supplemental Table 3), however, were significantly affected by transplantation (Figure 8).

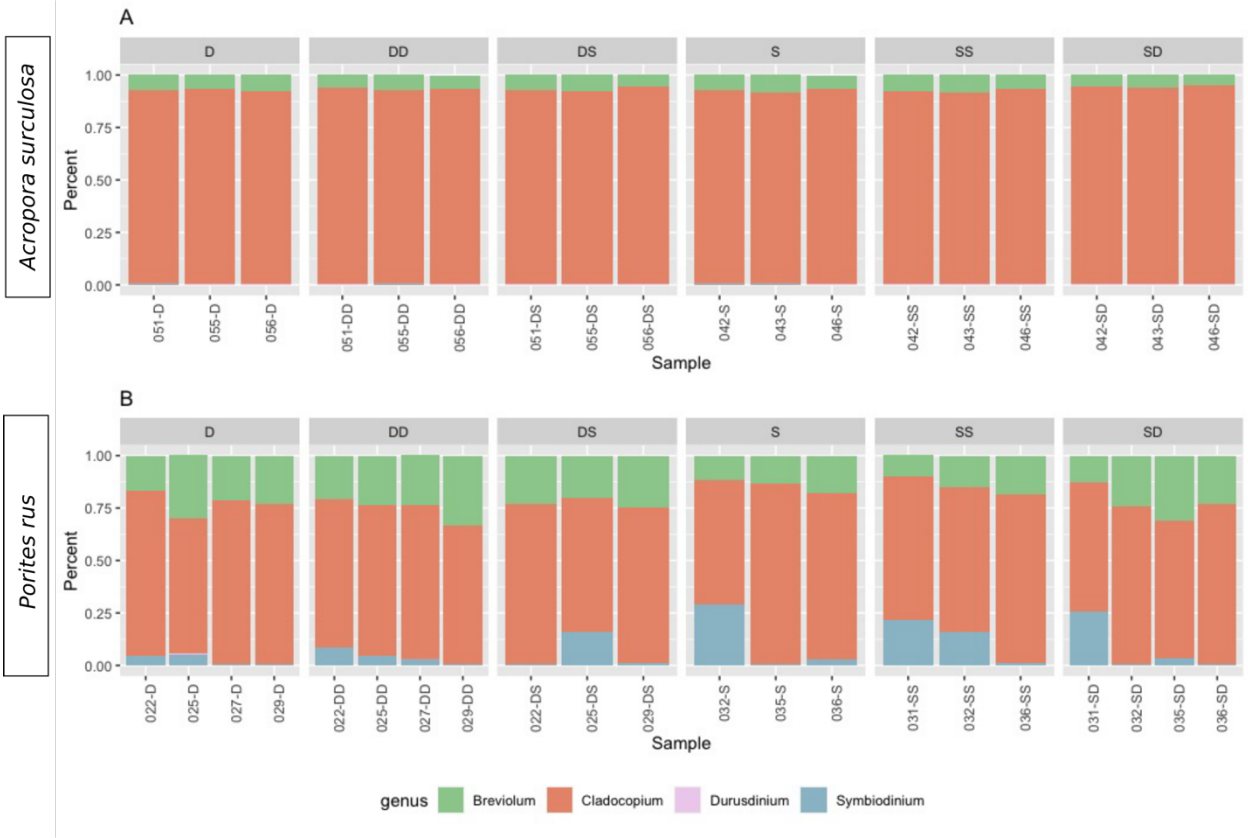


Figure 7. Proportion of various Symbiodiniaceae genera present in (A) *Acropora surculosa* and (B) *Porites rus,* grouped by transplant group and sample. Based on highly unique reads (mapping quality > 40) matching each Symbiodiniaceae genus. Sample names are labeled with field number followed by transplant group code. Treatment groups are as follows: shallow baseline (S), shallow control (SS), shallow transplanted to deep (SD), deep baseline (D), deep control (DD), deep transplanted to shallow (DS).

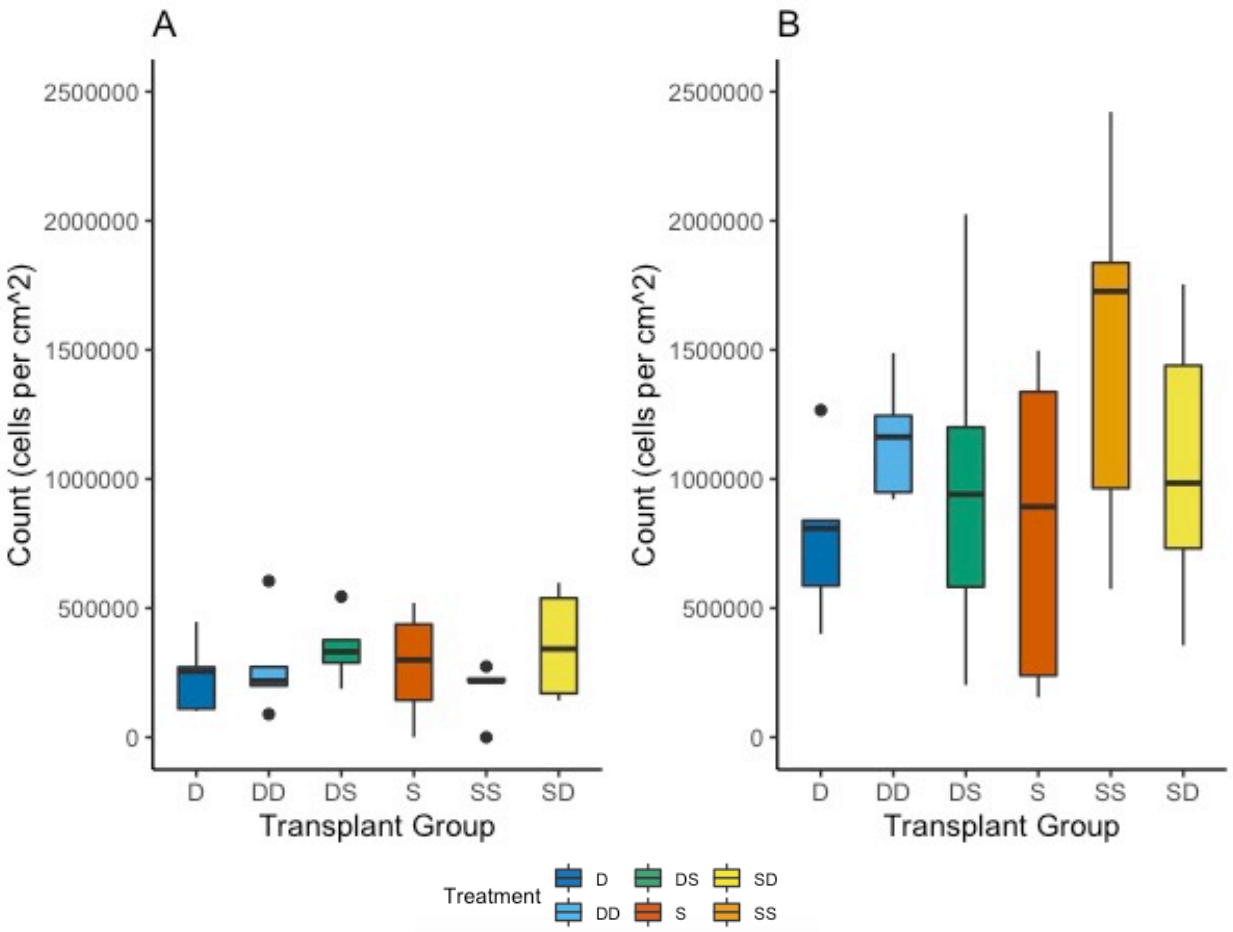


Figure 8. Symbiodiniaceae density in (A) *Acropora surculosa* and (B) *Porites rus* grouped by transplant group. The boundary of the box represents the 75th percentile, the black horizontal line within the box indicates the median, the whiskers above and below box represent 10th and 90th percentiles, and the points represent outliers. Treatment groups are as follows: shallow baseline (S), shallow control (SS), shallow transplanted to deep (SD), deep baseline (D), deep control (DD), deep transplanted to shallow (DS).

Sequencing yield, sample clonality and BUSCO analysis

Illumina sequencing resulted in an *A. surculosa* dataset containing a total of 550 million reads, with an average of 30,523,994 (±3,303,743 SE) reads per sample and a *P. rus* dataset containing a total of 534 million reads with an average of 25,434,060 (±5,422,559 SE) reads per sample. After filtering out adapters, low quality reads, rRNA, and Symbiodiniaceae, an average of 28 million and 19 million reads per sample remained for *A. surculosa* and *P. rus,* respectively. There were 298,618 transcripts in the final assembled *A. surculosa* reference transcriptome, with

91,281 coral genes and 1,866 symbiont genes. There were 156,977 transcripts in the final assembled *P. rus* reference transcriptome, with 166,839 coral genes and 1,976 symbiont genes. None of the parent colonies were clones (Supplemental Figure 2a, b). The BUSCO analysis indicated that 73.9% of the *A. surculosa* transcriptome had complete gene representation and 13.7% of the BUSCO groups were missing and 68.6% of the *P. rus* transcriptome had complete gene representation and 7.9% of the BUSCO groups were missing (Supplemental Table 4).

Differential gene expression analyses

Principal component analysis (PCA) conducted on significant differentially expressed genes in all *A. surculosa* samples revealed that samples' original depth explained 42% of the total variance (S, SS, SD vs. D, DD, DS) and a shift triggered by transplantation explained 26% of the total variance (S, D vs. SS, SD, DD, DS) (Figure 9A). The DAPC analysis showed a clear segregation of samples by depth of origin along the x axis, confirming the difference in coral gene expression patterns between corals that originated in the shallow (S, SS, SD) and corals that originated in the deep (D, DD, DS) (Figure 9C). When the baseline pre-transplant samples were removed from the PCA analysis, a more distinct clustering pattern between transplanted samples was revealed. There was clustering between original depth (59% of total variance; DD, DS vs. SS, SD) and between transplant groups for the shallow samples only (16% of variance; SS vs. SD) (Figure B).

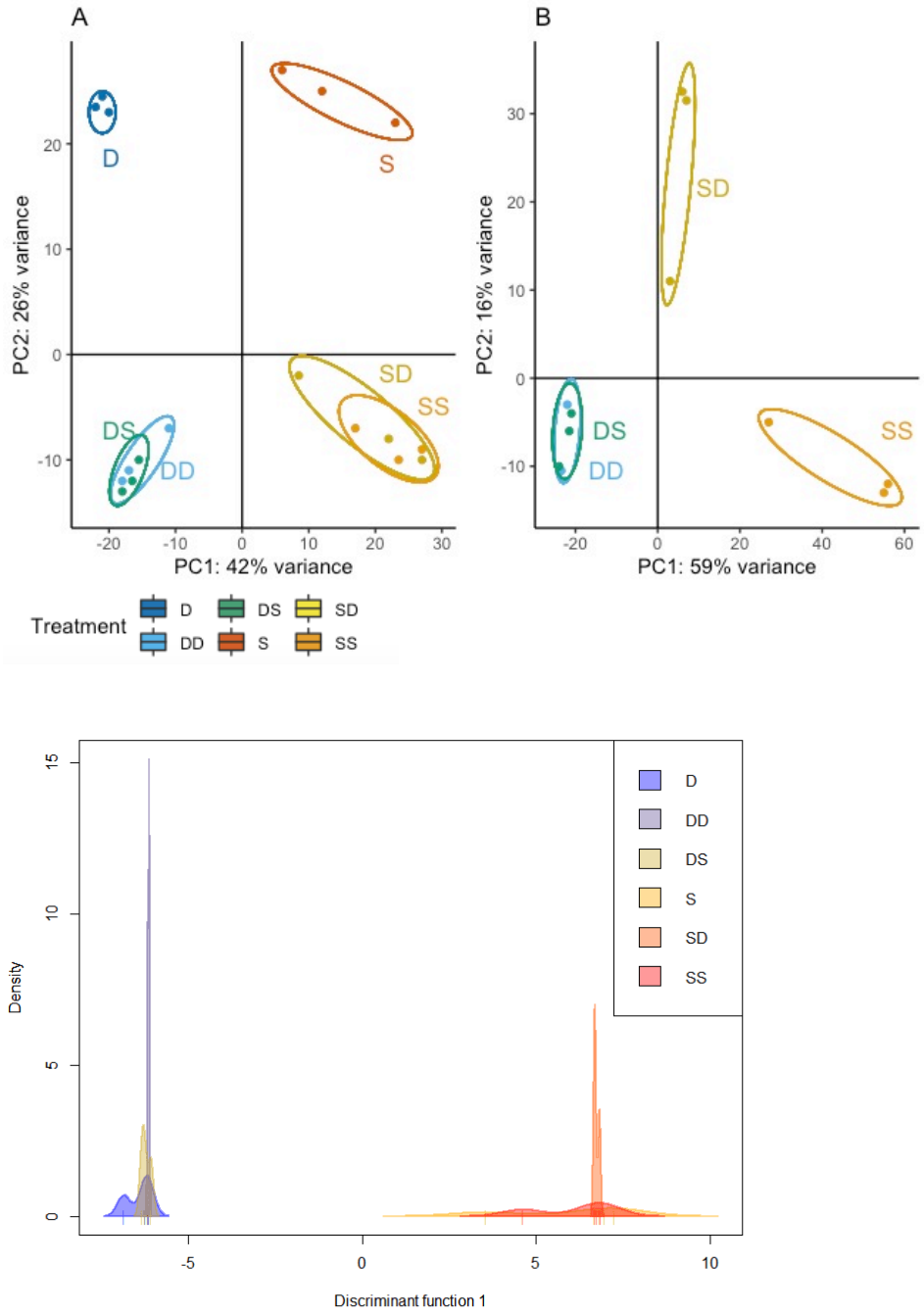


Figure 9. Principal component analysis (PCA) performed on significant differentially expressed genes (padj < 0.01, log fold change > 2) on (A) all *A. surculosa* samples and on (B) post-transplant samples. (C) Discriminant analysis of principal components (DAPC) performed on significant differentially expressed genes (padj < 0.01, log fold change > 2) in all *A. surculosa* samples, both before and after transplantation. The x-axis indicates the direction in multivariate gene expression space along which the difference between deep and shallow corals is maximized (deep--shallow discriminant function). Tick marks along the x-axis represent individual samples. Treatment groups are as follows: shallow baseline (S), shallow control (SS), shallow transplanted to deep (SD), deep baseline (D), deep control (DD), deep transplanted to shallow (DS).

Principal component analysis (PCA) conducted on significant differentially expressed genes in all *P. rus* samples revealed that samples' original depth explained 41% of the total variance (S, SS, SD vs. D, DD, DS) (Figure 10A). When the baseline pre-transplant samples were removed from the PCA analysis, clustering occurred between samples originating from the same depth (DD, DS vs. SS, SD, 57% of variance) (Figure 10B). The DAPC analysis illustrates the subtle differences within the gene expression of the corals originating from the same depth (Figure 10C). While the deep baseline (D) and control (DD) samples exhibit similar peaks, the deep to shallow (DS) transplanted samples shifted towards the shallow samples (S, SS, SD), indicating that the samples shifted their gene expression upon transplantation. There is also a more subtle shift of the shallow to deep (SD) transplanted fragments towards the samples that originated in the deep (D, DD, DS).

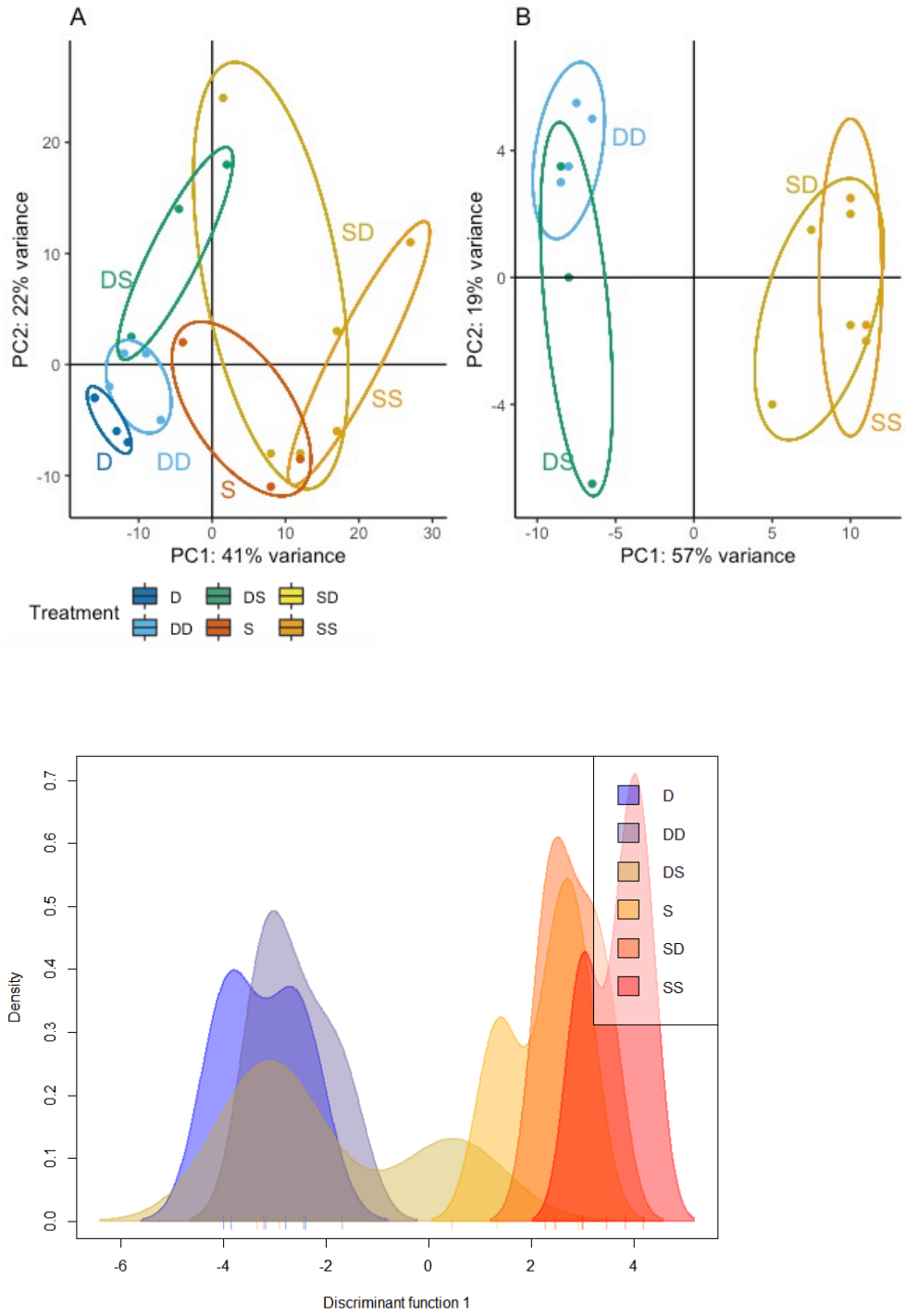


Figure 10. Principal component analysis (PCA) performed on significant differentially expressed genes (padj < 0.01, log fold change > 2) on (A) all *P. rus* samples and on (B) post-transplant samples. (C) Discriminant analysis of principal components (DAPC) performed on significant differentially expressed genes (padj < 0.01, log fold change > 2) in all *P. rus* samples, both before and after transplantation. The x-axis indicates the direction in multivariate gene expression space along which the difference between deep and shallow corals is maximized (deep--shallow discriminant function). Tick marks along the x-axis represent individual samples. Treatment groups are as follows: shallow baseline (S), shallow control (SS), shallow transplanted to deep (SD), deep baseline (D), deep control (DD), deep transplanted to shallow (DS).

Overall, there were significantly more differentially expressed genes (DEGs) ($p < 0.001$) for *A. surculosa* than for *P. rus* when comparing between the transplant groups (Table 2).

Table 2. Counts of differentially expressed genes (DEG) ($p < 0.01$, log fold change < 2) in *Acropora surculosa* (blue) and *Porites rus* (red) samples. Comparisons between different transplant groups. Treatment groups are as follows: shallow baseline (S), shallow control (SS), shallow transplanted to deep (SD), deep baseline (D), deep control (DD), deep transplanted to shallow (DS).

v.s.	S	SS	SD	D	DD	DS
S		26	9	20	11	9
SS	96		27	68	42	31
SD	102	276		3	28	4
D	76	264	215		0	3
DD	230	248	194	38		1
DS	176	302	173	33	10	

Gene Ontology Enrichment Analyses

To identify the functions of genes that were differentially expressed, we conducted a functional enrichment analysis, comparing the gene expression responses of different treatment groups.

Baseline difference (S vs. D)

Gene ontology (GO) enrichment analysis of the shallow baseline compared to the deep baseline detected significant GO terms within the "molecular function" category for both *A. surculosa* and *P. rus.* Most of the 11 significantly dysregulated (either 'up' or 'down' regulated) GO terms seen in the *A. surculosa* samples were related to protein synthesis and degradation, oxidative activity, and structural homeostasis (Table 3). For the *P. rus* baseline samples, the 11 dysregulated GO terms were mainly associated with cell signaling and transport (Table 4).

Transplantation stress (DD vs. D, SS vs. S)

In *A. surculosa,* there were only five significantly dysregulated GO terms associated with "molecular function" and two associated with "biological processes" when comparing deep baseline (D) samples to deep control (DD) samples. Most of the *A. surculosa* GO terms were associated with ion transport and developmental processes (Table 3). In contrast, more than twice the amount of GO terms were dysregulated in *P. rus* deep control samples, with functions relating to cell signaling and cell transport, similar to what was seen in the *P. rus* baseline comparison (Table 4).

When looking at transplantation stress for the *A. surculosa* shallow samples (S vs. SS), 12 GO terms associated with "molecular function" and three associated with "biological processes" were dysregulated. The GO terms were associated with calcium homeostasis, oxidative stress, protein synthesis, and extracellular matrix activity (Table 3). In the *P. rus* samples*,* the 12 dysregulated GO terms associated with shallow transplantation stress related to protein synthesis and cell signaling (Table 4).

Transplant effect (SS vs. SD, DD vs. DS)

When examining the effect of transplanting shallow *A. surculosa* fragments transplanted to the deep site (SS vs. SD) there were eight dysregulated GO terms associated with "molecular function" for *A. surculosa*, related to calcium homeostasis, structural homeostasis, and extracellular matrix (Table 3). For *P. rus* samples transplanted to the deep site (SS vs. SD), there were 13 dysregulated GO terms relating to calcium homeostasis, extracellular matrix, and cell transport (Table 4).

The greatest number of dysregulated genes (19) was found in the comparison of *A. surculosa* fragments transplanted from deep to shallow (DD vs. DS). In fragments transplanted from deep to shallow (DS), GO terms involving protein synthesis, calcium homeostasis, developmental processes, and structural homeostasis were dysregulated (Table 3). Only nine GO terms were significantly dysregulated in this comparison (DS vs. DD) for *P. rus.* They included cell signaling, protein activity, and ADP binding.

Symbiodiniaceae Gene Ontology

There were no significantly dysregulated Symbiodiniaceae genes in any of the transplant group comparisons for either coral species.

Table 3. Functional enrichment of GO categories for co-expressed gene clusters correlated with various transplant groups of *Acropora surculosa.* The table shows the GO term description, raw p-value, and direction of gene regulation, fraction (number of "good candidate" genes detected out of total number of genes belonging to that category) and ontology (MF = molecular function, BP = biological process). Treatment groups are shallow baseline (S), deep baseline (D), deep control (DD), deep transplanted to shallow (DS), shallow control (SS), shallow transplanted to deep (SD).

Group	Regulation Direction	Fraction	Description	p-value	Ontology
S vs. D	Downregulated in S	28/33	structural constituent of ribosome	>0.01	MF
		585/2316	oxidoreductase activity	> 0.01	MF
		254/1120	structural molecule activity	> 0.01	MF
		32/56	protease binding	> 0.01	MF
		530/2188	RNA binding	> 0.05	MF
		5/24	acid-thiol ligase activity	> 0.05	MF
		20/89	protein heterodimerization activity	> 0.05	MF
		8/27	triglyceride lipase activity	> 0.05	MF
	Upregulated in S	129/446	enzyme inhibitor activity	> 0.01	MF
		359/1307	molecular function regulator	> 0.01	MF
		79/236	ubiquitin-like protein ligase activity	> 0.01	MF
DD vs. D	Upregulated in DD	14/27	tumor necrosis factor receptor binding	> 0.05	MF
		36/324	ion transport	> 0.01	BP
		428/2604	calcium ion binding	> 0.01	MF
	Downregulated in DD	24/150	developmental process	> 0.01	MF
		16/89	animal organ development	> 0.01	MF
SS vs. S	Upregulated in SS	13/96	oxidoreductase activity, acting on the CH-CH group of donors	> 0.01	MF
		53/338	structural constituent of ribosome	> 0.01	MF
		76/335	divalent inorganic cation transmembrane transporter activity	> 0.05	MF
		34/86	cell communication	> 0.05	BP
		47/195	cell surface receptor signaling pathway	> 0.05	BP

	Downregulated in SS	154/446	enzyme inhibitor activity	> 0.01	MF
		108/415	extracellular matrix structural constituent	> 0.01	MF
		27/33	alpha-L-arabinofuranosidase activity	> 0.01	MF
		739/2604	calcium ion binding	> 0.01	MF
		346/1307	molecular function regulator	> 0.01	MF
		76/234	endopeptidase regulator activity	> 0.05	MF
		31/89	chitin binding	> 0.05	MF
SS vs. SD	Downregulated in SD	829/2123	catalytic activity, acting on DNA	> 0.05	MF
	Upregulated in SD	943/2604	calcium ion binding	> 0.01	MF
		28/33	alpha-L-arabinofuranosidase activity	> 0.01	MF
		133/415	extracellular matrix structural constituent	> 0.01	MF
		317/1120	structural molecule activity	> 0.01	MF
		148/445	hydrolase activity, acting on glycosyl bonds	> 0.05	MF
		143/446	enzyme inhibitor activity	> 0.05	MF
DD vs. DS	Downregulated in DS	64/338	structural constituent of ribosome	> 0.01	MF
		37/357	translation initiation factor activity	> 0.01	MF
		136/1120	structural molecule activity	> 0.01	MF
		93/811	ribonucleoside binding	> 0.01	MF
		9/38	protein domain specific binding	> 0.01	MF
		63/478	translation regulator activity	> 0.01	MF
		34/363	cysteine-type peptidase activity	> 0.01	MF
		18/185	cysteine-type endopeptidase activity	> 0.05	MF
		3/30	protein disulfide oxidoreductase activity	> 0.05	MF
		22/136	unfolded protein binding	> 0.05	MF
		1/16	ferroxidase activity	> 0.05	MF
		1/13	signal sequence binding	> 0.05	MF
		7/69	oxidoreductase activity, acting on a sulfur group of donors	> 0.05	MF
		7/43	bioluminescence	> 0.05	BP
	Upregulated in DS	5/170	mRNA binding	> 0.01	MF
		351/2604	calcium ion binding	> 0.01	MF
		18/33	alpha-L-arabinofuranosidase activity	> 0.01	MF
		156/2123	catalytic activity, acting on DNA	> 0.01	MF
		9/15	phosphatidylserine decarboxylase activity	> 0.05	MF

Table 4. Functional enrichment of GO categories for co-expressed gene clusters correlated with various transplant groups of *Porites rus.* The table shows the GO term description, raw p-value, and direction of gene regulation, fraction (number of "good candidate" genes detected out of total number of genes belonging to that category) and ontology (MF = molecular function, BP = biological process). Treatment groups are shallow baseline (S), deep baseline (D), deep control (DD), deep transplanted to shallow (DS), shallow control (SS), shallow transplanted to deep (SD).

Group	Direction	Fraction	Description	p-value	Ontology
S vs. D	upregulated in S	903/2786	passive transmembrane transporter activity	> 0.01	MF
		1821/5618	transporter activity	> 0.01	MF
		2267/6981	signaling receptor activity	> 0.01	MF
		389/1211	gated channel activity	> 0.01	MF

		872/2746	cation transmembrane transporter activity	> 0.01	MF
		266/917	transferase activity, transferring acyl groups	> 0.05	MF
		316/1001	voltage-gated ion channel activity	> 0.05	MF
		122/368	ligand-gated ion channel activity	> 0.05	MF
	downregulated in S	120/364	ADP binding	>0.001	MF
		57/158	transposase activity	> 0.01	MF
		2393/6956	transition metal ion binding	> 0.01	MF
DD vs. D	upregulated in DD	1436/6981	signaling receptor activity	> 0.01	MF
		576/2786	channel activity	> 0.01	MF
		1225/5618	transporter activity	> 0.01	MF
		599/2746	cation transmembrane transporter activity	> 0.05	MF
		301/1165	serine-type peptidase activity	> 0.05	MF
		231/1211	gated channel activity	> 0.01	MF
		68/316	exopeptidase activity	> 0.01	MF
		6/37	acid-amino acid ligase activity	> 0.01	MF
		4/21	glutamate-cysteine ligase activity	> 0.01	MF
	downregulated in DD	69/364	ADP binding	> 0.01	MF
		1374/6956	transition metal ion binding	> 0.01	MF
		274/1480	motor activity	> 0.05	MF
		297/1544	ligase activity	> 0.05	MF
		4/20	double-stranded telomeric DNA binding	> 0.05	MF
		131/612	obsolete signal transducer activity	> 0.05	MF
SS vs. S	upregulated in SS	405/1165	serine hydrolase activity	> 0.01	MF
		2065/6981	signaling receptor activity	> 0.01	MF
		1007/3353	peptidase activity	> 0.05	MF
	downregulated in SS	45/161	protein heterodimerization activity	> 0.01	MF
		74/197	protein homooligomerization	> 0.01	BP
		182/650	cellular component assembly	> 0.01	BP
		235/917	transferase activity, transferring acyl groups	> 0.01	MF
		9/22	phosphoenolpyruvate carboxykinase activity	> 0.05	MF
		16/47	organonitrogen compound biosynthetic	> 0.05	BP
		4/12	obsolete transcription factor activity, RNA polymerase	> 0.05	MF
		10/18	selenium binding	> 0.05	MF
		66/193	acetylglucosaminyltransferase activity	> 0.05	MF
SS vs. SD	upregulated in SD	1073/2786	channel activity	> 0.01	MF
		1801/4496	calcium ion binding	> 0.01	MF
		57/135	2-oxoglutarate-dependent dioxygenase activity	> 0.05	MF
	downregulated in SD	80/364	ADP binding	> 0.01	MF
		1050/3351	RNA binding	> 0.05	MF
DD vs. DS	upregulated in DS	1712/6981	signaling receptor activity	> 0.01	MF
		144/366	endopeptidase regulator activity	> 0.05	MF
	downregulated in DS	79/364	ADP binding	> 0.01	MF

	680/2586	hydrolase activity, acting on acid anhydrides	> 0.01	MF
	49/161	protein heterodimerization activity	> 0.01	MF

Chapter 4. Discussion

In this study, we aimed to characterize the acclimation abilities of *Acropora surculosa* and *Porites rus* at a molecular level by conducting reciprocal transplants between more- and less-variable habitats. With Illumina sequencing and gene expression analyses, we discovered contrasting transcriptomic signatures of stress and acclimation in the two coral species that will help us predict how these species will perform under future climate conditions. To our knowledge, this is the first study to simultaneously examine both a 'resistant' and 'susceptible' coral genus' acclimation abilities using reciprocal transplantation and transcriptomics.

Baseline transcriptomic differences between shallow and deep colonies highlight acclimation to native environment

The distribution of species and individuals within a coral reef is driven by major abiotic factors including light and temperature (Smith et al., 2008; Lesser et al., 2009). These parameters frequently vary by depth, so more tolerant species and individuals are often seen in more variable shallow depths. As the climate changes, we expect to see warmer, brighter and more variable oceanic conditions, such as those of our shallower (2 m) study depth (Figure 3), resembling future projections of abiotic factors and their environmental influences on Guam's reefs (Laffoley et al., 2016). Our shallow depth could potentially be used as a proxy for future climate-based predictions. Differences were also observed between sites; Pago Bay was warmer (2 m: $d = 0.553$, 10 m: $d = 0.746$) than Apra Harbor at both depths, which likely influences the transcriptomic differences between the coral species in each site.

Prior to transplantation, both *A. surculosa* and *P. rus* expressed distinct transcriptomic patterns based on depth providing us with baseline gene expression characteristics (Figures 9a and 10a). This was suggested by the shallow-dwelling *A. surculosa* colonies' upregulation of ubiquitin-related GO terms ($p < 0.01$) which are often used as biomarkers in more stressful environments. Ubiquitin is bound to damaged proteins and can be an indicator of endoplasmic reticulum stress, seen in corals living in more stressful, warmer, shallow environments (Barshis et al. 2010). Similarly, *Porites lobata* colonies acclimated to a more variable (in terms of temperature, salinity, irradiance, and flow) backreef environment also upregulated ubiquitin proteins (Barshis et al. 2010). The shallow-dwelling *P. rus* colonies also showed differential gene expression based on depth (Table 2). They upregulated terms associated with ion transport, seen in corals in warmer environments (Bernadet et al., 2019). Corals upregulate ions to supply to the calcifying fluid to sustain calcification in response to changing ocean chemistry (Hohn and Merico, 2015). This maintenance of calcium homeostasis suggests that the shallow colonies were able to respond and adjust to their variable environment. The corals' gene expression response is representative of the environmental conditions the colonies are acclimated to at each depth.

In addition to differences in gene expression, a coral's Symbiodiniaceae community can also be influenced by depth (Sivaguru et al., 2021), but we did not observe significant differences in either *A. surculosa* or *P. rus.* The lack of Symbiodiniaceae differences in the *A. surculosa* baseline colonies could indicate that *Cladocopium* are specific to *A. surculosa* regardless of environment, or that there was not a great enough difference in irradiance to cause a shift in density. *Cladocopium*-dominated (> 90%) *A. surculosa* colonies were also seen in a study by Moscato (2020), regardless of depth, in Pago Bay. *Durusdinium*, often associated with thermal tolerance (Fabricius et al., 2004) was not found in any *A. surculosa* baseline colonies, indicating

that it did not play a role in the resistance of the shallow-dwelling *A. surculosa* colonies as it had in other studies (Abrego et al., 2009; Jones et al., 2008). Similarly, the lack of variation in *P. rus* Symbiodiniaceae between depths could indicate that *Clacocopium, Breviolum,* and *Symbiodinium* are specific to *P. rus*. Our approach, however, was not targeted towards Symbiodiniaceae and may not have been powerful enough to detect minute shifts in Symbiodiniaceae species. Despite the lack of Symbiodiniaceae differences, we suggest that both coral species were acclimated to their native depths based on their gene expression patterns.

Transplantation stress is significant and long-lasting

Throughout the past few decades, there have been many studies on coral stress responses (Cziesielski et al., 2019; Mclachlan et al., 2020). Most of these studies involve the physical manipulation of corals, transplanting them and cutting and adhering corals in a new location, i.e. transplantation stress. About half of the coral heat stress studies from the past 30 years, however, did not report the time it takes to visually heal (meaning grow new tissue over a wound) from the handling (Mclachlan et al., 2020), let alone record what is happening at a molecular level. We found that after our ten-week transplant experiment, both *A. surculosa* and *P. rus* control fragments (SS and DD, which were transplanted to their original depths) exhibited signs of transplantation stress when compared to the baseline colonies (S and D, which were not transplanted but sampled directly from the parent colonies) at both a visual and transcriptomic level. To better interpret findings from coral stress response studies, we should account for transplantation stress as well.

We observed that transplantation stress in our control fragments actually resembled a ‘Type A’ general Environmental Stress Response (ESR) outlined by Dixon et al. (2020). In

response to environmental stress, corals often exhibit an increase of reactive oxygen species (ROS), causing oxidative stress (DeSalvo et al. 2008). The oxidative stress likely resulted in an ion imbalance in our control coral fragments. In a study on *Porites lobata,* Lock (2021) found an upregulation in ion transporters in response to fragmentation and outplantation in Guam, also seen in both of our coral species (Tables 3 and 4). Oxidative stress often causes a disruption in calcium homeostasis, as calcium is an important messenger in the cell (Loven, 1988). In fact, *calcium ion binding* (GO: 0005509) was dysregulated in the *A. surculosa* deep ($p < 0.01$) and shallow control ($p < 0.01$) fragments, suggesting a disruption in calcium homeostasis due to transplantation stress. Calcium homeostasis was also disrupted in *P. rus* control fragments at both depths. *P. rus* fragments exhibited upregulation of GO terms associated with general channel activity as well (Table 4). Calcium ion channels are involved in the release of calcium into the cytosol, so an increase in channel activity may suggest a calcium homeostasis disruption as well (Marshall et al. 2007). The disruption in calcium homeostasis results in endoplasmic reticulum (ER) stress causing the misfolding of proteins and thus an unfolded protein response (UPR) (Dimos et al. 2019). A UPR is meant to restore homeostasis; however, if the level of stress is too severe, it can cause damage beyond repair and finally apoptosis (Hetz et al., 2015). We suspect that in *P. rus,* the downregulation of *transition metal ion binding* ($p > 0.01$; GO: 0046914), which induces protein folding (Hoyer et al., 2019), indicates a UPR to ER stress. GO terms associated with an UPR were dysregulated in both coral species (Tables 3 and 4). In final stages of an ESR, corals express terms related to apoptosis, however, this was not observed in our corals, indicating that they may eventually recover from the transplantation stress. The impact of handling was clearly stressful on the corals and resembled many aspects of the 'Type A' ESR.

Although both coral species experienced a transplantation stress response that resembled an ESR, it is evident through the principal component analysis (PCA) (Figures 8 and 9) and the increased number of differentially expressed genes (DEGs) (Table 2) that *A. surculosa* exhibited a more pronounced stress response. This pronounced response was expected as *Acropora* is deemed a more stress-susceptible genus (Colgan et al., 1987). When outplanted for coral restoration, *Acropora* often exhibits lower survivorship and high growth rate whereas *Porites* exhibits high survivorship and low growth rate (Suggett et al., 2019). As expected, the *Acropora* control fragments downregulated GO terms associated with growth and development (Table 3). The downregulation of growth-related processes is part of the 'Type A' ESR, indicating that *A. surculosa* fragments were still stressed and focusing on survival rather than growth ten weeks into transplantation. Although certain factors that influence transplantation success including fragment/colony size, orientation and tissue injury size (Soong and Chen, 2003) were not taken into account in the present study, it should still be noted that even the fast-growing *Acropora* fragments exhibited a general stress response ten weeks after transplantation.

Prior to Lock's (2021) study and the present study, visible coral transplantation stress (i.e. wound healing) was reported to last no longer than 30 days (Lirman, 2000) and there were no known studies on transplantation stress at a molecular level. Although visible transplantation stress may not be closely related to the molecular activity, we would like to highlight the importance of taking transplantation stress into account when studying the impact of handling corals. Since our study demonstrates that a transplantation stress response resembles a 'Type A' coral ESR (Dixon et al., 2020), it could easily be confused with a response to environmental stressors. This may contribute to inaccurate conclusions of intensified coral environmental stress responses. Since corals undergo a recovery period when manipulated by humans, it is therefore

imperative that future studies either allow corals to undergo a healing period prior to the start of an environmental stress study or establish a control group to help parse out a transplantation stress effect.

Despite the widespread use of coral transplantation (Boström-Einarsson et al., 2020; Ferse et al., 2021), which inherently involves the human manipulation of corals, we still know very little about prolonged transplantation stress on corals. A lot of restoration studies target *Acropora* and *Porites*. For example, out of 407 coral restoration studies, 30% of them used *Acropora* and 10% used *Porites* (Boström-Einarsson et al., 2020), however none measured the effects of transplantation stress, which could impact the viability of some studies' results. Handling is necessary for active restoration, but as we plan future coral restoration projects, we must attempt to minimize the amount of handling of corals to reduce coral stress and enhance growth and survival.

Transplantation to deeper habitat is not stressful for *Acropora surculosa* or *Porites rus*

Since we have identified transcriptomic signatures of transplantation stress in both coral species, we can begin to parse out the environmental impact of transplantation. As mentioned previously, when corals are subjected to thermal stress, they typically exhibit downregulation of proteins associated with calcium binding as a result of homeostasis disruption (DeSalvo et al., 2008). We observed the same disruption of homeostasis in our shallow baseline (S) and control (SS) samples for both species. When corals recover from thermal stress, however, calcium homeostasis may rebound. We suspect that this is what we observed in our corals transplanted from the shallow to the deeper (SD), more stable sites. In fact, for both *A. surculosa* and *P. rus* samples transplanted to the deeper sites (SD), calcium ion binding and extracellular matrix

(ECM, in *A. surculosa* only) structural constituent GO terms were significantly upregulated ($p < 0.01$, Tables 2 and 3), suggesting calcium homeostasis and a recovery from thermal stress. In a similar study, overexpression of ECM structure associated genes was linked to corals that were transplanted to a more thermally stable environment in American Samoa (Bay and Palumbi, 2017), supporting our recovery hypothesis.

We suggest that both *A. surculosa* and *P. rus* acclimated to the deep depth during the ten-week transplant time frame. Corals can acclimate in as little as one week (Bellantuono et al., 2012) and one sign of acclimation is 'transcriptional dampening' (Bay and Palumbi, 2015), or a decreased transcriptomic response, which we saw in both our coral species. After ten weeks at the deep depth, rather than expressing terms related to an ESR, the *A. surculosa* deep transplants (SD) upregulated terms related to growth, indicating acclimation (Bay and Palumbi, 2017). Since the deep transplants were able to acclimate, they could be used as a form of 'genetic rescue', providing thermally tolerant alleles to the less-thermally tolerant deep coral population (Matz et al. 2018). This method was suggested by Schoepf et al. (2019) as a way of assisting less tolerant corals as ocean temperatures continue to rise.

Transplantation to shallow site is more stressful for *Acropora surculosa*

The act of transplantation from deeper to shallower depths simulates, at a much more rapid pace, the future changes corals may experience as ocean temperatures and irradiance levels increase and become more variable. As anticipated, *P. rus* fragments exhibited transcriptomic plasticity, or the ability to change their gene expression in response to transplantation to the shallower sites (Figure 9), a result also observed in 'resistant' *Montastrea* corals upon deep to shallow transplantation (Studivan and Voss, 2019). Gene expression plasticity is suggested to help corals

acclimate to more variable environments (Kenkel and Matz, 2016). With the ability to quickly recover after transplantation to the shallow depth, this study suggests that *P. rus* will be able to acclimate to the changing environment associated with climate change.

When transplanted to the more variable depth, *A. surculosa* fragments exhibited greater signs of stress than those of *P. rus* (Figure 8B). Although the transplantation was more stressful for *A. surculosa* than for *P. rus,* with 32 more DEGs (Seneca and Palumbi, 2015), it may not have been that intense overall. For example, the transcriptomic response of *A. surculosa* fragments when transplanted to the shallow depth resembled a milder 'Type B' ESR, which is observed in corals under low intensity stress (Dixon et al., 2020). In a meta-analysis of *Acropora* transcriptomic responses to various stressors, Dixon et al. (2020) found that milder stress events resulted in upregulation of growth-related factors and downregulation of protein folding, protein degradation and oxidative stress. Due to the significant difference in temperature and irradiance in the shallow depth ($p < 0.001$, $p < 0.001$), we expected a more severe stress response. The mild response may indicate that although *A. surculosa* was taking longer to acclimate compared to *P. rus,* it will eventually adjust to the more variable depth. In Guam, *A. surculosa* is already found naturally at shallow depths, suggesting that they are able to acclimate or adapt over time. While this is most likely due to *A. surculosa* being a more 'susceptible' species, we also found that Pago Bay was significantly warmer than Apra Harbor ($p < 0.001$), which could also play a role in the observed transcriptomic response.

One mechanism that may explain *P. rus's* increased plasticity is its ability to use both autotrophy and heterotrophy. Heterotrophic plasticity, or the capacity to modulate feeding methods, can make corals more resistant to stress (Grottoli et al., 2006). In general, *Acropora* is more reliant on autotrophy in comparison with *Porites,* which is a mixotroph, switching between

autotrophy and heterotrophy (Conti-Jerpe et al., 2020). When transplanted to the shallower site, *A. surculosa* fragments expressed various GO terms associated with oxidative stress, calcium homeostasis and metabolism, all of which were found in corals reliant on autotrophy under high light conditions (Levy et al., 2016). In contrast, *P. rus* did not express the same terms, indicating that perhaps they began to feed via heterotrophy. Transplantation to the more variable depth may have caused increased rates of heterotrophy in *P. rus*, which was also seen in a similar study on *P. rus* (Padilla-Gamiño et al., 2012). With increased heterotrophic plasticity, *P. rus* may be better equipped for acclimation under higher temperatures and irradiance levels than *A. surculosa.*

Although we did not detect statistically significant shifts in Symbiodiniaceae genera across treatment groups, overall, the variation in Symbiodiniaceae composition was greater for *P. rus* than for *A. surculosa* (Figure 7). This is surprising since *P. rus* vertically-transmits Symbiodiniaceae from parent to offspring (whereas *Acropora* is a horizontal transmitter), typically resulting in higher composition fidelity (Baker, 2003). With higher fidelity, one would not expect shifts in Symbiodiniaceae composition based on transplantation in *P. rus.* The slight flexibility in Symbiodiniaceae community composition in *P. rus* indicates that it may be quicker to acclimate than *A. surculosa.*

Although *Porites* is a dominant coral genus in Guam (Porter et al., 2005) and our study suggests that *P. rus* is more resistant to thermal and irradiance stress, it is slow to reproduce and grow relative to *A. surculosa* (Kuffner and Paul, 2004, Penland et al., 2004). Even if *P. rus* is able to acclimate to the changing climate, it may not reproduce and grow quickly enough to fill the niches left by corals that were unable to acclimate. *A. surculosa* is able to grow and reproduce quickly and may be able to acclimate to the changing environment using a ‘selective sieve’ in which poor survivors are filtered out from the more stressful environment (Bay and

Palumbi, 2017). It is likely that when the ocean temperatures become more variable, *P. rus* colonies will be able to acclimate, but will not be able to reproduce quickly, while more *A. surculosa* colonies will perish, leaving genotypes that can tolerate the harsher environment to quickly reproduce. Although *Porites* and *Acropora* are at opposite ends of the spectrum with regard to susceptibility, their life histories make them both competitive genera in the future of coral reefs.

Conclusion

The reciprocal transplantation of the more 'susceptible' *A. surculosa* in Pago Bay and more 'resistant' *P. rus* in Apra Harbor provided insights into how well each species may acclimate to the changing climate. Since our study was a long-term (70 days) stress experiment according to McLachlan (2020), it has increased ecological relevance, providing more accurate insights into future coral responses than a shorter-term transplant experiment (Grottoli et al., 2020). Shallow-dwelling colonies of both species were already exhibiting transcriptomic indicators of both thermal and irradiance acclimation compared to deep-dwelling colonies. When transplanted from the more variable shallow depth to the more stable deep depth, both coral species showed 'transcriptional dampening', indicating acclimation. When transplanted to the shallow depth, however, *A. surculosa* fragments exhibited a more pronounced 'Type B' ESR compared to *P. rus.* Surprisingly, after the ten-week transplantation, there was still evidence of transplantation stress that closely resembled a 'Type A' ESR in both coral species at both depths.

The present study adds to our growing knowledge of coral restoration and can help us better manage our existing coral reefs. The lasting impact of coral transplantation on both 'susceptible' and 'resistant' species in Guam can help guide restoration efforts. The shallow sites in this study

resemble the elevated irradiation and sea surface temperature projections seen with climate change (Laffoley et al., 2016). With the insight into how *A. surculosa* and *P. rus* respond to transplantation to a more variable shallow depth, we can begin to prioritize the management of certain more ‘resistant’ species in Guam to ensure the safety of future coral reefs.

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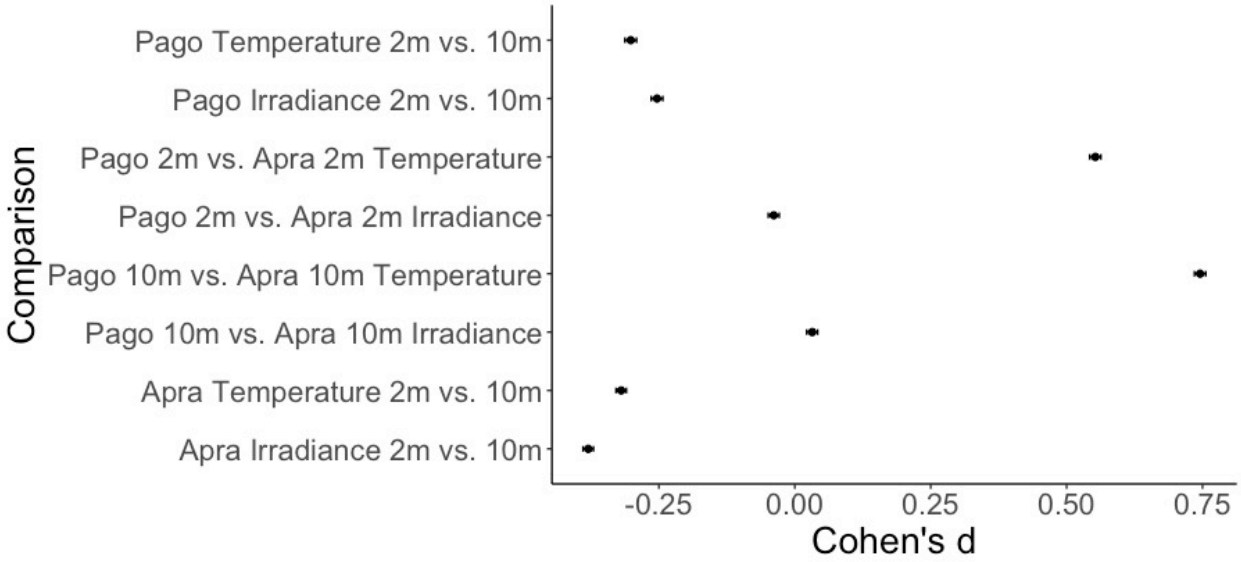
Supplemental Materials

Supplemental Table 1. Minimum, average, and maximum temperature and irradiance of Pago Bay (Feb-Oct 2019) and Apra Harbor (Jan-Nov 2019) at 2 m and 10 m sites. Data collected from HOBO Pendants® installed at each depth.

		Temperature (°C)			Irradiance (Lumens)		
	Depth	**Minimum**	**Average**	**Maximum**	**Minimum**	**Average**	**Maximum**
Pago	2m	27.76	29.73	32.29	0	3506.94	170,845.60
Bay	10m	27.67	29.51	31.27	0	1797.36	27,555.70
Apra	2m	26.88	29.20	31.88	0	3818.66	154,312.10
Harbor	10m	27.08	28.86	31.472	0	1692.25	24,800.20

Supplemental Figure 1. Effect size (Cohen's *d*, with 95% confidence interval) of temperature (°C) and irradiance (lumens) observed at different sites (Apra Harbor, Pago Bay) and depths (2 m, 10 m).

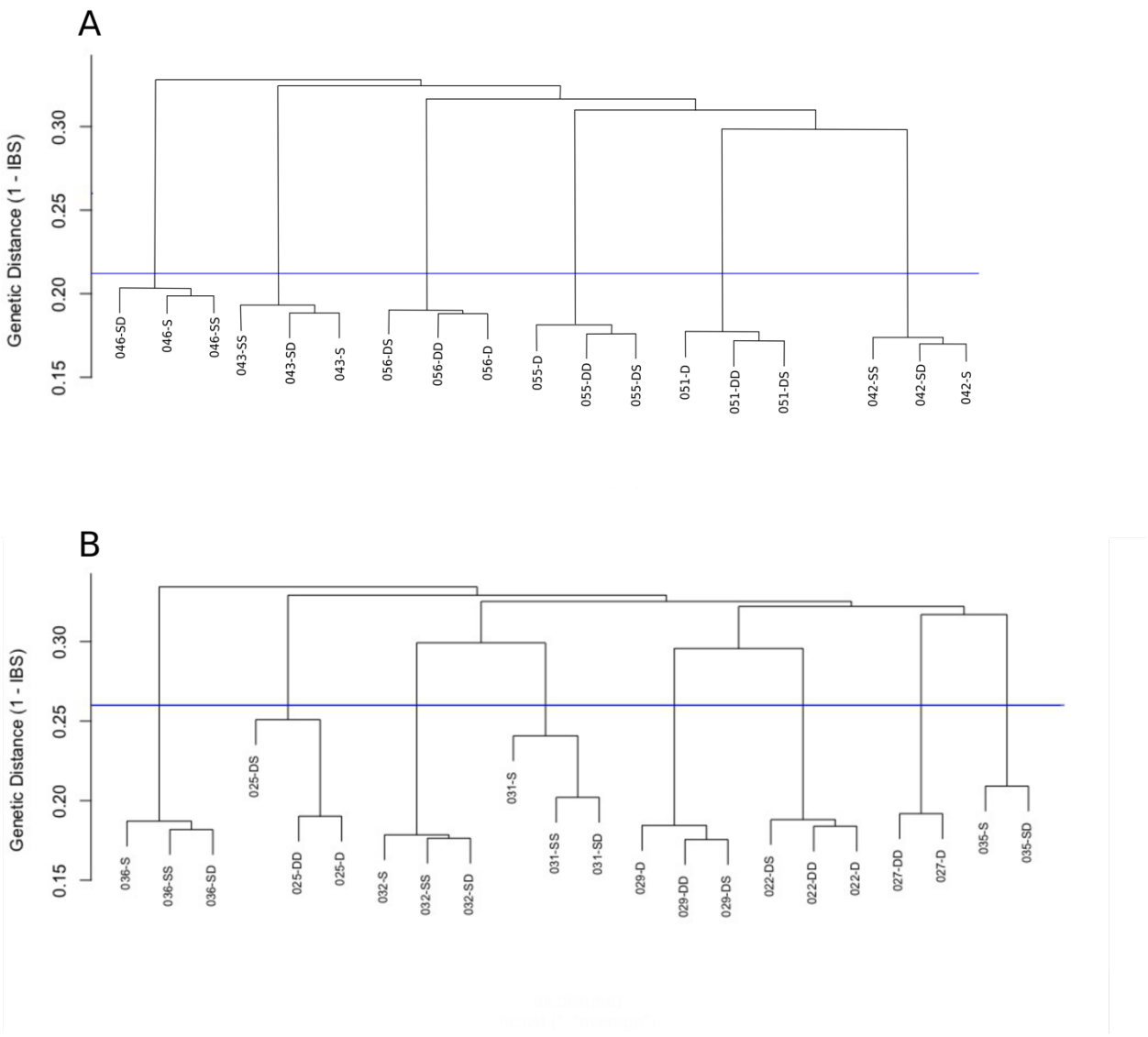


Supplemental Table 2. Kruskal-Wallis p-values for comparisons of color values between treatment groups before and during transplantation.

Comparison	***Acropora surculosa***	***Porites rus***
D vs. S	<0.001*	<0.001*
SD vs. SS	0.00432*	0.00479*
DS vs. DD	0.0.725	0.00650*

Supplemental Table 3. ANOVA p-values for symbiont density comparisons within coral species, between treatment groups. Treatment groups are as follows: shallow baseline (S), shallow control (SS), shallow transplanted to deep (SD), deep baseline (D), deep control (DD), deep transplanted to shallow (DS).

Comparison	***Acropora surculosa***	***Porites rus***
D vs. S	0.269	0.240
D vs. DD	0.779	0.070
S vs. SS	0.212	0.151
SD vs. SS	0.362	0.305
DS vs. DD	0.351	0.630



Supplemental Figure 2. (A) *Acropora surculosa* and (B) *Porites rus* clonality dendrograms based on ANGSD identity-by-state (IBS) matrices. Sample number indicates parent colony. Threshold line added above nodes of biological replicates (samples from same parent colony). Treatment groups are as follows: shallow baseline (S), shallow control (SS), shallow transplanted to deep (SD), deep baseline (D), deep control (DD), deep transplanted to shallow (DS).

Supplemental Table 4. *Acropora surculosa* and *Porites rus* transcriptome Benchmarking Universal Single-Copy Orthologs (BUSCO) analysis results. Compared with the Metazoa lineage.

Porites rus		***Acropora surculosa***		
Number of BUSCO groups	**Percent**	**Number of BUSCO groups**	**Percent**	**Description**
654	68.6%	705	73.9%	Complete BUSCOs
490	51.4%	537	56.3%	Complete and Single-copy BUSCOs
164	17.2%	168	17.6%	Complete and duplicated BUSCOs
224	23.5%	118	12.4%	Fragmented BUSCOs
76	7.9%	131	13.7%	Missing BUSCOs
954	100%	954	100%	Total BUSCO groups searched