PHENOTYPIC PLASTICITY IN ACROPORA PULCHRA UNDER VARIED ENVIRONMENTAL CONDITIONS

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PHENOTYPIC PLASTICITY IN ACROPORA PULCHRA UNDER VARIED ENVIRONMENTAL CONDITIONS

BY

DENNIS ANDREW CONETTA

A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN BIOLOGICAL AND ENVIRONMENTAL SCIENCES

UNIVERSITY OF RHODE ISLAND

2021
MASTER OF SCIENCE IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE

OF

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2021
ABSTRACT

The extent to which coral reefs have declined globally has triggered major scientific investment in coral restoration research. However, much of the predictions for reef futures do not include the capacity for coral acclimatization, or phenotypic plasticity, and how this plasticity varies across seasons. In light of this, we outplanted clonal replicates of distinct genotypes of the reef building coral *Acropora pulchra* from an existing coral nursery common garden site to three sites on the North Shore of Mo’orea, French Polynesia. After transplantation (October 2019), the outplanted colonies were sampled at all three sites in January and November of 2020, for the following physiological metrics; maximum photosynthetic rate (Am), photosynthetic efficiency (AQY), dark respiration (Rd), chlorophyll concentration, symbiont density, total protein, and ash free dry weight. Nursery genotypes and wild corals from two of the outplant sites were sampled at the outset of the experiment (October 2019) to provide a physiological baseline, which identified differences in coral physiology between the common garden and the wild corals from the two sites. Our results show that outplanted corals displayed significantly different phenotypes both through time and between sites. Our data show that plasticity score (calculated as the differences in multivariate space between October 2019 and each of the other timepoints for each genotype) was highest across all sites 3 months after transplantation (January 2020) and decreased across all sites by 13 months (November 2020) This identifies a capacity for site driven phenotypic plasticity that became more similar to the baseline common garden phenotype by 13 months, due to location acclimation, or seasonal environmental similarities. This study demonstrates that plasticity varies by site and through seasons,
highlighting the need for physiological time series research to interpret performance in following human interventions
ACKNOWLEDGMENTS

I would like to thank my advisor, Dr. Hollie Putnam, for her continual support, mentorship, guidance, grace and understanding throughout the entirety of this process. I have truly learned so much from you and could not be more grateful for all your help. I would also like to express the utmost gratitude to Ariana Huffmyer for taking time out of her schedule to specifically help me get to the finish line. I would also like to thank my labmates Kevin Wong, Emma Strand, Sam Gurr, Maggie Schedl, Jill Ashy, Lauren Zane, and Hannah Reich for their field support, writing support, and career advice. I would also like to thank my committee members Dr. Graham Forrester, and Dr. Carlos Prada for your encouragement and constructive criticism. I would like to thank my amazing family, partner, and friends for all the unwavering support and love that helped make this project possible. Lastly, I would like to dedicate this research to my grandfather Peter Salvatore Conetta. Thank you for always supporting me and doing it with a never-failing sense of humor. You are and will always be a source of motivation to me. I want you to know that I finally took your advice and I stopped going on vacation and I finally got a real job. I love and miss you every day.
PREFACE

This thesis has been prepared in Manuscript Format. Manuscript 1, entitled “Phenotypic plasticity in the reef building coral Acropora pulchra under varied environmental conditions”, will be prepared for submission to Coral Reefs. Additional authors for the manuscript are listed before the start of the body of the thesis. An appendix is presented at the end of the thesis and contains the supplementary material for the manuscript.
TABLE OF CONTENTS

ABSTRACT .........................................................................................................................ii
ACKNOWLEDGMENTS .....................................................................................................iv
PREFACE .......................................................................................................................... v
TABLE OF CONTENTS ................................................................................................. vi
LIST OF FIGURES .........................................................................................................viii
MANUSCRIPT 1 ............................................................................................................... 1

Abstract ......................................................................................................................... 2
Introduction ....................................................................................................................... 4
Methods ............................................................................................................................. 8
  Experimental Design
  Respirometry Trials
  Airbrushing
  Host Protein Assay
  Chlorophyll Measurements
  Symbiont Density Counts
  Surface Area (Wax Dipping)
  Ash Free Dry Weight (Biomass)
  Statistical Analysis
Results ............................................................................................................................16
  Baseline Univariate Analysis
    Max Photosynthesis (Am)
    Alpha (AQY)
    Respiration Rates (Rd)
    Total Chlorophyll per Fragment (ug/cm²)
    Total Chlorophyll per Symbiont (ug/cell)
    Host Protein (ug cm⁻²)
    Ash Free Dry Weight/Biomass (mg cm⁻²)
Timeseries Univariate Analysis

Max Photosynthesis (Am)

Alpha (AQY)

Respiration Rates (Rd)

Total Chlorophyll per Fragment (ug cm$^{-2}$)

Total Chlorophyll per Symbiont (ug cm$^{-2}$)

Host Protein (ug cm$^{-2}$)

Ash Free Dry Weight/Biomass (mg cm$^{-2}$)

Multivariate PCA

Plasticity Score

DISCUSSION........................................................................................................22

CONCLUSION......................................................................................................25

APPENDICES.......................................................................................................44
LIST OF FIGURES

FIGURE       PAGE

Figure 1. A) a map of the North Shore of the island Mo’orea, French Polynesia and the three outplant sites in the backreef lagoon chosen for this study. B) Colonies of known genotypes (n = 4) were tagged and sampled prior to and after transplantation to the three sites such that one colony of each genotype was present at each site. C) Environmental loggers deployed at each of the sites collecting light, pH, and temperature. These loggers were set at each of three transplant sites in October of 2019 and were offloaded and monitored throughout the experiment (Oct. 2019 - Nov. 2020) .......................................................... 38

Figure 2. series of strip plots depicting the baseline physiological metrics measured for the nursery genotypes (n=10) and wild genotypes (n=3) at two sites in the backreef lagoon of Mo’orea, French Polynesia in October of 2019 (prior to transplantation of nursery corals). The metrics observed are biomass (AFDW, mg cm\(^{-2}\)), host protein concentration (ug cm\(^{-2}\)), respiration rates (Rd), symbiont density (cells cm\(^{-2}\)), maximum photosynthesis (Am), apparent quantum yield (AQY), total chlorophyll per fragment (ug of chl a and chl c cm\(^{-2}\)), and total chlorophyll per symbiont cell (ug of chl a and chl c/cell). The black dots and bars represent the mean and standard deviation values for each metric at each site. Significant posthoc values (Tukey’s HSD) are indicated by unique letters in each plot.......................................................... 39

Figure 3. Radar plot displaying the ranked comparisons of multiple environmental metrics, with the circles indicating increasing values starting at
0.25 in the center and increasing by 0.25 increments until the outermost circle which is 1.0 …………………………………………………………………………………… 40

Figure 4. A series of boxplots depicting the physiological metrics of the 4 genotypes (Genotypes 4, 6, 8, 15) prior to transplantation (October 2019) and subsequent measurements of these physiological metrics for the same four genotypes at each of the three sites they were transplanted to both 3 months later (January 2020) and 13 months later (November 2020). The metrics measured were the same as those in Figure 1 (AFDW, Host Protein, Respiration Rate, Symbiont Density, Am, AQY, Total Chlorophyll per Fragment, Total Chlorophyll per Symbiont Cell). Significant posthoc values are indicated by unique letters on top of each boxplot…………………………………………………… 41

Figure 5. PCA of the distance metrics calculated from the averaged z-score of the combined univariate metrics for each genotype in both January and November of 2020. The centroid point, from which the distance metrics are calculated, represents the baseline phenotype which encompasses the eight univariate metrics collected in October of 2019 from the same four genotypes in the nursery. The length and direction of the lines correlate to the overall differences in phenotype (using the same 8 univariate metrics) at each of the transplant sites at both January and November 2020 time points.................................................. 42

Figure 6. Reaction norm plot displaying the plasticity scores of each genotype at each site. The averaged values for the genotypes for the three sites (n=4) are represented by the bolded colored lines and represent the overall trend of plasticity score throughout the sampling time points…………………………………43
Prepared for submission to Coral Reefs

**Phenotypic plasticity in *Acropora pulchra* under varied environmental conditions**

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Abstract

The extent to which coral reefs have declined globally has triggered major scientific investment in coral restoration research. However, much of the predictions for reef futures do not include the capacity for coral acclimatization, or phenotypic plasticity, and how this plasticity varies across seasons. In light of this, we outplanted clonal replicates of distinct genotypes of the reef building coral *Acropora pulchra* from an existing coral nursery common garden site to three sites on the North Shore of Mo’orea, French Polynesia. After transplantation (October 2019), the outplanted colonies were sampled at all three sites in January and November of 2020, for the following physiological metrics; maximum photosynthetic rate (Am), photosynthetic efficiency (AQY), dark respiration (Rd), chlorophyll concentration, symbiont density, total protein, and ash free dry weight. Nursery genotypes and wild corals from two of the outplant sites were sampled at the outset of the experiment (October 2019) to provide a physiological baseline, which identified differences in coral physiology between the common garden and the wild corals from the two sites. Our results show that outplanted corals displayed significantly different phenotypes both through time and between sites. Our data show that plasticity score (calculated as the differences in multivariate space between October 2019 and each of the other timepoints for each genotype) was highest across all sites 3 months after transplantation (January 2020) and decreased across all sites by 13 months (November 2020) This identifies a capacity for site driven phenotypic plasticity that became more similar to the baseline common garden phenotype by 13 months, due to location acclimation, or seasonal environmental
similarities. This study demonstrates that plasticity varies by site and through seasons, highlighting the need for physiological time series research to interpret performance in following human interventions.

**Keywords:** coral nursery, coral transplantation, phenotype, plasticity
Introduction

Coral reefs account for less than 0.1% of the global surface area, yet they account for more than 30% of all marine species (Reaka-Kudla 2005; Fisher et al. 2015). Coral reefs are created by their ecosystem engineers, scleractinian corals. These colonial cnidarians house photosymbiotic dinoflagellates in the family Symbiodiniaceae, which supply their coral hosts with the majority of their daily metabolic requirements (Muscatine, McCloskey, and Marian 1981) through the allocation of photosynthates. The coral in turn uses these substrates to generate energy needed to secrete a calcium carbonate skeleton (Tambutt et al. 2008), which ultimately creates the three-dimensional structure that is essential for fish and invertebrate habitat (Cesar 2002). In addition to the habitat that they provide, coral reefs are billion dollar commodities depended on by 500 million people globally (Global Coral Reef Monitoring Network 2004; Brander and van Beukering 2013) because they support fisheries, ecotourism businesses, and protect coastal and island communities. Although coral reefs are essential in many ways, today they face a myriad of local and global anthropogenic impacts that threaten their very existence (Hughes et al. 2017; Johnson et al. 2021; Tebbett et al. 2021; Donovan et al. 2020). Global climate change stressors such as rising sea surface temperatures are driving global coral mortality events (Hughes et al. 2017, 2018; Rodgers et al. 2017). Live coral cover has been reduced by 30-50% globally over the last 50 years (Bruno and Selig 2007; T. A. Gardner et al. 2003; De’ath et al. 2012), with major reductions in coral cover following major bleaching events (Miller et al. 2009; Pratchett et al. 2009; Babcock et al. 2020; Couch et al. 2017).
The global degradation of coral reefs has reached a point where the natural processes alone that lead to recovery are unlikely to assure that the biodiversity and ecological functions of coral reefs will be maintained in the future (Veron et al. 2009). This global decline in reefs has inspired active, coordinated, and effective human interventions (van Oppen et al. 2015; Anthony et al. 2017; National Academies of Sciences, Engineering, and Medicine et al. 2019). There are over 300 active coral restoration projects globally which are spread across 56 countries and collectively target 229 species from 72 coral genera (Boström-Einarsson et al. 2020). Although there are many proposed intervention practices for conserving coral reefs such as managed selection or genetic manipulation, (National Academies of Sciences, Engineering, and Medicine et al. 2019) the most developed and used practices to date take advantage of corals’ ability to asexually reproduce by fragmentation (Fabian, Beck, and Potts 2013; Young, Schopmeyer, and Lirman 2012). In corals if a branch or fragment of coral is broken off from a parent colony it will give rise to a genetic clone, thus allowing parent colonies to give rise to multiple genetically identical offspring. About half of current conservation plans to date utilize fragmentation to grow coral fragments in nurseries (Boström-Einarsson et al. 2020). Of the many species targeted for restoration purposes, approximately 30% are of the genus *Acropora* (Boström-Einarsson et al. 2020). Because of their high growth rates, natural use fragmentation for asexual reproduction, ability to rapidly heal wounds, comparatively high survivorship as fragments, and their endangered status in the Caribbean (Tunnicliffe 1981; Bak and Criens 1981; Gladfelter, Monahan, and Gladfelter 1978; Highsmith 1982; D. Lirman et al. 2010) this genus is a prime candidate for restoration projects.
However, despite the growing focus on reef conservation today, repeated temporal assessment of physiological plasticity following transplantation remains limited.

Most coral conservation programs and research are aimed at increasing survival and the biomass of outplants at restored sites because these are tangible results that are comparable and easy to mark success or failure. For example, (Schopmeyer et al. 2017), created a set of major benchmarks to measure the success of *Acropora cervicornis* restoration for six programs in Florida and Puerto Rico, which were centered on nursery and ouplant growth and survival rates. While growth and survival provide tangible benchmarks for conservation programs, these metrics do not fully assess the long-term physiological impacts of outplanted corals, or explain how they acclimate to these new environments. In fact, (Ware et al. 2020) found that while initial outplant survival remains high, after 2 years survival tends to decline. Thus, it is vital to understand the physiological capabilities of coral used in restoration and how they vary through time, to untangle the factors that can contribute to a successful or failed restoration effort.

Measuring a suite of targeted physiological metrics would provide insight to the plasticity and acclimation potential of key factors of metabolism, symbiont growth, symbiont productivity, stress tolerance and energetic content (Gardner et al. 2017; Wall et al. 2021). By integrating across biological scales in a set of genetic lines of a given species exposed to multiple environmental conditions, it is possible to gain a better understanding of the mechanisms driving phenotypic plasticity. Phenotypic
plasticity is the ability for a single individual or genotype to produce a wide array of phenotypes in response to their environment. The ability to acclimate to new environmental challenges varies by genotype (Jury, Delano, and Toonen 2019; Shaw et al. 2016) and the interaction with environmental history (Wong et al. 2021; Wall et al. 2018). Safaie et al. (Safaie et al. 2018) demonstrates that corals that experience high frequency temperature variability were less likely to experience bleaching even when temperatures would rise 1 °C above daily means. Similarly, (Morikawa and Palumbi 2019) demonstrates that growing nursery fragments under more variable temperature conditions displayed higher rates of bleaching resistance than the same genotypes grown in more stable temperature conditions. Therefore, characterizing the interplay of environment and genotype and its role in shaping coral phenotype is crucial to understanding of acclimatory mechanisms and the optimization of restoration success.

Here we examined phenotypic plasticity in corals from a common garden that were outplanted to sites ranging in environmental conditions. Using an existing coral nursery in Mo’orea as a common garden setting, clonal replicates of 10 Acropora pulchra genotypes were outplanted to three different reef sites located in the back reefs on the north shore of Mo’orea, French Polynesia (Fig. 1). These three sites represent an established nutrient gradient (Adam et al. 2021). Environmental conditions were also monitored in real time by deploying sensors to monitor temperature, light, and pH. Additionally, the locally occurring macroalgal species Turbinaria ornata was collected throughout the course of this study to provide
integrated environmental nutrient assessment (Adam et al. 2021). This research will provide meaningful insight to the physiological differences of nursery and wild type corals, metabolic impacts of nutrients to outplanted corals, and effectively quantify plasticity through time.

Methods

Experimental Design

Ten distinct genotypes of the reef building coral *Acropora pulchra* were identified within an existing coral nursery (17°29'01.1"S, 149°50'03.9"W) on the island of Mo’orea, French Polynesia at the outset of the experiment in October of 2019. *Acropora pulchra* was chosen for this experiment because it is a fast growing species reef building coral (Alcala, Alcala, and Gomez 1981; Soong and Chen 2003) that is found in the back reef lagoon environment in Mo’orea (Shaw et al. 2016; Veron 2000) in large thickets. These thickets provide essential habitat for other corals, reef invertebrates, and reef fishes. In addition, this species is among the top 20 species used in restoration programs globally (Boström-Einarsson et al. 2020). 3 replicates of these 10 genotypes were tagged with metal tags and outplanted to 3 sites along a naturally occurring nutrient gradient on the north shore (Adam et al. 2021): site 1 (17° 28' 36.7392"S, 149° 48' 21.384W), site 2 (17° 29' 13.56"S, 149° 53' 15.576"W), and site 3 (17° 28' 53.4"S, 149° 50' 56.688"W). At the outset of the experiment (October 2019), the 10 distinct nursery genotypes were sampled to provide baseline phenotypes. Simultaneously, 3 distinct wild *Acropora pulchra* colonies were sampled in October 2019 at site 1 and site 2 to provide baseline phenotypes for the wild corals at each of
these sites. Site 3 did not have high cover of live, wild occurring colonies at the time of the experiment, but sparse live colonies and skeletons evidenced that they can exist at this site. In addition to the physiological metrics, environmental sensors were deployed at the outset of the experiment (October 2019) to each of the three sites and measured temperature (HOBO v2 U22 Temperature Loggers), light (Odyssey Xtreem PAR Loggers), and pH (HOBO MX pH Logger). In addition to these environmental parameters, 100 fronds of the locally occurring macroalgae Turbinaria ornata were collected at each time point to characterize the nutrient conditions (%N) of each site (Fong, Donohoe, and Zedler 1994; Lin and Fong 2008; Adam et al. 2020). See Appendix 1 for site selection details.

In-situ sampling of each coral colony for every time point, which occurred in October 2019, January 2020, and November 2020, included taking a 2-3cm apical clipping of each Acropora pulchra colony which were transferred in quart (~946mL) ziplock bags filled with seawater from each site to the University of California Berkeley Gump research station on Mo’orea. These live fragments were immediately placed in flow-through seawater tables where they were exposed to ambient water from Cook’s Bay, Mo’orea for 2 days prior to physiological sampling. All colonies (nursery and outplant) were sampled for the following 8 physiological metrics; respiration (Rd), photosynthetic efficiency or apparent quantum yield (AQY), maximum rate of photosynthesis (Am), chlorophyll concentration, symbiont density, total protein, and ash free dry weight.
Photosynthesis: Irradiance curves

Each labeled coral was taken from the outdoor water table and placed into individual 620 ml acrylic respirometry chambers with a magnetic stir bar filled with seawater from the water tables. Chambers were sealed and placed in magnetic stir plate stands. The chambers were fully submerged in ambient temperature seawater, which was controlled by an aquarium heater (Finnex 300W Titanium Heater, Burnaby, British Columbia, Canada) and pump (Pulaco 400GPH Submersible Pump, Baiyun District, Guangzhou, China). A temperature sensor (PreSens Pt1000) and a fibre-optic oxygen probe [Presens dipping probe (DP-PSt7-10-L2.5-ST10-YOP)] was placed in each chamber to measure temperature corrected oxygen flux in µmol l$^{-1}$. The corals were exposed to ten different irradiance levels for 10 minutes each (~0, 18, 68, 113, 169, 243, 499, 709, 844, and 1025 µmol photons m$^{-2}$ s$^{-1}$) with one LED light (Prime™ 16HD Reef Aquarium Lights, Aquaillumination) positioned over each chamber. Each photosynthesis irradiance trial included both chambers filled with seawater and live corals and chambers filled with filtered seawater only (pore size 100µm) which served as a blank to account for background changes in oxygen. An underwater cosine corrected sensor (MQ-510 Quantum Meter, spectral range of 389–692±5 nm, Apogee Instruments, Logan, UT, USA) was used to determine the light levels at each chamber position prior to each respirometry trial. The oxygen concentrations at each light level were used to calculate light enhanced dark respiration (LEDR, denoted here as Rd), photosynthetic efficiency or apparent quantum yield (AQY), and maximum rate of photosynthesis (Am). Once all light levels were completed, the corals were removed from the chambers, transferred to 4oz sterile plastic bags (Grainger Industrial, Lake
Forest, Illinois, USA), the seawater was removed, fragments were snap-frozen in liquid nitrogen, and stored at -40°C until processing. The volume of water in each chamber was measured and used to correct for differences in coral displacement for each fragment. Rates of photosynthesis and respiration were calculated in final units of µmols O₂ cm⁻² h⁻¹.

*Tissue Removal for Assays*

Coral tissue from the snap frozen fragments was stripped from their skeletons using a pressurized airbrush (Iwata, Eclipse HP-BCS, Portland, Oregon, USA) and chilled 1X Phosphate Buffer Saline (PBS) solution. The resulting tissue and PBS slurry, or homogenate, was collected in a ziplock bag and transferred to labeled 50mL falcon tubes. The collected tissue slurry was homogenized for thirty seconds on high using a homogenizer (Pro Scientific Inc., PRO200 Homogenizer, Oxford, Connecticut, USA) that was cleaned between each use with 10% bleach solution, deionized water, and isopropanol wipes. The final homogenate volume was recorded. From the homogenized tissue slurry, two 1mL aliquots were transferred into 1.5mL microcentrifuge tubes and spun at 13,000g for 3 minutes in a microcentrifuge. The supernatant (host fraction) of the first tube was discarded and the pellet was stored at -40 °C for subsequent chlorophyll content assays. The supernatant of the second tube was removed, transferred to a new 1.5mL microcentrifuge tube, and stored at -40 °C for assessing host protein concentration assays. The remaining algal pellet was resuspended in 1 mL of ice-cold 1X PBS, with 500 µL of the resuspended pellet
separated into a third 1.5mL tube and stored in a 4 °C fridge for symbiont density counts within 48 hours.

**Surface Area Quantification**

The coral skeletons were placed in a drying oven (Fisher Scientific, Isotemp Oven, Waltham, Massachusetts, USA) at 60°C for 4 hours before surface area measurements were made. Following methods from (Veal et al. 2010), pre-weighed wooden dowels of known dimensions were used to determine a standard curve of mass change of wax dipped dowels against geometrically calculated surface area, with an $R^2 > 0.9$ for the relationship. Based on the wax dipping method of (Veal et al. 2010), a Minerva paraffin wax bath (Monroe, GA, USA) was heated to 65°C and used to single dip coral skeletons. These waxed skeletons were left at room temperature to dry and cool and then reweighed. The wax weight was calculated by subtracting the initial weight from the final weight and the standard curve (generated as change in wax weight of the standards as a function of surface area) was converted into surface area measurements in cm².

**Host Protein**

Host soluble protein was measured from tissue homogenate supernatant using the Pierce BCA Protein Assay Kit (Thermo Scientific, cat #23225, Waltham, Massachusetts, USA) according to manufacturer’s instructions. Host protein was normalized to homogenate volume and sampled coral fragment surface area for final units of µg cm⁻².
**Chlorophyll**

To calculate chlorophyll concentration in each sample, the algal pellet was thawed at room temperature and 1mL of 100% acetone was added to resuspend the cells. Following 24h in the dark at 4°C the samples were centrifuged at 13,000 rpm for 3 minutes to remove excess debris. 200µL of the supernatant was transferred to a 96-well quartz plate (Hellma Analytics, Müllheim, Baden Württemberg, Germany) in duplicate for each sample. This 96-well quartz plate also included duplicate acetone blanks. Absorbance was measured in a spectrophotometer (Synergy HTX Multi-Mode Reader, BioTek, USA) at 630, 663 and 750nm. Chlorophyll $a$ and $c_2$ concentrations were calculated using equations for dinoflagellates in 100% acetone described in (Jeffrey and Humphrey 1975) and corrected for path length of the 96-well plate (0.66cm; Hellma Analytics Quartz Microplate). Chlorophyll concentrations represent total chlorophyll ($a$ and $c_2$ values combined) standardized to sampled fragment surface area ($\mu$g cm$^{-2}$), and endosymbiont cell density ($\mu$g cell$^{-1}$).

**Symbiont Density**

To quantify the Symbiodiniaceae densities, repeated cell counts ($n = 8$) were conducted on the homogenate aliquots using an Improved Neubauer Haemocytometer (Marienfeld Superior, Lauda-Königshofen, Germany). The fixed volume within the defined area of the haemocytometer was used to calculate symbiont density of the sample and was normalized to the surface area of the coral skeleton (cells cm$^{-2}$).
**Ash Free Dry Weight (Biomass)**

Tissue biomass was quantified as Ash Free Dry Weight (AFDW) from both the host and symbiont fractions. An aliquot of 5 mL of coral tissue homogenate was centrifuged at 3500 rpm for 3 minutes. 4 mL of the supernatant (host) was removed and transferred into a pre-burned aluminum pan. The algal pellet (symbiont) was resuspended in 1 mL of cold 1X PBS before being placed in a separate pre-burned aluminum pan, and both pans for each sample were placed in a drying oven at 80 °C for 24 h and dry weight was measured. Pans were subsequently burned in a muffle furnace at 450 °C for 4-6 h. Dry mass was calculated as dry tissue and pan mass - initial pan mass. AFDW was calculated as dry tissue and pan mass - burned tissue and pan mass, normalized by homogenate volume and standardized surface area (mg cm⁻²).

**Statistical analysis**

To test that hypothesis that there was no difference in the physiology of the common garden (n=10) and wild coral colonies (n=3) from sites 1 and 2 (October 2019), we analysed each of the 8 physiological metrics using one-way ANOVA. Further, to test the hypothesis that each variable differed between sites (3 sites, fixed factor) and time point (January and November 2020, fixed factor), we analysed each of the aforementioned 8 physiological metrics using a two-way ANOVA. Site was used as a proxy for environmental conditions as the environmental data set did not capture data for all metrics for the entirety of the experiment. The environmental data was cleaned which resulted in n= 68 days of continuous data at all three sites for all metrics which
was used in the ANOVA analyses of all metrics used in Figure 3. Repeated measures were not considered for this analysis because additional replicates of the genotypes displaying reproductive viability were outplanted for separate reproduction work but were sampled at times due to loss of colonies (unidentifiable, lost, or died) so despite sampling the same genotypes throughout the study they do not always represent the same colony. Posthoc tests (Tukey’s HSD) were used to parse out significance of pairwise comparisons for both datasets.

The univariate data for all 8 physiological metrics were compiled to quantify multivariate physiology of each genotype at each site and time point using Principal Components Analysis (PCA). A plasticity score of multivariate physiology was calculated as the Euclidean distance between the PCA points (sensu (Barott et al. 2021; Abbott et al. 2018)) using the October 2019 multivariate PCA value for each genotype as a baseline. Specifically the X-Y location for each colony outplant was compared back to its associated baseline nursery genotype for both January and November 2020 time points, resulting in a plasticity score. Permutational multivariate analysis of variance or PermANOVAs were used to analyze factors used within the PCA (site and time point). The distances between the baseline genotype z-score and each of the time point’s values at each outplant site were used to calculate distance metrics which were compiled to create a PCA that showed the overall genotypic response by site through time (Fig. 4). These plasticity scores were then analyzed using a two-way ANOVA to test the significance of site and time point on multivariate plasticity, followed by posthoc tests (Tukey’s HSD). The assumptions of ANOVA
were tested with graphical analysis of the residuals and all variables were log transformed to meet the assumptions of normality and distribution.

**Results**

**Baseline Phenotype Analysis:**

*Coral Energy Production and Demand (Am, AQY, Rd)*

Analysis of P:I curves identified that Am was significantly different between nursery and wild sites ($F_{2,13} = 6.779$, $p = 0.01$), with the higher rates of photosynthesis at sites 1 and 2 in comparison to the nursery site (Table S1, Fig. 2). Apparent quantum yield (AQY) displayed high variation at each site and no significant differences were detected ($F_{2,13} = 2.2249$, p-value = 0.148). Dark respiration (Rd) were significantly different at each site ($F_{2,13} = 15.402$, $p < 0.0004$), with higher respiration rates at site 1 and site 2 in comparison to the common garden site (Table S1, Fig 2). These results show that energy production and demand are both higher at both wild sites than the nursery. Thus the environments at the wild sites and nursery are imposing different challenges that trigger differing energetic production and demand.

*Symbiont Variables [Total Chl (ug cm$^{-2}$, ug cell$^{-1}$), Symbiont Density (cells cm$^{-2}$)]*

Total chlorophyll concentrations of the sampled fragments (ug cm$^{-2}$) were significantly different between sites ($F_{2,13} = 36.524$, $p = 4.622e-06$) with all three sites being significantly different from one another (Table S2). Site 1 had the highest chlorophyll concentrations, site 2 had intermediate levels of chlorophyll, and the nursery had the lowest chlorophyll concentrations (Table S1, Fig 2). Total chlorophyll
concentrations per symbiont (ug cell\(^{-1}\)) were significantly different between sites as well (F\(_{2,13} = 15.642, p < 0.0004\)), with site 1 having the highest concentration of chlorophyll per symbiont than both site 2 and the common garden sites (Table S1, Fig. 2). Symbiont density was marginally different between sites (F\(_{2,13} = 3.774, p\)-value = 0.051). These results show that each site imposes varying environmental challenges that cause chlorophyll concentrations to fluctuate by site. The differences in chlorophyll concentration, therefore, are mostly attributed to the symbiont physiology and the degree to which they are concentrated with chlorophyll by site.

Host Variables [Host Protein (ug cm\(^{-2}\)), AFDW (mg cm\(^{-2}\))]

Host protein concentrations were significantly different between sites (F\(_{2,13} = 17.684, p < 0.0002\)), with higher concentrations of host protein at site 1 and site 2 in comparison to the common garden site (Table S1, Fig. 2). Ash free dry weight values were significantly different between sites (F\(_{2,13} = 11.51, p = 0.001\)), with site 1 having significantly higher concentrations than the nursery (Table S1, Fig. 2). Site 1 protein concentrations are not significantly different than site 2 and site 2 protein concentrations are not significantly different than the nursery (Table S1, Fig. 2) while site 2 was not significantly different than the nursery (TukeyHSD, p-value = 0.052). These results suggest that while the wild sites produce higher values of biomass than the nursery, the protein contribution to this biomass is not the only thing driving these differences between sites because site 1 and 2 were not both significantly higher in biomass and protein.
Transplant Timeseries Univariate Phenotype Analysis:

While 10 genotypes were outplanted, the full data set of values for all eight physiological metrics were limited to only four genotypes, due to either partial/full mortality of fragments, or not being able to locate a fragment in the field at a particular time point.

Environmental Data: From the data set we see that site 1 had the highest light intensity, was the warmest, the least variable in daily temperature and had relatively high pH but not the highest pH. Site 2 displayed moderate levels of light and was the most variable in temperature and pH of all three sites. Lastly, site 3 was characterized by having the lowest temperatures, pH values, light intensity, and was the least variable for pH and temperature. This data showed that out of the 12 metrics tested in Figure 3, 10 of them proved to be significant by site showing that each site had a unique environmental profile.

Coral Energy Production and Demand (Am, AQY, Rd)

Maximum photosynthesis rates (Am) were not significantly different between sites (F2, 18 = 2.566, p = 0.1046), timepoint (F1, 18= 4.119, p = 0.0575), nor the interaction of the two (F2, 18 = 0.105, p = 0.9007). Apparent quantum yield or alpha (AQY) values were not significantly different based on site (F2, 18 = 0.204, p = 0.817), timepoint (F1, 18= 2.818 , p = 0.110), nor the interaction of the two (F2, 18 = 0.362, p = 0.701). Respiration rates were not significantly different between sites (F2, 18 = 3.023, p = 0.073785), nor was the interaction of time point and site (F2, 18 = 0.017, p = 0.982903).
However, respiration rates were significantly different between timepoints \( (F_{1, 18} = 17.868, p = 0.000507) \), where the average respiration rates were higher at all three sites in January of 2020 compared to those in November of 2020 (Table S2, Fig. 3). These results suggest that photosynthetic capacity is unchanging spatiotemporally but coral energetic demands are higher in January compared to October/November. These higher metabolic demands could be the product of warmer austral summer temperatures or potentially a product of acclimatization, which requires more energetic input to help the corals overcome the stress of transplantation.

*Symbiont Variables [Total Chl (ug cm\(^{-2}\), ug cell\(^{-1}\), Symbiont Density (cells cm\(^{-2}\))]*

Total chlorophyll concentrations per coral fragment (ug cm\(^{-2}\)) were significantly different between sites \( (F_{2, 18} = 4.307, p = 0.0296) \), with site 3 having overall lower total chlorophyll concentrations than site 1 and significantly lower concentrations than site 2 (Table S2, Fig. 3). Total chlorophyll concentrations per fragment were also significantly different between time point \( (F_{1, 18} = 8.282, p = 0.0100) \), with January chlorophyll concentrations at site 1 and site 2 being significantly higher than the same sites sampled in November 2020 (Table S2, Fig. 3). Interestingly site 3 was virtually the same in both timepoints and did not contribute to the significance of time point. The interaction between site and time point for chlorophyll concentration per fragment was not significant \( (F_{2, 18} = 2.406, p = 0.1186) \). Chlorophyll concentrations per symbiont cell (ug cell\(^{-1}\)) were not significantly between sites \( (F_{2, 18} = 2.956, p = 0.07759) \), nor was the interaction of time point and site significant \( (F_{2, 18} = 0.518, p = 0.60453) \). However, chlorophyll concentration per symbiont was significant between
time points ($F_{1,18} = 11.730, p = 0.00302$), with overall higher chlorophyll values at all three sites in November 2020 compared to those in January 2020 (Table S2, Fig. 3). Symbiont density (ug cell$^{-1}$) values were not significantly different between sites ($F_{2, 18} = 1.237, p = 0.314$), nor was the interaction of time point and site significant ($F_{2, 18} = 1.102, p = 0.354$). However, there was a significant difference in symbiont densities between time points ($F_{1, 18} = 29.845, p = 3.45e-05$), with overall higher chlorophyll values at all three sites in January 2020 compared to those in November 2020 (Table S2, Fig. 3). These results suggest that while symbiont cells may be more abundant in corals in January, the few cells in November 2020 are much more concentrated with chlorophyll than those in January. Therefore, there is this physiological tradeoff where in the austral summer and earlier in the acclimatization period, corals increase the density of symbionts they house but overtime these cell densities revert back to common garden levels but increase the chlorophyll concentrations per symbiont.

**Host Variables [Host Protein (ug cm$^{-2}$), AFDW (mg cm$^{-2}$)]**

Protein concentrations (ug cm$^{-2}$) were significantly different at each site ($F_{2, 18} = 12.60, p = 0.000378$), with all three sites being significantly different from one another. Site 2 had the highest, site 1 had intermediate, and site 3 had the lowest protein concentrations (Table S2, Fig. 3). Protein concentrations were also significantly different between time points ($F_{1, 18} = 51.66, p = 1.09e-06$), with January 2020 having higher protein concentrations at all three sites (Table S2, Fig. 3). However, the interaction of site and time point was not significant ($F_{2, 18} = 0.86, p = 0.439673$) for protein concentration. Biomass values were not significantly different between sites.
(F\(_{2, 18}\) = 1.579, p = 0.233), time points (F\(_{1, 18}\) = 0.622, p = 0.440), nor the interaction of the two (F\(_{2, 18}\) = 0.139, p = 0.871). These results suggest that despite protein concentrations being higher in the austral summer months and earlier into the acclimatization period, biomass is ultimately consistent throughout the entire experiment. This could be the result of a commensurate matching of respiration rates (higher in January and lower or regression to common garden levels) to maintain consistent biomass throughout the year. Or this could mean that the host protein is a significant portion of the entire biomass with less contributions from other organic sources, whereas in November less of the biomass is made up of the host protein and so there are increased supplemental organic sources.

**Multivariate PCA**

Physiological profiles (multivariate analysis of all responses) of corals varied by site (PERMANOVA; p = 0.001) and time point (PERMANOVA; p = 0.002), while the interaction between the two were not significant (PERMANOVA, p = 0.785). Chlorophyll (ug cm\(^{-2}\) and ug cell\(^{-1}\)) and symbiont cell densities provided significant contributions to the variation seen in the phenotypes across all sites and time points (Fig. S1 and S2, Appendix 1).

**Plasticity Scores**

Here we used plasticity scores to indicate the change in the phenotypes between the baseline common garden and each site and time point. Plasticity score was not significantly different between sites (ANOVA; F\(_{2, 18}\), = 0.469 p = 0.633), nor was the
interaction between site and time point significant (ANOVA; \( F_{2, 18} = 1.406, p = 0.271 \)). However, the plasticity score was significant between time points (ANOVA; \( F_{1, 18} = 21.035, p < 0.0003 \)), where January 2020 (timepoint1) plasticity scores were significantly higher than November 2020 (timepoint4) plasticity scores. These results suggest that phenotypic plasticity varied across time and were greater at the January 2020 time point as compared to the November 2020 time point (Fig. 5). These results suggest that corals exhibit a more plastic or diverse phenotypic response to outplanting to various sites 3 months after transplantation (average plasticity value = 4.25) compared to 13 months after transplantation when the plasticity scores are about half (average plasticity value = 2.0) the January 2020 values.

**Discussion**

As human interventions become more widely adopted to address global declines of coral reefs, it is essential to investigate all possible mechanisms that drive restoration success and failures. While increasing biomass is important to restore ecological services for the reef communities, it is important to understand how restoration techniques like coral gardening or using coral nurseries will impact outplants. For example, understanding the impact of the environment of the nursery will be instrumental to predicting the outplants’ responses to future stressors and therefore dictate the restored coral community. [Morikawa and Palumbi 2019], demonstrated that temperature variability of a nursery site is a great predictor of bleaching resistance regardless of nursery genotype. In this case we saw the outplants thrived in conditions of low ambient nutrients, moderate light, variable pH and temperature whereas wild
corals seemed to thrive in high nutrient conditions, indicating that corals originating from this nursery were able to thrive in the most optimal growth settings while those found on the reef who acclimated to their environments.

*Initial differences between common garden and wild corals*

This study shows that nursery reared corals displayed differing phenotypic profiles compared to wild colonies naturally occurring on the backreef. Specifically across all 8 metrics, wild corals showed equal or higher values. This indicates that there was an environmental effect or inherent differences of nursery reared corals which should be further explored.

*Physiological plasticity occurs in space and time*

Outplanted corals acclimate to a new environment by inducing a more plastic phenotypic response and then revert back to their original nursery phenotype overtime. Phenotypic plasticity in this case serves as a short-term acclimation strategy but the long-term metabolic phenotype is dependent on the nursery environment.

These data provide insight to the biological complexities surrounding coral restoration techniques commonly used today on a commonly used restoration species (Boström-Einarsson et al. 2020). This work demonstrates the importance of nursery environments and elucidates how plasticity could be a short-term solution that drives high success in restoration programs, while the environment of the nursery these outplants are reared in can dictate the eventual metabolic phenotype. Coral gardening
and other restoration programs and methods have been hugely successful at increasing overall biomass of transplant to degraded reefs, which could be the result of programs primarily focusing on fast growing species that are poor competitors ecologically. Therefore, restoration outcomes may be successful in the short-term because of corals’ ability to acclimate to varied outplant environments, but lack the longevity. (Ware et al. 2020) indicates that coral outplant survivorship declines after 2 years, which is beyond the 18-month average outplant monitoring timeline of most conservation programs currently (Boström-Einarsson et al. 2020). I propose that this decline in survival of outplant to be the result of metabolic and environmental match or mismatch, in which after the acclimation period and corals revert back to their baseline phenotypes, these phenotypes are either compatible or at odds with the environmental challenges imposed by their outplant site. Thus leading to overall mortality over time. In fact, (Barott et al. 2021) corroborated this finding where the phenotype of outplants of Montipora capitata and Pocillopora acuta were more reflective of their origin site rather than the outplant site but those that came from a less challenging environment were more successful and more fecund because of their phenotype. Another important finding from (Barott et al. 2021), is that this response was consistent across widely disparate genera on the phylogenetic tree, suggesting that this could be a highly conserved phenomena across corals but future studies would need to investigate if this response is true outside of branching corals as well.
Conclusions

Nursery *Acropora pulchra* colonies displayed overall depressed metabolic phenotypes compared to wild *Acropora pulchra* colonies found in the backreef lagoon on the north shore of Mo’orea, French Polynesia. When outplanted to three sites spread across the north shore backreef, colonies within 4 fixed genetic lines displayed a highly plastic metabolic response 3 months post-transplantation whereas 13 months post-transplantation plasticity was significantly reduced, and their phenotype was more similar to their original nursery phenotypes. Plasticity in this case is the product of short-term acclimatization to a new environment but long-term phenotype is influenced more by environmental origin which was the nursery in this case.
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Figure 1. A) a map of the North Shore of the island Mo’orea, French Polynesia and the three outplant sites in the backreef lagoon chosen for this study. B) Colonies of known genotypes (n = 4) were tagged and sampled prior to and after transplantation to the three sites such that 1 colony of each genotype was present at each site. C) Environmental loggers deployed at each of the sites collecting light, pH, and temperature. These loggers were set at each of three transplant sites in October of 2019 and were offloaded and monitored throughout the experiment (Oct. 2019 - Nov. 2020).
Figure 2. Series of strip plots depicting the baseline physiological metrics measured for the nursery genotypes (n=10) and wild genotypes (n=3) at two sites in the backreef lagoon of Mo’orea, French Polynesia in October of 2019 (prior to transplantation of nursery corals). The metrics observed are biomass (AFDW, mg cm\(^{-2}\)), host protein concentration (ug cm\(^{-2}\)), respiration rates (Rd), symbiont density (cells cm\(^{-2}\)), maximum photosynthesis (Am), apparent quantum yield (AQY), total chlorophyll per fragment (ug of chl a and chl c cm\(^{-2}\)), and total chlorophyll per symbiont cell (ug of chl a and chl c/cell). The black dots and bars represent the mean and standard deviation values for each metric at each site. Significant posthoc values (Tukey’s HSD) are indicated by unique letters in each plot.
Figure 3. radar plot displaying the ranked comparisons of multiple environmental metrics, with the circles indicating increasing values starting at 0.25 in the center and increasing by 0.25 increments until the outermost circle which is 1.0.
Figure 4. A series of boxplots depicting the physiological metrics of the 4 genotypes (Genotypes 4, 6, 8, 15) prior to transplantation (October 2019) and subsequent measurements of these physiological metrics for the same 4 genotypes at each of the three sites they were transplanted to both 3 months later (January 2020) and 13 months later (November 2020). The metrics measured were the same as those in Figure 1 (AFDW, Host Protein, Respiration Rate, Symbiont Density, Am, AQY, Total Chlorophyll per Fragment, Total Chlorophyll per Symbiont Cell). Significant posthoc values are indicated by unique letters on top of each boxplot.
Figure 5. PCA of the distance metrics calculated from the averaged z-score of the combined univariate metrics for each genotype in both January and November of 2020. The centroid point, from which the distance metrics are calculated, represents the baseline phenotype which encompasses the 8 univariate metrics collected in October of 2019 from the same 4 genotypes in the nursery. The length and direction of the lines correlate to the overall differences in phenotype (using the same 8 univariate metrics) at each of the transplant sites at both January and November 2020 time points.
Figure 6. Reaction norm plot displaying the plasticity scores of each genotype at each site. The averaged values for the genotypes for the three sites (n=4) are represented by the bolded colored lines and represent the overall trend of plasticity score throughout the sampling time points.
APPENDICES

Supplementary Material for Manuscript 1

Phenotypic plasticity of the reef building coral Acropora pulchra under varied environmental conditions

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Methods

Calibration and Deployment of Environmental Sensors

Temperature

HOBO v2 U22 Temperature Loggers were manually cross calibrated at the outset of the experiment (October 2019) by tracking temperature of a bucket left outside in the sun at the GUMP research station in Mo’orea. Temperature was tracked using the Digital Traceable Thermometer to get instantaneous measurements to compare with launched and recording hobo loggers. Once the loggers were deemed acceptable (within ±0.01°C) they were deployed to each of the three sites attached to the cinderblock photographed in Figure 1 by multiple 8” zip ties. Each temperature logger was wrapped in white electrical tape to reduce marine biofouling on the actual sensor. The deployment periods were 10/28/2019 – 1/10/2020, 1/10/2020 – 03/06/2020, 03/06/2020 – 09/20/2020, 09/20/2020 – 11/20/2020. All three sites were sampled on separate days, and the respective site loggers were collected and brought back to GUMP research station and held in a free-flowing water table until they were all collected and read out on the same day.
**pH Logger**

HOBO MX pH Logger were manually calibrated using Bluetooth capabilities of the sensor and the HOBOmobile App on a smartphone. Sensor was calibrated by, first activating the bluetooth pairing between the smart device and the HOBO MX pH logger. The sync was completed by opening the HOBOmobile app. The correct device was selected by hitting the Devices icon and tapping the individual logger that appeared for the sync. Immediately the user was asked if they wanted to calibrate the sensor or the calibrate button is located at the bottom right of the screen. “Yes” was selected and then the two-point pH calibration option (using pH 7.00 and 4.01 standards) was selected. Using a squirt bottle the pH sensor was rinsed with deionized or distilled water prior to calibration and dried with a paper towel. The clear storage cap was then filled with Metrohm NBS buffer 7.00 before submerging the sensor in the solution, and secured onto the sensor by screwing on the cap. Once the top was secured, the 7.00 calibration was selected for the logger to take a reading. The reading then stabilized, and the ‘Confirm Buffer’ option was selected. This saved the calibration before calibrating for the 4.01 standard. Sensor was again rinsed and dried and the calibration process was repeated before finally selecting ‘Save Calibration’. Sensor was rinsed one last time with deionized or distilled water and then deployed. If deployment was not done immediately, logger was placed in pH electrode storage solution. During the March to September deployment, the sensors at sites 1 and 2 were damaged and were rendered unusable after (glass electrode was shattered).
Light Intensity

Odyssey Xtreem PAR Loggers were calibrated and deployed to capture light intensity at each site. Calibration required a two-step process requiring an Android device to connect to the logger via the Bluetooth capabilities of the logger via the Xtract App and then accessing the data through www.xpert.nz. Each logger was battery operated, so the loggers were filled with new AA batteries to activate the logger and connected to it using the Xtract App. The calibration was conducted on the Xpert website through the “Configure” tab. Here each logger, identified by its serial number, was labeled with a new name. Then the “Reset” option was selected on the Xpert website under the “Configure” tab, followed by selecting the “Submit” button. These loggers were then set in a bucket of seawater under a Prime™ 16HD Reef Aquarium Light set to a known light intensity alongside a LiCor to get discrete values alongside the loggers for 5-6 hours. The data was then off-loaded from the logger by re-opening the Xtract App and connecting to the loggers one at a time (connectivity based on proximity). The data was exported via .csv files sent to the Xpert account holder’s email by selecting the “Send Report” button under the “Data” tab. Using the “Calibration” tab in Xpert, select the “Test Duration” box and input the time of the calibration test for the loggers. The known light intensity value was then input to the “Reference Reading Average” box on the “Calibration” page and the submit option was selected. The calibrations were applied and the chart in Xpert was adjusted to the calibration. The loggers were recalibrated after each deployment period for the next. The logger at Site 1 unfortunately stopped working during the March – September
deployment (probably due to water seepage frying the circuit) and light was not obtained for this site past March.

**Table S1.** One way ANOVA (site) and post-hoc results for the 8 univariate metrics sampled on the nursery genotypes (n=10) and the wild type corals (n=3) at site 1 and 2 in October of 2019.

|                  | AFDW (mg/cm²) | Host Protein (µg/cm²) | Sym Dens (cells/cm²) | Total CHL (µg/cm²) | Total CHL (µg/cell) | Am   | AQY | Rd   |
|------------------|---------------|-----------------------|----------------------|--------------------|--------------------|------|-----|------|
| ANOVA p-value    | 0.00133       | 0.000195              | 0.05101              | 4.62E-06           | 0.000347           | 0.009624 | 0.1476 | 0.000372 |
| site1-nursery    | 0.0015647     | 0.000367              | 0.0522545            | 3.4E-06            | 0.000241           | 0.021492 | 0.183615 | 0.001182 |
| site2-nursery    | 0.0522102     | 0.006075              | 0.3596286            | 0.020365           | 0.340167           | 0.049243 | 0.386772 | 0.004064 |
| site2-site1      | 0.3091609     | 0.44027               | 0.6112591            | 0.002319           | 0.014027           | 0.930101 | 0.909858 | 0.849798 |
Table S2. Two-way ANOVA (site*timepoint) and post-hoc results for the 8 univariate metrics sampled on the outplanted genotypes at all three sites in January and November 2020 time points.

| ANOVA p-values | AFDW (mg/cm²) | Host Protein (ug/cm²) | Total CHL (ug/cm²) | Total CHL (ug/cell) | Sym Dens (cells/cm²) | Am | AQY | Rd |
|---------------|---------------|----------------------|-------------------|---------------------|---------------------|----|-----|----|
| site          | 0.233         | 0.000378             | 0.0296            | 0.07759             | 0.314               | 0.1046| 0.817 | 0.073785|
| timepoint     | 0.44          | 1.09E-06             | 0.01              | 0.00302             | 3.45E-05            | 0.0575| 0.11  | 0.000507|
| site*timepoint| 0.871         | 0.439673             | 0.1186            | 0.60453             | 0.354               | 0.9007| 0.701 | 0.982903|

| F-Values      |               |                      |                   |                     |                     |     |      |     |
|---------------|---------------|----------------------|-------------------|---------------------|---------------------|----|-----|----|
| site (F₂,₁₈)  | 1.579         | 12.6                 | 4.307             | 2.956               | 1.237               | 2.566| 0.204 | 3.023|
| timepoint (F₁,₁₈) | 0.622      | 51.66                | 8.282             | 11.73               | 29.845              | 4.119| 2.818 | 17.868|
| site*timepoint(F₁,₁₈) | 0.139 | 0.86                 | 2.406             | 0.518               | 1.102               | 0.105| 0.362 | 0.017|

| Post-hoc Results |               |                      |                   |                     |                     |     |      |     |
|------------------|---------------|----------------------|-------------------|---------------------|---------------------|----|-----|----|
| site2-site1      | 0.977289      | 0.0058392            | 0.917914          | 0.538229            | 0.9921067           | 0.79825| 0.958511| 0.410874|
| site3-site1      | 0.258756      | 0.4314068            | 0.078338          | 0.389702            | 0.4142604           | 0.288266| 0.802019| 0.495291|
| site3-site2      | 0.348809      | 0.000368             | 0.035965          | 0.063957            | 0.3529259           | 0.097766| 0.931618| 0.060244|

| tp_4-tp_1        | 0.440493      | 1.10E-06             | 0.010011          | 0.00302             | 3.45E-05            | 0.057458| 0.110479| 0.000507|

| site2:tp_1 - site1:tp_1 | 1    | 0.2000933 | 0.981411 | 0.992555 | 0.9753885 | 0.989645 | 0.998627 | 0.933512 |
| site3:tp_1 - site1:tp_1 | 0.754667 | 0.5214821 | 0.116186 | 0.577442 | 0.7750813 | 0.791213 | 1 | 0.934767 |
| site1:tp_4 - site1:tp_1 | 0.996082 | 0.0017462 | 0.32061 | 0.741136 | 0.0726608 | 0.834136 | 0.999591 | 0.173142 |
| site2:tp_4 - site1:tp_1 | 0.965801 | 0.3050966 | 0.265408 | 0.228057 | 0.0237251 | 0.945556 | 0.880842 | 0.612511 |
| site3:tp_4 - site1:tp_1 | 0.711841 | 0.0015878 | 0.131163 | 0.866159 | 0.0259561 | 0.321851 | 0.846617 | 0.046539 |
| site3:tp_3 - site2:tp_2 | 0.737322 | 0.0064587 | 0.030712 | 0.280423 | 0.3582783 | 0.443470 | 0.997876 | 0.458401 |
| site1:tp_4 - site2:tp_2 | 0.995511 | 0.0000136 | 0.102133 | 0.96081 | 0.0167035 | 0.493156 | 0.980435 | 0.029624 |
| site2:tp_4 - site2:tp_1 | 0.959781 | 0.0027177 | 0.080647 | 0.49897 | 0.0051281 | 0.677689 | 0.683224 | 0.168886 |
| site3:tp_4 - site2:tp_1 | 0.693713 | 0.0000125 | 0.035264 | 0.992273 | 0.0055459 | 0.119766 | 0.635127 | 0.006808 |
| site1:tp_4 - site3:tp_1 | 0.94645 | 0.0654489 | 0.988507 | 0.067261 | 0.5641126 | 0.999999 | 0.999785 | 0.618556 |
| site2:tp_4 - site3:tp_1 | 0.99245 | 0.9982805 | 0.959933 | 0.009326 | 0.270023 | 0.9985 | 0.895084 | 0.983276 |
| site3:tp_4 - site3:tp_1 | 1 | 0.0599929 | 1 | 0.108277 | 0.2856634 | 0.956429 | 0.86295 | 0.248409 |
| site2:tp_4 - site1:tp_4 | 0.999467 | 1.1398069 | 0.999995 | 0.922026 | 0.992399 | 0.999547 | 0.976798 | 0.937517 |
| site3:tp_4 - site1:tp_4 | 0.925679 | 0.993213 | 0.999833 | 0.9944452 | 0.935076 | 0.950556 | 0.978875 |
| site3:tp_4 - site2:tp_4 | 0.986559 | 0.1290349 | 0.998007 | 0.821087 | 1 | 0.81497 | 0.999999 | 0.596504 |
**Figure S1.** Principal component plot of centered and scaled values of 8 metrics for each colony at each site (n=4 Nursery; n=8 sites 1-3). Arrows are weighted by length and indicate the overall contribution of each metric to the spread of data. Site was significant in a two-way permutational multivariate analysis of variance of site and time point (PermANOVA, p-value = 0.001).

**Figure S2.** Principal component plot of centered and scaled values of 8 metrics for each colony at each site (n=4 Nursery; n=8 sites 1-3). Arrows are weighted by length and indicate the overall contribution of each metric to the spread of data. Site was significant in a two-way permutational multivariate analysis of variance of site and time point (PermANOVA, p-value = 0.001).