THE LARVAL SURVIVORSHIP AND POPULATION GENETICS OF *LEPTASTREA* IN THE SOUTHERN MARIANA ISLANDS

BY

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Abstract

Leptastrea purpurea, a hermaphroditic coral, thrives in temperatures temporarily exceeding 32°C, making it a model organism for studying stress tolerance. This thesis aimed to understand the population genetics of *Leptastrea purpurea* within the Mariana Island chain and provide insight on *L. purpurea*'s survivorship while facing climate change. For the population genetics study, *Leptastrea* colonies were collected from Guam, Rota, Tinian, and Saipan. Genotyping by Random Amplicon Sequencing Direct (GRAS-Di) data from 127 samples produced 270 million raw reads, with quality filtering retaining 115 samples and identifying 3864 single nucleotide polymorphisms (SNPs). Phylogenetic analyses revealed the presence of two different species segregated by habitat: one on the outer reef and the other on the reef flat. Morphological analyses supported these findings, showing significant differences in colony size and qualitative features such as corallite shape. Population genetic analyses indicated high clonality among Guam and between southern CNMI populations of the reef flat species and reduced genetic diversity in populations of the outer reef. I found that the reef flat species was composed of 4 genotypes due to clonality, while the outer reef species exhibited sexual reproduction.

Given its high stress tolerance, I also investigated the impact of elevated temperature on *Leptastrea purpurea* colonies and larvae. Adult colonies and larvae were exposed to elevated (32°C) compared to control (29°C) water temperatures for 5 weeks. Heat-treated colonies released higher abundances of smaller larvae with significantly better survivorship in elevated temperatures compared to control. *Leptastrea purpurea* thrive in extreme conditions and produce larvae better suited for higher temperatures with pre-exposure to elevated temperature. However, their high

clonality levels may prove fatal in the future due to lack of genetic recombination. This research provides valuable insights into the population dynamics and resilience of *Leptastrea* species, aiding predictions of future coral reef composition under climate change.

Key words: *Leptastrea*, climate change, clonality, population genetics, larvae

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Chapter 1: Introduction

Effects of Climate Change on Coral Reefs

Scleractinian corals fulfill essential ecological roles to coral reef communities (Wild et al. 2011). They provide shelter for various invertebrates and fishes, act as a barrier for wave action, and are economically beneficial for island communities through ecotourism (Burke et al. 2011). Because of anthropogenic disturbances, however, these fragile ecosystems have been degrading at alarming rates (Donner et al. 2005; Hughes et al. 2018, 2019).

After the Industrial Revolution in the 1800s, the excess use of non-renewable resources and meat production have caused an increase in CO_2 emissions into the atmosphere, leading to climate change and global warming (Petrovic et al. 2015; Manoli et al. 2016; Hoegh-Guldberg et al. 2017). These CO_2 emissions have resulted in increases in ocean acidity known as ocean acidification which can negatively affect calcifying organisms (Ellis et al. 2009; Chan and Connelly 2013; Duquette et al. 2017; Dickinson et al. 2021) and increases in global sea surface temperature (SST) (Hoegh-Guldberg et al. 2017). Periods of above average SST, known as marine heatwaves, have become more frequent and intense with the top ten highest global mean SSTs recorded in the last 10 years (Rohde 2024). By 2100, annual mean temperatures are projected to reach 34.7˚C in tropical regions within the Indo-Pacific (Descombes et al. 2014).

Increasing SST and marine heat waves have had negative impacts on marine ecosystems worldwide, particularly on coral reefs (Raymundo et al. 2019; Quigley et al. 2020). High temperatures can disrupt the symbiotic relationship between coral and microalgae, resulting in coral bleaching (Weiss 2008). Once bleaching occurs, algal recovery is possible, but without their symbionts, the coral will eventually succumb to starvation (Kleypas and Hoegh-Guldberg 2005).

Despite these devastating effects, some corals can tolerate heat stress thanks to frequent exposure to elevated temperature, symbiont species or community composition, bacterial

microbiome community, water flow, etc. (Barker 2018; Fifer et al. 2021; Rose et al. 2021). For example, in American Samoa, backreef pools can experience daily temperature fluctuations of 6°C (Bay and Palumbi 2014; Barker 2018; Thomas et al. 2018, Rose et al. 2021). Within these pools are thermo-sensitive corals (i.e. *Acropora*, *Pocillopora*) that have developed increased thermotolerance from daily short-term heat exposure and have filtered resistant genotypes into the population (Bay and Palumbi 2014; Barker 2018; Thomas et al. 2018; Rose et al. 2021). By developing this kind of resistance, coral species may be able to persist during future climate change.

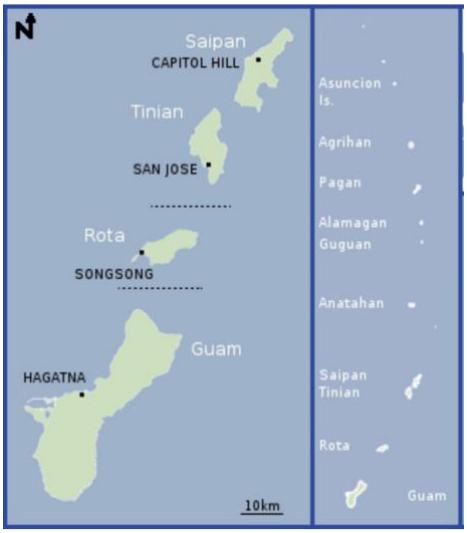


Figure 1. The location of Guam, Rota, and Saipan (A) within the Mariana Island Chain (B).

Effects of Climate Change on the Mariana Islands

The Mariana Island chain is 800 km long and is comprised of 15 islands; 14 islands form the Commonwealth of Northern Mariana Islands (CNMI) and the 15th being the U.S. territory of Guam (Cloud et al. 1956). Guam, the largest and southernmost, is 90 km southwest of Rota, and roughly 200 km southwest of Tinian and Saipan (Figure 1; Mortensen et al. 2008). Guam, Rota, Tinian, and Saipan make up the southern islands within the Mariana Island chain and are the focus of this study.

The windward-facing east sides of the CNMI and Guam are prone to increased wave action due to trade winds. The west side is sheltered from trade winds and therefore stays relatively calm year-long. Due to their shallow nature, inshore environments such as reef flats and lagoons are more prone to weather and temperature fluctuations, increased wave action, and light intensity

than deeper environments (De'ath and Fabricius 2010). The Guam coastline is surrounded by shallow lagoons and fringing reefs which host a variety of coral species that can experience temperature fluctuations, high wave energy, and other stressors in these environments. This habitat heterogeneity, even in small spatial scales with low structure, can lead to local adaptation (Selmoni et al. 2021).

Southern islands in the Marianas lie perpendicular within the westward-flowing Northern Equatorial Current (NEC) and north of the eastern-flowing Northern Equatorial Counter-Current (NECC) (Figure 2; Kendall and Poti 2015). Oceanographic models suggest that southern islands within the Marianas potentially supply larvae with longer pelagic larval duration (PLD) to northern islands within the archipelago, but larval transfer from north to south is unlikely due to their location in the NEC that promote northern flow. Larvae with short PLD (<20 days) are unlikely to leave their natal island and therefore self-seed (Kendall and Poti 2014). Guam is hypothesized to self-seed its own larvae from three eddies generated around the island due to its position in the NEC (Kendall and Poti 2014): one eddy is found in the north between Ritidian and Pati Point, another is a large oceanic eddy off the northwest, and finally in the south off Cocos Island (Wolanski et al. 2003). The presence of these eddies has caused local larval retention of *Acropora digitifera* (Davies et al. 2015), and likely other coral species. Currents within the Mariana Island chain can change in speed and direction during different seasons, weather patterns (i.e. typhoons, El Niño, La Niña), and global warming. As corals are reliant on ocean currents for seeding larvae to neighboring reefs, changes in current speed and direction can alter larval transportation pathways (Kendall and Poti 2015) and change larval distribution and connectivity between surrounding islands and populations.

In 2013, islands in the northern CNMI (Uracas, Maug, Asuncion, Pagan, Guguan, Sarigan, and Anathan) experienced a major bleaching event that caused 90% mortality in *Pocillopora* and *Acropora* species (Watch 2015). By mid 2014, *Pocillopora*, *Acropora*, *Astereopora*, and *Isopora* species in Maug had high bleaching and high mortality while severe, but less intense bleaching occurred in Saipan and Guam (Watch 2015). Between 2013-2017, Guam experienced marine heat waves, disease outbreaks, and abnormally low tides due to El Niño events (Raymundo et al. 2019). This resulted in bleaching events that decreased live coral coverage in reef flats along the west by 37% and seaward slopes island wide by about a third. Particularly devastated were east coast seaward slopes where live coral coverage decreased by 60%. Among the corals most affected by these bleaching events were acroporid coral species which had 36% decreased live cover island wide. However, the scleractinian coral *Leptastrea purpurea* remained almost unaffected by these disturbances (13±15% and 15±18% bleaching prevalence on seaward slopes and reef flat, respectively), and suffered almost 0% bleaching induced mortality (Raymundo et al. 2019).

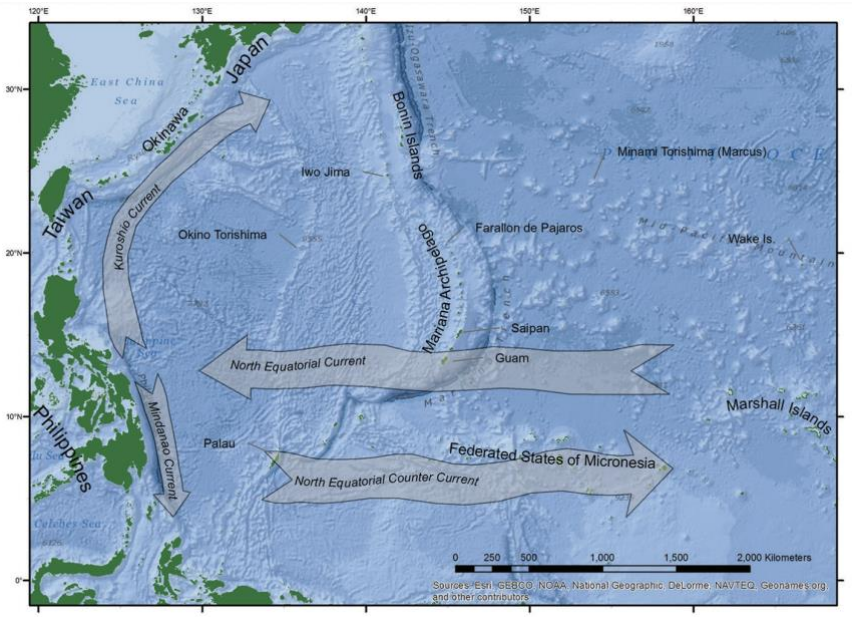


Figure 2. The location of Guam and the CNMI between the Northern Equatorial Current and the Northern Equatorial Counter Current.

The Stress Resistant Coral: *Leptastrea*

Leptastrea (Milne Edwards and Haime, 1849), is a genus of ahermatypic (i.e. non reef-building), encrusting scleractinian that are typically brown, yellow, or green (Veron 2000). There are currently eight recognized species of *Leptastrea,* which include *L. aequalis*, *L. inaequalis*, *L. bewickensis*, *L. bottae*, *L. transversa*, *L. gibbosa*, *L. magaloni*, and *L. purpurea* (Arrigoni et al. 2020). *Leptastrea* has a broad distribution throughout the Indo-Pacific and can be found from the Red Sea to Hawaii (Arrigoni et al. 2020).

My target species of interest was *Leptastrea purpurea*. *Leptastrea purpurea* has a high skeletal variation based on geographic location and environment and has been confused as other members within the genus due to its phenotypic plasticity (Todd 2008; Arrigoni et al. 2020). *L. purpurea*'s cryptic nature further complicates its identification due to its geographic distribution with other *Leptastrea* species, specifically *Leptastrea transversa*. The geographic ranges of *Leptastrea purpurea* and *L. transversa* have high overlap where both are found in the Red Sea and the Indian and Pacific Oceans (Arrigoni et al. 2020; Bahr et al. 2016) and within a variety of environments from reef flats down to 40m depth (Nietzer et al. 2018*). Leptastrea purpurea* and *L. transversa* are also both cerioid corals, meaning they have fused walls between neighboring irregularly shaped, hexagonal corallites (Veron 2000; Arrigoni et al. 2020). However, septa on *Leptastrea purpurea* are similar in size and tightly compact, whereas septa in *Leptastrea transversa* are not (Veron 2000).

Leptastrea purpurea has high resistance to increased SST, bleaching, and ocean acidification (Hughes et al. 2003; Bahr et al. 2016, 2018; Nietzer et al. 2018; Raymundo et al. 2019), and is also not normally targeted by predators, such as corallivorous *Drupella* snails (Morton et al. 2002). Finally, *Leptastrea purpurea* is commonly found on the reef flat where abiotic conditions can be extreme (high temperature fluctuation, air exposure at low tide, terrestrial

runoff, etc.). These harsh conditions might promote self-fertilization in some colonies, and it is probable that clonality is present within reef flat populations around Guam, Rota, Tinian, and Saipan.

In 2020, Galanto and Sartor et al. (2022) compared survivorship and settlement of *Leptastrea purpurea* larvae in ambient temperature when parental colonies were exposed to elevated or ambient conditions. They found colonies in higher temperature released more larvae than colonies in ambient sea water (Galanto and Sartor et al. 2022). These larvae were, however, significantly smaller compared to controls. In addition, settlement assays conducted at ambient temperature with both larvae produced in elevated and ambient SST conditions revealed no differences in settlement rates between larval types. Interestingly, recruits obtained from heated larvae tended to have higher survivorship than recruits from control larvae (Galanto and Sartor et al. 2022). However, what was not tested was how these larvae performed in elevated temperatures. By exposing coral larvae to higher temperatures, we can foreshadow how this scleractinian may develop, survive, and thrive in future climate change.

Sexual Reproduction and Population Connectivity

Members of Scleractinia exhibit two modes of sexual reproduction: broadcast spawning and brooding (Richmond 1987; Wilson and Harrison 1998; Davies et al. 2015). Scleractinian larvae have various pelagic larval durations (PLD) and can settle hours to months after release (Richmond 1989; Wilson and Harrison 1998; Davies et al. 2015). Larval behavior, habitat availability, ocean currents, and other abiotic factors also influence dispersal (Connolly and Baird 2010), which can determine genetic connectivity and structure between populations.

Leptastrea purpurea is described as a hermaphroditic brooding coral in Guam and releases fully developed larvae that settle within 72 hours (Nishikawa et al. 2003; Nietzer et al. 2018;

Galanto and Sartor et al. 2022). The distance these larvae travel from the parental colony is unknown, but since they have a reduced PLD, it is likely that they do not disperse very far and that populations exhibit small scale genetic structure.

Shorter dispersal distance and reduced PLD resulting in local retention and self-seeding (Connolly and Baird 2010) can cause isolation of populations and promote high genetic structure between and within populations (Davies et al. 2015; Oleksiak and Rajora 2020). For example, short PLD in the brooding coral *Pocillopora damicornis* causes genetic divergence between reef flat and reef slope, though the distance between these environments is <100m horizontally and ~5m vertically (van Oppen et al. 2018).

Ocean currents (Connolly and Baird 2010), large areas of open water (Underwood et al. 2017), SST, salinity (Wood et al. 2014; Oleksiak and Rajora 2020), and other physical barriers can play a major role in influencing genetic diversity and structure between populations. Simulated larval dispersal models show that larvae with longer PLD are capable of traveling up to 4000km but are often inhibited by ocean size and physical parameters (i.e. salinity, temperature; Wood et al. 2014). However, islands can act as stepping-stones for dispersal over large geographic ranges (Palumbi 2003; Wood et al. 2014; Davies et al. 2015). For example, Micronesia acts as a stepping-stone for *Acropora hyacinthus* and *A. digitifera* between the Coral Triangle and Central Pacific islands in an isolation-by-distance fashion, where connectivity decreases and structure increases farther from dispersal sites (Davies et al. 2015). Having these stepping-stones for larval dispersal allows for larval exchange over greater distance and can provide distant reefs with recruits.

Increased SST can disrupt larval transportation pathways by altering current direction (Toggweiler and Russell 2008; Kendall and Poti 2015), inhibit larval swimming and settlement abilities (Hughes et al. 2019), and increase larval mortality (Nozawa and Harrison 2007). This, in

turn, can cause reefs to become increasingly isolated from one another and decrease reef recovery from stress (Figueiredo et al. 2014). Understanding population dynamics of coral species can help determine what reefs supply larvae to others, and how global warming could affect larval dispersal.

Asexual Reproduction and Genetic Diversity

For organisms living in ecosystems that frequently experience extreme conditions (Foighil and Smith 1995; Lirman 2000; Bassim et al. 2002; Liu et al. 2006; Barrett 2015; Rios 2020), have few mates in their vicinity (Barrett 2015), or lack mobility (Foighil and Smith 1995), sexual reproduction can be a challenge. However, some organisms have overcome these challenges by reproducing asexually. Asexual reproduction is the production of offspring without the need of another individual, resulting in clonal offspring. Asexual reproduction can be achieved by fragmentation, fission (Lirman 2000), polyp budding, parthenogenesis (Combosch and Vollmer 2013; Eyal-Shaham et al. 2020), or self-fertilization (Smith and Potts 1987), and can also be influenced by the environment (Richmond 1997; Liu et al. 2006; Rios 2020). For example, acroporid corals have the ability to spawn, yet spawning events typically happen only once during specific times of the year. Instead, many acroporid species' primary form of reproduction is caused by daily wave action, which causes pieces of coral to break off (Highsmith 1982). This breakage leads to the formation of a new, fully clonal coral colony. Clonality has been understudied in the animal kingdom but has been well recorded in plants. In plant systems with limited mates, lack of mobility, and absence of pollinators, asexual reproduction allows for producing offspring without outside help from a mate (Liu et al. 2006).

Most coral species are hermaphroditic where a coral colony produces both sperm and eggs (Richmond and Hunter 1990; Richmond 1997). In many cases, eggs and sperm are bundled together in clusters and are released at the same time. These clusters break up in a delayed fashion

where sperm is released first followed by eggs to avoid self-fertilization (Richmond 1997). However, some species have been reported to self-fertilize (Richmond 1997; Brazeau et al. 1998; Combosch and Vollmer 2013; Eyal-Shaham et al. 2020). By having the ability to reproduce asexually, larval recruitment can still persist within a population.

There are both advantages and disadvantages to self-fertilization (Wells 1978). Self-fertilization can promote homozygosity of deleterious recessive alleles (Darwin 1876; Morton et al. 1956) which can lead to population collapse. It can also cause low genetic diversity within a population (Wells 1978), making it suboptimal in changing environments or disease outbreaks due to preventing future adaptations. However, self-fertilization can act as an advantageous reproductive strategy in environments where fertilization is limited due lack of mates or sperm availability, absence of pollinators, and in variable-temperature environments (Goodwillie et al. 2005; Liu et al. 2006; Sherman 2008; Eyal-Shaham et al. 2020; Torres et al. 2020). It can also maintain local adaptation within populations, adding to survivorship success in unfavorable conditions (Wells 1978; Ayre and Miller 2004; Adjeroud et al. 2014). The effects of high clonality on survival and resilience of coral populations remain understudied to this day.

Objectives and Hypotheses

Objectives

As SST continues to increase, it is important to understand how increased temperature affects coral reproductive output and larval fitness. The objective of the larval heat experiment was to determine larval survivorship and settlement in increased SST when *Leptastrea purpurea* larvae were produced in ambient or elevated conditions. Due to their high heat tolerance and brooding nature, along with increased reproductive output, we can hypothesize that *Leptastrea purpurea*

and their larvae will have a higher settlement and survivorship advantage in predicted future elevated temperatures.

Studies on coral population genetics within the Marianas are limited and focus on populations between Guam and Saipan. These studies also focus on thermosensitive *Acropora* species (Boulay et al. 2014; Rios 2020), which sexually reproduce by broadcast spawning and asexually through fragmentation. As of now, there are no inter- or intra-island population genetics studies within the Marianas of brooding corals, and in particular, *Leptastrea.* It is important to study population genetics of *Leptastrea purpurea* because it will inform us on the genetic diversity and structure of this species within Guam and the CNMI and provide insight on where larvae migrate within this archipelago. This type of data is useful in the context of climate change to help predict the future of brooding coral populations in the Mariana Islands.

My study aimed to investigate the survivorship and settlement of *Leptastrea purpurea* in elevated temperature by tank experiments, along with the population genetics of *Leptastrea* around Guam, Rota, Tinian, and Saipan *in situ*. By running these tank experiments, we tested whether preconditioning has a positive influence on survivorship and settlement of *L. purpurea* larvae in elevated temperature, and therefore act as a predictor for how this species will fare in future climate change. Focusing on *Leptastrea purpurea*'s genetic diversity and distribution between individuals within populations can provide insights on how far *L. purpurea* larvae disperse from their parental colony (small-scale). Looking at the genetic structure and connectivity among populations on Guam and islands in the CNMI can provide insight on how this species, and possibly other brooding corals, are connected throughout this region (large-scale).

Hypotheses

Larval Survivorship and Settlement

H_0: *Leptastrea purpurea* larvae produced from parental colonies exposed to elevated temperature will have no difference in survivorship and settlement when raised in elevated temperature compared to larvae from parental colonies exposed to ambient temperature.

H_1: *Leptastrea purpurea* larvae produced from parental colonies exposed to elevated temperature will have higher survivorship and settlement when raised in elevated temperature compared to larvae from parental colonies exposed to ambient temperature.

H_2: Larvae from parental colonies exposed to elevated temperature will have lower survivorship and settlement when raised in elevated temperature compared to larvae from parental colonies exposed to ambient temperature.

Population Genetics

H_0: *Leptastrea purpurea* in Guam and the southern CNMI is composed of one large population, with high genetic diversity, low clonality, and no genetic structure.

H_3: *Leptastrea purpurea* in Guam and the southern CNMI is composed of multiple genetically distinct populations, with various to high levels of clonality, each exhibiting small-scale genetic structure within their site.

Chapter 2: Methods

Larval Survivorship and Settlement

Colony collection

Following Galanto and Sartor et al. (2022), 60 *Leptastrea purpurea* colonies (~8-10cm) were collected from Luminao Reef Flat (13˚27'N 144˚ 38'E). Whole colonies were collected to reduce coral damage and limit impact on larval production. Each colony was stored in individual plastic bags with sea water until arrival at the University of Guam Marine Laboratory where they were placed in a holding tank at ambient temperature for one week to allow them to recover from being handled prior to the experiment.

Experimental design

To test the survivorship and settlement of coral larvae, two treatments deployed across two flow-through water tables (dimensions 2.4m x 1.2m x 0.3m) – one with ambient seawater (28.7˚C ± 2.2˚C) and one with elevated temperature seawater (32.2˚C ± 1.0˚C) – were used over the course of a month (Figure 3). Both tables were covered by shade cloth to reduce and homogenize light levels, and two circulation pumps were placed in each table at opposite corners to enhance water flow and homogenize water temperature. In the elevated temperature table, two heaters (Finnex HC-810M) were set to 34°C and placed on opposite sides of the table for temperature uniformity. A thermometer (Aquaneat Digital Thermometer) was placed in the middle of each side of both water tables and temperature readings were done twice daily: once in the morning (10:00) and once in the afternoon (16:00).

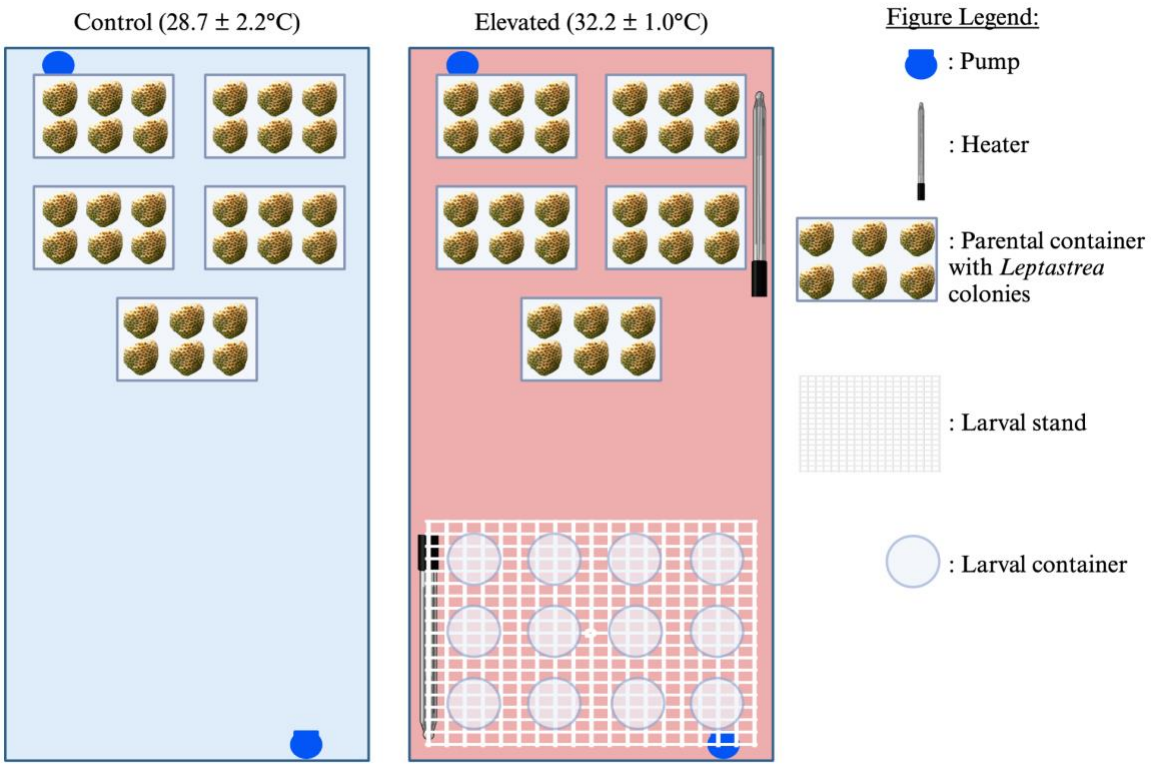


Figure 3. Schematic of experimental set-up with a figure legend. Two flow-through tables held 5 parental containers with 6 *Leptastrea purpurea* colonies each, totaling 30 colonies per treatment. A larval stand was placed in the heated table to expose larvae in larval settlement containers to the elevated temperature.

Ten 15 L plastic containers each with eight 3.2cm holes covered with 30- and 64-micron mesh were used to hold adult *Leptastrea purpurea* colonies for larval collection and divided equally between the water tables (5 containers per treatment; Figure 3). These containers allowed water flow and oxygen exchange while preventing larval escape. Six *L. purpurea* colonies were randomly assigned to each container within the 28.7°C or 32.2°C flow through tables, totaling to thirty colonies per treatment. Colonies were fed brine shrimp nauplii every three days in the late afternoon at 18:00. During the feeding, water flow was temporarily turned off to prevent brine shrimp from escaping and was turned back on in the morning.

Bleaching prevalence in adult colonies

To observe whether coral colonies in both treatments were paling or bleaching, each coral colony was assessed for color change with a CoralWatch Coral Health card for a month every 3 days. The CoralWatch Coral Health card color scale ranges from 1-6, where 1 indicates fully bleached and 6 was fully pigmented colony. A Kruskal-Wallis test using kruskal.test function in R was used to compare colony color between treatments throughout the study and at each observation.

Larval collection

Larvae and recruits were collected from the colony containers every three days, totaling 11 collection timepoints. Coral colonies were removed from the container, and each container was lifted out of the water tables one by one to allow water to drain through the eight 30- and 64-micron mesh covered holes until only about 200mL of water remained. The remaining content of each container was poured into an assigned 250mL glass jar. Containers were then sprayed down with sea water and emptied into their specified jar to ensure all larvae were collected. As a final precaution, each emptied container was inspected under fluorescent blue light at 460nm (GoBe Nightsea Light & Motion) to ensure no larvae were left behind. Containers were then wiped down and rinsed under tap water to remove waste and algal build-up, along with preventing larval count bias. Containers were placed back into the flow through tables and colonies were returned to their assigned container until the next larval collection.

Larval measurements and settlement assays

All larvae collected in each container from both treatments were counted and measured lengthwise using a built-in ruler in the left eyepiece of a dissecting scope. A Shapiro-Wilks test using the shapiro.test() function in R was used to determine normality for total larvae released between the heated and non-heated conditions overall and total larvae released between conditions at each collection. A linear mixed effects model was used to find if the total number of larvae released between treatments differed over the month-long study using the R packages lme4 (Bates et al. 2014) and nlme (Pinheiro and Bates 2000). A Welch T-Test using the t.test function in R was used to determine if there were differences in both larvae released at each collection time point and for overall larval size differences. A nested ANOVA using the function aov() in R was used to determine significance in total larval size between control and heated conditions overall and larval sizes between conditions at each collection timepoint. Graphs were visualized using ggplot2 (Wickham 2016).

Six larvae from each container per treatment were randomly chosen and distributed between three 1100mL containers (Pac-it Fresh© Food Storage Containers) for survivorship and settlement analyses totaling ten larvae per settlement container and thirty total larvae per collection. The bottoms of each settlement container had previously been cut and sanded, then completely covered with a 30-micron mesh affixed with thermoplastic glue to allow oxygen exchange and water flow within the container. Each container was placed in the 32°C water table and held chips of the crustose coralline alga *Hydrolithon reinboldii* to help trigger larvae settlement and metamorphosis (Nietzer et al. 2018; Moeller et al. 2019; Petersen et al. 2021; Galanto and Sartor et al. 2022). After 24 hours, containers were removed from the table and placed under a dissecting scope under fluorescent blue light (GoBe Nightsea Light & Motion) to assess and

quantify swimming, dead, and settled larvae. These observations began at collection 7 due to complications caused by the initial set up. Observations were repeated every day until all larvae had settled. Once all larvae settled, observations were conducted every other day for an additional 20 days.

Total survivorship and temperature influence on survivorship and settlement

A Kaplan-Meier curve was used to compare combined larval and recruit survivorship (referred to as "total survivorship" hereafter) for each treatment using the *survival* and *survminer* packages in R (Therneau and Grambsch 2000, Therneau and Lumley 2015). A Shapiro-Wilks test was used to determine normality of daily settlers. A Kruskal-Wallis test using the dplyr package in R was used to determine differences in settlement rate (Wickham et al. 2017) at 72 hours after release and overall. A two-way ANOVA using the aov() package in R was used to estimate the influence of temperature on larval settlement rate (R Core Team 2013; Galanto and Sartor et al. 2022).

Population Genetics

Sample collection

Samples were collected at five sites in Guam: four of which were on the reef flat and the fifth in the outer reef. The reef flat sites included Urunao in the north, Pago Bay in the east, the east side of Cocos Lagoon in the south, and Luminao Reef Flat in the west. The outer reef collection site was done in Luminao Outer Reef in the west. One collection on the reef flat was done on the northwest side for both Saipan and Rota at Pau Pau Beach and Teteto Beach, respectively. Collections were done in two outer reef sites in Tinian, which were First Cove and

Cute Beach. These two sites are 1.84km apart and were combined into one population due to small sample sizes at both sites (Figure 4, Table 1).

For collections done on Guam's reef flat, a 200m transect line approximately 200m offshore was laid parallel to the shoreline at each site. Colony fragments were sampled every 10m along the transect to test for small scale genetic structure within sites. GPS coordinates were recorded at the beginning and end of the transect, and for coral colonies collected greater than a meter off the transect line. Due to time and logistical constraints, along with the sparsity of colonies, *Leptastrea* samples in Saipan, Tinian, and Rota were collected haphazardly. All colonies sampled were photographed with a 3.5cm scale bar using an OLYMPUS Tough TG-6 camera and collected with a hammer and chisel. Colony fragments were placed in a 50mL falcon tube with sea water for the duration of the collection. Upon arrival at the University of Guam Marine Laboratory (UOGML), coral skeletons were broken up with a plier and hammer to obtain coral tissue and stored in 5mL tubes with 95% ethanol in a -20˚C freezer until DNA extraction.

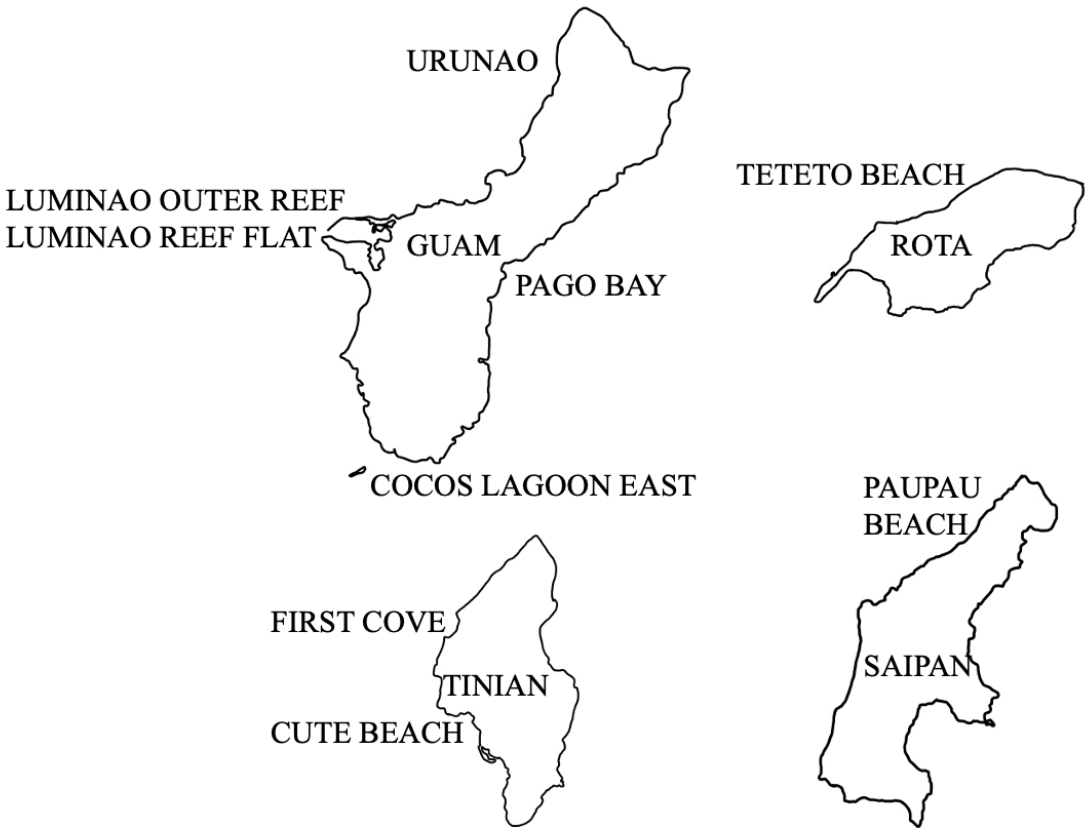


Figure 4. Islands and locations where *Leptastrea* collections were conducted (clockwise from top left: Guam, Rota, Tinian, and Saipan).

Table 1. Islands, site names, total samples collected, and final counts after quality filtration.

ISLAND	SITE	TOTAL	GRAS-Di	RETAINED AFTER FILTRATION
GUAM	URUNAO	20	18	10
	PAGO BAY	20	18	18
	COCOS LAGOON EAST	19	14	13
	LUMINAO REEF FLAT	19	16	16
	LUMINAO OUTER REEF	20	13	12
ROTA	TETETO BEACH	20	12	12
TINIAN	FIRST COVE	4	3	3
	CUTE BEACH	14	14	13
SAIPAN	PAU PAU BEACH	20	19	18
TOTAL:		**156**	**127**	**115**

DNA extraction and COI barcoding

DNA was extracted using Qiagen Blood and Tissue Kit™, and single stranded RNA was removed using 10uL RNAse A after digestion. Double stranded DNA was quantified using a Qubit Fluorometer (Invitrogen) and DNA fragmentation levels were checked through agarose gel electrophoresis.

As cryptic and closely related species are morphologically difficult to differentiate, a fragment of the mitochondrial cytochrome c oxidase subunit I marker (COI) was used to distinguish *Leptastrea* species with morphological ambiguity. The universal primers LCO1490 (*forward*) and HCO2198 (*reverse*) (Folmer et al. 1994) were used for PCR amplification of the COI fragments following Willman and Macken (2012). In brief, each PCR reaction contained 24µL of mastermix (16.25mL MiliQ water, 5mL buffer (Kappa 5X HiFi Fid with Mg), 0.5mL $MgCl_2^-$ (Kappa 25mM), 0.75mL dNTP (Kappa 10mM), 0.5mL LCO1490 and 0.5mL HCO2198 primers, and 0.5mL Taq (Kappa) plus 1µL of DNA. PCR cycling parameters had an initial denaturation step at 94°C for 2 minutes, followed by 30 cycles of denaturation at 94°C for 45 seconds, primer annealing at 50°C for 45 seconds, and extension at 72°C for 90 seconds. The last step was a final extension at 72°C for 5 minutes.

PCR products were sequenced by Epoch Life Sciences. Additional sequences of *Leptastrea purpurea* and other *Leptastrea* species from Arrigoni et al. (2020) were aligned against our sequences to determine if obscure specimens were *Leptastrea purpurea*. All sequences (Genbank + Guam) were aligned with MUSCLE and used to make a phylogenetic tree using a Maximum Likelihood inference.

GRAS-Di sequencing and data filtering

Genotyping by Random Amplicon Sequencing, Direct (GRAS-Di) was used to genotype samples that contained less than 10% RNA. GRAS-Di is a recently developed PCR based genotype-by-sequencing (GBS) technique that can be used to genotype organisms without the need for a reference genome, making it an ideal tool for non-model organisms (Hosoya et al. 2019; Miki et al. 2020). Other techniques used for population genetic analyses such as Restriction Site Associated DNA sequencing (RADseq) and Double-Digest RADseq (ddRADseq) use restriction enzymes that digest DNA into fragments (Davey and Blaxter 2010). However, RADseq and ddRADseq require high-quality, non-degraded DNA due to using restriction enzymes which break down DNA into smaller strands. GRAS-Di, on the other hand, does not break down DNA but uses random primers that can amplify parts of the genome for both high- and low-quality DNA (Enoki et al. 2018). *Leptastrea* DNA extractions were plated and sent to the UC Davis Technologies and Expression Analysis Core for GRAS-Di. Libraries were prepared and pair-end sequenced at UC Davis using an Illumina NovaSeq following Nomura et al. (2022).

STACKS pipeline v2.2 (Catchen et al. 2013) was used to filter, assemble, and identify loci. Briefly, raw reads were demultiplexed and cleaned by removing adaptors, discarding low quality reads, filtering reads with less than 60 bp, and removing samples that had <80% shared loci. Parameters m, M, and n were optimized following Paris et al. (2017). We ran the denovomap.pl script increasing the parameters' values in each new run and compared the number of final loci shared among 80% of the samples (r80 rule described in Paris et al. 2017). SNPs data were used for species identification alongside estimating clonality levels, population connectivity and structure between Guam, Rota, Tinian, and Saipan (Andrews et al. 2016; Arrigoni et al. 2020).

Subsequently, the STACKS pipeline was rerun as described above but separated outer reef and reef flat samples to obtain two independent datasets.

Species identification using phylogeny and morphology

A phylogenetic tree was constructed with the SNP data provided by STACKs by converting genepop files generated from STACKs into Phylip format using PGDSpider2.1.1.5. Phylip files were run through IQTREE 2 (Minh et al. 2020) using the GTR model with the ascertainment bias correction (GTR+ASC) for SNPs and using 1000 bootstraps to determine Maximum Likelihood Inference. Phylogenetic trees were visualized using FigTree v1.4.4 (Rambaut 2018).

19 *in situ* photographs with a scale bar of both reef flat and outer reef colonies were used to measure colony size, and 27 reef flat and 14 outer reef coral fragments were used to quantify septa. Fragments were soaked in bleach for 18 hours to preserve the coral skeleton but remove tissue. Fragments were washed, dried, and placed on a piece of polymer clay in a petri dish. The petri dish was put under a dissecting scope and photographed using the microscope setting on an OLYMPUS Tough TG-6 camera. The average number of septa was counted from 1 to 3 intact corallites from each coral fragment and used to compare differences between clusters using a Welch's t-test. Our phylogenetic and morphological analyses clearly identify two genetically distinct species that were then analyzed separately in the following steps.

Identity By State, relatedness, and clonality

Identity By State (IBS) and Dissimilarity matrices were calculated to determine differences in genotypes between individuals using SNPRelate in R (Zheng et al. 2012) and visualized as a dendrogram using ggplot2 (Wickham 2016). A threshold of 0.08 was determined by hierarchical

clustering by IBS distances which helped determine clonal vs. sexually reproducing individuals at IBS distances of 0.12-0.36 and 0.02-0.08, respectively. Relatedness between all individuals was estimated using VCFtools (--relatedness2) (Manichaikul et al. 2010) and visualized using pheatmap in R (Kolde and Kolde 2015). VCFtools (--het) was used on the full SNP dataset to determine if clonality was a result of parthenogenesis or selfing by comparing the proportion of heterozygous sites on the reef flat to the outer reef.

Clonal diversity and distribution on reef flat sites

We used GenoDive v. 3.06 (Meirmans 2020) to calculate clonal diversity for the reef flat populations. Following Meirmans et al. (2004), clones were assigned using a Stepwise Mutation Model (SMM), resulting in a threshold of 386 which yielded 4 clones. A corrected Nei's diversity index was used to test the probability of observed clonal diversity under random mating with 1000 permutations.

Distribution of clonal lineages across Guam, Rota, and Saipan was calculated by dividing the number of individuals found within each clonal lineage to the total number of individuals collected at each island, then visualized by pie charts using the pie() function in R. This was repeated for reef flat sites in Guam, and a runs test was used to determine if clonality within Guam sites was randomly distributed across sites using extraDistr in R (Wolodzko and Wolodzko 2020).

Genetic distance, diversity and population genetics of outer reef sites

Pairwise F_{st} with 1000 bootstrap replicates was used to look at the genetic distance between outer reef sites using the StAMPP package (Pembleton et al. 2013). Genetic diversity in the outer reef populations was calculated using Genodive v. 3.06 (Meirmans 2020). DartR (Gruber et al. 2018) and diveRsity (Keenan et al. 2013) packages in R were used to create a principal component

analysis (PCA) to identify structure between Guam and Tinian (McVean 2009), followed by a Discriminant Analysis of Principal Components (DAPC) using the adegenet package in R (Jombart et al. 2010) to further analyze genetic structure between islands. STRUCTURE (Pritchard et al. 2000) was used to determine admixture and population structure between the outer reef sites. STRUCTURE results were input into structureHarvester (Earl and vonHoldt 2012) to determine the number of K genetic clusters present using the Evanno deltaK approach followed by CLUMPAK (Kopelman et al. 2015) for graphical representation of the results.

Chapter 3: Results

Larval Survivorship and Settlement

Parental colony color differs between treatments

Twenty-four hours after starting the experiment, *L. purpurea* colonies in the elevated temperature treatment already showed signs of paling (Figure 5). Overall coral colony coloration was significantly different between the two treatments over the course of the experiment ($p = 0.013$; Figure 5).

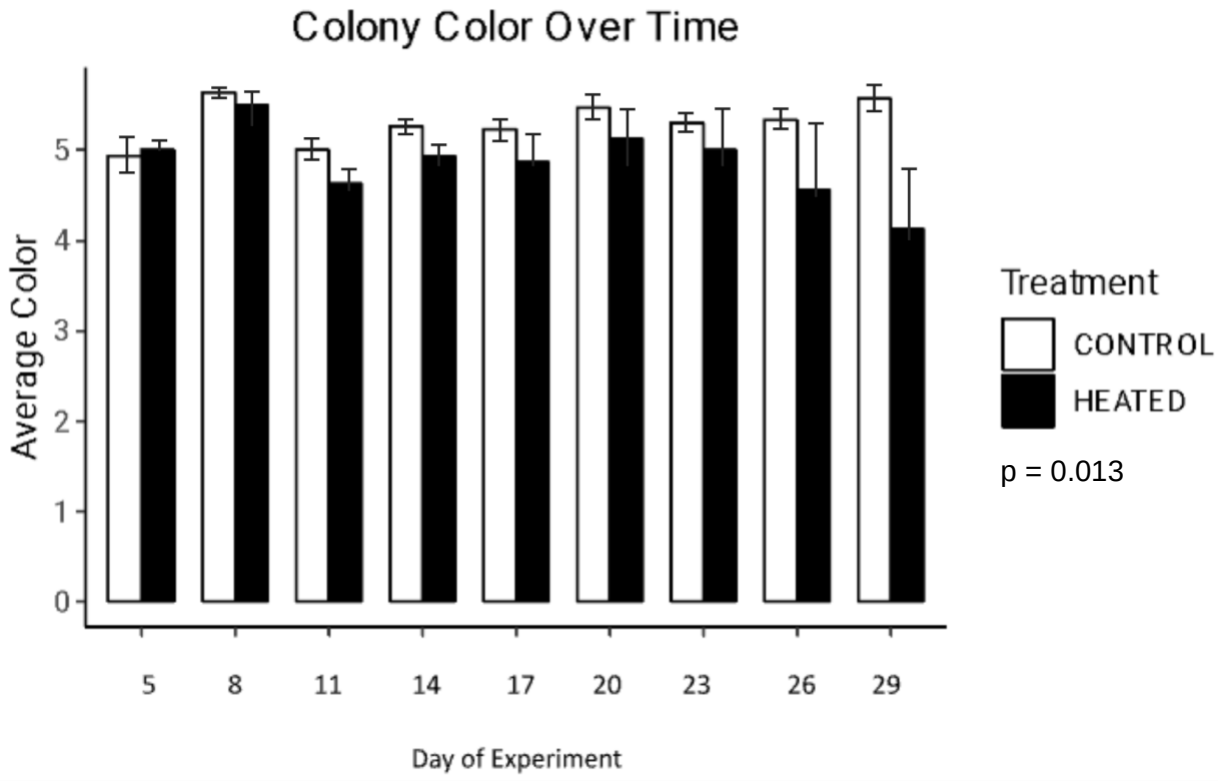


Figure 5. Comparison of parental colony color between the control and heated tables over the duration of the experiment. Colony color was found to differ between treatments ($p = 0.013$) over the month-long period.

Temperature influences the abundance of larvae released and their size

Overall, heat-treated *L. purpurea* colonies released significantly higher abundances of smaller sized larvae than colonies in control conditions over the month-long period of the experiment (p-value = 0.043; Table 2). Significant differences in larvae released between control and heated colonies were also seen on collections 2, 3, 6, and 7 (p-value = 0.01 - 0.05; Table 2). Insignificant differences in larval release were seen for all other collections (p-value = 0.17 – 0.93; Table 2). In collection 8, control colonies released higher numbers of larvae than heat-treated, likely due to decreased larval production due to colony die-off in one of the heated tanks.

Table 2. P-values comparing total larvae released at each treatment between control and heated tables. Collection 2, 3, 6, and 7 had significantly different larvae released, along with total larvae released at each collection.

COLLECTION	DAY OF EXPERIMENT	HEAT LARVAE TOTAL	CONTROL LARVAE TOTAL	P-VALUE
2	**7**	**250**	**132**	**0.01**
3	**10**	**365**	**142**	**0.02**
4	13	269	188	0.28
5	16	265	189	0.28
6	**19**	**337**	**163**	**0.05**
7	**22**	**269**	**140**	**0.04**
8	25	176	274	0.17
9	28	240	100	0.11
10	31	185	190	0.93
11	34	100	78	0.61
Total		2456	1596	**0.043**

Overall, larvae released by heat-treated colonies were significantly smaller (0.37mm on average) than the controls (0.48mm on average) (p-value = 0.003; Figure 6A). Significantly smaller larvae from heated colonies were observed on collections 2, 3, 6, and 7 (Figure 6B). Higher variation in size was observed in heated larvae on collections 4, 5, 8, 9, 10, and 11 likely contributing to non-significant size differences between treatments.

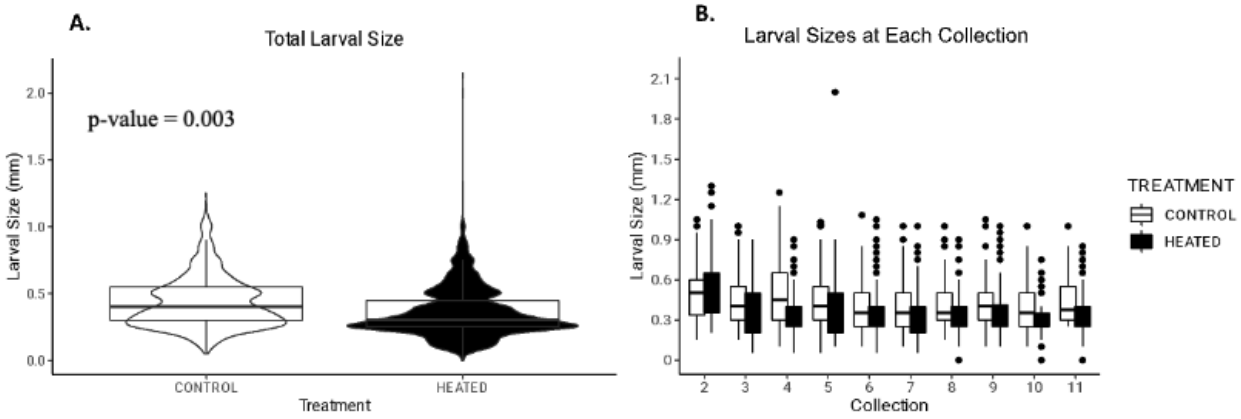


Figure 6. Total average larval size over the course of the experiments (A) and larval sizes from each collection (B). Heat-treated colonies (heated) released significantly smaller larvae than control (p = 0.003; A.

Heat-treated larvae have better total survivorship in elevated temperature

Total survivorship of larvae and recruits was the same between each collection time point within each treatment (p = 0.68 and p = 0.79, respectively; Figure 7A and 7B). When placed in elevated temperature, total survivorship of larvae released by heat-treated colonies was significantly higher than that of the control larvae overall (p = 0.001; Figure 7C).

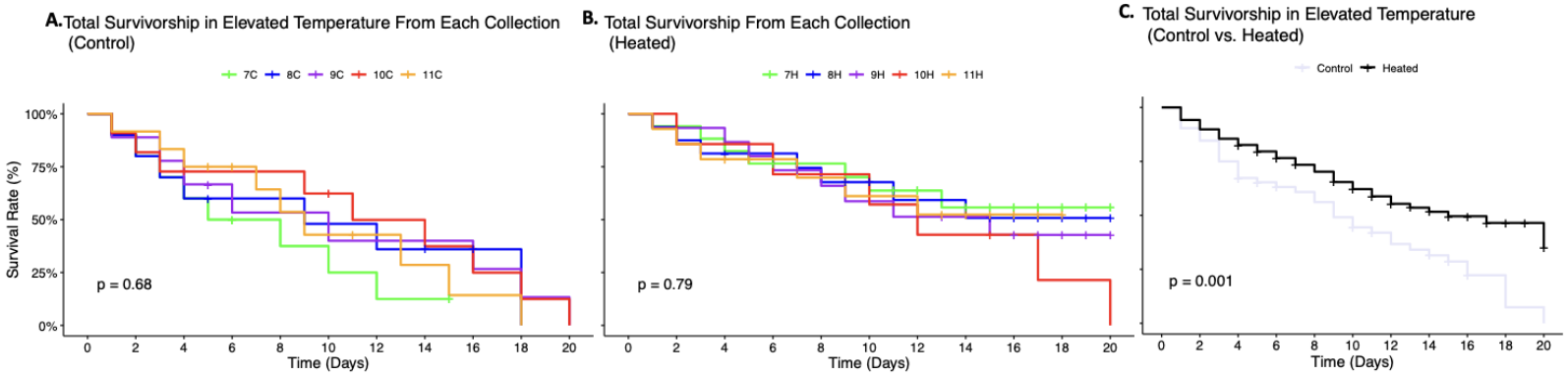


Figure 7. Total survivorship at each collection of control (A), heated (B), and total survivorship of control and heated (C) in elevated temperature. Control and heated total survivorship at each collection didn't differ, regardless of when larvae were collected, but were significantly different when compared amongst groups throughout the month.

Larval settlement is affected by temperature

Larvae released from control- and heat-treated colonies all had low but similar settlement rates after 72 hours (50% for controls and 45% for treated samples, p-value = 0.302; Figure 8). However, overall settlement was significantly different between the two treatments where heat treated larvae had 75% settlement rate (p-value = 0.005; Figure 8), likely due to the lack of replicates for the heat-treated larvae over time.

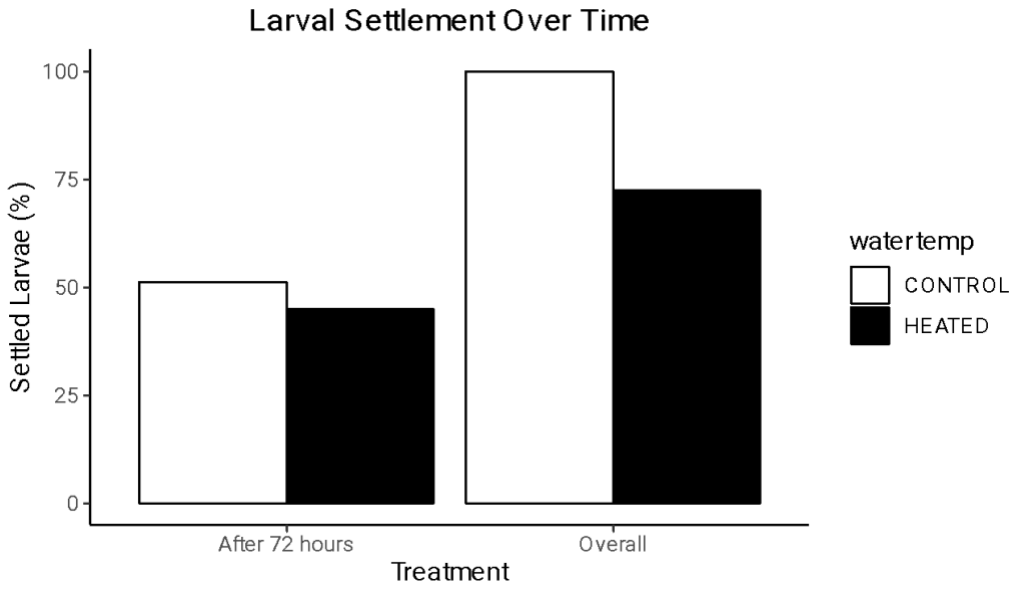


Figure 8. Percent of larval settlement from control and heat-treated colonies after 72 hours and overall settlement rates between treatments. Settlement between the two groups was the same after 72 hours but differed overall (p-value = 0.302 and p-value = 0.005, respectively).

Population Genetics

COI barcoding results

After sequence analyses of COI PCR products of a subset of *Leptastrea* samples (n = 13; 5 for outer reef, 8 for reef flat) with morphological ambiguity, all sequences blasted to *Leptastrea purpurea* from GenBank with high percent similarity (> 77%). COI sequences were also blasted against nearly complete mitochondrial *L. purpurea* (n = 33), *L. transversa* (n = 19), *L. inaequalis* (n = 8), *L. gibbosa* (n = 4), and *L. bottae* (n = 6) from Arrigoni et al. 2020. These sequences clustered with *L. purpurea* on a Maximum Likelihood tree (Supplemental Figure 1), indicating that all samples appeared to be *Leptastrea purpurea*. However, due to its highly conserved nature, the COI region in coral is not suitable to use for species differentiation (Arrigoni 2017). Therefore,

these PCR results were discarded and only data generated from GRAS-Di samples were used for this study.

Over-all genetic results

A total of 156 *Leptastrea* samples were collected from Guam, Rota, Tinian, and Saipan. After DNA extractions, 127 samples contained less than 10% RNA and were sent to UC Davis for GRAS-Di library preparation and sequencing and generated 270 million raw reads. After quality filtering, samples with >80% missing loci were removed, resulting in a final dataset of 115 individuals with 1,800,000 ± 1,280,000 reads. SNP calling and filtration steps in STACKs resulted in the parameters M=7, r=0.8, n=6, m=3 (Paris et al. 2017), resulting in 6553 loci, and 3864 variant sites or SNPs.

Phylogenetic analyses with SNPs

Phylogenetic analyses using IQTree produced a tree composed of two main branches supported by high bootstrap values (99.9-100) (Figure 9). Interestingly, the two branches provided a distinct separation of *Leptastrea* collected from the reef flat and outer reef sites. The reef flat branch was broken up into two main clades with two subclades each. One of the two main reef flat clades consisted of a subclade with 75% of all Guam samples and the other subclade had only three Guam samples. The second main reef flat clade had one subclade consisting of six Guam samples and the other containing 75% of all Rota samples and 58% from Saipan. The outer reef branch split into two main clades with both Luminao Outer Reef and Tinian interspersed between them. This phylogenetic structure suggests the presence of at least two distinct species: one that occupies the reef flat and another that is solely found in the outer reef (Figure 9).

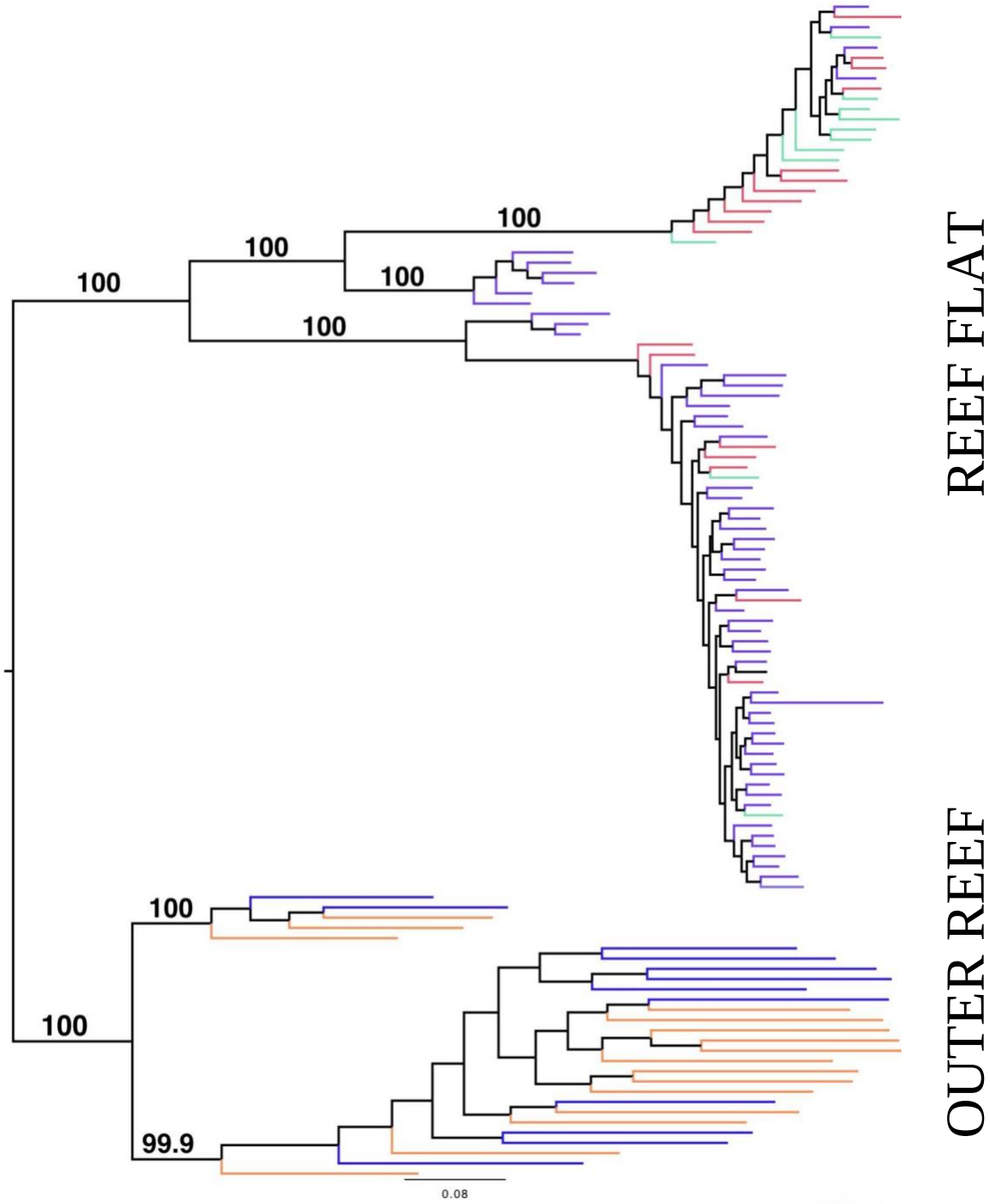


Figure 9. Maximum likelihood tree generated using IQtree. Outer reef and reef flat *Leptastrea* formed their own distinct clusters. Luminao Outer Reef (purple) and Tinian (orange) clustered in one branch (bottom), while Guam reef flat sites (Urunao, Pago Bay, Cocos Lagoon East, and Luminao Reef Flat), Rota (teal), and Saipan (red) clustered in the other branch (top).

Clonality, clonal proportions, and diversity

Like the phylogenetic tree, the IBS and dissimilarity matrix resulted in a dendrogram that separated *Leptastrea* from the outer reef and reef flat into two distinct branches: 28 samples from the outer reef made up one branch, and 87 samples from the reef flat made up the other branch (Figure 10). All reef flat individuals (n = 87) fell below the 0.08 cutoff line below which is considered clonal (IBS value = 0.05 - 0.06), and all outer reef samples (n = 28) were above the cutoff line and considered sexually reproducing (IBS value = 0.12 - 0.19; Figure 10).

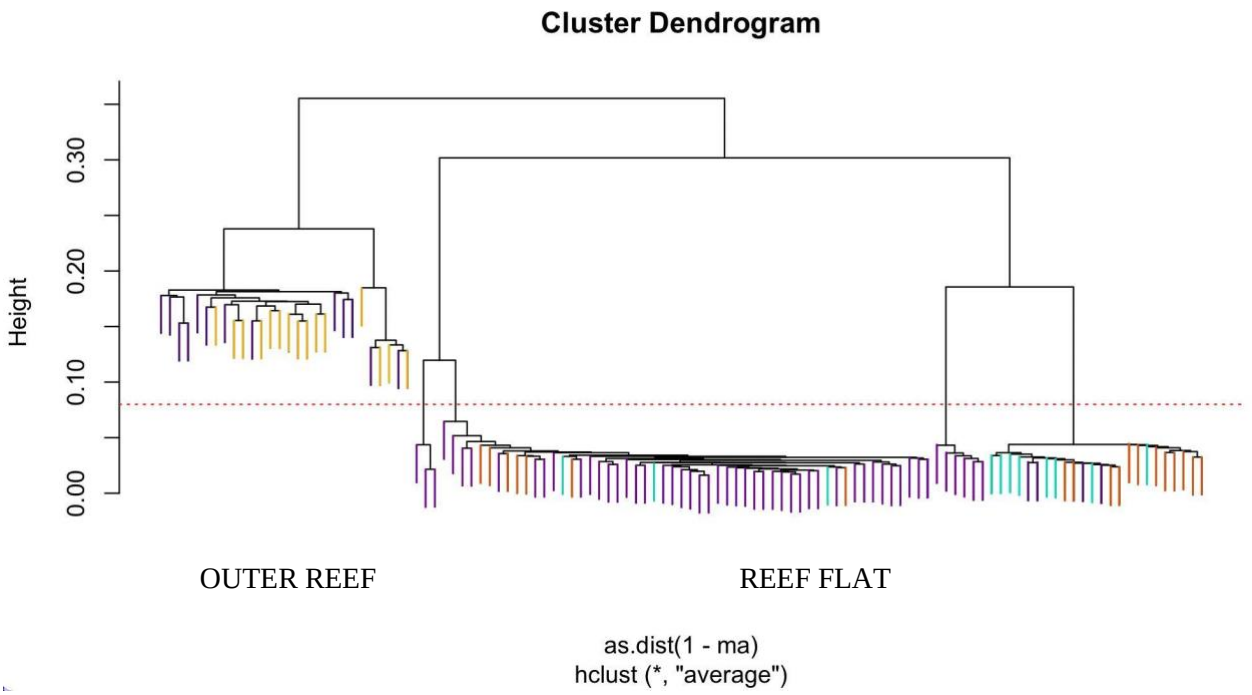


Figure 10. Cluster dendrogram created after running an IBS and Dissimilarity matrix. A cutoff line set to 0.08 indicates that any individual below this threshold is considered clonal. All outer reef populations (Luminao Outer Reef and Tinian, purple and orange, respectively) supersede the clonal threshold. All reef flat populations from Guam (Urunao, Pago Bay, Cocos Lagoon East, and Luminao Reef Flat indicated in purple), Rota (teal), and Saipan (orange) are found under 0.08, indicating clonality of reef flat individuals.

In relatedness heatmaps, individuals above 0.35 are considered sexually reproducing, whereas individuals falling below 0.35 are considered clonal (Manichaikul et al. 2010). The relatedness heatmap for all the *Leptastrea* samples confirmed the reproductive types of outer reef and reef flat individuals. The 28 rows and columns in the top left corner represent individuals found on the outer reef and had relatedness values between approximately -0.6 - 0. The remaining 87 rows and columns represent the reef flat branch. Following the dendrogram on top of the heatmap, distinct clonal structure can be seen where clones 1, 2, 3, and 4 had higher relatedness values to one another than to other lineages (approximately 0.35 - 0.5; Figure 11).

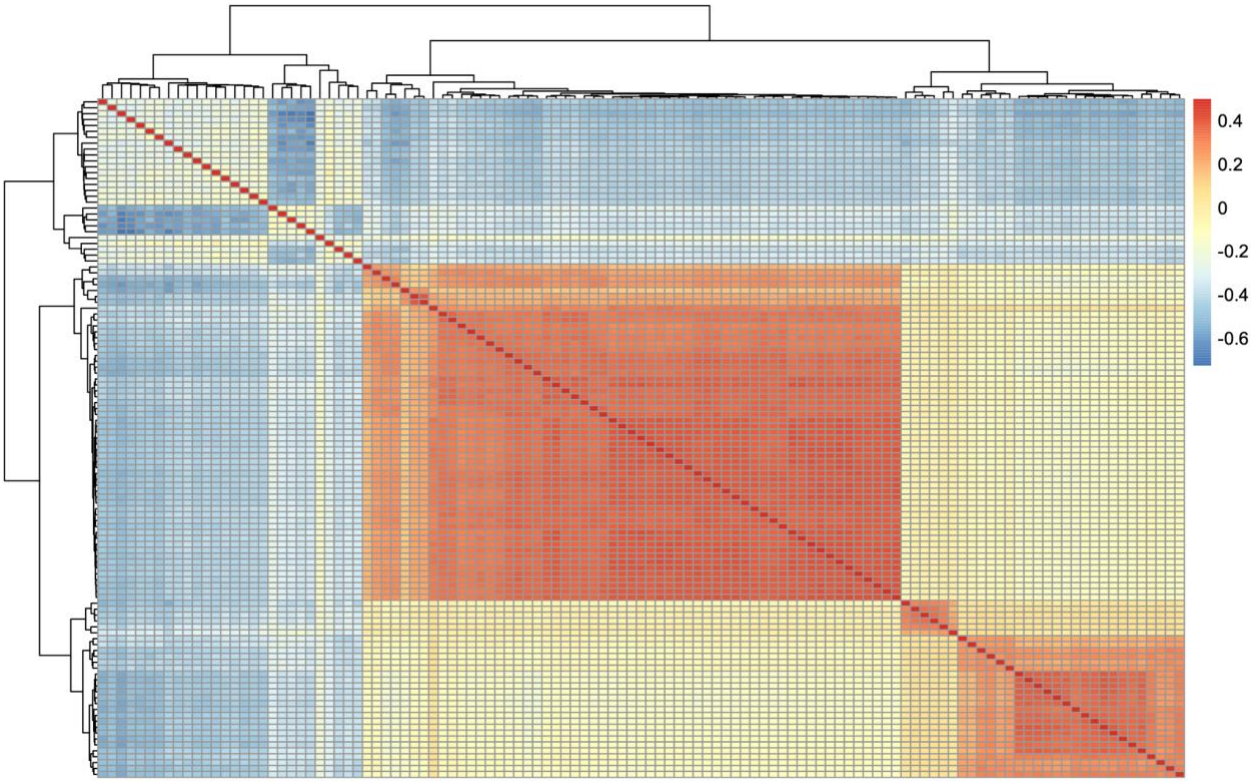
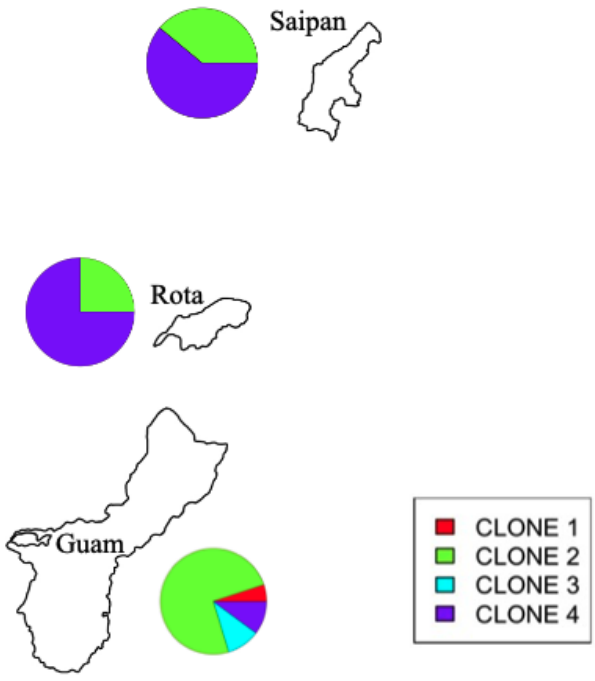


Figure 11. Heat map showing relatedness of all reef flat and outer reef samples. The left branch and top 28 rows and columns represent *Leptastrea* from the outer reef with a relatedness of approximately -0.6 - 0. The right branch and remaining 87 rows and columns represent *Leptastrea* from the reef flat. Clonal lineages in the reef flat sites can be observed within this cluster and had relatedness values between approximately 0.35 - 0.5.

Interestingly, all clonal samples originated from the reef flat sites, whereas the sexually reproducing were restricted to the outer reef. Based on the dendrogram, there were four clonal lineages (Figure 10). The Guam *Leptastrea* population on the reef flat was made up of all four clonal lineages: clone 1 represented 5%, clone 2 at 77%, clone 3 consisted of 11%, and finally clone 4 represented 7% of the population (Table 3). The Rota and Saipan reef flat *Leptastrea* populations were made up of exclusively clonal lineages 2 and 4 with clone 4 being the dominant lineage. For Rota, clone 2 made up 25% of the population and the remainder being clone 4 (75%), and Saipan's population was made up of 39% clone 2 and 61% clone 4 (Table 3, Figure 12).



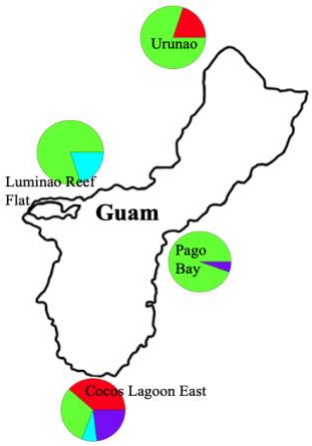


Figure 12. Clonal proportions found in each island and within sites on Guam. Reef flat *Leptastrea* populations in Saipan and Rota consisted of clones 2 and 4, whereas the population of Guam was made up of all four clonal lineages. Within Guam sites, *Leptastrea* in Urunao, Luminao Reef Flat, and Pago Bay consisted of two clonal lineages with clonal lineage 2 being the dominant clone. The population at Cocos Lagoon East was made up of all four clonal lineages with clone 2 and clone 1 making up the majority of the population.

Table 3. Proportion of clones found within each island.

ISLAND	CLONE 1	CLONE 2	CLONE 3	CLONE 4	TOTAL
GUAM	5%	77%	11%	7%	57
ROTA	NA	25%	NA	75%	12
SAIPAN	NA	39%	NA	61%	18

Clonal proportions and distribution were calculated per population on Guam (Figure 13, Table 4). Clones 1 and 2 made up 20% and 80% of the population in Urunao, respectively. Pago Bay had clones 2 and 4 make up 94% and 6% of the population, respectively. The population in Luminao Reef Flat was made up of two clonal lineages, which were 2 and 3 at 69% and 31%, respectively. Cocos Lagoon East had the most clonal variation, where 38% of the population was made up of clone 1, 31% was from clone 2, 8% from clone 3, and 23% from clone 4 (Figure 13,

Table 4). Interestingly, Cocos Lagoon East was also the only site with random clonal distribution after performing a runs test (p-value = 0.029; Table 4).

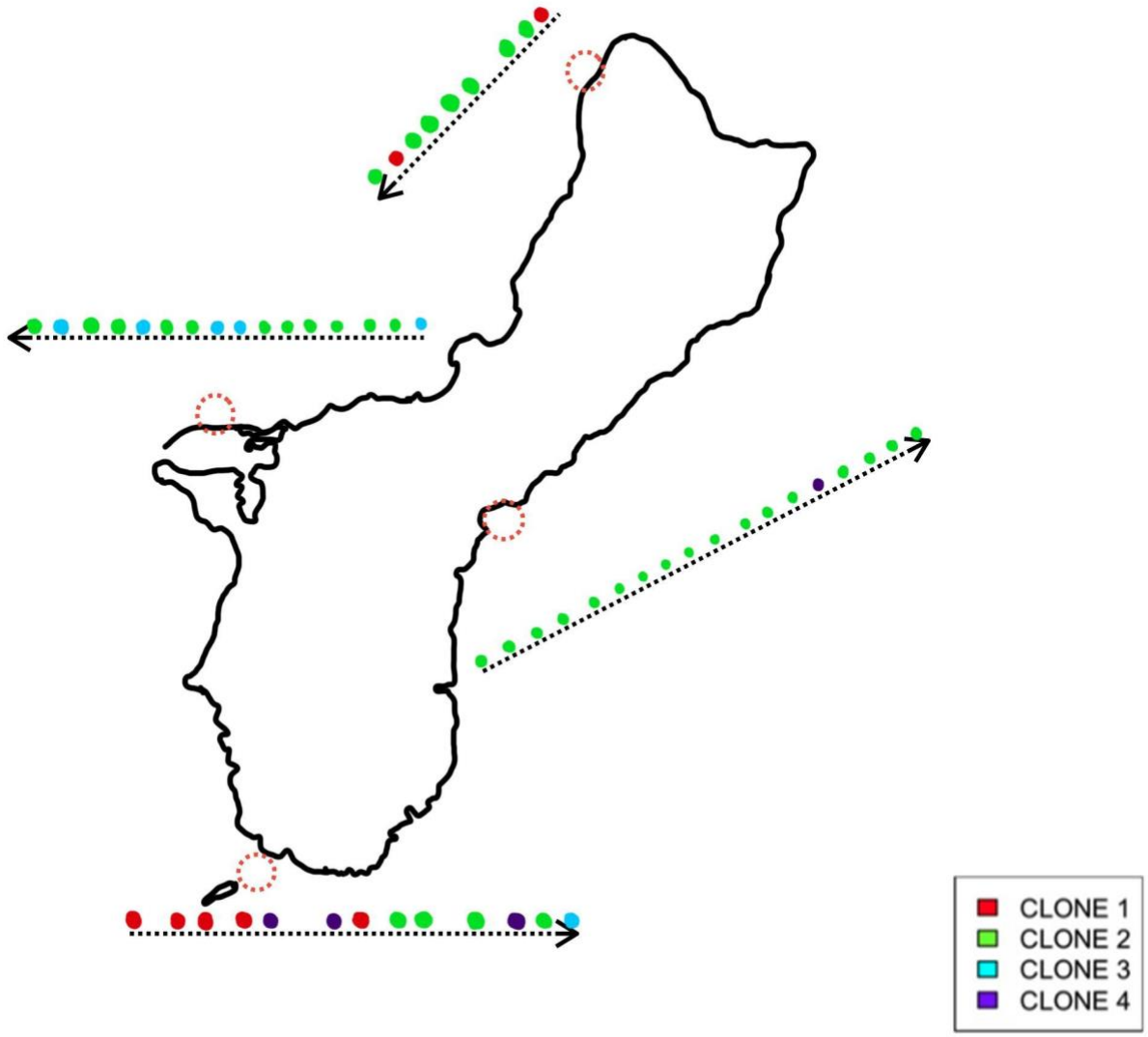


Figure 13. Clonal distributions along transects at each site in Guam. Colored circles represent individual clones collected along each transect, while uneven spaces between circles indicate missing data due to sample omission. The distribution of clones along the transects were analyzed using a runs test to assess whether clones were randomly or non-randomly distributed.

Table 4. Percent of clones and clonal distribution patterns in Guam. Cocos Lagoon East was the only site where clones were randomly distributed (p-value = 0.029).

SITE	CLONE 1	CLONE 2	CLONE 3	CLONE 4	RUNS	MEAN	STDEV	Z-VALUE	P-VALUE
COCOS LAGOON EAST	38%	31%	8%	23%	7	10.077	1.412	-2.179	**0.029**
LUMINAO REEF FLAT	-	69%	31%	-	8	7.875	1.641	0.076	0.939
PAGO BAY	-	94%	-	6%	3	2.889	0.314	0.354	0.724
URUNAO	20%	80%	-	-	3	4.2	0.884	-1.357	0.175

Clade-specific results

After determining that the outer reef and reef flat *Leptastrea* were two different species based on the phylogenetic tree, dendrogram, and relatedness heatmap, STACKs was rerun for the reef flat samples (n = 87) and 28,019 loci and 13,437 SNPs were retained. Overall clonal diversity among reef flat sites was 0.539. Pago Bay was found to have the lowest clonal diversity compared to all other reef flat sites (div_obs = 0.111; Table 5), and Cocos Lagoon East had the highest (div_obs = 0.603; Table 5).

Table 5. Clonal diversity of each reef flat site. Overall observed diversity (DIV_OBS) was 0.539. Pago Bay had the lowest observed diversity at 0.111, and Cocos Lagoon East was found to be the highest at 0.603.

POPULATION	DIV_OBS
OVERALL	0.539
COCOS LAGOON EAST	0.603
LUMINAO REEF FLAT	0.458
PAGO BAY	0.111
PAUPAU BEACH	0.503
ROTA	0.409
URUNAO	0.356

VCFtools (--het) showed that the proportion of heterozygous sites of reef flat *Leptastrea* was 31%, while outer reef *Leptastrea* was 16% (Table 6). Reef flat *Leptastrea* had approximately 50% higher heterozygous proportions than outer reef individuals. Interestingly, negative F_{is} was seen in all but 22 individuals from the reef flat, whereas all outer reef *Leptastrea* had positive F_{is} (Supplemental Material Table 1).

Table 6. Heterozygosity proportions of *Leptastrea* from the outer reef and reef flat.

OUTER REEF HET PROPORTION OR %	REEF FLAT HET PROPORTION OR %
-0.159721164	-0.309186164
16%	**31%**

Genetic structure and diversity of outer reef

Re-running STACKs for the outer reef (n = 28) resulted in 7,705 loci and 15,410 SNPs. Based on the dendrogram, all outer reef *Leptastrea* samples were found above the 0.08 threshold (Figure 10), indicating non-clonal, sexual reproduction. A large gap between the six samples in the second branch indicated the possibility of clonality, but no samples exceeded the threshold of 0.35 in the relatedness analysis indicative of clones (Figure 11).

Genetic diversity indices revealed that outer reef *Leptastrea* from Luminao Outer Reef and Tinian were genetically the same (Table 7). Both sites had observed heterozygosities of 0.2. Inbreeding was present at both sites as well, where Luminao Outer Reef had an inbreeding coefficient of 0.3, and Tinian with 0.3 (Table 7). The low observed heterozygosity between the two sites may be a result of sexually reproducing with genetically similar conspecifics.

Table 7. Genetic diversity of outer reef sites.

POPULATION	NUM	EFF_NUM	H_o	H_s	G_{is}
LUMINAO OUTER REEF	1.969	1.4	0.2	0.3	0.3
TINIAN	1.381	1.4	0.2	0.3	0.3

Pairwise F_{st} comparing the two populations revealed a very low pairwise F_{st} (F_{st} = 0.00), indicating that there was no significant genetic difference between the two sampling sites. DAPC supported low pairwise F_{st} which showed high overlap between the two sites (Figure 14). STRUCTURE analysis revealed that two ancestral genotypes made up the outer reef population (K=2; Figure 15) and also showed that there was no distinct genetic structure between the two outer reef sites. Together, all these results indicated that outer reef *Leptastrea* are a highly connected, single population.

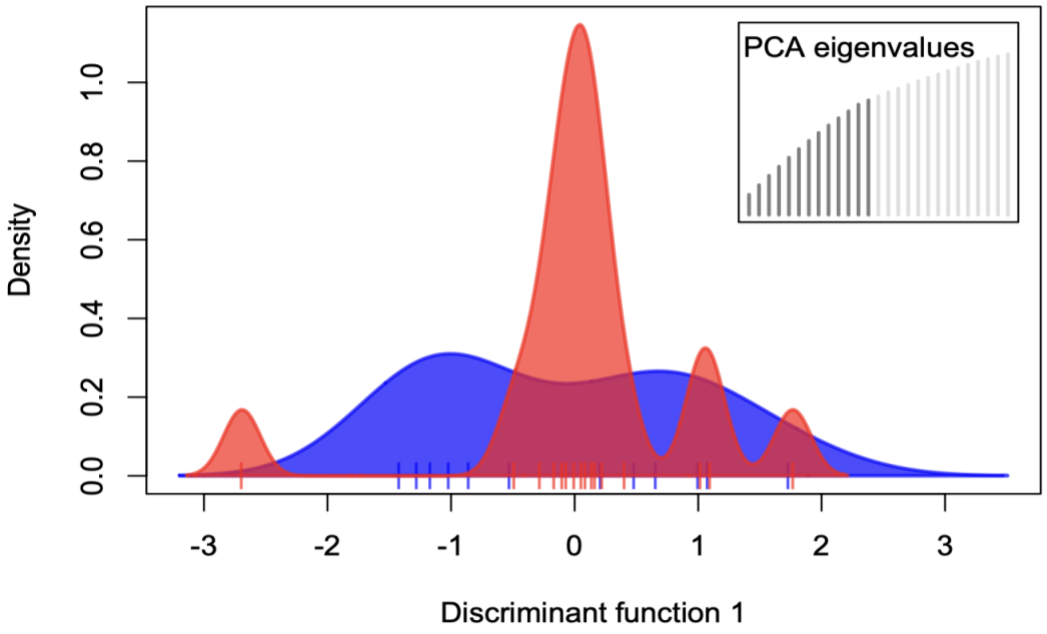


Figure 14. Discriminant Analysis of Principal Component (DAPC) for Luminao Outer Reef (blue) and Tinian (red) show high overlap between the outer reef *Leptastrea* population and support low pairwise F_{st}.

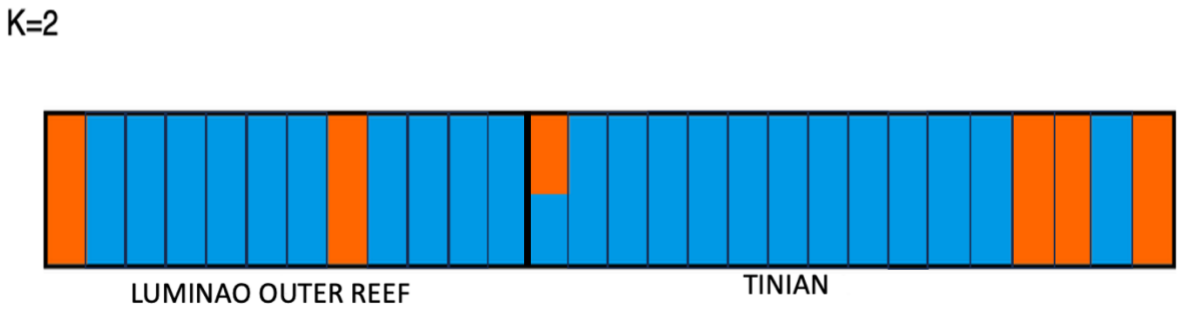


Figure 15. STRUCTURE plot between Luminao Outer Reef and Tinian. Low structure was seen between the two sites, indicating that *Leptastrea* from Luminao Outer Reef and Tinian are one highly connected population.

Morphological analyses

Morphological analyses showed differences between *Leptastrea* collected on the reef flat and outer reef (Table 8). Initial quantitative analyses found that *Leptastrea* colonies collected from the reef flat (n = 39) were on average 3.3cm and had about 18 septa per corallite whereas *Leptastrea* from the outer reef (n = 9) were on average 12.2cm and had approximately 16 septa per corallite. Colony size was found to be significantly different (p-value = 2.34e-06; Table 8) whereas the average number of septa per corallite was not (p-value = 0.085; Table 8).

Table 8. Morphological analysis. Average number of septa, colony size, and p-values.

HABITAT	SAMPLE SIZE	COLONY SIZE (cm)	AVG NUMBER SEPTA PER CORALLITE
OUTER REEF	9	12.2	16
REEF FLAT	39	3.3	18
p -value		**2.34e-06**	0.085

Qualitative observations showed that the first order of septa in outer reef samples extended higher than the corallite wall compared to reef flat individuals where septa and corallite wall height were generally uniform (Figure 16). Costae in outer reef samples were observed to be more prominent and steeply dropped into the groove separating corallites, whereas costae in reef flat individuals were less prominent and gently sloped and extended further into the corallite. Corallite shape also differed between the outer reef and reef flat, where corallites in outer reef *Leptastrea* were more symmetrical and uniform, whereas the reef flats were more irregularly shaped. These qualitative differences between outer reef and reef flat *Leptastrea* fell in line with descriptions of *Leptastrea immersa* and *L. purpurea*, respectively. Outer reef *Leptastrea* will therefore be referred to as *Leptastrea* cf. *immersa* for the remainder of this thesis.

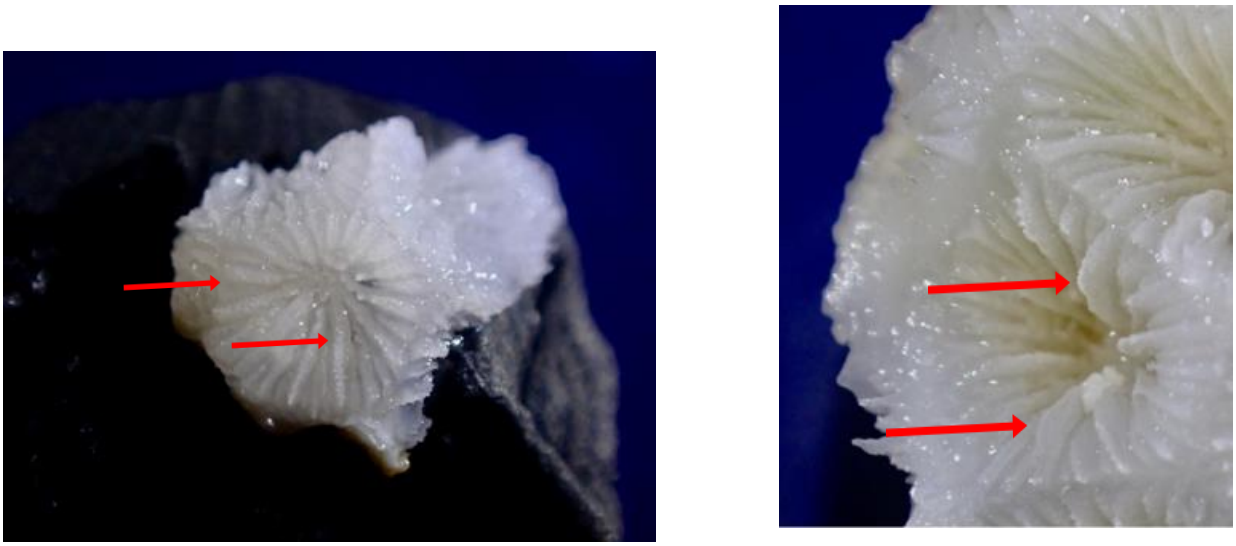


Figure 16. Comparison of corallite morphology of *Leptastrea* from the reef flat (left) and outer reef (right). The majority of first order of septa (indicated by arrows) from the reef flat were found to be more uniform in height to the corallite wall, whereas the height of septa in the outer reef extended higher than the corallite wall.

Chapter 4: Discussion

Effect of heat stress on reproduction, settlement and survival

The results from the larval experiment study showed that pre-exposure to elevated sea water temperature impacts *Leptastrea purpurea* at multiple life stages. Despite being a bleaching-tolerant coral (Nietzer et al. 2018, Raymundo et al. 2019, Galanto and Sartor et al. 2022), adult *L. purpurea* colonies in elevated temperature at 32.2°C paled and bleached at a greater rate than ones in ambient over the month-long period, indicating that sustained heating can negatively affect stress-tolerant corals (Figure 5). During the bleaching events from 2013-2017 on Guam, SST reached 31.5°C (Raymundo et al. 2019) which caused *L. pupurea* populations on seaward slopes and reef flats to bleach at 13 ± 15% and 15 ± 18%. However, this elevated temperature was not sustained for a full month like in our experiment, which resulted in a bleaching prevalence of 23%.

Elevated temperature can cause early gamete release and an increase in larval production (Edmunds et al. 2001; Randall and Szmant 2009a). Galanto and Sartor et al. (2022) found that *Leptastrea pupurea* released higher abundances of smaller sized larvae when exposed to temperature at 30.5°C, and this observation was confirmed at 32.2°C in our current study. Smaller sized larvae tend to have decreased lipid content which can cause accelerated metamorphosis and increased metabolism (Edmunds et al. 2001; Harii et al. 2002), leading to decreased pelagic larval duration (Edmunds et al. 2001).

Heated larvae had similar settlement to non-heated larvae after 72 hours but diminished over time in elevated temperature (Figure 8). However, both heated and non-heated larvae settle at similar rates when put in ambient temperature (Galanto and Sartor et al. 2022). Increased temperature can inhibit larval motility by decreasing ciliary movement (Bassim et al. 2002),

making it difficult for larvae to search for settlement area. Heat has been shown to inhibit settlement abilities in other coral, such as *Favia fragum*, which had 40% less settlement success at 31°C compared to larvae exposed to 28°C (Randall and Szmant 2009a). Reduced settlement rate combined with reduced larvae size suggests that as SST continues to increase, dispersal capabilities of coral larvae could decrease, and therefore, limit their connectivity and increase population isolation.

Despite having lower overall settlement success, total survivorship of heat-exposed larvae and recruits had higher survivorship after settlement in elevated temperature than larvae from control conditions (Figure 7). However, both heat- and ambient-produced larvae had similar survivorship after settlement in cooler temperature (Galanto and Sartor et al. 2022). The somewhat increased fitness observed in larvae and recruits produced in elevated temperature may be attributed to a maternal effect, where parental colonies precondition their offspring to the environmental conditions they were produced in (Marshall and Uller 2007). A maternal effect has also been hypothesized to affect other brooding coral, such as *Pocillopora damicornis* (Putnam and Gates 2015; Putnam et al. 2020) and *Montipora capitata* (Lenz et al. 2023). When exposed to low pH, *P. damicornis* larvae acclimate better to similar conditions than ones without this pre-exposure (Putnam and Gates 2015; Putnam et al. 2020). However, this phenomenon is very understudied and needs to be examined further. As of now, this study shows as temperatures continue to rise, *L. purpurea* larvae will have lower settlement capabilities, but the ones that do settle will be able to survive and thrive in suboptimal conditions.

Cryptic species in the Southern Mariana Islands

Using morphology alone to identify coral species has its setbacks. Pairing genetics alongside morphology can help differentiate species and provide better judgment for implementing

different management strategies in future global warming scenarios (Schmidt-Roach et al. 2014). Prior to this study, it was believed that *Leptastrea* from both the outer reef and reef flat were phenotypically plastic *Leptastrea purpurea* due to its previously described depth range (Nietzer et al. 2018) and appearance. However, genetic analyses revealed a distinct separation between *Leptastrea* corals from the two environments (Figures 9, 10, and 11), which was also supported by quantitative measurements of colony size paired with qualitative observations (Figure 16, Table 8). The genetic and morphological analyses provided in this study revealed that the two habitats most likely host two different cryptic species of *Leptastrea*.

The discovery of cryptic species in this study is hardly a novelty to coral research (Bongaerts et al. 2021; Johnston et al. 2022; Prada and Hellberg 2021). For example, species in the genus *Pocillopora* are almost indistinguishable from one another (Johnston et al. 2022). However, Johnston et al. (2022) sampled *Pocillopora* colonies at 5, 10, and 20m in Mo'orea, French Polynesia, and used gel-based restriction fragment length polymorphism (RFLP) assays for species determination. They found that *Pocillopora meandrina* was the dominant species at 5m, whereas *Pocillopora verrucosa* was dominant at 20m (Johnston et al. 2022). These findings highlight the importance of genetic tools in uncovering species-level differences that are otherwise undetectable through morphology alone.

Supporting our phylogenetic results, we found clear morphological differentiation between *Leptastrea* species in reef flat vs. outer reef (Figure 16, Table 8). The corallite shape, septa depth, and steepness of septa sloping toward the columella within the corallite for *Leptastrea* on the reef flat and outer reef fit the descriptions for *Leptastrea purpurea* and *L.* c.f. *immersa* (Burdick, unpublished), respectively. Corallites in *L. purpurea* can be hexagonal to irregularly shaped (Arrigoni et al. 2020), and reef flat *Leptastrea* matched this description better than outer reef

individuals whose corallites were more symmetrical (Randall, unpublished). Colony size was also different between reef flat and outer reef *Leptastrea*. *Leptastrea purpurea* colonies have been described to grow no larger than 10 to 12cm (Nietzer et al. 2018). The average size of reef flat colonies fell well below that size range at 3.3cm (Table 8), though one colony was approximately 10cm. Outer reef samples were found to be 12.2cm on average, which was significantly larger than reef flat samples (p-value = 2.34e-06; Table 8).

The Marianas were so far known to host only *L. purpurea* and *L. transversa (*Arrigoni et al. 2020). Unpublished documents claim that a third species, *Leptastrea immersa*, is also present in Guam (Burdick and Randall, unpublished). The morphological description of *L. immersa* closely fits the outer reef *Leptastrea* collected for this study. However, further morphometric analyses using intact colonies and larger sample sizes are necessary to confidently discern these two species and to definitively label them as *Leptastrea immersa* and *L. purpurea*.

Genetic diversity of Leptastrea purpurea and Leptastrea c.f. immersa

Leptastrea purpurea was found to show varying degrees of clonal diversity among sites (Div_obs = 0.111 - 0.603; Table 6). *L. purpurea* had similar clonal diversity to other clonal coral species, such as *Acropora cervicornis*. *A. cervicornis* in Florida and the Dominican Republic have clonal diversities of 0.141 - 0.538 and 0.221 - 0.619, respectively (Drury et al. 2019). *L. purpurea* at all sites, with the exception of Pago Bay, had similar clonal diversity to *A. cervicornis* (Div_obs = 0.356 - 0.603; Table 6). However, *L. purpurea* in Pago Bay had lower clonal diversity than all other sites (Div_obs = 0.111; Table 6), which may be due to its location on the east side of Guam that is prone to wave action throughout the year.

Leptastrea c.f. *immersa* was genetically the same between Luminao Outer Reef and Tinian

and had low genetic diversity (Table 7). Other sexually reproducing coral species, such as *Galaxea fascicularis* have moderate genetic diversity in the South China Sea (H_o = 0.367-0.586; Huang et al. 2023). *Porites lutea* and *Platygyra daedalea* sexually reproduce and have moderate genetic diversity in the Guangdong Province in the South China Sea (0.4474 and 0.4781, respectively; Jigui et al. 2018). Compared to *Galaxea fascicularis*, *Porites lutea*, and *Platygyra daedalea*, *Leptastrea* c.f. *immersa* had much lower genetic diversity (H_o = 0.2; Table 7) over hundreds of kilometers which may be detrimental in future climate change.

Differences in predominant reproductive strategy between clades/species

Leptastrea purpurea and *L.* cf. *immersa* appeared to differ not only environment type, but also reproductive strategy. *Leptastrea purpurea* populations were extremely clonal whereas *L.* cf. *immersa* populations were sexually-reproducing with no clonality present among the 28 *L.* cf. *immersa* samples collected. Different reproductive strategies between species in the same genus have been observed in a variety of organisms, notably the sea anemones *Anthopleura xanthogrammica* and *A. elegantissima* (Smith and Potts 1987), *Lasaea* clams (Foighil and Smith 1995), and members of *Timema* stick bugs (Freitas et al. 2023). *Porites astreoides* have been observed to produce fully developed planula larvae asexually alongside broadcast spawning, which ensures reproductive success if fertilization fails (Vollmer 2018). This difference in sexual and asexual reproduction between different species from the same genus may be attributed to the environment in which these organisms may be found (Binder et al. 2024).

This reproductive distinction between environments is interesting and could be related to their distinct habitat preferences. Reef flats are known to have increased light levels, higher wave action, and warmer temperatures (Bay and Palumbi 2014; Barker 2018; Nietzer et al. 2018) whereas the outer reef is relatively sheltered from these stressors due to depth. Many branching

coral species found in the reef flat persist in such conditions due to their ability to break apart from wave action (Highsmith 1982; Lirman 2000; Lizcano-Sandoval et al. 2018). By having the ability to reproduce clonally in harsh environments, coral populations can persevere in suboptimal conditions.

Due to *Leptastrea purpurea*'s encrusting morphology paired with negative F_{is} which reflects the lack of sexual reproduction (Balloux et al. 2003; Adjeroud et al. 2014), and high proportion of heterozygous sites (Table 4; Balloux et al. 2003), it is unlikely that clonality in *L. pupurea* was a result of fragmentation but is more realistic that parental colonies reproduce asexually due to their hermaphroditic nature (Nietzer et al. 2018). Asexual larval reproduction may have been observed in a *L. purpurea* colony that was placed in a display tank alone and a new visible colony formed 2-3 months later (pers. obs.).

It is possible that *Leptastrea purpurea* is highly specialized for life in the shallows due to their high stress tolerance, whereas *L.* cf. *immersa* is better fit for deeper water environments, which allows for greater connectivity due to underwater currents (Lodé 2013). For organisms living in environments that frequently experience stress or have limited sperm availability, selfing can prove to be advantageous to maintain populations (Foighil and Smith 1995; Lirman 2000; Liu et al. 2006; Lodé 2013; Barrett 2015; Rios 2020). For example, *Halcoglossum amesianum*, a high-altitude orchid found in China, relies solely on self-fertilization during the drought season when pollinators are scarce and wind is absent (Liu et al. 2006). On the shallow reefs of Guam, temperatures can exceed 34°C (Nietzer et al. 2018). As *L. purpurea* is known to be highly stress-resistant (Nietzer et al. 2018; Raymundo et al. 2019), it can be advantageous to produce larvae with exact copies of parental DNA through clonality to succeed in unfavorable conditions by the direct transfer of stress resistant genotypes to offspring (Bassim et al. 2002; Lodé 2013; Binder et

al. 2024). Future reciprocal transplant studies on *Leptastrea purpurea* from the reef flat to the outer reef or *L.* cf. *immersa* from the outer reef to reef flat could provide insight on how adapted these species are to each environment.

Self-fertilization is rare within the animal kingdom (Lodé 2013) and has not been well documented in corals (Brazeau et al. 1998). The solitary, gonochoristic coral *Fungia fungites* has been observed to release fully developed larvae in the absence of males (Eyal-Shaham et al. 2020). However, it is unknown if larval production is a result of self-fertilization, parthenogenesis, or sperm storage (Eyal-Shaham et al. 2020). *Pocillopora damicornis* produces the majority of its larvae asexually in French Polynesia (94%; Combosch and Vollmer 2013). However, these asexually produced larvae are from parthenogenesis where sperm is unnecessary to produce offspring. Two self-fertilizing corals in Florida, *Favia fragum* and *Porites asteroides*, naturally self-fertilize at rates of 49% and 34%, respectively (Brazeau et al. 1998). As of now, *L. purpurea* is the only coral to be almost uniquely made up of clonal individuals due to its hermaphroditism, and few studies have shown as high rates of self-fertilization as the one presented in this study. Though *Leptastrea purpurea* is a very thermotolerant and stress resistant coral species at present, clonality and reduced genetic diversity renders them susceptible to pathogens (Nietzer, pers. obs.), and may become disadvantageous in future predicted climate change.

Clonal distribution of Leptastrea purpurea

Eddies off the coast of Guam may lead to self-seeding and larval retention (Kendall and Poti 2014). Urunao and Luminao Reef Flat on the west side of Guam were found to host two clonal lineages: one that was only shared with Cocos Lagoon East, and the other being present in all reef flat populations (Figure 12 and 13). The clonal lineages within Urunao and Luminao Reef Flat that

were shared with Cocos Lagoon East could be a result of larval retention due to offshore eddies (Clone 1 and 3, respectively; Wolanski et al. 2003).

Cocos Lagoon East had the highest clonal diversity of all reef flat sites, and was the only site to host all four clonal lineages (Figure 12, 13; Table 4, 5). This high clonal diversity may be due to lunar phases that influence current direction, along with the currents and southern eddy off Cocos Lagoon (Wolanski et al. 2003; Kawahigashi 2021). Drifter data from a recent thesis from University of Guam showed that currents can shift southward due to lunar phase (Kawahigashi 2021). During the first quarter/waxing gibbous (lunar days 8-14), drifters released from the west side of Guam at Apra Harbor Station were found as far southwest as Umatac where currents with a southern directionality can be found (Wolanski et al. 2003; Kawahigashi 2021). This suggests that currents influenced by lunar phase contribute to larval distribution, and possibly why all four *Leptastrea purpurea* clonal lineages were found in Cocos Lagoon East.

The location of Guam and southern CNMI within the NEC promotes northern larval transfer with an east to west directionality (Figure 2; Kendall and Poti 2014). In larval dispersal simulations, larvae were released southeast of the Marianas and were observed to travel west between Guam and Saipan due to becoming embedded in the westward flowing NEC (Kendall and Poti 2014). The eastern sites in Guam share clonal lineages 2 and 4 with the western site in Rota and Saipan, mimicking the larval dispersal simulations in the Marianas. The east side of the Marianas experiences high wave action and wind throughout the year, whereas the west side is relatively sheltered (Kendall and Poti 2015). Because clone 4 was the only other clonal lineage to be found on the east side, it may be better equipped for such weather patterns due to its pre-exposure to stress compared to other clonal lineages found on the west side in Guam. Clonal

lineage 2 is the dominant lineage and found in every population, which may be due to a longer pelagic larval duration or high stress resistance.

Conclusion

There are three species of *Leptastrea* found in the Marianas, which are *Leptastrea purpurea*, *L.* c.f. *immersa*, and *L. transversa*. Two out of the three species, *L. purpurea* and *L.* c.f. *immersa*, were studied around Guam, Rota, Tinian, and Saipan, and were present in the reef flat and outer reef, respectively.

Leptastrea purpurea dominates the reef flat and has high stress resistance, making it well-adapted for hot environments. *L. purpurea* has similar clonal diversity to other clonal corals (Drury et al. 2019), and has only four genotypes on the reef flat (Figure 9, 10). *Leptastrea purpurea* larvae also have lower settlement success in elevated temperature (Figure 8), which raises concern for how this species may fare in future climate change. However, parental *L. purpurea* colonies when exposed to elevated temperature produce large quantities of larvae, which have higher survivorship success in elevated temperature (Figure 6, 7). These observed features tend to indicate that as temperatures and extreme environmental disturbances continue to increase, there may be smaller *L. purpurea* populations, but the colonies present will be able to survive in the elevated temperatures.

Leptastrea c.f. *immersa* is sexually reproducing and therefore has genetic recombination. Also, *L.* c.f. *immersa* is a highly connected population that spans from at least Guam to Saipan in the Marianas (Figure 15). However, because they have not been formally described and live in deeper environments which experience less stress, along with its low genetic diversity, it is unknown what *Leptastrea* c.f. *immersa*'s stress tolerance is and how their population genetics may shift in future climate change.

Finally, though *Leptastrea transversa* is present in the Marianas, it was not recorded or observed in this study, so it is unclear what its population genetics or stress tolerance is. However, if stress resistance is a uniform phenotype amongst *Leptastrea*, then all three species will likely survive in future global warming predictions in the Marianas and throughout their geographic range.

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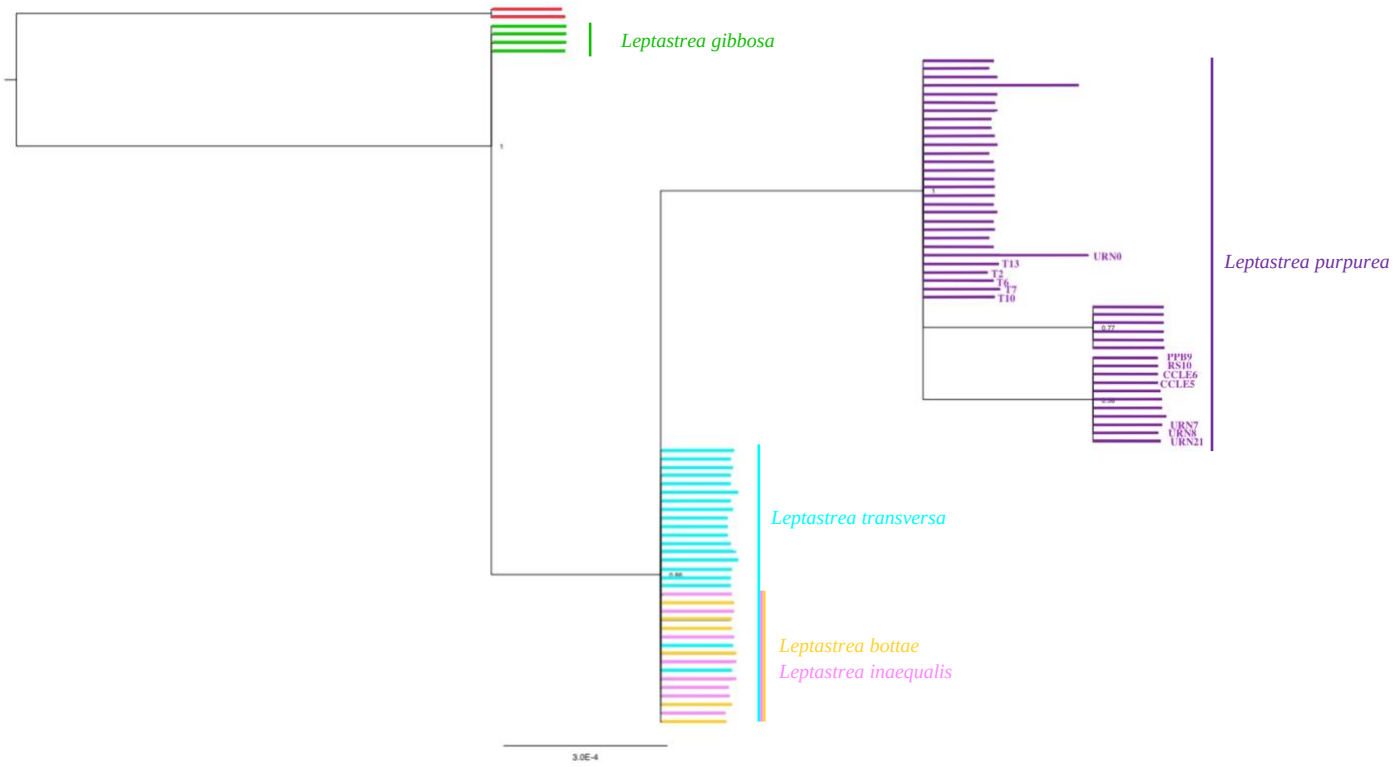
Supplemental Material



Supplemental Figure 1. Five outer reef and eight reef flat *Leptastrea* samples, indicated by sample name, using the cytochrome c oxidase subunit I marker (COI) were blasted against nearly complete mitochondrial *Leptastrea purpurea* (n = 33; purple), *L. transversa* (n = 19; light blue), *L. inaequalis* (n = 8; pink), *L. gibbosa* (n = 4; green), and *L. bottae* (n = 6; yellow). All samples clustered within the *Leptastrea purpurea* clade.

Supplemental Table 1. Individual heterozygosities of *Leptastrea* colonies from the reef flat and outer reef. Negative F are indicative of asexual reproduction, whereas positive is sexual and indicated in bold. All outer reef individuals had positive F, along with 22 reef flat colonies.

INDV	O(HOM)	E(HOM)	N_SITES	O(HOM)-N_SITES	F (INBREEDING)	HET PROPORTION
LORF1_S169	2459	2021.4	2855	-396	**0.52494**	-0.138704028
LORF3_S171	2249	1900	2687	-438	**0.44346**	-0.163007071
LORF4_S172	2301	1936.8	2739	-438	**0.45402**	-0.159912377
LORF5_S173	2279	1914.7	2716	-437	**0.45462**	-0.16089838
LORF7_S174	2252	1892	2684	-432	**0.45457**	-0.1609538
LORF8_S175	2129	1797.2	2552	-423	**0.4396**	-0.165752351
LORF9_S176	2311	1958.2	2773	-462	**0.43297**	-0.166606563
LORF10_S177	2268	1850.8	2617	-349	**0.54451**	-0.133358808
LORF11_S178	2121	1780.2	2532	-411	**0.45332**	-0.162322275
LORF12_S179	2230	1898.9	2693	-463	**0.41697**	-0.171927219
LORF14_S180	2124	1771.4	2512	-388	**0.47609**	-0.154458599
LORF15_S181	2323	1954.1	2771	-448	**0.4516**	-0.161674486
T1_S140	849.3	849.3	1222	-372.7	**0.51707**	-0.304991817
T2_S141	2436	2019.3	2857	-421	**0.49746**	-0.147357368
T4_S142	1248	1051.9	1505	-257	**0.43**	-0.17076412
T7_S144	1330	1112.9	1574	-244	**0.47078**	-0.15501906
T8_S145	2059	1712.2	2424	-365	**0.48725**	-0.150577558
T9_S146	2232	1909.4	2707	-475	**0.40446**	-0.175471001
T10_S147	1303	1074.4	1538	-235	**0.49307**	-0.152795839
T11_S148	1933	1579.1	2253	-320	**0.52513**	-0.142032845

T13_S149	1665	1353.8	1912	-247	**0.55752**	-0.1291841
T14_S150	1602	1331.9	1885	-283	**0.48832**	-0.150132626
T15_S151	1848	1522.9	2174	-326	**0.4993**	-0.149954002
T16_S152	2130	1798	2543	-413	**0.44566**	-0.162406606
T17_S153	2378	1932	2728	-350	**0.56029**	-0.12829912
T18_S154	2310	1900.1	2691	-381	**0.51826**	-0.141583055
T19_S155	2165	1841.4	2620	-455	**0.41562**	-0.173664122
T20_S156	2036	1657.6	2363	-327	**0.53645**	-0.138383411
CCLE0_S107	2161	2267.6	3221	-1060	-0.11176	-0.329090345
CCLE2_S109	2353	2458.6	3477	-1124	-0.10369	-0.323267184
CCLE3_S110	1814	1889.5	2687	-873	-0.09462	-0.324897655
CCLE5_S111	1745	1789.2	2548	-803	-0.05821	-0.315149137
CCLE6_S112	2114	2161.8	3085	-971	-0.05175	-0.314748784
CCLE9_S113	2089	2144.7	3044	-955	-0.06195	-0.313731932
CCLE10_S114	1765	1643.6	2321	-556	**0.17926**	-0.239551917
CCLE12_S115	1683	1619.7	2296	-613	**0.09361**	-0.266986063
CCLE13_S116	2336	2445.8	3459	-1123	-0.10834	-0.324660306
CCLE16_S117	2288	2399.3	3391	-1103	-0.11222	-0.325272781
CCLE18_S118	2149	2197.2	3131	-982	-0.05159	-0.313637815
CCLE19_S119	2205	2229	3161	-956	-0.02578	-0.302435938
CCLE20_S120	1748	1611.2	2276	-528	**0.20582**	-0.23198594
LRF1_S55	2191	2075	2933	-742	**0.13518**	-0.252983294
LRF2_S56	2137	2182.6	3094	-957	-0.05002	-0.309308339
LRF3_S57	2286	2371.7	3357	-1071	-0.08697	-0.319034853

LRF5D_S58	2198	2292.4	3257	-1059	-0.09786	-0.32514584
LRF6D_S59	2388	2469.8	3499	-1111	-0.07953	-0.317519291
LRF7_S60	2193	2269.8	3217	-1024	-0.08106	-0.318308984
LRF8_S61	921	891.5	1272	-351	**0.07741**	-0.275943396
LRF9_S62	1936	1842.4	2613	-677	**0.12151**	-0.25908917
LRF10_S63	1480	1421.4	2039	-559	**0.09492**	-0.274153997
LRF11_S64	2098	2154.3	3054	-956	-0.0626	-0.313032089
LRF12_S65	2363	2475.7	3506	-1143	-0.10942	-0.32601255
LRF14_S66	1973	1875.8	2665	-692	**0.12315**	-0.259662289
LRF15D_S67	2382	2495.8	3534	-1152	-0.1096	-0.325976231
LRF17_S68	1721	1654.4	2351	-630	**0.09564**	-0.267971076
LRF19_S69	856	798.8	1148	-292	**0.16388**	-0.254355401
LRF20_S70	2261	2379.2	3375	-1114	-0.11868	-0.330074074
PGO1A_S71	2314	2395	3393	-1079	-0.08112	-0.318007663
PGO2A_S72	2363	2457.5	3485	-1122	-0.09193	-0.32195122
PGO3A_S73	2339	2463.3	3491	-1152	-0.12095	-0.329991406
PGO4A_S74	2359	2459.3	3484	-1125	-0.09788	-0.322904707
PGO6A_S75	2306	2430.6	3434	-1128	-0.12413	-0.328479907
PGO7A_S76	2370	2462.4	3485	-1115	-0.09037	-0.319942611
PGO8A_S77	1826	1878.1	2679	-853	-0.0651	-0.318402389
PGO9A_S78	2382	2477.1	3506	-1124	-0.09238	-0.320593269
PGO10_S79	2347	2443.2	3452	-1105	-0.09535	-0.320104287
PGO11_S80	2354	2467	3492	-1138	-0.11019	-0.325887743
PGO13_S81	2323	2455.7	3473	-1150	-0.1304	-0.331125828

PGO14_S82	1897	2144.6	2999	-1102	-0.28984	-0.367455819
PGO15_S83	2294	2410.9	3411	-1117	-0.11691	-0.32746995
PGO16_S84	2076	2162	3068	-992	-0.0949	-0.323337679
PGO17_S85	2305	2440.8	3456	-1151	-0.1338	-0.333043981
PGO18_S86	2310	2423.9	3435	-1125	-0.11266	-0.327510917
PGO19_S87	2276	2405.8	3395	-1119	-0.13121	-0.329602356
PGO20_S88	2313	2449	3468	-1155	-0.13343	-0.333044983
PPB1_S121	2198	2245.8	3194	-996	-0.05041	-0.31183469
PPB2_S122	2170	2246.7	3188	-1018	-0.08153	-0.319322459
PPB3_S123	1542	1515.9	2175	-633	**0.03965**	-0.291034483
PPB4_S124	1834	1858.2	2664	-830	-0.02999	-0.311561562
PPB5_S125	2192	2270.2	3227	-1035	-0.08173	-0.320731329
PPB6_S126	1371	1356.5	1956	-585	**0.02421**	-0.299079755
PPB7_S127	1010	987	1416	-406	**0.05363**	-0.286723164
PPB8_S128	2236	2245.2	3181	-945	-0.00982	-0.297076391
PPB9_S129	1893	1934.9	2759	-866	-0.05079	-0.313881841
PPB10_S130	1820	1843.6	2615	-795	-0.03053	-0.304015296
PPB12_S132	2156	2215	3146	-990	-0.06333	-0.314685315
PPB13_S133	2337	2477.7	3514	-1177	-0.13572	-0.334945931
PPB14_S134	2078	2168.6	3086	-1008	-0.09879	-0.326636423
PPB15_S135	1273	1280.6	1822	-549	-0.01401	-0.301317234
PPB16_S136	1503	1479.2	2122	-619	**0.03706**	-0.291705938
PPB17_S137	1568	1540.1	2206	-638	**0.04191**	-0.289211242
PPB19_S138	1600	1572.8	2252	-652	**0.04003**	-0.289520426

PPB20_S139	1240	1209.4	1738	-498	**0.05792**	-0.286536249
RS3_S157	2200	2258.3	3210	-1010	-0.06125	-0.314641745
RS5_S158	1024	973.9	1406	-382	**0.11595**	-0.271692745
RS8_S159	1827	1805.2	2590	-763	**0.02778**	-0.294594595
RS9_S160	2275	2302.2	3261	-986	-0.02832	-0.302361239
RS10_S161	2174	2230.5	3175	-1001	-0.05987	-0.315275591
RS11_S162	2336	2437.5	3450	-1114	-0.10025	-0.322898551
RS12_S163	2169	2194.3	3128	-959	-0.02707	-0.306585678
RS13_S164	2179	2237.5	3183	-1004	-0.06183	-0.315425699
RS14_S165	2166	2200.3	3131	-965	-0.03687	-0.30820824
RS15_S166	2109	2093.6	2997	-888	**0.01708**	-0.296296296
RS18_S167	2035	2039.9	2903	-868	-0.00564	-0.299001033
RS19_S168	2347	2444.6	3457	-1110	-0.09643	-0.321087648
URN9_S97	2287	2406.9	3408	-1121	-0.11973	-0.328931925
URN11_S98	2251	2221.3	3134	-883	**0.03257**	-0.281748564
URN12_S106	2291	2402.9	3397	-1106	-0.11254	-0.325581395
URN13_S99	1651	1684.8	2398	-747	-0.04734	-0.311509591
URN16_S100	2000	2110.6	2999	-999	-0.12451	-0.333111037
URN17_S101	2298	2405.3	3394	-1096	-0.10855	-0.322922805
URN18_S102	2165	2316.5	3288	-1123	-0.15596	-0.341545012
URN19_S103	1732	1823.7	2602	-870	-0.1178	-0.334358186
URN20_S104	2364	2325.2	3278	-914	**0.04074**	-0.278828554
URN21_S105	2295	2420.5	3425	-1130	-0.12498	-0.329927007