ISLAND-SCALE GENETIC DIVERSITY AND CONNECTIVITY OF THE OCTOCORAL *HELIOPORA COERULEA* ON GUAM

BY

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ABSTRACT OF THE THESIS of Abram L. Townsend for the Master of Science in Biology presented May 2nd, 2022

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Dr. Bastian Bentlage, Chair, Thesis Committee

Coral reefs are considered one of the most vulnerable ecosystems on the planet. In the Western Pacific, coral reefs in various regions have been experiencing an annual 1-2% decline in coral cover since the 1980's. Reefs around the island of Guam are no exception to the degradative effects of global climate change, and the anthropogenic stressors that are associated with a deteriorating reef habitat. The hermatypic octocoral known as "blue coral", *Heliopora coerulea* is a unique example of one of the notable reef-building corals, with little research having been conducted regarding this species in Guam. *H. coerulea* displays more than one distinct morphology, some of which exist around Guam. Populations of reef associated organisms are influenced by prevailing sea surface currents and eddies, which have the potential to heavily contribute to larval dispersal and genetic connectivity. In this study, multiplexed inter simple sequence repeat (ISSR) Genotyping by sequencing (MIG-seq) was utilized to determine the population genetic structure of 110 *H. coerulea* samples representing four major isolated populations surrounding Guam were assessed, in addition to examining the occurrences of different morphologies between populations. *H. coerulea* samples were genotyped and analyzed. Genetic analysis revealed limited population structure and low genetic diversity, indicating elevated levels of genetic connectivity amongst populations. Morphological analysis revealed no clear correlation between sites and morphologies, indicating other factors are influencing morphology amongst populations. Guam's reproductively connected populations of *H. coerulea* promote genetic exchange between conspecifics,

which may help mitigate the negative effects a changing climate would have on a small or genetically unconnected population.

Keywords: blue coral, *Heliopora coerulea*, MIG-seq, population genetics, morphology, coral reef, conservation management

TO THE OFFICE OF GRADUATE STUDIES

The members of the committee approve the thesis of Abram L. Townsend presented May 02, 2022.

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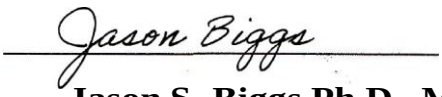

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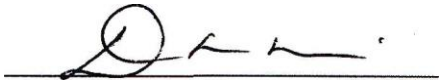

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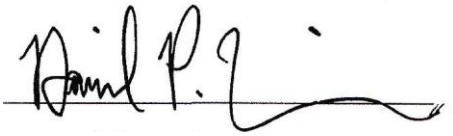

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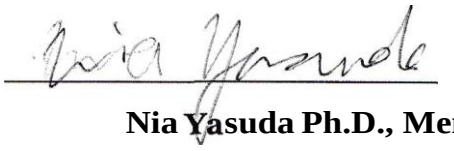

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Introduction

Coral Reef Importance

Regarded as the most important of all bioconstructions in the world (Allemand et al. 2004), coral reefs are distributed over an area of about 527,072 km^2 (Mora et al. 2006) throughout warm, shallow-water photic zones of the tropical oceans. These vast biological structures are built over centuries by continued calcium carbonate deposition from hermatypic, stony corals in the class Scleractinia, as well as by other calcium carbonate-producing organisms, such as coralline algae. In total, coral reef ecosystems occupy a miniscule (0.14%) portion of area within the global ocean. And yet, the biomass produced within these marine ecosystems accounts for nearly 2-5% of fish consumption on the planet (Pauly et al. 2003). In addition, coral reefs contribute substantially to the livelihood and socioeconomics of millions of people who live along coasts throughout the world (Golden et al. 2016; Albert et al. 2015; Cinner 2014; Sumaila et al. 2013; Sadovy 2005; Salvat 1992). It is estimated that 1 km^2 of viable coral reef has the ability to support 300 people with their daily protein needs (Jennings and Polunin 1996). Within the Indo-Pacific, coral reef fisheries amass about 25% total fish catch in the region (Cesar 1996).

Reefs provide additional economic impacts. For example, tourism offers an additional economic benefit to communities that are able to maintain healthy reef ecosystems (M. Spalding et al. 2017; M. D. Spalding, Ravilious, and Green 2002), as they are attractive to visitors who enjoy nature (Salvat 1992). Tourism is the primary source of revenue for private industry on Guam, which generated $1.85 billion in revenue during FY2019 (Guam Visitors Bureau). The discovery of compounds with biotechnological potential are also supplied by coral reefs (Motuhi et al. 2016), as a number of marine-derived medicines have been discovered (Carté 1996). In addition, many other natural resources such as building materials, minerals, oils, and gases generated by physicochemical processes are extracted from the remnants of previously living reefs (Moberg and Folke 1999).

The complex topography of coral reefs serves as a physical buffer zone for coastal areas, allowing shallow nearshore reefs to dissipate up to 97% of wave energy impacting shorelines (Ferrario et

al. 2014), and reduces annual expected damages from storms by more than an estimated $4 billion (Beck et al. 2018). Guam shorelines are protected by fringing and barrier reef formations, both of which are comprised of various assemblages of marine plants and animals. One ability all reef-building species share with hermatypic corals is the ability to incrementally accrete calcium carbonate onto physical structures within their immediate vicinity, adding volume and stability to the reef framework (Allemand et al. 2004; Jean-Pierre Gattuso 1999).

Dynamic, three-dimensionally complex reef systems also constitute an ecologically important and biologically diverse habitat for a multitude of marine organisms on the planet (Ecology and Bv 2017; Fisher et al. 2015; Graham and Nash 2013; Stefansson, Rich, and Rae 1954).

Concerns for Reef Health

Yet, despite obvious importance of coral reefs to the quality of human life, a combination of local and global anthropogenic and natural stressors threaten the very existence of coral reef ecosystems worldwide (Bellwood et al. 2004; Gray 1997; Hoegh-Guldberg 2011; Mumby and Steneck 2008; Pandolfi et al. 2003; Salvat 1992). Indeed, coral reefs are considered one of the most vulnerable ecosystems on this planet. In the Western Pacific, coral reefs are in decline, with an annual decrease of 1-2% in area since the 1980's (Hoegh-Guldberg 2011). Data collected from studies of ancient reefs suggest that coral reef ecosystems have been subjected to a continually escalating variability in climate since the Last Glacial Maximum, about 21ka BP (Montaggioni 2005). Exposure to a changing climate spanning centuries highlights the resilience and adaptive nature of coral reefs worldwide today. Regardless of climate variability, research also suggests that acute and chronic anthropogenic influences negatively affect coral reef health (Sweet and Brown 2016; Muthukrishnan and Fong 2014; Hoegh-Guldberg 2011). Pollution (Bruno and Selig 2007; M. D. Spalding, Ravilious, and Green 2002), unsustainable resource extraction (Becerro, Bonito, and Paul 2006; Hughes 2003), sedimentation and storm water run-off (Maina et al. 2013; Wolanski, Richmond, and McCook 2004) are all stressors that negatively affect reefs. In

addition to these more drastic outcomes, even when stressors are less severe they can increase coral susceptibility to infectious pathogens and disease outbreaks (Lesser et al. 2007; Pandolfi et al. 2003).

Threats to Coral Reefs of Guam

Reefs around the island of Guam are no exception to the degradative effects of global climate change, and the anthropogenic stressors such as overfishing, sedimentation and pollution that are associated with a deteriorating reef habitat (Burdick et al. 2008). For example, Fouha Bay on the southwest coast of Guam is perpetually subjected to sedimentation in the rainy season, resulting from land clearance in the adjacent catchment system (Wolanski et al. 2003). The rainy season coincides with the recruitment event of many juvenile corals, which are susceptible to sediment settling on reefs in calm ocean water (Wakwella et al. 2020; DeMartini et al. 2013; Wolanski et al. 2004; Wolanski et al. 2004). An increase in nitrogenous pollution derived from synthetic fertilizer since the 1950's, and until more recently from increasing sewage input from the expansion of coastal populations, is increasing the impacts diseases have on corals in Guam (Redding et al. 2013). Elevated water temperature associated with climate change caused initially recorded large-scale bleaching events in 1994 and 1996 (Paulay 1999) occurred in part by sustained higher than average sea surface temperatures (SST). Over a decade later, observations in 2006 and 2007 (Burdick et al. 2008), as well as in 2013, 2014, 2016 and 2017 (Reynolds et al. 2014; Eakin et al. 2019; Raymundo et al. 2019), confirmed more large-scale coral bleaching which coincided with elevated SST. Climate-induced and local-scale anthropogenic stressors, together with chronic crown-of-thorns seastar outbreaks, have negatively impacted reef health in Guam in recent decades, compromising the reef system's ability to provide goods and services to the island's human community.

General Information on Target Species

Even though the majority of reef-building corals are classified within the subclass Hexacorillia, other subclasses of coral (e.g., Octocorallia) can also be significant contributors (Yasuda et al. 2012).

The blue coral *Heliopora* spp. forms a massive aragonite calcium-carbonate skeleton like Scleractinians, which is a unique structural composition for an octocoral. Another unique characteristic is the blue pigmentation derived from iron salts incorporated within its trabecular skeleton of fibrous aragonite (Hill 1960). With a preferred, temperature range of 28-29 °C, *H. coerulea* populations reach denser concentrations and larger sizes in warmer water environments (Bolton 1985). Only a few documented cases of large *H. coreulea* stands exist within the extent of its range (Bolton 1985; Takino et al. 2010), suggesting reduced fecundity that may lead to small patchily colonies. In 2008, *H. coerulea* was classified as 'threatened' by the International Union for Conservation of Nature's Red List Criteria after studies determined a measured population decline over time throughout its geographic range (Bruckner et al. 2008). *Heliopora coerulea* is a conspicuous hermatypic octocoral on Guam's reefs, Although scleractinian corals have received the majority of scientific attention over the years, *H. coerulea* which is a non-scleractinian coral, could react similarly to environmental and anthropogenic pressures (Bruckner et al. 2008; Hughes 2003).

Cryptic Species of *Heliopora coerulea*

H. coerulea was considered to be the only species member of the family Helioporidae of the order Coenothecalia, until the discovery of a new species *Nanipora kamurai* (Miyazaki and Reimer 2015), and more recently *Heliopora hiberniana* (Richards et al. 2018). In previous studies examining cryptic speciation in *H. coerulea,* genetic analyses revealed the presence of two morphologically distinct lineages along Kuroshio Current, coexisting within the same geographical locales (Villanueva 2016; Yasuda et al. 2014) These morphologies are distinguished from each other by branch structure and encrusting growth forms. Lineage HC-A (within the *Heliopora* sp. group) exhibits a small-branch, columnar morph and is generally found in cooler temperatures on seaward slopes and forereefs, located in fringing reef environments, found in the Kuroshio current regions (Taninaka et al. 2021; Yasuda et al. 2014). Lineage HC-B exhibits a flattened undulating branching morph and is usually associated with

shallower and warmer water environments such as back-reefs of fringing reefs, belonging to the *H. coerulea* group (Taninaka et al. 2021; Yasuda et al. 2014).

More recently, three genetically distinct *Heliopora* groups have been identified throughout the Pacific Ocean based on a phylogeographic study: *H. coerulea* group, *H. hiberniana* group, and a new undescribed *Heliopora* sp. group (Taninaka et al. 2021). Populations along the Kuroshio Current stretching from the Philippines to Japan have been genetically investigated, revealing multiple gross morphologies specific to each of the *Heliopora* groups with some morphological overlap between the *H. hiberniana* and *H. coerulea* groups (Taninaka et al. 2021; Yasuda et al. 2014). Gene flow among sampled colonies was higher within members of the same lineage, rather than between lineages, which suggests these lineages are reproductively isolated (Yasuda et al. 2014). Depth is not correlated with the presence of any one specific lineage as both species coexist within the same depth ranges from 1 - 10 m (Yasuda et al. 2014). The previous discovery of discrete genetic lineages along the Kuroshio Current above (Yasuda et al. 2014, Yasuda et al, 2015), can aid in assessing the genetic diversity and population structure of *H. coerulea* in the reefs of Guam. Visual observation in Guam confirms the existence of at least two different morphologies present on the island.

Reproductive Strategy of *Heliopora* Including PLD

Heliopora coerulea is a gonochoric brooder, with separate male and female colonies. During its annual gametogenic cycle, fertilization and incipient larval development occurs internally (R. Babcock 1990). Subsequent development stages occur within 6 to 14 days as the female broods its larvae on the external surface of the colony (Liu et al. 2005; R. Babcock 1990). The planulae brooding reproductive strategy of *H. coerulea* generally suggests larval dispersal within a smaller geographic range from the parent colony compared to broadcast spawning corals (Harii and Kayanne 2003). Planula larvae were observed to possess average lengths of 3.7 mm, with restricted mobility and negative buoyancy, possessing a lower lipid content when compared to other species of planulae larvae (Harii et al. 2002). Low lipid content in addition to restricted movement supports short distance recruitment patterns from *H.*

coerulea. Limited initial dispersal potentially results from the planulae's short competency period in the water column, averaging approximately 30 days after release from parent colonies (Harii et al. 2002) and on average larvae of *H. coerulea* settle on substrate between 1- 6 hours after initial release from the parent colony and crawl across the substrate before locating a suitable area to recruit (Harii and Kayanne 2003). Generally, *H. coerulea* are capable of producing small amounts of planulae with a maximum competency period of > 70 days (Harii et al. 2002). However 98% of released planulae are competent up to 20 days post release of the parent colony and there after survival rates diminish to approximately 50% after 70 days post release (Harii et al. 2002).

Observations from previous studies indicate dispersal of *H. coerulea* planulae is directly related to planulae competency periods, position in the water column and currents at the time of release from the parent colony (Harii and Kayanne 2003). However, with the ability to remain competent for > 70 days in combination with the aid of contingent ocean currents and eddies present around the island of Guam, viable planula from other islands can disperse toward Guam over larger spatial scales.

During asexual reproduction, a fragment of the living portion of a coral colony is physically separated and thus becomes a new individual colony. Its ability to reproduce both sexually, and asexually through fragmentation, are considered to be its two reproductive strategies (Yasuda et al. 2012).

Importance and Pervious Research of Assessing Gene Flow and Genetic Diversity of *Heliopora*

Given populations of *Heliopora* spp. are maintained by larval recruitment, examining spatial scales of genetic homogeneity are of great important for coral conservation. A high rate of gene flow, both within and among populations, is intrinsically important to maintaining genetic diversity of populations. A genetically diverse population has greater adaptive capability (Selkoe et al. 2016; Frankham 2005; Gates and Edmunds 1999) and thus genetic diversity decreases the likelihood of extinction events associated with changing selective pressures (Noreen et al. 2009; Bijlsma et al. 2000). Although direct observation and recording of larval dispersal and genetic diversity that are under

influence of fluctuating environmental factors tidal/ current pattern are rather difficult, gene flow analysis can indirectly estimate the range of the larval dispersal and its genetic diversity (Harii and Kayanne 2003; Harii et al. 2002; Harrison and Wallace 1990). Previous genetic studies in Japan demonstrated limited larval dispersal of Heliopora both within fringing reef scale (Mokodongan et al. 2021) and within barrier reef scales (Taninaka et al. 2019).

There is, however, potential for the genetic structure of the blue coral to be connected between two regions within the North Pacific Ocean by the North Pacific Gyre, the prevailing North Equatorial Current and adjoining Kuroshio Current, inferring the possibility of between archipelago gene flow.

Relevance of the Technology and Methods

In order to assess gene flow of *H. coerulea* between populations, molecular data that can discern resolute genetic patterns is integral. Certain types of molecular data are not always able to provide enough resolution, such as more traditional mitochondrial markers (Bilewitch and Degnan 2011). High-Throughput Sequencing (HTS) using genome-wide polymorphisms is powerful enough to delimit species of non-model organisms, including corals via Multiplexed inter simple sequence repeat (ISSR) genotyping by sequencing (MIG-seq) (Hirai 2020; Park et al. 2019; Tamaki et al. 2017). MIG-seq is a novel method that is easy and economical in obtaining moderate numbers of single nucleotide polymorphisms (SNPs) from non-model organisms coupled with HTS and polymerase chain reaction (PCR) technology. MIG-seq can be performed using small amounts and/or low-quality DNA, making it and advantageous method in genetic research.

Hypothesis/Objectives

Understanding island scale genetic diversity of *H. coerulea*, will provide a basis for conservation strategies of *H. coerulea* on Guam, and determine gene flow among reefs. Under these conditions, I hypothesized genetic structure between populations of *H. coerulea* on Guam is strong. The reproductive strategy of the brooding coral *Heliopora coerulea* is mostly associated with very short pelagic larval

durations (PLD) of only a few hours, which bolsters the claim of highly structured localized populations on Guam. These populations adapt to specific local environments, and because the larvae of these octocoral coral exhibit very short PLDs, there will be limited gene flow between populations of *H. coerulea* on Guam. In addition, I presume genetic structure is reflected by the distribution of phenotypes. It is well documented that *H. coerulea* possesses several different growth forms, and I predict that certain growth forms are more advantageous in different environments. I also tried to discover phenotypic association with certain populations inhabiting differing environments.

The objectives of this study are to identify genetic connectivity patterns of *H. coerulea* to determine population genetic structure between study sites and to assess the gross morphology as it relates to geography around Guam.

Methods

Study Area

Four collection sites were chosen for this study (Fig. 1): Pago Bay (Eastern), Luminao (Western), Ritidian Point- Pati Point (Northern), Cocos Area (Southern). These four sites represent furthest regions of Guam separated according to the four cardinal directions. Guam's geographic location, size and orientation within the Northern Equatorial Current generates meandering island induced eddies and variable current patterns. The combination of these research initiatives, in examining the population genetic structure of hermatypic corals, creates a basis for assessing the genetic diversity and connectivity, and ultimately strengthens sustainable resource management plans on an island scale for Guam.

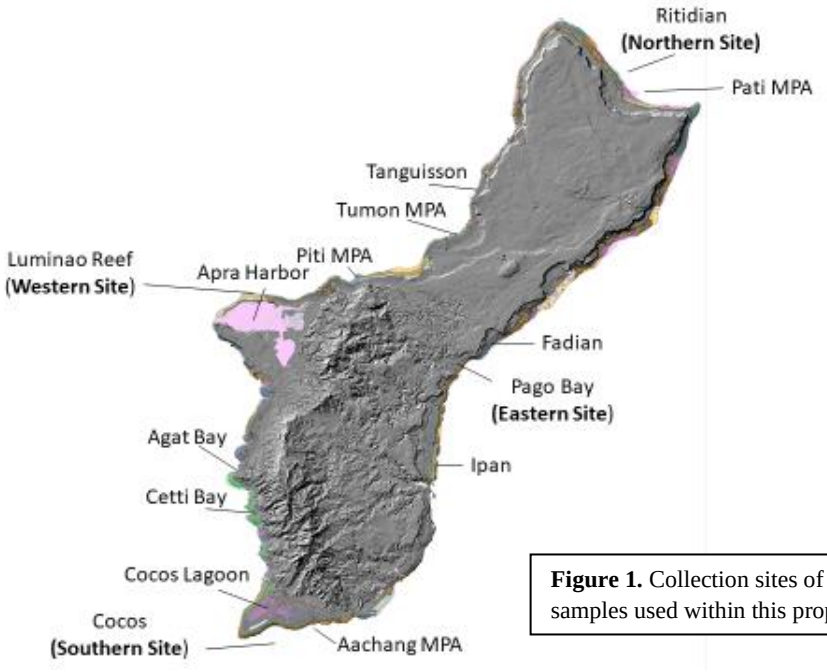

Figure 1. Collection sites of *H. coerulea* samples used within this proposed study.

In-field Sample Collections

Living coral fragments were collected from individual coral colonies sampled in the field. Thirty or more ($n \geq 30$) samples from different individual colonies per study site were chosen at random and collected via S.C.U.B.A. and/or snorkeling at depths between 1-8 m between June 2017 and November 2019. 2.5 cm fragments from each colony were stored in 50 ml collection vials containing sea water. Approximately 50 kick-cycles were completed between each sampled coral colony to increase sampling distribution and the probability of sampling non-clonal individuals. A photo of each sampled colony was taken during the sampling process and a time stamp of each photo was synchronized with the time stamp of a surface floating GPS unit, located directly above the sampled colony's location. Thus, each sampled colony was photo documented with GPS location coordinates. Seawater was replaced with 99.9% ethanol for tissue preservation and coral fragments were stored at -80°C. A total of one hundred and forty (*n =140*) *H. coerulea* coral samples were collected for this study.

Isolation of gDNA

Genomic DNA was isolated from preserved coral tissue, either using the DNeasy Blood & Tissue Kit (Qiagen; Hilden, Germany) following the Animal Tissues: Spin-Column Protocol (Qiagen; Hilden, Germany) or the GenCatch Blood & Tissue Genomic Mini-Prep kit following the manufacture's protocol (Epoch Life Science; Texas, USA). After DNA extraction, samples were loaded into a 0.8% agarose gel

to visually asses the presence and quality of extracted genomic DNA. Next, the DNA was quantified with the use of a NanoDrop spectrophotometer (Thermo Fischer Scientific Co.; Waltham, Massachusetts, USA) to determine sample DNA concentration.

gDNA Amplification and Library Preparation

PCR procedures were conducted using protocols from the University of Miyazaki, Miyazaki, Japan (Taninaka et al. 2021). Eight pairs of multiplex ISSR primers were used from Suyama and Matsuki (2015). The Multiplex PCR Assay Kit Ver.2 was used to amplify fragments using a total reaction volume of 7 µl in a thermocycler. The following parameters for the PCR protocol were input into the thermocycler as such: 94°C for 1 min, followed by 29 cycles at 94°C for 30 sec, 38°C for 1 min, 72°C for 1 min, and final extension at 72°C for 10 min. The initial PCR product was diluted fifty-fold and was used as template DNA for the second, tailed-PCR which incorporated the Illumina adapter sequence and individual indexes for each sample. PrimeSTAR GXL DNA polymerase (TaKaRa Bio Inc., Otsu, Shiga, Japan) w.as used for the secondary PCR procedure with a total reaction volume of 12 µl. The following parameters were input as follows: 20 cycles at 98°C for 10 s, 54°C for 15 s and 68°C for 1 min. 1 µl of each secondary PCR product was pooled in a single library mixture. The sample mix was visualized on a 0.1% agarose gel to verify amplification. Amplified gDNA ranging from 350 to 800bp was excised from the agarose gel on a transilluminator and extracted using the FastGene Gel/PCR Extraction kit (Nippon Genetics, Tokyo, Japan). A Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, United States) was used to measure library concentration. After library preparation, the sample DNA library was sequenced on a MiSeq Sequencer (sequencing control software v2.0.12, Illumina, San Diego, CA, Unites States) using a MiSeq Reagent Kit v3 (150 cycles; Illumina). Real-time analysis software v1.17.21 (Illumina) was used for image analysis and base calling, and in addition altering the DarkCycle option from “Amplicon-dark 17-3” to “Amplicon-dark 17-17” on the “Chemistry” line (Suyama and Matsuki 2015).

Sequence Processing to SNP Genotyping

To first eliminate low-quality reads and primer sequences from the raw MIG-seq data, the FASTX-toolkit version 0.0.14 (Gordon, A., and Hannon 2012), with a FASTQ-quality-filter setting of -Q 33 -q 30 -p 40, was used. Cutadapt version 2.10 (Martin 2011) was used to remove Illumina MiSeq adapter sequences from both the 5' and 3' ends. Reads < 80 bp were filtered out with local python script. These filtered raw reads were then mapped against the reference genome of *Heliopora coerulea*. which was derived from *H. coerulea* larvae, free of Symbiodiniacea-DNA (10.6084/m9figshare.14356418). Stacks v.2.2.0 (Catchen et al. 2011; Rochette et al. 2019) was used to align reads to the reference genome, following the reference aligned pipeline for discovering single nucleotide polymorphisms and genotypes. Variant calling of genotypes was done by first excluding unpaired reads from the data using the BBtools' repair.sh software (Bushnell, 2019). The remaining reads were aligned to the reference genome using Burrows-Wheeler Aligner (BWA) v. 0.0.17—r118 with default indexing parameters (Li and Durbin 2009). SAMtools v.1.10 (Li et al. 2009) was then used to index, sort and compress alignments. The *gstacks* program with parameters configured to the default settings within the Stacks software package were used to identify SNP genotypes for individuals. Stacks' *populations* program with option *-r* was used to filter the minimum proportion of individuals per locus for SNP identification. For phylogenetic analysis, a minimum of 50% of genotyped SNPs were required for individuals, while for clonal and population genetic analysis, 90% of the genotyped SNPs in individuals was required. The option *-ordered_export* was invoked to ensure only one overlapping SNP was output from aligned reads. PGDSpider version 2.1.1.5 (Lischer and Excoffier 2012) was used to convert data to the proper formatting for downstream genetic analysis.

Clonal Assessment

Genetically similar individuals possessing the same multilocus genotypes (clones), were identified and excluded from the data set before genetic analysis. Technical replicates were not included within the data set to determine clone identity. To first identify possible clones, NgsRelate was used to

infer relatedness between individuals. Although all reads used for analysis retained a base quality score of > 30, < 50% of all samples retained a 5X sequencing coverage depth. Since there was low coverage (< 10X) for a majority of the samples, genotype likelihoods were used to infer relatedness instead of called genotypes. To determine relatedness of individuals, population allele frequencies and genotype likelihoods were obtained using ANGSD. Coefficient of relatedness (r), between individuals with $r \geq 0.5$ were obtained from identity-by-decent (IBD) probabilities using NgsRelate, then noted for elevated relatedness. To further support clone identification, identity-by-state distance probabilities between individuals were generated using ANGSD and subsequently visualized via dendrogram in R studio. Possible clone mates were noted for longer branch lengths and appearing below the clonal threshold of 0.1 in the distance dendrogram. In addition, GenoDive v. 3.05 was used to determine possible clones based on the infinite allele model (IAM) within each population and omitting all missing data. A clonal threshold setting for differences (d) in multi-locus genotypes respective to each geographic population was used to identify clones upon visual estimation of the pairwise distance histogram generated in GenoDive: Cocos-East (d = 26), Luminao (d = 24), Pago Bay (d = 22), Ritidian (d = 42). Prior to clonal identification, the data set consisted of 140 individuals and 991 SNPs (r = 0.8) were used. A summation of combined clonal analysis identified 30 individuals likely to be clones, which were excluded from the data set, while the remaining 110 individuals were used for subsequent genetic analysis.

Phylogenetic Analysis

A variable site sequence alignment derived from the data set was used for phylogenetic tree construction of individual *H. coerulea* samples collected in Guam. To enable SNP ascertainment bias, variable site sequence alignments were used instead of entire sequences. The *populations* program in Stacks (Catchen et al. 2013) was used to produce the variable site sequence alignment, obtaining different minimum proportions of genotyped individuals per locus for phylogenetic analysis (*-r* = 0.5). Phylogenetic tree construction was based on the Maximum likelihood (ML) method and was generated in the program IQ-Tree2 v. 2.0.6 (Minh et al. 2020) using model PMB+F+ASC+R5 . Model

PMB+F+ASC+R5 was the best fit model selected based on the Bayesian Information Criterion (BIC). Non-parametric bootstrap analysis was performed with 1000 iterations using IQTree2. FigTree v.1.4.4 (Rambaut 2012) was used to visualize final tree construction.

Population Genetic Analysis

To examine population genetic structure of *H. coerulea* around Guam, an identity-by-state (IBS) covariance matrix was generated in ANGSD using filters *-uniqueOnly 1 -remove_bads 1 -minMapQ 20 -minQ 25 -dosnpstat 1 -doHWE 1 -hetbias_pval 1e-5 -skipTriallelic 1 -minInd 112 -minMaf 0.05*. The IBS matrix was then used to conduct Principal Coordinates Analysis (PCoA) and was visualized in R Studio (RStudio Team 2020) to determine genetic structure between and among study sites. A Minimum Spanning Network/Haplotype Network was also generated to discern the genetic relatedness within and between populations of *H. coerulea* on Guam.

Morphological Analysis

Morphological analysis was conducted up visual inspection in the field and additional visual inspection of photographic documentation of each individual colony. For this study, 3 different morphotypes were described: branching, lobate, and hybrid (an intermediate morphotype between the branching and lobate morphologies). Branching and lobate morphological classifications were assessed based on previous studies (Taninaka et al. 2021 and Villanueva 2016). Morphology of individuals in relation to study site was assessed to determine if there were any morphological correlations with geography.

Results

Clonal Analysis

After clonal assessment of the data set, 30 clones were identified and removed, leaving the remaining 110 *H. coerulea* samples to be further processed downstream (Table 1.). Figure 2. illustrates a histogram that uses a clonal threshold to determine possible clones within the data set.

Table 1: Population genetic summary statistics for each population within each study site.

Study Site	Number of Clones Identified
Ritidian (Northern)	10
Cocos (Southern)	3
Pago Bay (Eastern)	6
Luminao Reef (Western)	11

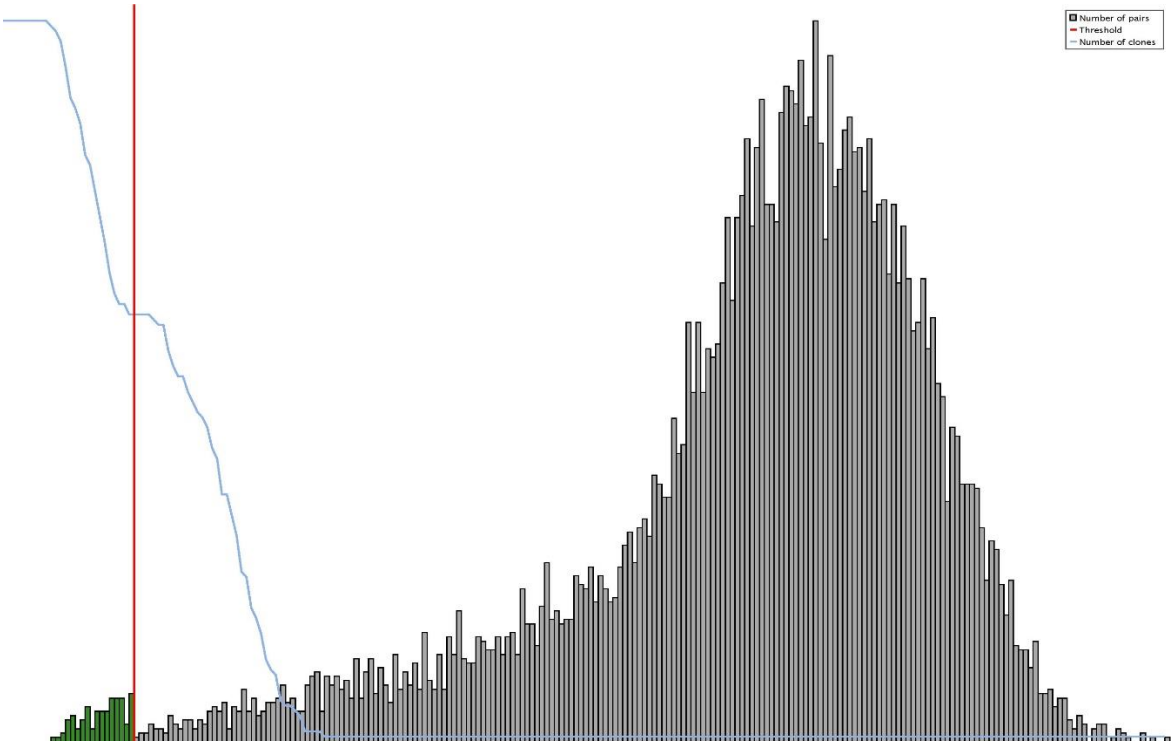

Figure 2: Histogram estimating potential clones. The *x*-axis indicates genetic distances between a pair of individuals and the y-axis indicates the number of pairs calculated by GenoDive.

Read Counts and SNPs from MIG-seq Analysis

A total of 44,259,008 raw reads were obtained from MIG-seq analysis with an average of 316,135 reads per sample. After quality filtering, removing index and adapter and short reads and unmapped reads a total of 11,042,749 reads with an average of 78,877 reads (SEM ± 3012) per 140 individuals were obtained. A total of 9,592,424 reads were mapped to the *H. coerulea* reference-genome with an average of 68,517 reads per sample. 991 SNPs obtained from 140 individuals (r = 0.8) were used to identify clonal individuals within the data set. 30 individuals from the data set were identified as clones and removed. A remaining 8,581,169 reads were kept with an average of 78,011 reads (SEM ± 3496 reads) per 110 remaining individuals over 4 study sites around the island of Guam. These 110 individuals were used for subsequent phylogenetic analysis (991 SNPs, r = 0.8) and population genetic analyses (430 SNPs, r = 0.9).

Genetic Diversity

After excluding a total of n = 30 samples from analysis because of potential clonality, the remaining n = 110 samples had extremely low traces of inbreeding (G_{IS} = -0.001 ± 0.018), as clonal individuals can induce biases into population genetic analyses. There was marginal inbreeding within the Pago Bay study site (G_{IS} = 0.008), and the Ritidian study site (G_{IS} = 0.013). Table 2. shows overall observed genetic heterozygosity among populations was low (H_o = 0.109 ± 0.005), with similar expected heterozygosity levels within populations (H_e = 0.108 ± 0.004). The average number of alleles amongst all 4 study sites was 2.0 ± 0.007 per locus. The number of alleles within each population were very similar, with the average number of alleles in Cocos Island (K = 1.60) and Ritidian (K = 1.59) slightly higher than the Pago Bay (K = 1.53) and Luminao Reef (K = 1.551) sites.

Table 2: Population genetic summary statistics for each population within each study site.

Population	Number of Samples (N)	Number of Alleles (K)	Effective number of Alleles (N_e)	Observed Heterozygosity (H_o)	Expected Heterozygosity (H_e)	Inbreeding Coefficient (G_{is})
Ritidian	26	1.594	1.164	0.109	0.110	0.013
Cocos Island	31	1.595	1.171	0.113	0.113	-0.002
Pago Bay	29	1.533	1.152	0.099	0.100	0.008
Luminao Reef	26	1.551	1.168	0.113	0.110	-0.021

Population Genetic Structure

Significant levels of population genetic structure revealed via AMOVA amongst *H. coerulea* populations was determined ($p = < 0.001$), along with low levels of genetic structuring among populations ($F_{ST} = 0.073 \pm 0.004$). Allelic diversity between all populations within study sites utilizing pairwise F_{ST} was low to moderate ($F_{ST} = 0.056 – 0.083$), but highly significant ($p = < 0.001$). The highest differentiation between study sites was Luminao Reef and Ritidian ($F_{ST} = 0.083$ Table 3.), and the most similar populations were Luminao Reef and Cocos Island ($F_{ST} = 0.056$ Table 3.). All populations maintained a similar average of allelic diversity values ($F_{ST} = 0.067 – 0.075$), with Cocos Island appearing to have the lowest average value ($F_{ST} = 0.067$ Table 3.) and Ritidian having the highest value ($F_{ST} = 0.075$ Table 3.) within the range. Both Luminao Reef and Pago Bay populations had identical averaged differentiation values ($F_{ST} = 0.070$) amongst populations.

Table 3: Pairwise F_{ST} values calculated via GenoDive between all populations. All comparisons have a significant p value ($p < 0.05$) determined after Bonferroni correction.

	Cocos_Island	**Luminao_Reef**	**Pago_Bay**	**Ritidian**
Cocos_Island	--	0.001	0.001	0.001
Luminao_Reef	0.056	--	0.001	0.001
Pago_Bay	0.071	0.070	--	0.001
Ritidian	0.074	0.083	0.068	--

NGSAdmix was used to perform admixture analyses on genotype likelihood calls generated in Angsd from SNP data. The admixture proportions of all individuals were plotted to determine the most parsimonious number of genetic clusters (K) of K= 2 (Figure 3.) though K = 6. Upon visual inspection of the admixture plots (Manzello et al. 2019), K = 2 (Figure 3.) was determined to display the most genetic clusters, consisting of a genetically distinct split between North-East and South-West populations. K = 4 plots (Figure 4.) were also generated to visualize sub-structuring of genotypes within all sites. Overall, admixture analysis revealed limited levels of population genetic structure amongst study sites, and differentiation between Northeastern and Southwestern populations of a single population of *H. coerulea*

on Guam.

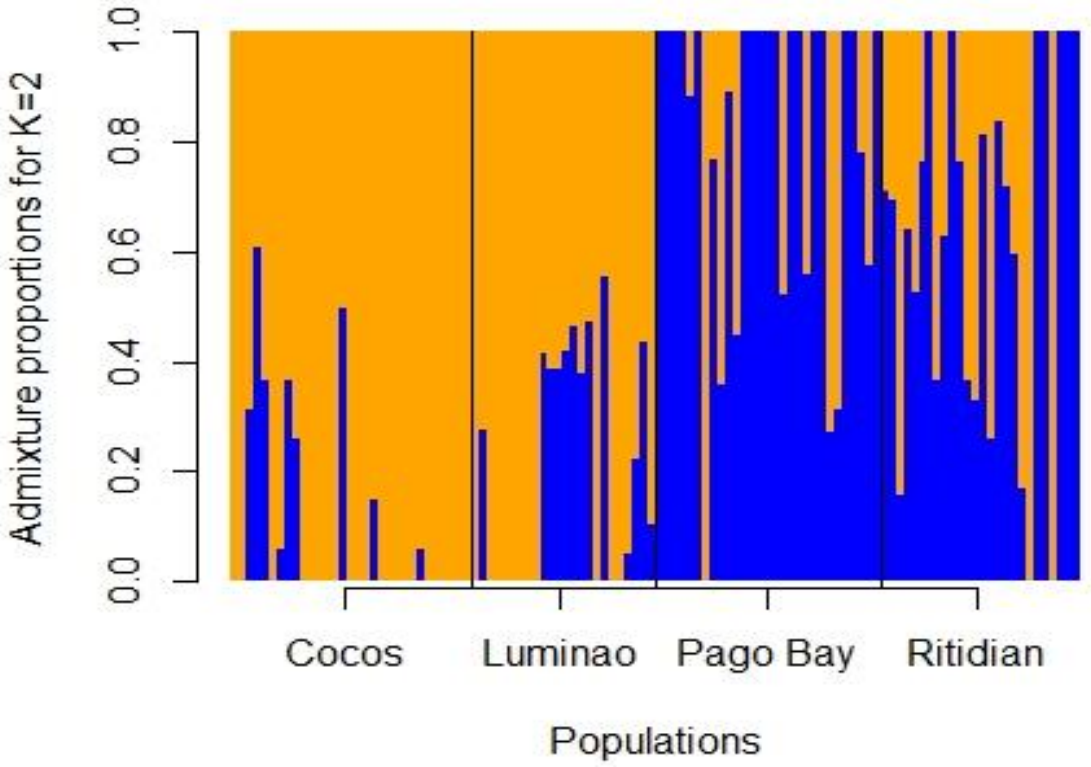

Figure 3: Admixture plot depicting K=2 genetic clusters indicated by both orange and blue colors. Each individual sample is denoted by a vertical bar within each study site. Colors also denote the proportion of genetic cluster or lineage within an individual sample

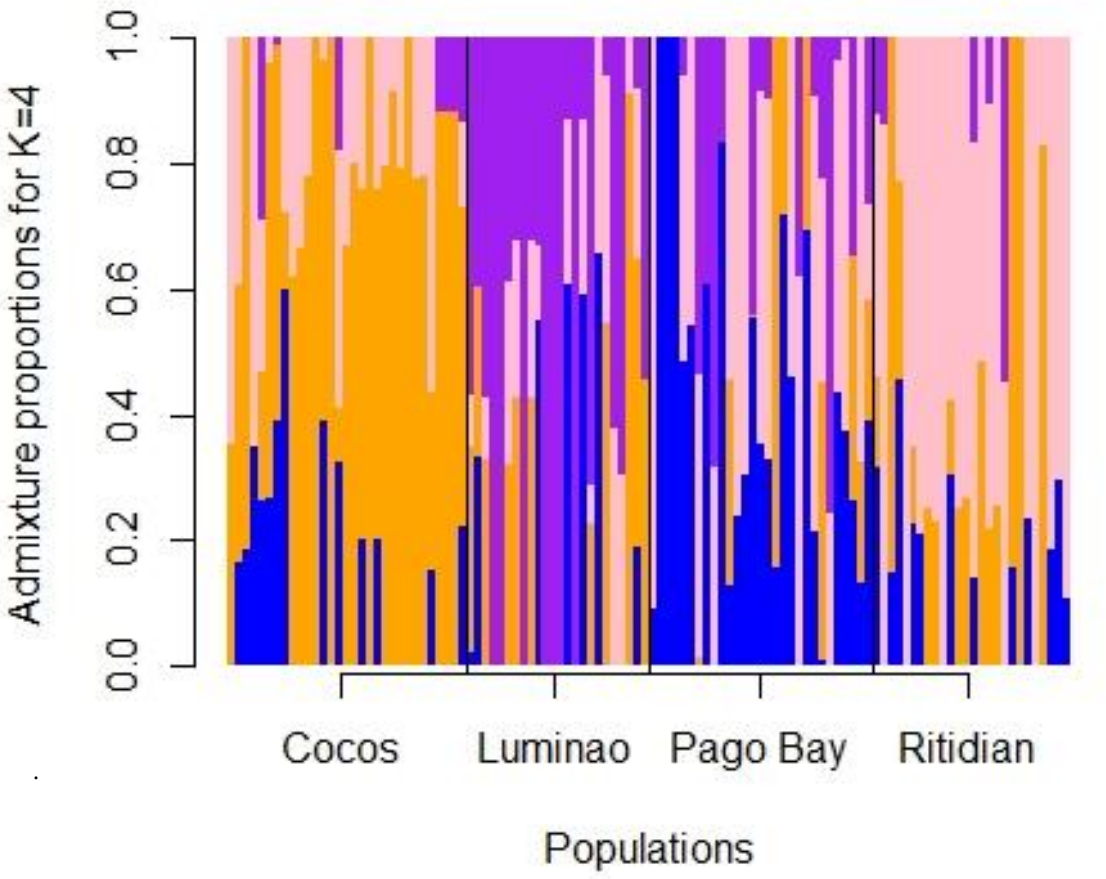

Figure 4: Admixture plot depicting K=4 genetic clusters to visualize sub-structuring, indicated by orange, pink, purple and blue colors. Each individual sample is denoted by a vertical bar within each study site. Colors also denote the proportion of genetic cluster or lineage within an individual sample.

Morphology

Out of the total sample size analyzed in this study (n = 110), just over half (approximately 51%) of the coral colonies were identified as a branching morphotype, regarding it as the overall dominant morphotype amongst the four study sites. The lobate and hybrid (a combination of both the branching and lobate morphotypes) morphotypes occurred in almost equal proportions of roughly 20% and 21% respectively amongst all the study sites. Approximately 8% of coral samples were not used for morphological analysis due to a lack of photographic documentation. The morphology of corals within the Northern-Ritidian study site was the least variable of all sites, with only the branching and hybrid growth forms existing in relatively equal proportions. Within the Eastern-Pago Bay site, only one sample

was identified as hybrid, while the majority were defined as branching, as lobate was only half as prevalent. The Western-Luminao site was the most variable overall, even though, as with all other sites the dominant branching morphotype occurred approximately three times more than the other two morphologies. The Southern-Cocos Island site was also dominated by the branching morph, and subsequently the lobate form which comprised most of the growth forms, only two corals displayed a hybrid morphology. Gross morphologies of classified individuals are depicted in Figure 5.

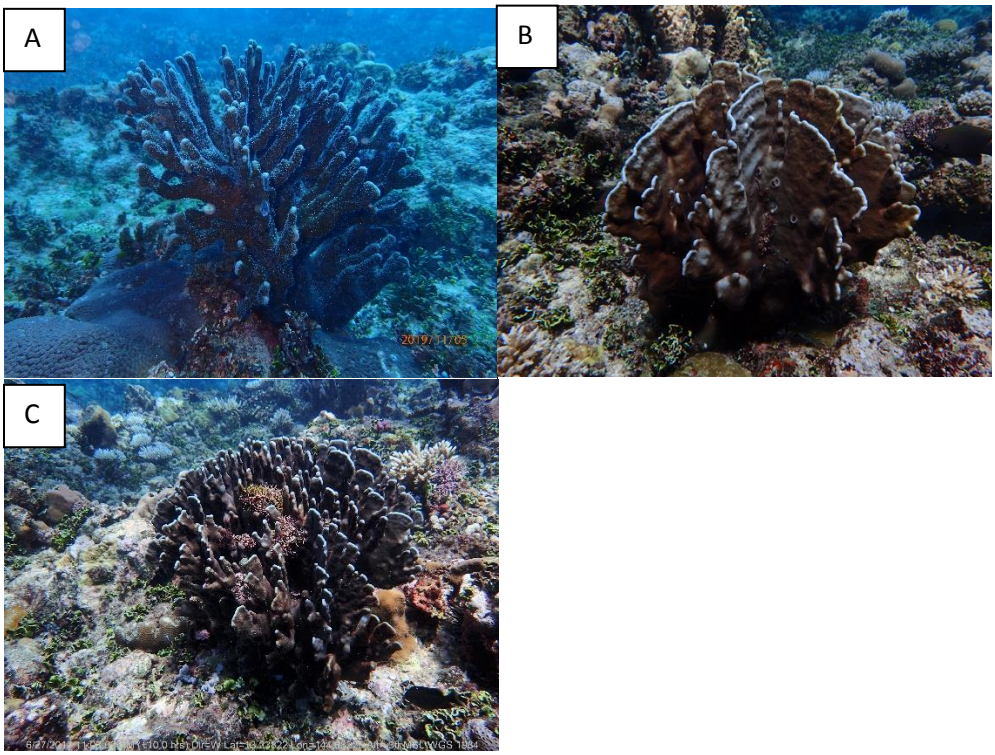

Figure 5. Examples of branching (A), lobate (B) and hybrid (C) of *Heliopora coerulea* morphotypes on Guam.

Discussion

It is well known that genetic diversity, especially as it pertains to a coral reef ecosystem, plays a major role maintaining the health and longevity of a multitude of reef associated organisms (Frankham 2005). Genetic diversity of coral reefs is bolstered by the ability for populations to exchange genetic information between genetically distinct and often geographically separated populations. Distance,

geographical features and ocean currents can form genetic barriers, which heavily influence the population structure of reef associated organisms, especially sessile coral colonies (Cavalcante, Feary, and Burt 2016; Limer, Bloomberg, and Holstein 2020; Sabatés et al. 2013; Wolanski, Richmond, and Golbuu 2021). This includes the brooding coral, *Heliopora coerulea*, a prominent hermatypic octocoral. In this study the population genetic structure of *H. coerulea* on Guam was assessed by utilizing MIG-Seq and analyzing SNP data from four geographically distinct populations. Limited to moderate population structure was observed between all four study sites, with the Northern-Ritidian site and the Southern-Cocos Island site identified as the most genetically distinct between all four sites. Genetic differentiation amongst all study sites was low, indicating a high level of overall gene flow. Genetic connectivity patterns of *H. coerulea* on Guam have the potential to be strongly influenced by the oceanic and island-generated eddies that prevail throughout the year (E. Wolanski et al. 2003). These eddies are an integral conduit for island scale genetic connectivity, trapping and returning eggs and larvae to their native reef or between other reefs.

Population Genetic Structure

Population genetic analysis of the octocoral *H. coerulea* revealed moderate to high genetic structuring amongst populations around the island of Guam. Genetic diversity between all populations studied was generally low (Fst = 0.056 – 0.083), exemplifying limited connectivity between study sites. These findings support the initial hypothesis of strong genetic structuring of populations as a result of geographic distance and innate reproductive biology as a brooding coral. In contrast, the strong genetic population structure of *H. coerulea* between all study sites indicates there is limited gene flow between populations, an artifact of its reproductive strategy as a brooding coral (Harii et al. 2002; Harrison and Wallace 1990; Nozawa and Harrison 2008). Shortened dispersal abilities commonly associated with brooding corals increase the instances of coral larvae settling closer to natal reefs (R. Babcock 1990; R. C. Babcock 1998; Harriott 1992; Harriott and Banks 1995; T. P. Hughes, A. H. Baird, N. A. Moltschaniwskyj*, M. S. Pratchett, and Willis 1999), thus decreasing the chances of dispersal to more

distant reefs. As a result of this limited gene flow, brooding corals in isolated populations have the potential to be more genetically structured with very limited genetic diversity (Ayre and Hughes 2000; Goodbody-Gringley et al. 2010; Hellberg 1996; Lasker and Porto-Hannes 2015; Nishikawa, Katoh, and Sakai 2003; J. N. Underwood et al. 2007; Jim N Underwood et al. 2009). In some cases, however, hydrogeographic forces such as sea surface currents and oceanic and island generated eddies can have a dramatic effect on the dispersal potential of brooded coral larvae, and their ability to travel over larger spatial ranges (Limer, Bloomberg, and Holstein 2020; E. Wolanski et al. 2003). Amongst all study sites, the Northern-Ritidian and Eastern-Pago Bay sites had more genetic similarity between each other, and the Southern-Cocos Island and Western-Luminao Reef sites shared more genetic similarity (Table 3.). There is evidence of overall moderate to high population structure amongst sites, including a clear genetic separation between the Northeastern and Southwestern populations. The genetic connectivity patterns amongst these study sites provide some insight into the population structure and potential larval distributing mechanisms at play for *H. coerulea* on Guam.

Hydrodynamic Forces and Larval Recruitment

The interaction between the westward-flowing NEC and the eastward-flowing NECC generates current loops and eddies that mostly travel North-Northwestwardly around Guam throughout the year (Cheng et al. 2014; Matthew S. Kendall and Poti 2014). Oceanographic forces that originate from the NEC heavily influence the formation of island-generated eddies in the Mariana Archipelago (Kendall and Poti 2015; Golbuu et al. 2012). A study from Wolanski et al. (2003) estimating near surface ocean currents around Guam using current meters, satellite-derived surface topography and a numerical model, revealed various transient eddies on the leeward side and tips of the island. Oceanic drifter data collected from 2004–2012 were used to model computer simulations that tracked cohorts of virtual coral larvae transported on ocean currents throughout the Marianas archipelago. (Kendall et al. 2016; Kendall et al. 2015). Guam was found to have the highest incidence of self-seeding larval recruits of any island, a consequence of Guam's size, geographic location, and ocean currents and eddies trapping and returning

coral larvae to Guam's reefs. This phenomenon of self-seeding potentially contributes to Guam's genetically distinct *H. coerulea* subgrouping revealed in a phylogeographic study of *H. coerulea* in the Pacific (Taninaka et al. 2021).

While eddies provide a means for larval distribution and increasing genetic connectivity, they could, conversely, become genetic barriers. For example, pelagic larvae can be prevented from being exported to certain areas as they become entrapped and retained, theoretically reducing gene flow to certain areas (Limer, Bloomberg, and Holstein 2020). Clear genetic clusters were identified that separate the Northern (Ritidian)-Eastern (Pago Bay) sites from the Southern (Cocos)-Western (Luminao) sites. Eddies inferred previously by Wolanski et al. (2003; Figure 6.) between the sites in the present study are viable mechanisms for coral larval dispersion and retention between reefs, possibly contributing to the stronger genetic connectivity between certain study sites. For example, the strongest genetic connectivity pattern between any two sites in this study were between the Cocos and Luminao, a phenomenon likely facilitated by a southwestern eddy formation (Fst = 0.056 and Table 3.). The second most notable connectivity pattern realized was between Ritidian and Eastern Pago Bay (Fst = 0.068 and Table 3.), which was likely facilitated by Northeastern eddy formation. These Northern and Northeastern eddies forming off the points of Guam, which exhibited a clockwise rotation (E. Wolanski et al. 2003), support the assumption that populations in the North of the island are the most genetically distinct. Decreased gene-flow between the Northern site and the rest of the island suggests the potential for increased larval retention. The genetic data presented here further bolsters Wolanski et al.'s (2003) suggestion that these eddies act as a mechanism for coral larval retention and distribution.

Identifying "source-sink" populations of local coral is an important component of effective management planning, helping to support and facilitate biodiverse populations via genetic connectivity between key habitat areas (Botsford et al. 2009; Mumby and Harborne 1999). Identifying and protecting vital source reef areas can help ensure the longevity and resilience of other areas through the distribution of genetically distinct organisms, thus mitigating the effects of decreased biodiversity in disturbance-

prone areas (Kool et al. 2011). The Southern-Cocos Island site had the lowest average genetic differentiation between all other study sites, which suggests elevated levels genetic connectivity between Cocos Island and the rest of the study sites and that the Southern-Cocos Island site is a major contributing "Source" population of larval recruits for Guam's *H. coerulea* populations. Reef areas, such as those near Cocos Island, that contribute to the maintenance of biodiversity on Guam's reefs can be considered as marine protected areas.

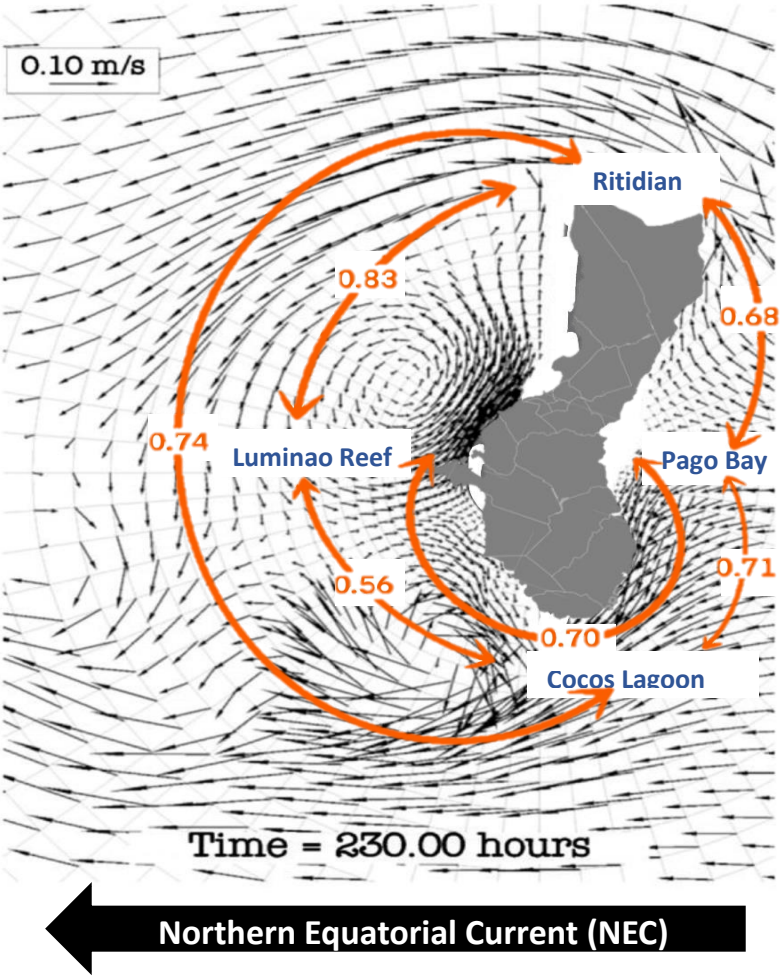

Figure 6. Fst values overlaid on Wolanski et al.'s (2003) example of predicted synoptic distribution of the predicted near-surface currents and eddies impinging on Guam.

Not only can prevailing eddies have an impact on genetic connectivity patterns, but stochastic eddies, such as those generated from cyclones, can also contribute to the randomized mixing and distribution of coral larvae amongst populations. During Wolanski et al.'s (2003) study, a cyclonic oceanic eddy 200 km in diameter, impinged on Guam, creating anticlockwise currents and disrupting the pre-existing current patterns around Guam. Even though this oceanic eddy appeared in August 2003, it is crucial to note that oceanic eddies occurring within the spawning season of *H. coerulea* are probable mechanisms for variable distribution of coral larvae around the island. Varied current patterns have the potential to contribute to the strong genetic connectivity and weak genetic structure of *H. coerulea* populations around Guam, as competent larvae are advected between populations. Oceanic and island generated eddies originating from Guam, as estimated in the Wolanski et al. (2003) study, were shown to have enough energy to suspend coral larvae for abnormally longer spatial and temporal measures, allowing the larvae to recruit to distant and otherwise more isolated reefs. Even though the maintenance of genetic diversity within a population of coral depends on many variables, such as abiotic forces and innate biological characteristics of each species population, it is estimated that only one recruitment event containing just a few larvae is enough to sustain adequate allelic diversity within an isolated population of coral (Adjeroud, Kayal, and Penin 2016; Ayre and Hughes 2000; Cowen et al. 2000). Not only do cyclonic derived oceanographic forces contain enough energy to contribute to the stochastic distribution of coral populations via the advection of pelagic coral larvae, but they can also physically fragment and translocate adult coral colonies in a form of asexual reproduction (Ayre and Hughes 2000; Nyström et al. 2000; Highsmith 1982;). Coral population dynamics are not influenced by one sole factor, but by many. It is vital to further understand how coral populations are regulated by mechanisms that influence dispersal and recruitment of coral larvae between reefs.

Morphology

Heliopora coerulea displays more than one gross morphology throughout the Pacific (Collins 2002; Taninaka et al. 2021; Villanueva 2016) and has proven difficult to assign an individual to a

particular *Heliopora* lineage based solely on morphology (Taninaka et al. 2021), likely due to phenotypic plasticity and hybridization (Taninaka et al. 2018; Yasuda et al. 2014). Assessment of these morphotypes occurring within populations around Guam revealed minor correlations between morphology and site location except for Ritidian the northern site. The lobate morphology was absent in the northern Ritidian site, which may be due to the Ritidian site being exposed to predominately high wave energy throughout most of the year. This high-wave energy environment may be putting selective pressure on certain *H. coerulea* morphologies. This study suggests that the lobate morphology is less advantageous within a high wave energy environment. Environmental variables such as water movement and light intensity are major contributors to phenotypic plasticity and morphological variation within the same species and closely related species (Todd 2008). Such variables are most likely responsible for the phenotypic morphology displayed by *H. coerulea* in Guam's waters.

The *H. coerulea* complex, detailed in a previous study (Taninaka et al. 2021), was found to be comprised of three genetically distinct subclades that correspond to geographically distinct regions: Guam, Northwestern Australia and Japan-Taiwan. The *H. coerulea* subclade from Guam was the most genetically distinct out of the three, having diverged first from the Northwestern Australia and Japan-Taiwan subclades. Despite spatial separation, the Japan-Taiwan and Northwestern Australia subclades are more closely related to each other than the geographically closer Guam subclade, which may be a result of genetic isolation events through geologic time (Taninaka et al. 2021). All 3 subclades within the *H. coerulea* group, including the Guam clade, generally displayed similar morphologies (branching and lobate) amongst all study sites. However, a hybrid morphology was found in most all study sites in Guam, displaying lobate and branching characteristics within the same colony, making it difficult to assign these colonies to a single morphological group. Further examination of *H. coerulea* morphologies on Guam, using finer spatial scales and oceanographic data can be conducted from a larger sample size, to determine potential factors influencing phenotype-environmental associations.

Research Significance

Gene flow, both within and among populations, is intrinsically important to maintaining genetic diversity of populations. A genetically diverse population has greater adaptive capability (Selkoe et al. 2016; Frankham 2005; Gates and Edmunds 1999) and thus genetic diversity decreases the likelihood of extinction events associated with changing selective pressures (Noreen et al. 2009; Bijlsma et al. 2000). Direct sequencing of *H. coerulea* genomic DNA and identification of Single Nucleotide Polymorphisms was used to delineate the genetic structure of *H. coerulea* populations existing in Guam. Through continued research, discovering genotypes prevalent in particular environmentally challenged habitats around Guam can result in the identification of key genotypic traits that can be advantageous to *H. coerulea* survival. With a high frequency of particular alleles, occurring within a population in a specific habitat, one may assume these alleles to have a significant role in individual survivorship.

Effective local management strategies are the key to maintaining the resiliency of coastal ecosystems for future generations (Gouezo et al. 2019; Mumby and Steneck 2008; West and Salm 2003; Bruno and Selig 2007) . Since *H. coerulea* is a common brooding coral on Guam, understanding how populations are structured could identify areas that serve as primary source and sink populations.

Understanding the population structure of key coral representatives on Guam could offer the ability to manage coral reef ecosystems more effectively. For example, understanding the population structure of one species could lead to the creation of a plan that could extend to many other corals exhibiting similar life history traits (Wolanski et al. 2004; Mumby and Harborne 1999; Gray 1997; Caselle and Warner 1996; Cornell et al. 1996). In addition, understanding the genetic diversity and connectivity of local populations can further management initiatives by identifying those key areas where the greatest diversity resides. Recent studies highlight the critical importance of the Micronesian islands in maintaining coral genetic diversity throughout the Pacific (Davies et al. 2015). By sheer virtue of their unique geographic location, Guam and the other 2,200 Micronesian islands serve as stepping stones for dispersing organisms, linking populations within the Coral Triangle (Indo-West Pacific Ocean) to those

throughout the Central Pacific Ocean (Davies et al. 2015). Therefore, conservation efforts on Guam have great value for maintaining the genetic diversity of corals across the Pacific.

The aims of this study were to identify genetic connectivity patterns of *H. coerulea* to determine population genetic structure between study sites and to assess the gross morphology as it relates to geography around Guam.

Conclusion

With average global temperatures increasing, coupled with an increase in anthropogenic disturbances that influence the health of coral reef ecosystems globally, a greater understanding of the dispersal patterns of key coral species is essential to effective management of Guam's reefs. Research on the *H. coerulea* spawning season in Guam would allow for a more accurate estimation of larval distribution and recruitment patterns as they relate to the influences of hydrodynamic forces. With its reproductive strategy as a brooding coral, indicative of short dispersion potential and strong population structure, *H. coerulea* could serve as a model organism for comprehending the connectivity patterns of many other coral species within the reefs of Guam. Since limited gene flow amongst populations within the study areas is present, it can be suggested that existing marine management regimes are sufficient in maintaining the genetic variability in populations of *H. coerulea* in Guam. If a management plan specifically for *H. coerulea* was implemented, for example, if this species were to suffer drastic population declines and was listed as Threatened or Endangered under the U.S. Endangered Species Act, or otherwise designated a priority species for local or federal management activities, preference to extend management protection in reef habitat adjacent to the southern tip of Guam (Cocos Island) is suggested since Cocos is likely a 'source' population. In this study, understanding gene flow between spatially distant populations of the octocoral *H. coerulea* located in Guam could serve as a proxy for understanding the genetic connectivity of other organisms, living in close proximity with similar reproductive strategies.

This would allow for an understanding of Guam reef ecology at a higher resolution on a sub-island scale, thus promoting genetic diversity distributed through-out Guam and the remainder of the Pacific.

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