WHY STUDY JUST ONE REEF: SPATIAL PATTERNS OF ENVIRONMENTAL HETEROGENEITY AND GENETIC RELATEDNESS FOR THE CORAL, *POCILLOPORA DAMICORNIS*

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAI‘I AT MĀNOA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

ZOOLOGY

MAY 2013

By

Kelvin D. Gorospe

Dissertation Committee:

Stephen A. Karl, Chairperson
David B. Carlon
Megan J. Donahue
Matthew McGranaghan
Robert J. Toonen

Keywords: landscape genetics, spatial genetic structure, molecular ecology, population genetics, adaptation, recruitment

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

ACKNOWLEDGEMENTS

I would like to thank all the volunteers who helped with fieldwork for this project, including P. Bool, T. Burgess, M. Castrence, S. Churchill, M. Coloma, C. Demyan, M. DeLisle, K. Duncan, L. Furuto, M. Gaither, M. Hagedorn, R. Haverkort, L. Hedouin, H. Hilmer, M. Iacchei, D. Jayewardene, R. Lambrix, D. Lin, R. Lindemann, M. Lurie, B. Noedl, M. Noedl, A. Pacini, A. Pan, C. Pequignet, H. Putnam, C. Rivera, T. Sauvage, J. Schultz, D. Smith, S. Soon, M. Sudek, M. Takahashi, K. Tice, C. Tran, N. Umphress, S. Webb, and K. Withy-Allen. Funding for this research was provided to S.A. Karl by the National Science Foundation Grant (NSF) OCE 06-27299 and Arcadia Wildlife, Inc. and to K. D. Gorospe by the NSF Graduate Research Fellowship Program, the East-West Center (EWC) Graduate Degree Fellowship Program, the University of Hawai‘i Edmondson Research Grant, and the NSF Hawai‘i Experimental Program to Stimulate Competitive Research (EPSCoR) Program EPS-0903833. In addition, the NSF Graduate Research Program, the EWC Graduate Degree Fellowship Program, the Office of National Marine Sanctuaries, and the Research Corporation for the University of Hawai‘i provided salary support. Coral sampling for this research was covered under Hawai‘i DLNR-DAR Special Activity Permits to the Hawai‘i Institute of Marine Biology (HIMB). Six anonymous reviewers greatly improved the manuscripts for Chapter 1 and 2. For Chapter 2, I thank S. Arnaud-Haond for constructive comments as well as O. Hardy for help with spatial autocorrelation analyses. For laboratory support, I thank C. Rocha, V. Baranets, the R. Gates Lab for providing *Symbiodinium* clade C DNA extractions, Z. Forsman for providing mtDNA ORF primers, and the HIMB NSF-EPSCoR Core Genetics Lab Facility for DNA fragment analysis support. Finally, I extend my sincere thanks to my advisor, S. A. Karl, and committee members, D. B. Carlon, M. J. Donahue, M. McGranaghan, and R. J. Toonen for their support and guidance throughout my graduate training.

ABSTRACT

Genetic isolation-by-distance describes a pattern driven by dispersal-limiting processes, whereby genetic variants sampled closer together in space are, on average found to be more genetically related than variants sampled at further distances. Many studies of population genetic patterns in corals, however, do not fit this pattern. Furthermore, increasing evidence on the primacy of local retention of larvae for marine systems as well as for the existence of locally adaptive genetic variation in corals warranted a new sampling and statistical approach. To gain insight into this "coral population genetic paradox", I present a near-exhaustive (n = 2352) assessment of individual-level spatial genetic patterns for the widely-studied, cosmopolitan, pan-Pacific coral, *Pocillopora damicornis*, within a single coral reef (Reef 19; diameter ~40 m) in Kāneʻohe Bay, Oʻahu, Hawaiʻi. Genetic and spatial data from three neighboring reefs are also included to allow for cross-scale comparisons. As environmental variation could potentially be influencing genetic patterns on this scale, I also present a reef-wide characterization of environmental heterogeneity in terms of depth, habitat cover, and temperature, drawing from a two-year dataset of *in situ* temperature variation across a 4 m grid on Reef 19. Overall, I demonstrate: (1) the existence of intra-reef, biologically-significant environmental heterogeneity, (2) that for this species, spatial patterns of individual-level genetic relatedness found within a reef do not scale up to an inter-reef level, (3) the importance of intense sampling efforts in assessing genetic diversity and revealing spatial genetic patterns in highly clonal species, and (4) the possibility of intra-reef, depth-dependent adaptation in this species. Throughout, results are discussed within the context of past studies of *P. damicornis*' genetic diversity as well as within the context of global climate change and coral reef conservation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1. INTRODUCTION

The utility of molecular tools for studying the ecology and evolution of species has proven essential, particularly in marine systems, where the challenges of tracking the dispersal of microscopic larvae and uncovering patterns of connectivity between and among populations may not have otherwise been possible. By assessing patterns of genetic diversity, scientists are able to infer the genealogical histories between individuals, populations, and higher taxa. Inferences, however, can sometimes be complicated by the fact that multiple processes (i.e., drift, dispersal, selection, mutation) work differently and on different spatial and temporal scales to produce the observed genetic patterns. Thus, patterns of genetic diversity observed on one scale do not necessarily hold on other scales (e.g., Avolio et al. 2012). This highlights the relevance of studies that bridge across scales to investigate how patterns and processes change. In fact, it has been argued that "the problem of relating phenomena across scales is the central problem in biology and in all of science" (Levin 1992).

In molecular ecology, researchers often test for a pattern of isolation-by-distance, whereby genetic variants sampled closer together in space are, on average, found to be more genetically related than variants sampled at further distances. Such a pattern can be explained by dispersal processes, whereby the genetic homogenizing effects of migration are distance-limited. Several examples from the marine environment, however, do not fit this expectation (Selkoe et al. 2010), either because the patterns are anisotropic (i.e., geographically asymmetric) or stochastic, leading researchers to give *ad hoc* explanations for the paradoxical pattern. For corals, in particular, geographic patterns of genetic variation have often proven difficult to interpret (Adjeroud & Tsuchiya 1999; Ayre & Hughes 2000; Magalon et al. 2005; Baums et al. 2006; Severance & Karl 2006; Souter et al. 2009). The interest of this dissertation is to gain insight into this coral population genetic paradox.

The original paradigm of dispersal in the sea was one of demographically open populations connected by planktonic larvae capable of dispersing long-distances across open ocean. Subsequent genetic studies, however, uncovered genetic differentiation on much smaller than expected scales. In fact, for corals, several studies demonstrate the potential for adaptive genetic variation (e.g., D'Croz & Maté 2004; Vermeij et al. 2007;

Barshis et al. 2010). Corals also share several terrestrial plant-like characteristics (e.g., dispersing propagule stage, sessile adult stage, and the ability to reproduce clonally) that are also believed to enhance the adaptive capacities of plants to small-scale environmental heterogeneity (Vekemans & Hardy 2004). Taken together, these observations warrant the study of coral genetic and environmental variation on an intra-reef scale, a scale for which there has been little interest. In ecology, it is widely recognized that patterns are scale-dependent. Population genetic sampling designs, however, rarely bridge across scales and have only recently been subject to explicitly spatial analyses whereby the spatial coordinates of individual sampling units are examined alongside genetic data (Storfer et al. 2007). Thus, taking a landscape-genetics approach, combining spatial analysis with landscape ecology and population genetics, will be important for making inferences on the processes driving patterns of genetic variation on an intra-reef scale.

The dataset for this dissertation is a near-exhaustive assessment of individual-level spatial genetic patterns for the widely-studied, cosmopolitan, pan-Pacific coral, *Pocillopora damicornis* within a single coral reef as well as a reef-wide characterization of environmental heterogeneity. The focal study site, Reef 19, is a single patch reef (diameter of ~40 m) in Kāne‘ohe Bay (21.45767°N, 157.80677°W) with a depth of between 1 and 5 m. Using a two year temperature dataset taken across a 4 m grid, Chapter 2 describes the environmental heterogeneity of Reef 19 in terms of temperature, depth, and habitat cover (Gorospe & Karl 2011). Chapter 3 (Gorospe & Karl, accepted) introduces the genetic and spatial data for *P. damicornis*, with an interest in dispersal and colonization on this scale. These data included a near-exhaustive (n=2352) genetic sampling and spatial mapping of *P. damicornis* throughout Reef 19, as well as a much smaller, stratified random sampling effort on three neighboring reefs. Rarely, however, is it practical to sample so intensively. Thus, the consequences of sampling effort and design on the characterization of reef genetic diversity are explored in Chapter 4. Finally, in Chapter 5, environmental, genetic, and spatial data are combined in a landscape genetics approach to tease apart the influence of spatially- versus environmentally-mediated processes on intra-reef patterns of genetic variation. Throughout, results are discussed from the perspective of *P. damicornis*’ breadth of literature, within the

framework of the coral population genetics paradox described above, as well as within the context of global climate change and coral reef conservation.

CHAPTER 2. SMALL-SCALE SPATIAL ANALYSIS OF IN SITU SEA TEMPERATURE THROUGHOUT A SINGLE CORAL PATCH REEF

Abstract

Thermal stress can cause geographically widespread bleaching events, during which corals become decoupled from their symbiotic algae. Bleaching, however, also can occur on smaller, spatially patchy scales, with corals on the same reef exhibiting varying bleaching responses. Thus, to investigate fine spatial scale sea temperature variation, temperature loggers were deployed on a 4 m grid on a patch reef in Kāneʻohe Bay, Oʻahu, Hawaiʻi to monitor in situ, benthic temperature every 50 minutes at 85 locations for two years. Temperature variation on the reef was characterized using several summary indices related to coral thermal stress. Results show that stable, biologically significant temperature variation indeed exists at small scales and that depth, relative water flow, and substrate cover and type were not significant drivers of this variation. Instead, finer spatial and temporal scale advection processes at the benthic boundary layer are likely responsible. The implications for coral ecology and conservation are discussed.

Introduction

As one of the most biodiverse ecosystems in the world, coral reefs are generally limited to tropical, shallow waters and cover less than 0.1% of the Earth's surface (Spalding & Grenfell 1997). Their restricted distribution, sessile adult stage, and narrow habitat preferences all point to corals as being vulnerable to global climate change and threatened by projected increases in sea temperature (Baker et al. 2008; Hoegh-Guldberg et al. 2007; Hughes et al. 2003). While corals, like many aquatic organisms, have physiological mechanisms for dealing with thermal stress (Brown et al. 2002; Shick & Dunlap 2002), the fact that they already inhabit waters that often are within a few degrees Celsius of their tolerance limits (Berkelmans & Oliver 1999; Jokiel & Brown 2004) underscores the scientific and conservation urgency to understand how global climate change will affect sea temperatures and how corals will respond.

A major response to thermal stress exhibited by corals is the phenomenon known as bleaching, whereby intracellular symbiotic algae, *Symbiodinium* spp., either leave or are expelled from the coral host. For corals, bleaching has been shown to result in decreased skeletal growth and reproductive output (Mendes & Woodley 2002), reduced resistance to disease (Ben Haim et al. 2003; Harvel et al. 1999; Lesser et al. 2007), as well as local extirpation and shifts in community composition (Burt et al. 2008; Sheppard & Obura 2005). While multiple environmental factors acting in concert result in bleaching (Brown 1997), thermal stress causing protein damage and disrupting photosynthetic reactions in *Symbiodinium* spp., has been implicated as the primary stressor in recent mass bleaching events (Warner et al. 1999; Jones et al. 1998). It is also the most important parameter in predicting when events will occur (Berkelmans 2002). One curiosity with regards to bleaching, however, is that it often appears as spatially patchy phenomena, such that bleached and unbleached corals of the same species can be found adjacent to one another on the same reef (Figure 2.1). Yet, despite observing variation among corals on the scale of a single reef, very little is known about how sea temperature may vary at the same scale. Thus, while the threat of climate change puts corals at risk on a global scale, their narrow range of environmental tolerance implies that habitat differences that exist on much smaller geographic scales may also prove to be biologically and ecologically significant. Indeed, one of the major challenges in understanding ecological processes is the quantification of physical and biological patterns at appropriate spatial scales (Fortin & Dale 2005).

On large geographic scales, mass bleaching episodes have been shown to correlate with anomalously high sea surface temperatures (reviewed in Eakin & Lough 2009). For example, the largest documented coral bleaching event occurred between mid-1997 and the end of 1998 and affected reefs in the Caribbean, Mediterranean, Persian Gulf, Red Sea, Indian Ocean, and throughout the Pacific Ocean. This event coincided with elevated sea temperatures caused by a strong El Niño-Southern Oscillation (Berkelmans & Oliver 1999). Such macrogeographic scale (e.g., ocean basin wide) bleaching events have captured the interests of conservation scientists because they offer possible clues as to how reefs will be affected by widespread rising sea temperatures caused by global climate change. This interest, combined with technological advances in

satellite remote sensing of the marine environment (Mumby et al. 2004), has driven scientific focus towards studying these ecosystems on broad geographic scales. Unfortunately, while a macrogeographic approach may be appropriate for the prediction of mass bleaching events, it may also inadvertently obscure smaller-scale ecological processes affecting individual reef organisms that are equally important.

Some attention has been turned to characterizing environmental variation among reefs and reef systems at a more regional scale (Marshall & Baird 2000). This has provided insight into explaining mesoscale (i.e., 10s–100s km) patterns of coral bleaching in the field. For example, experimental evidence shows that high and intermediate water flow environments can help to increase coral resistance to bleaching, presumably by increasing diffusion rates between the coral and external environment and thus, preventing the build up of toxic free radicals (Nakamura & van Woesik 2001). Some field studies, however, point to the opposite effect, whereby low water flow may expose corals to greater thermal fluctuations, possibly allowing them to acclimatize to temperature extremes (McClanahan et al. 2005; Castillo & Helmuth 2005). Other studies have shown that the thermal history of a reef (e.g., lagoon versus fringing reefs) may produce colonies better able to cope with high temperature stress (McClanahan et al. 2005). These studies of regional variability highlight some of the myriad of factors contributing to bleaching and point to the need for additional data to tease apart multiple, bleaching-related environmental factors. From a conservation standpoint, investigating bleaching on a variety of spatial scales may help to identify individual reefs that have proven to be more resistant to bleaching. Prioritizing such reefs as marine reserves could be the best, preemptive strategy for protecting reefs in the face of global climate change (West & Salm 2003).

Identifying processes that affect individual reef organisms within reefs, however, requires that microspatial (i.e., on the scale of meters or centimeters) environmental variation be measured. For example, despite the observation that corals of the same species separated by just a few centimeters can exhibit variable bleaching responses (Figure 2.1), sea temperature variation in the context of bleaching potential has largely been ignored at this spatial scale. Furthermore, laboratory methods, from which our current models of bleaching thresholds are derived, have primarily been limited to

prolonged, shock-based experiments that mimic the conditions of mass-bleaching episodes (Berkelmans & Oliver 1999; Warner et al. 1999; Jones et al. 1998). Rarely do these experiments take into account the temperature variability that corals are exposed to in situ. In addition, while numerous temperature indices have been described as good predictors of coral bleaching events (Winter et al. 1998), our understanding of temperature-sensitivity in corals has recently become more nuanced with the recognition that bleaching susceptibility may also be dependent upon the individual coral colony's past environmental experience and recent thermal history (Brown 1997; Castillo & Helmuth 2005; Middlebrook et al. 2008). Thus, characterization of temperature variation as it relates to coral bleaching will require an investigation of not only spatial but temporal variation over a range of scales.

Here, I present a spatiotemporal analysis of a two-year, in situ, microspatial scale dataset of benthic sea temperature variation in the context of coral bleaching on a single patch reef in Kāneʻohe Bay, Oʻahu, Hawaiʻi. While some studies suggest the possible existence of microspatial environmental variability on reefs—even as a potential mechanism for explaining spatial patterns of bleaching (Penin et al. 2007)—here, I describe such variation in detail. Our goal is not to implicate microspatial sea temperature variation as the sole or even primary mechanism by which patchy bleaching may occur, but instead, to investigate whether or not biologically relevant variation in temperature exists on small spatial scales across a reef.

Materials & Methods

Study site

A circular, patch reef in Kāneʻohe Bay, Oʻahu (Reef 19; 21.45767°N, 157.80677°W) was selected for its accessibility (snorkeling depth between one and five meters), size (~40 m in diameter), and appropriateness to the spatial scale of interest. This reef is typical of the type of reefs found in Kāneʻohe Bay as well as at some of the atolls of Northwestern Hawaiian Islands (e.g., Pearl and Hermes). Labeled reinforcing bars were placed at 2 m intervals, running north to south down the center of the reef, as well as at several points on the reef edge to be used as orientation markers during deployment and recovery of temperature data loggers (Figure 2.2).

Temperature data collection

High-Resolution Thermochron iButton temperature and time data loggers (model DS1921H; Maxim Integrated Products, Inc., Sunnyvale, CA, USA) were used to monitor spatiotemporal temperature at the study site. The iButton has a manufacturer-specified temperature range of 15°C to 46°C, an accuracy of $\pm$1°C, a resolution of 0.125°C, and a lifetime of seven to eight years (when used in 20°C–30°C environments and at a sampling rate of 10 minutes).

A total of 85 sites, situated 4 m apart in a grid pattern, were monitored for temperature (Figure 2.2). The loggers were waterproofed using liquid electrical tape and secured to the benthic substrate—rock, sand, rubble, or dead coral—using either an aluminum wire or nail. Before each deployment, all loggers were time-synchronized and programmed to begin to take temperature readings simultaneously at a user-defined time delay and sampling rate. Each logger is capable of storing 2048 temperature readings after which, data must be downloaded and cleared from memory before redeployment. Thus, loggers were collected every two to eight weeks and replaced with newly programmed loggers to ensure a virtually continuous time series. Due to the need to redeploy loggers on a regular basis throughout the study period, small gaps in the temperature time series were inevitably created. In order to minimize the duration of these time series gaps, the reef was divided into a western and eastern half, and loggers from each half were set to different redeployment schedules. As a result, time series gaps due to redeployments only lasted between one and four hours (i.e., from 1 to ~5 readings). Two other sources of time series gaps in the data resulted from (1) data saturation of the loggers occurring in situ before they could be replaced and (2) when individual loggers were lost or damaged during deployment.

Before their first deployment, all loggers were calibrated at room temperature for at least 24 hours by placing them in a sealed plastic container, free from air circulation. To account for any systematic logger-to-logger differences, I determined a calibration coefficient for each logger by dividing the individual logger's average calibration temperature by the global average temperature over all loggers. Field-recorded temperatures then, were adjusted by multiplying the raw temperature by the logger-specific calibration coefficient. As loggers were lost in the field, new loggers were

calibrated in batches as described above on an as-needed basis. Midstudy and poststudy calibration tests also were conducted on loggers that had previously been deployed. Furthermore, each logger has an internal counter that tallies the total number of sampled points it has recorded over its lifetime. This data was used to determine if there was any significant drift in the loggers' measurements over the course of the study period.

Temperature recordings began in June 2007 for the western-half and October 2007 for the eastern-half of the reef and continued until October 2009. Initially, the sampling rate was set to 25 minutes (June 2007 to July 2008 for the western-half and October 2007 to May 2008 for the eastern-half). Subsequently, and for the remainder of the study period, the sampling interval was increased to 50 minutes to allow for longer deployments and greater flexibility in the logger redeployment schedule.

Other environmental variables

Depth, relative water flow, and substrate type and cover were measured for each of the 85 monitoring sites as potential explanatory variables to be used in modeling spatial variation in temperature. For the shallow portions of the reef, a metric-labeled PVC pipe and a bubble level were used to measure depth. A standard dive computer depth gauge (IQ700, Tabata Co., Ltd., Tokyo, Japan) was calibrated using a pressurized instrument test chamber with 0.25% accuracy to 90 m of sea water (no. 48310, Global Manufacturing Company, West Allis, WI, USA) and used to measure the deeper portions of the reef.

To obtain estimates of relative water flow, half-sphere casts of plaster of Paris (100 mL of water per 250 mg of calcium sulfate hemihydrate) were deployed at each temperature-monitoring site and allowed to dissolve. This "clod card" approach (Thompson & Glenn 1994), although limited, is an elegant field method that provides time-averaged measurements of multidirectional, water flow. Clod cards were deployed on the reef for several days on each of two occasions (October 2009 and May 2010). The mass of each clod card was weighed before and after deployment in the field, and the percent loss in mass due to dissolution, averaged between the two deployments, was used as a measure of relative water flow between sites.

Finally, characterizing benthic coverage was accomplished by taking digital photographs of 30 cm x 30 cm quadrats centered at each temperature-monitoring site.

The program CPCe (Kohler & Gill 2006) was used to generate 90, spatially stratified random points on each photograph and to calculate percent coverage statistics at each location. There were four major benthic cover categories: coral, macroalgae, other substrates (e.g., dead coral, rubble, or sand), and miscellaneous (Table 2.1). Each category was subdivided into subcategories along taxonomic lines or substrate composition (Table 2.1). The two miscellaneous subcategories were other invertebrates (OINV), which primarily consisted of sponges and feather duster worms (*Sabellastarte spectabilis*), and "unknown" (UNK) for substrates that could not be identified. A Shannon diversity index was calculated for each temperature-monitoring site.

Data analysis

All spatiotemporal analyses of temperature variation were accomplished using the open source computer programming languages, Python (Python Software Foundation, http://www.python.org/) and R (R Development Core Team, http://www.R-project.org/). After logger calibration, the time series data for each monitoring site was smoothed by calculating the average hourly temperature. This created a more consistent dataset since the data consisted of both the initial 25- and subsequent 50-minute sampling intervals. In addition, this allowed for a simpler comparison of eastern versus western reef data loggers, which were not time-synchronized to each other due to different deployment schedules. Since temperature generally remained stable over the course of several hours (within the 0.125°C resolution of the iButton data loggers), it is unlikely that small-scale temporal variation is being lost as a result of smoothing.

Fourier transforms were used to decompose time series data into a linear combination of sinusoids each representing different frequencies present in the original signal (Shumway & Storfer 2006). There were a total of 15 monitoring sites that had complete time series datasets (see Results for details), thus making it possible to investigate the various periodicities of temperature variation and compare across sites. Before spectral analysis, each dataset was detrended (i.e., linear trend and mean removed) and smoothed by twice applying a modified Daniell kernel (bandwidth [L/n] = 0.000689; Shumway & Storfer 2006). The resulting time series was then used to perform a Fourier Transform and create a scaled periodogram. The periodogram can be regarded as a measure of the squared correlation between the original time series data and sinusoids

oscillating at all possible frequencies between 0.0 and 0.5 (here, frequency represents cycles per hour). The powers of the dominant frequencies as well as the 95% confidence intervals associated with each frequency were calculated. Furthermore, the same calculations described above were performed on a time series of standard deviation across all monitoring sites. Finally, to increase the resolution at low frequencies (i.e., between seasonal and diurnal periods), spectral analysis was repeated after daily averaging both the mean temperature and standard deviation across all monitoring sites.

Since the goal is to look for biologically relevant temperature variation, a series of temperature indices were developed to encompass one or more of the following temperature characteristics that have previously been implicated as potential thermal stress indicators in corals: (1) absolute temperature stress (e.g., temperature above some threshold), (2) duration at a particular temperature or cumulative stress, and (3) temperature variability or rate of change in temperature. In addition, some of the analyses performed here were modeled after techniques developed by the National Oceanic and Atmospheric Administration as part of Coral Reef Watch's satellite observation and coral bleaching monitoring program. Two products developed by them—Hotspots and Degree Heating Weeks (Gleeson & Strong 1995)—were modified slightly in my analysis. I define relative Hot- and Coldspots as any monitoring site whose temperature is more than one standard deviation above or below, respectively, the average temperature for the entire reef at a given hour. I define relative Hot- and Coldhours for each site as any hour the temperature is one standard deviation above or below, respectively, the average temperature experienced at that particular location in the past twelve hours. Twelve hours were chosen because this was the smallest cyclical period of temperature variation indicated in the spectral analysis. Thus, relative Hot- and Coldspots are based on spatially averaged temperatures, while relative Hot- and Coldhours are based on site-specific temporally averaged temperatures. The number of times that each site was a Hot- or Coldspot or a Hot- or Coldhour was tallied and normalized by the total number of logged hours for that site. Furthermore, the dataset was truncated to exactly two years (31 October 2007 to 31 October 2009) to minimize any seasonal bias by avoiding the pre-October 2007 (i.e., summer) data.

The approach described above of summarizing temperature variation over two

years effectively obscures my ability to look for temporal variation. Thus, to assess temporal variation, the Hot- and Coldspot and Hot- and Coldhour analyses were repeated for the following seasonal subsets: Winter 2007 (22 November 2007 to 13 January 2008), Summer 2008 (13 June 2008 to 13 August 2008), Winter 2008 (22 November 2008 to 13 January 2009), and Summer 2009 (13 June 2009 to 13 August 2009). Sites whose loggers were lost or broken during these seasonal data subsets were excluded from subsequent analysis and small, redeployment gaps linearly interpolated. In addition, the seasonal subsets were used in calculating average daily maximum and minimum temperatures, average daily temperature ranges, as well as overall average temperature.

Finally, the Degree Heating Hours (DHHs in units of °C-hour) were used to simultaneously characterize both the duration and intensity of heating. DHHs are calculated by tallying the number of hour equivalents the temperature at a site exceeds the maximum monthly mean (MMM) sea surface temperature of 27°C (as defined for Hawai‘i by Coral Reef Watch's Degree Heating Weeks Index; http://www.osdpd.noaa.gov/ml/ocean/cb/virtual_stations.html). Thus, for example, 2°C-hours are equivalent to two hours at 28°C or one hour at 29°C. The number of DHHs was summed per monitoring site for Summer 2008 and Summer 2009.

Overall, there were a total of 9 temperature indices: Hotspot, Coldspot, Hothour, and Coldhour (two-year and four seasonal subdatasets); overall, daily minimum, daily maximum, and daily range temperature averages (four seasonal subdatasets); DHHs (Summer 2008 and Summer 2009).

Ordinary kriging using *gstat* (Pebesma & Wesseling 1998), an extension package of R, was used to create spatially interpolated maps of the various temperature summary indices and environmental predictors. Kriging is a geostatistical spatial interpolation method that models the relationship between distance and variance of sampled points to predict values at unsampled locations. Depth, water flow, and benthic coverage data were tested for correlation with each temperature summary index using Dutilleul's modified *t*-test. The modification corrects for spatial autocorrelation in the data by adjusting the variance of the test statistic as well as the degrees of freedom. This correction is necessary because tests of significance using an unmodified *t*-test are subject to inflated rates of type I error when both the response and explanatory variables are spatially

autocorrelated (Legendre et al. 2002).

Furthermore, in order to explain spatial variation in each temperature summary index, spatial filters were generated by performing principal coordinates of neighbor matrices analysis (PCNM, also known as spatial eigenvector mapping or SEVM) on the distance matrix of the temperature monitoring sites. The resulting eigenvectors serve as spatial descriptors of the temperature monitoring sites (Borcard et al. 2004). Finally, the eigenvector-based spatial filters that were statistically significant at a $P < 0.1$ level were used, along with the potential explanatory environmental variables as part of a partial regression model in determining the relative contributions of pure environmental, pure spatial, and shared (i.e., environmental and spatial) variation in explaining temperature variation on the reef. Incorporating location as an explanatory variable in the partial regression model also reduces or eliminates spatial autocorrelation among the residuals, thus minimizing type I error rates. For each of the temperature summary indices, five models were tested: (1) a regression of the temperature summary index on depth, (2) temperature on location, (3) temperature on depth and location, (4) temperature on depth and substrate, and (5) temperature on depth, location, and substrate. Models were chosen based on minimizing Akaike's Information Criterion. Both the principal coordinates and the partial regression analyses were performed in the computer program, Spatial Analysis in Macroecology (SAM, ver. 3.1; Rangel et al. 2006).

Results

Calibration data

Over the course of the study period, a total of 201 loggers, or approximately 16.5% of the data, were lost in the field. For any given location on the reef, the percent of missing data (not including saturation or redeployment gaps) ranged from 0.3% to 49.1%. Fifteen of the sites, however, had only 0.3% missing data entirely due to the unavoidable gaps during redeployment and are considered to be complete data sets.

Calibration tests revealed that among logger variation in recorded temperatures ranged from 0.79 to 1.35°C ($\bar{x}$ = 0.80°C) and calibration coefficients ranged from 0.97868 to 1.01991 ($\bar{x}$ = 1.00004). Comparing pre-, mid-, and poststudy calibration tests showed that the mean change in calibration coefficients across 171 possible comparisons was 0.00021, indicating that loggers did not show significant drift in their calibration

coefficients. A linear regression, however, indicated a small but significant relationship between the magnitude of the change in calibration coefficient (i.e., with no regard to the direction of change) and the number of sampled points between calibration tests (r^2 = 0.03; $P < 0.05$). The amount of change, however, is negligible with a regression slope of 2.666×10^{-8}. Thus, the maximum possible calibration drift a logger could have experienced (i.e., 10 data-saturated deployments with 2048 sampled points per deployment) would be 0.00287. To put this into perspective, using the maximum observed calibration coefficient of 1.01991, the difference in pre- and postdrift calibration of a 25°C raw data point would only be 0.07°C. Therefore, drift can be safely ignored.

Spectral analysis

Figure 2.3 shows the temperature time series, averaged daily and across all monitoring sites, with shaded bars indicating the various data subsets used in creating the temperature summary indices described above. Figure 2.4 shows a representative scaled periodogram for the hourly averaged temperature time series at a single monitoring site. The periodograms point to five dominant frequencies corresponding to both solar- and lunar-driven periodicities: seasonal (365 days), principal lunar diurnal or O1 (24.83 hours), principal solar diurnal or P1 (24 hours), principal lunar semidiurnal or M2 (12.42 hours), and principal solar semidiurnal or S2 (12 hours). Peaks at eight- and six-hour periods (between 0.1 and 0.2 cycles per hour) most likely are harmonics of the principal lunar (O1) and solar diurnal (P1) components. Most notable, an expected ~28 day lunar monthly periodicity (between the seasonal and O1 peaks) is not present. Repeating the spectral analysis for the daily averaged temperature across all monitoring sites revealed no additional periodicities. Furthermore, comparing the periodograms for each of the 15 monitoring sites with complete time series datasets revealed that the relative distribution of power over the major driving periodicities was the same across the reef (seasonal signal P1 >> S2 >> O1 > M2). Finally, the scaled periodogram for the hourly standard deviation time series revealed the same five periodicities as well as an additional periodicity at 2.5 hours (frequency = 0.4 cycles per hour; see Figure 1S in APPENDIX A) Smoothing by calculating daily averages, as above, failed to reveal additional periodicities.

Modeling spatial variation

Ordinary kriging interpolation of depth (Figure 2.2) shows a smooth transition from the center, shallow areas of the reef to the outer, deeper areas and, as such, can be regarded as a representation of the reef's bathymetry. The map of water flow shows broad similarities with depth such that high and low relative flows correspond to shallow and deep sites, respectively (Figure 2.5A). In fact, depth also correlated with many of the substrate variables (discussed below). On the other hand, maps for the various temperature summary indices ranged from being visually consistent with depth, such as the proportion of time spent as a Coldhour and Winter 2008 range in daily temperature (Figures 2AS and 2BS in APPENDIX A), to being strikingly patchy as is seen for the proportion of time spent as a relative Hotspot and Summer 2008 DHHs (Figures 2.5B and 2.5C, respectively).

Tukey boxplots of the temperature summary indices reveal considerable microspatial temperature variation on the patch reef. For example, sites ranged from spending 0% to nearly 60% of the two years of temperature monitoring as a relative Hotspot (Figure 2.6A). In addition to the expected temporal variation seen in average daily minimum and maximum temperatures (Figure 3S in APPENDIX A), the boxplots also indicate seasonal and annual variation with regards to Hothours and Coldhours (Figure 4S in APPENDIX A), with a greater proportion of time being spent as a Hothour or Coldhour in the summer as opposed to the winter months (Figures 4S in APPENDIX A). Finally, Summer 2008 resulted in a considerably larger number of DHHs as compared to Summer 2009 (Figure 2.6B).

Spatial correlation analyses of substrate type using Dutilleul's modified *t*-test revealed that depth was significantly correlated ($P < 0.1$) with PCO, EKSP, RUB, SAN, and OINV (Table 1S in APPENDIX A). In addition, depth correlated with all coral (all subcategories combined) and all macroalgae (all subcategories combined). Variables that correlated significantly with depth were excluded from the PCNM and partial regression analyses to avoid colinearity among variables. The results of the partial regression analyses—reported as the percent of variation explained by location, environment (i.e., depth and/or substrate), and shared environment and location—can be found in Table 2S (APPENDIX A). For each temperature summary index, only the best model (i.e., the one

with the lowest AIC value) is reported. Thus, if a standard linear regression model (i.e., models with no shared component) fits better than a partial regression model (i.e., models that include a shared environmental and location component), then only the standard linear regression model is reported. Among the competing models, location, as described by the PCNM-generated eigenvector filters, was a component of the best model in 35 out of the 38 temperature index regressions and was the dominant (i.e., greatest explanatory power) component in 25 (Table 2S in APPENDIX A). The percent of temperature variation explained by purely spatial factors ranged from 4.6% to 48.9% (Table 2S in APPENDIX A). On the other hand, environmental variables (i.e., depth, substrate, etc.) were included in the model for 22 regressions (Table 2S in APPENDIX A). Compared with location, environmental variables had a much greater range in explanatory power (from 2.3% to 75.5%) when comparing across temperature datasets, but was a dominant component only five times (Table 2S in APPENDIX A). Finally, while depth was always included among the 22 regressions with environmental variables, AIC-based model comparisons only included substrate as part of the best model for two temperature summaries: DHHs and average daily temperature range for Summer 2009.

Discussion

Temperature variation in time and space

It is often stated that the environment is neither perfectly regular nor entirely random and that spatial patterns are, therefore, a common characteristic of the natural world (Legendre & Fortin 1989). Visual inspection of the spatially interpolated maps of water flow, depth, and by extension, most substrate variables, which correlated significantly with depth, reveal that my choice of environmental variables cannot by themselves account for the observed variation in temperature across the reef. Furthermore, results from the regression analyses indicate that location was the most ubiquitous statistically significant explanatory variable and, when compared to the environmental variables, was more commonly found to be the dominant component. Thus, location was the most important explanatory variable in accounting for temperature variation at the spatial scale of this study. To many, my finding that location was a significant predictor of observed temperature will not be surprising. What is unexpected, however, is how the explanatory power of location compared with that of the other

environmental variables were tested. Depth, water flow, and substrate were chosen specifically because they incorporate one or several processes and characteristics that should affect benthic sea temperature (i.e., advection, insolation, light absorption and reflection of the substrate, etc.). Yet, most of the temperature summary indices were not significantly predicted by these environmental explanatory variables or, when they were included in the model, were only a small fraction. In other words, these results indicate that at microspatial scales, two locations as little as 4 m apart on a reef may have similar depth, water flow, and substrate coverage and yet still exhibit different temperature profiles. Conversely, two locations that differ with respect to depth, water flow, and substrate coverage may in fact have very similar temperature profiles. Thus, while temperature variation at large spatial scales might be explained by certain intuitive environmental variables, the importance of these variables at much smaller spatial scales is diminished and in fact eclipsed by microspatial considerations.

It is important to note, however, that even though the calibrations applied were small (i.e., no more than 0.07°C), calibration can only correct any systematic bias present. The reported accuracy of the loggers is $\pm$1.0°C, and it is possible that some of the among site temperature differences that were found were due to poor logger accuracy. This is unlikely to be a major factor because all sites had at least two different loggers due to alternating deployments and logger loss. It is also difficult to imagine a stochastic process favoring specific sites and asymmetrically erring either too high or too low for long periods of time (e.g., up to 60% of the two-year recording period for the relative Hotspot analysis). The absolute temperature also is unimportant to many of my temperature indices because they are relative measures. If spatially patchy temperature variation were being caused by stochastic, among logger inaccuracies, one would expect all of my temperature indices to be affected equally by this. Each temperature index, however, exhibited distinct overall spatial patterns, with some indices (e.g., Coldhour and Winter 2008 range in daily temperature; Figures 2AS and 2BS in APPENDIX A, respectively) even exhibiting nonpatchy patterns.

Although I present data from only a single reef, I have no reason to suspect that these findings are particular to my field site. Overall diversity and coral cover vary from reef to reef in Kāneʻohe Bay (as elsewhere), but the monitored reef is typical of what is

found in the bay and at some of the atolls in the Northwestern Hawaiian Islands. It is located in the center of the bay and does not appear to be uniquely influenced by external inputs (e.g., river outflow, oceanic currents, etc.). Even so, further testing on other reefs is necessary to indicate how universal these results are.

Interpreting location as an explanatory variable can be abstract. Environmental variables themselves have an inherent spatial structure, which is why in a partial regression analysis, there is a distinction made between pure environment, pure location, and shared environment and location components. In other words, the pure location component is separate from the common spatial structure that is shared by the environment and response variable (i.e., the shared environment and location component). This does not exclude the possibility, however, that the pure location component in my analysis is comprised of other environmental features that were not captured by my choice of explanatory variables (Borcard et al. 2004). It is also possible that my choice of environmental variables is sound, but that the spatiotemporal scale at which they were measured was too large, and thus, limited their explanatory power. This is most likely to be true for my measurement of water flow, since relative depth and substrate cover measurements are less likely to significantly change at finer spatiotemporal scales. That location helps to explain sea temperature variation at a microspatial scale simply means that locations that are close together in space have more similar temperature characteristics than points farther apart. Thus, the most parsimonious explanation here is that location, in this study, likely refers to microscale water flow processes that were not captured by the clod card measurements.

Relative water flow was negatively correlated with depth (high flow in the shallow portions of the reef and low flow in the deeper portions)—a pattern that is consistent with the decay of oscillatory wave-driven flow with increasing depth. Water flow in the benthic boundary layer, or the layer of water at which flow is influenced by reef structure (Shashar et al. 1996), can be very different from wave- or tide-driven flow seen in the water column in terms of direction and magnitude as well as net transport, and these microscale differences can be lost as a result of time-averaging (Koehl & Hadfield 2004; Reidenbach et al. 2007). Thus, finer-scale processes (spatial or temporal) not captured by clod card measurements should not be excluded as a driver of temperature

variation at this scale. For example, water flow velocity influences the thickness of the coral thermal boundary layer, which in turn influences the rate of heat transfer between the substrate and the surrounding water (Jimenez et al. 2008). Coral pigmentation can also affect temperature such that darker-pigmented corals experience greater temperatures, but the effect is mediated by differences in water flow (Fabricius 2006). Furthermore, temporally stable temperature variation, such as that observed in this study, points to the possible influence of reef bathymetry in channeling warm or cold water across a reef (e.g., from internal waves; Leichter et al. 2005). Finally, small-scale temperature heterogeneity may be influenced by the movement of water into and through the interstitial structure of whole reefs, as evidenced by dye transport experiments (Koehl, Cooper, and Hadfield, unpublished data). All of these studies suggest that fine-scale water flow heterogeneity could be a potential driver of fine-scale temperature heterogeneity. That the observed temperature differences could not be explained by wave-driven water flow as measured by clod cards highlights the need for future research to focus on even finer spatial and temporal scale flow patterns across reefs in explaining microspatial temperature variation at the benthos.

The results of spectral analysis also lend insight into the temporal scale of the processes that are driving temperature variation. Scaled periodograms of the 15 monitoring sites with complete time series datasets reveal the same power distribution pattern across the same lunar- and solar-associated periodicities. This suggests that large temporal scale processes such as tides are not causing the observed temperature difference among sites. Interestingly, in addition to the lunar- and solar-associated signals, the periodogram for the time series of standard deviation of temperature data revealed a high frequency peak at 0.4 cycles per hour corresponding to 2.5-hour periodicity. Unfortunately, it is beyond the scope of this study to identify the specific processes involved, but other studies have pointed to high-frequency internal waves and internal tides as being potential drivers of high-frequency, subsurface temperature variation (Leichter et al. 2005; Leichter et al. 2006).

If depth, relative water flow, and substrate composition are not significant predictors of temperature variation within a single coral reef, then what is? These results suggest that benthic temperature differences on microspatial scales are likely due to finer

spatial and temporal scale advection processes. Specifically, these advection processes are on a spatial scale finer than oscillatory wave-driven flow and a temporal scale finer than tide-associated frequencies. Furthermore, my finding that biologically significant, benthic temperature variation exists on a microspatial scale demonstrates the need for future research to further explore the physical drivers of this variation as well as its ecological significance for benthic organisms.

Implications for corals

Since the 1970s, research on coral reef ecosystems has been shifting from small-scale research focused on ecological processes (e.g., competition and herbivory) to large-scale conservation and management driven research. This expansion in spatial scale was also accompanied by a paradigm shift, from one that viewed coral reefs as stable ecosystems to one that began to emphasize the vulnerability and decline of coral reefs due to a host of environmental stressors (Mumby & Steneck 2008). As our understanding of coral reefs progresses, it is important to acknowledge the rapid pace of these shifts and consider the possibility that coral reef scientists may have been pushed to scale up prematurely, obscuring the importance of ecological processes that may be occurring more locally and creating a knowledge gap in our understanding of these threatened ecosystems. It should be acknowledged, however, that high-density studies at much smaller geographical scales may involve a considerable amount of effort and expense and in some cases prohibitively so. It also is important to note that reliable, accurate data loggers for other important and interesting parameters (salinity, current, irradiance, etc.), which are necessary for this type of study, often do not exist. Even so, it is clear from this study that even reasonable outputs of money and effort can result in interesting and unexpected findings and deepen our understanding of coral reefs.

This study demonstrated the existence of microscale temperature heterogeneity on a single patch reef based on temperature summary indices that were developed specifically to investigate different aspects (e.g., absolute temperature, duration of temperature, and temperature variability) of coral thermal stress. But what relevance do microscale studies have in helping conservationists to better understand coral biology? For example, remotely sensed sea surface temperature (SST) data have been crucial to providing the first evidence of thermal stress as a primary environmental driver of

bleaching. Even so, satellite-derived SST data are spatially and temporally averaged and are based on the reflective properties of just the top few mm of the water column. They therefore do not reflect thermal stress levels experienced by individual coral colonies (Manzello et al. 2009).

As mentioned above, our current understanding of coral bleaching thresholds are based on either correlations between macrogeographic bleaching episodes and SST data or on laboratory-based experiments that ignore the actual temperature variability experienced by corals in situ. The former hides finer-scale variability that may be important for refining our bleaching models, while the latter suffers from temporal isolation that may obscure the importance of long-term acclimatization of corals to environmental conditions. In both cases, in situ temperature data at the scale of the individual colony can provide the environmental context for interpreting results and gaining a more comprehensive understanding of the spatial heterogeneity seen in bleaching patterns and thresholds of thermal stress for coral.

A full understanding of the spatial patterns of bleaching is further obscured by the biological complexity of the coral holobiont (a term used to describe the coral animal host, intracellular *Symbiodinium* spp., as well as associated bacterial and viral communities). For example, it is now well known that the genus *Symbiodinium* spp. is composed of several evolutionarily distinct clades and that these different clades have distinctive physiological tolerances and ecological roles (Baker 2003; Stat et al. 2008). The patchy distribution and zonation of *Symbiodinium* spp. have been implicated as a possible explanation for patchy bleaching (Rowan & Knowlton 1995; Rowan et al. 1997). Some evidence also points to the possibility that symbiont communities can be reshuffled after recovering from bleaching events to include more heat-tolerant clades (Baker et al. 2001; Berkelmans & van Oppen 2006). Other studies, however, report stable algal symbiont communities before and after bleaching episodes (Goulet & Coffroth 2003) or after transplant experiments (Iglesias-Prieto et al. 2004). Some evidence has pointed to *Symbiodinium* spp. clade D to be heat tolerant (reviewed in Baker 2003), but as with our understanding of coral bleaching patterns, this conclusion is based on data following severe bleaching episodes. Uncovering finer-scale differences in the physiological roles and tolerances of the remaining *Symbiodinium* spp. clades may require a consideration of

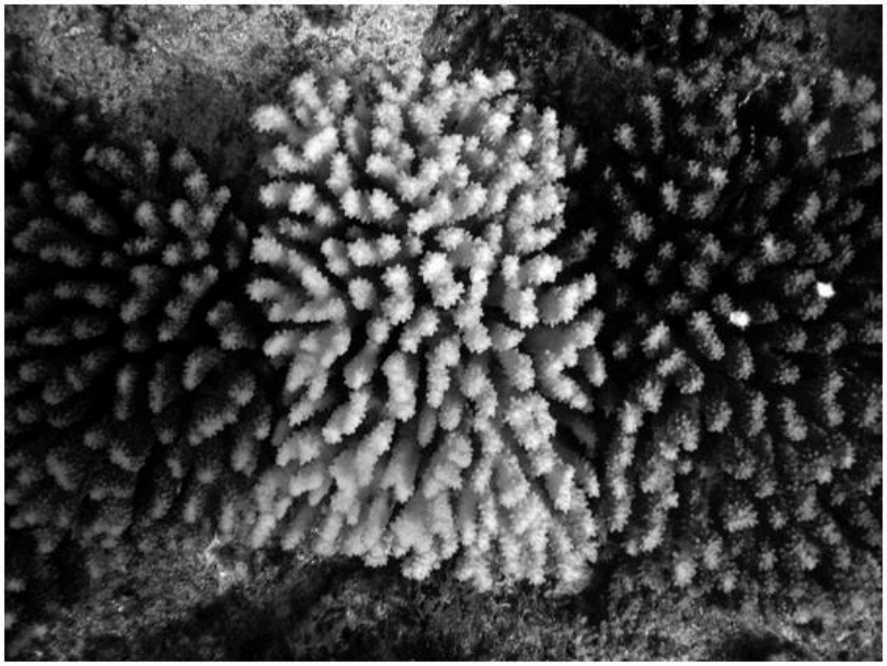
more finer temporal and spatial scale environmental differences. Nevertheless, what is clear is that there are other sources of small spatial scale or even individual-level variation on a reef. This study allows me to conclude that temperature variation can exist on the scale of meters and that this environmental heterogeneity is yet another source of individual-level variation that could explain why bleaching is patchy. My finding that a significant amount of temperature variation was not explained by depth also is consistent with the fact that patchy bleaching is not generally described as a depth-associated phenomenon.

It is possible that temperature is acting as an organizing force at small spatial scales, influencing the distribution of individuals and species within a single reef. Thus, while climate change is a global-scale phenomenon, the appropriate spatial scale at which coral reefs should be managed to cope with this threat remains an open question and underscores the need for coral studies to proceed at multiple scales. As genetic or species diversity is thought to lend stability and resilience to communities (Hooper et al. 2005), habitat heterogeneity may likewise structure reefs in ways that increase their ability to respond to climate change. In such a scenario, reefs that offer more intra-reef microhabitat heterogeneity may also harbor heterogeneous communities acclimated to a range of environmental conditions and might therefore be prioritized as marine reserves. Microspatial scale environmental heterogeneity is information that probably should be incorporated into evolving coral reef management strategies. This study demonstrates that while corals continue to be threatened on a global scale, integrating across multiple spatial scales is essential to understanding the ecological processes relevant to their survival.

Table 2.1. Categories and abbreviations used in benthic characterization.

Category	Abbreviation
Coral	
Porites compressa	PCO
Montipora sp.	MSP
Pocillopora damicornis	PDA
other coral	OCOR
Macroalgae	
Dictyosphaeria sp.	DSP
Eucheuma sp. or *Kappaphycus* sp.	EKSP
other macroalgae	OMAC
Other substrate	
dead coral with algae	DCA
recently dead coral	RDC
rubble	RUB
sand	SAN
Miscellaneous	
other invertebrate	OINV
unknown	UNK

A



B

Figure 2.1. Microspatial heterogeneity in degree of coral bleaching seen in *Pocillopora* sp. (photograph by K. Gorospe) (A) and *P. meandrina* (photograph by K. Tice) (B) on a reef at Kure Atoll, Hawai‘i.

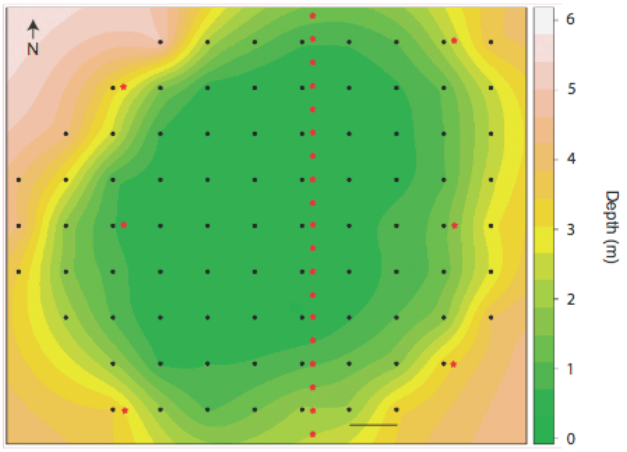


Figure 2.2. Ordinary kriging interpolation of depth across the reef. Small black dots are the locations of 85 temperature data loggers. Red stars are the locations of rebar placed for orientation. Scale bar in the lower right is 4 meters. Green areas are shallow, and peach ones are deep.

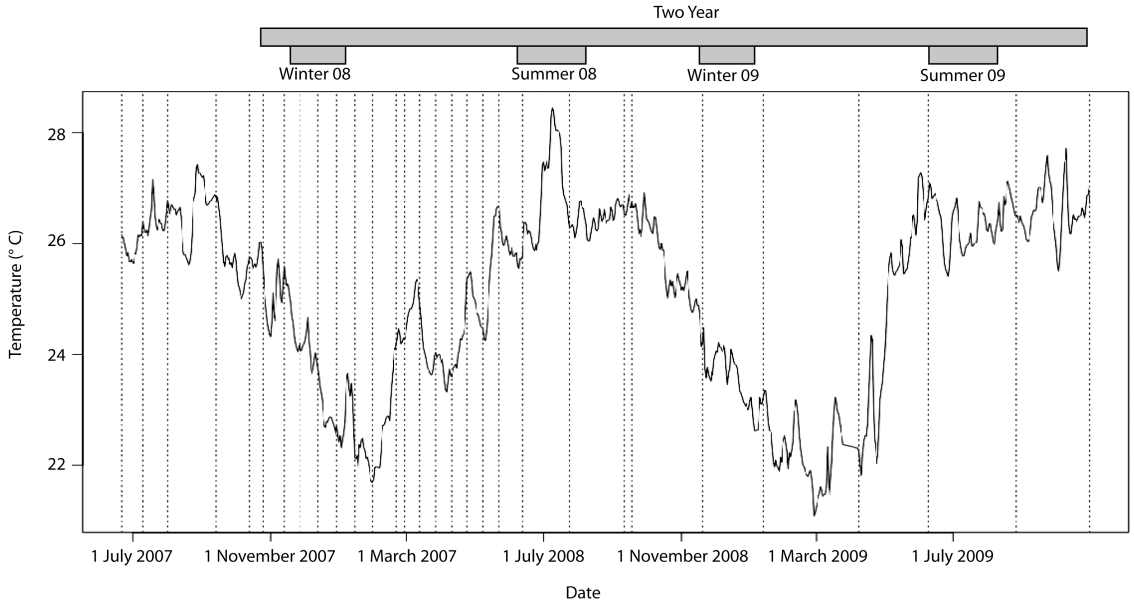


Figure 2.3. Average time series for the entire temperature data set (June 2007 to October 2008). Dashed vertical lines indicate deployment days when temperature data loggers in the field were collected and replaced. Bars above graph indicate the two-year and seasonal subsets that were used in the analyses.

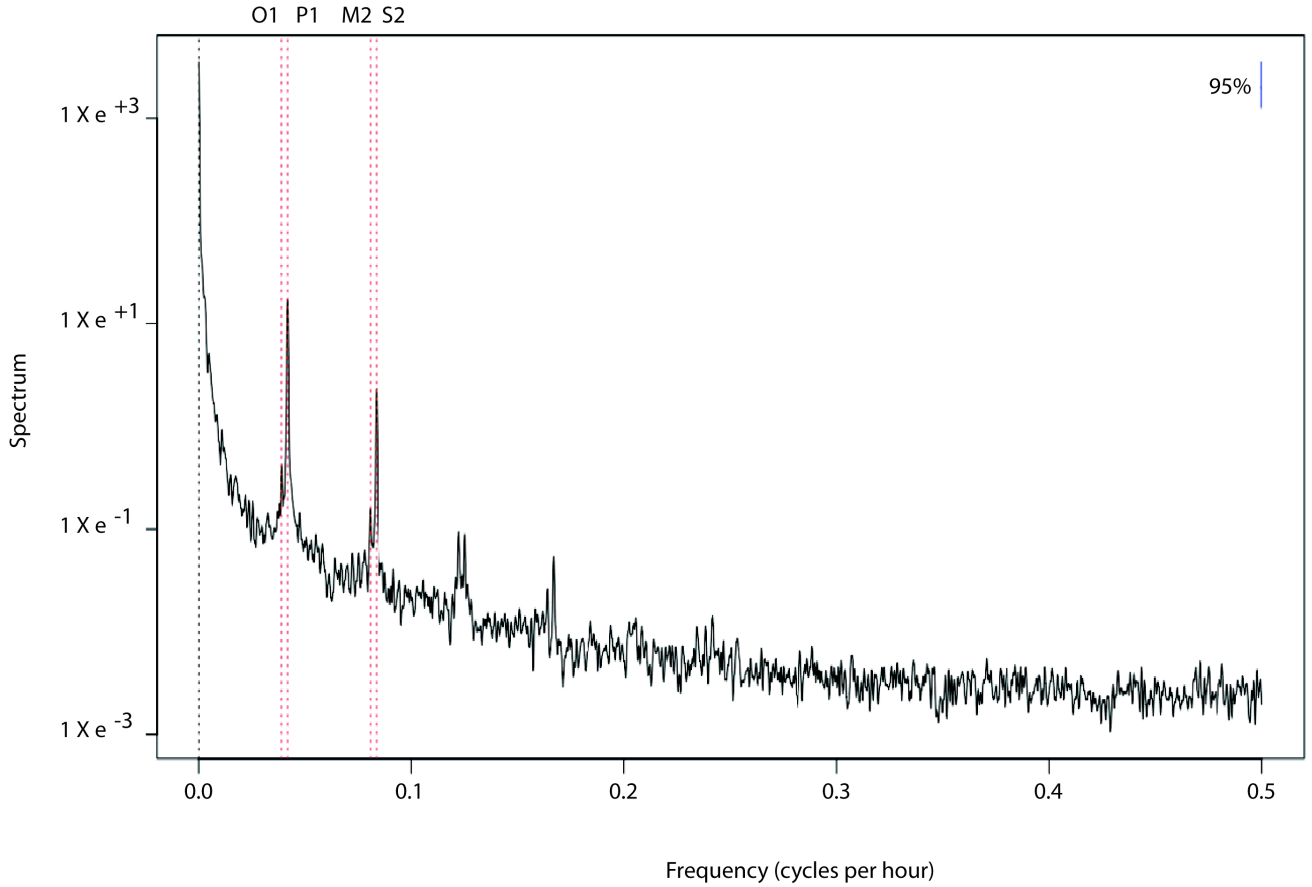
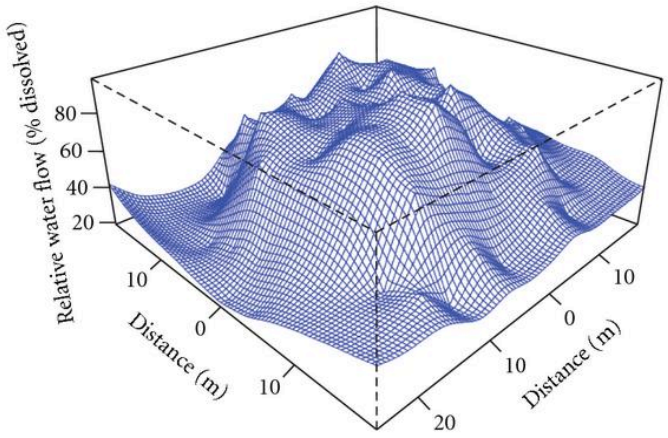
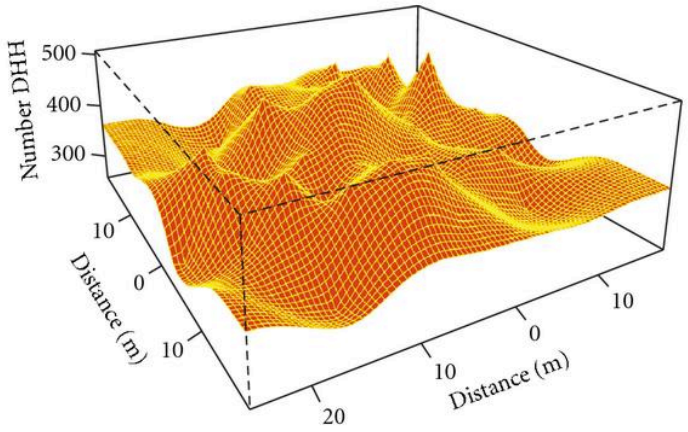


Figure 2.4. Fourier transform scaled periodogram of two years of temperature data at one monitoring site. Principal frequencies are marked with vertical, dashed lines and correspond to (from left to right) seasonal (365 days), lunar (O1, 25.82 hours), solar (P1, 24 hours), lunar semidiurnal (M2, 12.42 hours), and solar semidiurnal (S2, 12 hours). Other peaks between 0.1 and 0.2 cycles per hour are likely echoes of O1 and P1. Scale bar in the upper right corner is the 95% confidence limit.

A



B



C

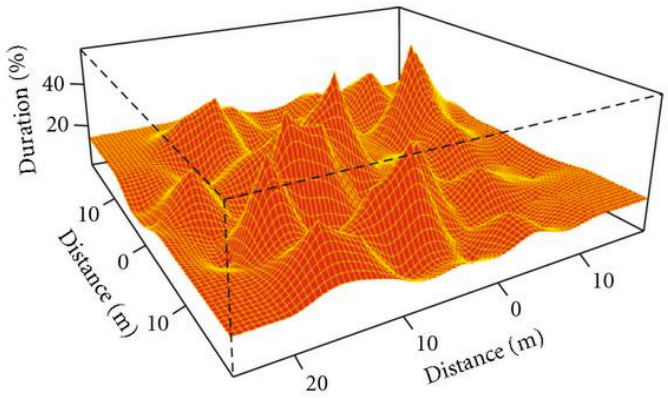


Figure 2.5. Ordinary kriging interpolation of water flow (A), percent of time spent as a relative Hotspot over two years (B), and number of Degree Heating Hours for Summer 2008 (C).

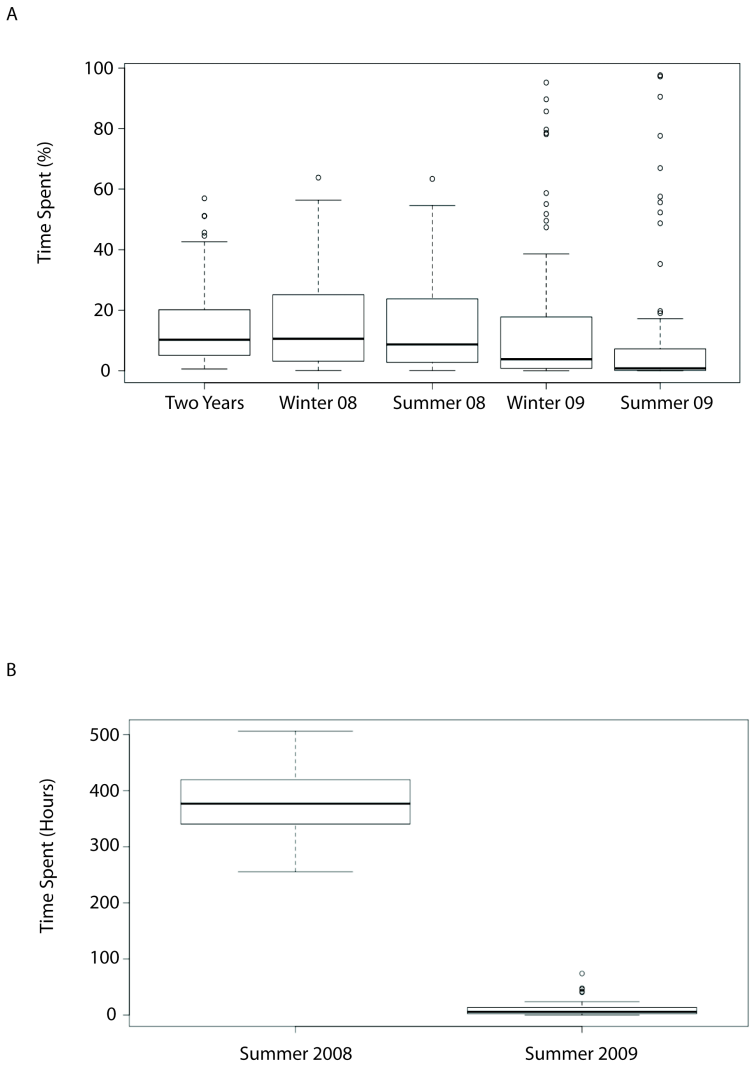


Figure 2.6. Tukey boxplots of the seasonal and annual variation in the proportion of time spent as a relative Hotspot (A) and total Degree Heating Hours for Summer 2008 and 2009 (B). The rectangle represents the interquartile range (i.e., the 25th percentile, median, and 75th percentile), the "whiskers" represent all values that are within 1.5 times the interquartile range, and the open circles represent outliers, defined as data points that lie outside the whiskers. Paired Welch's -tests were used to test for statistically significant temperature differences between seasonal subdatasets. One-tailed tests were used for comparing winter versus summer or two-year versus summer or winter datasets. Two-tailed tests were used for comparing winter versus winter or summer versus summer datasets.

CHAPTER 3. GENETIC RELATEDNESS DOES NOT RETAIN SPATIAL PATTERN ACROSS MULTIPLE SPATIAL SCALES: DISPERSAL AND COLONIZATION IN THE CORAL, *POCILLOPORA DAMICORNIS*

Everything is related to everything else, but near things are more related than distant things: Tobler's first law of geography (Tobler 1970)

Abstract

Patterns of isolation-by-distance are uncommon in coral populations. Here, I depart from historical trends of large-scale, geographic genetic analyses by scaling down to a single patch reef in Kāneʻohe Bay, Hawaiʻi, and map and genotype all colonies of the coral, *Pocillopora damicornis*. Six polymorphic microsatellite loci were used to assess population genetic and clonal structure and to calculate individual colony pairwise relatedness values. My results point to an inbred, highly clonal reef (between 53 and 116 clonal lineages out of 2352 genotyped colonies) with a very skewed genet frequency distribution (over 70% of the reef was composed of just seven genotypes). Spatial autocorrelation analyses revealed that corals found close together on the reef were more genetically related than corals further apart. Spatial genetic structure disappears, however, as spatial scale increases and then becomes negative at the largest distances. Stratified, random sampling of three neighboring reefs confirms that reefs are demographically open and inter-reef genetic structuring was not detected. Attributing process to pattern in corals is complicated by their mixed reproductive strategies. Separate autocorrelation analyses, however, show that the spatial distribution of both clones and non-clones contribute to spatial genetic structure. Overall, I demonstrate genetic structure on an intra-reef scale and genetic panmixia on an inter-reef scale indicating that, for *P. damicornis*, the effect of small- and large-scale dispersal processes on genetic diversity are not the same. By starting from an inter-individual, intra-reef level before scaling up to an inter-reef level, this study demonstrates that isolation-by-distance patterns for the coral *P. damicornis* are limited to small scales and highlights the importance of investigating genetic patterns and ecological processes at multiple scales.

Introduction

Scale is of central concern in ecology because it sets the spatial and temporal context of the natural phenomenon being described (Wiens 1989; Levin 1992) . The importance of scale can be illustrated by the phenomenon of coral bleaching whereby corals and the symbiotic algae from which they derive most of their nutrients become decoupled. On a large-scale, mass-bleaching events affecting whole reefs and atolls can be explained by large-scale (e.g., ocean basin wide) elevated sea temperatures. On a smaller scale (e.g. meters or centimeters), however, bleaching can manifest itself as a patchy phenomenon, affecting some colonies on a reef but not others. Explanations of small scale bleaching, therefore, cannot be based on large-scale measurements of sea temperature increase, but instead require a reassessment of biological and environmental variation that might also occur at small scales (Gorospe & Karl 2011). The issue of scale originates from the fact that patterns and processes observed on one scale do not necessarily hold at other scales.

Ultimately, the potential to relate processes occurring on different spatial or temporal scales is what allows scientists to integrate across seemingly separate disciplines, arguably making scale a "fundamental conceptual problem in ecology, if not in all of science" (Wiens 1989; Levin 1992). In molecular ecology and evolution, the issue of scale has primarily been viewed in a temporal context whereby ecological and evolutionary time scales are bridged by employing molecular markers with different rates of evolution, thus allowing for varying degrees of genetic resolution between related individuals, populations, and taxa. Alternatively, spatial scale is addressed in genetic studies mostly in terms of isolation-by-distance analyses between populations using sample estimates of parameters (e.g., allele frequencies). Analyses that bridge across multiple spatial scales and that use spatially explicit data (i.e., spatial coordinates and genotypes of individuals), however, also have tremendous potential for explaining spatial patterns of genetic diversity (Storfer et al. 2007). As molecular ecologists set out to describe patterns in nature, it is important that their data be spatially contextualized just as with any other ecological variable.

Patterns of neutral genetic diversity typically are explained by processes of mutation, migration and drift, such that populations that are geographically distant are

genetically more differentiated than populations that are geographically proximate (i.e., Tobler's first law of geography). Although this pattern should hold equally well for corals, it appears to be an elusive one to capture empirically (Adjeroud & Tsuchiya 1999; Ayre & Hughes 2000; Magalon et al. 2005; Baums et al. 2006; Severance & Karl 2006; Souter et al. 2009; however, see Polato et al. 2010). Instead, these studies and many other studies of marine organisms (e.g., Selkoe et al. 2010 and references therein) reveal a genetic patchiness, such that connectivity among populations is anisotropic (i.e., geographically asymmetric) or stochastic. The uncoupling of the correlation between genetic and geographic distances is what I coin here as the coral population genetic paradox. Explanations for this paradox have ranged from an examination of local oceanographic anomalies that may alter the expected pattern of gene flow (Baums et al. 2006; Severance & Karl 2006) to speculation on historical, ecological events, such as widespread bleaching and local extirpation or recruitment history, that could undo the evolutionary equilibrium required to make inferences about spatial patterns in gene dispersal (Magalon et al. 2005; Maier et al. 2005).

As we look for patterns in population genetic differentiation, however, the specific process driving these patterns may change depending on the spatial scale under consideration. This contrasts with recent trends in the coral population genetic literature that implicitly assume genetic differentiation to be patterned by larval dispersal processes at all spatial scales and require *ad hoc* explanations for instances when the data do not support these assumptions. Here, I emphasize that causative processes that generate patterns of genetic differentiation may not necessarily bridge across spatial scales and suggest that coral population geneticists may, in fact, be scaling up too broadly in their attempt to attribute pattern to process. In other words, observed spatial inconsistencies in patterns of genetic differentiation may be due to inappropriately attributing processes that operate at one spatial scale to patterns that are produced on a different spatial scale. I think a logical strategy to address this, therefore, would be to first consider coral genetic diversity on a small scale (i.e., intra-reef scale) before scaling up to broader scales (i.e., inter-reef and beyond).

The ability to characterize spatial genetic patterns is enhanced by using the individual (defined here as a physically-discrete, coral colony) as the fundamental unit of

focus and not the sample (i.e., collection of individuals from a single geographical location). Doing so allows me to address how genetic patterns may differ between individual colonies. For example, what contributions, if any, do clonal versus non-clonal coral colonies have on spatial genetic patterns? On what spatial scale or scales are these patterns found? Another advantage of assessing individual as opposed to population data, is a more unbiased description of spatial genetic structure (SGS) without having to *a priori* decide the boundaries of populations, as has often been done in population genetic analyses (Heywood 1991; Manel et al. 2003; Karl et al. 2012). To provide a comprehensive description of both clonal structure and spatial genetic patterns, I near exhaustively sampled and mapped all coral individuals within a single patch reef.

Here, I present a small-scale spatial analysis of genetic relatedness of the widely-studied, cosmopolitan coral, *Pocillopora damicornis*. Some of the first studies of fine-scale coral population genetics were pioneered using allozyme markers on this species (e.g., Stoddart 1983a; Benzie et al. 1995). Interest in *P. damicornis* has been perpetuated by the apparent geographic variation in genetic diversity reported throughout its range (Stoddart 1984; Benzie et al. 1995; Ayre et al. 1997; Adjeroud & Tsuchiya 1999; Miller & Ayre 2004; Sherman et al. 2006; Whitaker 2006; Yeoh & Dai 2009; Starger et al. 2010). *P. damicornis* also was one of the first species to reveal inconsistencies in the relationship between geographic and genetic distances (Stoddart 1984). Furthermore, the use of allozymes in these previous studies apparently does not appear to be the root cause of these inconsistencies, as subsequent development of microsatellite markers for *P. damicornis* (Starger et al. 2008) did not resolve the coral population genetic paradox for this species (e.g., Souter et al. 2009). Other studies have found strong genetic differentiation at small (10 km) (Miller & Ayre 2004) as well as large (900 km) scales (Whitaker 2006), but genetic distance did not appear to scale with geographic distance, thus limiting the ability to make generalizations regarding spatial genetic patterns from the literature. Production of asexual planulae (Stoddart 1983a; Adjeroud & Tsuchiya 1999; Yeoh & Dai 2009) and their limited dispersal ability (Tioho et al. 2001), both of which have been suggested for *P. damicornis*, warrants that a more in-depth study of intra-reef patterns of genetic variation be conducted for this species. My interests are three-fold: (1) What can be learned from studying the genetic resources of a single reef?

(2) What spatial patterns of genetic relatedness, if any, exist at the scale of a single reef? (3) What insight into the coral population genetic paradox can be gained by analyzing genetic relatedness at an individual-based, small-scale level before scaling up to a larger scale?

Materials and methods

Study sites and sample collection

For this study, I focus on the same reef described in Chapter 2 (Reef 19; Figure 3.1 and Table 3.1). Labeled rebar rods were installed every 2 m, running north to south down the center of the reef, as well as at several points on the reef edge to be used as orientation markers during coral sampling and mapping (Figure 3.2). This was the reef where near complete sampling of all *P. damicornis* colonies occurred (see below). In addition to Reef 19, three additional patch reefs within Kāneʻohe Bay (Reefs 12, 16, and 20; Figure 3.1 and Table 3.1) were chosen based on their similarity to Reef 19 in depth, size, *P. damicornis* density, and overall coral cover. In contrast to Reef 19, however, these neighboring sites were not exhaustively sampled (see below).

Coral sampling and genotyping

The location of each coral colony on Reef 19 was recorded as X-Y coordinates using standard line transect methods. A small sample of each colony was collected and preserved in DMSO buffer (20% dimethylsulfoxide, 0.25 M EDTA, NaCl to saturation, pH 8.0). Coral colonies less than 2 cm in diameter were not sampled to minimize sampling-related mortalities, although these were relatively few in number. In addition, coral fragments that were unattached to the reef substrate, also an uncommon occurrence, were avoided. All mapping and sampling for Reef 19 was completed between June 2007 and June 2008.

At the three neighboring reefs, colonies of *P. damicornis* were sampled using a stratified-random method (Baums et al. 2005). Briefly, a transect tape was secured to a rebar positioned near the center of the patch reef. Random compass headings and distances from the center were generated using the open source computer programming language, R (http://www.R-project.org, R Development Core Team), and used to locate approximately 20 colonies within each of three nested circles (between 0 and 2 m, 2 and 4 m, and 4 and 6 m) for a target total of 60 individuals. If no *P. damicornis* colony was

found at a particular compass heading and distance, the nearest colony was sampled and its coordinates recorded. Compass headings were rounded to the nearest 5˚ and distances to the nearest 5 cm. If only a single coral colony was found closest to two or more of the randomly generated coordinates, the colony was only sampled once and new random coordinates were chosen. This primarily happened at the outer most sampling distances (between 4 and 6 m), where the density of *P. damicornis* was noticeably lower. Neighbor reef collections were completed within the month of June 2010.

DNA was extracted from all coral samples using a HotSHOT protocol (Meeker et al. 2007). For each sample, a 4 mm^3 fragment was placed in 200 µl of 50 mM NaOH, heated (95° C), pH-neutralized with 1 M Tris-HCl, pH 8.0 and centrifuged. Extracts were diluted 1:20 with water. Generally, final DNA concentrations were between 1 and 10 ng μl^{-1} (based on NanoDrop ND-1000, Thermo Fisher Scientific, Waltham, MA). For each 6 µl PCR reaction, 1 µl of the diluted DNA solution was used as template.

QIAGEN® Multiplex PCR Kits (QIAGEN, Hilden, Germany) and fluorescently-labeled primers were used to amplify five published microsatellite loci (Starger et al. 2008); Pd2-001, Pd3-004, Pd3-005, Pd2-006, and Pd3-010) and an additional locus, Pd2-AB79 (F: 5'-GGAGATGGATGGAGACTGCT-3', R: 5'-CGAGTGCACGCACTAGATAGA-3') taken from GenBank (accession number AB214379, unpublished). For Pd3-010, I used a different primer annealing region from that published in (Starger et al. 2008) based on the sequence deposited in GenBank (accession number EF120465). Thus, in this study, I refer to the Pd3-010 locus as Pd3-EF65 (F: 5'-TGTGCAGGTGTTGTGACTGA-3', R: 5'-TGTCTTTTTCACTTTTGCTTCAA-3'). In addition, extractions of *Symbiodinium* clade C DNA were used to verify that all primers were coral-specific. The thermocycling protocol was 15 min at 95˚ C then 27 cycles of 94˚ C for 3 sec, 57˚ C for 3 min and 72˚ C for 30 sec with a final 30 min at 60˚ C. Alleles were scored on a 3100 Genetic Analyzer with GENEMAPPER 4.0 software (Applied Biosystems, Foster City, CA). Furthermore, in light of the recent debate of species delineation within *P. damicornis* (Pinzón & LaJeunesse 2010; Combosch & Vollmer 2011), I used the program GENODIVE (Meirmans & van Tienderen 2004) to perform a Principal Components Analysis (PCA) on the matrix of covariance between pairs of allele frequencies to see if this would suggest the presence

of cryptic species in my dataset. Furthermore, one ramet from each genet group was randomly selected and sequenced at a recently discovered mtDNA open reading frame of unknown function (Flot & Tillier 2007) to check for conspicuously divergent haplotypes.

Lastly, I should clarify here my use of the terms ramet and genet. I use the term genet to refer to a group of individuals with identical multi-locus genotypes (MLGs) and ramet to refer to each individual coral colony, irrespective of their MLG. Likewise, I define clones as individuals with identical MLGs and non-clones as individuals with different MLGs (Harper 1977; Arnaud-Haond et al. 2007). The terms genet, ramet and clone, therefore, are not interchangeable, as genet refers to a group of individuals with identical MLGs, while two ramets may have identical MLGs (i.e., clones) or different MLGs (i.e., non-clones).

Data analysis

Hardy-Weinberg equilibrium and locus-level analyses

The program, GENCLONE 2.0 (Arnaud-Haond & Belkhir 2007), was used to determine the number of different alleles between each genet pair. If genet pairs were found to have only one or two allelic differences between them (i.e., 236 out of 3003 comparisons; 8%), the chromatograms of all ramets belonging to these genets were double checked for scoring errors and were re-extracted, re-amplified and re-scored as necessary.

Both single and multi-locus data were used to perform Hardy-Weinberg equilibrium (HWE) exact tests. In addition, for each pair of loci, I tested the null hypothesis that alleles at one locus are independent from alleles at the other locus (i.e., linkage equilibrium). A Markov chain algorithm (Guo & Thompson 1992; Raymond & Rousset 1995) was used to compute the log likelihood ratio statistic (*G*-test) and test for statistically significant linkage disequilibrium between loci. Sequential Bonferroni adjustments (Rice 1989) were applied to account for false positives due to multiple, non-independent comparisons. I also calculated traditional population genetic parameters, including: allele frequencies and Wright's inbreeding coefficient (F_{IS}) for each locus (Weir & Cockerham 1984), as well as observed and expected heterozygosities. Tests for linkage equilibrium were performed both before and after the removal of repeated MLGs. All computations were made using the web-based version of GENEPOP 4.0 (Raymond &

Rousset 1995; Rousset 2008), based on only unique MLGs (i.e., one ramet per genet), and reported separately for each reef.

Individual-level spatial genetic patterns

I used the program, Spatial Pattern Analysis of Genetic Diversity version 1.3 (SPAGeDi: Hardy & Vekemans 2002) to conduct spatial autocorrelation analyses, a technique that calculates a genetic relatedness coefficient for all pairs of individuals and bins individuals into a set of predefined distance intervals to test for spatial dependency in the distribution of individual genotypes (Epperson & Li 1996). The resulting correlogram defines the relationship between genetic relatedness and physical distance between coral colonies. The Hardy and Vekemans coefficient of relationship (Hardy & Vekemans 1999), based on Moran's *I* measurement of spatial autocorrelation (Moran 1950), is reported here. This particular index was chosen because it is not based on an assumption of HWE, thus allowing me to compare spatial genetic structure between my sampled reefs while accounting for potentially different inbreeding levels (Hardy & Vekemans 1999).

Three different spatial genetic autocorrelation analyses were performed. The first, which I refer to as the ramet-level analysis, included all individuals (i.e., all genets and all ramets in each genet), thus describing the overall pattern of SGS without regard to clonal *versus* non-clonal relationships. The second, which I refer to as the genet-level analysis, only allowed for pairwise comparisons between ramets from different genets, thus, if present, removing the effect of spatially-clumped clones on SGS and thus, focusing only on the effect of SGS patterns due to non-clonal pairs (Alberto et al. 2006). Finally, for a third analysis, a new dataset was created to address the potential spatial genetic structuring driven specifically by spatially-clumped clones. Here, I convert the MLG of each individual into a haploid, single-locus genotype whose allelic identity was unique for each genet group. For example, all colonies in MLG 1 were assigned the genotype "01" and all colonies in MLG 2 were assigned the genotype "02" and this was done for all MLGs (i.e., MLGs 1 to 78 were assigned the genotypes "01" to "78", respectively). In contrast to the original dataset, where positive spatial genetic autocorrelation can be attributed to both clones and closely related non-clonal genotypes, the haploid dataset contains only completely identical pairs of genotypes (ramets within genets) and

completely different pairs of genotypes (ramets among genets). Thus, by removing the effect of closely related genotypes, this analysis, which I refer to as the clonal distribution analysis, describes the spatial genetic autocorrelation due primarily to clonality. Furthermore, comparisons between the clonal distribution and other analyses are possible because Hardy & Vekemans' relationship coefficients are not influenced by ploidy level (Hardy & Vekemans 1999). Together, the three analyses are meant to describe, respectively, the SGS due to all ramets (i.e., clones and non-clones), ramets between genets (i.e., non-clones), and ramets within genets (i.e., clones).

For the neighboring reef dataset, polar coordinates were first transformed into X-Y coordinates, but due to their smaller sample sizes, only the ramet-level analysis was performed. Finally, to allow for comparisons of the SGS analyses on Reef 19 with those performed separately for the neighboring reefs, I simulated subsampling Reef 19 using the same stratified-random methodology used for the neighboring reefs and repeat spatial autocorrelation analysis on this subsampled dataset.

The dataset was binned into 12 distance classes such that the number of pairwise comparisons within each distance interval was approximately equal. This was also done for the neighboring reefs, but due to the smaller sample sizes and the smaller spatial scales at these sites, there were only six distance classes. Statistical significance was based on permuting individual locations among all individuals 200 times and calculating upper and lower 95% confidence intervals for each distance class. Note, however, that for the genet-level analysis, significance values based on spatial permutations are invalid because by randomizing locations the spatial structures both between and within each clonal group are broken down, when in fact, within-group spatial structures should be retained (Hardy & Vekemans 2009). Thus, for these analyses, jackknifing across loci was used to calculate one standard error around relationship coefficients as an approximation of statistical significance.

Lastly, in a two-dimensional model of isolation-by-distance, kinship coefficients between individuals are expected to have a linear relationship with the logarithm of distance (Rousset 2000). While the genetic relationship coefficient described above for my spatial autocorrelation analyses are based on the proportion of genes shared between individuals, the Loiselle kinship coefficient used here is based on probabilities of identity

in state for randomly sampled genes between individuals (Loiselle et al. 1995; Hardy & Vekemans 2002). Using the slope of this linear relationship (Rousset 2000; Vekemans & Hardy 2004), I use SPAGeDi to estimate both gene dispersal distance, σ (defined as ½ the average parent-offspring distance) and neighborhood size, N_b, (defined as $N_b = 4\pi D_e \sigma^2$, where D_e is the effective population density accounting for variance (σ^2) in reproductive success among individuals; Wright 1943).

Population-level spatial genetic patterns

In order to perform a population-level, isolation-by-distance analysis, pairwise genetic distances (F_{ST}) based on unique MLGs for each reef were first calculated using an analysis of molecular variance (AMOVA; Excoffier et al. 1992) as implemented in GENODIVE (Meirmans & van Tienderen 2004). Each reef's GPS coordinates were then used to calculate pairwise geographic distances. Finally, in order to test for a significant relationship between the genetic and geographic distance matrices, a Mantel test was performed using the web-based program, IBDWS (http://ibdws.sdsu.edu/~ibdws/; Jensen et al. 2005). Tests were performed both pre- and post-logarithmic transformation of raw genetic and geographic distances.

Clonality

Maps of the locations of all corals sampled on each of the four reefs were produced in R (http://www.R-project.org; R Development Core Team). A custom R-script also was written to create maps that highlight the locations of all ramets within each genet among the four sampled reefs (e.g., Figure 3.3).

Due to my intensive sampling effort and use of highly variable markers, I used my dataset to estimate the levels of various reproductive strategies in my study area. First, I estimate the rate of selfing for this species by using the program RMES (David et al. 2007), which models this based on among-locus correlations independent of inbreeding levels. Next, I use the program GENODIVE (Meirmans & van Tienderen 2004) to calculate a variety of clonal diversity indices, including: clonal richness, N_G/N, (Ellstrand & Roose 1987) where N_G is the total number of unique multi-locus genotypes and N is the number of genotyped samples; genotypic diversity, G_O/G_E (Stoddart 1983b), where G_O and G_E are observed and expected genotypic diversities, respectively; as well as Simpson's (Simpson 1949; Pielou 1969) and Shannon's (Pielou 1966; Chao & Shen

2003) diversity indices, both corrected for sample size (Table 3.1). Lastly, I estimate clonality using a probability-based framework, thus enhancing my ability to discriminate between true clonal lineages. Unfortunately, the terms genet and ramet, become ambiguous here so I use the term multi-locus lineage (MLL; Arnaud-Haond et al. 2007) to refer to individuals that were statistically determined to be derived from the same clonal lineage (i.e., originated from the same ancestral zygote), regardless of their MLG. For example, individuals having identical MLGs may in fact have originated from distinct sexually-produced zygotes (i.e., not clonal reproduction) simply due to insufficient resolving power of the genetic markers used. To address this, I used the program MLGSIM 2.0 (http://www.rug.nl/fmns-research/theobio/downloads; updated by A.B.F. Ivens from Stenberg et al. 2003) to calculate the probability that the same MLG of two or more colonies was the result of sexual reproduction (P_{sex}) assuming Hardy-Weinberg equilibrium. Statistical significance of each P_{sex} value was determined by simulating a randomly mating population based on observed allele frequencies to serve as the null distribution. Thus, instead of presumptively equating identical MLGs to be the result of clonal reproduction, I conclude only those samples with statistically, significant low P_{sex} to be the result of clonal reproduction (i.e., part of the same MLL). Otherwise, identical MLGs that do not pass these criteria are considered to be the result of sexual reproduction. This procedure results in an upper bound for the number of distinct MLLs on the reef. Conversely, two individuals originating from the same ancestral zygote (i.e., clonal reproduction) may have distinct MLGs due to somatic mutation (i.e., cryptic clones; van Oppen et al. 2011). To address this, I used the program GENCLONE 2.0 (Arnaud-Haond & Belkhir 2007) to identify pairs of MLGs that differed by a single microsatellite repeat unit, removed this locus from the analysis, and recalculated new P_{sex} values. If the new P_{sex} value, as recalculated by MLGSIM 2.0, was low and statistically significant, then the two MLGs were lumped into a single MLL, thus resulting in a lower bound for the number of distinct MLLs on the reef.

Results

Coral sampling and genotyping

A total of 2882 individual colonies were sampled and mapped considering all reefs (Figures 3.2 and 3.3 and Table 3.1). Of these, 2493 (86.5%) had complete, six-locus genetic data (Table 3.1) and could be assigned to a genet based on sharing identical MLGs. Due to the small allelic distances between many of the samples, I was unable to confidently assign individuals with incomplete genotypes to a genet, so these individuals were dropped from most of the analyses. Furthermore, repeated multiplex amplifications and re-extraction and re-amplification of these individuals failed to recover the missing locus data.

One locus in particular, Pd3-EF65, presented problems with allele scoring. Out of the 2882 genotyped samples, 199 (~6.9%) revealed three alleles with similar peak heights (i.e., fluorescence intensity, which is proportional to amplicon concentration). A 189 base pair allele was shared among all 199 of the three peaked samples. This allele at this locus also was common among the rest of the samples (present in 2143 samples or 74.3% of the entire dataset). Excluding or including this allele changes neither the total number of MLGs found in the dataset nor the distribution of individuals among those genotypes. Interestingly, similar findings of more than two copies of a gene per individual (although not for the same locus reported here) have been described by others working with *P. damicornis* (Flot et al. 2008).

Finally, an open reading frame of unknown function in the mtDNA (Flot & Tillier 2007) was sequenced and revealed two haplotype clusters within my dataset separated by ~10 bp. Nearly all (79 of 81; 98.8%) of the genets in my dataset belong to the same *P. damicornis* haplotype cluster revealed by Flot et al. (2008). Furthermore, based on the results of PCA, the entire dataset of microsatellite MLGs appears to be a single cluster (Figure S1 in APPENDIX B). Thus, all samples, were retained in my analyses, as molecular delineation for this species (Pinzón & LaJeunesse 2010; Combosch & Vollmer 2011; Schmidt-Roach et al. 2012), and indeed, for many coral species (Stat et al. 2012), is still undergoing debate.

Hardy-Weinberg equilibrium and locus-level analyses:

The overall F_{IS} value for Reef 19 based on unique MLGs only was 0.17, but the variance among loci was high (Table 3.2). Concordant results were found for each of the neighboring reefs. For Reef 19, significant heterozygote deficits were seen at four loci (Pd3-005, Pd2-006, Pd2-AB79, Pd3-EF65), and heterozygote excesses at two loci (Pd2-001 and Pd3-004). When all ramets are used in the analysis, all 15 pairwise locus comparisons revealed significant linkage disequilibrium and 12 comparisons were significant after sequential Bonferroni adjustment (Rice 1989). After removal of repeat MLGs, however, linkage equilibrium was restored to three of the pairwise locus comparisons. Lack of recombination, non-random mating in inbred populations, or admixture of genetically-distinct populations (i.e., Wahlund effect) can cause loci to appear statistically linked. Given the small spatial scale of my study, however, disequilibrium at my study site is likely due to inbreeding rather than Wahlund effect, physical linkage among loci or admixture. Thus, all loci were retained for analyses. Note that the results reported here include samples with incomplete genotypes, as excluding them did not change the overall interpretation of the data.

Individual-level spatial genetic patterns

In the tests for spatial autocorrelation of relatedness, I used only those samples with complete six-locus genotypes so that ramets could be assigned to genet groups without ambiguity. Furthermore, my original analysis based on equalizing the number of pairwise comparisons among distance classes revealed that positive spatial autocorrelation extended only up to the first few distance classes, prompting a reanalysis based on new distance classes that would allow for higher resolution at smaller scales. This did not affect the overall pattern of spatial autocorrelation, so I only report on the latter set of distance classes here (Figure 3.4). When all individuals are included (i.e., ramet-level analysis), Reef 19 showed statistically significant, positive genetic spatial autocorrelation at small distances and negative genetic spatial autocorrelation at larger distances (Figure 3.4A). Correlograms using the genet-level (Figure 3.4B) and clonal distribution (Figure 3.4C) datasets show the same overall pattern but the magnitude is smaller than the ramet-level analysis. This is expected since both the genet- and clonal-level distribution datasets are components of the ramet-level dataset. Interestingly, for the

three neighboring reefs, intra-reef SGS was not detected (i.e., genetic relatedness was spatially random). Furthermore, SGS was no longer detectable on Reef 19 after sub-sampling from the dataset using the simulated stratified random strategy approach implemented on the neighboring reefs (data not shown).

Lastly, for the estimation of N_b and σ, I used two different approximations for the effective population density of Reef 19. My upper limit (2.34 corals per m^2) assumes equal reproductive success among all coral and thus, is based on the total census size, while my lower limit (1.42 corals per m^2) assumes that only the seven most common genets are reproductively successful and thus, is based on the total number of individuals (1657) in common genets (see Clonality results below). Using these approximations, I report a range of neighborhood sizes of between 240 and 257 corals and a σ of between 2.86 and 3.80 meters.

Population-level spatial genetic patterns

All pairwise reef F_{ST} values were not statistically significantly different from zero ($0.441<P<0.984$). Concomitantly, all Mantel tests based on 1000 permutations failed to show a significant relationship between geographic and genetic distances both pre- and post-logarithmic transformation ($0.74<P<1.00$).

Clonality

Based on an analysis that takes inbreeding into account (see Materials and Methods), I rule out the possibility of selfing in my dataset. Next, I calculated indices of clonal diversity for all four reefs (Table 3.1) and indicate both a high clonal input and highly skewed clonal frequency distribution. For Reef 19, a total of 78 unique genets was found in the dataset of complete six-loci genotypes. The frequency distributions of genets for Reef 19 (Figure 3.5A) and the three neighboring reefs (Figure 3.5B) indicate broadly similar genet compositions for all four reefs with the neighboring reefs revealing three novel MLGs not seen on Reef 19. Seven of the genets on Reef 19 were numerically dominant having between 125 and 362 ramets per MLG. Together, these dominant MLGs accounted for 70.45% of the colonies on the reef. Ramets of all seven common genets were found throughout reef 19 (e.g., Figure 3.3A). On the other hand, 24 MLGs (genets 1 to 24 in Figure 3.5A) were only represented by a single colony. Randomly stratified sampling of the neighboring reefs did not produce any samples that correspond

with any of the 24 genetically unique corals on Reef 19. All seven of the most common MLGs on Reef 19, however, were also found on, at least, one of the neighboring reefs (e.g., Figure 3.3).

Based on the probability framework described above I found the lower and upper bounds for the number of multi-locus lineages (MLLs) to be 53 and 116, corresponding to a clonal richness index of between 0.023 and 0.049. It should be noted, however, that inbreeding at my study site may cause an underestimation of the lower and upper bounds for the number of MLLs, because the calculation of P_{sex} values are based on the assumption of random mating (Arnaud-Haond et al. 2007).

Discussion

What can be learned from studying a single reef?

Given that ~70% of Reef 19's coral cover was dominated by just seven MLGs, my data point to the importance of clonal recruitment in maintaining coral cover for this species in Hawai‘i. This, however, was not surprising as similar patterns were found in Kāne‘ohe Bay, Hawai‘i previously (Stoddart 1988). While this study confirms the extremity of this species' clonal nature, it also points to a highly skewed distribution of genet recruitment. Why are some MLGs so numerically dominant on the reef while others are represented by just one or two ramets? It is possible that the frequency of MLGs present in the population fluctuates through time such that abundant genets outnumber rare ones simply due to recruitment history (i.e., early recruits that subsequently reproduced asexually). Alternatively, it may be due to differential fitness of the genets. Local adaptation of corals has been proposed both inferentially as indicated by the spatial scale of genetic population differentiation (van Oppen & Gates 2006) as well as experimentally (D'Croz & Maté 2004; Vermeij et al. 2007; Baums 2008).

These data also suggest that *P. damicornis* at my study site is characterized by populations of highly clonal individuals that occasionally reproduce sexually. For example, after the removal of repeat MLGs, I observed a decrease in linkage disequilibrium among loci, which is predicted for populations of mixed reproductive strategies where some clones have become widespread (Smith et al. 1993; Halkett et al. 2005). Furthermore, I observe high among-loci variance in F_{IS} values, which is expected

for a species with mixed sexual and asexual reproductive strategy as the accumulation of mutations in clonal lineages affect loci randomly (Balloux et al. 2003).

When averaged across loci, however, F_{IS} values for each of the four reefs are positive, indicating an overall reduction in heterozygosity at the individual level. I rule out the Wahlund effect as a potential source given the small spatial scale of my study site. My genetic data also allows me to rule out the possibility of selfing as the cause of inbreeding (see results). Null alleles, on the other hand, could potentially account for a portion of the observed inbreeding levels. The putative complex mating system of this species, however, does not meet many of the assumptions of the available statistical frameworks for the detection of null alleles. While I cannot rule out the possibility of null alleles, their presence would likely only cause one or a few loci to appear inbred, and not four loci, as observed here. Another explanation is that inbreeding levels are derived from the highly skewed genet composition of these reefs. Although there are 78 unique genets on the reef, any sexual reproduction that might occur is likely to involve the common genets as parents resulting in the production of half-sibs. Whatever the case, random mating in such a system is highly unlikely.

While this study focused on just a few localized reefs, corals are being threatened worldwide by rising sea temperatures and ocean acidification (Hughes et al. 2003). What role, therefore, do small-scale studies have in the face of global threats? Data have shown the importance of genetic diversity in increasing the ability of populations to persist in the face of environmental change (reviewed in Jump et al. 2009). While genetic variation in *P. damicornis* has been studied extensively, predicting this species' response to climate change requires a better understanding of the adaptive *versus* acclimating physiological capacities of corals and their symbionts (Brown et al. 2002; Baker et al. 2004; Berkelmans et al. 2006; Chown et al. 2010). In addition to predictive modeling approaches, however, monitoring programs that not only assess genetic diversity, but track changes in genetic diversity over time may also become an important management strategy in the future (Schwartz et al. 2007). This, coupled with the mounting data indicating that recruitment of coral propagules may be occurring primarily on a local-scale (Cowen et al. 2000, Palumbi 2003, Hellberg 2007, Cowen & Sponaugle 2009), underscores the need for investigating the baseline genetic resources of individual reefs

so that future studies can monitor their genetic response to rising threats. It is still uncertain if corals will be able to adapt to future stresses (Hughes et al. 2003), but ignoring small-scale genetic diversity may result in missed opportunities for better understanding how to manage coral reef resilience.

Spatial patterns of clonality and genetic relatedness on a single reef

Interpreting spatial genetic patterns in corals is complicated by their complex reproductive strategies where they reproduce both sexually and asexually (Hall and Hughes 1996). More importantly, the ability to make inferences on what specific processes are driving spatial genetic patterns is limited due to the multiple dispersal alternatives available to corals, including polyp-bailout, propagative fragmentation, gamete broadcasting, and larval brooding. An important first step, however, is to address how genetic patterns may differ between clonal and non-clonal colonies. Previous studies that have attempted to tease apart the effects of reproductive mode on spatial genetic patterns tended to either average the spatial locations of identical MLGs (Calderón et al. 2007; Billingham et al. 2007; Blanquer et al. 2009) or removed identical MLGs from the dataset all together (Alberto et al. 2005). Here, I retained all samples with complete spatial and genetic data to give a comprehensive description of SGS for my study site.

My analyses point to significant SGS for *P. damicornis* on an intra-reef scale, indicating that, on average, coral colonies that are close together on the reef are more genetically related to each other than coral colonies that are farther apart. As seen in my genet-level analysis (Figure 3.4B), this is true even after removing clonal comparisons. Although all the correlograms have the same basic shape, the spatial autocorrelation for clone distribution (Figure 3.4C) shows a more positive association over a greater distance than the correlogram for the genet-level analysis (Figure 3.4B). In terms of magnitude and spatial extent, therefore, the main process leading to SGS on Reef 19 is likely spatially-dependent recruitment of asexually derived planulae or fragmentation. That significant SGS is retained in the dataset in the genet-level analysis (Figure 3.4B), however, indicates that part of the observed SGS is also due to the spatial distribution of non-clones. Dependence on spatial distance for the genetic relatedness of non-clones means that sexually produced planulae tend to recruit near their brooding parent and that sperm is dispersal limited (i.e., non-random mating is occurring).

In general, the direct interpretation of correlograms can be limited because they can be sensitive to the sampling scheme and the information content of the markers (Vekemans & Hardy 2004). Here, however, I have nearly exhaustively sampled a single reef and used highly variable (i.e., informative) microsatellite markers. By sampling so intensively, my estimates of population parameters, therefore, are not technically estimates but are a measure of the true, population value. In other words, due to my sampling strategy, my calculated genetic relationship values are the actual population statistic, making these results less susceptible to over-interpretation (Vekemans & Hardy 2004).

It should also be noted, that intensively sampling a single reef allowed me to detect SGS on Reef 19, but not on the three neighboring reefs where I used much smaller sample sizes that are typical of coral genetic studies (~50 individuals). I also did not find SGS on Reef 19 when I subsampled this dataset in a similar fashion as the neighboring reefs. While it is true that other studies have found variability in SGS among different sites (Miller 1998), it is unlikely that the SGS pattern observed on Reef 19 is unique to that reef. The neighboring reefs were chosen not only because of their proximity to Reef 19, but also because of their similar size, coral cover, and depth. In addition, the inability to detect SGS after reducing the Reef 19 dataset to match the sampling effort on the neighboring reefs further indicates that sampling strategy, and not site-specific considerations, is likely accounting for the observed differences in SGS. This highlights the importance of decreasing spatial scale and increasing sample size in order to reveal ecological patterns that would otherwise be missed. One implication of these results to future population genetic studies, therefore, is that fine-scale sampling is necessary, at least in part, to detect the full extent of genotypic diversity present for this species. To avoid underestimating levels of genetic diversity, patterns of SGS, and other population level parameters, I recommend a combination of stratified random sampling across a reef and intensive, exhaustive sampling of a portion of the area.

The coral population genetics paradox: a matter of scale

Coral population genetic research has primarily focused on explaining reef connectivity and spatial genetic patterns in terms of larval dispersal potential and clonal distributions and these are unquestionably important issues. Indeed, many studies have

attributed patterns of genetic diversity to local or small-scale recruitment in coral reefs (Stoddart 1988; Hellberg 1995; Palumbi 2003 and reviewed in Hellberg 2007). When interpreting multi-scale patterns of genetic diversity, however, local patterns of genetic diversity are often framed as being at odds with larger scale patterns. Perhaps a more appropriate paradigm would be to consider that multiple processes are at work simultaneously and on different scales (Cowen & Sponaugle 2009).

Here, I observe SGS for both clonal and non-clonal colonies of *P. damicornis* within the scale of a single patch reef. I also observe that the strength of this SGS begins to decay towards spatial randomness within a few meters (Figure 3.4). Furthermore, the calculated gene dispersal distance, σ, ranged between 2.86 and 3.80 meters and the calculated neighborhood size, N_b, ranged between 240 and 257 corals. These parameters, however, primarily describe the balance between drift and gene flow on a local scale and do not exclude the possibility of rare, longer distance dispersal events (Fenster et al. 2003). In fact, similar genet compositions for all reefs (Figure 3.5, AMOVA results, and lack of an isolation-by-distance pattern between reefs) indicate that planulae are capable of dispersing hundreds of meters between the four sampled reefs. Yet, if planulae are dispersing both within and between reefs, why is the same genetic homogeneity found on an inter-reef scale not found within Reef 19? In fact, my results are exactly that posed by the population genetic paradox described previously: why, if genetic patterns of isolation-by-distance occur on a small scale, do I not see an extension of the genetic patterns of isolation-by-distance on a larger scale?

If limited dispersal coupled with occasional inter-reef dispersal were the only process at work, then I would expect genetic relatedness to decay towards spatial randomness (i.e., genetic relationship coefficients not significantly different from zero) and remain there throughout the spatial extent of the reef. Instead, however, I observe a continued decay towards negative genetic spatial autocorrelation for the largest distance classes on an intra-reef scale, indicating that corals at this scale are more genetically unrelated to each other than expected from random. Larval dispersal alone cannot account for this observed pattern. One explanation is that this pattern of genetic patchiness was created by chance recruitment events, whereby genetically distinct groups of coral recruits settled in different parts of the reef. If mating between individuals at this

distance is rare and if planulae tend to recruit near the brooding parent, this could potentially create the observed negative genetic spatial autocorrelation for extreme distances. Indeed, previous studies (Johnson & Black 1982; 1984; Watts et al. 1990; Veliz et al. 2006) have implicated larval recruitment history as a major contributor to unusually high levels of genetic variation at short distances despite long-distance larval dispersal potential.

Another explanation, however, is that some other process besides larval dispersal is at work on this scale. My previous study of Reef 19 found significant microhabitat variation in benthic sea temperature on the scale of a single reef (Chapter 2; Gorospe & Karl 2011). If microhabitat variation is influencing coral recruitment or survival, it is also possible that it is influencing intra-reef patterns of individual-level genetic relatedness but not among reefs. Teasing apart the effects of dispersal- (i.e., spatial) versus microhabitat-influenced (i.e., environmental) processes, however, requires a more landscape genetics-based approach beyond the scope of this chapter (Storfer et al. 2007).

Ecological processes such as dispersal and recruitment are important for informing conservation efforts, but have sometimes proven difficult to infer from genetic data due to paradoxical geographic patterns. Here, my cross-scale, explicitly-spatial analyses allowed for a clearer understanding of SGS and demonstrated that the genetic patterns produced by larval dispersal and recruitment processes are scale-dependent. In ecology, widespread acknowledgement of scale as a fundamental scientific issue constituted what some considered to be a paradigm shift (Golley 1989). The appropriate scale at which to sample genetic diversity in the sea is also shifting as data from genetic studies of natural populations, laboratory studies of larval settlement cues, and oceanographic models of larval transport all point to the increasing relevance of local-scale recruitment events (reviewed in Cowen & Sponaugle 2009). A renewed emphasis of small-scale genetic diversity and SGS analyses in marine systems, therefore, may shed light on ecological and evolutionary processes occurring at this scale (Alberto et al. 2005; Underwood et al. 2007; Calderón et al. 2007; Billingham et al. 2007; Blanquer et al. 2009; Ledoux et al. 2010; Combosch & Vollmer 2011). I do not necessarily advocate my approach of exhaustive sampling: this was an immensely time consuming approach. It is possible, however, that having a more explicitly spatial approach to the study of genetic

systems that includes multiple scales may allow greater insights and a more nuanced view of genetic variation across the seascape.

Table 3.1. GPS coordinates, total number of samples (N_{TOT}); number of samples with complete multi-locus genotypes (N); clonal richness (N_G/N), where N_G is the total number of unique multi-locus genotypes; genotypic diversity (G_O/G_E), where G_O is the observed genotypic diversity and G_E is the expected genotypic diversity; and sample size corrected Simpson's and Shannon-Wiener diversity indices for all reefs. The row labeled 19S refers to the average values for 50 simulated data sets of 50 individuals selected randomly from the Reef 19 data.

Reef	GPS coordinates	N_{TOT}	N	N_G/N	G_O/G_E	Simpson's	Shannon's
19	21.45767°N, 157.80677°W	2741	2352	0.033	0.005	0.915	1.309
19S	N/A	50	50	0.37	0.195	0.912	1.104
12	21.45051°N, 157.79773°W	50	50	0.28	0.102	0.820	1.007
16	21.45447°N, 157.80390°W	46	46	0.39	0.271	0.940	1.277
20	21.46063°N, 157.80970°W	45	45	0.40	0.238	0.927	1.255

Table 3.2. Number of alleles (*n*), number of individual genets (*N*), observed (H_O) and expected (H_E) heterozygosity, Weir & Cockerham's estimator of Wright's inbreeding coefficient (*f*), and significance level (* $P \leq 0.05$, ** $P \leq 0.01$, and *** $P \leq 0.001$) using only unique multi-locus genotypes from all four reefs.

Reef	Locus	*n*	*N*	H_E	H_O	*f*
19	Pd2-001	3	104	0.52	0.61	-0.16**
	Pd3-004	6	104	0.37	0.43	-0.16***
	Pd3-005	8	103	0.49	0.47	0.05**
	Pd2-006	6	88	0.52	0.19	0.63***
	Pd2-AB79	7	100	0.64	0.45	0.30***
	Pd3-EF65	9	101	0.54	0.36	0.34***
	Average	6.50	100	0.51	0.42	0.17
12	Pd2-001	2	14	0.48	0.71	-0.54
	Pd3-004	2	14	0.42	0.43	-0.01
	Pd3-005	6	14	0.66	0.71	-0.09
	Pd2-006	2	14	0.49	0.07	0.86**
	Pd2-AB79	4	14	0.63	0.50	0.22
	Pd3-EF65	4	14	0.59	0.29	0.52*
	Average	3.3	14	0.54	0.45	0.16
16	Pd2-001	3	18	0.52	0.61	-0.18
	Pd3-004	2	18	0.44	0.50	-0.15
	Pd3-005	6	18	0.57	0.56	0.03
	Pd2-006	2	18	0.41	0.11	0.77**
	Pd2-AB79	4	18	0.65	0.39	0.41
	Pd3-EF65	4	18	0.52	0.28	0.48**
	Average	3.5	18	0.52	0.41	0.23
20	Pd2-001	2	18	0.51	0.56	-0.08
	Pd3-004	2	18	0.39	0.39	-0.01
	Pd3-005	6	18	0.63	0.61	0.02
	Pd2-006	2	18	0.51	0.22	0.57*
	Pd2-AB79	4	18	0.65	0.50	0.23
	Pd3-EF65	4	18	0.42	0.22	0.48*
	Average	3.3	18	0.52	0.42	0.20

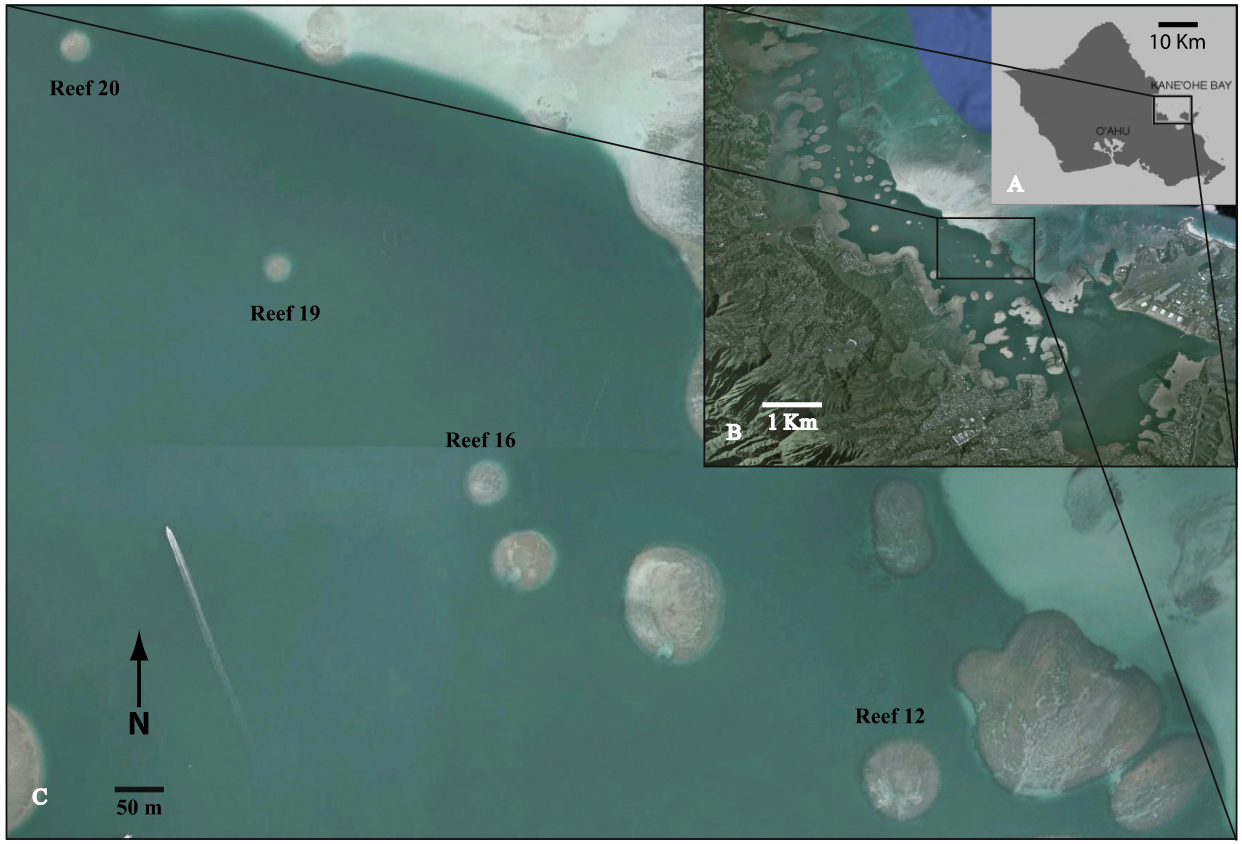


Figure 3.1. Aerial view of Kāneʻohe Bay, Oʻahu, Hawaiʻi showing the locations of Reefs 19, 12, 16, and 20. Inset A – map of Oʻahu, Hawaiʻi showing location of Kāneʻohe Bay. Inset B – close up of Kāneʻohe Bay showing location of reefs.

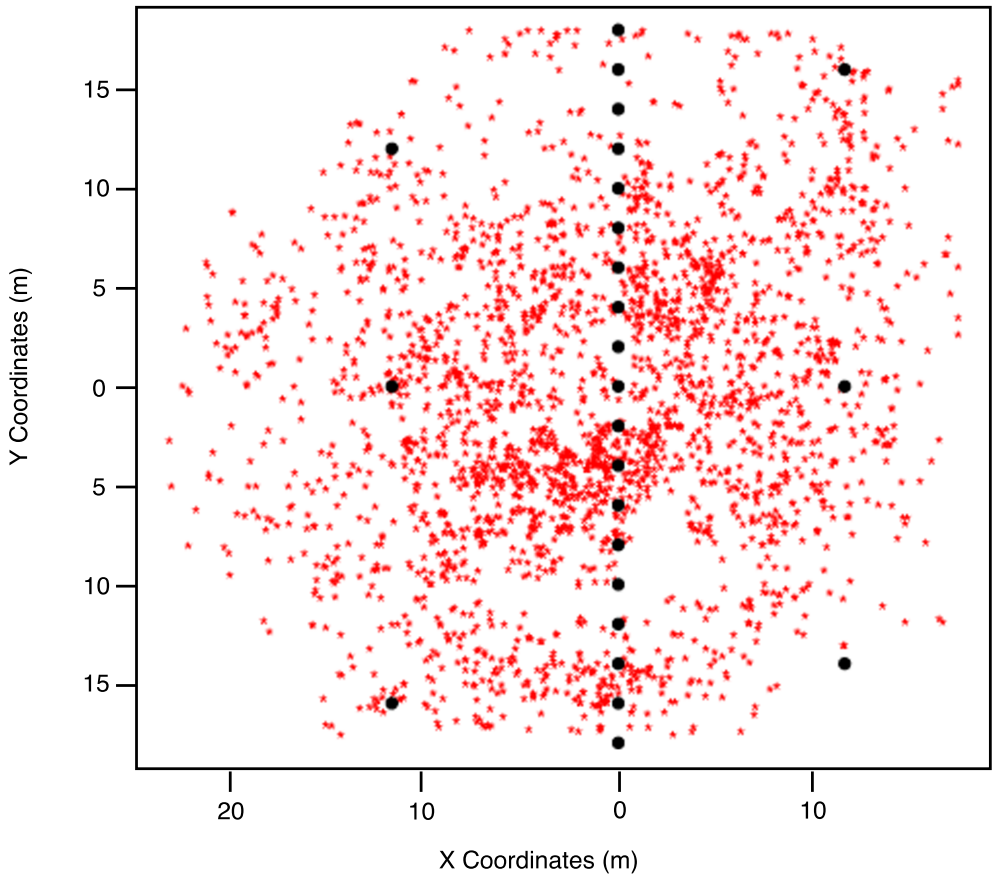


Figure 3.2. Location of the 2741 *Pocillopora damicornis* colonies on Reef 19. Each asterisk depicts the location of a colony. Circles depict the location of rebar markers used for orientation while sampling.

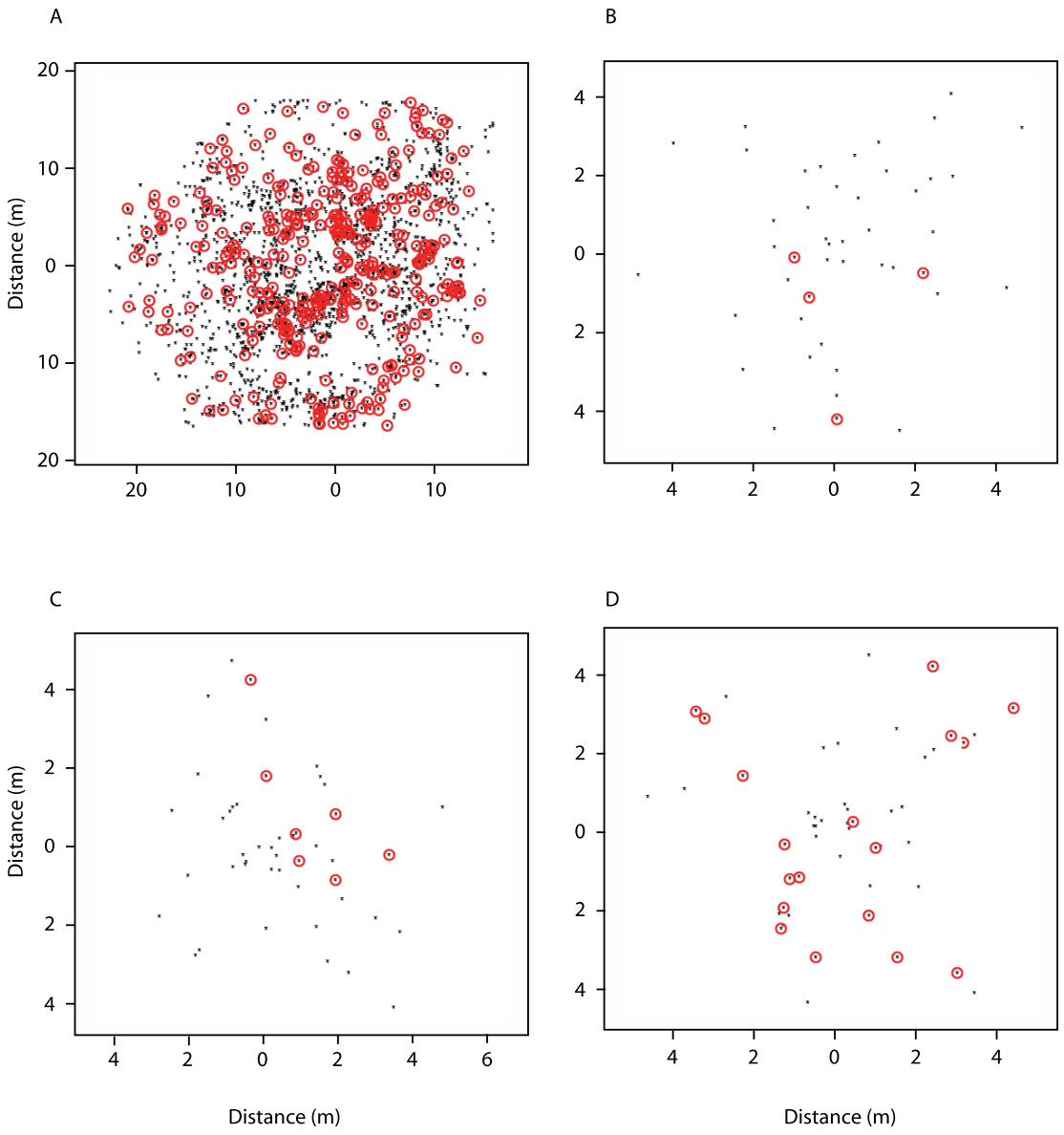


Figure 3.3. Spatial plots, showing the locations of all corals sampled at A) Reef 19, B) Reef 20, C) Reef 16, and D) Reef 12 (dots). The locations of the most abundant genet (i.e., genet 78) are circled.

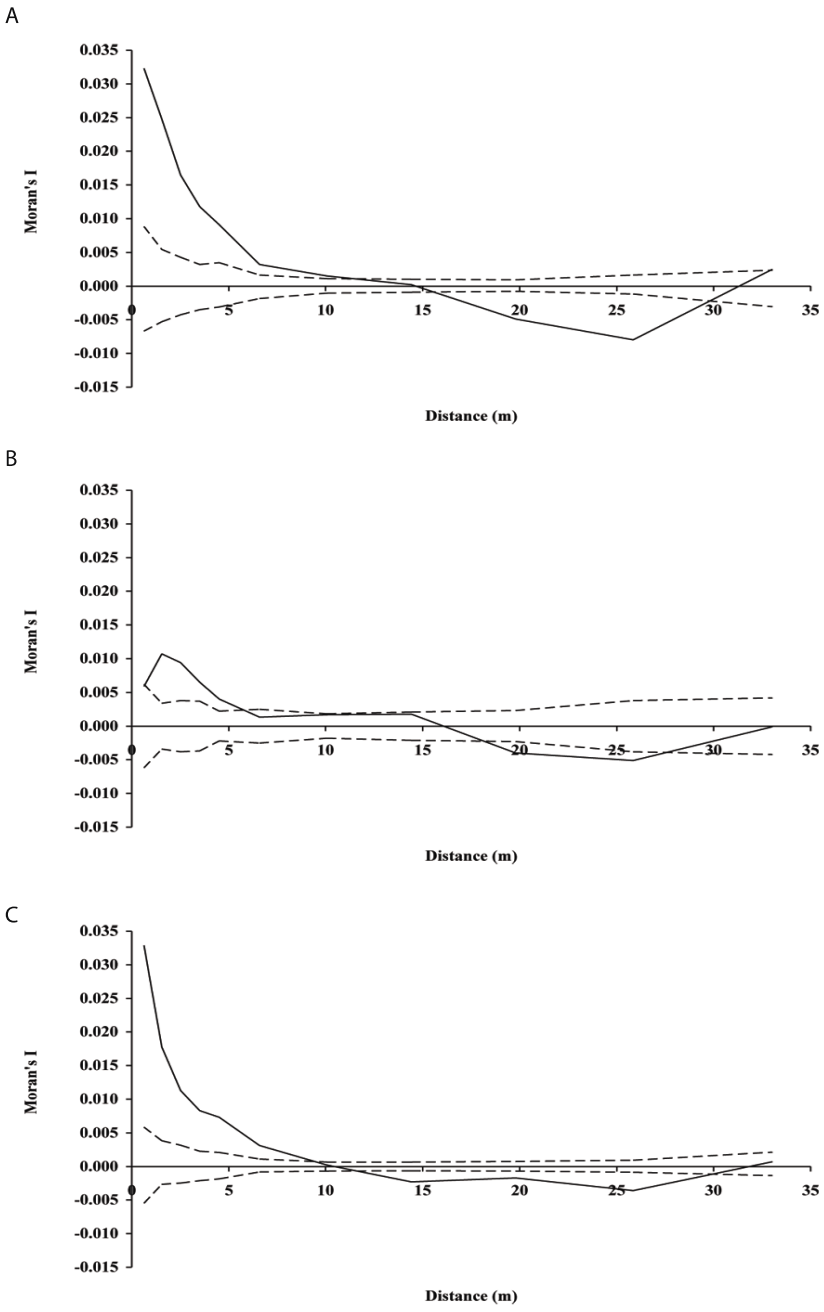


Figure 3.4. Results of spatial autocorrelation analyses for Reef 19 using A) ramet-level, B) genet-level, and C) clonal distribution analyses depicted as correlograms. Distance classes were chosen to provide higher resolution at smaller spatial scales. Dashed lines in A and C show 95% confidence intervals based on 200 permutations of individual locations among all individuals and in B show one standard error based on jackknifing across loci.

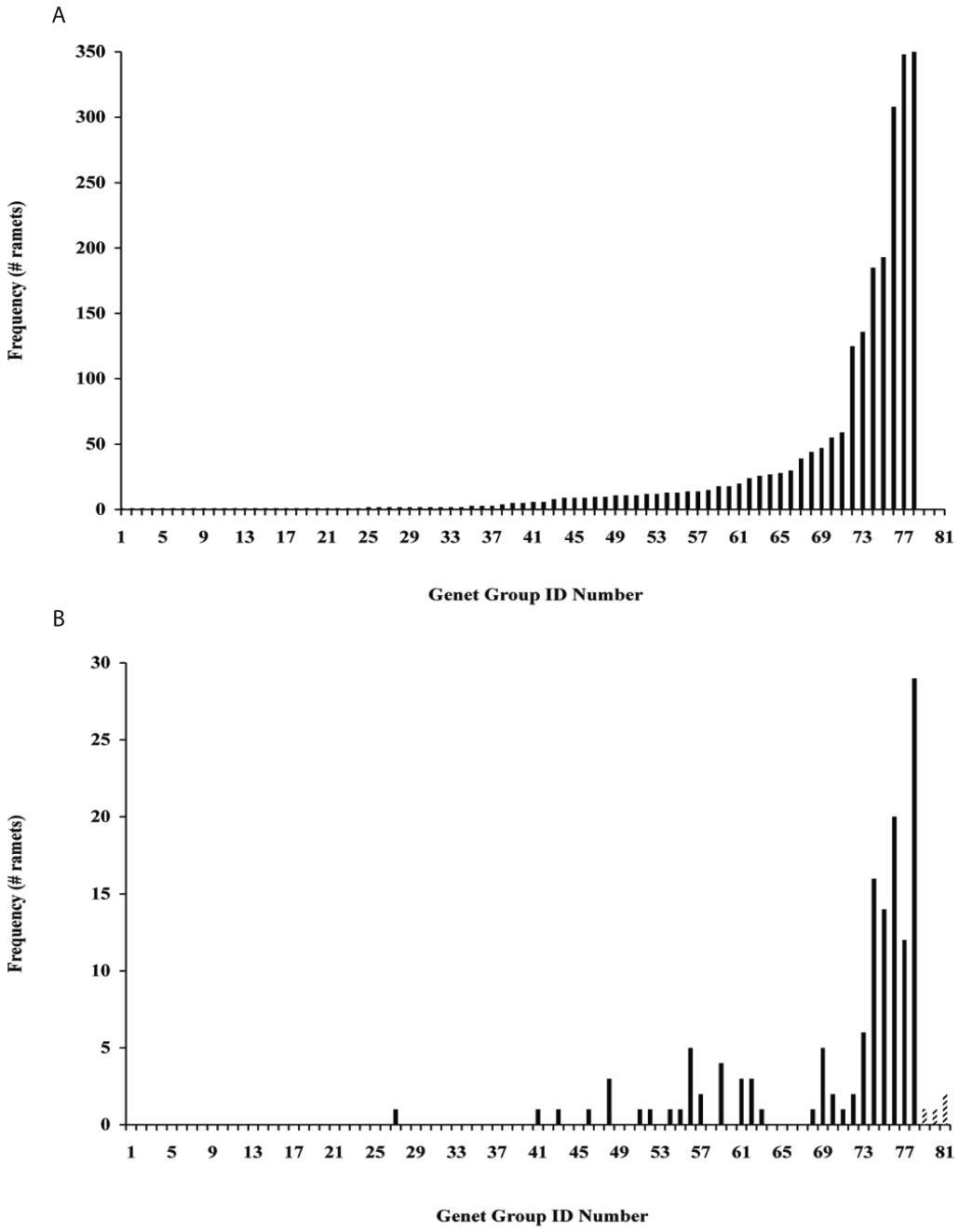


Figure 3.5. Histograms showing the frequency distribution of ramets for each genet on Reef 19 (A) and the neighboring reefs combined (B). The three novel genets found on the neighboring reefs, but not Reef 19, are hatched bars (i.e., genet numbers 79-81).

CHAPTER 4. HOW TO SAMPLE A CORAL REEF: THE IMPORTANCE OF SAMPLING DESIGN, SPATIAL PATTERNS, AND DEGREE OF CLONALITY ON CHARACTERIZING GENETIC DIVERSITY

Abstract

The interpretation of coral genetic variation on and among reefs has rarely been contextualized in an explicitly spatial framework where each individual has both genetic and spatial parameters. Here, I explore interactions between sampling effort, sampling strategy, and the presence of spatial genetic structure on the ability to effectively characterize genetic diversity on a reef. To do this, I take my original dataset of 2352 genotypes of *Pocillopora damicornis*, a dataset of near-exhaustive sampling of a single patch reef in Kāneʻohe Bay, Hawaiʻi, and subsample from it using two different strategies: (1) saturation sampling a portion of the reef and (2) random sampling throughout the entire reef. My results demonstrate large variation (e.g., 0.35 to 0.46) in estimates of observed heterozygosity (H_O) using a more typical sample size of 50 and that in the presence of spatial genetic structure, non-random sampling schemes can give biased estimates of genetic diversity. Furthermore, my results indicate that over 1000 samples are required to reveal the true pattern of spatial genetic structure at my site. I also demonstrate, by rarefaction analysis, that the bias in estimating clonal richness (i.e., the proportion of unique genotypes in a given sampling area relative to the total number of samples surveyed) for small sample numbers is due to the predominance of clones (i.e., high level of clonality), and not skew in genet frequency distribution. I argue that intense to near-exhaustive sampling schemes may be important for uncovering true patterns of genetic diversity in highly clonal populations.

Introduction

Intraspecific genetic diversity is necessary for evolution and adaptation. Characterizing intraspecific variation and preserving processes responsible for it, therefore, may be important for predicting how populations will be able to persist in the face of global climate change (Pauls et al. 2013). The assessment of genetic diversity, however, is particularly difficult for clonal organisms as standardized methods are yet to be fully codified (Halkett et al. 2005; Arnaud-Haond et al. 2007). In particular, corals are

one example of a clonally reproducing organism for which there is great interest in assessing genetic diversity in natural population (van Oppen & Gates 2006). For example, hierarchical analyses of genetic diversity are one of the primary methods for inferring patterns of connectivity between reefs (Palumbi 2003). While many advances have been made in the population genetic theory of clonal organisms (De Meeûs et al. 2006; Arnaud-Haond et al. 2007), less attention has been paid to the standardization of methods regarding the appropriate density, strategy, and scale at which to sample genetic diversity of coral in the field. Addressing these issues is an essential component to better understanding how corals will be impacted by climate change (van Oppen & Gates 2006).

Corals, even predominantly sexually reproducing ones, have the ability to reproduce clonally through fragmentation or polyp bailout or as in some species, through the production of asexually-derived larvae. One metric for characterizing the diversity of a reef is clonal richness (G/N) which is the proportion of unique genotypes in a given sampling area (G) relative to the total number of samples surveyed (N). This metric has historically been employed to understand the population biology of corals and the relative importance of sexual versus asexual reproduction in structuring coral reefs (Hunter 1993; McFadden 1997; Baums et al. 2006).

An important method that many plant population geneticists use to understand the clonal structure of populations is spatial autocorrelation analysis. The resulting correlogram plots the similarities between pairs of samples at various spatial distance classes, thus identifying the spatial scale or scales at which samples are more genetically related to each other than random (i.e., positive spatial autocorrelation), less genetically related to each other than random (i.e., negative spatial autocorrelation), or simply spatially random (i.e., no spatial autocorrelation). The detection of positive or negative spatial autocorrelation indicates the existence of spatial genetic structure (SGS). Characterizing these patterns can provide insight into the microevolutionary processes that cause them (Epperson 1993; Sokal et al 1997). For example, historical recruitment factors and natural selection were found to be important processes organizing local patterns of SGS in a flowering plant (Kalisz et al. 2001). Furthermore, failure to incorporate spatial patterns both in the detection of population structure as well as loci

under selection can lead to high rates of type I error (i.e., false positives: incorrectly inferring population structure or signals of selection when there is none; Meirmans 2012). Yet while standard in population genetic studies of terrestrial plants (Heywood 1991), spatial autocorrelation analyses have only recently been employed in the study of genetic diversity in marine ecosystems.

The importance of sampling scheme, while well known among community ecologists, is often overlooked by molecular ecologists (Arnaud-Haond et al. 2008; Schwartz & McKelvey 2008). Estimates of coral genetic diversity for individual reefs, generally, are based on relatively small sample sizes (usually around 50 individuals or less) and have often relied on opportunistic as opposed to systematic sample acquisition. In fact, one survey (Arnaud-Haond et al. 2008) found that only 35% of genetic marine invertebrate studies disclosed their sampling strategy or the spatial scale of the sampling area. Yet the characterization of certain types of genetic variation can be highly influenced by sampling design and spatial scale. For example, in the presence of genetic spatial autocorrelation, the number of distinct genetic populations detected by genetic clustering analyses will be different depending on the sampling scheme used (Schwartz & McKelvey 2008). As with any ecological variable (Legendre et al. 2002), the interpretation of genetic variation should be contextualized in an explicit spatial framework and sampling design.

As described in Chapter 3 (Gorospe & Karl, accepted), I near exhaustively sampled colonies of *Pocillopora damicornis* from a single patch reef in Kāneʻohe Bay, Hawaiʻi and uncovered the presence of intra-reef SGS whereby coral colonies closer together on the reef were more genetically related to each other than corals at the extremes of separation. In addition, I reported a highly clonal reef with a skewed genet frequency distribution (i.e., just seven genets accounted for nearly 70% of the reef's coral abundance for this species). My extensive sampling provided greater confidence in my description of genetic diversity and spatial genetic patterns on the reef, but rarely is it practical to sample this intensively. Here, by subsampling from my original, near-exhaustive dataset, I investigate the effect of sample size, degree of clonality and genet frequency skew on my ability to estimate clonal richness (i.e., *G/N*). Furthermore, I compare the estimates from two different sampling strategies (random sampling

throughout the reef versus saturation sampling a portion of the reef) for: (1) several Hardy-Weinberg based genetic diversity parameters, of which I focus on observed heterozygosity (H_O) and (2) spatial autocorrelation (i.e., SGS). Lastly, to explore the potential interaction of sampling strategy and spatial patterns, I investigate how the estimation of genetic diversity may be affected by the presence of spatial autocorrelation.

Methods

Sampling and subsampling

I used custom R-scripts (R Development Core Team, http://www.R-project.org/) to subsample from my original dataset using two different strategies: (1) saturation sampling a portion of the reef and (2) random sampling throughout the entire reef. These two strategies are meant to represent two common, contrasting approaches that researchers choose to sample a population's genetic characteristics. Saturation sampling a portion of the reef provides for a complete inventory of that portion of the reef at the expense of potentially overlooking genetic diversity in non-sampled areas. On the other hand, randomly sampling throughout the reef broadens the spatial extent of the sampling effort while potentially missing finer-scale patterns (e.g., SGS).

For the saturation sampling strategy, a circular area with a radius of 4 m was randomly selected on the reef, and all corals found within the area were used to create a single, subsampled genetic dataset (i.e., subset). This was repeated until a total of 20, separate, subsets were created in this manner. The sampling area was then systematically increased to circles of radii 6, 8, 10, 12, 14, and 16 m, each time repeating the subsampling 20 times, for a total of 140 saturation sampled subsets. In all cases, if any part of the circle fell outside the edge of the reef (Figure 4.1A), that sampling area was discarded and another random location chosen. Next, to simulate a sampling strategy using the entire reef, I randomly sampled without replacement 50 and 100 to 2300, in increments of 100, colonies from throughout the reef (Figure 4.1B). I generated 20 subsets for each sample size increment, thus making a total of 480 randomly sampled subsets.

Finally, I wanted to explore whether my overall findings were specific to the presence of spatial autocorrelation in my original dataset. To address this, I remove spatial autocorrelation from my original dataset by randomizing the locations of corals,

thus creating a new, spatially randomized genetic dataset. Using this dataset, I repeat the same subsampling process described above, generating an additional 140 saturation sampled subsets and 480 randomly sampled subsets. Thus, a total of 1240 subsets were analyzed in this study.

Genetic and clonal diversity

For each of the 1240 subsets, I calculated several traditional population genetic diversity estimates after removing repeated multi-locus genotypes (MLGs) from each dataset (i.e., only one ramet from each genet was used). Here, I define a ramet as a single coral colony and a genet as a group of ramets with identical MLGs. The genetic diversity estimates reported here include: Wright's inbreeding coefficient (F_{IS}) averaged across all six loci (Weir & Cockerham 1984) as well as observed (H_O) and expected (H_E) heterozygosities. All computations were made using the web-based version of GENEPOP 4.0 (Raymond & Rousset 1995; Rousset 2008). Furthermore, I use the program GENODIVE (Meirmans & van Tienderen 2004) to report on additional, widely-used clonal diversity indices including: the ratio of observed to expected genotypic diversity (G_O/G_E), evenness (E_{VE}), a corrected Shannon-Wiener diversity index (H_{SW}; Chao & Shen 2003) and Nei's (1987) corrected genetic diversity, also known as Simpson's diversity index (D).

To compare how sampling strategy (random versus saturation) interacted with spatial patterns (spatially autocorrelated versus spatially random data) and affected the estimation of genetic diversity, I performed weighted least squares (WLS) linear regressions of observed heterozygosity (H_O) on the number of samples in each subset using the *nlme* package in R (Pinheiro et al. 2006). An exponential variance function was used to improve parameter estimation and account for heteroskedasticity (i.e., unequal variances in H_O for different sampling intensities). I did this separately for subsets: (1) randomly sampled from my original, spatially autocorrelated dataset, (2) saturation sampled from my original, spatially autocorrelated dataset, (3) randomly sampled from the spatially randomized dataset, and (4) saturation sampled from the spatially randomized dataset.

A custom R-script was written to perform a rarefaction analysis on my original dataset's clonal richness statistic, G/N (Ellstrand & Roose 1987). This was done to allow

me to compare the clonal richness of my study site to estimates found in the literature for the same species from other locations. This analysis calculates *G/N* from a subset of individuals randomly sampled without replacement from my original dataset. Subsets consisted of 1 to 2352 individuals, in increments of 1, with 100 replicates for each subset. Lastly, in order to investigate how the estimation of *G*/*N* is affected by the predominance of clones (i.e., level of clonality) and skewed genet frequency distribution of my study site (see Chapter 3; Gorospe & Karl, accepted), I simulated additional datasets of 2000 individuals with 20, 50, 80, and 250 genets (i.e., different levels of clonality). Here, it is important to note that the dataset with 20 genets (*G/N* = 0.010) has a higher level of clonality (i.e., greater number of clones per genet) than the dataset with 250 genets (*G/N* = 0.125; i.e., fewer number of clones per genet). Furthermore, I varied the genet distributions to be either even (i.e., equal number of ramets per genet) or skewed (i.e., 70% of ramets were distributed evenly among 10% of the genets while the remaining 30% of ramets were distributed evenly among the remaining 90% of genets). When the total number of ramets (i.e., 2000) did not evenly divide into the number of genets, the leftover number was divided evenly among the least frequent genets. For each of the datasets, rarefaction curves are then generated as described above.

Spatial autocorrelation analysis

The program, Spatial Pattern Analysis of Genetic Diversity (*SPAGeDi* version 1.3; Hardy & Vekemans 2002), was used to conduct spatial autocorrelation analyses. While these analyses are meant to describe the spatial dependency of genetic relatedness (i.e., SGS), interpreting the relative contributions of clonal versus non-clonal input to these patterns can be challenging. In Chapter 3 (Gorospe & Karl, accepted), I described the details of teasing apart these components to the SGS, but here, I focus my attention on the overall (i.e., combined clonal and non-clonal) pattern of SGS, referred to as the ramet-level analysis. I use Hardy and Vekemans' (Hardy & Vekemans 1999) coefficient of relationship, which is a measurement of pairwise genetic relatedness based on the Moran's *I* measurement of spatial autocorrelation (Moran 1950). Spatial autocorrelation analyses were performed on all subsets taken (n=620) from the original, spatially autocorrelated dataset, producing for each, a correlogram of pairwise distances versus Moran's *I*. For the 480 randomly sampled subsets as well as the original dataset, distance

classes for the spatial autocorrelation analyses were set such that the number of pairwise comparisons per distance class was equal. Since the spatial scale of these datasets was the same (i.e., reef-wide), the distance class bins differed by less than 1 meter, allowing for correlogram comparisons between the randomly sampled subsets and the original dataset. Varying spatial scales (i.e., circles of 4, 6, 8 m, etc. radii) between my 140 saturation sampled subsets, however, means that correlograms across datasets will not be comparable if distance bins are set to have equal numbers of pairwise comparisons across distance classes. Therefore, I analyze these datasets by setting the distance class bins to the same ones used in the original datasets, thus allowing for correlogram comparisons across datasets. In all cases, statistical significance at each distance class was determined by calculating 95% confidence intervals based on 200 permutations of spatial locations. To summarize these analyses, all subset correlograms were then compared to the full dataset correlogram using Pearson's correlation coefficient. Furthermore, I calculate for each subset, the proportion of data subsets that correctly detected (i.e., based on the full dataset's correlogram) statistically significant autocorrelation at each distance class when it is present.

Results

Genetic and clonal diversity

After removing repeated MLGs, my original dataset of 2352 samples was reduced to 78 unique MLGs. Values for all indices (G, N, G/N, F_{IS}, H_O, H_E, G_O/G_E, E_{VE}, H_{SW}, and D), based on the original dataset and simulated subsets, are in Table 4.1. I also include previously published indices from other studies of *P. damicornis* for comparison. Observed heterozygosities for all saturation sampled (Figure 4.2A) and randomly sampled (Figure 4.2B) subsets are summarized as Tukey boxplots. In all cases, increased sampling intensity led to increased precision (i.e., decreased variance) of the estimate. Special attention should be drawn to the datasets of 50 randomly sampled individuals, as this size is most characteristic of sampling effort in the literature (Ruzzante 1998; van Oppen & Gates 2006). Notice, that when estimating H_O with 50 randomly sampled individuals, while the median value (0.405) is close to the true value (0.397), there is a large range associated with this sampling intensity (0.35 to 0.46). Similar patterns were found for H_E and F_{IS} and other diversity measures (Table 4.1).

Results from the four WLS linear regressions show a significant relationship between H_O and sample number (Figure 4.3; p=0.0001), but only when saturation sampling a portion of the reef in the presence of reef-wide spatial autocorrelation. In contrast, randomly sampling, both from the original spatially autocorrelated dataset as well as from a spatially-randomized dataset showed no significant relationship between H_O and sample number.

The rarefaction curves (Figure 4.4) demonstrated the influence of sampling effort, clonal richness, and genet frequency distribution skew on the estimation of the clonal richness (G/N) statistic. Displayed in Figure 4.4A are 100 overlaid rarefaction curves, each representing the calculated clonal richness statistic for all possible sampling intensities between 1 and 2352. Notice that G/N does not come close to the true value (0.033) until at least 500 samples are used in the estimation. The rarefaction curves comparing the effects of clonal richness (i.e., different levels of G/N) and genet distribution (i.e., even versus skewed) are displayed in Figure 4.4B. Here, rarefaction curves are normalized to the true G/N value for each dataset such that all curves asymptote at 1.0. Notice that datasets with high levels of clonality (e.g., my simulated dataset using 20 genets) are more biased (i.e., farther from 1.0) for all sample numbers than datasets with low levels of clonality (e.g., my simulated dataset using 250 genets). On the other hand, skewed genet distributions are less biased than even genet distributions.

Spatial autocorrelation analysis

For reference, the true correlogram based on performing a ramet-level spatial autocorrelation analysis on all 2352 samples is shown in Figure 4.5. Note that unlike in Chapter 3 (Gorospe & Karl, accepted), here I use equal distance classes as opposed to finer-scale distance classes presented in that study. The correlations between the resulting correlograms of all subsampled datasets to the correlogram of the original dataset were calculated and summarized in Figure 4.6. To compare the results from the two subsampling strategies, I reflect the results of the saturation sub-sampled sets as average sample sizes rather than as radii of the sampling area. In general, as sampling intensity increases so do the correlations between the subsampled dataset correlogram and the original, true correlogram (Figure 4.6). Random subsampling (Figure 4.6A) reached a 0.9

correlation with the true correlogram at 1000 sampled individuals (42.5% of the original data), while saturation subsampling (Figure 4.6B) required an average of 1883 sampled individuals (80.1% of the original data; i.e., a sampling area of radius 16m) to reach the same level of correlation.

I also summarize my findings based on each dataset's ability to detect statistically significant spatial genetic autocorrelation at each distance class. Here, however, I only focus on the results concerning the first and second distance classes, which, for the original dataset's correlogram, were both statistically significant for positive spatial autocorrelation (Figure 4.5). Figure 4.7 shows the proportions of datasets, for each sampling intensity, that were able to detect statistically-significant spatial autocorrelation for the first and second distance classes. For the saturation sampling subsets, the first distance class reached ~90% detection for significant spatial autocorrelation once the average sample size was 933 ± 134 (40% of the original data; i.e., a radius of 10m). On the other hand, for the randomly sampled subsets, the first distance class reached 90% detection once the sampling intensity reached 700 individuals (Figure 4.7A). Overall, autocorrelation in the first distance class was the easiest to detect (lowest sampling intensity), and in general, the larger the magnitude of Moran's *I* in the true correlogram, the easier it was to detect statistical significance during subsampling (Figure 4.7A versus 7B). Saturation sampling, however, reached 90% detection in the second distance class (i.e., smaller Moran's *I*) before random sampling (Figure 4.7B)

Discussion

Caution in interpreting geographic patterns of genetic and clonal diversity

Patterns of population genetic differentiation and isolation by distance can give insight into the spatial scale of dispersal and extent of genetic connectivity (e.g., Polato et al. 2010). When spatial patterns fail to arise or conflicting results are encountered, however, interpreting these data becomes difficult (see Selkoe et al. 2010). Misinterpretation of the data can occur when one attempts to explain patterns of genetic differentiation between populations before carefully considering processes that are occurring within populations (Chapter 3; Gorospe & Karl, accepted). Here I demonstrate a large amount of variance in my estimates of genetic diversity patterns, particularly for small sample sizes (Figure 4.2, Table 4.1). Furthermore, my rarefaction curve (Figure

4.4A) demonstrates that the oft-reported clonal diversity estimate, *G*/*N*, is consistently overestimated for small sample sizes and that this is a general symptom of clonal populations and not exclusive to those with skewed genet frequency distributions. More surprising, however, is what constitutes an appropriate sample size for obtaining a good estimate.

As confirmed by the weighted least squares regression, the estimation of H_O when considering all sub-datasets, is not strongly affected by sample size as long as a random sampling strategy was used. Instead, the main obstacle to characterizing the genetic diversity of Reef 19 with small subsamples is the predominance of clones on the reef. There is an appreciable amount of variance in estimating H_O (Figure 4.2A) for subsets consisting of 50 samples. As Hardy-Weinberg based genetic diversity estimates are mainly concerned with unique MLGs, however, 50 samples from my study site only equates to 18.25 ± 2.17 unique MLGs out of a total of 78 (~20%; Table 4.1). Rare genotypes are the cause and not until 500 individuals are subsampled, are 50 unique MLGs obtained (~67%; Table 4.1) with which to effectively estimate reef-wide allele frequencies and reduce the variance associated with estimating H_O (Figure 4.2A). Note that this same pattern would hold for any genetic parameter that relies on accurate estimation of allele frequencies.

Similarly, 500 individuals are needed to come within an order of magnitude of the true clonal richness (*G*/*N*) value for the reef (Figure 4.4A, Table 4.1). Based on the rarefaction curves calculated from simulated datasets of varying clonal richness and genet frequency distributions (Figure 4.4B), the calculation of *G*/*N* is more biased for datasets characterized by high levels of clonality than those with lower levels of clonality. Bias in *G*/*N* estimates, however, was actually reduced by genet frequency distribution skew (Figure 4.4B). Recall that *G* represents the number of distinct MLGs, while *N* represents the total number of samples. Therefore, sampling a new MLG increases both *G* and *N*, and causes the estimate of *G/N* to decay only gradually to the actual value. On the other hand, sampling a previously sampled MLG only increases *N*, and the estimate of *G/N* decays more rapidly to the actual value. The latter scenario (i.e., sampling a previously sampled MLG) is more likely to occur when the genet frequency distribution is skewed and the reef is dominated by just a few MLGs. Thus, the difficulty in sampling rare

genets, which is primarily a characteristic of skewed genet frequency distributions, actually facilitated my ability to estimate *G/N*. It is important to note, however, that in all but the most extreme case (i.e., having a low level of clonality and a highly skewed genet frequency distribution), large sample sizes (i.e., >100) are needed to accurately estimate *G/N*.

In summary, hundreds of coral colonies are needed to accurately estimate both genetic (e.g., H_O) and clonal (e.g., *G*/*N*) diversity. Overall, these results suggest that studies for this species that have not sampled near-exhaustively, as I did, may have also inaccurately estimated genetic diversity (Table 4.1).

SGS, an elusive yet biologically important pattern

My results indicate that SGS likely would not have been found at my study site had I not embarked on such an intense sampling effort. While random sampling throughout the reef was more efficient than saturation sampling a portion of the reef in detecting the whole SGS pattern (Figure 4.6), both strategies required an extremely intense sampling effort before the true spatial autocorrelation pattern emerged. If I set a correlation level of 0.9 as my threshold for detecting the true pattern, then uncovering this pattern required sampling over 900 individuals for random sampling and even more for saturation sampling. Even when I relax this criterion and consider only the number of samples needed to have a 90% detection success at the first distance class (Figure 4.7), I still find that a large sampling effort (i.e., ~750 randomly sampled individuals or ~900 saturation sampled individuals) is needed. Given the presence of fine-scale SGS at my site, it may seem counterintuitive that saturation sampling outperformed random sampling. For example, saturation sampling performed nearly equally as well as random sampling when considering only the first distance bin (Figure 4.7A) and outperformed random sampling in the second distance bin where the signal of SGS was weaker (Figure 4.7B). The SGS pattern, however, is reef-wide (i.e., positive relatedness for the smallest distance classes and negative relatedness for the largest distance classes). Thus, by focusing on only a small portion of the reef, saturation sampling misses these larger-scale patterns. Saturation sampling, however, appears to be better able to detect lower levels of SGS than random sampling.

While other studies have simulated the effect of increasing loci and sample numbers in the detection of SGS (Sokal et al. 1997; Cavers et al. 2005), computer simulations may not be realistic of patterns found in natural populations. My study places these past studies in context using an empirical approach. For terrestrial plants, patterns of SGS arise largely due to their sedentary nature and dispersal-limited gametes (Vekemans & Hardy 2004). Reports of SGS, however, for benthic marine organisms are less common (but see Alberto et al. 2005; Underwood et al. 2007; Calderón et al. 2007; Billingham et al. 2007; Blanquer et al. 2009; Ledoux et al. 2010; Combosch & Vollmer 2011). The fact that many benthic marine species have long-distance dispersal mechanisms that may preclude them from small-scale patterns of genetic diversity (however, see Weersing & Toonen 2009) and the intense sampling effort required to detect SGS are both likely explanations for why SGS has only rarely been reported in marine systems.

If SGS is weak or rare, particularly in marine systems, why study it? What is the biological relevance, after all, of weak patterns that require large datasets in order to be detected (Björklund & Bergek 2009)? Even for plants with limited gene dispersal, where population genetic theory predicts spatial genetic patterns to be widespread, empirical results tend to show only weak SGS when it is found (Heywood 1991). Likely, the reason for this is that SGS is affected by several sources of variation stemming from both population genetic, stochastic, and spatial processes (Slatkin & Arter 1991). Yet despite the fact that multiple processes are involved in organizing patterns of SGS, spatial autocorrelation analysis is still a powerful tool for providing evidence for or against different alternative hypotheses regarding microevolutionary processes (Sokal et al. 1997). Weak spatial genetic signals, therefore, are not weak for being biologically irrelevant, but merely, subtle for being the cumulative result of multiple drivers. For example, if multiple, strong drivers act in opposite directions they could theoretically result in a weak overall SGS pattern. Understanding these drivers requires an understanding of the SGS they produce.

What is the best strategy of sampling a coral reef?

One important consideration that I investigate here is the effect of spatial autocorrelation on the ability to accurately characterize genetic diversity. When spatial

autocorrelation was removed from my dataset, random sampling and saturation sampling of a portion of the reef were equivalent and non-biased in estimating heterozygosity for small sample sizes. On the other hand, in the presence of spatial autocorrelation, saturation sampling appeared to be upwardly biased in the estimation of heterozygosity (Figure 4.3) while random sampling remained unbiased. In both cases, however, there remains a considerably wide range of estimates (i.e., high variance) for small sample sizes. My finding that, in the presence of spatial autocorrelation, non-random sampling methods are biased in estimating genetic diversity agrees with similar studies looking at other diversity indices (Fager 1972; Baltanás 1992; but see Brose et al. 2003).

The interaction between sampling strategy and spatial autocorrelation is well known in ecology, but largely overlooked in molecular studies (however, see Schwartz & McKelvey 2008). Ecologists are trained to sample randomly, particularly when there is a lack of *a priori* information about the spatial patterns in a system. This is often done to maintain statistical independence between sampling points, and thus, avoid non-independence (i.e., autocorrelation; Legendre et al. 2002). A distinction must be made, however, between sampling in order to avoid the occurrence of autocorrelated data and sampling in order to fully characterize the genetic diversity of a population and detect weak, but potentially biologically important patterns of spatial autocorrelation. It is also important to note, although commonly known (e.g., Jost 2008), that some diversity measures (e.g., Shannon-Wiener, Nei's diversity) performed well in all of my simulations resulting in accurate estimates with small variance (Table 4.1).

As I have demonstrated, coral genetic diversity estimates may be inaccurate for small sample sizes if populations have a moderate to high levels of clonality. Unfortunately, this inaccuracy is likely not inconsequential. The variance associated with these estimates may prove problematic for studies that attempt to interpret patterns of connectivity or diversity among populations. Other studies that have failed to produce obvious spatial patterns of genetic diversity (i.e., chaotic genetic patchiness) may simply be revealing the underlying variance associated with small samples sizes. For example, for the smallest sample size in Figure 4.3, one population's estimate could be the largest simulated H_O estimate (0.46) and another the smallest (0.36) even though the real values were identical. Clearly, conclusions about population genetic diversity in clonal

organisms can be quite misleading when what was traditionally considered to be a large sample (i.e., 50) is actually a quite small one.

Table 4.1. Diversity estimates for the complete and permuted dataset of *Pocillopora damicornis* and from the literature
Number of individuals (*N*), umber of unique multi-locus genotypes (*G*), Wright's inbreeding coefficient (F_{IS}; Weir & Cockerham 1984), expected (H_E) and observed (H_O) heterozygosities, observed genotypic diversity (G_O), the ratio of observed to expected genotypic diversities (G_O/G_E) genotypic diversities, evenness (E_{VE}), corrected Shannon-Wiener index (H_{SW}; Chao & Shen 2003), Nei's genotypic diversity (*D*), equivalent to Simpson's diversity index (Nei 1987). Values reported from this study are averaged across resampled datasets. Values reported from previous studies are averaged across sites. NR = Not reported; S = See original manuscript because data are available but inconsistent with this table (e.g., F_{IS} only calculated per locus or calculated based on pooling entire dataset instead of per site.

N	*G*	*G/N*	F_{IS}	H_E	H_O	G_O	G_O/G_E	E_{VE}	H_{SW}	*D*	Reference
50	18.25 (2.17)	0.37 (0.04)	0.19 (0.06)	0.50 (0.03)	0.40 (0.03)	9.75 (1.72)	0.20	0.53 (0.07)	1.23 (0.08)	0.91 (0.02)	This study
100	26.00 (2.97)	0.26 (0.03)	0.20 (0.03)	0.51 (0.02)	0.41 (0.02)	10.70 (1.27)	0.11	0.41 (0.05)	1.28 (0.06)	0.91 (0.01)	This study
500	49.00 (2.83)	0.10 (0.01)	0.21 (0.02)	0.51 (0.01)	0.40 (0.01)	11.67 (0.49)	0.02	0.24 (0.02)	1.31 (0.02)	0.92 (0.00)	This study
1000	60.75 (2.94)	0.06 (0.00)	0.22 (0.02)	0.51 (0.01)	0.40 (0.01)	11.78 (0.37)	0.01	0.19 (0.01)	1.31 (0.01)	0.92 (0.00)	This study
1500	66.90 (3.02)	0.05 (0.00)	0.22 (0.02)	0.52 (0.01)	0.40 (0.01)	11.71 (0.28)	0.01	0.18 (0.01)	1.30 (0.01)	0.92 (0.00)	This study
2000	74.45 (1.19)	0.04 (0.00)	0.22 (0.01)	0.52 (0.01)	0.40 (0.01)	11.72 (0.13)	0.01	0.16 (0.00)	1.31 (0.00)	0.92 (0.00)	This study
2352	78	0.03	0.22	0.52	0.40	11.72	0.01	0.15	1.31	0.92	This study
33.60 (15.30)	12.00 (4.4)	0.40 (0.17)	NR	NR	NR	6.096 (2.62)	0.27 (0.15)	NR	NR	NR	Stoddart 1984
49.20 (1.72)	44.00 (3.20)	0.89 (0.05)	0.022 (0.04)	NR	NR	39.22 (6.11)	0.91 (0.099)	NR	NR	NR	Benzie et al. 1995
45.20 (2.95)	36.00 (6.00)	0.80 (0.14)	NR	NR	NR	29.72 (9.27)	0.88 (0.16)	NR	NR	NR	Ayre et al. 1997

48.00 (0.00)	29.00 (4.40)	0.61 (0.09)	S	NR	NR	NR	0.42 (0.11)	NR	NR	NR	Adjeroud & Tsuchiya 1999
28.60 (1.81)	21.00 (3.30)	0.72 (0.01)	S	S	S	S	0.73 (0.13)	NR	NR	NR	Miller & Ayre 2004
29.50 (0.58)	24.00 (2.90)	0.81 (0.10)	S	NR	NR	20.08 (3.73)	0.81 (0.09)	NR	NR	NR	Ayre & Miller 2004
54.10 (16.40)	42.00 (5.90)	0.81 (0.12)	S	S	S	33.67 (8.13)	0.77 (0.19)	NR	NR	NR	Sherman et al. 2006
63.60 (34.20)	15.00 (9.40)	0.27 (0.12)	S	NR	NR	4.70 (2.28)	0.44 (0.26)	NR	NR	NR	Whitaker 2006
41 (0.00)	15.00 (3.5)	0.38 (0.09)	NR	NR	NR	1.96 (0.01)	0.05 (0.00)	NR	NR	0.50 (0.00)	Yeoh & Dai 2009
16.60 (7.86)	16.00 (7.80)	0.97 (0.09)	S	S	S	NR	NR	NR	NR	0.54 (0.12)	Starger et al. 2010

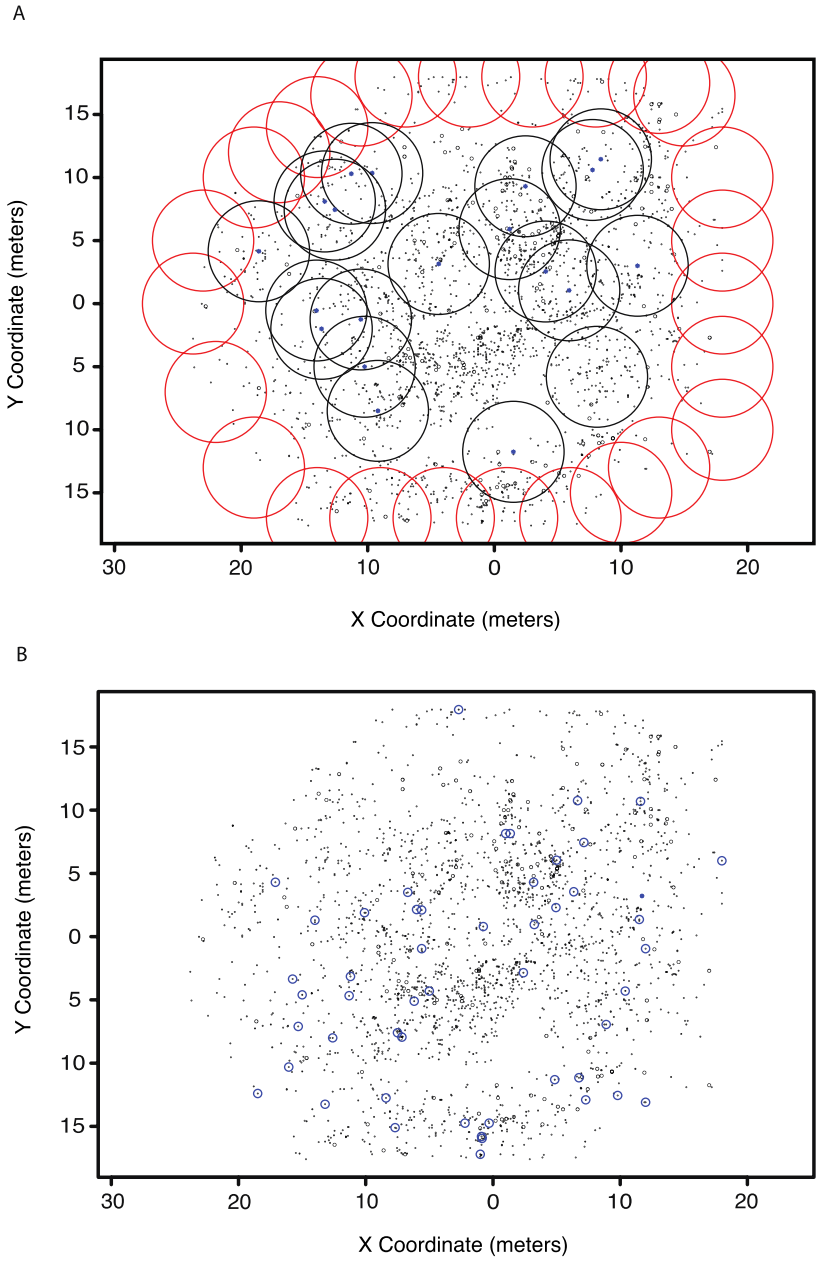


Figure 4.1. (A) Example of 20 saturation sampled subsets. Each black circle has a diameter of 8 m and the black dots within the circle are the locations of coral colonies, representing a subset. Red circles represent areas that could not be used as the center of a sampling area due to proximity to the edge. (B) Example of one randomly sampled subset of 50 individuals. Sampled colonies are circled.

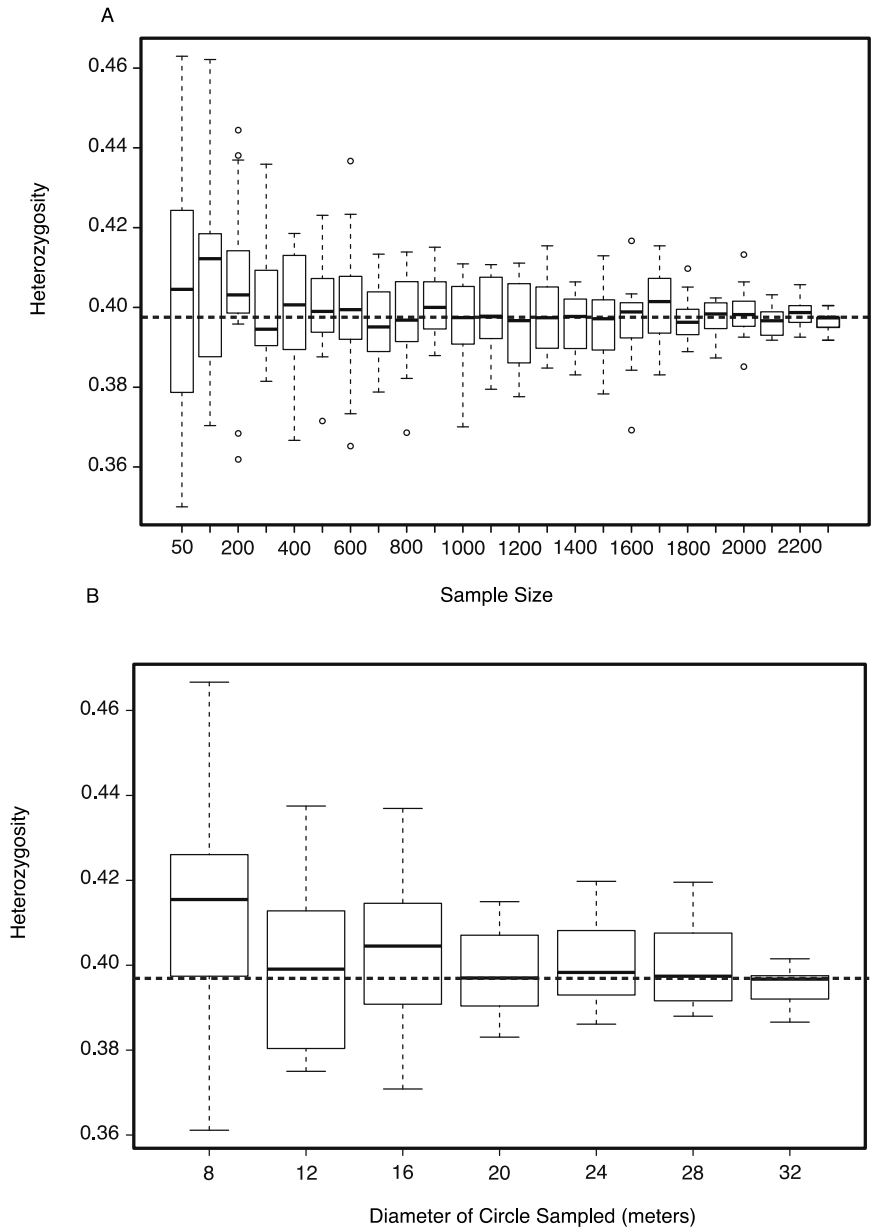


Figure 4.2. Estimated observed heterozygosity values (H_O) for increasing sampling numbers with random sampling (A) or increasing spatial area and exhaustive sampling (B). The rectangle represents the interquartile range (i.e., the 25th percentile, median, and 75th percentile), the whiskers represent all values that are within 1.5 times the interquartile range. Open circles represent outliers, defined as data points that lie outside the whiskers. The horizontal dashed line is the actual value.

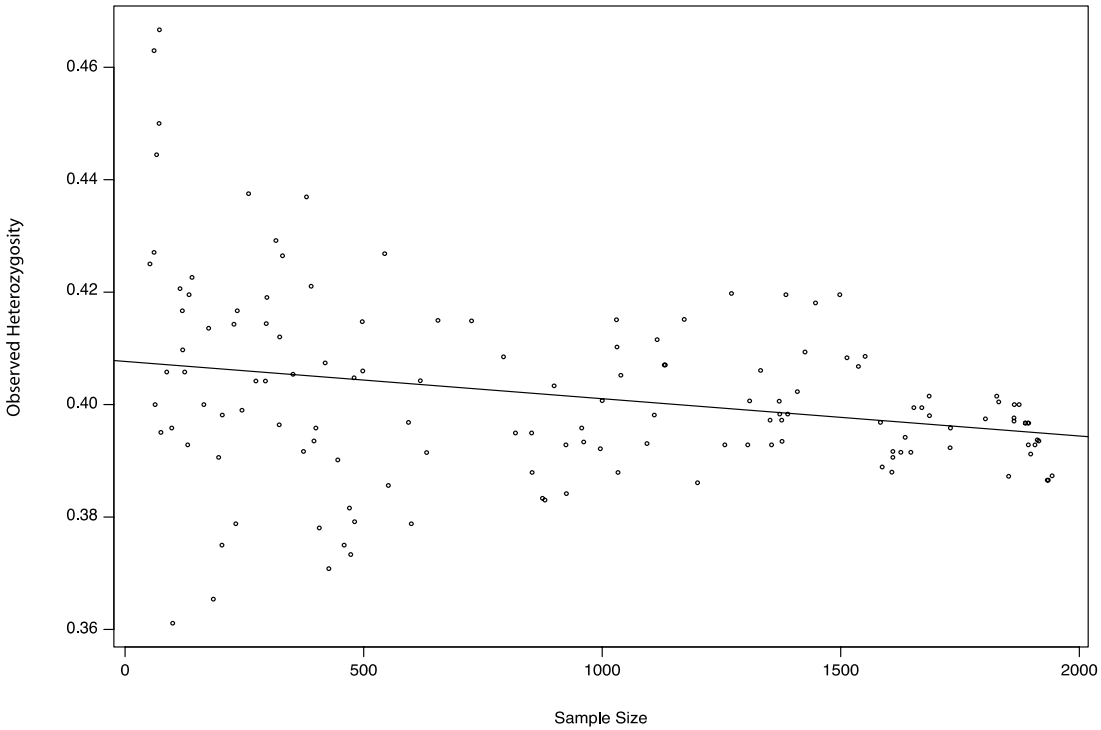


Figure 4.3. Weighted least squares linear regressions of observed heterozygosities (H_O) on the number of saturation sampled individuals taken from the original, spatially-autocorrelated dataset. Similar regressions based on randomly sampling from the original dataset as well as randomly sampling or saturation sampling from a spatially-randomized dataset were not statistically significant.

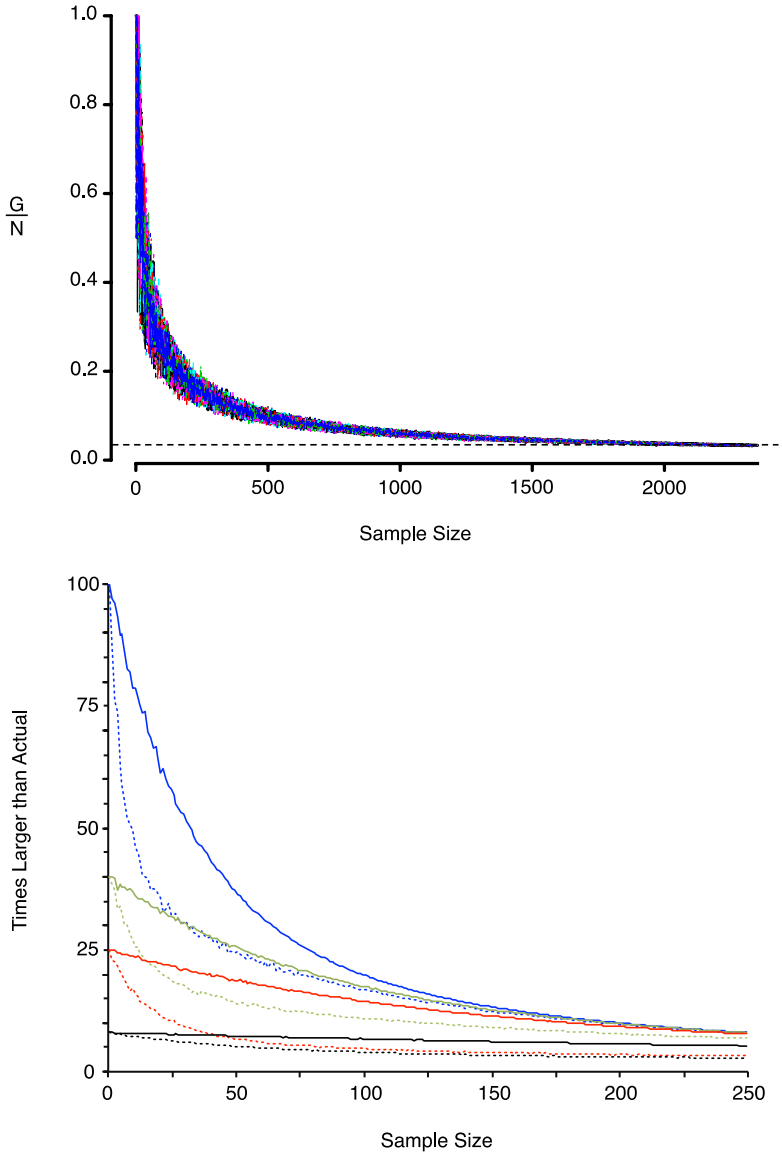


Figure 4.4. (A) 100 overlaid rarefaction curves, each representing the calculated clonal richness statistic (*G*/*N*) for all possible sample sizes between 1 and 2352. The horizontal, dashed line is at the actual value for the population. (B) Rarefaction curves based on the means of 100 replications of simulated datasets of 2000 ramets split evenly (solid lines) among 20 (blue), 50 (green), 80 (red), and 250 (black) genets or split unevenly (dashed lines) such that 70% of the ramets were distributed evenly among 10% of the genets while the remaining 30% of ramets were distributed evenly among the remaining 90% of the genets.

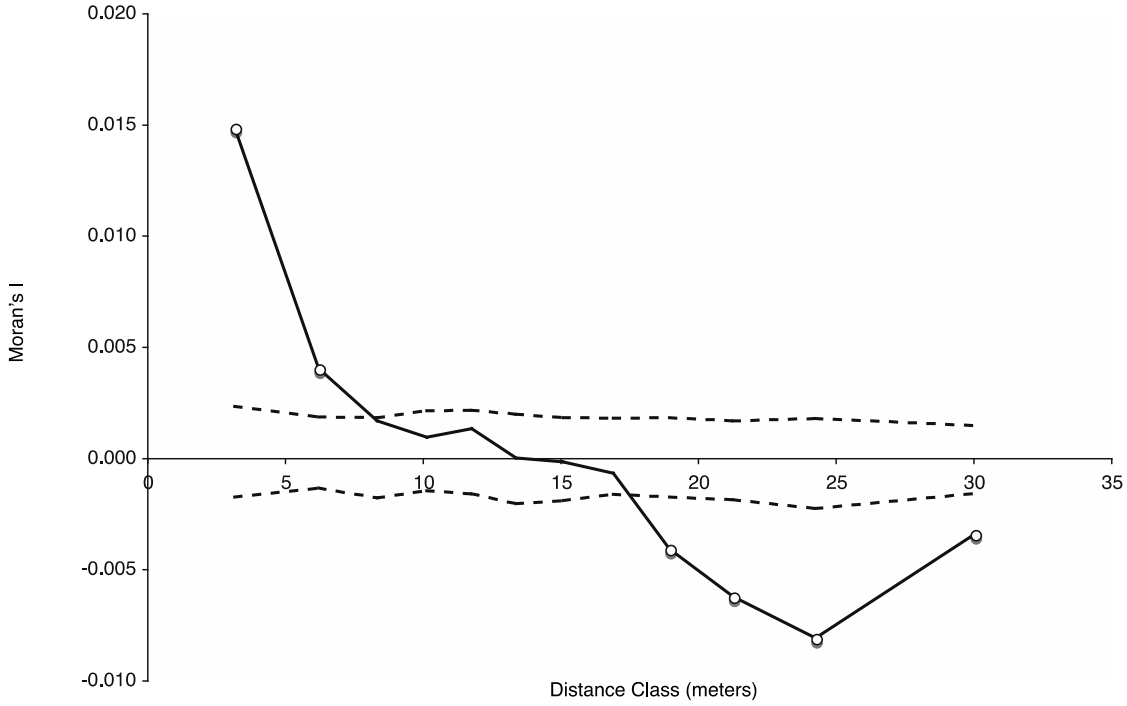


Figure 4.5. Spatial autocorrelation analysis of my complete dataset depicted as a correlogram of Moran's *I* relationship coefficient versus geographic distance. Dashed lines represent 95% confidence intervals based on permutating individual locations. Open circles on the graph highlight distance classes with statistically significant autocorrelation.

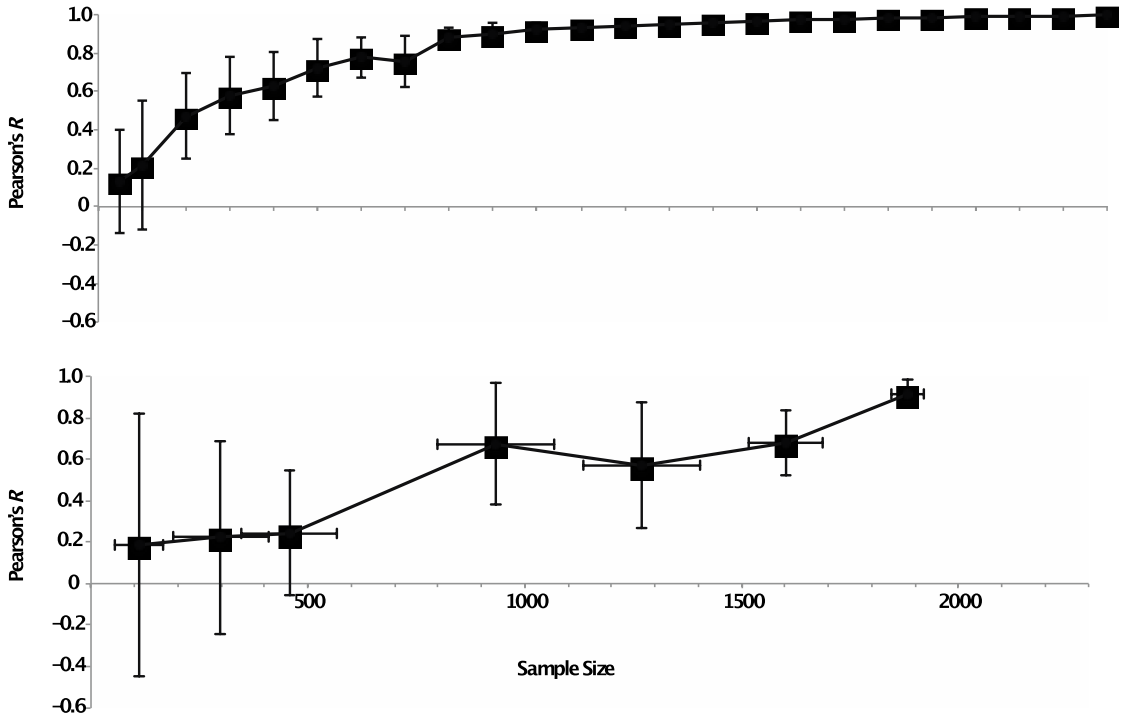


Figure 4.6. Average correlation coefficient with the original dataset's correlogram (Pearson's R) of all randomly sampled (top) and saturation sampled (bottom) subsets. Y axis error bars reflect one standard deviation in Pearson's R.

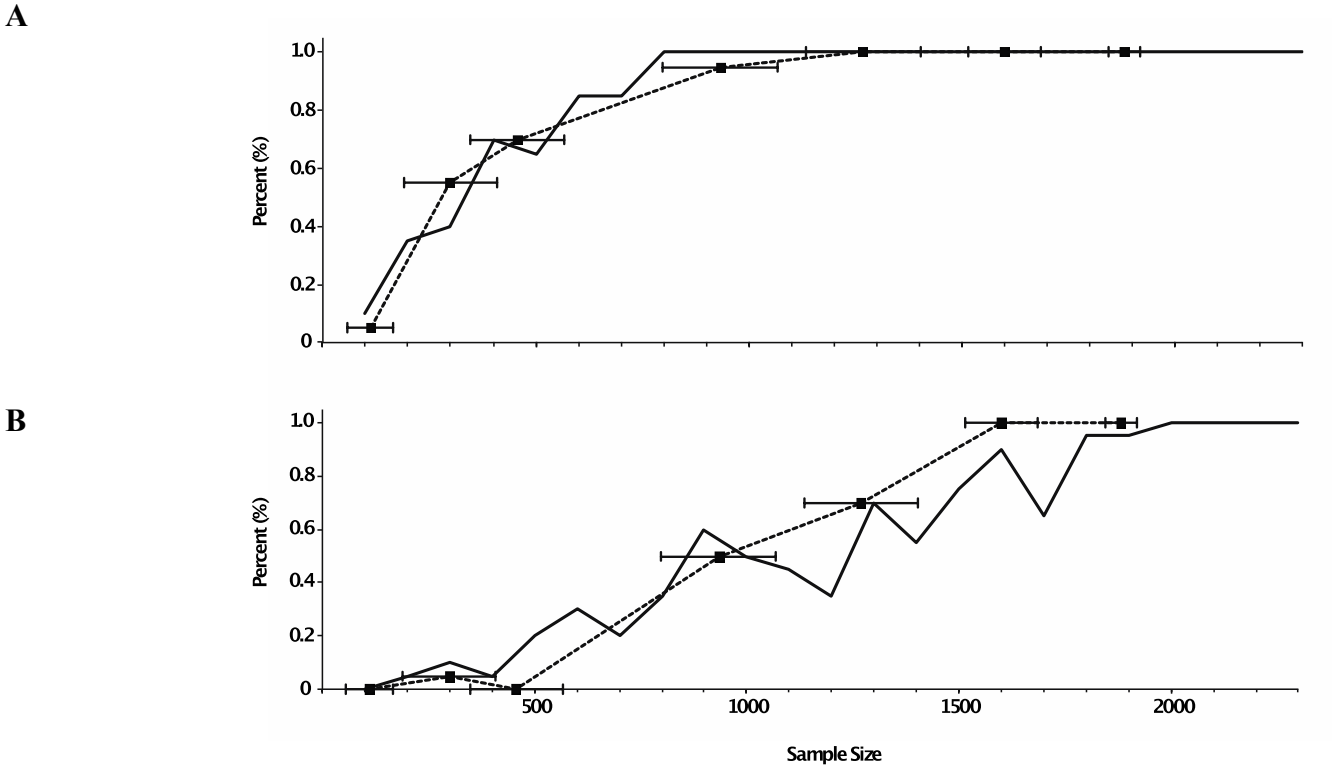


Figure 4.7. Percent of subsets, for each sampling intensity, that are able to detect statistically significant spatial autocorrelation for the first (A) and second (B) distance classes, respectively. Solid lines represent random sampling and the dashed lines represent exhaustive sampling.

CHAPTER 5. DEPTH AS AN ORGANIZING FORCE IN *POCILLOPORA DAMICORNIS* INTRA-REEF SPATIAL GENETIC STRUCTURING

Abstract

Relative to terrestrial plants, and despite similarities in life history characteristics, the potential for corals to exhibit intra-reef local adaptation in the form of genetic differentiation along an environmental gradient has received little attention. The potential for natural selection to act on such small scales is likely increased by the ability of coral larval dispersal and settlement to be influenced by environmental cues. Here, I combine genetic, spatial, and environmental data for a single patch reef in Kāneʻohe Bay, Oʻahu, Hawaiʻi in a landscape genetics framework to uncover environmental drivers of intra-reef genetic structuring. The genetic dataset consists of near-exhaustive sampling (n = 2352) of the coral, *Pocillopora damicornis* at my study site and genotyping at six microsatellite loci. In addition, three environmental parameters – depth and two depth-independent temperature indices – were collected on a 4 m grid across 85 locations throughout the reef. I use ordinary kriging to spatially interpolate my environmental data and estimate the three environmental parameters for each coral individual. Partial Mantel tests indicate a significant correlation between genetic relatedness and depth while controlling for space. These results are also supported by multiple regression of distance matrices as well as multi-model inference. Furthermore, spatial Principle Component Analysis was used to visualize spatial genetic patterns by optimizing spatial autocorrelation and genetic variance in the dataset. The results indicate a statistically significant genetic cline along a depth gradient. Binning the genetic dataset based on size-class information revealed that the correlation between genetic relatedness and depth was significant for new recruits and increased for larger size classes, suggesting a possible role of larval habitat selection as well as selective post-settlement mortality in structuring intra-reef genetic diversity. That both pre- and post-recruitment processes are involved points to the adaptive role of larval habitat selection in increasing adult survival. The conservation importance of uncovering intra-reef patterns of genetic diversity is discussed.

Introduction

Local adaptation occurs when populations become fine-tuned to their environment through the process of natural selection. Locally adapted genotypes, however, can be swamped out by the arrival of immigrants from populations differently adapted. This paradigm has served as the dominant one in terrestrial systems where physical isolation is considered important, if not essential for populations to diverge (Mayr 1942). In contrast, since many marine organisms begin their lives as planktonic larvae, it was believed that the diversifying effects of natural selection would be countered by the homogenizing effects of high gene flow connecting populations in the ocean (discussed in Hellberg 2009). This has since been largely abandoned as numerous examples from fish (Conover et al. 2006, Rocha & Bowen 2008) and various marine invertebrates (Bird et al. 2011, Sanford & Kelly 2011) demonstrate that not only can marine populations become locally-adapted, but that ecological differences between populations can, in some cases, be strong enough to foster speciation events in sympatry (Bowen et al. 2013).

In marine systems, indication of local adaptation comes from both experimental studies involving reciprocal transplant experiments (e.g., Sherman & Ayre 2008, Barshis et al. 2010) as well as from statistical inferences based on correlating population genetic data with environmental variables (e.g., Jørgensen et al. 2005) or in the detection of outlier loci that deviate from neutral expectations (e.g., Oetjen & Reusch 2007). Recently, researchers have uncovered both environmental (e.g., Gorospe & Karl 2011) and genetic heterogeneity (reviewed in Palumbi 2004) in marine systems at small spatial scales. As a result, local adaptation in marine systems has begun to receive increasing attention (Sotka 2005, Conover et al. 2006, Sanford & Kelly 2011).

The term “local adaptation” implies the existence of a pattern on a small spatial scale. This is often interpreted to mean adaptive differences between discreet demes existing in distinct habitats. It is important to note, however, that local adaptation can also refer to differences within continuous populations along a continuous environmental gradient (Kawecki et al. 2004). Small-scale, local adaptation in plants often can be found in the form of genetic differentiation being structured along environmental gradients (Vekemans & Hardy 2004, Leimu & Fischer 2008). This characteristic is believed to be

common in plants because they are: (1) sessile, and thus more susceptible to small-scale environmental heterogeneity (Linhart & Grant 1996) and, in some cases, (2) able to reproduce clonally, allowing selective factors to act on identical genotypes over multiple generations (van Kleunen et al. 2001; Knight et al. 2004). For both fish and marine invertebrates, experimental studies have found evidence of adaptive genetic divergence at spatial scales ranging from tens to thousands of kilometers (Sanford & Kelly 2011), however, this is likely biased by the fact that marine local adaptation has largely been viewed from a population standpoint. For example, while local adaptation has been demonstrated for corals on an inter-reef level, (e.g., D'Croz & Maté 2004; Vermeij et al. 2007), the potential for local adaptation on an intra-reef scale has rarely been investigated (however, see Barshis et al. 2010). Investigating patterns of individual-level genetic variation, as is advocated for in landscape genetic analyses (Storfer et al. 2007), may hold insight into the potential for natural selection to influence patterns of genetic diversity.

Corals share the same sessile and reproductive traits that predispose plants to local adaptation. Yet, while investigations that look at individual-level genetic variation across an environmental gradient are common in the terrestrial plant literature (e.g., Kalisz et al. 2001), similar investigations are rare for corals. Furthermore, it could be argued that corals may have an increased potential to exhibit similar patterns because unlike plants, the dispersing propagules of corals engage in active site selection. The ability of larvae in sessile marine invertebrates to sense and be attracted to specific settlement cues has been shown to be intra-specifically variable and heritable (Toonen & Pawlik 2001, Baums 2008, Meyer et al. 2009) as well as resulting in an increased probability of survival (Hurlbut 1993, Raimondi & Morse 2000) indicating an evolutionary potential for natural selection to act on this trait. Indeed, a variety of biotic and abiotic settlement cues are known to act on a range of spatial scales from long-distance cues (e.g., open ocean currents) that influence larval transport towards islands to those that influence larval site selection within a reef (e.g., sedimentation) to still others (e.g., depth, temperature, light intensity, and chemical cues) that likely influence larvae throughout their dispersing stage (McEdward 1995; Gleason & Hofmann 2011). Therefore, it is possible that adaptive genetic divergence in corals may be found on an intra-reef scale. It has long been recognized that reefs exhibit zonation patterns in the distribution of coral species (Goreau

1959) largely driven by environmental heterogeneity and inter-specific larval behavioral differences (Raimondi & Morse 2000, Baird et al. 2003), but it remains to be tested whether intra-specific, individual-level genetic variation at this scale can also be explained by similar processes.

As described in Chapter 3, (Gorospe & Karl, accepted) I genotyped and mapped nearly every individual of the coral, *Pocillopora damicornis*, within a single reef. I found non-random spatial patterns of genetic relatedness (i.e., spatial autocorrelation) and attributed intra-reef positive spatial autocorrelation of genetic relatedness to larval dispersal processes whereby both clonally and sexually produced planulae of *P. damicornis* tend to settle nearby their brooding parent. What remained speculative, however, was the causes of negative spatial autocorrelation whereby at extreme distances, corals were more unrelated to each other than expected from random. Indeed, a major question remaining unanswered is to what extent are geographic patterns maintained by drift (i.e., restricted dispersal and spatial processes) versus environmental heterogeneity (i.e., natural selection and larval recruitment behavior). In other words, there is a need to disentangle the contributions of spatial and environmental processes that underlie the observed pattern of genetic variation. By allowing for the control of spatial dependence in environmental variables, landscape genetic analyses move beyond simply describing patterns of genetic and environmental variation and attempt to elucidate the processes that are responsible for generating them (McIntire & Fajardo 2009, Schoville et al. 2012). Here, I integrate my spatial and genetic datasets (Gorospe & Karl, accepted) with depth and temperature datasets collected for the same reef (Gorospe & Karl 2011) to examine how environmental characteristics may be influencing intra-reef spatial genetic patterns.

Methods

Genetic, spatial, and size data collection

Coral sampling and mapping and microsatellite genotyping are described in Chapter 3 (Gorospe & Karl, accepted). In addition, the image analysis software, *ImageJ* (ver. 1.45s; Rasband 1997) was used to measure the surface area of each individual coral based on size-standardized photographs taken in the field. For my measure of genetic relatedness, I use Hardy and Vekemans' (Hardy & Vekemans 1999) coefficient of

relationship, which is a measurement of pairwise genetic relatedness based on the Moran's *I* measurement of spatial autocorrelation (Moran 1950). Finally, it should be noted that Hardy-Weinberg equilibrium and locus-level analyses as well as spatial and clonal structure analyses are highlighted elsewhere (Gorospe & Karl, accepted) such that I can focus on the landscape genetics analyses here.

Environmental data collection

High-Resolution Thermochron iButton temperature and time data loggers (model DS1921H; Maxim Integrated Products, Inc., Sunnyvale, CA, USA) were deployed on a 4 m grid across 85 locations throughout Reef 19 and depth measurements were made as described in Chapter 2 (Gorospe & Karl 2011).

Many characteristics of sea temperature are indeed correlated with depth (e.g., average daily temperature, average daily temperature range, average monthly temperature, etc.) and therefore, are not included in subsequent analyses to avoid colinearity among variables. As described in Chapter 2 (Gorospe & Karl 2011), however, I found two temperature indices that in fact could not be explained by depth based on partial regression analyses. The two temperature indices were Relative Hotspots and Relative Hothours (Gorospe & Karl 2011) and are included in this study. The Relative Hotspots Index is defined as the proportion of time over two years during which a location was one standard deviation hotter than the average temperature for the whole reef. The Relative Hothours Index, on the other hand, is defined as the proportion of time spent over the course of two years during which the temperature at a location was one standard deviation hotter than the average temperature for that same location in the past twelve hours. Thus, areas of the reef that have a high Relative Hotspots Index are frequently warmer than spatially averaged temperatures for the entire reef, while areas of the reef that have a high Relative Hothours Index are frequently warmer than site-specific temporally averaged temperatures.

I then combine the Hotspots and Hothours indices information of the 85 temperature monitoring sites with their individual spatial locations to spatially interpolate a Hotspots and Hothours map for the entire reef. To do this, I use the *gstat* package (Pebesma & Wesseling 1998) in R to perform ordinary kriging, a geostatistical spatial interpolation method that models the relationship between distance and variance of

sampled points to predict values at unsampled locations. My dataset of coral spatial coordinates was then layered on top of these maps to obtain an estimate for each coral colony's Hotspot and Hothour index. Lastly, the same statistical techniques were applied to my depth measurements to generate a bathymetric map of the reef (Gorospe & Karl 2011) and estimate the depth at which each coral colony resides.

Data analysis

First, I calculated pairwise Euclidean distances for each individual coral's spatial coordinates and environmental data (i.e., depth as well as Hothours and Hotspots indices). For the genetic data, coefficients of relationship based on Moran's *I* (Hardy & Vekemans 1999) were calculated using the program Spatial Pattern Analysis of Genetic Diversity (SPAGeDi; Hardy & Vekemans 2002) for all pairs of coral individuals. These five measures (i.e., spatial, Hothours, Hotspots, and depth distances as well as genetic relatedness) were then used for subsequent analyses investigating the relationship, if any, between genetic relatedness and one or a combination of the spatial or environmental variables.

In order to account for spatially autocorrelated environmental data, I performed separate partial Mantel tests (Mantel 1967, Smouse et al. 1986) to calculate the ranked correlation (i.e., analogous to a Spearman's correlation) between genetic relatedness and each of the three environmental variables (i.e., Hothours, Hotspots, depth), while controlling for the effect of spatial distances. The partial Mantel test calculates the correlation between two distance matrices, A and B, while controlling for the effect of a third, C, by calculating the correlation between the matrices of residuals between A and C and A and B. Furthermore, statistical significance is based on creating a null distribution by Monte Carlo randomization, whereby one of the matrices is unmanipulated and the other is randomly permuted (Smouse et al. 1986). While past attention has criticized the use of the permutation procedure for the partial Mantel test (Raufaste & Rousset 2001, Castellano & Balletto 2002), studies confirm that for sample sizes greater than 50, permutation procedures remain valid (Anderson & Robinson 2001, Legendre & Fortin 2010). In addition to treating depth as an environmental variable in which spatial autocorrelation must be controlled for, I also tested for the relationship between genetic

relatedness and spatial distance, while controlling for the effect of depth. This allows me to tease out the influence that depth may have on spatial location and vice versa.

Furthermore, to investigate how the genetic correlation with space and each of the environmental variables may have changed over time, I repeated the partial Mantel test analyses on several subsets of coral samples after incorporating each coral individual's size data (i.e., surface area) as a proxy for age. Note that while issues of partial mortality and individual variation in growth rate make the correlation between surface area and age less than perfect in *P. damicornis*, it is still true that older corals tend to be larger than younger corals (Hughes and Connell 1987), thus allowing me to bin corals into various size classes. Corals with surface area < 10 cm^2 (i.e., max diameter ~3.5 cm) were placed into the smallest size class and are considered to be relatively recent recruits on the reef (i.e., less than 2 years old; Harriott 1985). Defining a bin size for the oldest size class, however, is complicated by the fact that colonies may suffer from partial mortality or may be the result of fusion between two originally separate colonies (i.e., larger size classes will likely have a larger variance in age; Hughes & Connell 1987). To reflect this uncertainty, I vary the lower bound of the largest size class and define the oldest recruits on the reef to be those with surface areas greater than 30, 40, 60, or 90 cm^2. Partial Mantel tests investigating the relationship between genetics and depth versus space were then rerun separately for each bin. I also calculate pairwise genetic distances (F_{ST}) between all size class bins based on an analysis of molecular variance (AMOVA; Excoffier 1992) as implemented in the program, GENODIVE (Meirmans & van Tienderen 2004). For this analysis, I remove all repeated MLGs within each size class bin and calculate significance based on 9999 permutations.

Next, I used an information theoretic approach (Burnham & Anderson 2001) to decide which environmental variables to interpret further by modeling genetic relatedness as a function of each variable described above (i.e., spatial, depth, Hothours, and Hotspots distances) as well as all possible linear combinations of them. To do this, I use the package MUMIN (Bartón 2009) in R. Akaike weights (i.e., normalized likelihood values; *w*) were calculated for each model and all models were ranked based on Akaike's (1992) information criterion (AIC). For each model, i, if $AIC_i - AIC_{min} > 10$ this model was considered to be poorly supported and therefore not considered further (Burnham &

Anderson 2001). Finally, for each predictor variable, the AIC weights are summed for all models containing that variable. A predictor weight (w_+) is thus calculated for each variable, allowing the variables to be ranked in order of their importance. Thus, in contrast to simply selecting those variables that are contained in the single best model, inference is based on the entire set of models (i.e., multi-model inference; Burnam & Anderson 2001, Anderson et al. 2000).

To analyze the relationship of all variables simultaneously, I performed a multiple regression of distance matrices (MRDM; Manly 1986) using package ECODIST (Goslee & Urban 2007) in R. Unlike multiple regression on raw data, the parameter estimates of MRDM do not depend on the order of predictors inputted in the model, but only on which specific predictor variables are used (Legendre et al. 1994). In this analysis, the matrix of coefficients of genetic relationship is considered as a function of multiple independent distance matrices (predictor variables), and the statistical significance of regression coefficients for each predictor variable is tested based on matrix permutations (Legendre et al. 1994). Here, I perform MRDM using the four spatial and environmental distances described above as predictor variables, but then repeat the analysis selecting only those predictor variables that emerged from my multi-model inference framework.

Finally, as standard genetic differentiation analyses (i.e., F_{ST}) are too coarse for the fine-spatial scale patterns in which I am interested, I perform a spatial principal components analysis (sPCA; Jombart et al. 2008) using the adegenet package in R (Jombart 2008). Unlike PCA, which seeks new axes (i.e., principal components) to summarize the data based on maximizing the genetic variance among individuals, sPCA seeks new axes that optimize the product of genetic variance and their spatial autocorrelation as measured by Moran's *I*. The calculation of Moran's *I* requires that neighboring entities in the dataset be defined by a connection matrix. Here, I define neighbors as any set of coral individuals within a certain distance from one another (i.e., a neighbor by distance connection network). I use the program, SPAGeDi, to calculate the mean of the largest distance bin to exhibit positive autocorrelation and use this as the upper distance limit of neighbors. For the distance classes, I use bins that create equal numbers of pairwise comparisons per distance bin and use 200 permutations of the spatial locations to define statistically significant autocorrelation.

Using both the connection matrix and a matrix of individual allele frequencies, sPCA results in both positive and negative eigenvalues, corresponding to global (i.e., clines where neighbors tend to be genetically similar) and local (i.e., patches where neighbors tend to be genetically different) spatial structures, respectively (Thioulouse et al. 1995). Each principal component axis represents a different spatial structure, and the decision of which axes to retain for interpretation is based on inspection of the scatter plot of all eigenvalues decomposed into their genetic variance and spatial autocorrelation components (i.e., screeplot). The mean PC scores of each individual's neighbors (i.e., lagged or de-noisified scores) are then plotted back to that individual's spatial coordinates to reveal the genetic spatial structure of the reef. To test for significance of overall global and local structures, I use the Jombart et al. (2008) method involving spatial filters created by decomposing the connection matrix into a set of Moran's eigenvector maps (as is done in principal components of neighbor matrices; Griffith et al. 2006, Dray et al. 2006, see Gorospe & Karl 2011 for an example). The matrix of individual allele frequencies is then correlated separately for global and local filters and statistical significance based on a Monte Carlo randomization procedure in which I use 9999 matrix permutations to generate a null distribution. Here, the null hypothesis is that individual allele frequencies are randomly distributed throughout the neighbor by distance connection network, while the alternative hypothesis is that individual allele frequencies display at least one global or local spatial structure (Jombart et al. 2008).

Results

To highlight the small scale of my landscape genetic dataset, I provide boxplots (Figure 5.1) of all pairwise coefficients of genetic relationship as well as all spatial and environmental variables. A total of 2352 individuals had complete six-loci genotypes as well as spatial and environmental data and were included in these analyses. Notice for all datasets, there is a concentration of small distances with larger distances as outliers. For genetic relatedness (Moran's *I*), this indicates a concentration of closely related pairs of individuals, as was previously discussed in Chapter 3 (Gorospe & Karl, accepted). For spatial distances, this pattern of mostly small distances is expected for any sampling scheme as larger distances are confined to pairs of the relatively fewer outer sampling points (i.e., the number of individual separated by a certain distance decreases with

increasing distance). For the environmental distances of depth, Hotspots and Hothour indices, the pattern noted in the boxplots simply is a reflection of normally-distributed data, with points around the mean characterizing most of the small distances and outlying points characterizing the relatively fewer larger distances.

The results of all partial Mantel tests are shown in Tables 5.1 and 5.2. Here, a significant and negative Mantel *r* indicates a weak relationship between that variable and genetic relatedness, whereby closely related corals (high genetic relationship coefficients) tend to be found in similar environments (small environmental distance). In fact, all environmental variables displayed this relationship, but only depth and space were found to be statistically significant (Table 5.1). It should be noted, however, that partial Mantel tests are based on distances and not raw data values, and therefore Mantel *r* values cannot be interpreted as the percent of variance in the response variable explained by the predictor variable (i.e., the coefficient of determination; r^2) as is done in regression analyses (Legendre & Fortin 2010). Of the 2352 genotypes, 2229 also had surface area data based on size-standardized photographs taken in the field, and could therefore be binned into size classes. The results of partial Mantel tests comparing smaller (< 10 cm^2) versus larger (> 30, 40, 60, or 90 cm^2) corals are reported in Table 5.2. Once again, only depth and space were significantly correlated with genetic relatedness for smaller corals. For depth, the correlation with genetic relatedness increased for larger corals (-0.020 to -0.030) when compared to smaller corals (-0.014). In contrast, spatial distance and genetic relatedness was only significantly correlated for the smallest size class. Lastly, based on the AMOVA, all estimates of genetic differentiation (F_{ST}) between pairs of size class bins were not significantly different from zero ($0.948 < p < 1.00$), indicating a lack of genetic differentiation among recruiting cohorts.

After model ranking based on AIC, only eight models had $AIC_i - AIC_{min} < 10$, and were thus selected for further consideration (Table 5.3). The model with the most support (i.e., lowest AIC) only contained depth and space as predictors of genetic relatedness. The AIC values for many of the models including Hotspots and/or Hothours, however, were relatively close ($\Delta AIC < 2$) and thus hold considerable support of their own. Predictor weights calculated by multi-model inference for each environmental and spatial variable (w_+) are reported in Table 5.1. Based on multi-model inference of

predictor weights, only space and depth were highly supported variables. Furthermore, when using all four spatial and environmental variables, results of MRDM also indicated that only the regression coefficients for space (p=0.001) and depth (p=0.001) were statistically significant. Thus, MRDM was repeated using just depth and space as predictor variables, and results of this analysis are reported in Table 5.1.

In the sPCA analysis, since space and depth both appear to be significant drivers of genetic variation, I perform two separate analyses: one using each coral's depth coordinates and another using spatial (x, y) coordinates. Thus, for the connection networks, I performed two separate spatial autocorrelation analyses comparing genetic relatedness with spatial distances (Figure 5.2A) and genetic relatedness with depth distances (Figure 5.2B) to define the upper distance limit of neighbors. Although theoretically possible, it should be noted that I do not perform a three dimensional sPCA using x, y, and depth coordinates because in this study I consider depth and space as separate, potential drivers of genetic variation. In the interpretation of sPCA, I consider depth-driven genetic variation as an environmental process and space-driven genetic variation as a dispersal process, and thus analyze them separately.

Based on the Monte Carlo randomization test, the sPCA based on depth coordinates showed significant global structures (p=0.019), while the sPCA based on spatial coordinates did not (p=0.102). Neither analysis, however, showed significant local structures (p=0.936 for space and p=0.840 for depth), and therefore I only consider the global structures further. Based on the screeplot for the depth-based sPCA (Figure S1A in APPENDIX C), it appears that all global and local principal components contain relatively similar amounts of spatial autocorrelation, but only display results for the first global principal component (λ_1), as this contained most of the genetic variance in the data. Mapping the PC scores of each individual back to their x, y coordinates revealed a depth cline in genetic variation with corals in the shallow, center portion of the reef and corals on the outer and deeper edges of the reef generally showing negative and positive PC scores, respectively (Figure 5.3A). The pattern becomes even more pronounced when the lagged (i.e., de-noisified) PC scores are plotted (Figure 5.3B). Global structures of other components exhibited similar spatial structures (not shown). I also express the contribution of each allele to this first principal component as squared loadings (Figure

S2 in APPENDIX C), and notice appreciable loadings from five out of six loci, although alleles from two loci in particular (PD2-006 and PD2-AB79) were the largest contributors. I do not include the sPCA results based on spatial coordinates since neither global nor local structures in this analysis were statistically significant. For exploratory purposes, however, I translate each of the first three global components of my sPCA based on spatial coordinates into a color intensity (red, green, and blue) to visualize them simultaneously (Figure S3 in APPENDIX C). The resulting map displays what also appears to be a depth cline in genetic variation with colonies in the center portion of the reef appearing to have different combined PC scores from corals on the outer edges of the reef (Figure S3 in APPENDIX C).

Discussion

Depth as a selective factor

Uncertainty in explaining patterns of genetic diversity can originate from multiple processes converging, particularly at small-scales (e.g., Johnson & Black 1982, Johnson & Black 1984, Selkoe et al. 2010). I have attempted to reduce this uncertainty through my intense characterization of my study site as well as through the use of spatially-explicit analyses. Depth and spatial location are correlated such that corals found at the same location will also tend to have similar depths and vice versa. Furthermore, my relative Hotspot and Hothour temperature indices are correlated with space (Chapter 2; Gorospe & Karl 2011). I tease apart the effects of depth, temperature and space using my partial Mantel tests, and find only depth and space to be significantly correlated with genetic relatedness. This is also confirmed by multi-model inference as well as by the results of multiple regression on distance matrices. Since spatial processes were discussed previously (Chapter 3; Gorospe & Karl, accepted), here I focus on the causes and consequences of genetic diversity patterned along a depth gradient. This study joins only a few (Bongaerts et al. 2010; Carlon & Budd 2002) that have focused on the depth-associated distribution of the host coral's genetics.

Based on the Monte Carlo randomization procedure of the sPCA, allele frequencies at my study site show positive autocorrelation ($p = 0.019$) with regards to depth. In other words, genetic diversity exhibits a depth cline whereby individuals at similar depths tend to have more similar genotypes than individuals at different depths.

When I restrict the partial Mantel tests to the smallest size class, I see that this is true even for likely recent coral recruits. Planulae of *P. damicornis* have the ability to remain in the plankton for more than 100 days after release (Richmond 1987). While this might point to the potential for *P. damicornis* to disperse over long distances, it has also been shown that planulae are competent (i.e., able to respond to settlement cues and begin metamorphosis) as soon as 12 h after release (Isomura & Nishihira 2001). Given the weak, but significant correlations between genetic relatedness and depth distances (Tables 5.1 and 5.2), these data point to a detectable role for depth in larval habitat selection at this scale.

In addition to genetics and depth being correlated for the smallest size class, I also find that the magnitude of this correlation increases for increasing size classes (Table 5.2). Furthermore, based on the AMOVA, groups of corals in different size classes were genetically not different, thus ruling out the possibility that the temporal change in genetic-depth correlation could be due to genetic differences between recruiting cohorts (i.e., chance recruitment events). Put another way, since there are no genetic differences between newer and older coral recruits, the increasing correlation between genetic relatedness and depth for increasing size classes is being driven by differences in the depth distribution between older and younger corals, thus pointing to depth as a post-recruitment, selective factor. In particular, increased environmental dependence of genetic relatedness for larger size classes may be explained by selective mortality (e.g., Tonsor et al. 1993, Kalisz et al. 2001). Indeed, pre- and post-recruitment processes are linked for marine larvae as metamorphosis is seen as irreversible and behavioral selection of a settlement site is meant to increase adult survival. While depth has been shown to influence larval swimming behavior (Stake & Sammarco 2003), it is difficult to explain how depth alone (i.e., hydrostatic pressure) could play a selective role in structuring intra-reef genetic diversity.

It should be emphasized, that my Hotspot and Hothour temperature indices were specifically chosen for this analysis because they were independent of depth (Gorospe & Karl 2011). The use of depth as an explanatory variable, however, may potentially be serving as a proxy for other temperature characteristics that are in fact depth-dependent. For corals, temperature appears to be an important environmental selective factor, with

different populations exhibiting different bleaching responses (D'Croz & Maté 2004, Smith-Keune & van Oppen 2006), growth rates (Smith et al. 2007), or stress protein expression levels (Barshis et al. 2010) in different temperature regimes. The importance of temperature in adult survival, therefore, makes it a likely candidate as an important settlement cue. On the other hand, another potential cause of depth-dependent genetic structuring to consider is the role of light in affecting coral larval settlement (Mundy & Babcock 1998). Selecting a habitat with a suitable light regime is important for adult coral survival, especially given the role of their intracellular, photosynthetic *Symbiodinium* spp. from which they derive considerable energy. Indeed, the marine environment experiences considerable spatio-temporal variation in spectral quality and light intensity (Zepp et al. 2008), particularly at shallow depths where the need for light within the photosynthetically-active spectrum must be balanced by the potential damages of increased UV irradiance (Dunne & Brown 1996) as well as the potential for desiccation during exceptionally low tidal phases. In fact, it also has been shown that larvae originating from deeper colonies have lower survivorship when exposed to light spectra more typical from shallow depths (Gleason & Wellington 1995). Thus, while my results indicate a correlation between genetic relatedness and depth, it is important to keep in mind that the proximal, causative factor responsible for these patterns may in fact be any number of depth-dependent environmental variables. Confirmation of the specific mechanism by which depth could influence patterns of genetic diversity on an intra-reef scale requires further experimentation.

Conservation implications

That the environmental heterogeneity found within a reef is enough to structure genetic diversity patterns along a depth cline has important conservation implications. Recall that the lagged PC scores (Figure 5.3B) are obtained by averaging for each individual the scores of its neighbors as defined by the connection network. In this sense, the lagged sPCA map represents average spatio-genetic variance throughout the reef, while the non-lagged sPCA map (Figure 5.3A) represents the non-averaged, individual data. If the depth cline observed at my study site is due to the selective factors discussed above, then a comparison of the non-lagged versus lagged maps could help to identify individuals whose PC scores grossly differ from their neighbors. If the selective factor is

strong enough, then these individuals will eventually be weeded out by selective processes. This may even explain the occurrence of highly patchy phenomena, such as bleaching, whereby the coral host and *Symbiodinium* spp. relationship is broken down (i.e., bleaching) due to a combination of thermal and irradiance stress (Brown 1997, Jones et a. 1998, Warner et al. 1999). The occurrence of bleached and unbleached coral individuals of the same species found adjacent to one another may be explained by environmental heterogeneity (Figure 2.1) or due to selective mortality. This, however, is only conjecture as no phenotypic data was collected in this study.

Studying adaptation in the face of climate change has been difficult due to the uncertainty of how corals will respond to environmental differences. Conservation efforts have largely focused on maximizing population connectivity, however, connectivity refers not just to the transport of larvae but also their ability to recruit, survive, and seed the next generation in their new home. In other words, habitat unsuitability can decrease levels of connectivity even in the face of considerable population mixing. This has been termed phenotype-environment mismatch (Conover et al. 2006, Nosil et al. 2005, Marshall et al. 2010), and could potentially serve as a biological barrier to gene flow for organisms where the scale of environmental heterogeneity is smaller than the scale of larval transport and non-random mortality occurs after dispersal. Here, I show that this process may be occurring at the intra-reef scale as well. Predicting how species will respond to climate change, therefore, will require the incorporation of habitat suitability alongside studies of genetic connectivity.

A new paradigm for intra-reef coral genetic diversity

Marine genetic adaptive divergence can be seen as a continuum, with populations being pulled apart by selective forces to species whose reproductive isolation is maintained by ecological boundaries (Rocha et al. 2005, Bird et al. 2011, Bird 2011). What I suggest here is a new paradigm for individual-level, intra-reef patterns of coral genetic diversity. Selection-driven genetic divergence has historically been viewed as difficult to occur if gene flow is high. Here, however, I demonstrate that despite genetic homogeneity on an inter-reef scale (Chapter 3; Gorospe & Karl, accepted), genetic relatedness patterns within a reef are not random and instead, driven by both environmental and spatial factors. In other words, genetic differentiation may still arise

among populations connected by high gene flow (Nosil 2008). Unlike plant dispersal, marine larvae exhibit active microhabitat settlement choice and a renewed emphasis on the causes and consequences of larval retention has emerged (Levin 2006, Jones et al. 2009). In understanding the scale of gene flow in marine environments, therefore, one must move beyond purely spatial factors, and consider the influence of larval settlement behavior as well as post-recruitment selective mortality due to phenotype-environment mismatch.

It should be emphasized, however, that I only focus on inferring processes that can explain my observed depth cline in genetic diversity and multiple processes are likely co-occurring. Since most larval settlement studies have been conducted in the laboratory, it is still unknown how larvae in the field would respond to a suite of cues acting synergistically and on a variety of spatial scales (Levin 2006, Gleason & Hofmann 2011). Just as population genetic studies attempt to infer processes of population connectivity, landscape genetic studies that focus on explaining processes of diversification within a continuous landscape may help to explain patterns of recruitment and larval behavioral responses to multiple cues acting simultaneously.

Table 5.1. Various landscape genetic analyses comparing the relationship between genetic relatedness and each of four spatial or environmental predictor variables. Reported for each variable are Mantel *r* correlation coefficients from partial Mantel tests, predictor weights (w_+) from multi-model inference, and regression coefficients from multiple regressions on distance matrices (MRDM). Significant tests are only available for the partial Mantel tests and MRDM. Statistics with p-values < 0.05 are in bold.

Variable	Mantel *r* (p-value)	w_+	MRDM (p-value)
Depth (controlling for space)	**-0.011 (0.002)**	1.00	**-0.012 (0.002)**
Space (controlling for depth)	**-0.008 (0.001)**	0.97	**-0.001 (0.001)**
Hotspot Index (controlling for space)	-0.004 (0.07)	0.46	NA
Hothour Index (controlling for space)	-0.002 (0.13)	0.30	NA

Table 5.2. Mantel *r* correlation coefficients between genetic relatedness and each of four spatial or environmental variables for different size class bins based on surface area. *N* indicates the number of coral individuals. NS indicates non-significant correlations (* $p < 0.05$; ** $p < 0.01$; NS = not significant).

Size	*N*	Depth	Space	Hotspots	Hothours
< 10 cm^2	1037	-0.014**	-0.004*	NS	NS
> 30 cm^2	486	-0.020*	NS	NS	NS
> 40 cm^2	370	-0.022*	NS	NS	NS
> 60 cm^2	207	NS	NS	-0.027*	NS
> 90 cm^2	95	-0.030*	NS	NS	NS

Table 5.3. Model selection results on the response of genetic relatedness to all possible linear combinations of depth, space, Hotspots, and Hothours. Only those models with $\Delta AIC = AIC_i - AIC_{min} < 10$ for model i are shown. Number of parameters (*K*), log likelihood [log(L)], Akaike's information criterion (AIC), and Akaike weights (*w*) are reported for each model.

Landscape Model	*K*	log(L)	AIC	ΔAIC	*w*
$\beta_0 + \beta_1$ (Depth) + β_2 (Space)	4	-1832529	3665067	0	0.362
$\beta_0 + \beta_1$ (Depth) + β_2 (Space) + β_3 (Hotspots)	5	-1832529	3665067	0.27	0.317
$\beta_0 + \beta_1$ (Depth) + β_2 (Space) + β_3 (Hothours)	5	-1832529	3665068	1.64	0.159
$\beta_0 + \beta_1$ (Depth) + β_2 (Space) + β_3 (Hotspots) + β_4 (Hothours)	6	-1832528	3665069	1.96	0.136
$\beta_0 + \beta_1$ (Space)	3	-1832534	3665074	7.05	0.011
$\beta_0 + \beta_1$ (Space) + β_2 (Hotspots)	4	-1832533	3665074	7.74	0.008
$\beta_0 + \beta_1$ (Space) + β_2 (Hothours)	4	-1832534	3665076	8.99	0.004
$\beta_0 + \beta_1$ (Space) + β_2 (Hotspots) + β_3 (Hothours)	5	-1832533	3665076	9.64	0.003

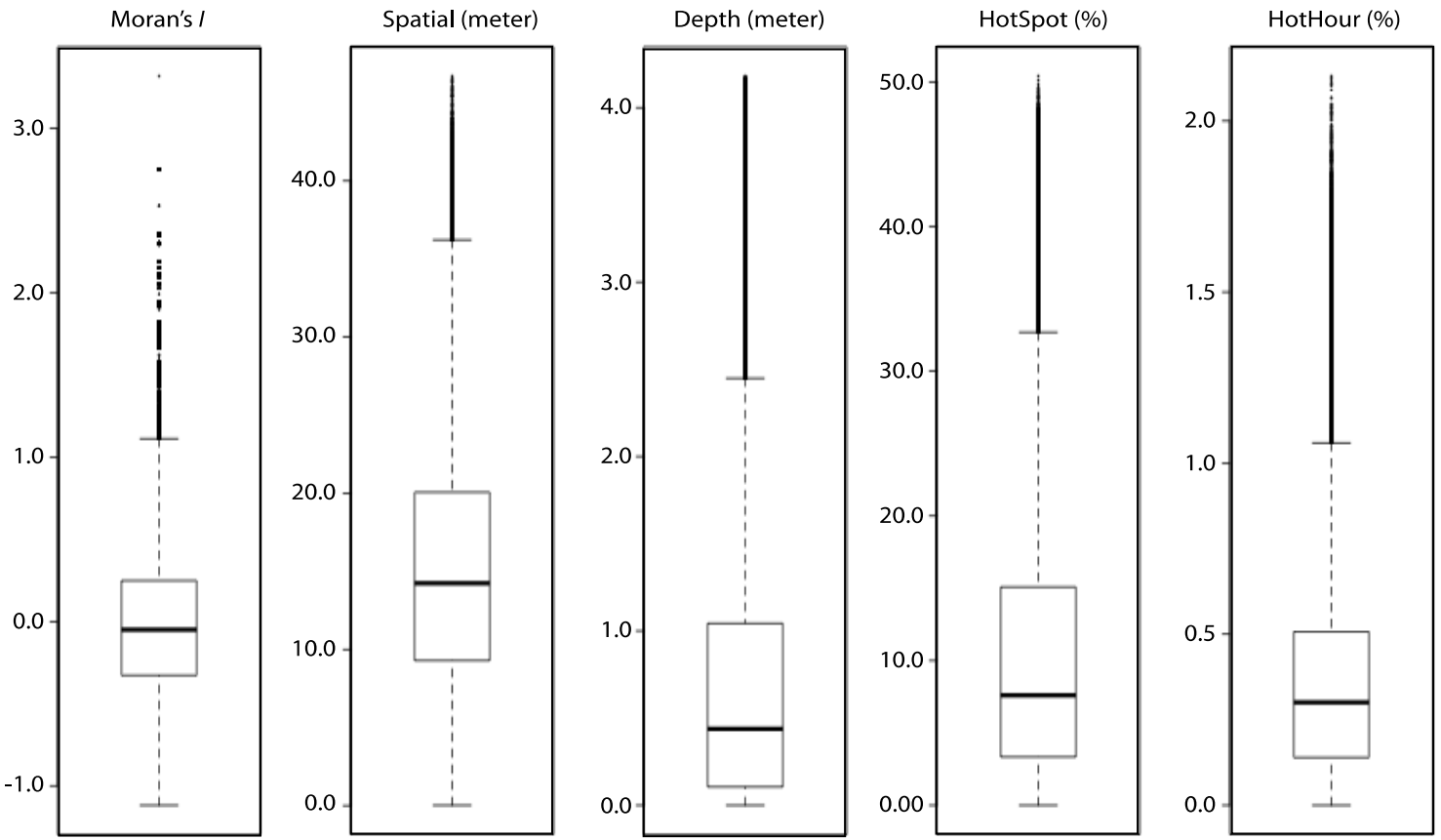


Figure 5.1. Boxplots of pairwise coefficients of genetic relationship as well as spatial and environmental distances. The rectangles represent the interquartile range (i.e., lower 25th percentile, median, and upper 75th percentile), the whiskers represent 1.5 times the interquartile range, and points represent outliers.

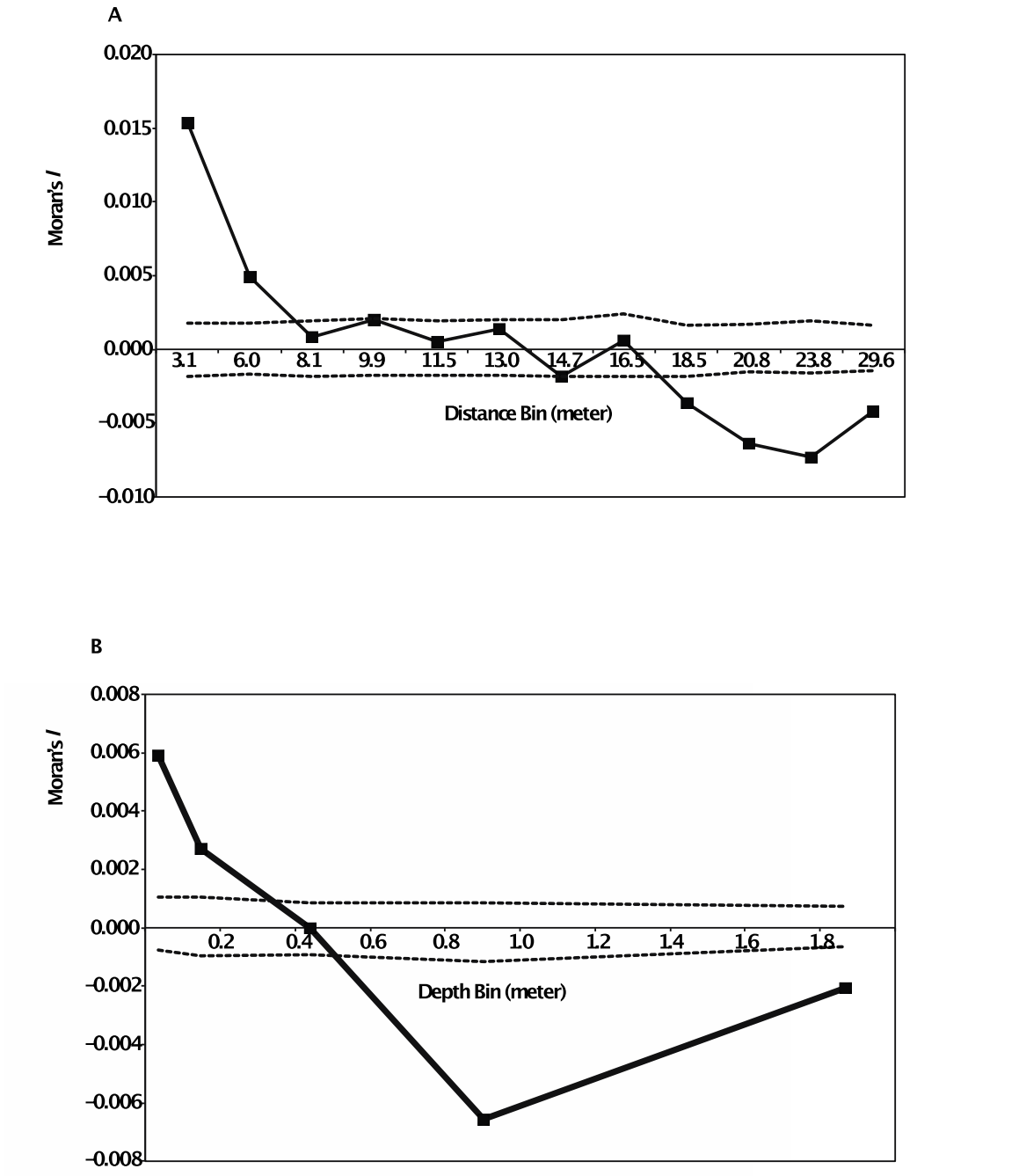


Figure 5.2. Results of spatial (A) and depth (B) autocorrelation analyses. Distance classes in both analyses are based on equalizing the number of pairwise comparisons across distance bins. Dashed lines represent 95% confidence intervals based on 200 permutations of individual spatial or depth coordinates among all individuals.

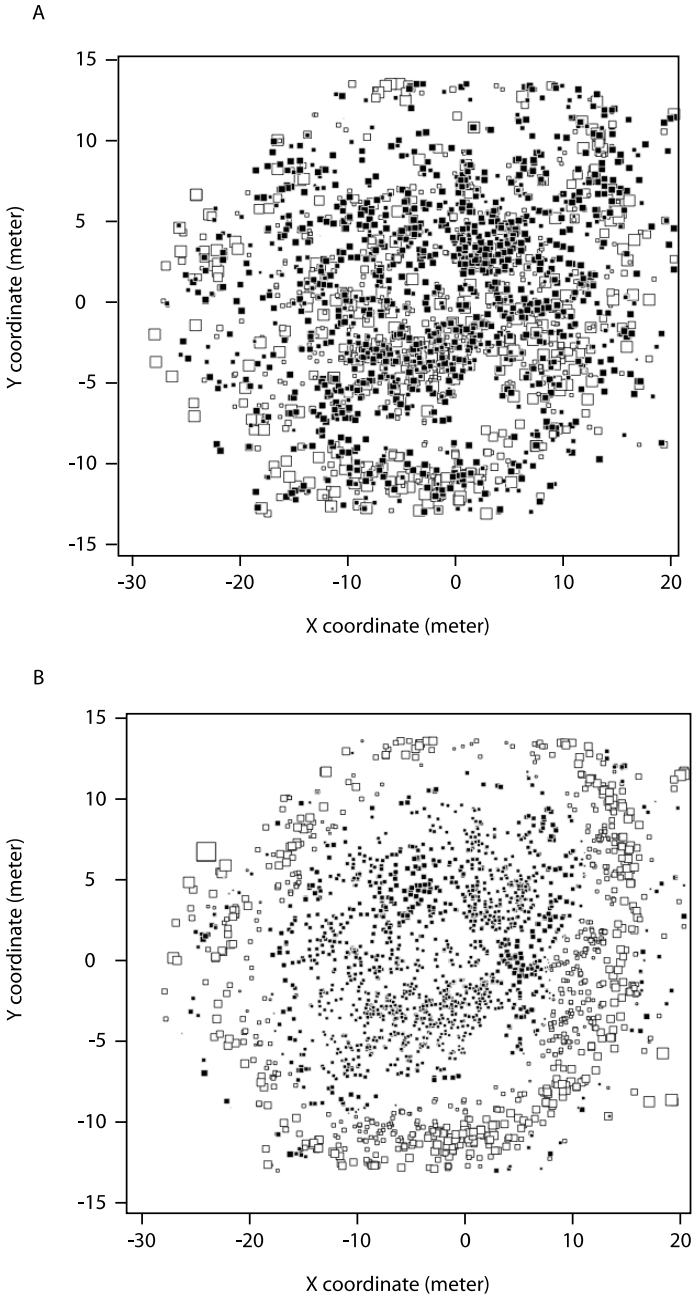


Figure 5.3. Spatial map of non-lagged (A) and lagged (B) scores from the first global principal component of sPCA based on depth. Each coral colony is represented by a box. The size of the box indicates the magnitude and filled boxes are positive and open boxes are negative PC scores, respectively.

CHAPTER 6. FINAL DISCUSSION

Diverging from past studies of coral genetic diversity, this dissertation intensely studied a single patch reef to fully characterize intra-reef patterns of coral genetic diversity and environmental heterogeneity. Chapter 2 (Gorospe & Karl 2011) emphasized the importance of biologically significant environmental variation on this scale. In Chapter 3 (Gorospe & Karl, accepted), using a cross-scale sampling approach and explicitly spatial analyses, allowed for a clearer understanding of dispersal and colonization processes for *P. damicornis* on this scale. It also demonstrated that for this species, spatial-driven processes (i.e., dispersal) only create non-random patterns of genetic relatedness within a reef and that these patterns do not scale up to an inter-reef level. The intense sampling effort of this study was in fact necessary, as discussed in Chapter 4, particularly for uncovering spatial patterns of genetic diversity, and highlights the importance of near-exhaustive sampling studies. These simulations also demonstrated a considerable amount of variance in the characterization of coral genetic diversity with small (~50) sample sizes (Chapter 4). Lastly, Chapter 5 revealed a genetic cline along a depth gradient, inferred to be created by a combination of depth-associated settlement behavior and selective mortality, and thus demonstrating local adaptation on an intra-reef scale.

This dissertation demonstrated that much can be gained from scaling down to a single reef and investigating inter-individual, cross-scale patterns of coral genetic diversity. In some cases, the coral population genetic paradox, therefore, may only be paradoxical within the context of broad-scale studies. Spatial analyses indicate that while *P. damicornis* was genetically homogenous on an inter-reef scale, non-random patterns of genetic relatedness could be found on an intra-reef scale, and that therefore, the effects of dispersal-driven patterns of genetic diversity are not retained across scales. On the other hand, it is clear that past studies using smaller sample sizes may have inaccurately estimated coral genetic diversity, thus potentially complicating the interpretation of geographic variation while also implicating sampling factors as another potential cause of the coral population genetic paradox. Furthermore, landscape genetic analyses described the organization of intra-reef coral genetic diversity along spatial and depth gradients,

and highlighted the importance of considering environmental heterogeneity in explaining spatial patterns of coral genetic diversity.

Coral reefs are threatened on a global scale by both rising sea surface temperatures and ocean acidification (Hughes et al. 2003). Studies that focus on coral genetic diversity on larger scales are important for understanding the evolutionary and ecological connectivity between populations, and thus the potential to maintain genetic diversity, the source of evolutionary change and adaptive capacity, through demographic processes. Understanding the impact of global climate change on intra-species genetic diversity, however, will require information on multiple processes occurring on multiple scales (Pauls et al. 2013). Here, the intense characterization of the genetic and environmental heterogeneity of a single reef allowed for the inference of processes of dispersal, recruitment, local adaptation, and selection occurring on an intra-reef scale. Gaining a better understanding of coral demographic processes occurring on different scales will be crucial to our ability to predict, and thus potentially mitigate, how global climate change will affect coral population persistence. Overall, while there is no one correct scale at which to study a species (Levin 1992), inattention to any one particular scale may inhibit our ability to understand how processes and patterns change across scales.

APPENDIX A

Table 1S – Degree of correlation of depth with biotic and abiotic factors and diversity. Bolded values are considered significant.

Test	Coefficient		Corrected	
	Pearson's	Spearman's	df	*P*
Relative Water Flow	**-0.837**	**-0.912**	9.789	< 0.001
All coral combined	**-0.548**	**-0.402**	24.951	0.003
Porites compressa (PCO)	**-0.625**	**-0.550**	19.561	0.002
Montipora sp. (MSP)	0.168	0.316	49.622	0.237
Pocillopora damicornis (PDA)	-0.164	-0.303	58.867	0.207
Other coral (OCOR)	-0.086	0.027	56.913	0.519
All macroalgae combined	**-0.344**	**-0.416**	29.825	0.055
Dictyosphaeria sp. (DSP)	-0.120	-0.132	72.709	0.306
Eucheuma sp. and Kappaphycus sp. (EKSP)	**-0.308**	**-0.532**	29.580	0.089
Other Macroalgae	-0.068	-0.213	87.026	0.526
All abiotic substrates combined	**0.621**	**0.481**	23.953	< 0.001
Dead coral with algae (DCA)	-0.189	-0.189	54.562	0.161
Recently dead coral (RDC)	-0.097	0.223	84.304	0.373
Rubble (RUB)	**0.564**	**0.524**	33.448	< 0.001
Sand (SAN)	**0.744**	**0.690**	15.917	< 0.001
Other invertebrates (OINV)	**0.448**	**0.346**	30.101	0.010
Unknown (UNK)	-0.127	-0.031	54.259	0.350
Shannon Diversity Index	**-0.280**	**-0.225**	32.998	0.103

Table 2S – Percent of the variance in the index explained by environmental variables (i.e., depth and/or substrate), location, and shared (environmental variables and location) in the regression of: (1) temperature on depth, (2) temperature on location, (3) temperature on depth and location, (4) temperature on substrate and depth, and (5) temperature on substrate, depth, and location. Dominant factors are bolded.

	Data Set									
Index	Two Years		Summer 2008		Summer 2009		Winter 2008		Winter 2009	
Relative Hotspots	**location**	**19.1**	**location**	**13.1**	**location**	**29.5**	**location**	**35.4**	**location**	**15.7**
Relative Coldspots	depth **location** shared	2.1 **29.6** 6.6	depth **location** shared	5.4 38.2 0.0	**location**	**28.9**	**location**	**39.9**	**location**	**17.4**
Relative Hothours	**location**	**28.1**	depth **location** shared	11.4 **33.9** 0.0	**location**	**30.9**	**location**	**48.9**	**location**	**9.3**
Relative Coldhours	depth location **shared**	24.1 4.6 **61.9**	depth location **shared**	23.1 15.2 **39.4**	depth location **shared**	9.7 17.9 **49.6**	depth location **shared**	19.2 5.8 **57.2**	depth location **shared**	20.5 6.7 **49.1**
Overall Average	NA		depth **location** shared	7.1 **17.0** 7.2	depth **location** shared	2.3 **34.9** 1.9	**location**	**39.7**	**location**	**17.5**
Average Daily Minimum	NA		**location**	**25.9**	**location**	**36.4**	depth **location** shared	4.2 **24.6** 20.9	depth **location** shared	4.1 **19.6** 10.9
Average Daily Maximum	NA		**depth** location shared	**21.4** 10.1 15.7	depth **location** shared	8.7 **25.4** 15.1	depth **location** shared	11.3 **33.4** 19.2	**depth**	**21.5**
Average Daily Range	NA		depth location **shared**	24.0 9.7 **29.7**	**substrate and depth**	**68.9**	depth location **shared**	19.4 9.9 **57.6**	**depth**	**75.5**
Total Heating Hours	NA		depth location **shared**	14.3 15.4 **15.8**	**substrate and depth** location shared	**25.9** 21.2 21.0	NA		NA	

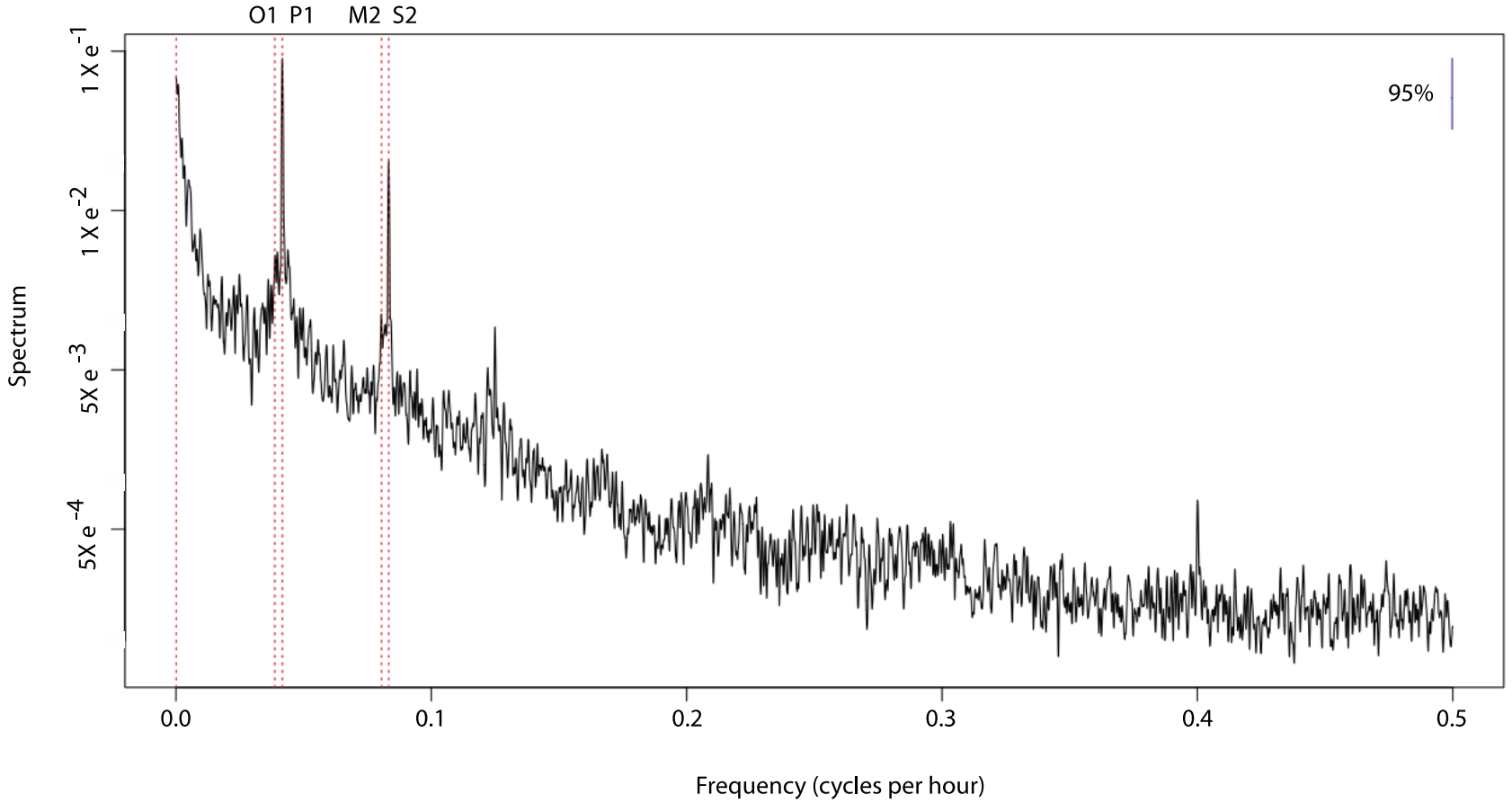


Figure 1S – Fourier transform scaled periodogram of the standard deviation in temperature for the two years of data. Principal frequencies are marked with vertical, dashed lines and correspond to seasonal (365 days), lunar (O1, 24.83 hours), solar (P1, 24 hours), lunar semi-diurnal (M2, 12.42 hours), and solar semi-diurnal (S2, 12 hours). Other peaks between 0.1 and 0.2 cycles per hour are likely echoes of O1 and P1. Scale bar in the upper right corner is the 95% confidence limit.

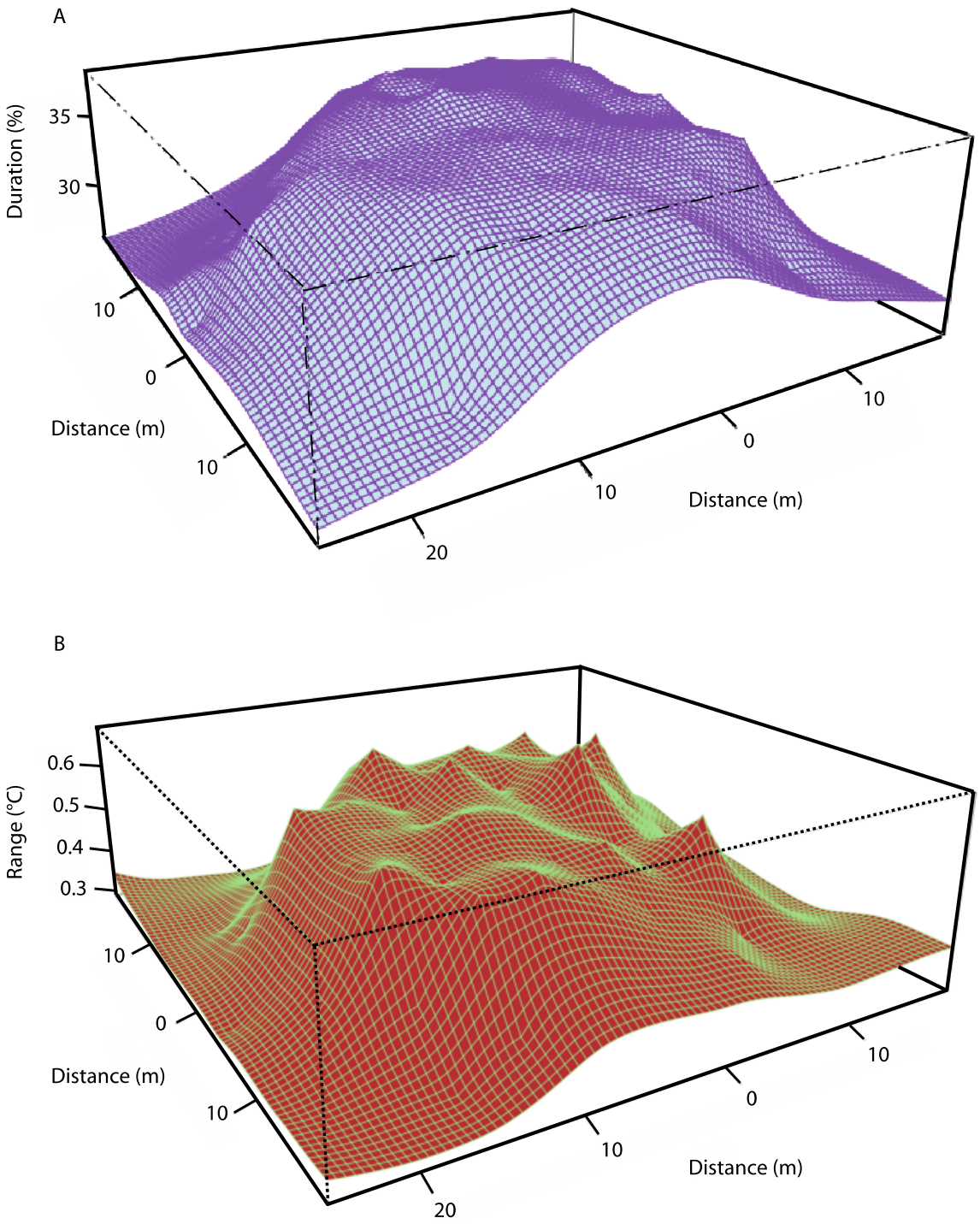


Figure 2S – Ordinary kriging interpolation of the proportion of two-year data set that were Coldhours (A) and the average daily temperature range for winter 2008 (B).

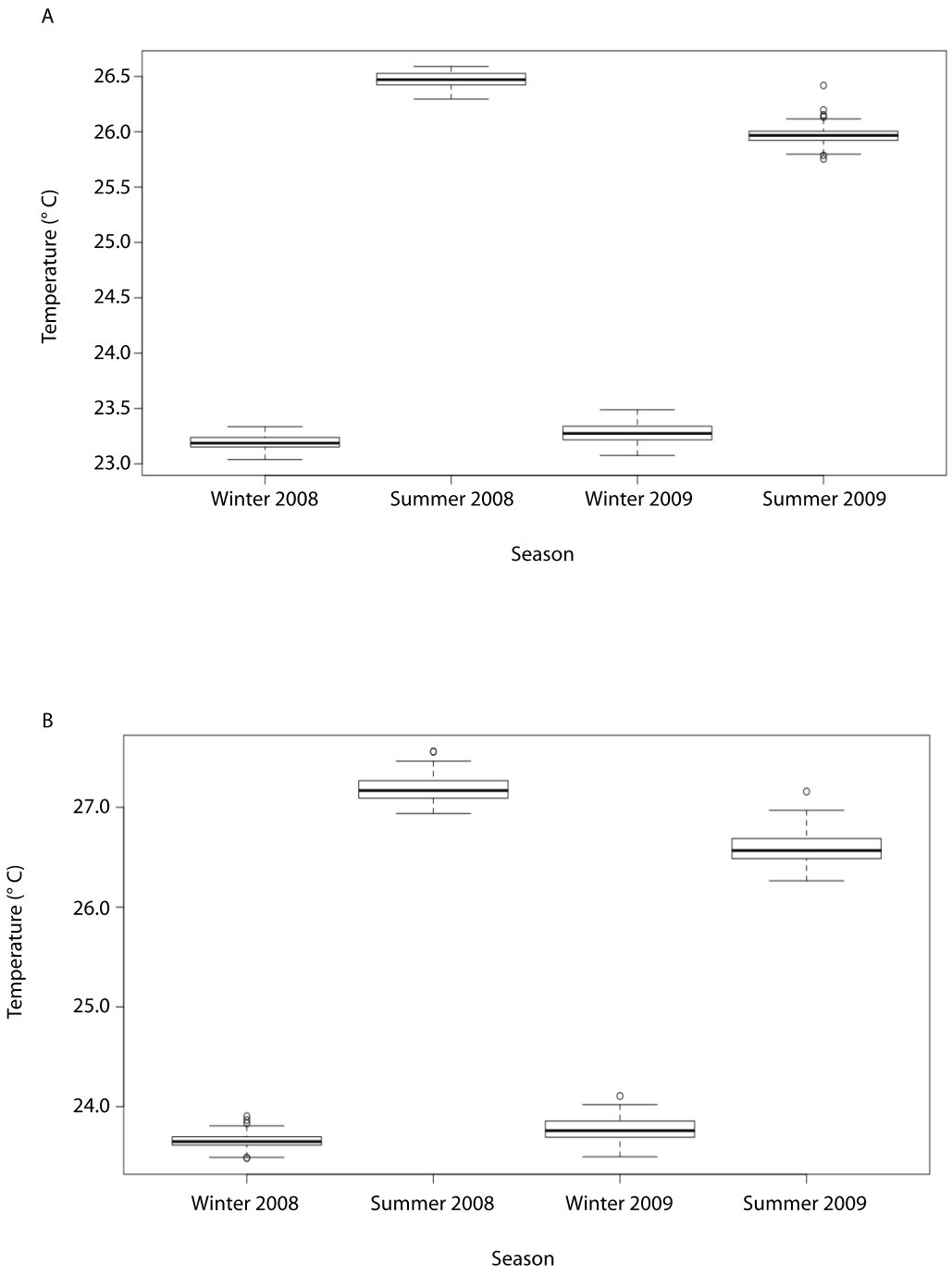


Figure 3S – Tukey boxplots of the seasonal and annual variation seen in daily minimum (A) and maximum (B) temperatures. The rectangle represents the interquartile range (i.e., the 25th percentile, median, and 75th percentile), the "whiskers" represent all values that are within 1.5 times the interquartile range, and the open circles represent outliers, defined as data points that lie outside the whiskers.

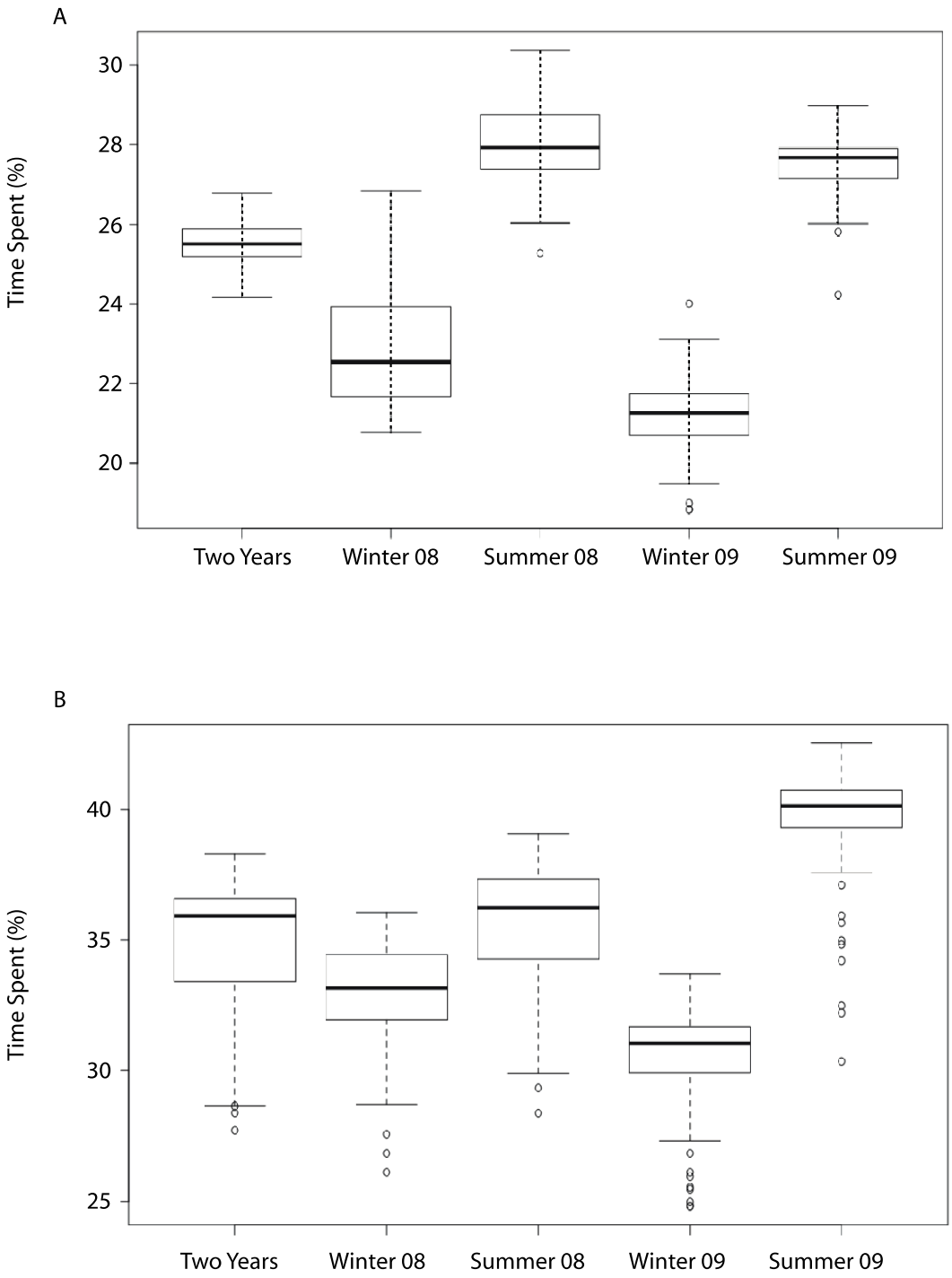


Figure 4S – Tukey boxplots of the seasonal and annual variation in percent of time sites spent as Hothours (A) and Coldhours (B). The rectangle represents the interquartile range (i.e., the 25th percentile, median, and 75th percentile), the "whiskers" represent all values that are within 1.5 times the interquartile range, and the open circles represent outliers, defined as data points that lie outside the whiskers.

APPENDIX B

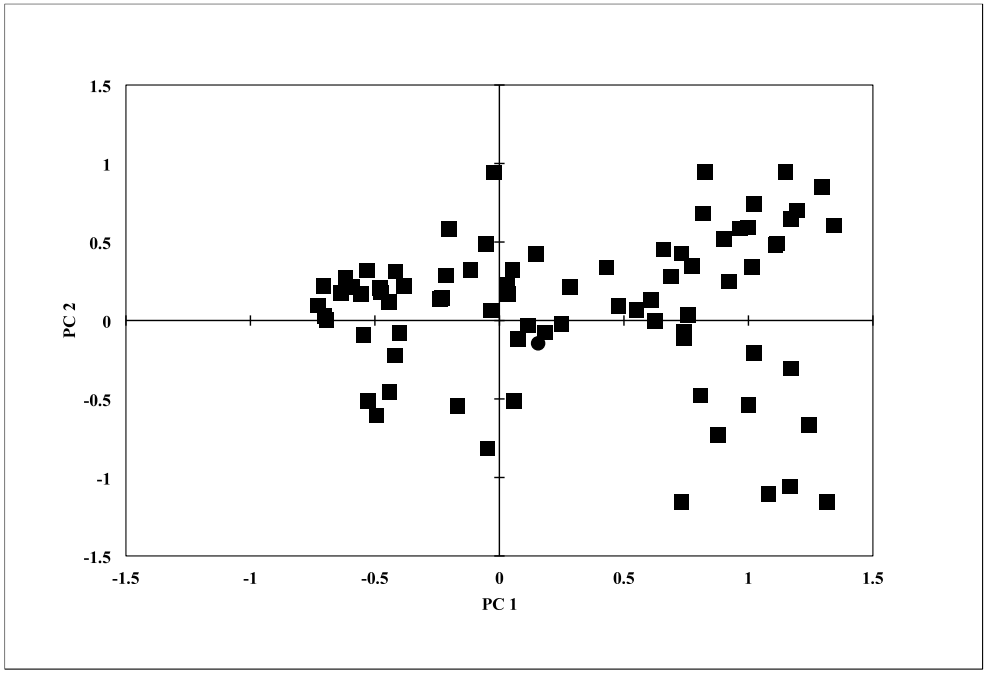


Figure S1. Principle component analysis of the microsatellite data. Each circle represents a genet and the filled circles are the ones with the uncommon mitochondrial DNA haplotypes.

APPENDIX C

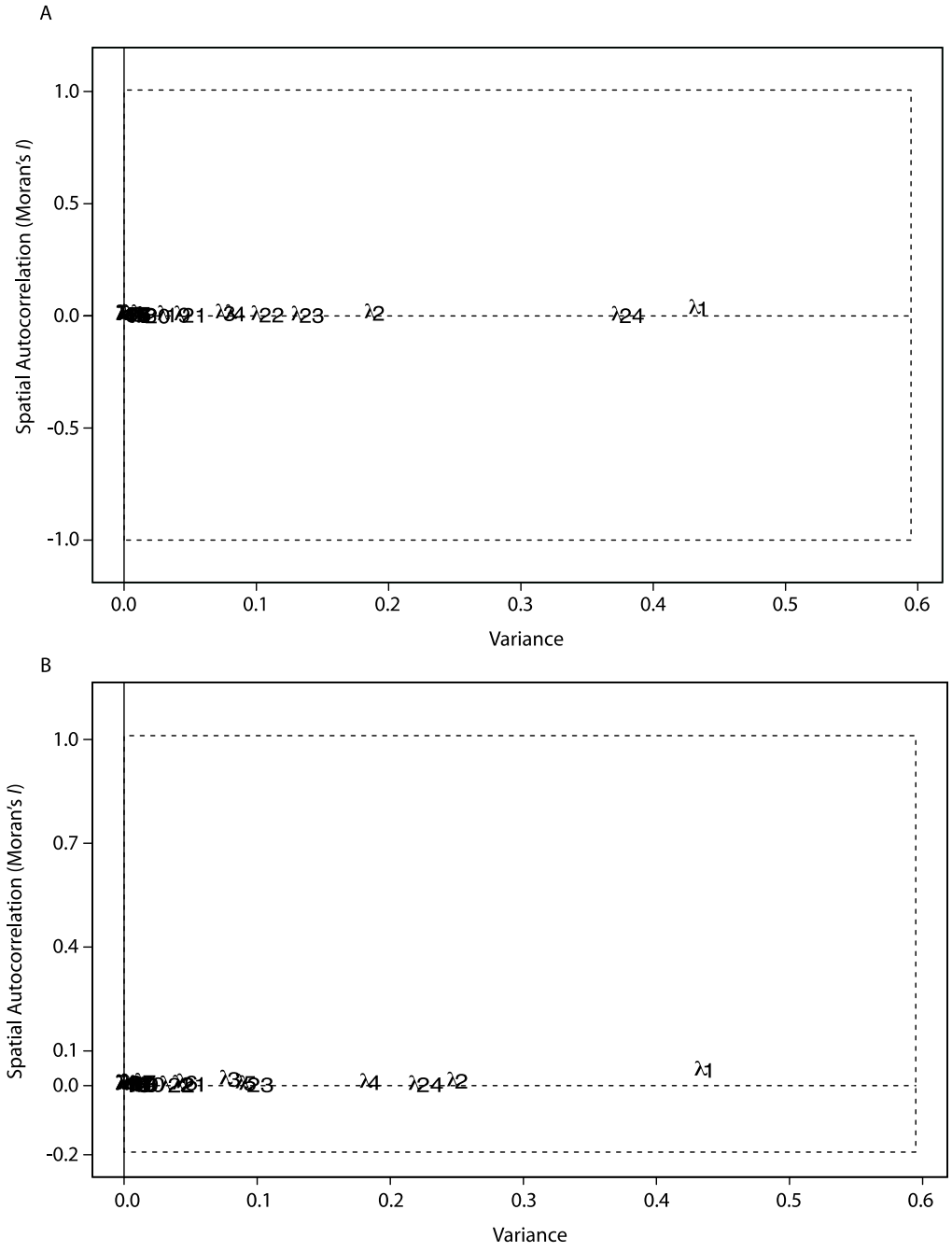


Figure S1. Screeplots representing the genetic variance and spatial autocorrelation components of each eigenvalue from spatial-based (A) and depth-based (B) sPCA.

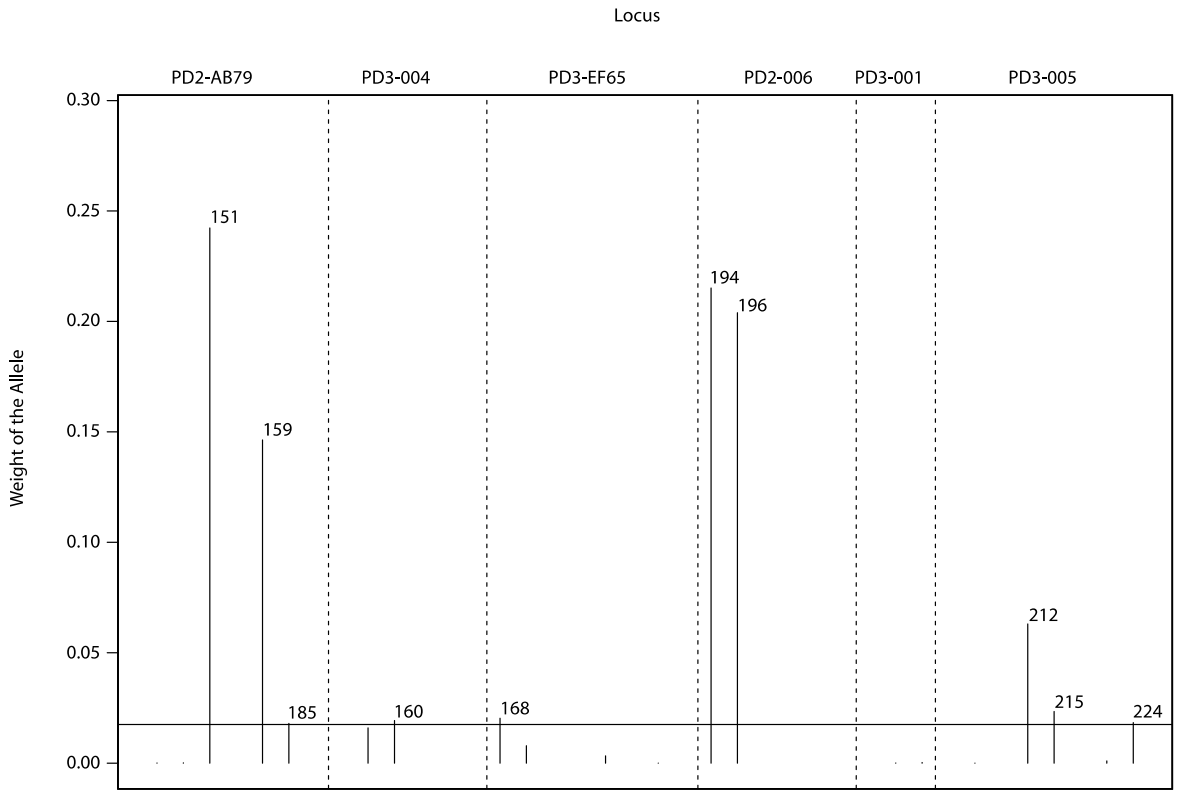


Figure S2. Squared loadings (i.e., contribution) of each allele to the first component of sPCA based on depth. Alleles with loadings in the upper third quartile are labeled on the graph.

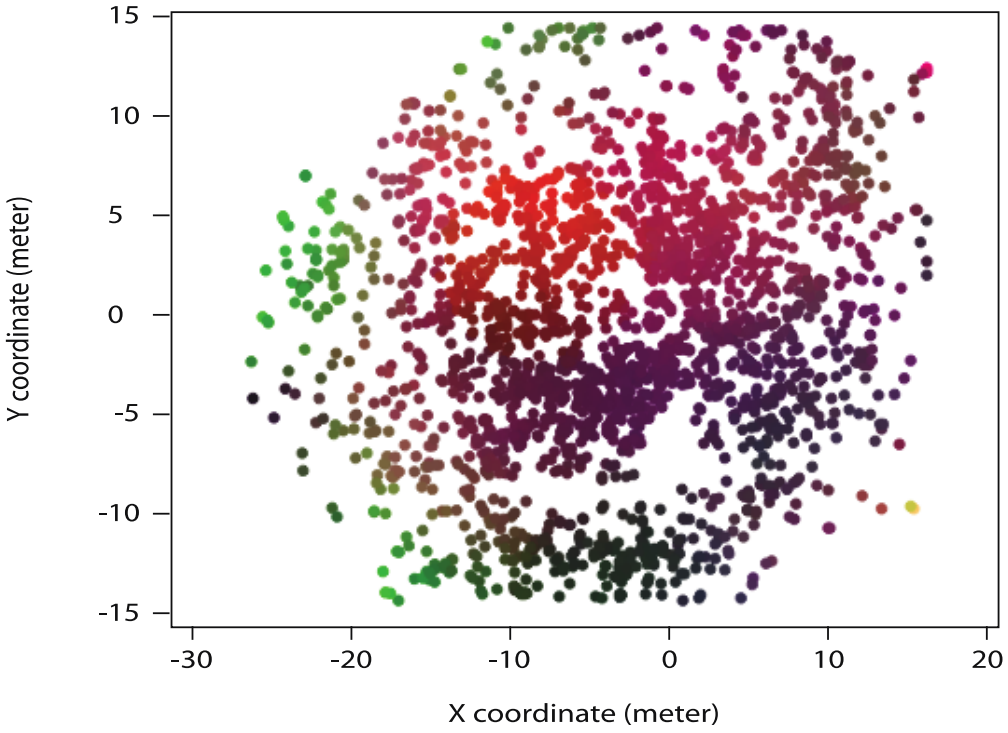


Figure S3. Spatial map of the lagged scores from the first three global principal components of sPCA based on spatial locations. Here, the three principal components are visualized simultaneously by translating each into a color intensity (red, green, or blue) and displaying the combined mixed colors.

REFERENCES

Adjeroud M, Tsuchiya M (1999) Genetic variation and clonal structure in the scleractinian coral *Pocillopora damicornis* in the Ryukyu Archipelago, southern Japan. *Marine Biology*, 134, 753–760.

Alberto F, Arnaud-Haond S, Duarte CM, Serrão EA (2006) Genetic diversity of a clonal angiosperm near its range limit: the case of *Cymodocea nodosa* at the Canary Islands. *Marine Ecology Progress Series*, 309, 117.

Alberto F, Gouveia L, Arnaud-Haond S, *et al.* (2005) Within-population spatial genetic structure, neighbourhood size and clonal subrange in the seagrass *Cymodocea nodosa*. *Molecular Ecology*, 14, 2669–2681.

Anderson TJ, Haubold B, Williams JT, Estrada-Franco JG, Richardson L, et al. (2000) Microsatellite markers reveal a spectrum of population structures in the malaria parasite *Plasmodium falciparum*, *Molecular Biology and Evolution*, 17:1467–1482.

Anderson MJ, Robinson J (2002) Permutation tests for linear models, *Australian & New Zealand Journal of Statistics*, 43:75–88.

Arnaud-Haond S, Belkhir K (2007) GENCLONE: a computer program to analyse genotypic data, test for clonality and describe spatial clonal organization. *Molecular Ecology Notes*, 7, 15–17.

Arnaud-Haond S, Duarte CM, Alberto F, Serrão EA (2007) Standardizing methods to address clonality in population studies. *Molecular Ecology*, 16, 5115–5139.

Arnaud-Haond S, Vonau V, Rouxel C, Bonhomme F, Prou J, Goyard E, Boudry P (2008) Genetic structure at different spatial scales in the pearl oyster (Pinctada margaritifera cumingii) in French Polynesian lagoons: beware of sampling strategy and genetic patchiness, *Marine Biology*, 155:147–157.

Avolio ML, Beaulieu JM, Smith MD (2012) Genetic diversity of a dominant C 4 grass is altered with increased precipitation variability, *Oecologia*, 171:571–581.

Ayre DJ, Hughes TP (2000) Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. *Evolution*, 54, 1590–1605.

Ayre DJ, Hughes TP, Standish RJ (1997) Genetic differentiation, reproductive mode, and gene flow in the brooding coral *Pocillopora damicornis* along the Great Barrier

Reef, Australia. *Marine Ecology Progress Series*, 159, 175–187.

Ayre DJ, Miller KJ (2004) Where do clonal coral larvae go? Adult genotypic diversity conflicts with reproductive effort in the brooding coral *Pocillopora damicornis*, *Marine Ecology Progress Series*, 277:95–105.

Baird AH, Babcock RC, Mundy CP (2003) Habitat selection by larvae influences the depth distribution of six common coral species, *Marine Ecology Progress Series*, 252: 289–293.

Baker AC (2001) Reef corals bleach to survive change, *Nature*, 411:765–766.

Baker AC (2003) Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of Symbiodinium, *Annual Review of Ecology, Evolution, and Systematics*, 34:661–689.

Baker AC, Starger CJ, McClanahan TR, Glynn PW (2004) Coral reefs: corals' adaptive response to climate change. *Nature*, 430, 741.

Baker AC, Glynn PW, Riegl B (2008) Climate change and coral reef bleaching: an ecological assessment of long-term impacts, recovery trends and future outlook. *Estuarine, Coastal and Shelf Science*, 80:435–471.

Balloux F, Lehmann L, de Meeûs T (2003) The population genetics of clonal and partially clonal diploids. *Genetics*, 164, 1635–1644.

Baltanás A (1992) On the use of some methods for the estimation of species richness, *Oikos*, 484–492.

Barshis DJ, Stillman JH, Gates RD, Toonen RJ, Smith LW, et al. (2010) Protein expression and genetic structure of the coral Porites lobatain an environmentally extreme Samoan back reef: does host genotype limit phenotypic plasticity?, *Molecular Ecology*, 19:1705–1720.

Bartón K (2009) *MuMIn:* Multi-model Inference, R package version 1.7.7, http://r-forge.r-project.org/projects/mumin.

Baums IB (2008) A restoration genetics guide for coral reef conservation. *Molecular Ecology*, 17, 2796–2811.

Baums IB, Hughes CR, Hellberg ME (2005) Mendelian microsatellite loci for the Caribbean coral *Acropora palmata*. *Marine Ecology Progress Series*, 288, 115–127.

Baums IB, Paris CB, Chérubin LM (2006) A bio-oceanographic filter to larval dispersal in a reef-building coral. *Limnology and Oceanography*, 51, 1969–1981.

Baums IB, Miller MW, Hellberg ME (2006) Geographic variation in clonal structure in a reef-building Caribbean coral, *Acropora palmata*, *Ecological monographs*, 76:503–519.

Ben-Haim Y, Zicherman-Keren M, Rosenberg E (2003) Temperature-regulated bleaching and lysis of the coral *Pocillopora damicomis* by the novel pathogen *Vibrio coralliilyticus*. *Applied and Environmental Microbiology*, 69:4236–4242.

Benzie J, Haskell A, Lehman H (1995) Variation in the genetic composition of coral (*Pocillopora damicornis* and *Acropora palifera*) populations from different reef habitats. *Marine Biology*, 121, 731–739.

Berkelmans R (2002) Time-integrated thermal bleaching thresholds of reefs and their variation on the Great Barrier Reef. *Marine Ecology Progress Series*, 229:73–82.

Berkelmans R, Oliver JK (1999) Large-scale bleaching of corals on the Great Barrier Reef. *Coral Reefs*, 18:55–60.

Berkelmans R, van Oppen MJH (2006) The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope' for coral reefs in an era of climate change, *Proceedings of the Royal Society B*, 273:2305–2312.

Billingham MR, Simões T, Reusch TBH, Serrão EA (2007) Genetic sub-structure and intermediate optimal outcrossing distance in the marine angiosperm *Zostera marina*. *Marine Biology*, 152, 793–801.

Bird CE (2011) Morphological and behavioral evidence for adaptive diversification of sympatric Hawaiian limpets (*Cellana* spp.), *Integrative and Comparative Biology*, 51:466–473.

Bird CE, Holland BS, Bowen BW, Toonen RJ (2011) Diversification of sympatric broadcast-spawning limpets (*Cellana* spp.) within the Hawaiian archipelago, *Molecular Ecology*, 20:2128–2141.

Björklund M, Bergek S (2009) On the relationship between population differentiation and sampling effort: is more always better?, *Oikos*, 118:1127–1129.

Blanquer A, Uriz M, Caujapé-Castells J (2009) Small-scale spatial genetic structure in *Scopalina lophyropoda*, an encrusting sponge with philopatric larval dispersal and

frequent fission and fusion events. *Marine Ecology Progress Series*, 380, 95–102.

Bongaerts P, Riginos C, Ridgway T, Sampayo EM, van Oppen MJH, et al. (2010) Genetic divergence across habitats in the widespread coral *Seriatopora hystrix* and its associated *Symbiodinium*, *PLoS ONE*, 5:e10871.

Borcard D, Legendre O, Avois-Jacquet C, Tuomisto H (2004) Dissecting the spatial structure of ecological data at multiple scales, *Ecology*, 85:1826–1832.

Brose U, Martinez ND, Williams RJ (2003) Estimating species richness: sensitivity to sample coverage and insensitivity to spatial patterns, *Ecology*, 84:2364–2377.

Brown BE (1997) Coral bleaching: causes and consequences. *Coral Reefs*, 16:S129–S138.

Brown BE, Downs CA, Dunne RP, Gibb SW (2002) Exploring the basis of thermotolerance in the reef coral Goniastrea aspera. *Marine Ecology Progress Series*, 242:119–129.

Burnham KP, Anderson DR (2001) Kullback-Leibler information as a basis for strong inference in ecological studies, *Wildlife Research*, 28:111–119.

Burt J, Bartholomew A, Usseglio P (2008) Recovery of corals a decade after a bleaching event in Dubai, United Arab Emirates. *Marine Biology*, 154:27–36.

Calderón I, Ortega N, Duran S, *et al.* (2007) Finding the relevant scale: clonality and genetic structure in a marine invertebrate (*Crambe crambe,* Porifera). *Molecular Ecology*, 16, 1799–1810.

Carlon DB, Budd AF (2002) Incipient speciation across a depth gradient in a scleractinian coral?, *Evolution*, 56:2227–2242.

Castellano S, Balletto E (2002) Is the partial Mantel test inadequate?, *Evolution*, 56:1871–1873.

Castillo KD, Helmuth BST (2005) Influence of thermal history on the response of Montastraea annularis to short-term temperature exposure, *Marine Biology*:148, 261–270.

Cavers S, Degen B, Caron H, Lemes MR, Margis R, Salgueiro F, Lowe AJ (2005) Optimal sampling strategy for estimation of spatial genetic structure in tree populations, *Heredity*, 95:281–289.

Chao A, Shen T-J (2003) Nonparametric estimation of Shannon's index of diversity when

there are unseen species in sample. *Environmental and Ecological Statistics*, 10, 429–443.

Chown SL, Hoffmann AA, Kristensen TN, Angilletta Jr MJ, Stenseth NC, Pertoldi C (2010) Adapting to climate change: a perspective from evolutionary physiology. *Climate Research*, 43, 3-15.

Combosch DJ, Vollmer SV (2011) Population genetics of an ecosystem-defining reef coral *Pocillopora damicornis* in the tropical eastern Pacific, *PLoS ONE*, 6:e21200.

Conover DO, Clarke LM, Munch SB, Wagner GN (2006) Spatial and temporal scales of adaptive divergence in marine fishes and the implications for conservation, *Journal of Fish Biology*, 69:21–47.

Cowen RK, Sponaugle S (2009) Larval dispersal and marine population connectivity. *Annual Review of Marine Science*, 1, 443–466.

Cowen RK, Lwiza KM, Sponaugle S, Paris CB, Olson DB (2000) Connectivity of marine populations: open or closed? *Science*, 287, 857–859.

David P, Pujol B, Viard F, Castella V, Goudet J (2007) Reliable selfing rate estimates from imperfect population genetic data. *Molecular Ecology*, 16, 2474–2487.

D'Croz L, Maté JL (2004) Experimental responses to elevated water temperature in genotypes of the reef coral *Pocillopora damicornis* from upwelling and non-upwelling environments in Panama. *Coral Reefs*, 23, 473–483.

Dray S, Legendre P, Peres-Neto PR (2006) Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM), *Ecological Modelling*, 196:483–493.

Dunne RP, Brown BE (1996) Penetration of solar UVB radiation in shallow tropical waters and its potential biological effects on coral reefs; results from the central Indian Ocean and Andaman Sea, *Marine Ecology Progress Series*, 144:109–118.

Eakin CM, Lough JM (2009) Climate variability and change: monitoring data and evidence for increased coral bleaching stress, in *Coral Bleaching: Patterns, Processes, Causes, and Consequences*, van Oppen M, Lough JM, eds., *Ecological Studies*, 205:41–68, Springer: Berlin, Germany.

Ellstrand NC, Roose ML (1987) Patterns of genotypic diversity in clonal plant species.

American Journal of Botany, 74, 123–131.

Epperson BK (1993) Spatial and space-time correlations in systems of subpopulations with genetic drift and migration, *Genetics*, 133:711–727.

Epperson BK, Li T (1996) Measurement of genetic structure within populations using Moran's spatial autocorrelation statistics. *Proceedings of the National Academy of Sciences*, 93, 10528–10532.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, 131, 479–491.

Fabricius KE (2006) Effects of irradiance, flow, and colony pigmentation on the temperature microenvironment around corals: implications for coral bleaching?, *Limnology and Oceanography*, 51:30–37.

Fager EW (1972) Diversity: a sampling study, *The American Naturalist*, 293–310.

Fenster CB, Vekemans X, Hardy OJ (2003) Quantifying gene flow from spatial genetic structure data in a metapopulation of *Chamaecrista fasciculata* (Leguminosae). *Evolution*, 57, 995-1007.

Flot J-F, Magalon H, Cruaud C, Couloux A, Tillier S (2008) Patterns of genetic structure among Hawaiian corals of the genus *Pocillopora* yield clusters of individuals that are compatible with morphology. *Comptes Rendus Biologies*, 331, 239–247.

Flot J-F, Tillier S (2007) The mitochondrial genome of *Pocillopora* (Cnidaria: Scleractinia) contains two variable regions: the putative D-loop and a novel ORF of unknown function. *Gene*, 401, 80–87.

Fortin M, Dale M (2005) *Spatial Analysis: A Guide for Ecologists*, Cambridge University Press: Cambridge, UK.

Gleason DF, Hofmann DK (2011) Coral larvae: from gametes to recruits, *Journal of Experimental Marine Biology and Ecology*, 408:42–57.

Gleason DF, Wellington GM (1995) Variation in UVB sensitivity of planula larvae of the coral *Agaricia agaricites* along a depth gradient, *Marine Biology*, 123:693–703.

Gleeson MW, Strong AE, Applying MCSST to coral reef bleaching, *Advances in Space Research*, 16:151–154.

Golley F (1989) Paradigm shift: Editor's comment. *Landscape Ecology*, 3, 65–66.

Goreau TF (1959) The ecology of Jamaican coral reefs I. Species composition and zonation, *Ecology*, 40:67–90.

Gorospe KD, Karl SA (2011) Small-scale spatial analysis of in situ sea temperature throughout a single coral patch reef. *Journal of Marine Biology*, 2011, 1–12.

Gorospe KD, Karl SA (2013) Genetic relatedness does not retain spatial pattern across multiple scales: dispersal and colonization in the coral, *Pocillopora damicornis*, *Molecular Ecology*, accepted.

Goslee SC, Urban DL (2007) The *ecodist* package for dissimilarity-based analysis of ecological data, *Journal of Statistical Software*, 22:1–19.

Goulet TL, Coffroth MA (2003) Stability of an octocoral-algal symbiosis over time and space, *Marine Ecology Progress Series*, 250:117–124.

Griffith DA, Peres-Neto PR (2006) Spatial modeling in ecology: The flexibility of eigenfunction spatial analyses, *Ecology*, 87:2603–2613.

Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics*, 48, 361–372.

Hall VR, Hughes TP (1996) Reproductive strategies of modular organisms: comparative studies of reef building corals. *Ecology*, 77, 950-963.

Halkett F, Simon J, Balloux F (2005) Tackling the population genetics of clonal and partially clonal organisms. *Trends in Ecology & Evolution*, 20, 194–201.

Hardy OJ, Vekemans X (1999) Isolation by distance in a continuous population: reconciliation between spatial autocorrelation analysis and population genetics models. *Heredity*, 83, 145–154.

Hardy OJ, Vekemans X (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes*, 2, 618–620.

Hardy OJ, Vekemans X (2009) SPAGeDi 1.3: User's manual. *http://ebe.ulb.ac.be/ebe/Software_files/manual_SPAGeDi_1-3.pdf*.

Harper JL (1977) *Population Biology of Plants*. Academic Press, London.

Harriott VJ (1985) Mortality rates of scleractinian corals before and during a mass bleaching event, *Marine Ecology Progress Series*, 21:81-88.

Harvell CD, Kim D, Burkholder JM, et al. (1999) Emerging marine diseases—climate

links and anthropogenic factors. *Science*, 285:1505–1510.

Hellberg ME (1995) Stepping-stone gene flow in the solitary coral *Balanophyllia elegans*: equilibrium and nonequilibrium at different spatial scales. *Marine Biology*, 123, 573–581.

Hellberg ME (2007) Footprints on water: the genetic wake of dispersal among reefs. *Coral Reefs*, 26, 463–473.

Hellberg ME (2009) Gene flow and isolation among populations of marine animals, *Annual Review of Ecology Evolution and Systematics*, 40:291–310.

Heywood JS (1991) Spatial analysis of genetic variation in plant populations. *Annual Review of Ecology and Systematics*, 22, 335–355.

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, et al. (2007) Coral reefs under rapid climate change and ocean acidification. *Science*, 318:1737–1742.

Hooper DU, Chapin III FS, Ewel JJ, et al. (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge, *Ecological Monographs*, 75:3–35.

Hughes TP, Baird AH, Bellwood DR, et al. (2003) Climate change, human impacts, and the resilience of coral reefs. *Science*, 301:929–933.

Hunter CL (1993) Genotypic variation and clonal structure in coral populations with different disturbance histories, *Evolution*, 1213–1228.

Hurlbut CJ (1993) The adaptive value of larval behavior of a colonial ascidian, *Marine Biology*, 115:253–262.

Iglesias-Prieto R, Beltrán VH, LaJeunesse TC, Reyes-Bonilla H, Thomé PE (2004) Different algal symbionts explain the vertical distribution of dominant reef corals in the eastern Pacific, *Proceedings of the Royal Society B*, 271:1757–1763.

Isomura N, Nishihira M (2001) Size variation of planulae and its effect on the lifetime of planulae in three pocilloporid corals, *Coral Reefs*, 20:309-315.

Jensen JL, Bohonak AJ, Kelley ST (2005) Isolation by distance, web surface. *BMC Genetics*, 6, 13.

Jimenez IM, Kühl M, Larkum AWD, Ralph PJ (2008) Heat budget and thermal microenvironment of shallow-water corals: do massive corals get warmer than branching corals?, *Limnology and Oceanography*, 53:1548–1561.

Johnson MS, Black R (1982) Chaotic genetic patchiness in an intertidal limpet,

Siphonaria sp. *Marine Biology*, 70, 157–164.

Johnson MS, Black R (1984) Pattern beneath the chaos: the effect of recruitment on genetic patchiness in an intertidal limpet. *Evolution*, 38, 1371–1383.

Jokiel PL, Brown EK (2004) Global warming, regional trends and inshore environmental conditions influence coral bleaching in Hawaii. *Global Change Biology*, 10:1627–1641.

Jombart T (2008) *adegenet*: a R package for the multivariate analysis of genetic markers, *Bioinformatics*, 24:1403–1405.

Jombart T, Devillard S, Dufour A-B, Pontier D (2008) Revealing cryptic spatial patterns in genetic variability by a new multivariate method, *Heredity*, 101:92–103.

Jones GP, Almany GR, Russ GR, Sale PF, Steneck RS, van Oppen MJH, Willis BL (2009) Larval retention and connectivity among populations of corals and reef fishes: history, advances and challenges, *Coral Reefs*, 28:307–325.

Jones RJ, Hoegh-Guldberg O, Larkum AWD, Schreiber U (1998) Temperature-induced bleaching of corals begins with impairment of the CO_2 fixation mechanism in zooxanthellae. *Plant, Cell and Environment*, 21:1219–1230.

Jørgensen HBH, Hansen MM, Bekkevold D, Ruzzante DE, Loeschcke V (2005) Marine landscapes and population genetic structure of herring (*Clupea harengus* L.) in the Baltic Sea, *Molecular Ecology*, 14:3219–3234.

Jost L (2008) G_{ST} and its relative do not measure differentiation, *Molecular Ecology*, 17:4015-4026.

Jump AS, Marchant R, Peñuelas J (2009) Environmental change and the option value of genetic diversity. *Trends in Plant Science*, 14, 51-58.

Kalisz S, Nason JD, Hanzawa FM, Tonsor SJ (2001) Spatial population genetic structure in Trillium grandiflorum: the roles of dispersal, mating, history, and selection, *Evolution*, 55:1560–1568.

Karl, SA, Castro AFL, Garla RC (2012) Population genetics of the nurse shark (*Giglymostoma cirratum*) in the western Atlantic. *Marine Biology*, 159, 489-498.

Knight TM, Miller TE (2004) Local adaptation within a population of *Hydrocotyle bonariensis*, *Evolutionary Ecology Research*, 6:103-114.

Koehl MAR, Hadfield MG (2004) Soluble settlement cue in slowly moving water within

coral reefs induces larval adhesion to surfaces, *Journal of Marine Systems*, 49, 75–88.

Kohler KE, Gill SM (2006) Coral point count with excel extensions (CPCE): a visual basic program for the determination of coral and substrate coverage using random point count methodology, *Computers and Geosciences*, 32:1259–1269.

Leberg PL (2002) Estimating allelic richness: effects of sample size and bottlenecks, *Molecular Ecology*, 11:2445–2449.

Ledoux JB, Garrabou J, Bianchimani O, Drap P, Féral JP, Aurelle D (2010) Fine-scale genetic structure and inferences on population biology in the threatened Mediterranean red coral, *Corallium rubrum*. *Molecular Ecology*, 19, 4204-4216.

Legendre P, Dale MRT, Fortin M-J, Gurevitch J, Hohn M, Myers D (2002) The consequences of spatial structure for the design and analysis of ecological field surveys, *Ecography*, 25:601–615.

Legendre P, Fortin M-J (1989) Spatial pattern and ecological analysis, *Vegetatio*, 80:107–138.

Legendre P, Fortin M-J (2010) Comparison of the Mantel test and alternative approaches for detecting complex multivariate relationships in the spatial analysis of genetic data, *Molecular Ecology Resources*, 10:831–844.

Legendre P, Lapointe FJ, Casgrain P (1994) Modeling brain evolution from behavior: a permutational regression approach, *Evolution*, 48:1487–1499.

Leichter JJ, Deane GB, Stokes MD (2005) Spatial and temporal variability of internal wave forcing on a coral reef, *Journal of Physical Oceanography*, 35, 1945–1962.

Leichter JJ, Helmuth B, Fischer AM (2006) Variation beneath the surface: quantifying complex thermal environments on coral reefs in the Caribbean, Bahamas and Florida, *Journal of Marine Research*, 64:563–588.

Leimu R, Fischer M (2008) A meta-analysis of local adaptation in plants, *PLoS ONE*, 3:e4010.

Lesser MP, Bythell JC, Gates RD, Johnstone RW, Hoegh-Guldberg O (2007) Are infectious diseases really killing corals? Alternative interpretations of the experimental and ecological data. *Journal of Experimental Marine Biology and Ecology*, 346:36–44.

Levin LA (2006) Recent progress in understanding larval dispersal: new directions and digressions, *Integrative and Comparative Biology*, 46:282–297.

Levin S (1992) The problem of pattern and scale in ecology: the Robert H. MacArthur award lecture. *Ecology*, 73, 1943–1967.

Linhart YB, Grant MC (1996) Evolutionary significance of local genetic differentiation in plants, *Annual Review of Ecology and Systematics*, 237–277.

Loiselle BA, Sork VL, Nason J, Graham C (1995) Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). *American Journal of Botany*, 82, 1420–1425.

Magalon H, Adjeroud M, Veuille M (2005) Patterns of genetic variation do not correlate with geographical distance in the reef-building coral *Pocillopora meandrina* in the South Pacific. *Molecular Ecology*, 14, 1861–1868.

Maier E, Tollrian R, Rinkevich B, Nürnberger B (2005) Isolation by distance in the scleractinian coral *Seriatopora hystrix* from the Red Sea. *Marine Biology*, 147, 1109–1120.

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology & Evolution*, 18, 189–197.

Mantel N (1967) The detection of disease clustering and a generalized regression approach, *Cancer research*, 27:209–220.

Marshall DJ, Monro K, Bode M, Keough MJ, Swearer S (2010) Phenotype-environment mismatches reduce connectivity in the sea, *Ecology Letters*, 13:128–140.

Marshall PA, Baird AH (2000) Bleaching of corals on the Great Barrier Reef: differential susceptibilities among taxa, *Coral Reefs*, 19:155–163.

Mayr E (1942) *Systematics and the Origin of Species, From the Viewpoint of a Zoologist*, Columbia University Press: New York, NY, USA.

McClanahan TR, Maina J, Moothien-Pillay R, Baker AC (2005) Effects of geography, taxa, water flow, and temperature variation on coral bleaching intensity in Mauritius, *Marine Ecology Progress Series*, 298:131–142.

McEdward LR (1995) The ecology of marine invertebrate larvae. CRC Press: Boca Raton, FL, USA.

McFadden CS (1997) Contributions of sexual and asexual reproduction to population structure in the clonal soft coral, *Alcyonium rudyi*, *Evolution*, 112–126.

McIntire EJB, Fajardo A (2009) Beyond description: the active and effective way to infer processes from spatial patterns, *Ecology*, 90:46–56.

Meeker ND, Hutchinson SA, Ho L, Trede NS (2007) Method for isolation of PCR-ready genomic DNA from zebrafish tissues. *BioTechniques*, 43, 610–614.

Meirmans PG (2012) The trouble with isolation by distance, *Molecular Ecology*, 21:2839-2846.

Meirmans P, van Tienderen P (2004) GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes*, 4, 792–794.

Mendes JM, Woodley JD (2002) Effect of the 1995-1996 bleaching event on polyp tissue depth, growth, reproduction and skeletal band formation in *Montastraea annularis*. *Marine Ecology Progress Series*, 235:93–102.

Meyer E, Davies S, Wang S, Willis BL, Abrego D, et al. (2009) Genetic variation in responses to a settlement cue and elevated temperature in the reef-building coral *Acropora millepora*, *Marine Ecology Progress Series*, 392:81–92.

Middlebrook R, Hoegh-Guldberg O, Leggat W (2008) The effect of thermal history on the susceptibility of reef-building corals to thermal stress, *Journal of Experimental Biology*, 211:1050–1056.

Miller KJ (1998) Short-distance dispersal of black coral larvae: Inference from spatial analysis of colony genotypes. *Marine Ecology Progress Series*, 163, 225–233.

Miller KJ, Ayre DJ (2004) The role of sexual and asexual reproduction in structuring high latitude populations of the reef coral *Pocillopora damicornis*. *Heredity*, 92, 557–568.

Moran PAP (1950) Notes on continuous stochastic phenomena. *Biometrika*, 37, 17–23.

Mumby PJ, Steneckn RS (2008) Coral reef management and conservation in light of rapidly evolving ecological paradigms, *Trends in Ecology and Evolution*, 23:555–563.

Mumby PJ, Skirving W, Strong AE, et al. (2004) Remote sensing of coral reefs and their physical environment. *Marine Pollution Bulletin*, 48:219–228.

Mundy CN, Babcock RC (1998) Role of light intensity and spectral quality in coral settlement: Implications for depth-dependent settlement?, *Journal of Experimental Marine Biology and Ecology*, 223:235–255.

Nakamura T, van Woesik R (2001) Water-flow rates and passive diffusion partially explain differential survival of corals during the 1998 bleaching event. *Marine Ecology Progress Series*, 212:301–304.

Nei M (1987) Molecular Evolutionary Genetics. Columbia University Press, New York

Nosil P (2008) Speciation with gene flow could be common, *Molecular Ecology*, 17:2103–2106.

Nosil P, Vines TH, Funk DJ (2005) Perspective: Reproductive isolation caused by natural selection against immigrants from divergent habitats, *Evolution*, 59:705–719.

Oetjen K, Reusch TB (2007) Genome scans detect consistent divergent selection among subtidal vs. intertidal populations of the marine angiosperm *Zostera marina*, *Molecular Ecology*, 16:5156–5157.

Palumbi SR (2003) Population genetics, demographic connectivity, and the design of marine reserves. *Ecological applications*, 13, S146–S158.

Palumbi SR (2004) Marine reserves and ocean neighborhoods: the spatial scale of marine populations and their management, *Annual Review of Environmental Resources*, 29:31–68.

Pauls SU, Nowak C, Bálint M, Pfenninger M (2013) The impact of global climate change on genetic diversity within populations and species, *Molecular Ecology*, 22:925–946.

Penin L, Adjeroud M, Schrimm M, Lenihan HS (2007) High spatial variability in coral bleaching around Moorea (French Polynesia): patterns across locations and water depths, *Comptes Rendus—Biologies*, 330:171–181.

Pebesma EJ, Wesseling CG (1998) *Gstat*: a program for geostatistical modelling, prediction and simulation, *Computers and Geosciences*, 24:17–31.

Petit RJ, Mousadik El A, Pons O (1998) Identifying populations for conservation on the basis of genetic markers, *Conservation Biology*, 12:844–855.

Pielou EC (1966) Shannon's formula as a measure of specific diversity: its use and misuse. *The American Naturalist*, 100, 463–465.

Pielou EC (1969) *An introduction to mathematical ecology*. Wiley Interscience, New York, New York.

Pinheiro JC, Bates DM, DebRoy S, Sarkar D (2006) NLME: linear and nonlinear mixed effects models, R package version 3.1-103.

Pinzón JH, LaJeunesse TC (2010) Species delimitation of common reef corals in the genus *Pocillopora* using nucleotide sequence phylogenies, population genetics and symbiosis ecology. *Molecular Ecology*, 20, 311–325.

Polato N, Concepcion GT, Toonen RJ, Baums IB (2010) Isolation by distance across the Hawaiian Archipelago in the reef-building coral *Porites lobata*. *Molecular Ecology*, 19, 4661–4677.

Raimondi PT, Morse ANC (2000) The consequences of complex larval behavior in coral, *Ecology*, 81:3193–3211.

Rangel TFLVB, Diniz-Filho JAF, Bini LM (2006) Towards an integrated computational tool for spatial analysis in macroecology and biogeography, *Global Ecology and Biogeography*, 15:321–327.

Rasband, W.S. (1997) *ImageJ*, U.S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/.

Raufaste N, Rousset F (2001) Are partial Mantel tests adequate?, *Evolution*, 55:1703–1705.

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, 86, 248–249.

Reidenbach MA, Koseff JR, Monismith SG (2007) Laboratory experiments of fine-scale mixing and mass transport within a coral canopy, *Physics of Fluids*, 19:Article ID 075107.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, 43, 223–225.

Richmond RH (1987) Energetic relationships and biogeographical differences among fecundity, growth and reproduction in the reef coral *Pocillopora damicornis*, *Bulletin of Marine Science*, 41:594–604.

Rocha LA, Robertson DR, Roman J, Bowen BW (2005) Ecological speciation in tropical reef fishes, *Proceedings of the Royal Society B: Biological Sciences*, 272:573–579.

Rocha LA, Bowen BW (2008) Speciation in coral-reef fishes, *Journal of Fish Biology*, 72:1101-1121.

Rousset F (2000) Genetic differentiation between individuals. *Journal of Evolutionary Biology*, 13, 58–62.

Rousset F (2008) GENEPOP'007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources*, 8, 103–106.

Rowan R, Knowlton N (1995) Intraspecific diversity and ecological zonation in coral-algal symbiosis, *Proceedings of the National Academy of Sciences*, 92:2850–2853.

Rowan R, Knowlton N, Baker A, Jara J (2001) Landscape ecology of algal symbionts creates variation in episodes of coral bleaching, *Nature*, 388:265–269.

Ruzzante D (1998) A comparison of several measures of genetic distance and population structure with microsatellite data: bias and sampling variance, *Canadian Journal of Fisheries and Aquatic Sciences*, 55:1–14.

Sanford E, Kelly MW (2011) Local adaptation in marine invertebrates, *Annual Review of Marine Science*, 3:509–535.

Schmidt-Roach S, Lundgren P, Miller KJ, *et al.* (2012) Assessing hidden species diversity in the coral *Pocillopora damicornis* from Eastern Australia. *Coral Reefs*.

Schoville SD, Bonin A, François O, Lobreaux S, Melodelima C, et al. (2012) Adaptive genetic variation on the landscape: methods and cases, *Annual Review of Ecology Evolution and Systematics*, 43:23–43.

Schwartz MK, McKelvey KS (2008) Why sampling scheme matters: the effect of sampling scheme on landscape genetic results, *Conservation Genetics*, 10:441–452.

Schwartz MK, Luikart G, Waples R (2007) Genetic monitoring as a promising tool for conservation and management. *Trends in Ecology and Evolution*, 22, 25-33.

Selkoe KA, Henzler CM, Gaines SD (2008) Seascape genetics and the spatial ecology of marine populations, *Fish and Fisheries*, 9:363–377.

Selkoe KA, Watson JR, White C, *et al.* (2010) Taking the chaos out of genetic patchiness: seascape genetics reveals ecological and oceanographic drivers of genetic patterns in three temperate reef species. *Molecular Ecology*, 19, 3708–

3726.

Severance EG, Karl SA (2006) Contrasting population genetic structures of sympatric, mass-spawning Caribbean corals. *Marine Biology*, 150, 57–68.

Shashar N, Kinane S, Jokiel PL, Patterson MR (1996) Hydromechanical boundary layers over a coral reef, *Journal of Experimental Marine Biology and Ecology*, 199:17–28.

Sherman CDH, Ayre DJ (2008) Fine-scale adaptation in a clonal sea anemone, *Evolution*, 62:1373–1380.

Sherman CDH, Ayre DJ, Miller KJ (2006) Asexual reproduction does not produce clonal populations of the brooding coral *Pocillopora damicornis* on the Great Barrier Reef, Australia. *Coral Reefs*, 25, 7–18.

Shick JM, Dunlap WC (2002) Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-protective functions in aquatic organisms. *Annual Review of Physiology*, 64:223–262.

Simpson EH (1949) Measurement of Diversity. *Nature*, 163, 688.

Slatkin M, Arter HE (1991) Spatial autocorrelation methods in population genetics, *The American Naturalist*, 138:499–517.

Smith JM, Smith NH, O'Rourke M, Spratt BG (1993) How clonal are bacteria? *Proceedings of the National Academy of Sciences*, 90, 4384–4388.

Smith LW, Birkeland C (2007) Effects of intermittent flow and irradiance level on back reef *Porites* corals at elevated seawater temperatures, *Journal of Experimental Marine Biology and Ecology*, 341:282–294.

Smith-Keune C, Van Oppen M (2006) Genetic structure of a reef-building coral from thermally distinct environments on the Great Barrier Reef, *Coral Reefs*, 25:493-502.

Smouse PE, Long JC, Sokal RR (1986) Multiple regression and correlation extensions of the Mantel test of matrix correspondence, *Systematic Zoology*, 35:627–632.

Sokal RR, Oden NL, Thomson BA (1997) A simulation study of microevolutionary inferences by spatial autocorrelation analysis, *Biological Journal of the Linnean Society*, 60:73–93.

Sotka EE (2005) Local adaptation in host use among marine invertebrates, *Ecology Letters*, 8:448–459.

Souter P, Henriksson O, Olsson N, Grahn M (2009) Patterns of genetic structuring in the coral *Pocillopora damicornis* on reefs in East Africa. *BMC Ecology*, 9, 1–13.

Spalding MD, Grenfell AM (1997) New estimates of global and regional coral reef areas. *Coral Reefs*, 16:225–230.

Sheppard C, Obura D (2005) Corals and reefs of Cosmoledo and Aldabra atolls: extent of damage, assemblage shifts and recovery following the severe mortality of 1998. *Journal of Natural History*, 39:103–121.

Shumway RH, Storfer DS (2006) *Time Series Analysis and Its Applications: With R Examples*, Springer: New York, NY, USA.

Stake JL, Sammarco PW (2003) Effects of pressure on swimming behavior in planula larvae of the coral *Porites astreoides* (Cnidaria, Scleractinia), *Journal of Experimental Marine Biology and Ecology*, 288:181–201.

Starger CJ, Barber PH, Ambariyanto, Baker AC (2010) The recovery of coral genetic diversity in the Sunda Strait following the 1883 eruption of Krakatau. *Coral Reefs*, 29, 547–565.

Starger CJ, Yeoh SSR, Dai C-F, Baker AC, DeSalle R (2008) Ten polymorphic STR loci in the cosmopolitan reef coral, *Pocillopora damicornis*. *Molecular Ecology Resources*, 8, 619–621.

Stat M, Morris E, Gates RD (2008) Functional diversity in coral-dinoflagellate symbiosis, *Proceedings of the National Academy of Sciences*, 105:9256–9261.

Stat M, Baker AC, Correa AMS, *et al.* (2012) Molecular delineation of species in the coral holobiont. In: *Advances in Marine Biology*, pp. 1–65. Advances in Marine Biology.

Stenberg P, Lundmark M, Saura A (2003) MLGsim: a program for detecting clones using a simulation approach. *Molecular Ecology Notes*, 3, 329–331.

Stoddart JA (1983a) Asexual production of planulae in the coral *Pocillopora damicornis*. *Marine Biology*, 76, 279–284.

Stoddart JA (1983b) A genotypic diversity measure. *Journal of Heredity*, 74, 489–490.

Stoddart JA (1984) Genetical structure within populations of the coral *Pocillopora*

damicornis. *Marine Biology*, 81, 19–30.

Stoddart JA (1988) Coral populations fringing islands: Larval connections. *Australian Journal of Marine and Freshwater Research*, 39, 109–115.

Storfer A, Murphy MA, Evans JS, *et al.* (2007) Putting the "landscape" in landscape genetics. *Heredity*, 98, 128–142.

Thioulouse J, Chessel D, Champely S (1995) Multivariate analysis of spatial patterns: a unified approach to local and global structures, *Environmental and Ecological Statistics*, 2:1–14.

Thompson TL, Glenn EP (1994) Plaster standards to measure water motion, *Limnology and Oceanography*, 39:1768–1779.

Tioho H, Tokeshi M, Nojima S (2001) Experimental analysis of recruitment in a scleractinian coral at high latitude. *Marine Ecology Progress Series*, 213, 79–86.

Tobler WR (1970) A computer movie simulating urban growth in the Detroit region. *Economic Geography*, 46, S234–S240.

Tonsor SJ, Kalisz S, Fisher J, Holtsford TP (1993) A life-history based study of population genetic structure: seed bank to adults in *Plantago lanceolata*, *Evolution*, 47:833–843.

Toonen RJ, Pawlik JR (2001) Foundations of gregariousness: a dispersal polymorphism among the planktonic larvae of a marine invertebrate, *Evolution*, 55:2439–2454.

Underwood JN, Smith LD, van Oppen MJH, Gilmour JP (2007) Multiple scales of genetic connectivity in a brooding coral on isolated reefs following catastrophic bleaching. *Molecular Ecology*, 16, 771-784.

van Kleunen M, Fischer M (2001) Adaptive evolution of plastic foraging responses in a clonal plant, *Ecology*, 82:3309-3319.

van Oppen MJH, Gates RD (2006) Conservation genetics and the resilience of reef-building corals. *Molecular Ecology*, 15, 3863–3883.

van Oppen MJH, Souter P, Howells EJ, Heyward A, Berkelmans R (2011) Novel genetic diversity through somatic mutations: Fuel for adaptation of reef corals? *Diversity*, 2011, 405–423.

Vekemans X, Hardy OJ (2004) New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology*, 13, 921–935.

Veliz D, Duchesne P, Bourget E, Bernatchez L (2006) Genetic evidence for kin aggregation in the intertidal acorn barnacle (*Semibalanus balanoides*). *Molecular Ecology*, 15, 4193–4202.

Vermeij MJA, Sandin SA, Samhouri JF (2007) Local habitat distribution determines the relative frequency and interbreeding potential for two Caribbean coral morphospecies. *Evolutionary Ecology*, 21, 27–47.

Warner ME, Fitt WK, Schmidt GW (1999) Damage to photosystem II in symbiotic dinoflagellates: a determinant of coral bleaching. *Proceedings of the National Academy of Sciences*, 96:8007–8012.

Watts RJ, Johnson MS, Black R (1990) Effects of recruitment on genetic patchiness in the urchin *Echinometra mathaei* in Western Australia. *Marine Biology*, 105, 145–151.

Weersing K, Toonen R (2009) Population genetics, larval dispersal, and connectivity in marine systems, *Marine Ecology Progress Series*, 393:1–12.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution*, 38, 1358–1370.

West JM, Salm RV (2003) Resistance and resilience to Coral Bleaching: implications for Coral Reef conservation and management, *Conservation Biology*, 17:956–967.

Whitaker K (2006) Genetic evidence for mixed modes of reproduction in the coral *Pocillopora damicornis* and its effect on population structure. *Marine Ecology Progress Series*, 306, 115–124.

Wiens J (1989) Spatial scaling in ecology. *Functional Ecology*, 3, 385–397.

Winter A, Appeldoorn RS, Bruckner A, Williams Jr. EH, Goenaga C (1998) Sea surface temperatures and coral reef bleaching off La Parguera, Puerto Rico (northeastern Caribbean Sea), *Coral Reefs*, 17:377–382.

Wright S (1943) Isolation by distance. *Genetics*, 28, 114-138.

Yeoh S-R, Dai C-F (2009) The production of sexual and asexual larvae within single broods of the scleractinian coral, *Pocillopora damicornis*. *Marine Biology*, 157, 351–359.

Zepp RG, Shank GC, Stabenau E, Patterson KW, Cyterski M, et al. (2008) Spatial and temporal variability of solar ultraviolet exposure of coral assemblages in the

Florida Keys: importance of colored dissolved organic matter, *Limnology and Oceanography*, 53:1909–1922.