Fine-scale population structure of two anemones (*Stichodactyla gigantea* and *Heteractis magnifica*) in Kimbe Bay, Papua New Guinea

Thesis by

Remy Gatins Aubert

In Partial Fulfillment of the Requirements

For the Degree of

Master of Science in Marine Science

King Abdullah University of Science and Technology, Thuwal,

Kingdom of Saudi Arabia

December 2014

The thesis of Remy Gatins Aubert is approved by the examination committee.

Committee Chairperson: Dr. Michael Berumen

Committee Member: Dr. Xabier Irigoien

Committee Member: Dr. Pablo Saenz-Agudelo

Committee Member: Dr. Anna Scott

© 2014

Remy Gatins Aubert

All Rights Reserved

ABSTRACT

Fine-scale population structure of two anemones (*Stichodactyla gigantea* and *Heteractis magnifica*) in Kimbe Bay, Papua New Guinea.

Anemonefish are one of the main groups that have been used over the last decade to empirically measure larval dispersal and connectivity in coral reef populations. A few species of anemones are integral to the life history of these fish, as well as other obligate symbionts, yet the biology and population structure of these anemones remains poorly understood. The aim of this study was to measure the genetic structure of these anemones within and between two reefs in order to assess their reproductive mode and dispersal potential. To do this, we sampled almost exhaustively two anemones species (*Stichodactyla gigantea* and *Heteractis magnifica*) at two small islands in Kimbe Bay (Papua New Guinea) separated by approximately 25 km. Both the host anemones and the anemonefish are heavily targeted for the aquarium trade, in addition to the populations being affected by bleaching pressures (Hill and Scott 2012; Hobbs et al. 2013; Saenz-Agudelo et al. 2011; Thomas et al. 2014), therefore understanding their biology is crucial for better management strategies. Panels of microsatellite markers were developed for each species using next generation sequencing tools. Clonality analyses confirm six pairs of identical genotypes for *S. gigantea* (n=350) and zero for *H. magnifica* (n=128), indicating presence/absence of asexual reproduction in this region. *S. gigantea* showed low structure between islands (F_{ST}= 0.003, p-value= 0.000), however, even if the majority of the individuals were unrelated (r~0), 81 families that shared 50% of their genetic material formed from two to four members were found. Out of these families,

45% were found with individuals only within Tuare Island, 11% only in Kimbe Island, and 44% were sharing individuals among islands. In comparison, *H. magnifica* showed no structure (F_{ST}= 0.002, p-value= 0.278), mean relatedness indicated the majority of individuals were unrelated, and 31 families were identified. Families again consisted from two to four members and were found within Kimbe Island 90% of the time, and shared between islands the remaining 10%. Results show the first genetic evidence of their reproductive characteristics, high levels of connectivity among islands and significant levels of genetic relatedness among individuals within islands.

Keywords: *Stichodactyla gigantea*, *Heteractis magnifica*, Microsatellites, Host anemone, Kinship, Connectivity

ACKNOWLEDGMENTS

I would first like to thank my advisor Dr. Michael Berumen for funding this thesis, as well as being supportive of all additional side projects. Dr. Pablo Saenz-Agudelo (Universidad Austral de Chile) was a key player during this project and mentored me from beginning to end in population genetics, always helping to put things into perspective. I thank Dr. Anna Scott (Southern Cross University) for constantly giving me feedback and insight on the biology of anemones. The KAUST Bioscience Core Lab did a great job carrying out the fragment analysis of all the samples. Additionally, I extend my gratitude to all those that were involved in collecting the samples, including: Dr. Geoff Jones, Hugo Harrison, Lisa Bostrom, Océane Salles, Mark Priest, Dr. Gerrit Nanninga, Dr. Mary Bonin, and Dr. Danielle Dixson. This project would not have been possible without their extensive time spent in the field. Moreover, I thank Alex Kattan for his help during the editing of the document, as well as May Roberts for providing a safe haven during my thesis period. Finally, I would like to thank my committee members for their constructive criticism and support during my thesis writing.

TABLE OF CONTENTS

LIST OF ABREVIATIONS

- 1. Introduction
  - NGO: Non governmental organization
  - MPA: Marine protected area
  - PLD: Pelagic larval duration
- 2. Methods
  - SCUBA: Self-Contained Underwater Breathing Apparatus
  - DNA: Deoxyribonucleic acid
  - PCR: Polymerase chain reaction
  - *Na*: Number of Alleles
  - *Ho*: Observed heterozygosity
  - *He*: Expected heterozygosity
  - *Fis*: Inbreeding coefficient
  - LD: Linkage disequilibrium
  - FDR: False Discovery Rate
  - HWE: Hardy-Weinberg Equilibrium
  - FL-PLS: Full likelihood-

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

1.1 Population genetics and marine conservation

There are at least 16 definitions of a population depending on whether one stands in an ecological, evolutionary, or statistical point of view (Waples & Gaggiotti 2006). For example, under an ecological perspective, a population can be defined as a group of organisms of the same species occupying a particular space at a particular time (Krebs 1994; Newman & Squire 2001). On the other hand, under an evolutionary perspective, a population can be defined as a group of individuals of the same species living close enough together for one to potentially mate with any other (Hartl & Clark 1988; Paetkau et al. 1995). Seeing as this study is focused on conservation genetics under an evolutionary approach, a population will therefore be defined as a group of individuals of the same species that coincide in a specific area and are capable of inter-breeding.

Darwin (1859) was the first to introduce the idea of evolution through means of natural selection by which genetic variability is accounted for. Evolution can lead to divergence in a species when gene flow between groups is limited or when one group is under selective pressure. When dealing with conservation biology, an ideal population maintains genetic variability, thereby enhancing adaptability to environmental changes (Frankham et al. 2010). Consequently, in order to assess the status of a population, or a species, it is essential to understand the distribution and changes of genetic variability found within the species and identify what evolutionary processes drive this (i.e. mutation, natural selection, gene flow, and genetic drift) (Frankham et al. 2010; Vandermeer & Goldberg 2013); this is the study of population genetics.

1.2 Population structure and conservation

Genetic barriers can drive the fragmentation of populations by limiting gene flow, leading to a range of minor to complex impacts. Different population structures may arise as a result (Allendorf & Luikart 2009; Frankham et al. 2010; Fox et al. 2001; Vandermeer & Goldberg 2003). The mainland-island situation occurs when a source ("mainland") provides input to a sink ("island") population (Cronin 2003). In addition, the island structure refers to when equal migration occurs between equal-sized islands (Wright 1931). The linear stepping-stone model occurs when exchange is present only between neighboring populations, commonly known as isolation by distance (Le Corre & Kremer 1998). Moreover, the two-dimensional stepping-stone model describes a scenario in which all neighboring populations exchange migrants (Maruyama 2007). Finally, a structure where regular extinction and recolonization events occur is referred to as a metapopulation (Hanski 1998).

Population isolation is often associated with short and long-term genetic consequences, with deleterious effects being most noticeable in the long-term (Hewitt 2001). Cessation of gene flow in small populations can lead to greater inbreeding, loss of genetic diversity, and greater genetic differentiation and is therefore associated with a high extinction risk or speciation (Frankham et al. 2010; Segelbacher et al. 2003). In other words, connectivity between populations is crucial from a genetic point of view; differentiation increases as gene flow decreases. In order to maintain high genetic diversity, conservation efforts should aim to maintain connectivity between spatially separated populations, especially when low population densities are present.

1.3 Reproduction and connectivity

A planktonic larval stage, followed by a sessile juvenile or adult phase, is a common characteristic in many marine species (Treml et al. 2008). Connectivity between populations in marine ecosystems is generally determined during this larval period and is subject to a variety of influences including biological characteristics of the larvae (e.g. fitness, dispersal duration, swimming ability, mortality, etc), physical factors of the environment (e.g. temperature, currents, salinity, etc.), and health of the source population (Cowen et al. 2006; Cowen & Sponaugle 2009; Saenz-Agudelo et al. 2009).

The ocean covers more than 70% of the earth's surface, less than 1% of it being coral reefs, which harbor approximately 25% of all marine life (www.noaa.gov). In order for these organisms to thrive, connectivity between populations must persist across large spatial scales and through harsh environments. Many marine organisms with a pelagic larval stage exhibit r-selection reproductive strategies, producing large numbers of young via broadcast spawning (Miller & Mundy 2003; Scott & Harrison 2005, 2007a) or the laying of demersal egg clusters (Doherty 1983; Moyer & Bell 1976), thereby increasing the probability of survival and maintaining gene flow over extensive areas.

1.4 Host anemones

Ten species of anemones are host to a total of 30 species of anemonefishes (Allen et al. 2008, 2010; Dunn 1981; Fautin & Allen 1992). Not only are host anemones crucial for the survival of these fishes (Fautin & Randall 1992; Mariscal 1970; Saenz-Agudelo et al. 2011b), but they also play an important role in the life history of other organisms, such as some species of crustaceans (Fautin et al. 1995; Guo et al. 1996), and the juvenile three-

spot dascyllus (*Dascyllus trimaculatus*) (Fautin & Randall 1992; Holbrock & Schmitt 2004; Mariscal 1970). Seeing as the anemonefish are iconic elements of coral reefs and popular among the general public, attention for their conservation is not hard to come by. Nonetheless, understanding the biology about the host anemones is necessary to protect these fish, given their dependence on the anemones.

The marine ornamental aquarium trade is a rapidly expanding global industry. Estimated import values of marine fish and invertebrates of US $24–40 million annually in the late 1980s (Wood 1985), increased to US $200–330 in the early 2000s (Wabnitz et al. 2003). Sea anemones that are symbiotic hosts for anemonefishes (hereafter "host anemones") are a major target of this industry. This is largely due to the global popularity of anemonefishes in the aquarium trade (Shuman et al. 2005). In addition, sea anemones contain zooxanthellae (Dunn 1981; Fautin & Allen 1997), making them susceptible to bleaching events (Hobbs et al. 2013). Decrease in the abundance and size of host anemones following bleaching events due to elevated sea temperatures has been recorded for some species (*Entacmaea quadricolor, Stichodactyla haddoni*), while others have been able to recover within a couple of months (*Cryptodendrum adhesivum, Heteractis magnifica, Stichodactyla mertensii*) (Hobbs et al. 2013). An increase in mortality (disappearance) has also been noted in some cases following these events, impacting both the population of host anemones and consequently their obligate symbionts, the anemonefish (Hattori 2002; Hobbs et al 2013; Saenz-Agudelo et al 2011b).

Reproduction in anthozoans can take place either by sexual or asexual means. The variant modes of reproduction found in sea anemones have been well studied, however, information on which mode each species utilizes is limited (Bocharova & Kozevich

2010). Sexual reproduction in these organisms can occur by broadcast spawning (e.g. *Metridium senile, Protanthea simplex, Entacmaea quadricolor, Heteractis crispa*) (‘Loseva, 1971; Scott & Harrison 2005, 2007) or brooding (e.g. *Urtcina crassicornis, Aulactina stella*) (Loseva 1971), the first being of greater prefecence (Bocharova & Kozevich 2010). Asexual reproduction in sea anemones can take place by different means: transverse or longitudinal fission, laceration, or autotomy of tentacles (Bocharova & Kozevich 2010. A host anemone utilizing asexual reproduction has been presumed in most cases where large clusters have been observed (Brolund et al. 2004; Fautin & Allen 1992; Scott & Baird 2014), however, a lack of genetic evidence has yet to corroborate these observations. Moreover, all host anemones appear to have separate sexes, with the exception of *Macrodactyla doreensis* that is hermaphroditic (Dunn 1981).

Information on embryonic and larval development for host anemones exists for *Entacmaea quadricolor* and *Heteractis crispa*, two gonochoric broadcast spawners (Scott & Harrison 2005, 2007a, 2007b). Spawning in these species occurs twice a year for *E. quadricolor* and up to four times for *H. crispa* (Scott & Harrison 2007a). Embryos first developed into planula 14 h ad 22 h after spawning, respectively, with the first directional movement being recorded at 36 h for both species.. *E. quadricolor* larvae had a survival rate higher than 90% during the first four days which then strikingly decreased to 50% at seven days. Only a few remained swimming when the larvae were 14 days old, however, at 59 days a small number of larvae were still actively swimming (Scott & Harrison 2007b). No information on the survival rates for *H. crispa* planulae were quantified.

1.5 Study Species: *Stichodactyla gigantea* and *Heteractis magnifica*

Stichodactyla gigantea inhabits shallow sandy areas and has a distribution from Micronesia west to the Red Sea and from Australia northward to the Ryukyu Islands. It is shown to be associated with eight anemonefishes (*Amphiprion akindynos, A. bicinctus, A. clarkii, A. melanopus, A. ocellaris, A. percula, A. perideraion, A. rubrocinctus*) (Fautin 1991; Fautin & Allen 1992; Ollerton et al. 2007).

Heteractis magnifica is distributed throughout the Indo-Pacific region, from French Polynesia to east Africa and from Australia northward to the Ryukyu Islands. This species is generally found fully exposed and attached to a solid substrate. Its cylindrical column is in bright colors (blue, green, red, white, brown), and the oral disc can reach up to one meter in diameter, although more commonly to 300-500 mm. It has been recorded in association with 13 anemonefish species (*A. akallopisos, A. akindynos, A. bicinctus, A. chrysogaster, A. chrysopterus, A. melanopus, A. clarkii, A. leucokranos, A. melanopus, A. nigripes, A. ocellaris, A. percula, A. perideraion)* (Fautin 1991; Fautin & Allen 1992; Ollerton et al. 2007), as well as the juvenile stage of the three spot damselfish (*D. trimaculatus*) (Holbrook & Schmitt 2005).

1.6 Study Site: Kimbe Bay, Papua New Guinea

Kimbe Bay is located in the Bismarck Sea on the north coast of New Britain Island, Papua New Guinea. Coastal communities of the area rely both on marine and terrestrial resources for their income and subsistence (TNC Pacific Island Countries Report No. 11/04). Since 1993 the Nature Conservancy, along with the government and a number of businesses, universities, and NGOs, have made efforts to increase awareness and participation of the community in conservation of their marine systems. Furthermore, for

approximately a decade now, actions have been made to establish a network of marine protected areas (MPAs) within the Kimbe Bay area. Tuare, Kappepa, and Kimbe Island, which are the focus sites for this study, form part of this larger MPA network (Green et al. 2009). Connectivity and self-recruitment between and within these sites have been recorded in previous studies comparing a benthic (*Amphiprion percula*) and a pelagic spawner (*Chaetodon vagabundus*). The pelagic spawner with a longer pelagic larval duration (PLD) showed lower self-recruitment rates but higher connectivity, however this varied over time presumably due to current patterns of the area (Berumen et al. 2012).

1.7 Aim of Study

The aim of this study is to assess the population structure of *Stichodactyla gigantea* and *Heteractis magnifica* at two reefs in Kimbe Bay, Papua New Guinea. Little is known of their reproduction and dispersal capabilities. Therefore, genetic tools were used to assess the fine-scale patterns and population structure at these islands. Results were compared and explained with the corresponding biology of each species, the physical environment, as well as compared with other connectivity studies done in the same area. It was hypothesized that *Stichodactyla gigantea* would show limited or no signs of asexual reproduction since there are no previous records of this. In comparison, *Heteractis magnifica*, considered mostly to reproduce clonally, should show a greater number of identical genotypes. What type of sexual reproduction is used by either of the species in question is unknown, however, if broadcast spawning were their mode of preference I would expect individuals to be found randomly in space, as this is considered long-range dispersal. If individuals that are genetically more similar are found closer together, this could be evidence of brooding as their sexual reproductive mode. In addition, previous

studies comparing *Chaetodon vagabundus* and *Amphiprion percula*, pelagic and benthic spawners, with relatively long (29–48 days) and short (10–13 days) PLDs, respectively, have shown connectivity between Tuare and Kimbe Island (Berumen et al. 2012). This study revealed greater self recruitment rates in *A. percula*, however *C. vagabondus* showed greater connectivity with more individuals dispersing to nearby islands. No information on the PLD of these specific host anemones exists, but based on evidence from *E. quadricolor* it seems that metamorphosis begins after seven days up to 14 days, with the exception that a few were still free-swimming at 59 days, indicating a lot of plasticity in the larvae(Scott & Harrison 2007b), I hypothesize that connectivity between islands 25 km apart will be present but not as common as recorded for *Chaetodon vagabundus*. This hypothesis is made based only on the comparisons of PLD, all other behavioral characters of the free swimming larvae are disregarded hence we're dealing with animals with a very different life history's, and cannot generalize behavior characters from larvae from different phylum's. All together, results will provide valuable information for the conservation of these species, as well as yield valuable knowledge on the connectivity patterns that occur within Kimbe Bay, PNG.

2. METHODS

2.1 Sample Collection

Anemones from Tuare Island (5°05’14.84”S, 150°11’39.85”E) and Kimbe Island (5°12’13.54”S, 150°22’32.69”E), were exhaustively tagged and mapped in 2011 (Figure 1 and 2). In April 2013, a group sampled individuals of *Stichodactyla gigantea* and *Heteractis magnifica* exhaustively at these two reefs over the course of six weeks. Tentacle were removed from each anemone were preserved in vials with 96% ethanol and transported to King Abdullah University of Science and Technology (KAUST), Saudi Arabia, where DNA extractions and genetic analysis took place. Depth and GPS coordinates were recorded.. A third reef was surveyed to add to the distribution and abundance patterns of the species, Kapepa Island (5°05’30.71”S, 150°12’07.15”E), located approximately 700 m away from Tuare Island. Only abundance and depth per species was recorded at this site.

Tissue samples were collected on SCUBA by 4 different buddy teams, consisting of 2-4 people, splitting up in an organized fashion to systematically cover as much area as possible, in order to circle each island completely. Every individual encountered from both species was sampled.

2.2 DNA extraction, sequencing, and characterization of microsatellite loci

Genomic DNA was extracted using Qiagen DNeasy kits according to the manufacturer’s protocol. Next-generation sequencing was done for one *S. gigantea* and one *H. magnifica*

sample using a Roche 454 GS-FLX (titanium) sequencer at the KAUST Bioscience Core Lab.

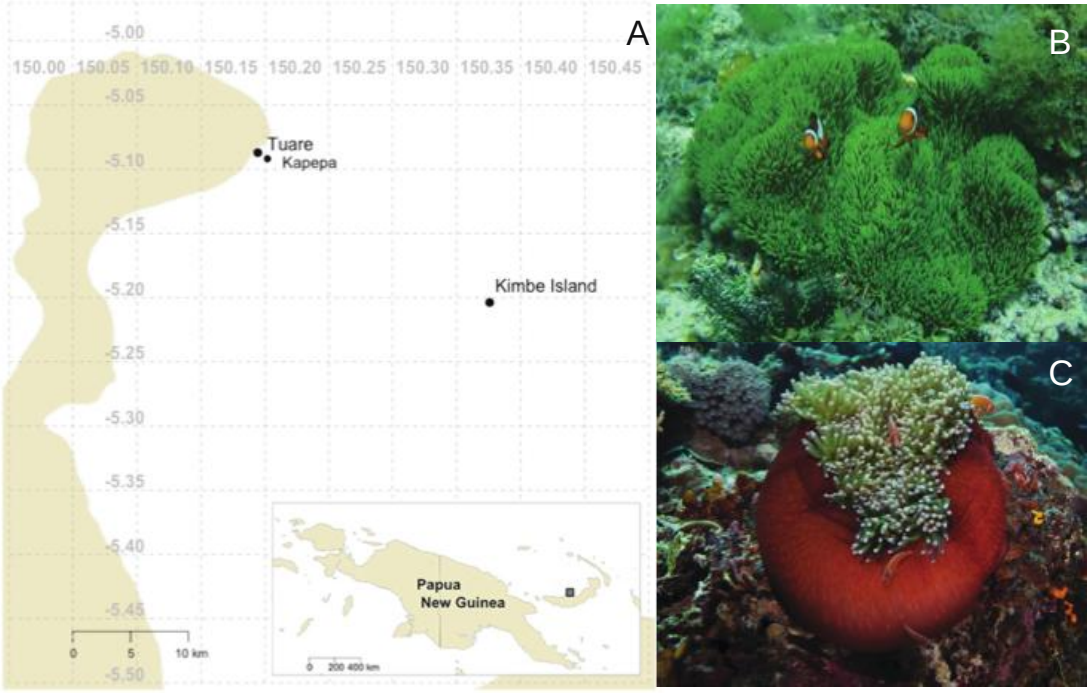

Figure 1. (A) Sampling sites in Kimbe Bay, Papua New Guinea. (B) *Stichodactyla gigantea* (www.aquaworldaquarium.com) and (C) *Heteractis magnifica* (photo by M. Priest) were exhaustively sampled at Tuare and Kimbe Island and used for all genetic analysis. Kapepa Island was only used to gather abundance data for the actinians.

The genomic library for each species was generated and raw unassembled reads were used to search for putative microsatellite loci. Microsatellites were identified from the library using the software MSATCOMMANDER v1.0.8 (Faircloth 2008) with Primer3 plug-in (Rozen & Skaletsky 2000). MSATCOMMANDER was set to default settings to generate perfect di-, tri-, and tetra-nucleotide repeats with a minimum length of 20 bp. A total of 90 microsatellite loci were selected per species and their respective primers were designed (using Primer3) for polymerase chain reaction (PCR) trials. The 90 loci were tested on four samples collected from Tuare and Kimbe Island following the Multiplex PCR kit protocol (Qiagen) with annealing temperatures ranging from 57 to 60

°C. The total PCR reaction volume was 10 µl; this corresponds to 5 µl of Multiplex Mix (Qiagen), 1 µl of primers (2µM), 3.3 µl water, and 0.7 µl of genomic DNA (50-150 ng/µl). PCR products were run on a Qiaxcel genetic analyzer (Qiagen) using a high-resolution cartridge to check for clear polymorphic loci. From 90 potential loci, 22 *S. gigantea* and 33 *H. magnifica* loci clearly amplified in at least 3 out of 4 individuals and were polymorphic. Forward primers for these elected loci were labeled with fluorescent tags (6-FAM, PET, NED, VIC), were placed accordingly into 3 multiplex mixes for each species (Table 1 and 2), and were tested on all samples of the species in question from both islands (*S. gigantea* n=368 , *H. magnifica* n=168). The same reaction volume as previously mentioned was followed using the subsequent thermal cycle: a denaturation step of 95 °C for 15 min, followed by 25 cycles at 94 °C for 30 s, annealing at a locus-specific temperature (57/60 °C) for 90 s and an extension at 72 °C for 60 s, with a final extension set at 60 °C for 30 min. PCR products were diluted with 130 µl of MilliQ water and mixed with Hi-Di formamide (Applied Biosystems) and GeneScan 500-LIZ size standard (Applied Biosystems). Furthermore, fragment analysis was conducted on a ABI 3730x1 genetic analyzer (Applied Biosystems). Allele sizes were manually scored using Geneious v 7.1.5 (Drummond 2011). Individuals with 4 or more pairs of alleles missing from the dataset were excluded from all genetic analysis.

Once scoring the alleles, allelic frequencies, number of alleles (*Na*), observed (*Ho*), and expected heterozygocities (*He*) were estimated for each population (Tuare & Kimbe Island) with Genalex v 6.5 (Peakall & Smouse 2012). For *H. magnifica*, because of having a low population size from Tuare (n=9), microsatellite loci was checked considering one total population by adding individuals from both sites (n=128). Presence

of linkage disequilibrium (LD), deviations from Hardy-Weinberg (HWE), and the inbreeding coefficient (*Fis*) were analyzed using Genepop (Raymond and Rousset 1995; Rousset 2008). False Discovery Rate (FDR) corrections were made for multiple testing according to Benjamin and Hochberg (1995). Finally, the presence of null alleles was tested using MICROCHECKER (Van Oosterhout et al. 2004) in order to check if this could explain deviations from HWE.

2.3 Genetic analysis

2.3a Clonality

Clones indicate the ability of a species to reproduce asexually; hence, clonality within a species was determined by measuring the amount of 100% multi locus matches and near matches using Genalex v 6.5. Results for near matches mismatching in one or two loci were reanalyzed by going back to the raw allele sizes to assess any possibility of scoring errors. The proportion of clones for each population was calculated by dividing the number of clones by the total number of individuals. Identified clones were then located on a map to assess their spatial distribution. In order to avoid over-representation of individual genotypes, one member of each pair of clones was removed for all subsequent analysis.

2.3b Dispersal patterns

A population (for the purposes of the present study) is defined as the spatial extent at which dispersal allows enough random mating to maintain genetic homogeneity in the allele frequencies between individuals. In order to determine dispersal patterns, the

following questions were addressed: a) are there one or more populations per species within the scale of the study? b) Are individuals that are geographically closer also genetically more similar as well? c) Are there differences between the population structure between species?

To answer the first question of genetic differentiation, F_{ST} values were calculated using running an AMOVA framework the infinite allele model with 100,000 permutations in GenoDive v 2.0b27. Due to current controversy of the correct metric to use when reporting differentiation (Bird et al. 2011; Jost 2008; Meirmans 2011), I also report values of G_{ST}, G'_{ST}, F'_{ST}, and D_{est}. Standardized fixation indices (G'_{ST} and F'_{ST}) are a corrected estimate of F_{ST} and G_{ST} in relation to its maximum achievable value (Hedrick 2005; Meimans 2006, 2011). G_{ST}, G'_{ST}, F'_{ST} and D_{est} were tested for significance in GenoDive v 2.0b27 under the same conditions previously mentioned.

2.3c Relatedness

Subsequently, an analysis of genetic relatedness was performed. Specifically, the genetic pairwise relatedness among individuals within and among islands was estimated and mean relatedness among groups (within vs among island) was compared. The null hypothesis here is that if random mating between sites is occurring, then mean relatedness between and within sites should not differ. Pairwise relatedness was calculated using the program KINGROUP v2_090501 (Konovalov et al. 2004), by taking into account the KINSHIP relatedness estimator (based upon Queller & Goodnight 1989). Relatedness for each species within and between islands was viewed in a boxplot to observe the general relatedness trend, as well as exceptions (outliers possibly due to

highly related pairs of individuals like parent-offspring, full-, and half-siblings) in the system. Relatedness values vary from negative one to one, generally anything less than or equal to zero indicate no relatedness, 0.25 and 0.5 indicate half and full siblings, respectively, and the value of one being an identical genotype match (in this case any clones would have already been taken out of the study system). Additionally, mean relatedness was calculated but now considering genetic distance between individuals (Genalex v 6.5, Queller & Goodnight 1989), with 9999 permutations to determine the 95% confidence intervals around the null hypothesis previously mentioned.

2.3d Spatial autocorrelation

In order to take a closer look at the system and check whether individuals genetically similar are spatially closer or further apart within sites, a spatial autocorrelation was examined by comparing genetic vs geographic distance between all individuals within each island. In this case our null hypothesis is that individuals are distributed randomly in space within the island. In other words, individuals that are geographically close are on average as genetically similar as individuals that are further apart. Genalex v 6.5 was used to generate one genetic distance and one geographic distance matrix for each island. Subsequently, an autocorrelation test was run using the same program by binning samples into 50m distance classes with 9,999 permutations and 9,999 boostraps to determine the 95% confidence intervals. In order to generate the geographic distance matrix, GPS coordinates for each sample are needed; therefore, samples lacking coordinates were removed for the purpose of this analysis (total remainder samples: *S. gigantea* n=244, *H. magnifica* n=80).

2.3e Kinship

Finally, since the number of related individuals might be too small to have an impact on the mean relatedness of the population(s) as well as the mean autocorrelation coefficient within distance bins, the focus of this analysis aimed at looking exclusively at those highly related individuals (full-siblings) found for each species, as well as to asses whether these full-sibs are found closer or further apart. The null hypothesis is that full-siblings are not necessarily located closer together, nor are they found only within an island. In other words, individuals sharing approximately 50% of their genotype will show no specific distribution structure. The program COLONY was used to determine full sibling pairs present in the entire data set of each species (n=337 *S. gigantea*, n=128 *H. magnifica*). It is important to point out that since our individuals could not be separated into adults and juveniles, the COLONY assigns pairs as full siblings but in reality these pairs can represent either full siblings or parent-offspring relationships. COLONY ran assuming a polygamous mating system without inbreeding in diploid organisms. A complex sibship prior was also presumed and the FL-PLS combined method was selected, using a medium length run and high likelihood precision with updating allelic frequencies. This program analyses the allelic frequencies of each individual and based on a full-likelihood approach, compares genotypes to either accept or reject them as full siblings. Once obtained the number of full sibs, these were plotted on a map to assess whether they showed any grouping pattern in certain locations or lagoons within the island. Moreover, the number of full sibs found within an island was compared with those that were shared between them. Kinship will help shed light on the fine-scale connectivity occurring in this study system. Results will be expressed in terms

of families, where a family refers to a group of members who all share on average 50% of their genotype with one another.

3. RESULTS

3.1 Species and study site

A total of 723 anemones, 576 *Stichodactyla gigantea,* and 147 *Heteractis magnifica*, were found at Tuare, Kimbe, and Kappepa Island (Figure 2). Abundance of *Stichodactyla gigantea* was approximately double in Tuare (n=245) and Kapepa (n=208), inshore reefs, than Kimbe Island (n=123), which is much further offshore (Figure 3). In addition, 78% of all individuals of this species showed a striking preference for shallow waters less than 1 m (Figure 4). *Heteractis magnifica* on the other hand, has a higher abundance in Kimbe Island (n=125) than in Tuare (n=22) and Kapepa (n=0) (Figure 3), and occurs randomly at depths ranging from 1 to 15 m (Figure 4).

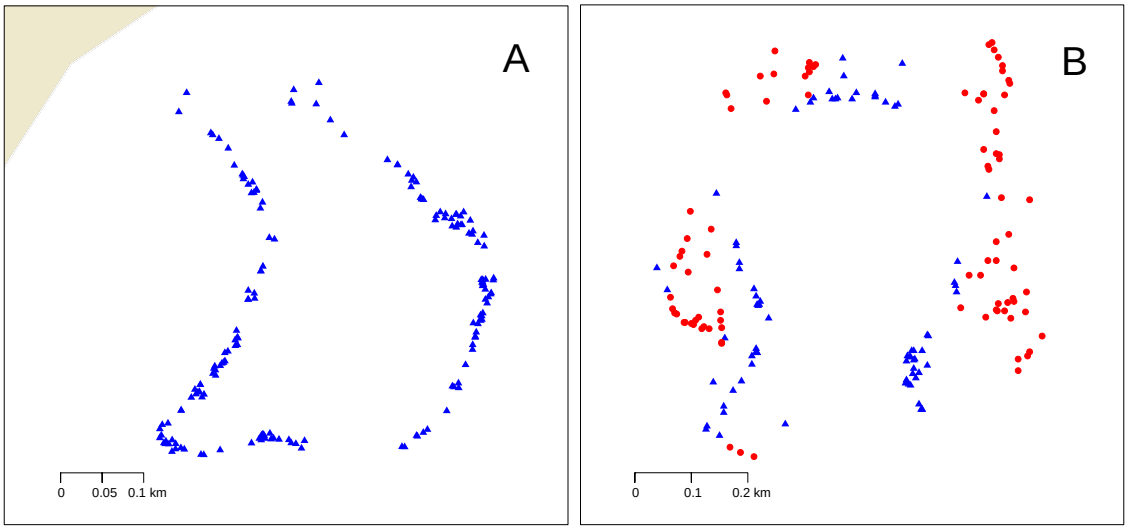

Figure 2. A) Map of Tuare Island plotting individuals of *Stichodactyla gigantea* (n=176) with blue triangles. B) Map of Kimbe Island plotting *Stichodactyla gigantea* (n=78) in blue triangles, and *Heteractis magnifica* (n=91) with red circles. More anemones than those shown on the map were used for the analysis, however coordinates were unavailable.

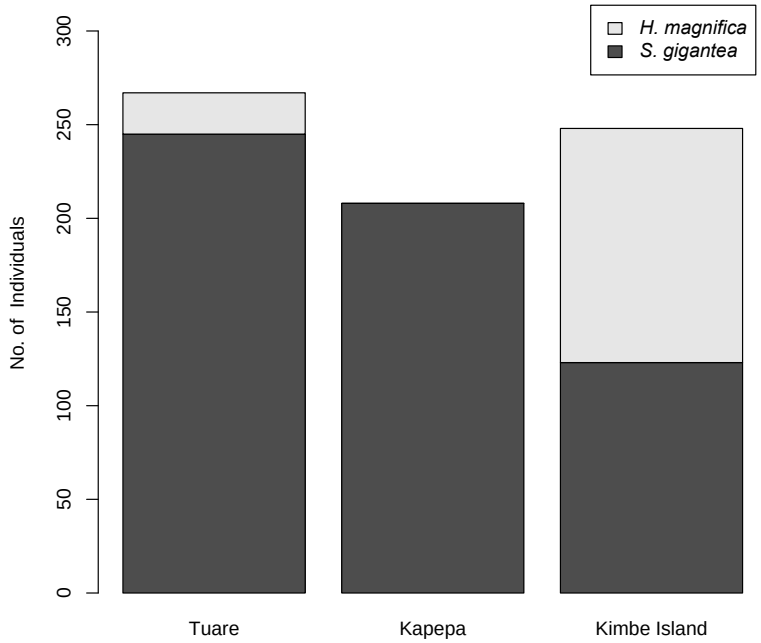

Figure 3. Total number of anemones in each reef. Dark grey represents *S. gigantea* and light grey indicates *H. magnifica*. Tuare and Kapepa are inshore reefs and Kimbe Island is approximately 30 km offshore.

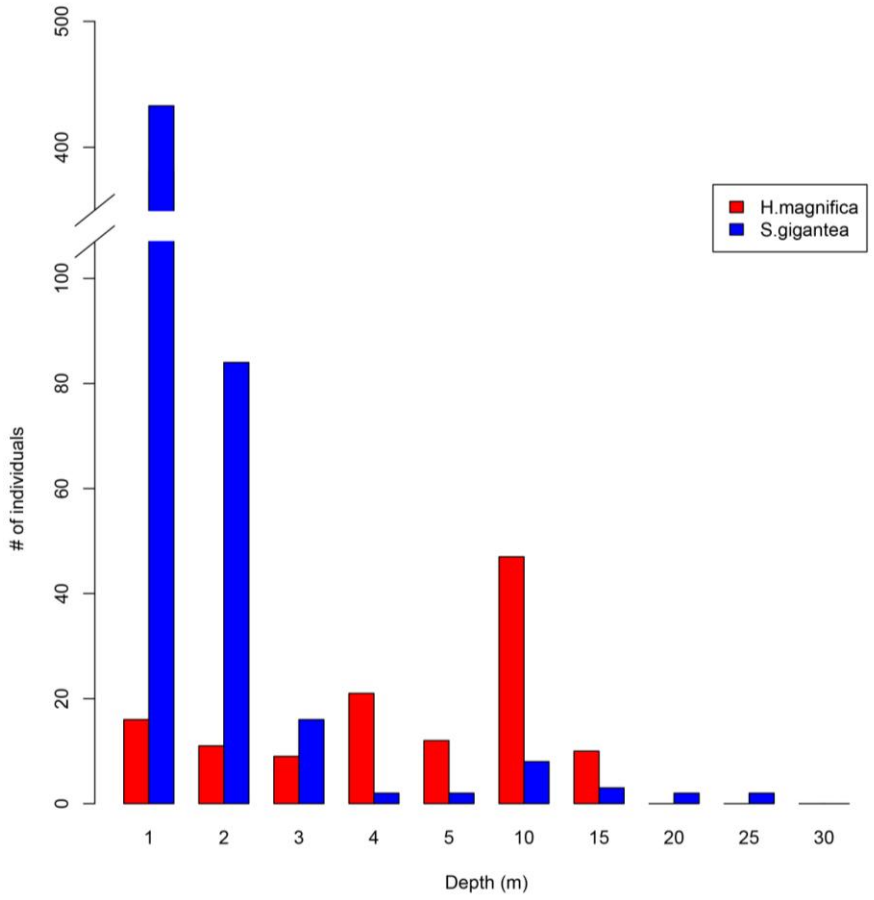

Figure 4. A comparison of the frequency distribution of the total number individuals per depth (m) of, *H. magnifica* (red) and *S. gigantea* (blue). Individuals from all three reefs (Tuare, Kapepa and, Kimbe Island) were taken into consideration. Depths from 0 to 5 m were pooled per meter due to high distinctions found in this zone, while depths from 5 to 30 m were compiled every 5 m.

3.2 Microsatellite statistics

Tissue samples for 368 *S. gigantea* and 136 *H. magnifica* samples were obtained and genotyped using 22 and 25 labeled loci, from which only 14 and 12, respectively, yielded clear fragment peaks for scoring. Samples with poor amplification due to low quality DNA were excluded from the analysis (*S. gigantea* n=19, *H. magnifica* n=10*)*, leaving a total of 350 and 128 samples, accordingly, to run the genetic analysis.

Observed and expected heterozygosity ranged from 0.802-0.913 and 0.763-0.903, for the 14 primers used for *S. gigantea* (Table 1). For each population, 8-27 alleles were found per loci resulting in an average of 168 alleles per population. Results from Genepop suggested that five loci showed deviations from HWE after correcting for FDR (Tuare: Sgig_45, Sgig_62, Sgig_75; Kimbe: Sgig_02, Sgig_61, Sgig_62, Sgig_75), However, only two loci diverged from HWE in both populations (Sgig_62, Sgig_75). Microchecker identified the presence of five null alleles (Tuare: Sgig_02, Sgig_45, Sgig_62, Sgig_75; Kimbe: Sgig_02, Sgig_51, Sgig_62, Sgig_75), three of these being consistent in both populations and were removed (Sgig_02, Sgig_62, Sgig_75). From the initial 14 loci scored, 3 previously mentioned were removed due to the presence of null alleles and one that amplified for multiple peaks and could have possibly affected scoring.

The 12 primer pairs of *Heteractis magnifica* yielded an observed heterozygosity from 0.344-0.977, and expected heterozygosity from 0.475-0.935 (Table 2). An average

of 14 alleles were found per loci, ranging between 7 and 32 alleles. Genepop suggested six loci deviated from HWE after FDR correction (Het06, Het15, Het19, Het21, Het23, Het46), from which three were identified as null alleles by Microchecker (Het15, Het19, Het23) and posteriorly removed. Deviations from HWE tend to occur in invertebrates, therefore loci that showed deviations but were not identified as null alleles were kept in analysis (Iacchei et al. 2013). Out of the initial 12 loci, 9 were used for the genetic analyses.

Table 1. Summary of 10 microsatellite markers isolated from *Stichodactyla gigantea* from two populations, Tuare and Kimbe Island, in Kimbe Bay, Papua New Guinea. Key: *Ta*-Annealing temperature, *N* - number of individuals, *Na* - number of alleles, *Ho* - observed heterozygosity, *He* - expected heterozygosity, *Fis* - inbreeding coefficient.

Locus		Primer sequence F and R (5´-3´)	Repeat motif	Ta (°C)	Size range	A	***TUARE***						***KIMBE***					
							N (/234)	*Na*	*Ho*	*He*	*Fis*	*p* value	*N (/116)*	*Na*	*Ho*	*He*	*Fis*	*p* value
Sgig23	F:	GCTCCTTTAATGTTGAGCACTC	$(AT)_{19}$	57	132-192	20	234	18	0.855	0.831	-0.026	0.025	116	18	0.905	0.865	-0.0423	0.043
	R:	CAACCTCCTCCACGCTTATG																
Sgig25	F:	AGCGATATCACCTTTGTCATCC	$(AG)_{25}$	57	239-297	17	230	17	0.835	0.788	-0.058	0.039	115	13	0.809	0.765	-0.053	0.032
	R:	TGTCAGCGGAATACTACCTGAG																
Sgig40	F:	AAATATCGGCAACCAAATCGAG	$(AG)_{19}$	57	242-258	9	234	8	0.876	0.782	-0.119	0.050	116	9	0.836	0.771	-0.080	0.039
	R:	GGTAACAGAGCTGCATGACG																
Sgig45	F:	CTGATCCTGGTCATGTGCAC	$(AC)_{18}$	57	246-310	27	229	27	0.803	0.895	0.104	0.004	116	22	0.879	0.882	0.007	0.029
	R:	ACAGGAGTGCACAGGTGATG																
Sgig49	F:	TCAAACTCATCTTGGCATGCC	$(AAT)_{28}$	60	139-190	15	234	12	0.872	0.851	-0.0218	0.043	116	15	0.836	0.874	0.048	0.025
	R:	AAACACGTCCGCACTACTTG																
Sgig51	F:	ATACTTGCAAACCTGGCTCG	$(AAT_{)25}$	57	146-192	16	233	16	0.867	0.901	0.040	0.018	116	15	0.802	0.878	0.091	0.018
	R:	AAAGCGCATTGAGACAGGTG																
Sgig54	F:	CGCGCGTCCATGTAATATCC	$(AAT)_{24}$	57	99-166	20	228	19	0.895	0.871	-0.025	0.029	115	18	0.913	0.898	-0.013	0.036
	R:	ACCACTAAAGATCAGTGTGCG																
Sgig59	F:	GCATAGCCTAGGACTAGTCTCG	$(AAT)_{28}$	60	213-279	21	234	19	0.889	0.903	0.018	0.032	116	17	0.853	0.896	0.052	0.021
	R:	ATTCGTTCCTTGACAACCGC																
Sgig61	F:	GTTTCGCGCCACCAAACG	$(AAG)_{25}$	57	193-283	24	234	21	0.833	0.879	0.054	0.021	116	21	0.853	0.869	0.022	0.011
	R:	GGGCTTCCTGTGTAAAGATTTG																
Sgig74	F:	ATAAACAGTGCCGACCTCCC	$(AAAT)_{21}$	60	108-184	17	233	17	0.841	0.818	-0.026	0.046	115	13	0.887	0.837	-0.056	0.050
	R:	AACGTATGTCAGATTGCGGG																

Table 2. Summary of 9 microsatellite markers isolated from *Heteractis magnifica* from two populations pooled together, Tuare and Kimbe Island, in Kimbe Bay, Papua New Guinea. Key: *Ta*-Annealing temperature, *N*-number of individuals, *Na*- number of alleles, *Ho*- observed heterozygosity, *He*- expected heterozygosity, *Fis*- inbreeding coefficient.

Locus		Primer sequence F and R (5´-3´)	Repeat motif	Ta (°C)	Size range	***Kimbe Bay***					
						N (/234)	*Na*	*Ho*	*He*	*Fis*	*p* value
Het_05	F:	GCTCCTTTAATGTTGAGCACTC	$(AT)_{19}$	57	132-192	128	12	0.9765	0.700	-0.392	0.050
	R:	CAACCTCCTCCACGCTTATG									
Het_06	F:	AGCGATATCACCTTTGTCATCC	$(AG)_{25}$	57	239-297	128	9	0.5781	0.595	0.032	0.025
	R:	TGTCAGCGGAATACTACCTGAG									
Het_21	F:	AAATATCGGCAACCAAATCGAG	$(AG)_{19}$	57	242-258	125	9	0.68	0.748	0.094	0.021
	R:	GGTAACAGAGCTGCATGACG									
Het_33	F:	CTGATCCTGGTCATGTGCAC	$(AC)_{18}$	57	246-310	120	32	0.9583	0.937	-0.019	0.029
	R:	ACAGGAGTGCACAGGTGATG									
Het_36	F:	TCAAACTCATCTTGGCATGCC	$(AAT)_{28}$	57	139-190	128	7	0.6093	0.543	-0.118	0.046
	R:	AAACACGTCCGCACTACTTG									
Het_38	F:	ATACTTGCAAACCTGGCTCG	$(AAT)_{25}$	57	146-192	128	7	0.6875	0.670	-0.023	0.042
	R:	AAAGCGCATTGAGACAGGTG									
Het_45	F:	CGCGCGTCCATGTAATATCC	$(AAT)_{24}$	57	99-166	128	12	0.5234	0.475	-0.097	0.038
	R:	ACCACTAAAGATCAGTGTGCG									
Het_46	F:	GCATAGCCTAGGACTAGTCTCG	$(AAT)_{28}$	57	213-279	128	12	0.8046	0.790	-0.014	0.017
	R:	ATTCGTTCCTTGACAACCGC									
Het_65	F:	GTTTCGCGCCACCAAACG	$(AAG)_{25}$	57	193-283	117	32	0.9401	0.935	-0.001	0.033
	R:	GGGCTTCCTGTGTAAAGATTTG									

3.3 Reproductive strategies and clonality

Out of 350 samples, seven pairs of clones were discovered for *Stichodactyla gigantea*, showing identical multilocus genotypes from Tuare Island. No additional clones were found in Kimbe Island. In addition, no clones were found for *Heteractis magnifica*.

3.4 Dispersal patterns

Population genetic structure for *S. gigantea* was low and significant (F_{ST} 0.003, p-value: 0.000; D_{est} 0.018, p-value 0.000), indicating the possibility of there being two populations present (p-value: 0.000), nonetheless, they do seem to be exchanging individuals which is why there is a low fixation index. Structure for *H. magnifica* was low but not significant (F_{ST} 0.002, p-value 0.316; D_{est} 0.009, p-value: 0.278), showing no structure. However, this could possibly be explained due to the low number of individuals found at Tuare (n=9).

Table 3. Summary of population structure statistics for both actinian species. F-satistics, G-statistics and Jost differentiation (D_{est}). F_{ST} , F'_{ST} (maximum F_{ST}), G_{ST} -Fixation index, G'_{ST} (Nei) -Nei, corrected fixation index, G'_{ST} (Hed) -Hedrick, standardized fixation index, G''_{ST} -Corrected standardized fixation index, D_{est} -Jost, differentiation.

	Stichodactyla gigantea		*Heteractis magnifica*	
		p-value		*p-value*
F_{ST}	0.003	0.000	0.002	0.316
F'_{ST}	0.02		0.08	
G_{ST}	0.001	0.000	0.002	0.273
G'_{ST} (Nei)	0.003	0.000	0.004	0.273
G'_{ST} (Hed)	0.019	0.000	0.011	0.277
G''_{ST}	0.021	0.000	0.012	0.276
D_{est}	0.018	0.000	0.009	0.278

3.5 Relatedness

The mean relatedness of *Stichodactyla* within and between reefs is approximately zero (within Tuare r= 0.00044 +/- 0.1262, within Kimbe r= -0.0022 +/- 0.1223, and between islands r= -0.0065 +/-0.1221) (Figure 7a). Interestingly, although all above values indicate no relatedness between the majority of individuals, groupings of outliers show possible half- (r=0.25) and full-siblings (r=0.5). Similar to *Stichodactyla*, *Heteractis* indicates that the average pairs of individuals are unrelated (within Tuare r= 0.0403 +/- 0.207, within Kimbe r= -0.012 +/- 0.1965, and between islands r= -0.0005 +/_0.2) (Figure 7b). In this case possible half- and full-sibs are found only within Kimbe Island and between reefs. No outliers are found within Tuare, however this might be due to the low sample size of this species at this reef (n=9).

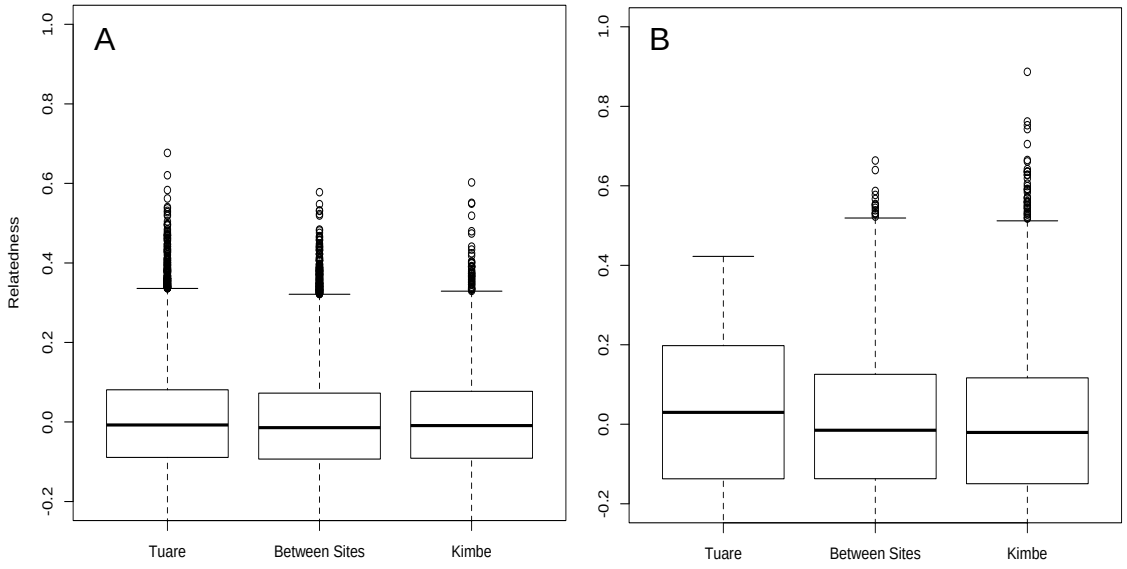

Figure 5. Comparison of pairwise relatedness of *S. gigantea* (A) and *H. magnifica* (B) individuals within and between sites. Relatedness values are read from zero (unrelated) to one (identical genotypes). Moreover, values less than or equal to zero are considered unrelated, half sibling are on average 0.25 and full sibling average r=0.5. Relatedness estimator is based upon Queller and Goodnight 1989 using KINGROUP. Boxplots show the mean relatedness as well as the upper and lower quartile of the data, th whiskers represent the 25% outside the middle 50% of the data, and the circles indicate the outliers within

the dataset). In average both species seem to be unrelated within and between sites, nonetheless, outliers indicate possible pairs of siblings within the dataset.

3.6 Spatial autocorrelation

For this test my null hypothesis is that individuals are distributed randomly within the island. The null hypothesis is accepted for *Stichodactyla*, but rejected for *Heteractis*. However, results show certain patterns of autocorrelation for each species within reefs. Spatial autocorrelation of genetic distance with geographic distance for *S. gigantea* in Tuare Island (Figure 6a) and Kimbe Island (Figure 6b) shows no significant structure. *Heteractis magnifica* had too few individuals for Tuare Island therefore this test could only be carried out for Kimbe Island. In this case individuals with higher relatedness dominated distance classes 0-50 m (r=0.022), 150-200 m (r=0.028) and 200-350 m (r=0.022), by falling outside the 95% confidence intervals (Figure 6c). The physical form of the island as well as currents in that area could possibly explain patterns and/or pockets of higher relatedness.

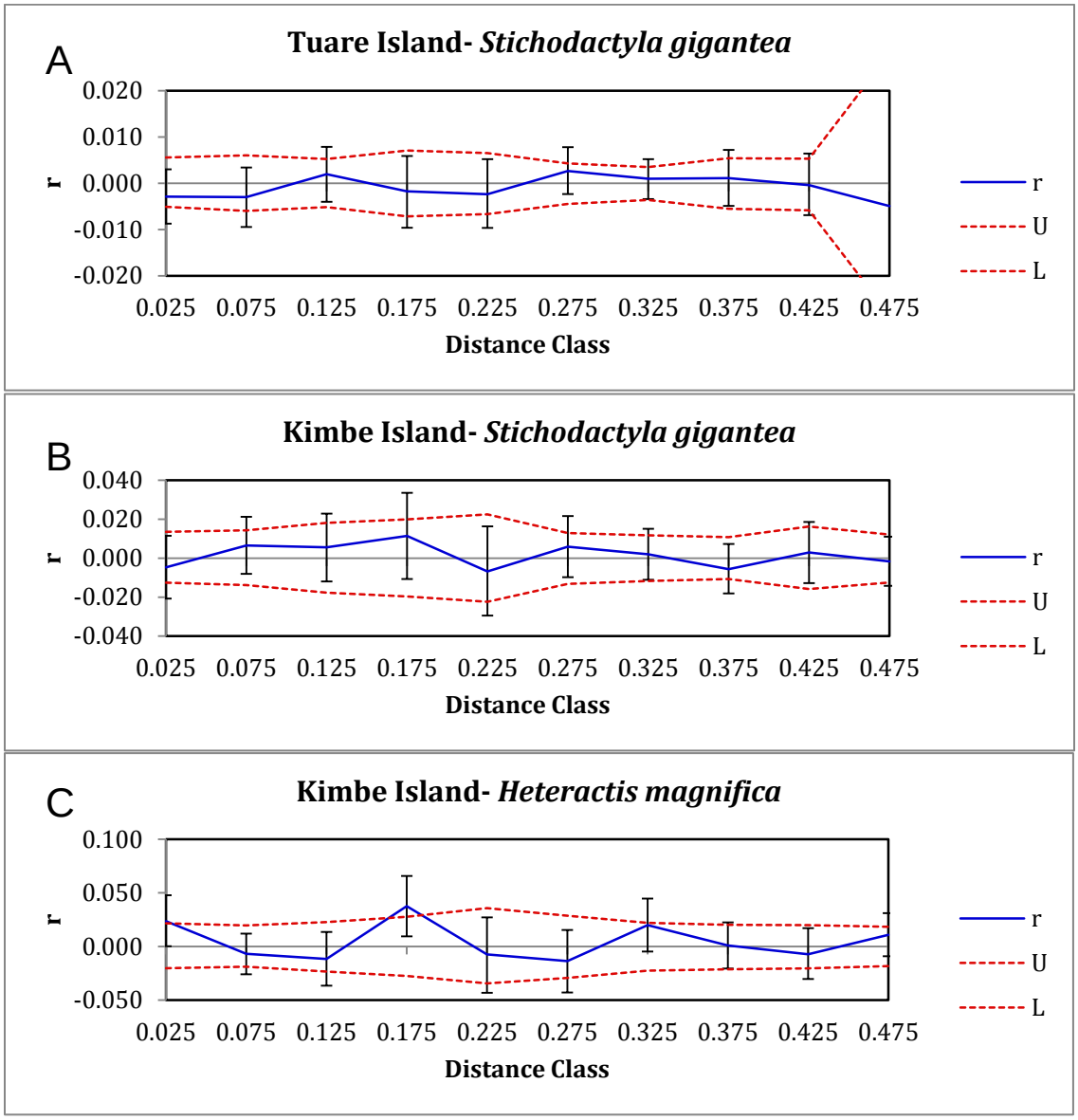

Figure 6. Spatial autocorrelation between genetic and geographic distance is shown for *S. gigantea* (a and b) and *H.magnifica* (c) at Tuare and Kimbe Island. Average relatedness estimation (r) is indicated by the blue solid line (Queller & Goodnight 1989, using Genalex v 6). Distance classes of 50m were selected. The dashed red lines represent 95% confidence intervals based on 9,999 permutations among all individuals.

3.7 Fine scale structure

COLONY results for full siblings in both species showed siblings composed up to four members. In some cases full siblings were not only found within the same island but between them, 25 km apart (Table 4 and 5). *Stichodactyla* (n=350) revealed a total of 82

families: two families with four members, nine with three members, and 71 with two members (Table 4). Out of the total number of families (n=82), 45% of those are found only within Tuare (n=37 families), 11% only within Kimbe (n=9 families), and 44% have individuals shared between both islands (n=36 families)

In addition, *Heteractis* (n=128) revealed a total of 31 families: one family of four members, ten with three, and twenty with two (Table 5). From these families, 90% were found only within Kimbe Island (n=28 families) and 10% were sharing members between islands (n=3 families). No families were found only within Tuare Island, but this might be due to the low sample size (n=9).

Table 4. Full sibling family assignments using COLONY from 337 individuals of *S. gigantea* found in Tuare and Kimbe Island. The program ran assuming a polygamous mating system without inbreeding in diploid organisms. A complex sibship prior was also presumed and the FL-PLS combined method was selected, using a medium length run and high likelihood precision with updating allelic frequencies. Missing data is represented by #N/A.

	Member 1					**Member 2**					**Member 3**					**Member 4**				
Fam #	***Reef***	***No.***	***Depth***	***Lat***	***Long***	***Reef***	***No.***	***Depth***	***Lat***	***Long***	***Reef***	***No.***	***Depth***	***Lat***	***Long***	***Reef***	***No.***	***Depth***	***Lat***	***Long***
1	K	290	1	-5.20735	150.3752333	T	4	1	-5.086339591	150.1932028	T	182	1	-5.087903487	150.1961667	T	182	1	-5.087903487	150.1961667
2	T	208	0.6	#N/A	#N/A	T	209	0.7	#N/A	#N/A	T	239	1	-5.088383267	150.1960828	T	246	1	-5.088334652	150.1961098
3	K	136	1	-5.205916667	150.37495	K	331	1	-5.207316667	150.3777	T	142	1	-5.087927207	150.1961327					
4	K	307	1	-5.20785	150.37765	K	334	1.8	-5.20745	150.37775	T	252	#N/A	#N/A	#N/A					
5	K	314	1.6	-5.2076	150.3777333	K	401	1	#N/A	#N/A	T	236	1	-5.088474797	150.196064					
6	T	13	1	-5.086876536	150.1935891	T	60	5.1	-5.085776243	150.1943551	T	192	1	-5.088288803	150.1961115					
7	T	21	1	-5.086939065	150.193674	T	136	1	-5.088530285	150.1934702	T	266	1	-5.089589087	150.1926452					
8	T	28	1	#N/A	#N/A	T	72	#N/A	#N/A	#N/A	T	143	1	-5.087907342	150.196119					
9	T	49	1	-5.089593278	150.193762	T	147	1	-5.08754449	150.1961511	T	232	1	-5.088610249	150.1960258					
10	T	68	1.6	-5.086666066	150.1952086	T	186	1	-5.088119656	150.1961637	T	186	1	-5.088119656	150.1961637					
11	T	140	1	-5.087944809	150.1961365	T	144	1	No GPS 2013	No GPS 2013	T	274	1	-5.089680701	150.192801					
12	K	302	1	-5.206016667	150.37495	K	514	1	-5.2033	150.3765167										
13	K	320	1	-5.206233333	150.3783833	K	572	1	-5.204816667	150.3745833										
14	K	438	4.5	#N/A	#N/A	K	310	1	-5.20825	150.37785										
15	K	444	1	#N/A	#N/A	K	502	1	-5.203433333	150.3774333										
16	K	449	1	#N/A	#N/A	K	482	1	#N/A	#N/A										
17	K	461	1	#N/A	#N/A	K	311	1	-5.208166667	150.3778167										
18	K	515	1	-5.203316667	150.3764833	K	550	12	-5.203233333	150.3771167										
19	K	525	1	-5.20565	150.3749	K	525	1	-5.20565	150.3749										
20	K	526	1	-5.206333333	150.3751833	K	261	1.8	-5.20825	150.3778667										
21	K	262	1.9	-5.207683333	150.3777167	T	128	1	-5.08878652	150.1933347										
22	K	265	1.4	-5.207466667	150.3777333	T	267	1	-5.089617083	150.192628										
23	K	268	1	#N/A	#N/A	T	50	1	-5.089630242	150.1938371										

24	K	269	1	-5.207483333	150.3776167	T	58	2.1	-5.085998951	150.194062
25	K	281	1	-5.206583333	150.3752333	T	111	1	-5.089459	150.1927172
26	K	286	1	-5.207333333	150.3752167	T	1	1	-5.086091068	150.1928348
27	K	287	1	-5.207283333	150.3752167	T	277	1	#N/A	#N/A
28	K	289	1	-5.20735	150.3752333	T	166	1	#N/A	#N/A
29	K	293	1	-5.2078	150.3749833	T	165	1	#N/A	#N/A
30	K	306	1	-5.207866667	150.3776833	T	127	1	-5.088802613	150.1933209
31	K	319	1	-5.206383333	150.3784167	T	248	1	#N/A	#N/A
32	K	325	1.3	-5.207066667	150.37795	T	235	1	-5.0885214	150.1960543
33	K	404	1	#N/A	#N/A	T	141	1	-5.087972134	150.1961333
34	K	410	1	#N/A	#N/A	T	244	1	-5.088335825	150.196126
35	K	411	1	#N/A	#N/A	T	139	1	-5.08793693	150.1961277
36	K	415	1	#N/A	#N/A	T	157	1	-5.089729652	150.1941666
37	K	417	1	#N/A	#N/A	T	135	1	-5.088539002	150.1934775
38	K	420	1	#N/A	#N/A	T	240	1	#N/A	#N/A
39	K	445	1	#N/A	#N/A	T	16	1	-5.087069152	150.1937432
40	K	447	1	#N/A	#N/A	T	79	1	-5.086902436	150.1953575
41	K	463	1	#N/A	#N/A	T	31	1	-5.08781313	150.1937253
42	K	481	9.6	#N/A	#N/A	T	6	1	-5.08648443	150.1933715
43	K	488	5	#N/A	#N/A	T	117	1	-5.089093968	150.1930095
44	K	511	1	-5.2034	150.3774833	T	153	1	-5.089638121	150.1940302
45	K	512	1	-5.20275	150.37755	T	119	1	-5.089116683	150.1930581
46	K	518	1	-5.2033	150.3761333	T	105	1	#N/A	#N/A
47	K	524	1	-5.2056	150.3749	T	264	1	-5.08947501	150.1926545
48	K	527	1	-5.206533333	150.3752833	T	139	1	-5.08793693	150.1961277
49	K	528	1	-5.206583333	150.3752667	T	154	1	-5.089671649	150.1940546
50	K	549	15	-5.203266667	150.3771167	T	273	1	-5.089737196	150.1927991
51	K	570	1	-5.208666667	150.3746333	T	125	1	-5.088836141	150.1932849
52	K	587	#N/A	#N/A	#N/A	T	148	1	-5.087416833	150.1960194
53	T	2	1	No GPS 2013	No GPS 2013	T	160	1	-5.089715654	150.1952873

54	T	3	1	-5.086319223	150.1931828	T	237	#N/A	#N/A	#N/A
55	T	5	1	#N/A	#N/A	T	5	1	#N/A	#N/A
56	T	7	1	#N/A	#N/A	T	30	1	-5.087812208	150.1937253
57	T	20	1	-5.08693127	150.1936787	T	32	1	#N/A	#N/A
58	T	22	1	-5.086939568	150.1936761	T	156	1	-5.089647761	150.1941997
59	T	27	1	#N/A	#N/A	T	134	1	-5.088558951	150.1934469
60	T	35	1	#N/A	#N/A	T	73	1.6	#N/A	#N/A
61	T	39	1	-5.089673661	150.1936236	T	124	1	-5.088841924	150.1932259
62	T	41	1	-5.089589674	150.1937319	T	190	1	-5.088267345	150.1961363
63	T	47	1	-5.089568384	150.1938244	T	97	1.7	-5.087311892	150.19591
64	T	55	2.4	#N/A	#N/A	T	181	1	-5.087960819	150.1961426
65	T	57	2.3	-5.085979253	150.1940548	T	236	1	-5.088474797	150.196064
66	T	64	1.5	#N/A	#N/A	T	191	1	-5.088299196	150.1961237
67	T	67	2	#N/A	#N/A	T	123	1	-5.088866232	150.1932154
68	T	69	1.4	-5.086663133	150.1952103	T	91	1.4	-5.087326979	150.1958032
69	T	74	1.8	-5.086832531	150.1953645	T	210	1.2	#N/A	#N/A
70	T	76	1.5	#N/A	#N/A	T	140	1	-5.087944809	150.1961365
71	T	86	1.5	#N/A	#N/A	T	276	1	-5.089740213	150.1928944
72	T	89	1.7	-5.087238969	150.1957223	T	235	1	-5.0885214	150.1960543
73	T	110	1	#N/A	#N/A	T	201	0.7	-5.087265288	150.196
74	T	115	1	-5.089041498	150.1930683	T	149	1	-5.087505514	150.1960812
75	T	122	1	-5.08888484	150.1932327	T	169	1	-5.089066895	150.1958227
76	T	132	1	-5.088620977	150.1934489	T	269	1	-5.08965346	150.1926944
77	T	140	1	-5.087944809	150.1961365	T	142	1	-5.087927207	150.1961327
78	T	143	1	-5.087907342	150.196119	T	269	1	-5.08965346	150.1926944
79	T	145	1	#N/A	#N/A	T	149	1	-5.087505514	150.1960812
80	T	151	1	-5.089621944	150.1938635	T	245	1	-5.088347308	150.1961127
81	T	155	1	-5.089681037	150.1941059	T	158	1	#N/A	#N/A
82	T	181	1	-5.087960819	150.1961426	T	244	1	-5.088335825	150.196126

Table 5. Full sibling family assignments using COLONY from 123 individuals of *H. magnifica* found in Tuare and Kimbe Island. The program ran assuming a polygamous mating system without inbreeding in diploid organisms. A complex sibship prior was also presumed and the FL-PLS combined method was selected, using a medium length run and high likelihood precision with updating allelic frequencies. Missing data is represented by #N/A.

	Member 1					**Member 3**					**Member 3**					**Member 4**				
Fam #	***Reef***	***No.***	***Depth***	***Lat***	***Long***	***Reef***	***No.***	***Depth***	***Lat***	***Long***	***Reef***	***No.***	***Depth***	***Lat***	***Long***	***Reef***	***No.***	***Depth***	***Lat***	***Long***
1	K	160	2.1	-5.203333333	150.3787667	K	116	6	-5.206966667	150.3744667	K	536	1	-5.208933333	150.3749667	K	820	#N/A	#N/A	#N/A
2	K	43	6.4	-5.202916667	150.3755	K	154	9	-5.202733333	150.3760667	K	220	4.1	-5.207083333	150.3797833					
3	K	62	3	-5.205466667	150.37925	K	228	9.8	-5.206783333	150.3743	K	219	3.3	-5.207333333	150.3795833					
4	K	157	2	-5.203216667	150.3747333	K	132	6	-5.205816667	150.374	K	827	#N/A	#N/A	#N/A					
5	K	167	8.8	-5.202866667	150.37915	K	235	6.6	-5.205733333	150.3740333	K	37	4.1	-5.202816667	150.37605					
6	K	183	2	-5.204183333	150.37905	K	233	2.8	-5.206733333	150.37395	K	194	1.3	-5.20655	150.3792333					
7	K	184	6	-5.204266667	150.3791	K	195	3	-5.206116667	150.3788	K	413	11	#N/A	#N/A					
8	K	190	1	-5.204116667	150.37885	K	50	5	-5.20325	150.37475	K	801	#N/A	#N/A	#N/A					
9	K	337	1	-5.205883333	150.37905	K	231	4.5	-5.20665	150.3738833	K	824	#N/A	#N/A	#N/A					
10	K	73	#N/A	#N/A	#N/A	K	144	#N/A	#N/A	#N/A	T	814	#N/A	#N/A	#N/A					
11	K	532	10.6	-5.206066667	150.3741333	T	192	#N/A	#N/A	#N/A	T	818	#N/A	#N/A	#N/A					
12	K	36	4.4	-5.2028	150.3761333	K	434	#N/A	#N/A	#N/A										
13	K	39	4.6	-5.20295	150.376	K	821	#N/A	#N/A	#N/A										
14	K	54	5.2	-5.20295	150.3752833	K	47	5.4	-5.20335	150.3753833										
15	K	98	3.9	-5.206483333	150.3793167	K	810	#N/A	#N/A	#N/A										
16	K	108	6.6	-5.205533333	150.3741167	K	834	#N/A	#N/A	#N/A										
17	K	112	6.5	-5.2051	150.3741667	K	35	3.2	-5.202766667	150.3761667										
18	K	158	3.2	-5.203016667	150.37925	K	125	3.3	-5.205783333	150.3744333										
19	K	159	7	-5.20325	150.3791833	K	421	6.6	#N/A	#N/A										
20	K	161	6.9	-5.20245	150.3789333	K	138	3.6	-5.207183333	150.3746667										
21	K	163	3.7	-5.202416667	150.3789833	K	808	#N/A	#N/A	#N/A										
22	K	164	9.7	-5.202533333	150.3790167	K	545	1	-5.20325	150.37605										
23	K	165	10.8	-5.20265	150.3790833	K	45	12.4	-5.20255	150.3755167										

24	K	226	8.6	-5.2069	150.3742167	K	197	3.4	-5.206683333	150.3791833
25	K	436	#N/A	#N/A	#N/A	K	822	#N/A	#N/A	#N/A
26	K	439	2	#N/A	#N/A	K	811	#N/A	#N/A	#N/A
27	K	521	1	-5.203066667	150.3792667	K	806	#N/A	#N/A	#N/A
28	K	523	1	-5.205883333	150.3789167	K	221	2.5	-5.2068	150.3792833
29	K	802	#N/A	#N/A	#N/A	K	812	#N/A	#N/A	#N/A
30	K	807	#N/A	#N/A	#N/A	K	829	#N/A	#N/A	#N/A
31	K	130	3.5	-5.20695	150.3746667	T	816	#N/A	#N/A	#N/A

4. DISCUSSION

This study shows the first genetic evidence of asexual reproduction in *S. gigantea*, as well as a lack of the same in *H. magnifica*, contrary to the initial hypothesis. *Stichodactyla* shows low structure between islands (p-value= 0.000), however, *H. magnifica* shows no structure. In addition, the vast majority of the individuals are unrelated, suggesting that many larvae are arriving from alternative sources other than Tuare and Kimbe, thereby indicating that these study sites are a smaller segment of a larger connectivity network. More than 30 full sibling groups were identified in both anemone species, within and between islands, raising various questions on whether this is the result of self-recruitment, dispersal between both sites, or cohorts arriving from alternative sources. Additionally, inquiries on whether these individuals are authentic full siblings or parent-offspring relationships arise. However, at an even greater resolution, irregularly spaced groups of related individuals within each island shed light on physical factors (i.e., currents, temperature, eddies, physical structure of the reef, etc.) that may play an important role in the connectivity, dispersal, and retention on the larvae stage of these animals in Kimbe Bay.

Giant sea anemones can reproduce sexually and asexually (Dunn 1981; Fautin & Allen 1992). Hattori & Kobayashi (2009) believed to have one individual of *S. gigantea* to have divided by asexual reproduction in their study, nonetheless, they could not be certain because of the lack of reproductive information for this species. In this study, I was able to confirm multiple instances of asexual reproduction using molecular techniques. Nonetheless, asexual reproduction does not seem to be the method of

preference, as the most common relationship I was able to document were groups of full siblings. In any case, this study shows that *S. gigantea* does have the ability to use this reproductive mode.

Interestingly, *H. magnifica* showed no signs of clonality, contrary to what was expected since it is found in big assemblages in some areas of its distribution (Brolund et al. 2004). Anemones sampled at Kimbe Bay generally did not exceed depths greater than 15 m, potentially shallower than a threshold size at which asexual reproduction becomes common (Brolund et al. 2004). Nonetheless, clusters in shallow depths within the Red Sea have been observed (*pers. obs*). Additionally, Brolund et al. (2004) illustrates a map pinpointing nine geographic regions within its distribution where large clusters of this species have either briefly been mentioned or recorded, suggesting that this species only forms clusters in certain areas. In addition, Scott and Baird (2014) found no clusters for this species in the central and southern mid shelf reefs of the Great Barrier Reef, supporting this theory. Other anemone species such as *Anthopleura elegantissima* (Hand 1955) and *Entacmaea quadricolor* (Dunn 1981) have also been observed as solitary individuals or in clusters in different geographic regions of their distribution. Whether this is due because of the presence or absence of asexual reproduction is uncertain. Additional factors may be driving this species to reproduce using both reproductive modes in some regions, while in other areas it only uses sexual reproduction. Further research is needed to underlie what is driving this distinctive reproductive pattern.

S. gigantea shows low structure between islands (F_{ST}=0.003, D_{est}=0.018, p-value=0.000), indicating some exchange but not enough to completely homogenize gene flow between populations. On the other hand, *H. magnifica* shows random mating

occurring between sites (F_{ST}=0.002, D_{est}=0.009, p-value= 0.278-0.316), nonetheless, this analysis could be biased because the sample size of Tuare is relatively small (n=9) from the overall dataset (n=119).

Mean relatedness within and between islands for both species is approximately zero, indicating that on average individuals within islands are unrelated. However, 82 and 31 family groupings of full siblings of *S. gigantea* and *H. magnifica*, respectively, were found both within and between the study sites. We encounter a few limitations when talking about full siblings identified in the area. The individuals we assume as full siblings could also be the product of a parent-offspring relationship since discriminating between parents and offspring in the field is not possible and in both cases individuals share 50% of their genetic material, precluding a molecular confirmation. Therefore, in the case of pairs of individuals that share 50% of their allelic frequencies (full sibs/parent-offspring) found between sites can fall into three different scenarios: a) The source of actinians is either Tuare or Kimbe Island, in which case we don't know which is the source, but one of the sources would retain part of the cohort (i.e., self-recruitment), while the other island would be receiving the dispersed individuals of the same cohort. b) Swapping between the two islands. c) Neither Tuare or Kimbe are the source of the cohort, therefore both islands share a common source (or sources) outside the area of this study. In the case of related individuals found within an island, this could indicate self-recruitment or a sibling cohort dispersing from the same initial source. All the information previously mentioned indicates that both anemone species from Tuare and Kimbe Island appear to fall into a bigger connectivity network beyond the scope of this study. Thereby, the population configuration of both anemone species is believed to be a

two-dimensional stepping-stone model, where all neighboring populations exchange migrants. The problem we face here is being able to identify the origin of dispersal. Nonetheless, connectivity between these islands seems to be present between these islands and probably more sites found at greater distances. Future work should include more reef sites spread further apart to understand possible dispersal limitations, in addition to figuring out a method to measure self-recruitment.

The spatial autocorrelation analyses between genetic and geographic distance gave insight of the possible existence of pockets of greater relatedness found within Kimbe Island for *H. magnifica* (Figure 6c). In order to take a closer look at the situation in both species, individuals grouped together by COLONY (Table 4 and 5), referred to as families, that had GPS coordinates were plotted to visualize the occurrence of individuals that share 50% of their allelic frequencies (Figure 7 and 8). *Stichodactyla* shows greater clustering on individuals on the eastern tip of Tuare Island when mapping families only found within the island (Figure 7.1). This could be driving the slightly greater relatedness value from 100-150m found in Figure 6a. Since the islands in question are relatively small (diameter of approximately 300m), and the autocorrelation analyses take into account direct distances (over land) instead of the shortest aquatic distance, the second patch of relatedness greater than 250 m could indicate individuals on opposite sides of the island. In the case of individuals only found within Kimbe Island for the same species in question, less number of families were found at Tuare possibly due to its lower abundance of individuals at this site (Figure 7.2). Nonetheless we can see a bit clearer that in some cases families tend to be near each other (approximately 150 m) or on opposite sides of the island (approximately 300 m away). There is no apparent pattern of

families being located in only certain areas, in other words, members of a family seem to be distributed at random, but those located close together might be because of local retention of the currents (i.e., eddies). Families shared between islands show no specific distribution patterns either (Figure 7.3a and 7.3b).

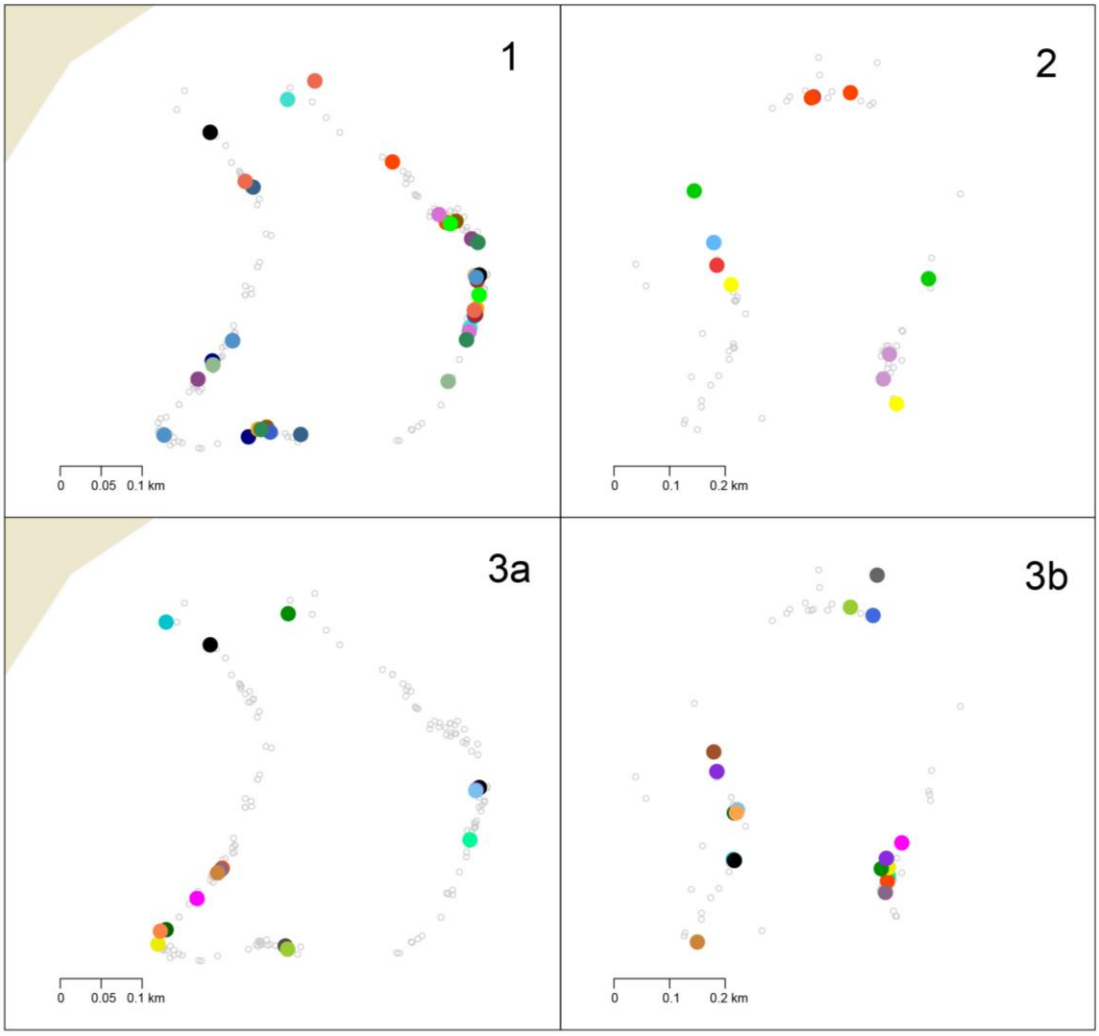

Figure 7. Map of related *Stichodactyla gigantea* individuals. Individuals sharing 50% of their genotype with one another are color coded (see Table 4). 1) Shows "full sib" map of "families" found only within Tuare. 2) Families found only within Kimbe Island. 3) Families that are shared between Tuare (3a) and Kimbe Island (3b).

In comparison, *H. magnifica* did show significant pockets of relatedness (Figure 6c). This can be driven because of the physical form of Kimbe Island and the presence of lagoons (Figure 8), which seems to be the habitat of preference of the species. The

distance between these lagoons is what might be accounting for the drop in relatedness between certain distance classes (Figure 6c).

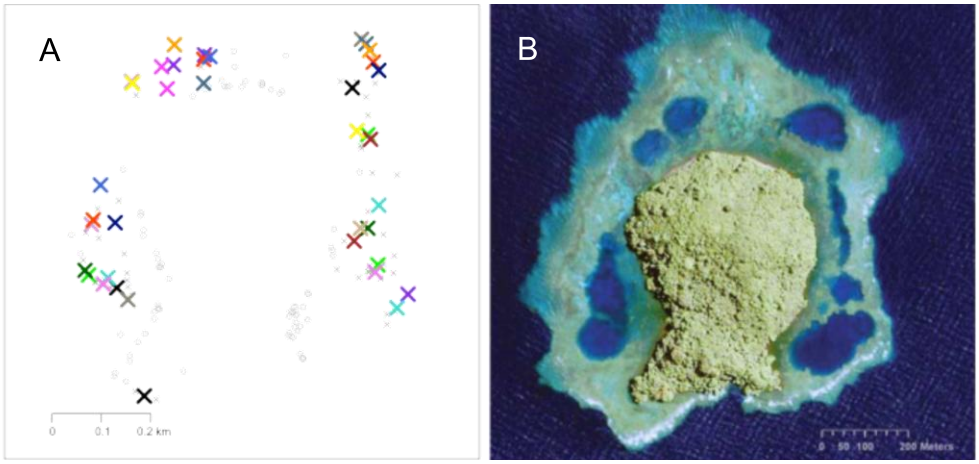

Figure 8. (A) Map of related *Heteractis magnifica* individuals at Kimbe Island, color coded to represent those individuals that share 50% of their genotype with one another (see Table 5). Grey circles represent individuals of *S. gigantea* used to delimitate the edge of Kimbe Island. Individuals shared between Kimbe and Tuare Island are not plotted because of lack of GPS coordinates. (B) Satellite image of Kimbe Island taken from Almany et al. 2007, to show the physical structure of the island and reef. Reef lagoons are clearly observed by the darker blue shades within the reef. These lagoons seem to play an important role in the distribution of *H. magnifica*.

S. gigantea shows greater preference for shallow and patchy reef substrates (Hattori & Kobashi 2009), while *H. magnifica* tends to occupy reef ridges (Brolund et al. 2004). Shallow habitat is most abundant at Tuare but occurs all around both islands. Therefore, the settlement of *S. gigantea* larvae randomly in space seems to be the product of the species large range dispersal mode (broadcast spawning), available habitat (shallow sandy patches), and currents of the area dispersing larvae around Kimbe Bay. Distance classes of pairs of individuals with slightly greater genetic similarity (Figure 6a and 6b) seem to be a random occurrence, not indicating any specific pattern. Additionally, *H. magnifica* seems to occur at random using the same sexual reproduction mode, nevertheless the presence of habitat where this species tends to occur might play a bigger role in its distribution. However, as previously mentioned, relatedness

distinguished by distance classes (Figure 6) takes into account direct distances, and future work should focus on interpreting shortest aquatic distances between highly related individuals.

5. CONCLUSIONS

By means of population genetics, this study provides a broader understanding of the reproductive and dispersal capabilities of host anemones, a vastly genetically understudied group. Fish have been a predominant subject of focus for the study of connectivity and dispersal, for the design of MPAs. Nonetheless, over 98% of the earth animals are invertebrates, more specifically, the basal structure of our coral reefs are formed by calcium carbonate depositing invertebrates. However, connectivity and dispersal of marine species cannot always be generalized in accordance to vertebrate patterns (Toonen et al. 2011). It is therefore important to understand the biology and capabilities of different invertebrate species, in order to identify possible alternative genetic barriers. Additionally, host anemones are heavily targeted for the aquarium trade and provide habitat for a variety of organisms. It is important to understand their biology to develop better management strategies.

This study underlies the first genetic evidence of asexual reproduction of *Stichodactyla gigantea* and the interesting absence of the same in *Heteractis magnifica*, in Kimbe Bay, PNG. Tuare and Kimbe Island show high levels of connectivity among islands and significant levels of genetic relatedness among individuals within islands. However, connectivity appears to be occurring at a larger network, beyond these study sites. As previously mentioned, anemonefish and their host anemones are heavily targeted in the aquarium trade (Madduppa et al. 2014), therefore understanding the dynamics of the biology of the host will help in the conservation both the anemones and the anemonefish. These findings support the decision that a large network of MPAs should be present in Kimbe Bay for its conservation and management since both

anemone populations cannot solemnly survive on the conservation of Tuare and Kimbe Island, but highly depend on the arrival of larvae from alternative sources.

REFERENCES

Allendorf FW, Luikart G (2009) Conservation and the Genetics of Populations. Blackwell Publishing

Allen GR, Drew J, Kaufman L (2008) *Amphiprion barberi*, a new species of anemonefish (Pomacentridae) from Fiji, Tonga, and Samoa. Aqua: International Journal of Ichthyology 14: 105–114. 24.

Allen GR, Drew J, Fenner D (2010) *Amphiprion pacificus*, a new species of anemonefish (Pomacentridae) from Fiji, Tonga, Samoa, and Wallis Island. Aquaculture 16: 129–138. 25.

Wood E (1985) Exploitation of coral reef fishes for the aquarium trade. Report to the Marine Conservation Society. Marine Conservation Society, UK

Benjamini Y, Hochberg Y (1995) Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society. Series B (Methodological) 57: 289 – 300

Bird CE, Karl SA, Smouse PE, Toonen RJ (2011) Detecting and measuring genetic differentiation. In: S. Koedemann, C. Held & C. Schubart (eds.) Phylogeography and Population Genetics in Crustacea. CRC Press Crustacean Issues Series 19: 31-55

Berumen ML, Almany GR, Planes S, Jones GP, Saenz-Agudelo P, Thorrold SR (2012) Persistence of self-recruitment and patterns of larval connectivity in a marine protected area network. Ecology and Evolution 2(2): 444-452

Brolund T, Tychsen A, Nielsen, LE, Arvedlund M (2004) An assemblage of the host anemone Heteractis magnifica in the northern Red Sea, and distribution of the resident anemonefish. Journal of the Marine Biological Association of the United Kingdom 84: 671-674

Cronin JT (2003) Movement and spatial population structure of a prairie planthoper. Ecology 84: 1179-1188.

Darwin C (1859) The Origin of Species by Means of Natural Selection. 1st ed. John Murray, London.

Doherty PJ (1983) Diel, lunar and seasonal rhythms in the reproduction of two tropical damselfishes: *Pomacentrys flavicauda* and *P. wardi*. Marine Biology 75: 215-224

Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Wilson A (2011) Geneious, version 5.4. Geneious, Auckland, New Zealand.

Dunn DF (1981) The clownfish sea anemone. Transactions of the American Philosophical Society 71: 2-115

Faircloth B (2008) MSATCOMMANDER: detection of microsatellite repeat arrays and automated, locus-specific primer design. Molecular Ecology Resources 8: 92–94

Fautin DG (1991) The anemonefish symbiosis: what is known and what is not. Symbiosis 10: 23-46

Fautin DG, Allen GR (1992) Field guide to anemone fishes and their host sea anemones. Western Australian Museum.

Fautin DG, Allen GR (1992) Anemonefishes and their host sea anemones. Western Australian Museum, Perth Grober-Dunsmore.

Fautin DG, Guo C, Hwang J (1995). Costs and benefits of the symbiosis between the anemoneshrimp *Periclimenes brevicarpalis* and its host *Entacmaea quadricolor*. Marine Ecology Progress *129*: 77–84

Fox CW, Roff DA, Fairbairn DJ (2001) Evolutionary Ecology: Concepts and Case Studies. Oxford University Press

Frankham R, Ballou JD, Briscoe DA (2010) Introduction to Conservation Genetics. Second Ed. Cambridge University Press.

Guo CC, Hwang JS, Fautin DG (1996) Host selection by shrimps symbiotic with sea anemones: a field survey and experimental laboratory analysis. Journal of Experimental Marine Biology 202: 165-176

Green A, Smith SE, Lipsett-Moore G, Groves C, Peterson N, Sheppard S, Lokani P, Hamilton R, Almany J, Aitsi J, Bualia L (2009) Designing a resilient network of marine protected areas for Kimbe Bay, Papua New Guinea. Oryx 43(4): 488-498

Hand C (1955) The sea anemones of central California, Part II. The Endomyarian and Mesomyarian anemones. Wasnian J. Biol. 13: 37-99

Hansky I (1998) Metapopulation dynamics. Nature 369: 41-49

Hartl DL, Clark AG (1988) Metapopulation dynamics, ecology, genetics, and evolution. Academic Press, New York.

Hattori A (2002) Small and large anemonefishes can coexist using the same patchy resources on a coral reef, before habitat destruction. Journal of Animal Ecology 71: 824–831

Hattori A, Kobayashi M (2009) Incorporating fine-scale seascape composition in an assessment of habitat quality for the giant sea anemone Stichodactyla gigantea in a coral reef shore zone. Ecology Resources 24: 415-422

Hedrick PW (1999) Highly variable loci and their interpretation in evolution and conservation. Evolution 53: 313–318

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

Hewitt GM (2001) Speciation, hybrid zones and phylogeography - or seeing genes in space and time. Molecular Ecology 10: 537–549

Hobbs JA, Frisch AJ, Ford BM, ThumsM, Saenz-Agudelo P, Furby KA, Berumen ML (2013) Taxonomic, spatial and temporal patterns of bleaching in anemones inhabited by anemonefishes. PLoS ONE 8:e70966

Holbrook SJ and Schmitt RJ (2005) Growth, reproduction and survival of a tropical sea anemone (Actiniaria): benefits of hosting anemonefish. Coral Reefs 24(1): 67-73

Iacchei M, Ben-Horin T, Selkoe KA, Bird CE, García-Rodríguez FJ, Toonen RJ (2013) Combined analyses of kinship and F_{ST} suggest potential drivers of chaotic genetic patchiness in high gene glow populations. Molecular Ecology 22: 3476-3494

Jones O, Wang J (2009) COLONY: a program for parentage and sibship inference from multilocus genotype data. Molecular Ecology Resources, 10: 551–555

Jost L (2008) GST and its relatives do not measure differentiation. Molecular Ecology 17: 4015-4026

Konovalov DA, Manning C, Henshaw T (2004) KINGROUP: a program for pedigree relationship reconstruction and kin group assignments using genetic markers. Molecular Ecology Notes 4: 779-782

Krebs CJ (1994) Ecology: The experimental analysis of distribution and abundance. Haper Collins, New York.

Le Corre V, Kremer A (1998) Cumulative effects of founding events during colonization on genetic diversity and differentiation in an island and stepping-stone model. Journal of Evolutionary Biology 11: 495-512.

Madduppa HH, con Juterzenka K, Syakir M, Kochzius M (2014) Socio-economy of marine ornamental fishery and its impact on the population structure of the clown anemonefish *Amphiprion ocellaris* and its host anemones in Spermonde Archipelago, Indonesia. Ocean & Coastal Management 100: 41-50.

Mariscal RN (1970) The nature of the symbiosis between Indo-Pacific anemone fishes and sea anemones. Marine Biology 6: 58-65

Maruyama T (2007) Analysis of population structure: II. Two-dimensional stepping stone models of finite length and other geographically structured populations. Annals of the human gene 35(2): 179-196

Meirmans PG (2006) Using the AMOVA framework to estimate a standardized genetic differentiation measure. Evolution 60: 2399–2402

Meirmans PG and Hedrick PW (2011) Assessing population structure: FST and related measures. Molecular Ecology Resources 11: 5–18

Moyer JT, Bell LJ (1976) Reproductive behavior of the anemonefish *Amphiprion clarkia* at Miyake-Jima, Japan. Japanese Journal of Ichthyology 23(1): 23-32

Miller K, Mundy C (2003) Rapid settlement in broadcast spawning corals: implications for larval dispersal. Coral Reefs 22: 99-106

Newman RA, Squire T (2001) Microsatellite variation and fine-scale population structure in the wood frog (*Rana sylvatica*). Molecular Ecology 10:1087-1100

Ollerton J, McCollin D, Fautin DG, Allen GR (2007) Finding NEMO: nestedness engendered by mutualistic organization in anemonefish and their hosts. Proceedings of the Royal Society doi: 10.1098/rspb.2006.3758

Paetkau D, Calvert W, Stirling I, Strobek C (1995) Microsatellite analysis of population structure in Canadian polar bears. Molecular Ecology 4: 347-354

Peakall R, Smouse P (2012) GenAlEx 6.5: genetic analysis in excel. Population genetic software for teaching and research–an update. Bioinformatics 28: 2537–2539

Planes S (2002) Biogeography and larval dispersal inferred from population genetic analysis. Coral Reef Fishes. Dynamics and Diversity in a Complex Ecosystem (ed. Sale P): 201–220. Academic Press, San Diego, California.

Pontash S, Scott A, Hill R, Bridge T, Fisher PL, Davy SK (2014) *Symbiodinium* diversity in the sea anemone *Entacmaea quadricolor* on the east Australian coast. Coral Reefs 33: 537-542

Queller DC, Goodnight KF (1989) Estimating Relatedness Using Genetic Markers. Evolution, 43: 258–275

Randall JE, Fautin DG (2002) Fishes other than anemonefishes that associate with sea anemones. Coral Reefs 2:188-190

Raymond M, Rousset F (1995) An exact test for population differentiation. Evolution 49(6): 1280–1283

Rousset F (2008) Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Molecular Ecology Resources 8(1): 103–106

Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics methods and protocols: Methods in Molecular Biology. Humana Press, Totowa, pp 365-386

Saenz-Agudelo P, Jones GP, Thorrold SR, Planes S (2009) Estimating connectivity in marine populations: an empirical evaluation of assignment tests and parentage analysis under different gene flow scenarios. Molecular Ecology 18: 1756-1776

Saenz-Agudelo P, Jones GP, Thorrold SR, Planes S (2011a) Connectivity dominates larval replenishment in a coastal reef fish metapopulation. Proceedings of the Royal Society 278: 2954-2961

Saenz-Agudelo P, Jones GP, Thorrold SR, Planes S (2011b) Detrimental effects of host anemone bleaching on anemonefish populations. Coral Reefs 30: 497-506

Scott A, Baird A (2014) Trying to find Nemo: low abundance of sea anemones and anemonefishes on central and southern mid-shelf reefs in the Great Barrier Reef. Marine Biodiversity DOI 10.1007/s12526-014-0245-0

Scott A, Harrison P (2005) Synchronous spawning of host sea anemones. Coral Reefs 24(2): 208-208

Scott A, Harrison P (2007a) Broadcast spawning of two species of sea anemone, *Entacmaea quadricolor* and *Heteractis crispa*, that host anemonefish. Invertebrate Reproduction and Development 50(3): 163-171.

Scott A, Harrison P (2007b) Embryonic and larval development of the host sea anemones Entacmaea quadricolor and Heteractis crispa. The Biological Bulletin 213: 110-121

Segelbacher G, Hoglund J, Storch I (2003) From connectivity to isolation: genetic consequences of population fragmentation in capercaillie across Europe. Molecular Ecology 12: 1773-1780

Shuman CS, Hodgson G, Ambrose RF (2005) Population impacts of collecting sea anemones and anemonefish for the marine aquarium trade in the Philippines. Coral Reefs 24: 564-573

Toonen RJ, Andrews KR, Baums IB, et al. (2011) Defining boundaries for ecosystem-based management: a multispecies case study of marine connectivity across the

hawaiian archipelago. Journal of Marine Biology, Article ID 460173, 13 pages, doi:10.1155/2011/460173

TNC Pacific Island Countries Report No 11/04. Designing a resilient network of marine protected areas in Kimbe Bay, West New Britain, Papua New Guinea. The Nature Conservancy

Treml EA, Halpin PN, Urban DL, Pratson LF (2008) Modeling population connectivity by ocean currents, a graph-theoretic approach for marine conservation. Landscape Ecology 23: 19-36

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro- Checker: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Resources 4: 535–538

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

Vandermeer JH, Goldberg DE (2003) Population Ecology: first principles. Princeton University Press.

Wabnitz C, TaylorM,Green E, Razak T (2003) From ocean to aquarium: the global trade in marine ornamental species. UNEP-WCMC, Cambridge

Waples RS, Gaggiotti O (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. Molecular Ecology 15: 1429-1439

Wright S (1931) Evolution in Mendelian populations. Genetics 16: 97-159.